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THE ANXIOMIMETIC PROPERTIES OF PENTYLENETETRAZOL IN THE RAT

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THE ANXIOMIMETIC PROPERTIES OF PENTYLENETETRAZOL IN THE RAT

BY

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ABSTRACT

Investigation of the biological basis of anxiety is hampered by the lack of an appropriate animal model for research purposes. There are no known drugs that cause anxiety in laboratory animals. Pentylenetetrazol (PTZ) produces intense anxiety in human volunteers (Rodin, 1958; Rodin and Calhoun, 1970). Therefore, it was the major objective of this dissertation to test the hypothesis that the discriminative stimulus produced by PTZ in the rat was related to its anxiogenic action in man. It was also an objective to suggest the neurochemical basis for the discriminative stimulus property of PTZ through appropriate drug interactions. In an operant procedure of lever pressing on a FR 10 schedule of food reinforcement, male hooded rats were trained to respond with a lever on one side of a food cup 15 min following a 20 mg/kg PTZ injection and to respond with a lever on the alternate side 15 min following a 1 ml/kg saline injection. All of the rats learned this discrimination reliably in a mean of 30 training sessions. The discriminative stimulus produced by PTZ was dose- and time-dependent. The anxiogenic stimulants cocaine, R05-3663 and strychnine generalized to the PTZ discriminative stimulus whereas yohimbine partially generalized.

The discriminative stimulus produced by cocaine, R05-3663 and yohimbine was antagonized by the anxiolytic drug, diazepam.

The discriminative stimulus produced by cocaine was not antagonized by haloperidol. The non-anxiogenic psychomotor stimulants d-amphetamine, methylphenidate or caffeine did not generalize to the PTZ discriminative stimulus. Bemegride was the only convulsant drug tested that generalized to the PTZ discriminative stimulus. Picrotoxin and 3-mercaptopropionic partially, but not significantly, generalized to the PTZ discriminative stimulus but bicuculline, gamma-hydroxybutyrate or nicotine did not generalize. The PTZ discriminative stimulus was dose-dependently antagonized by benzodiazepine-type, barbiturate-type, propanediol carbamate-type anxiolytics as well as valproic acid. Tolerance did not develop to antagonism of the PTZ of discriminative stimulus by diazepam or chlordiazepoxide. The discriminative stimulus produced by PTZ was not antagonized by an anticonvulsant or other central nervous system depressants that are not anxiolytics. There was a significant correlation between the potency of drugs effective in antagonizing the PTZ discriminative stimulus and their effective doses in a conflict test of anxiety as well as their clinically effective doses. The GABA antagonist R05-3663 generalized to the PTZ discriminative stimulus whereas picrotoxin and 3-mercaptopropionic acid partially generalized. The PTZ discriminative stimulus was antagonized by the GABA mimetic, valproic acid. The glycine antagonist, strychnine, generalized to the PTZ discriminative stimulus. Neither acetylcholine nor serotonin agonists or antagonists

generalized to or antagonized the PTZ discriminative stimulus. The catecholamine agonist, cocaine, generalized to the PTZ discriminative stimulus whereas yohimbine and apomorphine partially generalized. However, neither d-amphetamine nor methylphenidate generalized to the PTZ discriminative stimulus and catecholamine receptor antagonists did not antagonize the PTZ discriminative stimulus. Generalization to the PTZ discriminative stimulus by anxiogenic drugs and lack of generalization by non-anxiogenic psychomotor stimulants or convulsants as well as antagonism of the PTZ discriminative stimulus by anxiolytics but not non-anxiolytic anticonvulsant or depressants supports the hypothesis that the discriminative stimulus produced by PTZ in the rat is related to its anxiogenic action in man. Generalization to the PTZ discriminative stimulus by GABA antagonists and antagonism by GABA agonists but lack of generalization to or antagonism by drugs affecting other neurotransmitter systems suggests that the PTZ discriminative stimulus might be mediated through decreased GABA neuronal activity.

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INTRODUCTION

Anxiety is an unpleasant emotion for which millions of patients visit physicians each year seeking relief (Rickels et al., 1978). Anxiety can be defined as that experience or feeling of an unpleasantness or impending doom (Matz and Nash, 1979).

Although we suffer from and can define anxiety we do not understand its biological basis. This is partly because investigations of the biological basis of anxiety have been hampered by the lack of an appropriate animal model for research purposes. Presently, conflict paradigms such as those described by Geller and Seifter (1962) and Howard and Pollard (1977) are believed to provide the best animal models of anxiety. These procedures have been used to investigate the biological basis of anxiety as well as the neurochemical mechanisms underlying the anxiolytic action of anti-anxiety drugs (for reviews see Sepinwall and Cook, 1978; Lippa et al., 1979). However, with regard to elucidating the biological basis of anxiety these studies have not met with great success. In addition, conflict procedures are not without methodological problems that make interpretation of results difficult (Howard and Pollard, 1977). Therefore, a more appropriate animal model to investigate the biological basis of anxiety is needed.

Pentylenetetrazol (PTZ) has been reported to produce intense anxiety in human volunteers (Rodin, 1958; Rodin and Calhoun, 1970). In a personal communication, Rodin wrote "...within a matter of seconds [(after he had had himself injected with the drug by another physician)] I experienced catastrophic anxiety... It was a sense of utter distress and impending catastrophe. There is no doubt that this was one of the most anxiety producing events of my life."

Subjective effects of drugs cannot be measured directly in laboratory animals, however, it is believed that these effects are reflected in the discriminative stimulus properties of drugs in a number of cases (for review see Lal, 1977; Schuster and Balster, 1977; Colpaert and Rosecrans, 1978). Therefore it was the major objective of this dissertation to test the hypothesis that the discriminative stimulus produced by PTZ in the rat was related to its anxiogenic action in humans. It was also an objective to suggest a neurochemical basis of the discriminative stimulus property of PTZ in the rat.

It was hoped that characterization of the anxiomimetic property of PTZ in the rat utilizing the drug discrimination paradigm would provide a more appropriate animal model to investigate the biological basis of anxiety.

This research problem is of significance because it could contribute towards a better understanding of the biological basis of anxiety which could result in the development of better treatments for this unpleasant emotion.

LITERATURE REVIEW

BEHAVIORAL AND NEUROCHEMICAL ACTIONS OF PTZ

A. Behavioral Actions of PTZ

Most of the research to date on the behavioral actions of PTZ have been concerned with the effect of this drug on learning and memory. This section of the literature review will therefore concentrate on the effect of PTZ on these behaviors.

Facilitation of Learning

Several investigators have reported that subconvulsive doses of PTZ facilitate learning in a variety of experimental situations. Irwin and Benucazizi (1966) found that PTZ (1-30 mg/kg, p.o.) significantly facilitated one-trial learning and memory retention of CF 1 mice in a passive avoidance task. This effect occurred whether PTZ was injected before or immediately after the training trial and was greater than that observed with strychnine or picrotoxin.

Using a simple Y maze, Hunt and Krivanek (1966) and Krivanek and Hunt (1967) reported that post-trial injections of PTZ (20 mg/kg) improved learning of a black-white brightness discrimination. Animals treated with PTZ reached the criterion of learning earlier and made fewer errors than did saline treated animals.

Grossman (1969) bilaterally injected PTZ (5-10 ug) directly into the hippocamus of rats before as well as after training to perform a brightness discrimination. PTZ facilitated the learning of this discrimination and post-trial injections of PTZ were found to produce a significantly greater effect than pre-trial injections. It was suggested that PTZ facilitated learning by activating neural pathways concerned with memory consolidation processes.

Hunt and Bauer (1969) reported that subconvulsive doses (7.5-15 mg/kg) of PTZ, when injected immediately or 15 min following training of a black-white brightness discrimination, facilitated learning. Animals retested 24 h after training performed significantly better than saline treated animals. In a position discrimination task, Hunt and Bauer (1969) reported that PTZ (10 mg/kg) maximally facilitated learning when injected 10 min after training.

Bauer (1969) injected subconvulsive doses of PTZ daily for 20 days and reported this dosage regimen to facilitate learning of a shuttle box avoidance or a brightness discrimination task when testing occurred 24 h after the last PTZ injection. Interestingly, Bovet et al. (1966) had reported that whereas strychnine or picrotoxin enhanced shuttle avoidance learning, PTZ (5 or 10 mg/kg) did not. Buckholtz (1974) also reported that PTZ (20 mg/kg) did not facilitate shuttle avoidance learning. Using a wheelturn avoidance procedure, Krivanek (1971) reported subconvulsive doses of PTZ facilitated learning.

For the most part, studies employing subconvulsive doses of PTZ demonstrate that this drug facilitates learning. It has been suggested that PTZ produces this effect by facilitating memory consolidation.

Impairment of Learning

An impairment or loss of memory for events preceding a trauma is referred to as retrograde amnesia. Whereas subconvulsive doses of PTZ are reported to facilitate learning, convulsive doses of this drug produce retrograde amnesia or impair learning.

Palfai and Chillag (1971) reported that a convulsive dose (50 mg/kg) of PTZ, when injected up to 20 but not 60 min after training produced retrograde amnesia in mice for a step-through passive avoidance task. The degree to which PTZ produced retrograde amnesia was directly related to the intensity of the foot shock indicating that the nature of the original traumatic experience contributed towards the temporal characteristics of the retrograde amnesia. Because electroconvulsive shock had previously been reported to produce retrograde amnesia Palfai and Chillag (1971) suggested that a "massive neural explosion" causes retrograde amnesia.

Palfai and Kurtz (1973) reported that this convulsive dose of PTZ injected 15, 30 or 60 min but not 4 hr before a single step-through passive avoidance training impaired retention performance 24 h later. Interestingly, when this dose of PTZ was injected immediately after the training trial

it did not immediately produce retrograde amnesia, but rather the amnesia became apparent 4.5 h following the PTZ injection. These data indicate that a convulsive dose of PTZ can affect memory for a long (24 h) time after injection. Palfai and Kurtz (1973) suggested that PTZ may produce dissociated memory consolidation and that PTZ did not immediately produce amnesia because the drug state in which memory consolidation took place was still present when the rats were tested. However, when the animals were tested 4.5 h after the PTZ injection a different brain state may have existed that resulted in impaired retention.

Kurtz and Palfai (1973) tested for a dissociated or state-dependency hypothesis for the memory impairment produced by PTZ. Using the step-through passive avoidance task (Palfai and Kurtz, 1973), results were suggestive for the state-dependency hypothesis but PTZ produced a general depression of activity that may have confounded this interpretation. Therefore, Kurtz and Palfai (1973) used a discriminated escape paradigm to test for dissociative properties of PTZ. When reversal training and testing occurred under similar physiological states, i.e., both following PTZ injection (PTZ-PTZ) or both in the non-drug state (saline-saline), test performance was unimpaired. However, when PTZ was injected before reversal training but not before testing (PTZ-Saline) or vice versa (Saline-PTZ) test performance was impaired. These data suggest that retrograde amnesia produced by a convulsive dose of PTZ was related to its dis-

sociative or state-dependent effects. Furthermore, these data suggest the PTZ seizures may interfere with memory retrieval rather than memory consolidation.

To determine if convulsions were necessary for PTZ to produce state-dependent learning, Palfai and Kurtz (1976) tested for the ability of a subconvulsive dose (30 mg/kg) of PTZ to produce this phenomenon. Unlike the convulsive dose of PTZ that produced symmetrical dissociation, the subconvulsive dose produced asymmetrical dissociation, that is, whereas training in the non-drug state did transfer to the drug state during testing, training in the drug state did not transfer to testing in the non-drug state. Electroencephalographic (EEG) changes were still evident at the time when training and testing took place after injection of the convulsive dose but not the subconvulsive dose. Therefore, the EEG correlates could explain the asymmetrical dissociation produced by the non-convulsive dose of PTZ.

Whereas it was suggested (Weissman, 1968) that only convulsive doses of PTZ produce retrograde amnesia, van Buskirk and McGaugh (1974) reported that in Ha/ICR mice subconvulsive as well as convulsive doses of PTZ, injected after training of a passive avoidance task, impaired retention of the task. However, in C57 BL/6J mice convulsive doses of PTZ did not impair retention. Not only do these data point out species differences with respect to the effect of PTZ in producing retrograde amnesia, these data also indicate that convulsions are not a prerequisite for PTZ to induce retro-

grade amnesia. The data of Essman (1968) and Vinnitsky and Abulzade (1971) was cited by van Buskirk and McGaugh (1979) as supporting the suggestion that seizures are not required for the retrograde amnesia effect of PTZ. Essman (1968) reported that a post-trial convulsive dose (60 mg/kg) of PTZ produce retrograde amnesia even when the convulsions were blocked by lidocaine pretreatment. A similar finding was reported by Vinnitsky and Abulzade (1971) using other anesthetic agents. However, Palfai and Albula (1976a) criticized the data of Essman (1968), pointing out that lidocaine only partially blocked the seizures. Using a passive avoidance conditioning paradigm, Palfai and Albula (1976a) reported that convulsions were necessary for the development of PTZ-induced retrograde amnesia. These authors reported that subconvulsive doses (10 or 30 mg/kg) of PTZ did not produce retrograde amnesia and that animals pretreated with pentobarbital to block PTZ convulsions did not show retrograde amnesia.

Convulsive doses of PTZ have also been reported to produce retrograde amnesia for classically conditioned fear and taste aversions. Palfai and Cornell (1968) reported that seizures produced by PTZ caused amnesia for classically conditioned fear when PTZ was injected immediately following the conditioning trial. Subconvulsant doses of PTZ or convulsant doses injected 4 or 24 h (Palfai and Albula, 1976b) after training did not produce amnesia. Retrograde amnesia produced by PTZ was still evident 1 week after PTZ adminis-

tration. Ahlers and Best (1972) reported that convulsions induced by PTZ produced amnesia for a conditioned taste aversion to a novel taste produced by injections of apomorphine. Millner and Palfai (1975) injected PTZ (40 mg/kg) 2 min before, immediately or 3 min after injection of LiCl and reported conditioned aversion to the taste of saccharin. Kessler and Gellhorn (1943) had also reported that a convulsive dose of PTZ disrupted conditioned behavior.

Putney and McCoy (1976) found that a subconvulsive dose (10 mg/kg) of PTZ enhanced the ability of subthreshold electroconvulsive (ECS) shock to produce retrograde amnesia. Whereas ECS administration alone given 60 min after passive avoidance learning did not produce amnesia, injection of PTZ plus ECS produced amnesia.

Possible Chemical Basis of PTZ-Induced Retrograde Amnesia

Essman (1968) reported that a convulsive dose (60 mg/kg) of PTZ injected immediately following a single trial to establish a passive avoidance response produced retrograde amnesia for the task but did not significantly change the brain ribonucleic acid (RNA) concentration. Therefore whereas the amnesia effect of some agents has been attributed to their ability to decrease brain RNA (for review see Seiden and Dykstra, 1977), retrograde amnesia produced by PTZ could not be correlated with a change in brain RNA.

Palfai *et al*. (1974) reported a reduction in whole brain norepinephrine at 5 min but not one or 24 h after injection

of a convulsive dose (50 mg/kg) of PTZ in mice trained in a passive avoidance paradigm. Because the time course of anterograde amnesia reported by Palfai and Kurtz (1973) was similar to the time course of the brain norepinephrine decrease and recovery it was suggested that normal norepinephrine levels in brain during the early phase of memory storage may be necessary for retention. However, convulsions produced by PTZ have been reported to alter the brain levels of several other neurotransmitters (see next section: Neurochemical Action of PTZ) therefore these substances must also be looked at with respect to their possible involvement in mediating the amnestic effect of PTZ before any conclusion can be made concerning norepinephrine.

Iuvone et al. (1977) reported that immediate post-training administration of subconvulsive (50 mg/kg) or convulsive (60 mg/kg) doses of PTZ resulted in significant retention deficits on step-through inhibitory avoidance behavior. These doses also inhibited the incorporation of ^{3}H-lysine into brain protein during the first 10 min after training and PTZ injection. These data suggest a relationship between inhibition of protein synthesis and PTZ-induced retrograde amnesia.

Bookin and Pfeifer (1977) injected lysine vasopressin (LVP) one hour prior to the acquisition trial and retention trial in a passive avoidance task and reported that LVP facilitated passive avoidance retention and antagonized amnesia for retention of the task produced by PTZ (50 mg/kg). Because PTZ has been proposed to exert its amnestic effect

by interfering with retrieval process (Kurtz and Palfai, 1973) it was proposed that LVP may exert its anti-PTZ effect by facilitating retrieval processes or blocking the PTZ-induced interference with retrieval.

PTZ-Induced Amnesia as a Tool to Screen Anti-Petit Mal Drugs

Clincke and Wauquier (1979) reported that a subconvulsive dose (40 mg/kg) of PTZ, injected 30 min before testing, impaired retention of behavior in a step-down passive avoidance procedure. Pretreatment, 5 min before PTZ, with the anti-petit mal drugs ethosuximide, trimethadione or clonazepam antagonized amnesia produced by PTZ. In contrast drugs ineffective against petit mal epilepsy including phenobarbital, diphenylhydantoin, haloperidol, amphetamine, valproic acid or carbamazepine did not antagonize the amnestic effect of PTZ. These data suggest that this paradigm might be useful to screen effective anti-petit mal drugs from other antiepileptic and nonspecific drugs.

Conclusion

In a number of different paradigms subconvulsive dose of PTZ injected before or after training of a learning task facilitate learning or enhance retention whereas convulsive doses of this drug impair retention or produce amnesia. These effects are time dependent for the task. The exact mechanism for these effects of PTZ are unclear. However, it has been suggested that subconvulsive doses may facilitate learning

by facilitating memory consolidation whereas convulsive does impair retention by interfering with the retrieval.

B. Neurochemical Actions of Pentylenetetrazol

Introduction

Pentylenetetrazol (1,5-pentamethylenetetrazole, Metrazol) is an analeptic drug which stimulates all levels of the neuroaxis. Pentylenetetrazol (PTZ) has been the most extensively studied analeptic, however its mechanism of action is poorly understood. Good reviews of the early literature (Hildebrandt, 1937; Hahn, 1960) and the chemistry of the tetrazole derivatives (Benson, 1947) are available. The present review will concern the effect of PTZ on CNS acetylcholine, GABA and monoamine metabolism as well as the effect of manipulation of brain monoamine levels on PTZ seizure threshold.

Effect of PTZ on Acetylcholine

Acetylcholine Levels

Giarman and Pepeu (1962) sacrificed rats 12 minutes after a convulsant (75 mg/kg) dose of PTZ and reported a significant (23%) reduction in whole brain acetylcholine. Beani *et al.* (1969) sacrificed guinea pigs 30 sec., 3 min. and 6 min. after convulsions produced by PTZ (80 mg/kg) and measured total brain acetylcholine. The animals sacrificed 6 minutes post PTZ were almost dead at sacrifice with clear signs of post-seizure neurodepression. Determination of

acetylcholine content in brains of guinea pigs sacrificed at 30 seconds or 3 minutes post-PTZ showed that the total amount of acetylcholine was significantly below control values. The reduction in brain acetylcholine produced by PTZ concerned only bound acetylcholine not free acetylcholine.

In contrast to the reports of Giarman and Pepeu (1962) and Beani et al. (1969), Nistri and Pepeu (1974) reported that a subconvulsant (80 mg/kg) or a convulsant (160 mg/kg) dose of PTZ produced a statistically significant elevation of acetylcholine content in the frog spinal cord after 30 and 15 minutes, respectively. However, no changes in spinal cord acetylcholine levels were noted if the convulsions, interrupted by periods of depression, persisted for 90 minutes. Total frog brain acetylcholine content was not altered 15 minutes following the administration of the convulsant (160 mg/kg) dose of PTZ.

The discrepant findings on the effect of PTZ on acetylcholine levels may be due to a different effect of PTZ on the mammalian and amphibian central nervous system.

Acetylcholine Release

Mitchell (1963) reported that intravenous administration of PTZ (150 mg/kg) increased acetylcholine output from the left and right parietal cortex of sheep. In immobilized, anesthetized cats Beleslin et al. (1965) found that intravenous infusion of PTZ (20-80 mg/kg) produced a dose-dependent increase in the output of acetylcholine into the cerebral

ventricles. Acetylcholine output also increased (2-7 fold) from the cerebral cortex. Intraperitoneal injection of PTZ (150 and 300 mg/kg) produced a 2.9 and 7.5 fold increase in the release of acetylcholine from the cerebral cortex of anesthetized rats (Hemsworth and Neal, 1968). The increased release of acetylcholine was nearly maximal during the first 30 minute period of collection after injection and did not appreciably decrease over a period of 3 hours. The increase in release of acetylcholine was associated with increased electrical activity of the cerebral cortex.

Gardner and Webster (1973) found that intraperitoneal or intravenous administration of PTZ produced a dose-dependent increase in Ca^{++} dependent acetylcholine efflux from the cerebral cortex of anesthetized rats which was proportional to EEG activation and convulsive activity. A single intraperitoneal injection (250 mg/kg) produced a 12-fold increase in acetylcholine efflux together with convulsive EEG activation. Trimethadione reduced the PTZ-induced convulsive activity, EEG activation and acetylcholine release. Phenobarbital, however, reduced the PTZ-induced EEG and convulsive activity but left the acetylcholine release relatively unaffected. Therefore the effect of PTZ on acetylcholine efflux may not be related to its convulsant effect.

Assuming that increased release of acetylcholine from brain leads to a reduction in its acetylcholine content the studies demonstrating PTZ-induced acetylcholine release are in agreement with the reports of Giarman and Pepeu (1962)

and Beani et al. (1969) demonstrating a PTZ-induced reduction in brain acetylcholine.

Acetylcholinesterase

Mahon and Brink (1970) reported that PTZ produced a concentration-dependent ($K_i = 4.7 \times 10^{-3}$ M) competitive inhibition of acetylcholinesterase in crude homogenates of rat brain. These authors point out, however, that kinetic constants calculated from their data indicate that the quantity of PTZ necessary to cause 50 percent inhibition of acetylcholinesterase in vitro is much higher than that amount likely to be present in the brain after administration of a convulsant dose of the drug. Furthermore inhibition of acetylcholinesterase by PTZ would elevate acetylcholine which is contradictory to existing data (Giarman and Pepeu, 1962; Beani et al., 1969).

Nistri et al. (1974) administered PTZ (160 mg/kg) subcutaneously to frogs. Twenty minutes after PTZ administration, when convulsive symptoms were present, the animals were killed and spinal cord acetylcholinesterase activity was measured. No significant difference was found between controls and animals treated with PTZ. Nistri et al. (1974) suggested that the difference between the effect of PTZ on acetylcholinesterase in mammalian brain homogenates and amphibian spinal cord in vivo could reflect a higher resistance of the amphibian enzyme to PTZ.

Choline Uptake

When rats were sacrificed immediately after the onset of the flexor stage of the convulsion, Simon et al. (1976) reported that the intravenous administration of PTZ (75 mg/kg) produced a 45% increase in sodium-dependent high affinity choline uptake in rat hippocampal synaptosomes. This increase was not found, however, when cholinergic afferents to the hippocampus were interrupted by septal lesion prior to PTZ administration. Injection of a lower, non-convulsant (10 mg/kg) dose of PTZ did not result in a change in choline uptake.

Addition of PTZ (10^{-4}M) to hippocampal synaptosomal preparation from saline treated animals did not alter high affinity choline uptake (Simon et al., 1976). Because in vitro addition had no effect, Simon et al. (1976) suggested that the effect of PTZ is not a direct one synaptosomes but may be due to an increase in impulse flow.

PTZ increased only the Vmax and not the Km of high affinity choline uptake (Simon et al., 1976). PTZ is not alter the conversion of [^{3}H]-choline to [^{3}H]-acetylcholine suggesting that the alteration in uptake is not a secondary effect consequent to a change in choline acetyltransferase (Simon et al., 1976).

Jenden et al. (1976) and Klemm and Kuhar (1979) also reported that convulsant doses of PTZ activated high affinity choline uptake in hippocampal synaptosomes. Choline uptake

rapidly returned toward normal in post-mortem tissue indicating the importance of measuring this process immediately after sacrificing (Klemm and Kuhar, 1979).

In summary, results of experiments investigating the effect of PTZ on acetylcholine function suggest that PTZ activates cholinergic neurons. Such an activation results in acetylcholine release, choline uptake and decreased brain acetylcholine levels.

EFFECT OF PTZ ON GABA

GABA Levels

Kamrin and Kamrin (1961) sacrificed mice during the tonic extension component of a PTZ-induced seizure and reported no difference in the concentration of brain GABA from control treated mice. There was also no difference between the cerebral concentration of glutamic acid, glycine, taurine or aspartic acid in PTZ or control treated mice (Kamrin and Kamrin, 1961). Tews *et al.* (1963) failed to detect any change in brain GABA concentration of dogs following tonic-clonic seizures produced by intravenous administration of PTZ (40 mg/kg). Similarly, Sytinskii and Priyatkina (1966) found no alteration of GABA concentration in rat brain following convulsions produced by PTZ (100 mg/kg). Wood and Peesker (1975) determined mouse brain GABA concentration at the onset of seizures induced by PTZ and found no difference between PTZ treated and saline-treated mice.

Nahorski et al. (1970) examined rat brain GABA levels at various times after intraperitoneal administration of a convulsant dose (70 mg/kg) of PTZ and found no change in the levels of this amino acid until tonic extension when a significant rise was observed. A similar increase in GABA levels was also observed when the rats were sacrificed after the convulsion. These authors point out that the increase in GABA levels may be due to anoxic conditions which existed at the time of sacrifice.

In contrast to the above reports indicating either no change or an increase in cerebral GABA during convulsions induced by PTZ, Maynert and Kaji (1962) reported that mice given PTZ (85 mg/kg) and sacrificed during the first tonic seizure showed a small but significant decrease in brain GABA concentration. Wood et al. (1966) reported a slight but not statistically significant reduction brain GABA levels when rats were sacrificed 5 minutes after a convulsant dose (60 mg/kg) of PTZ.

GABA Release

Johnston and Mitchell (1971) found that PTZ (10^{-4}-10^{-6}M) inhibited the resting but not the electrically evoked release of [^{3}H]-GABA from rat brain cerebral cortical slices. By examining the release of [^{3}H]-GABA from feline cortical slabs, Reiffenstein (1979) tested the hypothesis that seizure susceptibility of the chronically denervated cortex was due to interruption of recurrent inhibitory pathways. PTZ-induced seizures evoked a small increase in [^{3}H]-GABA efflux from

both the epileptic and the normal cortex. Because interruption of recurrent inhibitiory pathways would result in decreased resting and seizure-evoked release of [^{3}H]-GABA the above hypothesis was not supported.

GAD/GABA-T

Wood et al. (1966) reported that PTZ (60 mg/kg) did not significantly reduce GAD activity in rat brain when rats were sacrificed 5 minutes after PTZ-induced seizures. Similarly, Syntinskii and Priyatkina (1966) found no alteration in GAD activity produced by PTZ either in vivo or in vitro. Tapia et al. (1969) also reported a lack of effect of PTZ on GAD activity.

Sytinskii and Priyatkina (1966) reported that GABA-T activity in rat brain was not changed either by in vivo administration of a convulsant dose of PTZ or by the in vitro addition of PTZ. However, Wood et al. (1966) reported that PTZ (60 mg/kg) produced a significant increase in GABA-T activity. The increase in GABA-T activity was not correlated, however, with decreased GABA levels.

Uptake

Johnston and Mitchell (1971) reported that PTZ (10^{-4} - 10^{-6} M) did not alter [^{3}H]-GABA uptake into rat cerebral cortical slices.

Effectiveness of GABA and GABA-Mimetic Drugs Against PTZ

Hawkins and Sarett (1957) found that orally adminis-

tered GABA protected mice from PTZ-induced convulsions. Wood et al. (1966) reported that GABA but not glycine in a dose of 120 mmoles/kg) administered intraperitoneally produced a slight protection against PTZ-induced convulsions in the rat. However, several investigators (Purpura et al., 1958 a,b; Gulati and Stanton, 1960; were unable to demonstrate an anti-convulsant action of GABA following systemic administration.

Kobrin and Seifter (1966) reported that intravenous administration of GABA (25-1000 mg/kg) to 1 day old chicks in which the blood-brain barrier was not complete produced a dose-dependent protection against convulsions induced by PTZ (35 mg/kg) also administered intravenously. Several other w-amino acids including glycine and B-alanine were ineffective. When GABA was administered to older chicks in which the blood-brain barrier was developed it was ineffective in protecting against PTZ-induced convulsions. Schlesinger et al. (1969) found that intracranial injection of GABA protected mice against PTZ-induced seizures.

Elevations of brain GABA levels with the GABA-T inhibitors, amino-oxyacetic acid (Roa et al., 1969; Kuriyama et al., 1966; Wood and Peesker, 1975), hydroxylamine (Roberts et al., 1960) or valproic acid (Simler et al., 1973) affords protection against PTZ-induced convulsions. However, Maynert et al. (1962) showed that a three-fold elevation of brain GABA produced by hydrazine did not protect mice against PTZ convulsions. Similarly, Schecter et al. (1977) reported that a

six-fold elevation of brain GABA produced by gamma-acetylenic GABA did not protect mice against seizures induced by PTZ.

Frey et al. (1979) examined the role of GABA mechanisms in PTZ convulsions by investigating the effect of inhibition of high affinity GABA uptake on PTZ-induced convulsions. The ability of inhibitors of high affinity GABA uptake blockers to increase the PTZ seizure threshold correlated well with the ability of these drugs to inhibit GABA uptake in vitro. (-)-Nipecotic acid had the most pronounced effect. This effect of the GABA uptake blockers was not correlated with alteration in brain GABA levels or changes in GAD or GABA-T activities.

The GABA receptor agonist muscimol also elevated the PTZ convulsant threshold and this was accompanied by decreased brain GABA concentration and GAD activity (Frey et al., 1979).

Effectiveness of PTZ Against GABA

Presynaptic inhibition is thought to be mediated by GABA (Barker and Nicoll, 1972; Curtis and Johnston, 1974; Davidoff, 1972; Davidson and Southwick, 1971) and several investigations have been directed towards examining the effect of PTZ on GABA mediated presynaptic inhibition. Boyd et al. (1966) reported that PTZ blocked GABA mediated presynaptic inhibition in the cuneate nucleus. Similarly, Banna and Hazbun (1969) and Hill et al. (1974) found that PTZ reduced GABA-mediated presynaptic inhibition. Nicoll and Padjen (1976) found that PTZ in concentration of $>10^{-2}$M antagonized

the action of GABA at primary afferents of the isolated frog spinal cord.

While it appears clear that PTZ blocks GABA-mediated presynaptic inhibition, evidence on the effect of PTZ on GABA mediated post-synaptic inhibition is contradictory. Krnjevic et al. (1966) reported that PTZ did not block cortical inhibition mediated by GABA and Hill et al. (1973a) reported that slow intravenous infusion of PTZ to cats produced seizure activity without antagonizing the effect of iontophonetic application to the cortex.

Microiontophoretic application of PTZ (1M) to single feline cortical neurons failed to antagonize the inhibitory effects of GABA (Hill et al., 1973b). However, the authors cautioned that a sufficient concentration of PTZ to produce GABA antagonism may not have been achieved in the area of the neuron when PTZ was applied iontophoretically.

In contrast to the above studies showing lack of an effect of PTZ on GABA-mediated post-synaptic inhibition, MacDonald and Barker (1977) reported that iontophoretically applied PTZ (0.3M) competitively inhibited GABA-mediated post-synaptic responses in cultured mammalian spinal cord neurons. Similarly, Scholfield (1979) found that PTZ antagonized the post-synaptic action of GABA on guinea pig olfactory cortical neurons.

In summary, while PTZ does not alter brain GABA concentration, GAD or GABA-T activity it has been demonstrated that GABA and some GABA-mimetic drugs antagonize the convulsant

action of PTZ. Furthermore, PTZ has been reported to block both pre- and post-synaptic inhibition mediated by GABA.

EFFECT OF PTZ ON MONOAMINE METABOLISM

Bonnycastle et al. (1957) reported that a convulsant dose (75 mg/kg) of PTZ failed to alter rat brain serotonin levels. However, Bertaccini (1959) found that rat brain serotonin concentration was elevated 20-30% when the animals were sacrificed 15-60 minutes after convulsions. Garattini et al. (1960) also reported that PTZ increased the brain serotonin content and that this effect was independent of convulsions.

Kato et al. (1967) looked at the effect of acute and 11 day treatment of PTZ (50 mg/kg) in rats. When the animals were sacrificed 5 minutes after an acute injection there was an increase in 5-HT levels. At 2 hours 5-HT levels had returned to normal but were elevated 3-fold at 24 hours. Sacrificing the rats 5 minutes or 24 hours after 11 day PTZ treatment resulted in marked elevation of 5-HT levels at all times.

Diaz (1970) injected a subconvulsant (30 mg/kg) dose of PTZ to rats and reported a 42% increase in brain serotonin level and a 34% decrease in 5-HIAA level when the animals were sacrificed 30 minutes after injection. Administration of a convulsive (50 mg/kg) dose of PTZ produced an 18% increase in 5-HT level but no change in 5-HIAA level. PTZ (30 mg/kg) produced a significant (48%) decrease in the rate of 5-HT synthesis, however, 50 mg/kg had no significant

effect. The rate of depletion of 5-HT following reserpine was significantly decreased by PTZ (30 mg/kg) but not the convulsant dose. These data show that PTZ alters brain 5-HT metabolism and that this effect is not dependent on seizures.

McMillen and Isaac (1974) reported the effect of PTZ (10-50 mg/kg) on serotonin and dopamine metabolism in the cat. Intraperitoneal administration of PTZ (10 mg/kg) produced no change in the CSF concentration of either 5-HIAA or HVA. A larger, also non-convulsant dose (20 mg/kg) increased the 5-HIAA concentration by 103% four hours after injection and 5-HIAA levels remained elevated for 24 hours. HVA levels were not significantly altered by this dose of PTZ. When the cats were administered 30 mg/kg they exhibited a "pseudoconvulsion" which was characterized by clonus and vocalization. This dose of PTZ increased 5-HIAA levels 44% after 4 hours and the level remained elevated for 24 hours. HVA levels were found to be increased 27% after 2 hours but this did not remain elevated for 24 hours as did 5-HIAA levels. Clonic convulsions occurred following the administration of a convulsant dose (50 mg/kg) of PTZ. This dose produced elevation of 5-HIAA and HVA which lasted 24 hours. These data show that non-convulsant doses of PTZ increased metabolic activity in the 5-HT system for up to 24 hours while not affecting dopamine systems and that convulsions induced by PTZ cause a non-specific increase in the metabolism of dopamine systems as well.

McMillen and Isaac (1978) later reported that the increase in 5-HIAA levels reported to occur after administration

of 20 mg/kg PTZ (McMillen and Isaac, 1974) was probably secondary to a reduction in body temperature produced by the drug since maintaining body temperature prevented the increase in 5-HIAA produced by this dose of PTZ. Injection of a convulsant dose (40 mg/kg) of PTZ increased 5-HIAA levels and this effect was not prevented by controlling body temperature. This indicates that convulsant doses of PTZ can increase 5-HT metabolism but after non-convulsant doses the increase in 5-HT metabolism is secondary to the hypothermic effect of PTZ.

Determination of PTZ concentration in plasma and CSF showed that PTZ was not present to exert an effect at 24 hours, therefore the increased levels of 5-HIAA and HVA at this time was not due to the presence of PTZ (McMillen and Isaac, 1978).

PTZ (20 mg/kg) did not alter plasma tryptophan levels and a 40 mg/kg dose decreased total plasma tryptophan. This change is opposite to the change observed in CSF 5-HIAA (McMillen and Isaac, 1978). These data suggest that the 24 hour increase in CSF 5-HIAA levels does not result from an increase in plasma tryptophan levels and may be a direct effect of PTZ on 5-HT neurons.

Pretreatment of cats with trimethadione (200 mg/kg) blocked the convulsions but not the EEG excitation produced by PTZ (40 mg/kg). This dose of PGZ elevated 5-HIAA levels whether or not the animals were pretreated with trimethadione suggesting that PTZ can increase 5-HT metabolism without causing convulsions (McMillen and Isaac, 1978).

The results of Diaz (1970) and McMillen and Isaac (1974, 1978), with respect to the effect of PTZ on 5-HIAA levels, are opposite. This discrepancy can best be explained by differences in the period of measurement of 5-HT metabolism. In addition to its effect on 5-HT and dopamine metabolism, Kato et al. (1967) reported that acute administration of PTZ (50 mg/kg) resulted in elevated levels of brain histamine 24 hours after injection. Epinephrine levels were decreased at 5 minutes and 2 hours after PTZ, not changed after 1 hour and elevated after 24 hours. Norepinephrine levels were increased at 5 minutes, 1 hour and 2 hours but had returned to normal at 29 hours. Eleven day treatment with PTZ (50 mg/kg) produced marked elevation in brain histamine but not epinephrine or norepinephrine (Kato et al., 1967).

In summary, the above studies show that PTZ affects monoamine metabolism at both non-convulsant and convulsant doses. Furthermore, these effects can last long after the immediate action of PTZ has stopped.

Effect of Monoamine Manipulation on PTZ Seizure Threshold

Bonnycastle et al. (1957) reported that elevation of rat brain 5-HT levels by administration of either iproniazid or 5-HTP failed to protect rats against the convulsant or lethal effect of PTZ (75 mg/kg). However, Frey and Kilian (1973) found that 5-HTP increased the PTZ seizure threshold of both mice and rats. Rudzik and Johnson (1970) reported that administration of tranylcypromine, 5-HTP or 3,4-dihydroxyphenyl-

alanine (DOPA) did not alter the PTZ seizure threshold. However, when 5-HTP was combined with tranylcypromine a significant elevation in the PTZ convulsive threshold occurred. In contrast the combination of tranylcypromine and DOPA did not increase the threshold for PTZ seizures.

The survival time of mice infused intravenously with PTZ is shortened by reserpine (Chen et al., 1954; Lessin and Parkes, 1959; Pfeifer and Galambos, 1967) or tetrabenazine (Lessin and Parkes, 1959) pretreatment. Administration of the MAO inhibitor, iproniazid, prevented the reduction in survival time produced by reserpine (Chen and Bohner, 1961; Lessin and Parkes, 1959; Pfeiffer and Galambos, 1967) or tetrabenazine (Lessin and Parkes, 1959). 5-HTP lengthened the survival time of mice pretreated with iproniazid but had no effect alone. In contrast, DOPA did not alter the survival time of mice pretreated with iproniazid (Lessin and Parkes, 1959). LSD but not 2-bromo-LSD antagonized the effect of reserpine on PTZ survival time. LSD did not alter the survival time of control mice (Lessin and Parkes, 1959). These data suggest a relationship between sensitivity to PTZ and brain 5-HT levels. Chen and Bohner (1961) reported that in addition to 5-HTP, 5-HT, DOPA and dopamine reversed the lowering effect of reserpine on PTZ seizure threshold in iproniazid treated mice.

Jones and Roberts (1968) found that intracerebroventricular injection of noradrenaline antagonized reserpine-induced

facilitation of PTZ seizures but had no effect alone. Whereas small amounts of intraventricularly administered dopamine lowered the threshold for PTZ convulsions and were without effect on the reserpine facilitative effect, higher doses showed anti-convulsant action and antagonized the facilitative effect of reserpine. Schlesinger et al. (1969) reported that the combined intracranial injection of norepinephrine plus 5-HT protected mice against PTZ-induced seizures.

Pfeiffer and Galambos (1967) suggested that norepinephrine has a more important role in the change of susceptibility to PTZ seizures than 5-HT or dopamine. These investigators found that prenylamine decreased the PTZ convulsive threshold without altering 5-HT levels. Also guanethidine decreased only brain norepinephrine levels without altering dopamine or 5-HT and lowered the threshold for PTZ-induced convulsions.

Alexander and Kopeloff (1970) found that pretreatment of rats with p-chlorophenylalanine (PCPA) to deplete brain serotonin lowered the threshold for PTZ-induced seizures. Frey and Kilian (1973) pretreated mice with PCPA or cyproheptadine and found no change in the threshold for PTZ-induced clonic seizures. However, PCPA lowered the threshold in rats confirming the data of Alexander and Kopeloff (1970).

Rudzik and Johnson (1970) reported the PTZ convulsive threshold in mice was lowered only by drugs which decreased whole brain 5-HT and was not altered by catecholamine depleting drugs. Reserpine and P-CPA were found to lower the seizure threshold but U-14,624 and alpha-methyl-para-tyrosine produced no chage.

In contrast to these data, Frey and Kilian (1973) reported that alpha-methyl-para-tyrosine, disulfarim, FLA-63 and propranolol but not haloperidol or phentolamine lowered the threshold for PTZ-induced clonic seizures. L-dopa elevated this threshold in mice but had no significant effect in rats. Because dopamine B-hydroxylase inhibitors were as effective as alpha-methyl-para-tyrosine and because of the lack of effectiveness of haloperidol these data suggest a more important role for norepinephrine than dopamine.

The threshold for PTZ-induced tonic extension was depressed by FLA-63, PCPA, cyproheptadine and propranolol but not alpha-methyl-para-tyrosine or disulfarim (Frey and Kilian, 1973).

Corcoran et al. (1973, 1974) found that selective destruction of catecholamine but not 5-HT neurons by intraventricular injection of 6-OHDA to rats pretreated with an MAO inhibitor increased the duration and intensity of convulsions induced by PTZ (70 mg/kg). The severity of seizures in rats with depletion of both norepinephrine and dopamine was not different from rats with preferential depletion of norepinephrine, suggesting that norepinephrine and not dopamine is important for the 6-OHDA induced exacerbation of PTZ convulsions.

A number of investigations have looked at the effect of amphetamines on PTZ seizure threshold. Kobinger (1958) found that methamphetamine raised the PTZ seizure threshold when administered 15 minutes before PTZ. Methamphetamine also antagonized the decreased PTZ seizure threshold produced by

reserpine. Turner and Spencer (1968) however, reported that d-amphetamine had a pro-convulsant effect on PTZ convulsions in mice when administered 30-90 minutes before PTZ but had an anticonvulsant effect when administered 6 hours before PTZ. BLockade of dopamine but not norepinephrine or 5-HT synthesis antagonized the pro-convulsant action of d-amphetamine (Spencer and Turner, 1969) indicating the importance of dopamine for this action.

Rudzik and Johnson (1970) reported that neither d-amphetamine or methamphetamine significantly altered the PTZ convulsive threshold but administration of the chloroderivatives which have a unique effect on 5-HT disposition (Fuller et al., 1965) produced a significant increase of the PTZ seizure threshold. Gerald and Riffee (1973) found that acute administration of d- or l-amphetamine increased susceptibility to PTZ-induced convulsions. d-Amphetamine was about twice as potent as the l-isomer. Whereas l-amphetamine increased tonic seizure susceptibility, d-amphetamine decreased susceptibility to this seizure. Tolerance developed to these effects of amphetamine after seven consecutive daily injections (Gerald and Riffee (1973)).

Finally, Bhattacharya and Sanyal (1978) reported that prostaglandin E, (PGE_1) induced inhibition of PTZ-induced seizures in rats was antagonized by drugs which reduce brain 5-HT activity but not by drugs which decrease catecholamine activity. Pretreatment with reserpine, P-CPA, methysergide or 5,6-DHT significantly antagonized PGE_1 induced inhibition

of convulsions produced by PTZ (60 mg/kg). Alpha-methyl-para-tyrosine, diethyldithiocarbamate, phenoxybenzamine, propranolol or haloperidol pretreatment did not significantly alter the effect of PGE_1. These data suggest that this effect of PGE_1 is not a direct one but is mediated through serotoninergic mechanisms.

In summary, manipulation of CNS monoamines has provided information regarding the involvement of these neurotransmitters in the convulsant action of PTZ. The majority of evidence suggests that reduction of 5-HT or noradrenergic activity increases the susceptibility to PTZ seizures.

CONCLUSION

Studies to elucidate the action of PTZ on neurotransmitter metabolism have demonstrated that this drug affects the metabolism of acetylcholine, GABA and monoamines. A uniform neurochemical mechanism for the pharmacological actions of PTZ, however, is not apparent.

In addition to its effect on neurotransmitter function, PTZ has also been reported to have a direct effect on membrane excitability (Gross and Woodbury, 1972; Klee et al., 1973; David et al., 1974; Suguya and Onozuka, 1978) which may contribute to its action.

METHODS

Subjects

A total of 44 male hooded rats of the Long-Evans strain (Charles River Breeding Laboratories, Wilmington, MA) weighing between 250 and 300 g at the beginning of the investigation were used as subjects. Animals were housed in single cages in a large colony room thermostatically maintained at 21 $\pm$ 1°C. Room lights were turned off from 8:00 p.m. to 8:00 a.m. Water was continuously available in the home cages but food was restricted to 20 g a day made available approximately 4 hours following each operant session.

Apparatus

The behavioral apparatus consisted of conventional Skinner boxes 24 x 30 cm and 26 cm high enclosed in lightproof, sound-attenuated, and fan-ventilated chambers. Each Skinner box contained two response levers which were 2.5 cm wide and which extended 2.0 cm into the operant boxes. One lever was on either side of a food cup which was installed in the center of the short wall equidistant from each lever. Scheduling of behavioral contingencies and recording of data was made by a combination of electromechanical (Lehigh Valley Electronics, Lehigh Valley, PA) and solid-state (Coulbourn Instruments, Lehigh Valley, PA) programming equipment.

PTZ-Saline Discrimination Training

All of the rats were first magazine trained and shaped to lever press for food reinforcement. When the rats began to lever press at a rate of one response per minute they were shaped to learn a progressively increasing fixed ratio (FR) schedule of reinforcement until they consistently responded on an FR 10 schedule (10 lever presses for each 45 mg Noyes food pellet). In the beginning only responses with one of the levers was reinforced while responding with the other lever was not reinforced. When the rats began to respond with only one lever at a rate of 20 responses per minute the lever with which responses were reinforced was changed to the alternate lever. When the rats began to respond with this lever at a rate of 20 responses per minute, the lever with which responses were reinforced was again changed. After 4-5 such alternations without any injection, a PTZ-saline injection schedule was introduced. In this phase of training, daily 15 minute sessions were preceded by injection of either PTZ (20 mg/kg) or saline (1 ml/kg). Each session began with one food pellet in the food cup so as to orient the rats to the manipulanda. The animals were trained to respond with one of the levers 15 minutes following a PTZ injection and the other lever 15 minutes following a saline injection (Figure 1). Every tenth response (FR 10) with the appropriate lever was reinforced by delivery of a 45 mg Noyes food pellet. Responses with the incorrect lever (i.e., saline

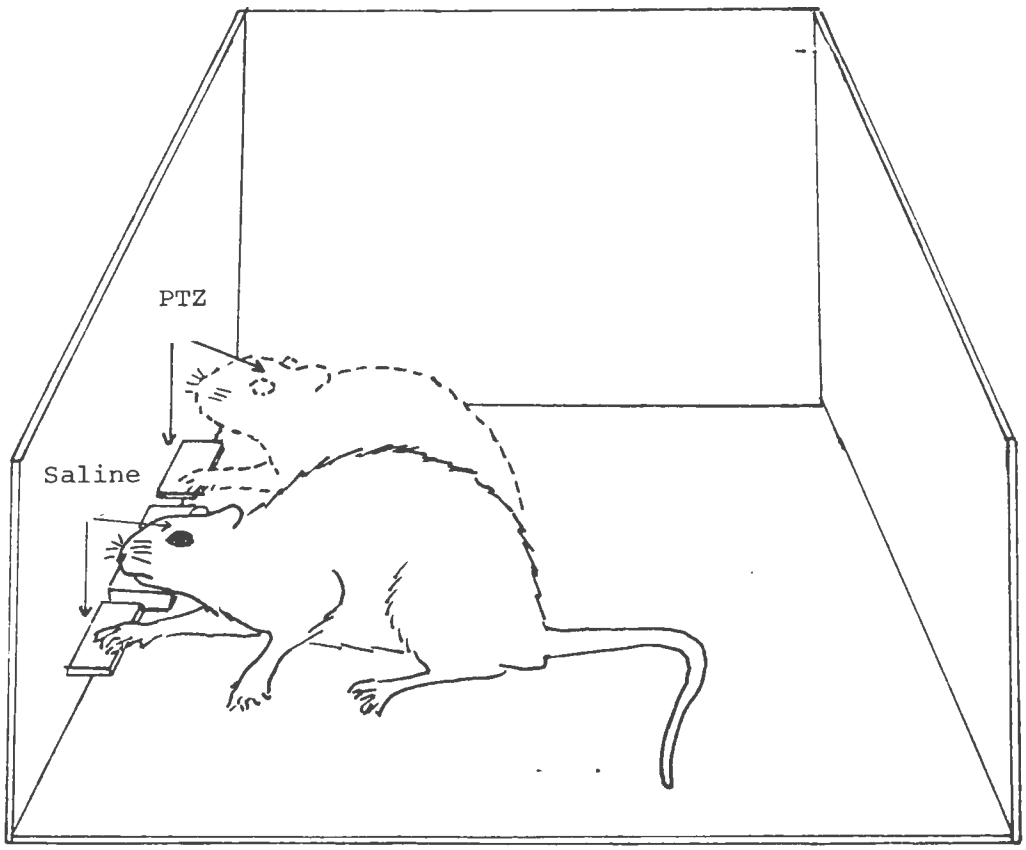

Figure 1. Diagrammatic representation of the PTZ-saline discrimination procedure. On an FR 10 schedule of food reinforcement, hungry rats were trained to respond with a lever on one side of a food cup 15 min following a 20 mg/kg PTZ injection and to respond with the lever on the alternate side 15 min following a 1 ml/kg saline injection.

lever following PTZ injection or PTZ lever following saline injection) were recorded but were not reinforced by delivery of food.

To guard against a possible effect of lever or position preference the lever with which responses were reinforced following a PTZ injection was randomly assigned to be the lever on the right side of the food cup for half of the rats and the lever on the left side of the food cup for the remaining rats. For each rat, the position (i.e., right or left) of the PTZ lever remained constant on each subsequent session. To avoid the possibility that olfactory cues associated with the correct lever for rats previously tested in the Skinner boxes could serve as a cue for lever selection (Weissman, 1976), the sequence of PTZ-saline injections was varied separately for each group of rats trained successively on the same day.

Initially, the sequence of PTZ-saline injections alternated. This training continued until five such alternations were achieved and responding was stabilized with the appropriate lever. Following this the rats entered the final phase of training where the PTZ-saline sessions were carried out seven days a week according to an irregularly alternating sequence of PTZ-saline injections. In this and all subsequent phases of the experiment, the session length was fixed at 10 minutes. The rats were trained to a criterion of emitting four or less responses with the incorrect lever

prior to the first reinforcement (10 responses with the correct lever) on nine out of ten consecutive sessions.

For each session data were recorded automatically to include the number of responses emitted with the incorrect lever prior to the first reinforcement as well as the total number of responses emitted with the correct and incorrect lever during the entire 10 minute session.

Discrimination Testing

When the rats reached the criterion described above they were continuously and repeatedly used for generalization and antagonism testing. These tests consisted of ten-minute sessions separated by at least five practice sessions in which saline and PTZ were correctly discriminated. For half of the rats, the sessions were preceded by a practice session in which saline was injected whereas for the remaining rats test sessions were preceded by a practice session in which PTZ was injected. If the rats' performance on these practice sessions seemed to deteriorate with respect to the number of responses on the incorrect lever prior to the first reinforcement, further training sessions were given before testing was reinstated. For generalization and antagonism testing, doses of each drug were administered in an irregular order.

A. Generalization Testing - Following injection of the test drug (for pretreatment time see Table 3) each rat was

placed in its assigned Skinner box and allowed to respond with the levers until ten nonreinforced responses were completed with one of the levers. The lever with which ten responses were completed first was considered the selected lever and was subsequently fixed to be reinforced (FR 10) for the remainder of the session. Responses emitted with the other lever were recorded but not reinforced.

B. Antagonism Testing - Animals were injected with the appropriate dose of the test drug or saline. Following this pretreatment (for pretreatment time see Table 5), the rats were injected with pentylenetetrazol (20 mg/kg). Fifteen minutes after the pentylenetetrazol injection the animals were placed in their assigned Skinner boxes and tested for lever selection as described above.

C. Antagonism Testing with Diazepam and Chlordiapepoxide After Their Chronic Administration - The rats that were tested acutely with diazepam (2.5 or 10 mg/kg) for antagonism of the PTZ discriminative stimulus were treated with diazepam (10 mg/kg) for ten consecutive days. Similarly, the animals tested acutely with chlordiazepoxide (2.5 or 10 mg/kg) were treated with chlordiazepoxide (10 mg/kg) for ten consecutive days. Other rats that were tested acutely with solvent were treated with solvent for ten consecutive days. Results of previous studies (Cook and Sepinwall, 1975; Goldberg et al., 1967; Margules and Stein, 1968; Stein et al., 1975) suggest

this treatment regimen to be more than sufficient to develop tolerance to the sedative effects of benzodiazepines. No discrimination training or test trials were administered during this period. On the eleventh, twelfth and thirteenth days, animals were injected with the appropriate dose of either diazepam, chlordiazepoxide or solvent and tested for antagonism of the PTZ discriminative stimulus as described above for the acute study. To maintain tolerance for these days the rats were injected daily with the appropriate drug four hours after each operant session so that the chronic dosing schedule was maintained.

D. Test for Diazepam Antagonism of the Discriminative Stimuli Produced by Cocaine, Yohimbine and R05-3663 in Rats Trained to Discriminate Between PTZ and Saline - On these tests rats were treated with diazepam (5 mg/kg) 30 min. before injection of yohimbine (2.5 mg/kg) or R05-3663 (5-20 mg/kg). Thirty min or 1 h after the cocaine injection and 30 min after yohimbine or R05-3663 injection the rats were tested for lever selection as described previously.

E. Test for Diazepam and Haloperidol Antagonism of Discriminative Stimulus Produced by Cocaine in Rats Trained to Discriminate Between PTZ and Saline - On these tests rats were injected with diazepam (5 mg/kg) or haloperidol (0.16 or 0.69 mg/kg) 30 or 60 min before injection of cocaine (20 mg/kg), respectively. Fifteen min after the cocaine injection the rats were tested for lever selection as described previously.

Drugs

The drugs used in this study (listed in alphabetical order) were aceperone (Janssen Pharmaceutica N.V., Beerse, Belgium); amitriptyline hydrochloride (Merck and Co. Inc., Rahway, NJ); d-amphetamine sulfate (Smith, Kline and French Laboratories, Philadelphia, PA); apomorphine hydrochloride (Eli Lilly and Co., Indianapolis, IN); atropine sulfate (Merck and Co. Inc. Inc., Rahway, NJ); bemegride (Aldrich Chemical Co. Inc., Milwaukee, WI); bicuculline methiodide (Pierce Laboratories, Rockford, IL); caffeine citrate (The New York Quinine and Chemical Works, Inc., New York, NY); carbachol (Aldrich Chemical Co. Inc., Milwaukee, WI); chlordiazepoxide hydrochloride (Hoffman LaRoche Inc., Nutley, NJ); chlorpromazine (Smith, Kline and French Laboratories, Philadelphia, PA); clobazam (Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ); clonazepam (Hoffman LaRoche, Inc., Nutley, NJ); clonidine hydrochloride (Boehringer Ingelheim Ltd., Ridgefield, CT); cocaine hydrochloride (Merck and Co. Inc., Rahway, NJ); diazepam (Hoffman LaRoche Inc., Nutley, NJ); diphenylhydantoin (K and K Laboratories, Plainview, NY); ethanol (U.S. Industrial Chemical Co., New York, NY); ethosuximide (Parke, Davis and Co., Detroit, MI); etomidate sulfate (Janssen Pharmaceutica N.V., Beerse, Belgium); fluoxetine (Eli Lilly and Co., Indianapolis, IN); flurazepam dihydrochloride (Hoffman LaRoche Inc., Nutley, NJ); gamma-acetylenic GABA (Merrell International Research Center, Strasbourg, France); gamma-hydroxybutyrate (Aldrich Chemical Co., Milwaukee, WI); gamma-vinyl GABA (Merrell

International Research Center, Strasbourg, France), haloperidol (McNeil Laboratories Inc., Fort Washington, PA); 5-DL-hydroxytryptophan (Aldrich Chemical Co., Milwaukee, WI); meprobamate (Wallace Laboratories, Cranbury, NJ); 3-mercaptoproprionic acid (Sigma Chemical Co., St. Louis, MO); methylphenidate hydrochloride (Ciba-Geigy Corp., Summit, NJ); methysergide maleate (Sandoz Pharmaceuticals, Hanover, NJ); morphine sulfate (Merck and Co. Inc., Rahway, NJ); naloxone hydrochloride (Endo Laboratories, Garden City, NJ); nicotine (Aldrich Chemical Co. Inc., Milwaukee, WI); oxotremorine (Aldrich Chemical Co., Milwaukee, WI); pentobarbital (American Pharmaceutical Co., Bronx, NY); pentylenetetrazol (Sigma Chemical Co., St. Louis, MO); phenobarbital (American Pharmaceutical Co., Bronx, NY); physostigmine sulfate (Merck and Co. Inc., Rahway, NJ): picrotoxin (Aldrich Chemical Co. Inc., Milwaukee, WI); propranolol hydrochloride (Ayerst Laboratories, Inc., New York, NY); R05-3663 (Hoffman LaRoche Inc., Nutley, NJ); scopolamine hydrobromide (Robbins Research Laboratories, Richmond, VA); strychnine sulfate (Mallinckrodt Chemical Works, St. Louis, MO); trimethadione (Abbott Laboratories, North Chicago, IL); valproic acid (Abbott Laboratories, North Chicago, IL); and yohimbine hydrochloride (Sigma Chemical Co., St. Louis, MO).

Dizepam, clobazam, clonazepam and meprobamate were homogenized in 0.9% saline containing 13% propylene glycol and 1% Tween 80. Haloperidol was dissolved in 0.3% tartaric acid. Trimethadione was dissolved in 0.9% saline containing 40%

propylene glycol and 10.5% ethyl alcohol. All other drugs were dissolved in 0.9% saline.

All drugs were administered intraperitoneally in a volume of 1 ml/kg except caffeine citrate, clobazam, diazepam, diphenylhydantoin, ethanol, ethosuximide, gamma-hydroxybutyrate, naloxone hydrochloride, R05-3663, trimethadione and valproic acid and yohimbine hydrochloride which were administered in a volume of 2-8 ml/kg. Drug doses were calculated in terms of the salt.

Statistical Analyses

Lever selection data was expressed as a percentage of rats selecting the PTZ lever following each drug treatment. The Fisher Exact Probability Test was used to determine statistical significance of results. Drug treatments were considered to significantly generalize to the PTZ discriminative stimulus if the Fisher Exact Probability Test revealed that such treatments resulted in a percentage of rats selecting the PTZ lever that was not significantly ($p > 0.05$) different from the percentage of rats selecting the PTZ lever after injection of PTZ (20 mg/kg). Drug pretreatments were considered to significantly antagonize the PTZ discriminative stimulus if the Fisher Exact Probability Test revealed that such treatments resulted in a percentage of rats selecting the PTZ lever that was significantly different ($p < 0.05$) from the percentage of rats selecting the PTZ lever after saline pretreatment.

Regression analysis was performed to determine whether generalization to or antagonism of the PTZ discriminative stimulus by a drug treatment was a dose-related effect. Drug treatments were considered to produce a significant dose-related effect if by regression analysis $p < 0.05$. ED 50s and 95% confidence limits were calculated according to Finney (1952).

To determine whether a drug treatment significantly affected response rate, One Way Analysis of Variance (ANOVA) was performed to compare mean test session response rate with mean response rate of the PTZ and saline sessions preceding the test. A drug treatment was considered to significantly affect response rate if One Way ANOVA revealed a statically significant ($p < 0.05$) difference among the treatment means. If One Way ANOV revealed a statistically significant difference among treatment means, a Duncan's Multiple Range Test (Duncan, 1955) was performed to determine which particular mean differed from another particular mean. Mean response rates were considered to significantly differ from each other by Duncan's vultiple Range Test if $p < 0.05$.

RESULTS

Acquisition of the PTZ-Saline Discrimination

All of the rats acquired the PTZ-saline discrimination to the required criterion. The number of sessions needed to reach this criterion ranged from 16 to 49, the mean (±S.E.M.) being 30± 1.1. Acquisition of the PTZ-saline discrimination is shown in Figure 2. On the first training session, 43% of the rats injected with PTZ selected the PTZ lever and 55% of the animals injected with saline selected the PTZ lever. On the twentieth training session, 73% of the rats injected with PTZ selected the PTZ lever and 28% of the rats injected with saline selected the PTZ lever. By the thirty-sixth training session, 95% of the animals injected with PTZ selected the PTZ lever and 10% of the animals injected with saline selected the PTZ lever.

The number of responses emitted with the incorrect lever prior to correct lever selection during acquisition of the PTZ-saline discrimination is shown in Table 1. On the first PTZ training session, the rats emitted 31±11.1 responses with the saline lever before selecting the PTZ lever. On the first saline session the rats emitted 34±6.8 responses with the PTZ lever before selection of the saline lever. On the twentieth training session the rats injected with PTZ emitted 13±4.0 responses with the saline lever prior to

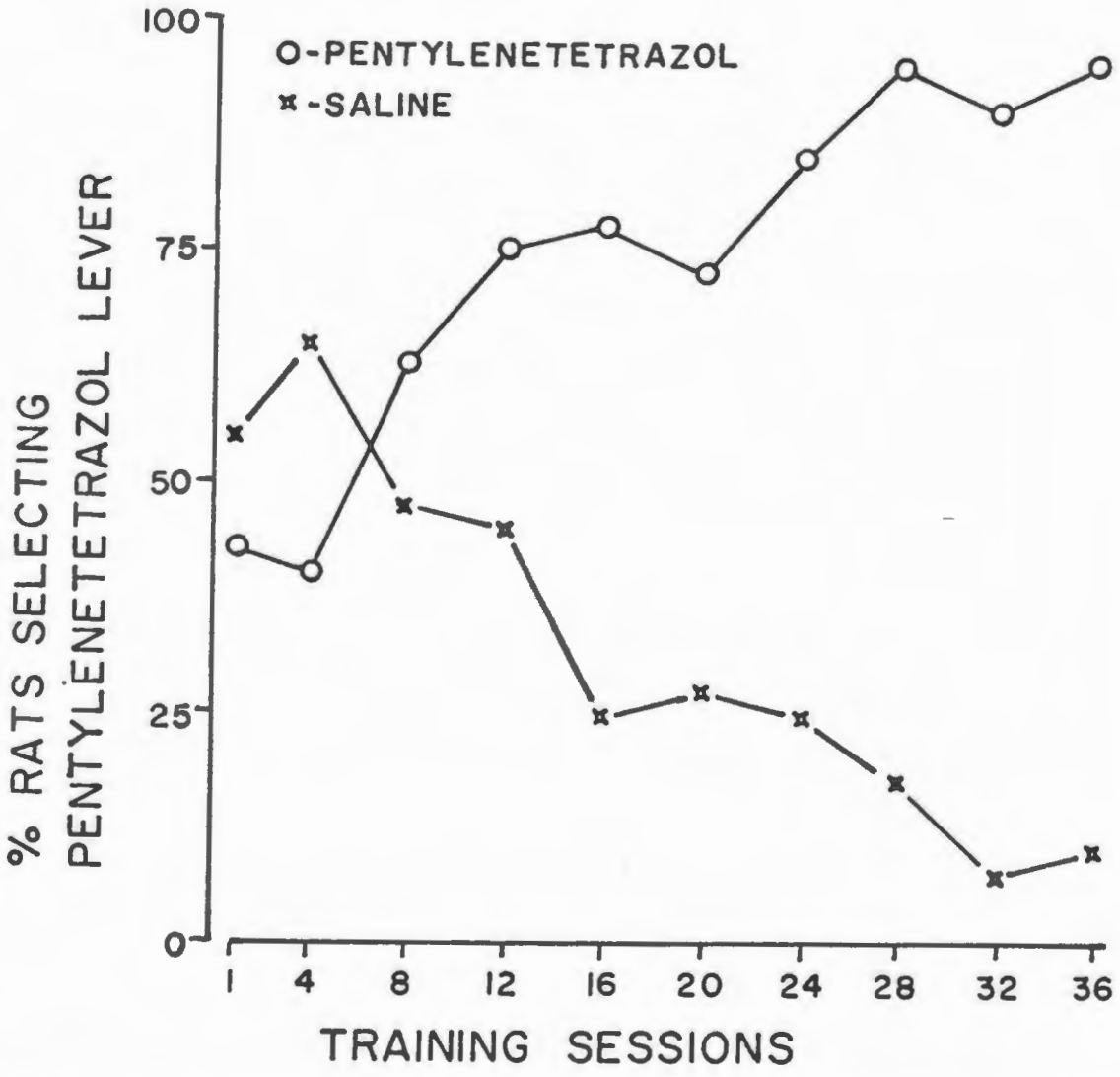

Figure 2. Acquisition of PTZ-saline discrimination. Values are the percentage of rats selecting the PTZ lever or the indicated training session when an injection of PTZ or saline was given. Data are based on 44 rats.

TABLE 1. Responses Emitted with the Incorrect Lever Prior to Lever Selection During Acquisition of the Pentylenetetrazol (20 mg/kg) - Saline Discrimination

Training Session Injection Number[1]	Responses with Saline Lever Following PTZ (20 mg/kg) Injection (Mean ± S.E.)[2]	Responses with PTZ Lever Following Saline Injection (Mean ± S.E.)[3]
1	31±11.1	34±6.8
4	18±4.7	39±9.1
6	22±5.5	27±4.7
8	15±3.5	21±6.0
10	10±1.9	22±5.5
12	8±2.2	16±5.1
14	7±1.7	14±3.1
16	8±2.6	11±3.5
18	7±2.1	11±3.6
20	13±4.0	8±2.3
22	5±1.6	8±3.2
24	6±2.7	9±2.6
26	11±3.3	9±2.0
28	4±1.3	7±2.0
30	6±2.3	2±0.5
32	3±1.0	5±1.6
34	4±1.7	5±1.7
36	1±0.6	3±1.2

[1]PTZ or saline were administered daily in an irregularly alternating sequence.

[2]Responses (Mean±S.E.) emitted with the saline lever prior to selection of the PTZ lever (10 responses with PTZ lever) on days when PTZ (20 mg/kg) was injected.

[3]Responses (Mean±S.E.) emitted with the PTZ lever prior to selection of the saline lever (10 responses with saline lever) on days when saline (1 ml/kg) was injected.

selection of the PTZ lever and following saline injection the rats emitted 8±2.3 responses with the PTZ lever before selection of the saline lever. By the thirty-sixth training session the rats injected with PTZ emitted 1±0.6 responses with the saline lever before PTZ lever selection and following saline injection the rats emitted 3±1.2 responses with the PTZ lever before saline lever selection. During acquisition of the PTZ-saline discrimination there was a significant ($R=0.82$ and 0.91; $p < 0.05$) decrease in the number of responses emitted with the saline lever prior to PTZ lever selection and vice versa. During the last ten sessions before generalization and antagonism testing began these rats emitted only 2.5 (±0.44) responses with the saline lever after PTZ injection and 3.1 (±0.43) responses with the PTZ lever after saline injection. During subsequent testing the rats continued to reliably discriminate between PTZ and saline (Table 3).

Effect of Varying Pentylenetetrazol Pretreatment Time on Lever Selection in Rats Trained to Discriminate Between Pentylenetetrazol and Saline (Table 2). - One hundred percent of the rats selected the PTZ lever when the training dose (20 mg/kg) of PTZ was administered 15 min before testing for lever selection. Ninety percent of the rats tested for lever selection 5 or 30 min after PTZ (20 mg/kg) injection selected the PTZ lever and 80% selected the PTZ lever when tested for lever selection 60 min following the PTZ injection. When tested for lever selection 2 h after PTZ injection 63% of the

TABLE 2. Effect of Varying Pentylenetetrazol Treatment Time on Lever Selection in Rats Trained to Discriminate Pentylenetetrazol from Saline

Pretreatment Time[1]	N[2]	% Selecting PTZ Lever[3]
5 min	10	90
15 min	10	100
30 min	10	90
60 min	10	80
2 h	8	63
4 h	8	13
24 h	10	0

[1]Rats were injected i.p. with PTZ (20 mg/kg). Following the specified pretreatment time the rats were placed in their assigned Skinner box and allowed to make a lever selection. The lever with which ten responses were completed first was considered the selected lever.

[2]Number of rats tested.

[3]Percent of rats selecting PTZ lever.

rats selected the PTZ lever. This was not significantly different (Fisher Exact Probability; $p > 0.05$), however, from 100% PTZ lever selection when lever selection was tested 15 min after PTZ (20 mg/kg). In contrast PTZ lever selection 4 or 24 h after PTZ injection was significantly different (Fisher Exact Probability, $p < 0.05$) from lever selection 15 min after PTZ injection as only 13% and none of the rats tested selected the PTZ at these times.

Generalization Testing (Tables 3 and 4)

1. Anxiogenic CNS Stimulants.

The training dose of PTZ generalized to lower doses of PTZ in a dose dependent ($R=0.98$, $p < .05$) manner. Whereas, none of the rats selected the PTZ lever following a 2.5 mg/kg PTZ injection, 28, 50 and 100% of the rats selected the PTZ lever following injection of 5, 10 and 20 mg/kg of PTZ (Table 3). The ED50 was 8 (5.9-10.8) mg/kg. The rats emitted 0.3 ± 0.25, 0.5 ± 0.37, 0.7 ± 0.39 and 1.1 ± 0.51 responses with the nonselected lever prior to lever selection following injection of 2.5, 5, 10 and 20 mg/kg of PTZ, respectively (Table 3). These doses of PTZ did not produce overt convulsions or myoclonus which were observed to occur when PTZ (40 mg/kg) was administered to naive rats. Following approximately 6 months of the PTZ-saline discrimination, however, some of the rats began to develop myoclonus after PTZ (20 mg/kg) injection. When PTZ (2.5-20 mg/kg) was administered to these rats the percentage selecting the PTZ lever was not significantly

different (Fisher Exact Probability, $p > 0.05$) from the percentae of rats selecting the PTZ lever following the acute administration of these doses of PTZ. Response rates during test sessions when PTZ (2.5-20 mg/kg) was administered were not significantly different (One-Way Analysis of Variance, $p > 0.05$) from the response rates of these rats on the PTZ or saline sessions preceding these tests.

Following injection of 5, 10 and 20 mg/kg of cocaine 0, 17 and 86% of the rats selected the PTZ lever ($R=0.94$, $p<0.05$). The ED50 was 13.9 (9.5-20.3) mg/kg. These rats emitted 0.3 ± 0.20, 1.5 ± 0.81 and 1.7 ± 1.12 responses with the nonselected lever prior to lever selection. One rat injected with cocaine (20 mg/kg) did not make a lever selection. Response rate during the cocaine (5 mg/kg) generalization test was significantly greater than (Duncan's Multiple Range Test, $p<0.05$) response rate during the preceding PTZ and saline sessions. After cocaine (10 mg/kg) the rats emitted 1046 ± 77 responses. This was significantly greater (Duncan's Multiple Range Test, $p<0.05$) than the 806 ± 53 responses emitted during the preceding PTZ session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from the 1064 ± 66 responses emitted the preceding saline session. Following the highest dose of cocaine (20 mg/kg) response rate was not significantly different ($F_{d.f.\ 2,15}=3.04$, $p>0.05$) from response rate during the preceding PTZ or saline session.

None of four rats injected with yohimbine (0.16 mg/kg) selected the PTZ lever. One out of five rats selected the

PTZ lever after 0.64 mg/kg yohimbine and one out of four rats selected the PTZ lever after 10 mg/kg yohimbine. However, 50% of twelve rats selected the PTZ lever after injection of 2.5 mg/kg yohimbine. This was significantly different (Fisher Exact Probability, $p<0.05$) however from 100% PTZ lever selection after 20 mg/kg PTZ. During the yohimbine (0.16-10 mg/kg) generalization tests the rats emitted 3 ± 1.9, 3 ± 1.6, 1.7 ± 0.91 and 3 ± 3.0 responses with the nonselected lever prior to lever selection. Response rate during the 0.16 and 0.64 mg/kg yohimbine sessions were not significantly different (F d.f. 2,6=0.44, $p>0.05$, F d.f. 2,12=1.13, $p>0.05$) from response rate during the PTZ or saline sessions preceding these tests. Response rate during the 2.5 mg/kg generalization test was significantly lower (Duncan's Multiple Range Test, $p<0.05$) than response rate during the preceding saline session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from response rate during the preceding PTZ session. During the 10 mg/kg yohimbine session response rate was significantly lower (Duncan's Multiple Range Test, $p<0.05$) than response rate during the preceding PTZ and saline sessions.

None of six rats injected with strychnine (1.25 mg/kg) selected the PTZ lever, however, three out of five rats injected with 2.5 mg/kg strychnine selected the PTZ lever. One rat did not make a lever selection after injection of the highest dose of strychnine. Whereas PTZ lever selection after the lowest dose of PTZ was significantly different (Fisher Exact Probability, $p\leq0.05$) from lever selection after 20 mg/kg

PTZ, PTZ lever selection after the highest dose of strychnine was not significantly different (Fisher Exact Probability, $p \geq 0.05$) from PTZ lever selection after the training dose of PTZ. The rats injected with these doses of strychnine emitted zero and 0.2 ± 0.20 responses with the nonselected lever prior to lever selection. Response rates during the strychnine (1.25 and 2.5 mg/kg) generalization tests were not significantly different ($F > d.f.\ 2,15=3.45$, $p>0.05$; F d.f. $2,12=2.76$, p 0.05) from response rates during the PTZ or saline sessions preceding the tests.

R05-3663 (0.64-5 mg/kg) dose-dependently ($R=0.99$, $p<0.05$) generalized to the discriminative stimulus produced by PTZ. The ED50 was 2.3 (1.8-2.8) mg/kg. Whereas none of the rats selected the PTZ lever following injection of 0.64 mg/kg R05-3663, 67 and 100% of the rats selected the PTZ lever following injection of R05-3663 at doses of 2.5 and 5 mg/kg, respectively. During these tests 1.3 ± 0.94, 0.5 ± 0.34 and 1.2 ± 0.80 responses were emitted with the nonselected lever prior to lever selection. Response rates during the R05-3663 (0.64-5 mg/kg) generalization tests were not significantly different (F d.f. $2,6=0.52$, $p>0.05$; F d.f. $2,15=3.11$, $p>0.05$; F d.f. $2,15=1.58$, $p>0.05$) from response rates during the PTZ or saline sessions preceding the tests.

2. Non-Anxiogenic Stimulants

One out of six rats injected with d-amphetamine (0.64 lmg/kg) selected the PTZ lever. This was significantly

different (Fisher Exact Probability, $p<0.05$) from 100% PTZ lever selection after injection of PTZ (20 mg/kg). These rats emitted 0.8 ± 0.54 responses with the nonselected lever prior to lever selection. Response rate after this dose of d-amphetamine was not significantly different (F d.f. 2,15=1.16, $p>0.05$) from response rate during the PTZ or saline session preceding the test. Three rats injected with d-amphetamine (2.5 mg/kg) did not make a lever selection.

None of the rats injected with methylphenidate (2.5-10 mg/kg) selected the PTZ lever. All of these rats selected the saline lever. Prior to saline lever selection these rats emitted 0.5 ± 0.34 and zero responses with the PTZ lever. Response rate after methylphenidate (2.5 or 10 mg/kg) was not significantly different (F d.f. 2,15=2.31, $p>0.05$; F d.f. 2,9=1.99, $p>0.05$) from response rate during the preceding PTZ or saline session.

None of the rats injected with caffeine (5 and 20 mg/kg) selected the PTZ lever, whereas 20% of the rats injected with 80 mg/kg of caffeine selected the PTZ lever. PTZ lever selection following caffeine was significantly different (Fisher Exact Probability, $p<0.05$) from 100% PTZ lever selection after PTZ (20 mg/kg). After caffeine injection the rats emitted zero, 0.8 ± 0.48 and 0.2 ± 0.20 responses with the nonselected lever before lever selection. During the caffeine (5 or 20 mg/kg) generalization tests response rates were not significantly different (F d.f. 2,9=1.30, $p>0.05$; F d.f. 2,9=3.44, $p>0.05$) from response rates during the pre-

ceding PTZ saline sessions. After caffeine (80 mg/kg) the rats emitted 228±73 responses. This was significantly less (Duncan's Multiple Range Test, $p<0.05$) than the 796±121 responses emitted during the preceding saline session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from the 540±133 responses emitted during the preceding PTZ session.

3. Convulsants

Bemegride (1.25-5 mg/kg) dose dependently ($R=1.00$, $p<0.05$) generalized to the PTZ discriminative stimulus. Whereas none of the rats selected the PTZ lever after a bemegride injection, 50 and 100% selected the PTZ lever after injection of 2.5 and 5 mg/kg bemegride, respectively. The ED50 was 2.5 (2.1-2.9) mg/kg. Whereas zero responses were emitted with the nonselected lever before lever selection during the generalization test with bemegride (1.25 and 2.5 mg/kg), 0.6±0.33 responses were emitted with the saline lever prior to PTZ lever selection during the 5 mg/kg bemegride generalization test. Response rates during the bemegride (1.25-5 mg/kg) generalization test sessions were not significantly different (F d.f. 2,6=0.41, $p>0.05$; F d.f. 2,18=2.87, $p>0.05$; F d.f. 2,15=2.92, $p>0.05$) from rates of responding during the PTZ or saline sessions preceding these tests.

Fifteen and sixty percent of the rats tested selected the PTZ lever following injection of 0.32 and 1.25 mg/kg picrotoxin, respectively. This was significantly different

(Fisher Exact Probability, $p< 0.05$) however, from 100 percent PTZ lever selection following PTZ (20 mg/kg) injection. During the picrotoxin generalization tests the rats emitted 1.1 ± 0.77 and 1.0 ± 0.63 responses with the nonselected lever prior to lever selection. Response rate following picrotoxin (0.32 or 1.25 mg/kg) was not significantly different (F d.f. 2,18=0.83, $p>0.05$; F d.f. 2,27=1.60, $p>0.05$) from the rate of responding during the PTZ or saline session preceding the picrotoxin test. Three rats injected with 2.5 mg/kg picrotoxin did not make a lever selection. Higher doses of picrotoxin produced convulsions in naive rats and therefore were not tested.

Following injection of 3-mercaptoproprionic acid (5 mg/kg), 40% of the rats selected the PTZ lever. This was significantly different (Fisher Exact Probability, $p<0.05$) however from 100% PTZ lever selection after injection of PTZ (20 mg/kg). These rats emitted 1 ± 1.0 responses with the nonselected lever prior to lever selection. During the 3-mercaptopropionic acid (5 mg/kg) test session the rats emitted 898 ± 94 responses. This was significantly greater (Duncan's Multiple Range Test, $p<0.05$) than the 608 ± 50 responses emitted during the preceding PTZ session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from the 958 ± 84 responses emitted during the saline session preceding the test session. Four rats injected with a 40 mg/kg dose of 3-mercaptopropionic acid died following convulsions produced by the drug.

Bicuculline methiodide (1.25-2.5 mg/kg) failed to produce PTZ lever selection when administered to the rats trained to discriminate between PTZ and saline. All of the rats selected the saline lever when tested for lever selection after these doses of bicuculline. Before saline lever selection these rats emitted zero and 0.5±0.50 responses with the PTZ lever. Response rates during the bicuculline (1.25 mg/kg and 2.5 mg/kg) generalization test sessions were significantly different (Duncan's Multiple Range Test, $p<0.05$) from response rates during the PTZ sessions preceding the tests but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from rate of responding during the preceding saline sessions.

None of six rats injected with gamma-hydroxybutyrate (GHB) at a dose of 160 mg/kg selected the PTZ lever whereas 17% of the rats selected the PTZ lever following a 320 mg/kg GHB injection. The percent PTZ lever selection produced by GHB was significantly different (Fisher Exact Probability, $p<0.05$) from 100% PTZ lever selection after PTZ (20 mg/kg). Higher doses of GHB are hypnotic and therefore were not tested. The rats treated with GHB 160 and 320 mg/kg, respectively, emitted 0.2±0.17 and 0.3±0.21 responses with the incorrect lever prior to lever selection. Response rate during the GHB (160 mg/kg) generalization test was not significantly different (F d.f. 2,15=0.90, $p>0.05$) from response rate during the preceding PTZ or saline session, however, during the GHB (320 mg/kg) test response rate was significantly different (Duncan's Multiple Range Test, p 0<05) from response

rate during the preceding PTZ and saline session.

Eleven and twenty-five percent of the rats injected with nicotine, 0.64 and 1.25 mg/kg, respectively, selected the PTZ lever. This was significantly different (Fisher Exact Probability, $p<0.05$) from 100% PTZ lever selection after PTZ (20 mg/kg). One rat injected with nicotine (1.25 mg/kg) did not make a lever selection. The rats making a lever selection emitted 1.3 ± 0.90 and 1.6 ± 0.90 responses with the nonselected lever prior to lever selection. Response rates during the nicotine (0.69 or 1.25 mg/kg) test sessions were not significantly different (F.d. f. 2,24=0.92, $p>0.05$; F. d.f. 2,21=2.33, $p>0.05$) from response rates during the PTZ or saline sessions preceding the tests.

4. Cholinergic Drugs

None of the rats injected with carbachol (0.32-0.64 mg/kg) selected the PTZ lever. All of the rats selected the saline lever. Prior to lever selection 0.2 ± 0.17 and zero responses were emitted with the nonselected lever. Three rats injected with 0.64 mg/kg carbachol did not make a lever selection. The rats emitted 803 ± 203 responses during the carbachol (0.32 mg/kg) generalization test. This was significantly less (Duncan's Multiple Range Test, $p<0.05$) than the 1317 ± 52 responses emitted during the preceding saline session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from the 1117 ± 36 responses emitted during the preceding PTZ session.

Thirty-six percent of eight rats injected with physostigmine (0.64 mg/kg) selected the PTZ lever when this drug was administered 30 minutes prior to testing. This was significantly different (Fisher Exact Probability, $p<0.05$) from 100% PTZ lever selection following injection of PTZ (20 mg/kg). Six rats did not make a lever selection. Four rats injected with physostigmine (1.25 mg/kg) and tested 30 minutes after injection did not make a lever selection. However when tested 75 minutes after injection, three rats selected the saline lever and one rat did not make a lever selection. Zero responses were made with the nonselected lever prior to lever selection in all generalization tests with physostigmine. Response rates during the physostigmine (0.64-1.25 mg/kg) generalization tests were significantly different (Duncan's Multiple Range Test, $p<0.05$) from response rate during the PTZ and saline sessions preceding the tests.

The rats injected with oxotremorine (0.32 mg/kg) selected the saline lever and these rats did not emit any responses with the PTZ lever before saline lever selection. Three other rats did not make a lever selection after 0.32 mg/kg oxotremorine. Response rate after oxotremorine was not significantly different (F d.f. $2,6=2.65$, $p>0.05$) from response rate during the preceding PTZ or saline session.

Thirty-eight percent of eight rats injected with scopolamine (0.64 mg/kg) selected the PTZ lever. This was significantly different from 100% PTZ lever selection after PTZ

(20 mg/kg) (Fisher Exact Probability Test, $p<0.05$). These rats emitted 0.8 ± 0.75 responses with the nonselected lever prior to lever selection. One rat did not make a lever selection. Response rate during the scopolamine (0.64 mg/kg) generalization test was significantly less (Duncan's Multiple Range Test, $<p$ 0.05) than response rate during the PTZ and saline sessions preceding the scopolamine test.

5. Drugs Affecting Monoaminergic Activity

Thirteen and fifty percent of eight rats selected the PTZ lever following administration of 0.16 and 0.64 mg/kg apomorphine, respectively. The percent PTZ lever selection after these doses of apomorphine was significantly different (Fisher Exact Probability Test, $p<0.05$) from 100% PTZ lever selection after PTZ (20 mg/kg). After apomorphine (1.25 mg/kg) one of two rats selected the PTZ lever and four rats did not make a lever selection. Zero, 0.4 ± 0.38 and 0.3 responses were emitted with the nonselected lever prior to lever selection following these doses of apomorphine. Response rate after apomorphine (0.16 mg/kg) was not significantly different (F d.f. $2,21=1.85$, $p>0.05$) from response rate during the preceding PTZ or saline sessions. After injection of 0.64 mg/kg apomorphine, however, response rate was significantly less (Duncan's Multiple Range Test, $p<0.05$) than response rate during the preceding saline session but not significantly different from response rate during the preceding PTZ session (Duncan's Multiple Range Test, $p>0.05$).

Thirty-three percent of six rats injected with propranolol (20 mg/kg) selected the PTZ lever. This was significantly different from 100% PTZ lever selection after PTZ (20 mg/kg) (X^2 d.F.1=7.87, $P<0.05$). These rats emitted 1.0±0.82 responses with the nonselected lever prior to lever selection. Response rate during the propranolol test session was significantly less (Duncan's Multiple Range Test, $p<0.05$) than response rate during the PTZ and saline session preceding this test.

All of the rats injected with aceperone (0.64 or 2.25 mg/kg) selected the saline lever and these rats did not emit any responses with the PTZ lever before saline lever selection. These rats were removed from the Skinner boxes after lever selection and therefore response rate is not given.

Twenty-five percent of eight rats selected the PTZ lever after a 100 mg/kg injection of DL-5-hydroxytryptophan (5-HTP). This was significantly different (Fisher Exact Probability, $p<0.05$) however, from 100% PTZ lever selection after PTZ (20 mg/kg). These rats emitted 0.3±0.25 responses with the nonselected lever before making a lever selection. Two rats did not make a lever selection after this dose of 5-HTP. Response rate after 5-HTP was significantly less (Duncan's Multiple Range Test, $p<0.05$) than rates of responding during the previous PTZ and saline sessions.

After fluoxetine (5 mg/kg), 33 percent of six rats selected the PTZ lever and these rats did not emit any responses with the nonselected lever prior to lever selection.

Following injection of a higher (10 mg/kg) dose of fluoxetine, two rats selected the saline lever and two rats did not make a lever selection. The rats emitted 566±97 responses during the fluoxetine (5 mg/kg) generalization test. This was significantly less (Duncan's Multiple Range Test, $p < 0.05$) than the 1120±82 responses emitted by these rats during the previous saline session but not significantly different (Duncan's Multiple Range Test, $p > 0.05$) from the 822±76 responses emitted during the PTZ session preceding the test. Response rate after 10 mg/kg fluoxetine was lower than the preceding PTZ and saline sessions but the small number of rats prohibited statistical analysis.

6. Opiate Agonist and Antagonists

None of the four rats injected with morphine (2.5 mg/kg) selected the PTZ lever and these rats did not emit any responses with the PTZ lever prior to saline lever selection. Response rate after morphine was not significantly different (F d.f.2,6=1.71, $p>0.05$) from rates of responding during the PTZ and saline sessions preceding the morphine test.

Thirty-three percent of six rats injected with naloxone (80 mg/kg) selected the PTZ lever and this was significantly different (Fisher Exact Probability, $p<0.05$) from 100% PTZ lever selection after PTZ (20 mg/kg), however. These rats did not emit any responses with the incorrect lever prior to lever selection. One rat did not make a lever selection after

TABLE 3. Generalization tests with rats trained to discriminate between pentylenetetrazol (20 mg/kg) and saline.[1]

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	% Selecting PTZ Lever[3]	Mean Responses[4] (±S.E.) with Non-Selected Lever Before Lever Selection
ACEPERONE	0.64	30	5	0	0
	2.5		5	0	0
d-AMPHETAMINE SULFATE	0.64	15	6	17	0.8±0.54
	2.5		6	No Selection	
APOMORPHINE HYDROCHLORIDE	0.16	15	8	13	0
	0.64		8	50	0.4±0.38
	1.25		2	50	0.3
			4	No Selection	-
BEMEGRIDE	1.25	15	3	0	0
	2.5		6	50	0
	5		7	100	0.6±0.33
BICUCULLINE METHIODIDE	1.25	10	6	0	0
	2.5		6	0	0.5±0.50
CAFFEINE CITRATE	5	30	4	0	0
	20		4	0	0.8±0.48
	80		5	20	0.2±0.20
CARBACHOL	0.32	15	6	0	0.2±0.17
	0.64		1	0	0
			3	No Selection	-
COCAINE HYDROCHLORIDE	5	15	6	0	0.3±0.20
	10		6	17	1.5±0.81
	20		7	86	1.7±1.12
			1	No Selection	-
FLUOXETINE	5	15	6	33	0
	10		2	0	1.5
			2	No Selection	-

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	% Selecting PTZ Lever	Mean Responses[4] (±S.E.) with Non-Selected Lever Before Lever Selection
GAMMA-HYDROXYBUTYRATE	160	7	6	0	0.2±0.17
	320		6	17	0.3±0.21
3-MERCAPTOPROPIONIC ACID	10	30	5	40	1.0±1.0
	40		4	lethal	-
METHYLPHENIDATE	2.5	15	6	0	0.5±0.34
	10		4	0	0
MORPHINE SULFATE	2.5	20	4	0	0
NALOXONE HYDROCHLORIDE	80	15	6	33	0
			1	No Selection	0
	160		3	100	
NICOTINE	0.64	15	9	11	1.3±0.90
	1.25		8	25	1.6±0.90
			1	No Selection	-
OXOTREMORINE	0.32	15	3	0	0
			3	No Selection	-
PENTYLENETETRAZOL	2.5	15	4	0	0.3±0.25
	5		14	28	0.5±0.37
	10		14	50	0.7±0.39
	20		14	100	1.1±0.51
PHYSOSTIGMINE SULFATE	0.64	30	8	36	0
			6	No Selection	-
	1.25		4	No Selection	-
	1.25	75	3	0	0
			1	No Selection	-
PICROTOXIN	0.32	15	7	14	1.1±0.77
	1.25		10	60	1.0±0.63
	2.5		3	No Selection	-

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	% Selecting PTZ Lever	Mean Responses[4] (±S.E.) with Non-Selected Lever Before Lever Selection
PROPRANOLOL	20	30	6	33	1.0±0.82
R04-4602 + DL-5-HYDROXYTRYPTOPHAN	25	60			
	100	30	8	25	0.3±0.25
			2	No Selection	-
R05-3663	0.64	30	4	0	1.3±0.94
	2.5		6	67	0.5±0.34
	5		6	100	1.2±0.80
SALINE	--	15	14	0	0.4±0.25
SCOPOLAMINE HYDROBROMIDE	0.64	30	8	38	0.8±0.75
			1	No Selection	-
STRYCHNINE SULFATE	1.25	15	6	0	0
	2.5		5	60	0.2±0.20
			1	No Selection	-
YOHIMBINE HYDROCHLORIDE	0.16	30	4	0	3±1.9
	0.64		5	20	3±1.6
	2.5		12	50	1.7±0.91
	10		4	25	3±3.0

[1]Following pretreatment with the test drug the rats were placed in their assigned Skinner boxes and allowed to make a lever selection. The lever with which ten responses were completed first was considered the selected lever.

[2]Number of rats tested.

[3]% of rats selecting the pentylenetetrazol lever. "No Selection" indicates that 10 responses were not completed with either lever in the 10 min session due to behavioral toxicity of the drug.

[4]Responses (Mean±S.E.) emitted with nonselected lever before lever selection. Where N <3 mean is given.

TABLE 4. Generalization Tests: Effect of test drugs on response rate in rats trained to discriminate between pentylenetetrazol (20 mg/kg) and saline.

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	Responses/10 Min; Mean±S.E.[3]			% ± S.E.[4]	
				PTZ	Saline	Test Drug	% PTZ	% Saline
d-AMPHETAMINE SULFATE	0.64	15	6	760±66	918±80	898±92	118±5	98±4
	2.5		3			0	0	0
APOMORPHINE HYDROCHLORIDE	0.16	15	8	789±45	1033±95	900±114	112±10	74±11
	0.64		8	850±64	1164±59	739±148	86±17	62±12
	1.25		2	1045	1085	830	78	75
			4	863±96	1085±86	0	0	0
BEMEGRIDE	1.25	15	3	437±104	523±43	540±57	136±26	80±37
	2.5		6	753±49	967±97	955±55	129±9	102±8
	5		7	726±55	965±91	834±60	116±7	91±9
BICUCULLINE METHIODIDE	1.25	10	6	658±84	1087±35	1038±59	171±22	96±4
	2.5		6	623±72	1003±48	977±61	168±22	97±3
CAFFEINE CITRATE	5	30	4	428±98	640±247	743±140	198±40	122±10
	20		4	508±65	805±68	785±124	158±23	98±12
	80		5	540±133	796±121	228±73	48±14	28±7
CARBACHOL	0.32	15	6	1117±36	1317±52	803±203	72±17	59±14
	0.64		1	670	690	710	106	103
			3	977±19	1193±28	0	0	0
COCAINE HYDROCHLORIDE	5	15	6	923±61	1209±35	1075±47	121±10	88±4
	10		6	806±53	1064±66	1046±77	130±6	99±6
	20		7	843±38	991±74	767±77	93±12	82±13
			1	890	950	0	0	0

TABLE 4 continued

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	Responses/10 Min; Mean±S.E.[3] PTZ	Saline	Test Drug	% ± S.E.[4] % PTZ	% Saline
FLUOXETINE	5	15	5	822±76	1120±82	566±97	73±12	51±10
	10		2	970	1110	300	31	28
			2	795	1170	0	0	0
GAMMA-HYDROXYBUTYRATE	160	7	6	1050±81	1163±65	1053±55	93±7	89±3
	320		5	842±44	1136±60	508±127	58±14	44±10
3-MERCAPTO-PROPIONIC ACID	10	30	5	608±50	958±84	898±94	148±13	96±10
METHYLPHENIDATE	2.5	15	6	938±78	1070±48	1133±66	123±6	106±3
	10		4	928±34	1173±51	1005±14	107±12	85±10
MORPHINE SULFATE	2.5	20	3	927±52	933±37	837±32	90±2	90±7
NALOXONE HYDROCHLORIDE	80	15	6	897±87	1093±91	452±152	61±27	44±14
			1	590	670	0	0	0
	160		1	780	1120	510	65	46
			3	1056±119	1183±127	0	0	0
NICOTINE	0.64	15	9	820±58	971±106	954±88	126±8	112±10
	1.25		8	836±75	1040±95	805±80	96±3	79±4
			1	700	860	0	0	0
OXOTREMORINE	0.32	15	3	1030±140	1293±32	1223±18	124±18	95±1
			3	873±55	1060±72	0	0	0

TABLE 4 continued

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	Response/10 Min; Mean±S.E.[3] PTZ	Saline	Test Drug	% ± S.E.[4] % PTZ	% Saline
PENTYLENETETRAZOL	2.5	15	4	918±92	1068±122	1105±106	121±7	104±2
	5		14	691±63	856±67	834±78	106±12	100±4
	10		14	720±39	859±51	825±47	112±7	98±4
	20		14	725±43	847±56	718±45	100±4	88±7
PHYSOSTIGMINE SULFATE	0.64	30	8	815±96	956±98	446±153	56±20	36±14
			1			0	0	0
	1.25		4			0	0	0
	1.25	75	3	1060±15	1277±55	170±95	15±7	14±8
			1	820	1030	0	0	0
PICROTOXIN	0.32	15	7	759±78	916±108	924±118	121±11	102±5
	1.25		10	810±59	992±97	829±77	106±11	91±11
PROPRANOLOL	20	30	6	858±70	953±70	465±86	54±10	50±9
R04-4602+DL-5-HYDROXYTRYPTOPHAN	25	60						
	100	30	8	980±89	1103±12	267±39	27±4	24±4
			2	680	750	0	0	0
R05-3663								
	0.64	30	4	520±147	653±62	667±111	142±35	101±9
	2.5		6	433±60	673±83	702±103	163±18	104±9
	5		6	396±64	587±140	370±67	92±9	69±10
SALINE		15	14	805±49	981±74	1004±70	126±8	104±2

TABLE 4 continued

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	Response/10 Min; Mean±S.E.[3]			% ± S.E.[4]	
				PTZ	Saline	Test Drug	% PTZ	% Saline
SCOPOLAMINE HYDROBROMIDE	0.64	30	8	853±54	1173±47	479±125	55±13	33±10
			1	670	910	0	0	0
STRYCHNINE SULFATE	1.25	15	6	818±69	1037±63	1032±69	129±13	100±5
	2.5		5	908±44	1024±51	620±206	68±21	62±20
			1	880	1110	0	0	0
YOHIMBINE HYDROCHLORIDE	0.16	30	4	490±110	600±65	567±72	129±30	94±2
	0.64		5	426±102	600±88	438±84	114±15	72±9
	2.5		8	569±40	828±41	643±75	112±9	67±6
	10		3	556±145	853±213	117±92	18±11	18±15

[1]Test drugs were administered intraperitoneally. Following pretreatment with the test drugs the rats were placed in their assigned Skinner box and allowed to respond with the levers for 10 min.

[2]Number of rats tested.

[3]Total responses (Mean±S.E.) emitted during the 10 min session following test drug treatment and during the preceding pentylenetetrazol (20 mg/kg) and saline session.

[4]Values were obtained by dividing the total number of lever presses emitted following test drug by the total number of responses during the preceding pentylenetetrazol (20 mg/kg) and saline session.

this dose of naloxone. After naloxone (160 mg/kg), one rat selected the PTZ lever and three rats did not make a lever selection. Response rate after naloxone (80 mg/kg) was significantly less (Duncan's Multiple Range Test, $p<0.05$) than the response rate during the PTZ and saline sessions preceding the naloxone test.

Antagonism Testing (Tables 5 and 6)

1. Effect of Benzodiazepines, Meprobamate and Barbiturates

Pretreatment of rats trained to discriminate PTZ (20 mg/kg) from saline with either saline or 86% saline, 1% Tween and 13% propylene glycol failed to antagonize the discriminative stimulus produced by PTZ. All of the rats pretreated with the solvents selected the PTZ lever. Rats pretreated with saline emitted 0.5 ± 0.37 responses with the saline lever prior to PTZ lever selection whereas rats pretreated with 86% saline 1% Tween 80 and 13% propylene glycol emitted 0.3 ± 0.29 responses with the saline lever before PTZ lever selection. Rate of responding during the saline antagonism test was not significantly different (F d.f. $2,39=0.38$, $p>0.05$) from response rate during the PTZ or saline session preceding the test. Response rate after 86% saline, 1% Tween 80 and 13% propylene glycol treatment however, was significantly less (Duncan's Multiple Range Test, $p<0.05$) than response rate during the preceding saline session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from response rate during the preceding PTZ session.

Pretreatment with chlordiazepoxide (0.64-10 mg/kg) produced a dose-dependent ($R=0.93$, $p<0.05$) antagonism of discriminative stimulus produced by PTZ. WHereas 80% of the rats pretreated with chlordiazepoxide (CDP) at a dose of 0.64 mg/kg 30 minutes before PTZ (20 mg/kg) injection selected the PTZ lever, 30 and 20% of the rats selected the PTZ lever after pretreatment with 2.5 and 10 mg/kg CDP. The ED50 was 1.8 (0.67-4.96) mg/kg. The rats pretreated with CDP (0.64 mg/kg) emitted 0.4 ± 0.22 responses with the nonselected lever prior to lever selection whereas the rats pretreated with CDP (2.5 or 10 mg/kg) did not emit any responses with the nonselected lever prior to lever selection. Response rate during the CDP (0.64-10 mg/kg) antagonism test was not significantly different (One-Way Analysis of Variance, $p>0.05$) from rates of responding during the saline or PTZ sessions preceding the tests.

Clonazepam (0.04-0.64 mg/kg) pretreatment antagonized the discriminative stimulus produced by PTZ in a dose-dependent ($R=0.96$, $p<0.05$) manner. Whereas 50% of teh rats pretreated with clonazepam at a dose of 0.04 mg/kg 30 minutes prior to PTZ (20 mg/kg) selected the PTZ lever, 38 and 0% of the rats pretreated with clonazepam (0.16 and 0.64 mg/kg) selected the PTZ lever. The ED50 was 0.05 (0.015-0.17) mg/kg. The rats pretreated with clonazepam (0.04 mg/kg) emitted 0.2 ± 0.16 responses with the nonselected lever prior to lever selection whereas the rats pretreated with 0.16 and 0.64 mg/kg of clonazepam did not emit any responses with the non-

selected lever prior to lever selection. Response rates of rats pretreated with clonazepam (0.04 mg/kg) were significantly greater (Duncan's Multiple Range Test, $p<0.05$) than rates of responding during the preceding saline session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from rates of responding during the preceding PTZ session. Response rates after clonazepam (0.16 and 0.64 mg/kg) pretreatment, however, were not significantly different (F d.f. 2,15=1.45, $p>0.05$; F d.f. 2,15=1.94, $p>0.05$) from rates of responding during the preceding PTZ or saline sessions.

Diazepam (1.25-10 mg/kg) injection 30 minutes prior to administration of PTZ (20 mg/kg) produced a dose-dependent ($R=0.99$, $p<0.05$) antagonism of the discriminative stimulus produced by PTZ (20 mg/kg). Whereas 83% of the rats pretreated with diazepam (1.25 mg/kg) selected the PTZ lever, 67, 33 and 17% of the rats selected the PTZ lever after pretreatment with 2.5, 5 and 10 mg/kg of diazepam. The ED50 was 3.5 (1.6-8.0) mg/kg. These rats emitted 0.8 ± 0.54, 1.5 ± 1.33, 0.7 ± 0.49 and 2.0 ± 0.77 responses with the nonselected lever prior to lever selection after pretreatment with diazepam 1.25, 2.5, 5 and 10 mg/kg, respectively. Response rate during the diazepam (1.25 mg/kg) antagonism test was significantly less (Duncan's Multiple Range Test, $p<0.05$) than rates of responding during the preceding saline session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from rates of responding during the preceding PTZ

session. Rates of responding after diazepam (2.5 and 5 mg/kg) pretreatment were not significantly different (F d.f. 2,6=0.47, $p>0.05$; F d.f. 2,15=1.60, $p>0.05$) from rates of responding during the saline or PTZ sessions preceding tests with these doses of diazepam. However, rates of responding after pretreatment with the highest dose of diazepam (10 mg/kg) were significantly less (Duncan's Multiple Range Test, $p<0.05$ than rates of responding during the preceding PTZ and saline sessions.

Clobazam (0.64-40 mg/kg) 30 minutes prior to PTZ (20 mg/kg) antagonized the discriminative stimulus produced by PTZ in a dose-dependent manner (R=0.99, $p<0.05$). Whereas 100% of the rats pretreated with clobazam (0.64 mg/kg) selected the PTZ lever, 83, 33 and 0% of the rats selected the PTZ lever after treatment with 2.5, 10 and 40 mg/kg clobazam, respectively. The ED50 was 6.2 (2.4-16.0) mg/kg. The rats pretreated with clobazam (0.64 and 2.5 mg/kg) did not emit any responses with the nonselected lever prior to lever selection whereas the rats pretreated with 10 and 40 mg/kg clobazam emitted 0.2 ± 0.17 responses with the nonselected lever prior to lever selection. Response rates during the clobazam (2.5 and 40 mg/kg) antagonism tests were not significantly different (F d.f. 2,5=1.61, $p>0.05$; F d.f. 2,9= 2.05, $p>0.05$) from rates of responding during the saline or PTZ sessions preceding the tests. Response rates after clobazam (10 mg/kg) was significantly less (Duncan's Multiple Range Test, $p<0.05$) than rate of responding during

the preceding saline session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from rates of responding during the preceding PTZ session.

Flurazepam (1.25-20 mg/kg) antagonized the PTZ discriminative stimulus in dose-dependent fashion ($R=0.96$, $p<0.05$). Whereas 100% of the rats pretreated with flurazepam (1.25 mg/kg) 30 minutes before PTZ (20 mg/kg) selected the PTZ lever, 50 and 33% of the rats selected the PTZ lever after pretreatment with 5 and 20 mg/kg flurazepam. The ED50 was 8.6 (3.1-13.7) mg/kg. The rats pretreated with flurazepam emitted 0.3 ± 0.25, 0.2 ± 0.17 and zero responses with the non-selected lever prior to lever selection after 1.25, 5 and 20 mg/kg flurazepam, respectively. Rate of responding of rats pretreated with flurazepam (1.25 mg/kg) was significantly greater (Duncan's Multiple Range Test, $p<0.05$) than rate of responding during the preceding saline session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from response rate during the preceding PTZ session. After flurazepam (5 mg/kg) pretreatment the rats emitted 963 ± 78 responses. This was not significantly different (F.d. f.2,15= 1.36, $p>0.05$) from the 1105 ± 57 emitted during the preceding saline session or the 963 ± 74 responses that were emitted during the preceding PTZ sessions. Response rate after flurazepam (20 mg/kg) pretreatment, however, was significantly less (Duncan's Multiple Range Test, $p<0.05$) than rate of responding during the preceding PTZ and saline sessions.

Meprobamate (5-80 mg/kg) antagonized the PTZ discriminative stimulus in a dose-dependent ($R=0.98$, $p<0.05$) manner. Whereas 100% of the rats treated with meprobamate (5 mg/kg) 30 minutes prior to PTZ (20 mg/kg) selected the PTZ lever, 67 and 0% of the rats injected with meprobamate 20 and 80 mg/kg selected the PTZ lever. The ED50 was 22.5 (16.6-30.7 mg/kg. The rats treated with meprobamate 5 and 80 mg/kg emitted 0.2 ± 0.17 responses with the nonselected lever prior to lever selection whereas the rats treated with 20 mg/kg meprobamate did not emit any responses with nonselected lever before lever selection. Response rates after meprobamate (5-80 mg/kg) pretreatment were significantly less (Duncan's Multiple Range Test, $p<0.05$) than rates of responding during the preceding saline sessions but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from response rates during the preceding PTZ sessions.

Pentobarbital (2.5-10 mg/kg) pretreatment dose-dependently ($R=0.98$, $p<0.05$) antagonized the discriminative stimulus produced by PTZ (20 mg/kg). Whereas 100% of the rats treated with pentobarbital (2.5 mg/kg) 30 minutes before PTZ (20 mg/kg) selected the PTZ lever, 33 and 0% selected the PTZ lever after pretreatment with 5 and 10 mg/kg pentobarbital, respectively. These rats emitted 2.0 ± 1.98 zero and 0.9 ± 0.80 responses with the nonselected lever prior to lever selection after pretreatment with pentobarbital. Response rates during the pentobarbital (2.5-10 mg/kg) test sessions were not significantly different (F d.f. $2,15=3.21$, $p>0.05$;

F d.f. 2,15=0.01, $p>0.05$; F d.f. 2,15=0.76, $p>0.05$) from rates of responding during the saline or PTZ sessions preceding the pentobarbital antagonism tests.

Phenobarbital (2.5-40 mg/kg) treatment 30 minutes prior to PTZ (20 mg/kg) produced a dose-dependent ($R=1.00$, $p<0.05$) antagonism of the PTZ discriminate stimulus. Whereas 100% of the rats pretreated with 2.5 mg/kg phenobarbital selected the PTZ lever, 50 and 0% of the rats pretreated with 10 and 40 mg/kg phenobarbital selected the PTZ lever. The ED50 was 10 (7.4-13.5) mg/kg. The rats pretreated with 2.5 and 40 mg/kg phenobarbital emitted 0.7 ± 0.42 responses with the nonselected lever before lever selection whereas the rats pretreated with 10 mg/kg phenobarbital emitted zero responses with the nonselected lever prior to lever selection. Rates of responding after pretreatment with phenobarbital (2.5 and 10 mg/kg) were not significantly different (F d.f. 2,12=1.43, $p>0.05$, F d.f. 2,12=3.29, $p>0.05$) from rates of responding during the PTZ or saline sessions preceding these phenobarbital antagonism tests. After pretreatment with 40 mg/kg phenobarbital the rats emitted 632 ± 91 responses. This was significantly less (Duncan's Multiple Range Test, $p<0.05$) than 966 ± 63 responses that were emitted during the preceding saline session but not significantly different (Duncan's Multiple Range Test, p 0.05)>from 778 ± 94 responses emitted during the preceding PTZ session.

2. Effect of Non-Anxiolytic Anticonvulsant Drugs

Trimethadione (TMD) failed to significantly (Fisher

Exact Probability, $p>0.05$) antagonize the discriminative stimulus produced by PTZ. Seventy-eight percent of the rats pretreated with TMD (160 mg/kg) selected the PTZ lever when TMD was administered 30 minutes before PTZ (20 mg/kg) and 67% of six rats selected the PTZ lever after 320 mg/kg TMD pretreatment. Two rats pretreated with the latter dose of TMD did not make a lever selection. After TMD 160 and 320 mg/kg pretreatment the rats making a lever selection emitted 0.3 ± 0.24 and 2.0 ± 1.29 responses, respectively, with the nonselected lever prior to lever selection. On the TMD (160 mg/kg) test session the rats emitted 553 ± 54 responses. This was significantly less (Duncan's Multiple Range Test, $p<0.05$) than the 803 ± 71 responses emitted during the preceding saline session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from 623 ± 75 responses emitted during the preceding PTZ session. During the TMD (320 mg/kg) test session, however, rate of responding was significantly less (Duncan's Multiple Range Test, $p<0.05$) than response rate during the preceding PTZ and saline sessions.

Ethosuximide (100-200 mg/kg) treatment 30 minutes prior to injection of PTZ (20 mg/kg) failed to significantly (Fisher Exact Probability, $p>0.05$) antagonize the PTZ discriminative stimulus. Seventy-eight percent of the rats pretreated with ethosuximide (100 mg/kg) selected the PTZ lever and 100% of the rats injected with 200 mg/kg ethosuximide selected the PTZ lever. Two rats pretreated with the

highest dose of ethosuximide did not make a lever selection. The rats pretreated with 100 mg/kg ethosuximide emitted 0.9±0.51 responses with the nonselected lever prior to lever selection, whereas the rats pretreated with 200 mg/kg ethosuximide that made a lever selection emitted 0.4±0.29 responses with the saline lever prior to PTZ lever selection. Response rates during the ethosuximide (100 and 200 mg/kg) test sessions were significantly less (Duncan's Multiple Range Test, $p<0.05$) than rates of responding during the preceding PTZ and saline sessions.

Diphenylhydantoin (30-60 mg/kg) administered 15 minutes before injection of PTZ (20 mg/kg) failed to significantly (Fisher Exact Probability, $p>0.05$) antagonize the PTZ discriminative stimulus. Eighty-three percent of the rats pretreated with either 30 or 60 mg/kg diphenylhydantoin (DPH) selected the PTZ lever. Zero and 0.2±0.17 responses were emitted with the nonselected lever prior to lever selection during the test sessions when the rats were pretreated with 30 and 60 mg/kg (DPH), respectively. Response rates during the antagonism test sessions were significantly less (Duncan's Multiple Range Test, $p<0.05$) than response rates during the saline and PTZ sessions preceding the tests.

3. Effect of Some GABAmimetic Drugs

Treatment of rats trained to discriminate PTZ (20 mg/kg) from saline with valproic acid (40-320 mg/kg) produced a dose-dependent ($R=0.98$, $p<0.05$) antagonism of PTZ

discriminative stimulus. Whereas 100% of the rats treated with valproic acid (40 mg/kg) selected the PTZ lever, 75 and 33% of the animals selected the PTZ lever after pretreatment with 160 and 320 mg/kg valproic acid, respectively. The ED50 was 244 (156-382) mg/kg. Three rats pretreated with 640 mg/kg valproic acid did not make a lever selection. Rats pretreated with 40 mg/kg valproic acid did not emit any responses with the saline lever prior to PTZ lever selection whereas the rats pretreated with 160 and 320 mg/kg valproic acid emitted 0.4 ± 0.26 and 0.7 ± 0.49 responses with the incorrect lever prior to lever selection. Rats pretreated with valproic acid (160 mg/kg) emitted 600 ± 59 responses during this valproic acid test session. This was significantly less (Duncan's Multiple Range Test, $p<0.05$) than 903 ± 80 responses emitted during the preceding saline session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from 614 ± 80 responses emitted during the preceding PTZ session. Response rate during the valproic acid (320 mg/kg) test session, however, was significantly less (Duncan's Multiple Range Test, $p<0.05$) than rates of responding during the preceding PTZ and saline sessions.

Administration of etomidate sulfate (5 mg/kg) 5 minutes before injection of PTZ (20 mg/kg) failed to significantly (Fisher Exact Probability, $p>0.05$) antagonize the PTZ discriminative stimulus. Eighty-three percent of the rats treated with this dose of etomidate selected the PTZ lever.

The rats pretreated with etomidate (5 mg/kg) emitted 1.2±1.17 responses with the nonselected lever prior to lever selection. Five out of six rats treated with etomidate (10 mg/kg) 5 minutes before PTZ (20 mg/kg) did not make a lever selection. The one rat that made a lever selection selected the PTZ lever and emitted one response with the saline lever before PTZ lever selection. The rats emitted 793±77 responses during the etomidate (5 mg/kg) test session. This was significantly less (Duncan's Multiple Range Test, $p<0.05$) than 1087±50 responses emitted during the preceding saline session but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from 810±49 responses emitted during the preceding PTZ session.

Treatment of rats with etomidate (10 and 40 mg/kg) two hours before PTZ (20 mg/kg) injection also failed to antagonize the discriminative stimulus produced by PTZ. All of the rats pretreated with 10 mg/kg etomidate and tested 2 hours later for lever selection selected the PTZ lever. These rats emitted 0.8±0.48 responses with the saline lever before PTZ lever selection. Response rate during this test session was significantly different (Duncan's Multiple Range Test, p 0≤05) from rates of responding during the preceding PTZ and saline sessions. After a 40 mg/kg etomidate injection two out of four rats selected the PTZ lever and two selected the saline lever. This was not significantly different (Fisher Exact Probability, $p>0.05$) from 100% PTZ lever selection after PTZ (20 mg/kg). These rats emitted 2.0±1.68 responses

with the nonselected lever prior to lever selection. Two rats did not make a lever selection after pretreatment with 40 mg/kg etomidate. Response rate during the etomidate (40 mg/kg) test session was significantly different (Duncan's Multiple Range Test, $p<0.05$) from response rates during the preceding PTZ and saline sessions.

Gamma-hydroxybutyrate (GHB) treatment 7 minutes prior to PTZ (20 mg/kg) injection failed to antagonize the PTZ discriminative stimulus. One hundred percent of the rats pretreated with GHB (80-160 mg/kg) selected the PTZ lever. These rats emitted 1.3 ± 0.95 and 0.5 ± 0.29 responses with the saline lever before PTZ lever selection. Response rates during the GHB antagonism tests were not significantly different (F d.f. 2,9=1.65, $p>0.05$; F d.f. 2,9=1.86, $p>0.05$) from response rates during the PTZ and saline sessions preceding these tests. Four rats pretreated with 320 mg/kg GHB did not make a lever selection.

Administration of gamma-acetylenic GABA (100 mg/kg) 2-24 hours prior to PTZ (20 mg/kg) injection did not significantly (Fisher Exact Probability, $p<0.05$) antagonize the discriminative stimulus produced by PTZ. When tested for lever selection 2, 4, 8 and 24 hours after gamma-acetylenic GABA (GAG), 80, 100, 100 and 86% of the rats making a lever selection selected the PTZ lever. These rats emitted zero, 0.4 ± 0.40, 0.5 ± 0.50 and 1.4 ± 0.81 responses with the nonselected lever prior to lever selection. Response rates during all GAG

antagonism tests were significantly lower (Duncan's Multiple Range Test, $p<0.05$) than response rates during the PTZ and saline sessions preceding these tests. One, two and four rats tested for lever selection at four, eight and 24 hours after GAG did not make a lever selection.

Gamma vinyl GABA (1000 mg/kg) did not angagonize the PTZ discriminative stimulus when the rats were tested for lever selection 72 hours after gamma-vinyl GABA (GVG) injection. Three out of four rats tested at this time selected the PTZ lever and these rats did not emit any responses with the saline lever before PTZ lever selection. One rat did not make a lever selection. When tested at 2 and 8 hours after GVG the rats did not make a lever selection. One rat selected the PTZ lever when tested 24 hours after GVG, seven rats did not make a lever selection. Response rate for the three rats making a lever selection 72 hours after GVG was significantly less (Duncan's Multiple Range Test, $p<0.05$) than the rate of responding of these rats during the preceding PTZ and saline sessions.

4. Other CNS Depressants

Ethanol (250-1000 mg/kg) treatment 15 minutes prior to injection of PTZ (20 mg/kg) failed to antagonize the discriminative stimulus produced by PTZ. All of the rats pretreated with ethanol selected the PTZ lever and these rats did not emit any responses with the saline lever before PTZ lever selection. Two rats pretreated with 1000 mg/kg ethanol

did not make a lever selection. Response rates during the ethanol test sessions were significantly less (Duncan's Multiple Range Test, $p<0.05$) than rates of responding during the saline sessions preceding the tests but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from response rates during the PTZ sessions preceding the tests.

Morphine (10 mg/kg) pretreatment did not antagonize the PTZ discriminative stimulus when administered 15 minutes prior to PTZ (20 mg/kg). Three of eight rats that made a lever selection selected the PTZ lever and these rats emitted 0.7 ± 0.67 responses with the saline lever prior to PTZ lever selection. Response rate during the morphine antagonism test session was significantly less (Duncan's Multiple Range Test, $p<0.05$) than rate of responding during the preceding PTZ and saline sessions.

5. Effect of Caffeine: A Phosphodiesterase Inhibitor

Eighty percent of five rats treated with caffeine (20 mg/kg) 30 minutes before PTZ (20 mg/kg) injection selected the PTZ lever (Fisher Exact Probability, $p>0.05$). These rats emitted 0.4 ± 0.24 responses with the nonselected lever prior to lever selection. Response rate during the caffeine antagonism test session was not significantly different ($F_{2,9}=1.05$, $p>0.05$) from rate of responding during the preceding PTZ or saline sessions.

6. Effect of Drugs Affecting Catecholaminergic Transmission.

Clonidine (0.04-0.64 mg/kg) treatment 30 minutes

before injection of PTZ (20 mg/kg) failed to antagonize the discriminative stimulus produced by PTZ. All of the rats pretreated with these doses of clonidine selected the PTZ lever. Three rats pretreated with 0.64 mg/kg clonidine did not make a lever selection. After 0.04 mg/kg pretreatment the rats emitted 0.8±0.75 responses with the saline lever before PTZ lever selection and after 0.16 mg/kg clonidine pretreatment the rats emitted 1.8±0.97 responses with the saline lever before PTZ lever selection. Response rates during the clonidine test sessions were significantly less (Duncan's Multiple Range Test, $p<0.05$) than the rate of responding during the PTZ and saline sessions preceding the clonidine antagonism tests.

Propranolol (10 mg/kg) pretreatment did not significantly (Fisher Exact Probability, $p>0.05$) antagonize the PTZ discriminative stimulus. Eighty percent of five rats pretreated with propranol selected the PTZ lever and these rats did not emit any responses with the nonselected lever prior to lever selection. During the propranol test session the rats emitted 588±121 responses. This was significantly less (Duncan's Multiple Range Test, $p<0.05$) than the 1008±66 responses emitted during the saline session preceding the test but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from 668±97 responses emitted during the PTZ preceding the test.

Aceperone (0.64 or 2.5 mg/kg) did not antagonize the PTZ discriminative stimulus. All of four rats pretreated with

Haloperidol (0.16-0.64 mg/kg) also failed to antagonize the discriminative stimulus produced by PTZ. All of six rats treated with haloperidol (0.16 mg/kg) 30 minutes prior to PTZ (20 mg/kg) injection selected the PTZ lever. These rats emitted 0.4±0.33 responses with the saline lever before selection of the PTZ lever. During the haloperidol (0.16 mg/kg) test session the rats emitted 265±97 responses. This was significantly less (Duncan's Multiple Range Test, $p<0.05$) than the 975±86 and 1132±71 responses that were emitted during the PTZ and saline sessions preceding the haloperidol antagonism test.

Of two rats pretreated with 0.64 mg/kg haloperidol, one rat did not make a lever selection and the other rat selected the PTZ lever.

7. Effect of Drugs Affecting Serotonergic Transmission

Five rats treated with methysergide (2.5 mg/kg) 30 minutes before injection of PTZ (20 mg/kg) selected the PTZ lever. These rats emitted 3.4±1.67 responses with the saline lever before PTZ lever selection. Response rate during the methysergide antagonism test session was significantly different (Duncan's Multiple Range Test, $p<0.05$) from response rate during the saline session preceding the test but not significantly different (Duncan's Multiple Range Test, $p>0.05$) from response rate during the PTZ session preceding the test.

Fluoxetine (5-10 mg/kg) treatment 15 minutes before injection of PTZ (20 mg/kg) failed to antagonize the discrimi-

native stimulus produced by PTZ. All of the rats pretreated with these doses of fluoxetine selected the PTZ lever and these rats did not emit any responses with the saline before selection of the PTZ lever. Response rate during the fluoxetine antagonism test sessions were significantly less (Duncan's Multiple Range Test, $p<0.05$) than response rate during the PTZ and saline sessions preceding the tests.

Amitriptyline (10-20 mg/kg) injection 30 minutes before PTZ (20 mg/kg) did not block the PTZ discriminative stimulus. All of six rats pretreated with 10 mg/kg amitriptyline selected the PTZ lever and these rats emitted 1.8 ± 0.87 responses with the saline lever before PTZ lever selection. These rats emitted 213 ± 75 responses during this amitriptyline test session. This was significantly less (Duncan's Multiple Range Test, $p<0.05$) than the 410 ± 72 and 853 ± 80 responses that were emitted during the PTZ and saline sessions preceding the tests. After 20 mg/kg amitriptyline pretreatment three rats did not make a lever selection while two selected the PTZ lever. The two rats that made a lever selection emitted an average of one response on the saline lever before selection of the PTZ lever. Response rate of these two rats appeared to be decreased compared to response rate during the PTZ and saline sessions preceding the test.

Treatment with R04-4602 plus DL-5-hydroxytryptophan (100 mg/kg) one hour before PTZ (20 mg/kg) injection did not antagonize the discriminative stimulus produced by PTZ. All of the rats pretreated with R04-4602 plus DL-5-hydroxytryp-

tophan (5-HTP) selected the PTZ lever and these did not emit any responses with the saline lever before selection of the PTZ lever. Response rate during the 5-HTP antagonism test session was significantly less (Duncan's Multiple Range Test, $p<0.05$) than response rate during the PTZ and saline sessions preceding the R04-4602 + 5-HTP tests.

8. Effect of Anticholinergics

Scopolamine (0.64 mg/kg) treatment 15 minutes before injection of PTZ (20 mg/kg) did not significantly (Fisher Exact Probability, $p>0.05$) antagonize the PTZ discriminative stimulus. Eighty-four percent of six rats selected the PTZ lever while one rat did not make a lever selection. The rats that made a lever selection emitted 1.0 ± 0.63 responses with the nonselected lever prior to lever selection. During the scopolamine antagonism test the animals emitted 124 ± 70 responses. This was significantly less (Duncan's Multiple Range Test, $p<0.05$) than the 906 ± 67 and 1166 ± 54 responses that were emitted during the PTZ and saline sessions preceding the test.

Atropine (5-10 mg/kg) also failed to antagonize the PTZ discriminative stimulus. All of four rats treated with 5 mg/kg atropine 30 minutes before injection of pTZ (20 mg/kg) selected the PTZ lever and these rats did not emit any responses with the saline lever before PTZ lever selection. Response rate of these rats during the test session was significantly less (Duncan's Multiple Range Test, $p<0.05$) than

TABLE 5. Antagonism tests with rats trained to discriminate between pentylenetetrazol (20 mg/kg) and saline.[1]

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	% Selecting PTZ Lever[3]	Mean Responses[4] (±S.E.) on Non-Selected Lever Before Lever Selection
ACEPERONE	0.64	30	4	100	0.75±0.75
	2.5		5	80	0.20±0.20
AMITRIPTYLINE HYDROCHLORIDE	10	30	6	100	1.8±0.87
	20		2	100	1.0
			3	No Selection	--
ATROPINE SULFATE	5	30	4	100	0
			1	No Selection	--
	10		2	100	0.5
CAFFEINE CITRATE	20	30	5	80	0.4±0.24
CHLORDIAZEPOXIDE HYDROCHLORIDE	0.64	30	10	80	0.4±0.22
	2.5		10	30	0
	10		10	20	0
CHLORPROMAZINE	1.25	30	4	100	0
	5		4	No Selection	--
CLOBAZAM	0.64	30	6	100	0
	2.5		6	83	0
	10		6	33	0.2±0.17
	40		5	0	0.2±0.17
CLONAZEPAM	0.04	30	6	50	0.2±0.16
	0.16		6	33	0
	0.64		8	0	0
CLONIDINE HYDROCHLORIDE	0.04	30	4	100	0.8±0.75
	0.16		5	100	1.8±0.97
	0.64		1	100	0
			3	No Selection	--
DIAZEPAM	1.25	30	6	83	0.8±0.54
	2.5		6	67	1.5±1.33
	5		6	33	0.7±0.49
	10		6	17	2.0±0.77

TABLE 5 continued

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	% Selecting PTZ Lever[3]	Mean Responses (±S.E.) on Non-Selected Lever Before Lever Selection[4]
DIPHENYLHYDANTOIN	30	15	6	83	0
	60		6	83	0.2±0.17
ETHANOL	250	15	3	100	0
	1000		4	100	0
			2	No Selection	--
ETHOSUXIMIDE	100	30	9	78	0.9±0.51
	200		5	100	0.4±0.24
			2	No Selection	--
ETOMIDATE SULFATE	5	5	6	83	1.2±1.17
	10		1	100	1.0
			5	No Selection	--
	10	2 h	4	100	0.8±0.48
	40		4	50	2.0±1.68
			2	No Selection	--
FLUOXETINE	5	15	3	100	0
	10		4	100	0
FLURAZEPAM DIHYDROCHLORIDE	1.25	30	6	100	0.3±0.25
	5		6	50	0.2±0.17
	20		6	33	0
GAMMA-ACETYLENIC GAMA	100	2 h	5	80	0
		4 h	5	100	0.4±0.40
			1	100	0.5±0.50
		8 h	4	100	0.5±0.50
			2	No Selection	--
		24 h	7	86	1.4±0.81
			4	No Selection	--
GAMMA-HYDROXY-BUTYRATE	80	7	4	100	1.3±0.95
	160		4	100	0.5±0.29
	320		4	No Selection	--

TABLE 5 continued

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	% Selecting PTZ Lever[3]	Mean Responses[4] (±S.E.) on Non-Selected Lever Before Lever Selection
GAMMA-VINYL GABA	1000	2	4	No Selection	--
		8	4	No Selection	--
		24	1	100	0
			7	No Selection	--
		72	3	100	0
			1	No Selection	--
HALOPERIDOL	0.16	45	6	100	0.4±0.33
	0.64		1	100	0
			1	No Selection	--
MEPROBAMATE	5	30	6	100	0.2±0.17
	20		6	67	0
	80		6	0	0.2±0.17
METHYSERGIDE	2.5	30	5	100	3.4±1.67
MORPHINE SULFATE	10	15	3	100	0.7±0.67
			5	No Selection	--
PENTOBARBITAL	2.5	20	6	100	2.0±1.48
	5		6	33	0
	10		6	0	0.9±0.8
PHENOBARBITAL	2.5	45	5	100	0.7±0.42
	10		6	50	0
	40		6	0	0.7±0.42
PROPRANOLOL	10	30	5	80	0
R04-4602+DL-5-HYDROXYTRYPTOPHAN	25	1 h			
	100	30	4	100	0
SALINE	--	15-60	14	100	0.5±0.37
SCOPOLAMINE HYDROBROMIDE	0.64	15	6	84	1.0±0.63
			1	No Selection	--
TRIMETHADIONE	160	30	9	78	0.3±0.24
	320		6	67	2.0±1.29
			2	No Selection	--

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	% Selecting PTZ Lever[3]	Mean Responses[4] (±S.E.) on Non-Selected Lever Before Lever Selection
1% TWEEN 80 + 13% PROPYLENE GLYCOL	--	30	10	100	0.3±0.29
VALPROIC ACID	40	45	6	100	0
	160		8	75	0.4±0.26
	320		6	33	0.7±0.49
	640		3	No Selection	--

[1]Following pretreatment with the test drug, the rats were injected with pentylenetetrazol (20 mg/kg). 15 min following the pentylenetetrazol injection, animals were placed in their assigned Skinner box and allowed to make a lever selection. The lever with which ten responses were completed first was considered the selected lever.

[2]Number of rats tested.

[3]% of rats selecting the pentylenetetrazol lever. "No selection" indicates that ten responses were not completed on either lever in the 10 min session due to behavioral toxicity of the drugs.

[4]Responses (Mean±S.E.) emitted with nonselected lever before lever selection. Where $N < 3$ mean is given.

TABLE 6. Antagonism Tests: Effect of Test Drugs on Response Rate in Rats Trained to Discriminate Between Pentylenetetrazol and Saline

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	Responses/10 Min[3] Mean+S.E.			% + S.E.[4]	
				PTZ	Saline	Test Drug	% PTZ	% Saline
ACEPERONE	0.64	30	4	895±72	1125±80	615±76	68±5	54±4
	2.50		5	444±69	858±70	422±63	95±4	48±4
AMITRYPTYLINE HYDROCHLORIDE	10	30	6	410±72	853±80	213±75	51±13	24±6
	20		2	725	1325	70	10	6
			3	200±90	590±171	0	0	0
ATROPINE SULFATE	5	30	4	543±58	843±74	157±34	28±5	18±3
			1	290	960	0	0	0
			2	360	655	95	26	19
CAFFEINE	20	30	5	385±117	668±170	543±120	163±25	85±18
CHLORDIAZEPOXIDE HYDROCHLORIDE	0.64	30	10	660±56	809±61	703±45	111±7	90±6
	2.5		10	709±48	880±58	767±60	108±5	88±4
	10		10	807±95	951±90	776±58	100±7	84±7
CHLORPROMAZINE	1.25	30	4	443±94	620±21	258±31	68±18	45±16
	5		4	638±105	1080±125	0	0	0
CLOBAZAM	2.5	30	6	885±82	1072±83	858±108	102±21	83±14
	10		6	872±100	1138±63	907±50	112±16	80±3
	40		5	728±116	853±94	540±134	88±36	64±14

TABLE 6 continued

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	Responses/10 Min[3] Mean±S.E. PTZ	Saline	Test Drug	% ± S.E.[4] % PTZ	% Saline
CLONAZEPAM	0.04	30	6	513±95	987±69	702±99	145±19	73±11
	0.16		6	492±75	655±50	610±81	128±13	91±9
	0.64		6	457±73	700±106	595±80	139±17	89±10
CLONIDINE HYDROCHLORIDE	0.04	30	6	678±162	743±55	210±63	33±10	28±7
	0.16		5	782±48	1106±63	56±22	7±3	5±2
	0.64		1	830	950	60	7	6
			3	663±157	1197±81	0	0	0
DIAZEPAM	1.25	30	6	1002±47	1178±49	903±87	91±8	76±7
	2.5		6	743±48	757±67	820±62	114±7	110±12
	5		6	827±42	983±99	827±62	101±8	90±13
	10		6	933±84	1073±66	588±154	65±19	53±13
DIPHENYLHYDANTOIN	30	15	6	770±149	973±100	332±75	52±12	33±7
	60		6	690±90	865±148	267±101	40±17	34±13
ETHANOL	250	15	3	553±60	907±38	500±110	93±25	56±14
	1000		4	630±172	893±49	300±46	67±24	33±4
			2	595	1025	0	0	0
ETHOSUXIMIDE	100	30	9	747±92	1001±111	643±91	84±4	64±7
	200		5	768±125	913±132	325±91	49±18	41±15
			2	560	675	0	0	0

TABLE 6 continued

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	Responses/10 Min[3] Mean ± S.E. PTZ	Saline	Test Drug	% ± S.E.[4] % PTZ	% Saline
ETOMIDATE	5	5	6	810±49	1087±50	793±77	99±9	73±6
SULFATE	10		1	380	990	310	82	31
			5	320±90	708±164	0	0	0
	10	2 h	4	595±115	870±310	125±55	20±5	14±7
	40		4	648±96	773±68	243±159	40±24	29±18
			2	295	780	0	0	0
FLUOXETINE	5	15	3	893±74	1240±29	730±15	83±5	59±1
	10		4	810±86	1128±54	370±97	44±9	32±7
FLURAZEPAM	1.25	30	6	480±83	860±89	625±24	148±34	76±9
DIHYDROCHLORIDE	5		6	963±74	1105±57	963±78	100±4	87±4
	20		6	1053±33	990±33	845±52	80±3	86±7
GAMMA-ACETYLENIC	100	2 h	5	1046±97	1116±59	195±125	22±13	18±10
GABA		4 h	5	906±62	1048±83	214±112	22±10	23±13
			1	890	1000	0	0	0
		8 h	4	1085±85	1203±110	610±206	61±23	57±22
			2	710	1180	0	0	0
		24 h	7	927±109	1110±101	400±91	46±15	38±12
			4	1225	1125	0	0	0
GAMMA-HYDROXY-	80	7	4	615±199	923±130	548±127	96±9	58±7
BUTYRATE	160		4	538±160	660±155	328±94	75±18	47±5
	320		4	810±390	775±275	0	0	0

TABLE 6 continued

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	Responses/10 Min 3 Mean±S.E. PTZ	Saline	Test Drug	% ± S.E.[4] % PTZ	% Saline
GAMMA-VINYL GABA	1000	4 h	4	658±112	975±106	0	0	0
		8 h	4	717±31	1102±39	0	0	0
		24 h	1	730	670	260	36	39
			7	681±63	1091±27	0	0	0
		72 h	3	807±168	963±149	320±25	41±8	34±3
			1	350	1010	0	0	0
HALOPERIDOL	0.16	45	6	975±86	1132±71	265±97	30±12	25±10
	0.64		1	1120	1160	80	7	7
MEPROBAMATE	5	30	6	733±125	1097±46	618±76	90±7	56±6
	20		6	803±45	1043±55	795±79	101±11	78±9
	80		6	798±112	1132±47	872±59	122±21	78±4
METHYSERGIDE	2.5	30	5	508±83	606±76	366±62	74±10	62±11
MORPHINE SULFATE	10	15	3	676±102	980±98	223±74	36±14	24±10
			5	502±96	966±97	0	0	0
PENTOBARBITAL	2.5	20	6	901±44	1060±43	997±46	111±5	94±2
	5		6	813±98	822±135	837±97	103±6	107±9
	10		6	733±74	890±105	817±88	113±11	93±5
PHENOBARBITAL	2.5	30	6	784±142	988±81	708±137	92±6	70±11
	10		6	596±137	976±75	740±100	138±19	75±7
	10		6	778±94	966±63	632±91	81±4	65±5

TABLE 6 continued

Test Drug	Dose (mg/kg)	Pretreatment Time (MInutes)	N[2]	Responses/10 Min[3] Mean±S.E. PTZ	Saline	Test Drug	% ± S.E.[4] % PTZ	% Saline
PROPRANOLOL	10	30	5	668±97	1008±66	588±121	88±12	54±12
R04-4602-DL-5-HYDROXYTRYPTOPHAN	4	1 h						
		30		838±54	1233±73	330±68	39±6	27±5
SALINE	--	15-60	14	755±102	975±155	815±124	107±3	86±12
SCOPOLAMINE HYDROBROMIDE	0.64	15	6	906±67	1166±54	124±70	14±8	11±6
			1	900	1030	0	0	0
TRIMETHADIONE	160	30	9	623±75	803±71	553±54	104±20	72±8
	320		6	602±74	783±110	305±76	46±9	37±7
			2	685	900	0	0	0
1% TWEEN 80 + 13% PROPYLENE GLYCOL	-	30	7	941±59	1243±30	941±81	103±10	75±6
VALPROIC ACID	160	45	8	614±80	903±80	600±59	111±20	73±14
	320		6	885±53	1080±88	380±34	44±4	36±4
	640		3	830±147	927±178	0±0	0±0	0±0

[1]Test Drugs were administered intraperitoneally. Following pretreatment with the test drug the rats were placed in their assigned Skinner box and allowed to respond with the levers for 10 min.

[2]Number of rats tested.

TABLE 6 continued

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[3]Total responses (mean±S.E.) emitted during the 10 min session following pretreatment with the test drug and during the preceding pentylenetetrazol (20 mg/kg) and saline session.

[4]Values were obtained by dividing the total number of lever presses emitted following test drug pretreatment by the total number of responses during the preceding pentylenetetrazol (20 mg/kg) and saline session.

response rate during the PTZ and saline sessions preceding the test session. One rat did not make a lever selection after pretreatment with 5 mg/kg atropine. Two rats pretreated with 10 mg/kg atropine selected the PTZ lever and these rats did not emit any responses with the saline lever before PTZ lever selection. Response rate during the 10 mg/kg atropine test session appeared to be decreased compared to the response rate during the PTZ and saline sessions preceding the test but the small number of animals prohibited statistical analysis.

Lack of Tolerance Development to Diazepam and Chlordiazepoxide in Antagonism of the PTZ Discriminative Stimulus (Table 7)

Chronic administration of solvent, diazepam or CDP to rats tested acutely with these drugs for antagonism of the PTZ discriminative stimulus did not alter their ability to antagonize the discriminative stimulus produced by PTZ. All of the rats pretreated acutely with solvent for antagonism of the PTZ discriminative stimulus selected the PTZ lever after chronic solvent administration. Whereas 67 and 17% of the rats pretreated acutely for antagonism of the PTZ discriminative stimulus with 2.5 and 10 mg/kg diazepam selected the PTZ lever, 67 and 33% of these rats selected the PTZ lever after chronic diazepam administration. Similarly, whereas 30 and 20% of the rats pretreated acutely for antagonism of the PTZ discriminative stimulus with 2.5 and 10 mg/kg CDP

selected the PTZ lever, 40 and 20% of these rats selected the PTZ lever after chronic CDP treatment.

Tests for Diazepam and Haloperidol Antagonism of the Discriminative Stimuli Produced by Cocaine, Yohimbine and R05-3663 in Rats Trained to Discriminate Between Pentylenetetrazol (20 mg/kg) and saline (Tables 8 and 9).

1. Cocaine

As stated previously (Table 3), 86% of the rats injected with cocaine (20 mg/kg) selected the PTZ lever when tested for lever selection 15 min after the cocaine injection. Diazepam (5 mg/kg) produced a significant (Fisher Exact Probability, $p<0.05$) antagonism of the discriminative stimulus produced by cocaine as only 17% of the rats selected the PTZ lever when cocaine (20 mg/kg) was preceded by a diazepam (5 mg/kg) injection. Prior to lever selection the rats emitted 2.3 ± 1.43 responses with the nonselected lever. During the diazepam plus cocaine test session the rats emitted 223 ± 93 responses. This was significantly less (Duncan's Multiple Range Test, $p<0.05$) than the 553 ± 88 responses that were emitted by these rats during the PTZ session preceding this test and the 658 ± 87 responses that were emitted during the saline session preceding the test.

Haloperidol (0.16 mg/kg) did not significantly (Fisher Exact Probability, p 0.05) antagonize the discriminative stimulus produced by cocaine. Whereas 86% of the rats selected the PTZ lever after cocaine, 75% selected the PTZ

TABLE 7. Antagonism Tests with Diazepam and Chlordiazepoxide After Their Chronic Administration.[1]

Drug	Dose (mg/kg)	N[2]	% Selecting PTZ Lever[3]	
			Acute	Chronic
SOLVENT	--	6	100	100
DIAZEPAM	2.5	6	67	67
	10.0	6	17	33
CHLORDIAZEPOXIDE	2.5	10	30	40
	10.0	10	20	20

[1]Rats were injected with the appropriate dose of either diazepam, chlordiazepoxide or solvent. 30 min later PTZ (20 mg/kg) was injected. 15 min following the PTZ injection, animals were placed in their assigned Skinner box and allowed to make a lever selection. The lever with which ten responses were completed first was considered the selected lever.

[2]Number of rats tested.

[3]Percent of rats selecting PTZ lever.

lever when haloperidol was injected before cocaine. Prior to lever selection the rats emitted 0.8±0.33 responses with the nonselected lever. Response rate during the haloperidol plus cocaine test session was significantly less (Duncan's Multiple Range Test, $p<0.05$) than response rate during the PTZ and saline session preceding the test.

2. R05-3663

R05-3663 (0.64-5 mg/kg) dose-dependently generalized to the discriminative stimulus produced by PTZ (Table 3). Diazepam (5 mg/kg) pretreatment antagonized the discriminative stimulus produced by R05-3663 so that higher doses of this drug were required to produce PTZ lever selection. Whereas 100% of the rats injected with 5 mg/kg R05-3663 selected the PTZ lever, none of the rats selected the PTZ lever when this dose of R05-3663 was preceded by a 5 mg/kg diazepam injection. However, 40 and 80% of the rats selected the PTZ lever when injected with 10 and 20 mg/kg R05-3663, respectively 30 min after diazepam (5 mg/kg) injection. Prior to lever selection these rats emitted 2.6±1.60, 0.8±0.80 and 0.6±0.60 responses with the nonselected lever. Response rates during the diazepam plus R05-3663 (5 and 10 mg/kg) test session were not significantly different (Duncan's Multiple Range Test, $p>0.05$ and F.d. $f.2,9=3.95$, $p>0.05$) from response rates during the PTZ and saline sessions preceding these tests. However, response rate during the diazepam plus R05-3663 (20 mg/kg) test session was significantly less (Duncan's

Multiple Range Test, $p < 0.05$) than response rate during the saline session but not the PTZ session preceding this test.

3. Yohimbine

Yohimbine (2.5 mg/kg) generalized to the PTZ discriminative stimulus in six of twelve rats (Table 3). Diazepam (5 mg/kg) pretreatment antagonized the yohimbine discriminative stimulus in three out of four of these rats that selected the PTZ leger after yohimbine alone.

TABLE 8. Tests for Diazepam and Haloperidol Antagonism of the Discriminative Stimuli Produced by Cocaine, Yohimbine and R05-3663 in Rats Trained to Discriminate Between Pentylenetetrazol (20 mg/kg) and Saline[1]

Test Drug	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	% Selecting PTZ Lever	Mean Responses[4] (±S.E.) on Non-Selected Lever Before Lever Selection
COCAINE	20	15	7	86	1.7±1.2
DIAZEPAM	5	30	6	0	0
DIAZEPAM + COCAINE	5 20	45-90 15-60	6	17	2.3±1.43
HALOPERIDOL	0.16	75	5	0	0.40±0.4
HALOPERIDOL + COCAINE	0.16 20	75 15	8	75	0.75±
YOHIMBINE	2.5	30	4	100	0
DIAZEPAM + YOHIMBINE	5 2.5	60 30	4	25	1±1.0
R05-3663	5	30	6	100	1.2±0.80
DIAZEPAM + R05-3663	5 5	60 30	5	0	2.6±1.60
	10	30	5	40	0.8±0.80
	20	30	5	80	0.6±0.60

[1]Following injection of the test drug the rats were placed in their assigned Skinner box and allowed to make a lever selection. The lever with which ten responses were completed first was considered the selected lever.

[2]Number of rats tested.

[3]% of rats selecting the PTZ lever.

[4]Responses (Mean±S.E.) emitted with nonselected lever before lever selection.

TABLE 9. Response Rates During Tests for Diazepam and Haloperidol Antagonism of the Discriminative Stimuli Produced by Cocaine and R05-3663 in Rats Trained to Discriminate Between Pentylenetetrazol and Saline

Test Drug[1]	Dose (mg/kg)	Pretreatment Time (Minutes)	N[2]	Responses/10 Min[3] Mean±S.E. PTZ	Saline	Test Drug	% S.E.±S.E.[3,4] % PTZ	% Saline
COCAINE	20	15	7	843±38	991±74	767±77	93±12	82±13
DIAZEPAM +	5	45-90						
COCAINE	20	15-60	6	553±88	658±87	223±93	42±19	33±15
HALOPERIDOL +	0.16	75						
COCAINE	20	15	8	500±70	820±99	315±55	83±24	40±8
R05-3663	5	30	6	396±64	587±140	370±67	92±96	69±10
DIAZEPAM +	5	60						
R05-3663	5	30	5	385±98	848±115	563±35	206±87	70±10
	10	30	5	605±103	850±94	450±106	72±9	51±10
	20	30	5	628±88	900±35	303±162	50±23	23±17

[1]Test drugs were administered intraperitoneally. Following pretreatment the rats were placed in their assigned Skinner box and allowed to respond with the levers for 10 min.

[2]Number of rats tested.

[3]Total responses (Mean±S.E.) emitted during the 10 min session following pretreatment with the test drug and during the preceding pentylenetetrazol (20 mg/kg) and saline session.

[4]Values were obtained by dividing the total number of responses emitted following test pretreatment by the total number of resonses during the preceding pentylenetetrazol (20 mg/kg) and saline sessions.

DISCUSSION

Acquisition of PTZ-Saline Discrimination

A number of drugs are known to function as discriminative stimuli which reliably control operant behavior (for reviews see Schuster and Balster, 1977; Lal, 1977; Colpaert and Rosecrans, 1978). Usually in these experiments laboratory animals are trained to emit one response when treated with a drug and an alternate response when treated with the drug vehicle, another dose of the same drug or a different drug. When acquisition of such response differentiation is reliably established, the drug is said to produce a discriminative stimulus which controls the differential response - emission in the trained subjects.

The present experiment demonstrates that rats can learn to reliably discriminate between the effect of PTZ (20 mg/kg) and saline. A mean of 30 sessions was required for the rats to reach the discrimination criterion. A similar number of trials to reach a similar criterion was previously reported for many other psychoactive drugs (Weissman, 1978). As was the case with other drugs (Lal, 1976; 1977; Shearman et al., 1978) the discriminative stimulus produced by PTZ was dose-dependent.

Previously PTZ was reported to produce state-dependent learning (Overton, 1966, 1973; Kurtz and Palfai, 1973).

Anxiogenic Property of PTZ as a Discriminative Stimulus

1. Time Course of PTZ Discrimination

In addition to its anxiogenic action, PTZ has also been reported to produce hallucinations in man (Rodin, 1958; Winter and Wallach, 1969). However, whereas the anxiogenic effect of PTZ diminishes soon after injection of PTZ is discontinued the hallucinations persist for up to 24 h after PTZ injection. In order to test the hypothesis that the discriminative stimulus produced by PTZ in the rat was related to its anxiogenic action and not its hallucinatory effect, discrimination of PTZ was tested at various times after injection of the drug.

Pharmacokinetic studies of PTZ in rats show that 70-95% of this drug appears in the urine within 24 h (Rowles et al., 1971; Vohland and Koransky, 1972). The cerebrospinal fluid and plasma half-life of a 20 mg/kg PTZ injection in cats has been reported to be approximately one hour (McMillen and Isaac, 1978).

In the present study the discrimination of PTZ diminished as the reported tissue levels of this drug decreased. A significant percentage of the rats no longer selected the PTZ lever when the reported blood levels of PTZ were significantly decreased. These data support the hypothesis that the discriminative stimulus produced in the rat is related to its anxiogenic property and not its hallucinatory effect.

2. Generalization Tests with Other Anxiogenics

To test the hypothesis that the discriminative stimulus produced by PTZ in the rat was related to its anxiogenic action in man (Rodin, 1958; Rodin and Calhoun, 1970; Winter and Wallach, 1969), other anxiogenic stimulants were tested for generalization to the PTZ discriminative stimulus.

Cocaine has been well recognized to induce anxiety in man (Cohen, 1975; Siegel, 1977; Wesson and Smith, 1977). Dose-dependent generalization by cocaine to the PTZ discriminative stimulus supports the hypothesis that the discriminative stimulus property of PTZ in the rat is related to its anxiogenic action in man. Additional support for this was provided by the finding that the anxiolytic drug, diazepam, antagonized the cocaine discriminative stimulus. Whereas the nonanxiolytic drug, haloperidol, did not antagonize the cocaine discriminative stimulus.

In addition to its anxiogenic action, however, cocaine is also a psychomotor stimulant. In order to determine that psychomotor stimulation was not the basis of the cocaine generalization to PTZ, nonanxiogenic psychomotor stimulants were tested for generalization to PTZ. Neither d-amphetamine, methylphenidate nor caffeine generalized to the PTZ discriminative stimulus suggesting that the psychomotor stimulant property of cocaine was not the basis of its discrimination to PTZ.

Several investigators (Holmberg and Gershon, 1961; Ingram, 1962; Gershon and Lang, 1962; Garfield et al., 1967) have

reported the anxiety-like effects produced by yohimbine in man. Amobarbital was reported to reduce the anxiety response to yohimbine while imipramine and epinephrine potentiated this effect (Holmberg and Gershon, 1961).

In the present study, yohimbine partially generalized to the discriminative stimulus property of PTZ. Furthermore, diazepam antagonized the yohimbine discriminative stimulus in those rats that perceived the yohimbine stimulus as similar to the PTZ discriminative stimulus. These data support the hypothesis that the discriminative stimulus property of PTZ in the rat is related to its anxiogenic action in man.

R05-3663 (1,3-dihydro-5-methyl-2H-1,4 benzodiazepin-2-one) is structurally related to the anxiolytic drug, diazepan (Schlosser and Franco, 1979). However, unlike diazepam, R05-3663 does not bind to benzodiazepine receptors (Speth et al., 1979); O'Brien and Spirt, 1980) and antagonizes the effect of diazepam (Schlosser and Franco, 1979). Because the action of R05-3663 is opposite to that of anxiolytic benzodiazepines this compound might be expected to be anxiogenic.

Recently, O'Brien and Spirt (1980) speculated that if the anxiolytic action of benzodiazepines were considered to be mediated through an antagonistic action then a molecule structurally similar to R05-3663 could exist in brain to function as an endogenous anxiety producing ligand. In the present study, R05-3663 dose-dependently generalized to the PTZ discriminative stimulus. Furthermore, the discriminative stimulus strength of R05-3663 was attenuated by diazepam.

These data provide behavioral evidence that R05-3663 may structurally resemble a naturally occurring anxiety-inducing ligand and support the hypothesis that the discriminative stimulus produced by PTZ in the rat is related to the anxiogenic action in man.

It has been reported that patients poisoned with strychnine are extremely apprehensive and fearful of impending death (Polson and Tattersall, 1969; Franz, 1975). Strychnine generalization to the discriminative stimulus produced by PTZ supports the hypothesis that the discriminative stimulus property of PTZ in the rat is related to its anxiolytic action in man.

In addition to the anxiogenic action of strychnine and R05-3663, however, these drugs are also convulsants. In order to determine that the convulsant action of strychnine and R05-3663 was not the basis of their generalization to the PTZ discriminative stimulus several nonaxiogenic convulsant drugs were tested for generalization to the PTZ discriminative stimulus.

Bemegride, a central nervous system stimulant and convulsant drug like PTZ (Hahn, 1960) dose-dependently generalized to the PTZ discriminative stimulus. This would suggest that the convulsant action of strychnine and R05-3663 was the basis of their generalization to the PTZ discriminative stimulus and that the discriminative stimulus produced by PTZ was related to its own convulsant action. However, none of the

other convulsant drugs tested, including picrotoxin, bicuculline, 3-mercaptoproprionic acid and nicotine, generalized to the PTZ discriminative stimulus.

Also, gamma-hydroxybutyrate that produces a hypersynchronous EEG in the chick (Osuide, 1972), rat (Marcus et al., 1967; Godschalk et al., 1976, 1977), cat (Winters and Spooner 1965), rabbit and man (Schneider et al., 1963), that is selectively antagonized by anti-petit mal drugs (Godschalk et al., 1976) and has been compared to petit mal epilepsy (Winters and Spooner, 1965; Snead et al., 1976; Godschalk et al., 1977), did not generalize to the PTZ discriminative stimulus. These data suggest that the convulsant action of strychnine and R05-3663 was probably not the basis of their generalization to the PTZ discriminative stimulus and that a subconvulsant or petit mal like brain state likely to be produced by these drugs was not the basis of the discriminative stimulus produced by PTZ. The finding that bemegride generalized to the PTZ discriminative stimulus suggests that this drug may be anxiogenic.

3. Blockade of the PTZ Discriminative Stimulus by Anxiolytics

As another approach to test the hypothesis that the discriminative stimulus produced by PTZ in the rat was related to its anxiogenic action in man, antagonism of the PTZ discriminative stimulus by clinically effective anxiolytic drugs was tested.

Several benzodiazepine anxiolytics, barbiturate type anxiolytics as well as a propanediol carbamate anxiolytic effectively antagonized the PTZ discriminative stimulus in a dose-dependent manner and in nonsedative doses. In addition, valproic acid, which was recently found to have anxiolytic properties in a conflict test (Lal et al., 1979) also dose-dependently antagonized the PTZ discriminative stimulus. These data support the hypothesis that the discriminative stimulus produced by PTZ in the rat is related to its anxiogenic action in man.

The potency of the various drugs effective in antagonizing the PTZ discriminative stimulus was highly correlated with their effective doses in a widely accepted conflict test used to measure anxiolytic activity (Figure 3) as well as their clinically effective doses (Figure 4). These data also support the hypothesis that the discriminative stimulus property of PTZ in the rat was related to its anxiogenic action in man.

In addition to anxiolytic activity, however, all of the drugs that antagonized the PTZ discriminative stimulus are also central nervous system depressants. Several investigators (Warner, 1965; Goldberg et al., 1967; Margules and Stein, 1969; Stein et al., 1975; Cook and Sepinwall, 1975) have reported that tolerance develops only to the depressant action benzodiazepines. Therefore, lack of tolerance development to other actions of these drugs in experimental animals

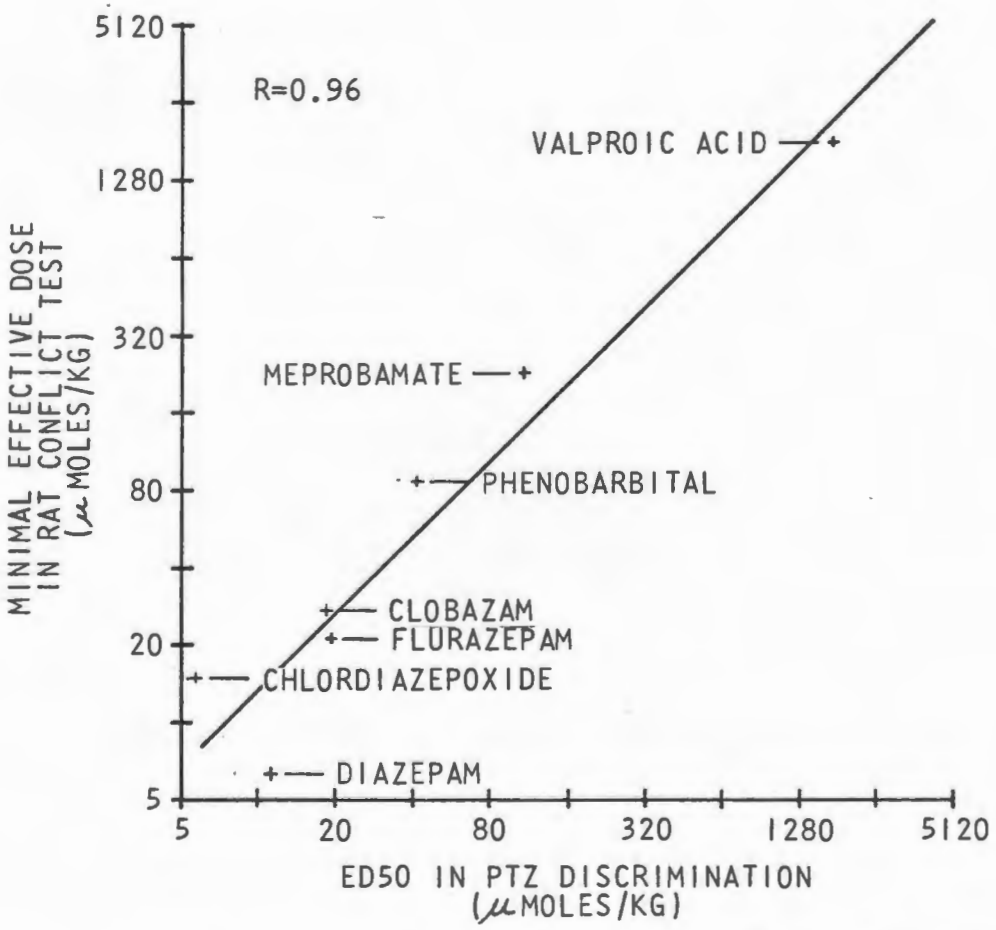

Figure 3. Correlation between the effective dose of anxiolytic drugs in antagonnzing the anxiogenic discriminative stimulus of PTZ and their effective doses in the conflict test.

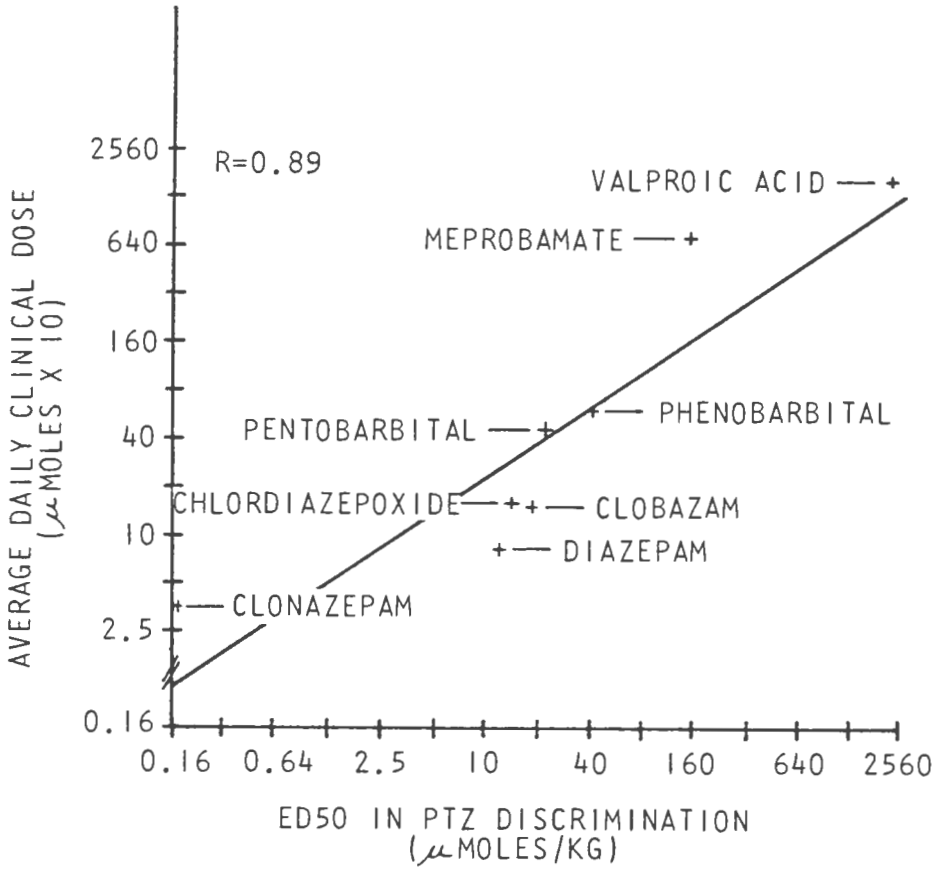

Figure 4. Correlation between the effective doses of anxiolytic drugs in antagonizing the anxiogenic discriminative stimulus of PTZ and their effect on clinical doses.

has often been employed as a tool to identify pharmacological actions for their predictive value for clinical efficacy (Fielding and Lal, 1979; Lippa et al., 1979). Because benzodiazepines have been the most widely studied anxiolytic drugs with respect to tolerance development to their anxiolytic actions, it was investigated whether tolerance would not develop to two prototype benzodiazepines, chlordiazepoxide and diazepam, in antagonism of the PTZ discriminative stimulus. It was hypothesized that if the antagonistic property of these benzodiazepines in the PTZ discrimination paradigm was related to their anxiolytic action tolerance would not develop. Previously, lack of tolerance development to the prevention of PTZ-induced convulsions by diazepam was reported (Fuxe et al., 1975; Juhasz and Dairman, 1977; Lippa and Regan, 1977).

The ability of either diazepam or chlordiazepoxide to antagonize the PTZ discriminative stimulus was not affected by their chronic administration even though this treatment was more than sufficient (Cook and Sepinwall, 1975; Goldberg et al., 1967; Margules and Stein, 1968; Stein et al., 1975) to produce tolerance to the depressant action of these drugs. The present data are consistent with previous findings (Fuxe et al., 1975; Jahasz and Dairman, 1977; Lippa and Regan, 1977) that show lack of tolerance development to prevention of PTZ induced convulsions by diazepam. Because tolerance develops to the depressant but not anxiolytic action of benzodiazepines these data support the hypothesis that antagonism

of the PTZ discriminative stimulus by these drugs is related to their anxiolytic action.

Additional data which supports the suggestion that antagonism of the PTZ discriminative stimulus was related to anxiolytic action and not to the central nervous system depressant activity of these drugs was provided through antagonism tests with nonanxiolytic central nervous system depressants. Etomidate is a nonbarbiturate hypnotic which is used for the induction of anesthesia because of its short duration of action (Jannsen et al., 1975). The anxiolytic action of etomidate has not been reported to date. Although ethanol is widely considered an anxiolytic, the anti-anxiety effect of this drug has not been clearly demonstrated (Sepinwall and Cook, 1978). Whereas morphine is known to alleviate anxiety during opiate withdrawal (Redmond, 1979) it does not relieve anxiety in opiate free individuals. Chlorpromazine and haloperidol, useful in the treatment of psychoses, do not decrease anxiety (Rickels et al., 1979).

None of these central nervous system depressants were effective in antagonizing the discriminative stimulus produced by PTZ. These data indicate that drugs that depress the central nervous system but do not possess anxiolytic activity do not antagonize the PTZ discriminative stimulus. Therefore these data support the hypothesis that the discriminative stimulus produced by PTZ in the rat is related to its anxiogenic action.

In addition to their anxiolytic and depressant actions, all of the drugs that were effective in antagonizing the PTZ discriminative stimulus are also effective against PTZ convulsions (Childress and Gluckman, 1964; Banziger, 1965; Swingard and Castellion, 1966; Ludwig and Potterfield, 1971; Dren et al., 1978; Lippa et al., 1979). Based upon these data, the finding that tolerance does not develop to diazepam in antagonizing PTZ convulsions (Lippa and Regan, 1977) as well as other considerations (Lippa et al., 1979), it has been suggested that the ability of drugs to prevent or antagonize or prevent PTZ convulsions may reflect their anxiolytic property (Hill and Tedeschi, 1971; Lippa and Regan, 1977; Lippa et al., 1979).

Therefore, to test the hypothesis that antagonism of the PTZ discriminative stimulus by these drugs was not related to their anticonvulsant action, antagonism of the PTZ discriminative stimulus with anticonvulsants that lack clinical anxiolytic actions was tested.

Trimethadione and ethosuximide are known to protect laboratory animals against PTZ produced convulsions (Everett and Richards, 1944; Toman and Goodman, 1948, Swinyard and Castellion, 1966; Woodbury and Fingl, 1975). These drugs are effective in the treatment of petit-mal epilepsy (Woodbury and Fingl, 1975) but not in the treatment of anxiety. In the present experiment neither trimethadione nor ethosuximide antagonized the discriminative stimulus produced by PTZ.

Etomidate like trimethadione and ethosuximide, is known to protect mice and rats against the convulsant action of PTZ (Desmedt et al., 1976). However, etomidate also did not antagonize the PTZ discriminative stimulus.

Unlike trimethadione, ethosuximide and etomidate, diphenylhydantoin is an anticonvulsant which is ineffective in petit mal epilepsy (Woodbury and Fingl, 1975) and does not protect against pentylenetetrazol-induced convulsions (Everett and Richards, 1944; Toman and Goodman, 1948; Swinyard and Castellion, 1966). Diphenylhydantoin did not block the discriminative stimulus produced by pentylenetetrazol.

There is a high correlation ($R=0.95$; $p < 0.05$) between the potency of drugs effective in antagonizing the PTZ discriminative stimulus and their effective doses against PTZ-induced convulsions (Figure 5). However, whereas all drugs that antagonize the PTZ discriminative stimulus prevent PTZ convulsions not all drugs that are effective against PTZ convulsions antagonize the PTZ discriminative stimulus. These data suggest that the anticonvulsant action of the drugs effective in antagonizing the PTZ discriminative stimulus is not the property that is responsible for this effect. These data also suggest that the mechanism of action of the PTZ in producing convulsions is different from that underlying its mechanism for producing a discriminative stimulus. Furthermore these data suggest that antagonism of the PTZ discriminative stimulus is a better measure of anxiolytic activity than is antagonism of PTZ seizures.

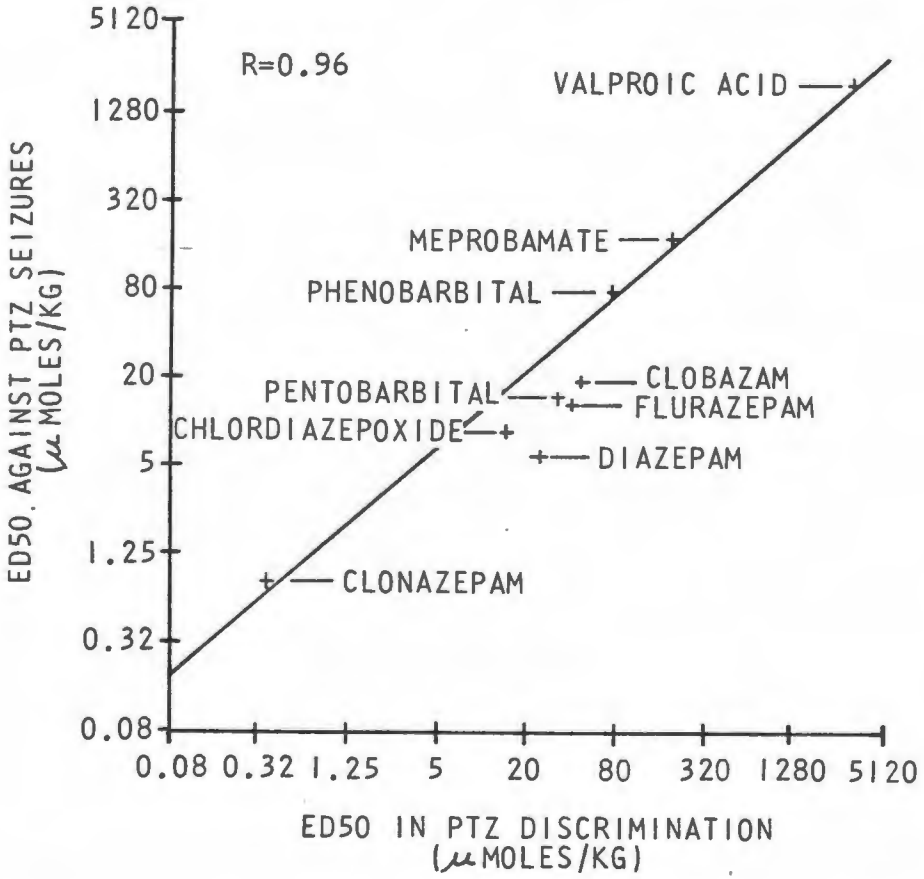

Figure 5. Correlation between the effective doses of anxiolytic drugs in antagonizing the anxiogenic discriminative stimulus of PTZ and their effective doses in antagonizing PTZ seizures.

Neurochemical Correlates of the Anxiogenic Discriminative Stimulus Property of PTZ

The exact neurochemical basis for anxiety is unknown. However, it is possible that some indication of this may be obtained from the postulated mechanism of action of anxiolytic drugs with the assumption that the neurochemical basis for anxiety is opposite to that for the anti-anxiety action of anxiolytic drugs.

Benzodiazepines are the most effective and thus most widely prescribed drugs for the treatment of anxiety (Howard and Pollard, 1977). Also, this class of anxiolytics has been the most extensively studied with respect to their mechanism of action (Costa and Greengard, 1975; Sepinwall and Cook, 1978; Koe, 1979).

In the present study, it was hypothesized that the neurochemical basis of the anxiogenic discriminative stimulus property of PTZ was opposite to that of the postulated neurochemical mechanism(s) responsible for the anxiolytic action of benzodiazepines. To test this hypothesis, drugs known to have effects opposite to those of benzodiazepines on the neurotransmitter systems postulated to mediate the anxiolytic action of benzodiazepines and drugs known to have a similar effect as benzodiazepines on these neurotransmitter systems were tested for generalization to and antagonism of the anxiogenic discriminative stimulus property of PTZ.

1. Gamma-aminobutyric Acid (GABA)

A functionally significant interaction between benzodiazepines and brain gamma-aminobutyric acid (GABA) became apparent from a variety of neurochemical (for reviews see Costa et al., 1978, Costa and Guidotti, 1979) and neurophysiological (for review see Haefly, 1978) experiments. However, the pharmacological significance of such an interaction is unclear. Whereas the anticonvulsant, sedative, and muscle relaxant actions of benzodiazepines seem to be related to GABA-ergic mechanisms (Cook and Sepinwall, 1975; Lippa et al., 1979), a similar relationship for the anxiolytic action of benzodiazepines is not clear.

To measure anxiolytic actions of drugs in experimental animals, one test that is considered quite specific is the conflict paradigm where reduction of response suppression by "conflictful" stimuli is measured (Lippa et al., 1979, Sepinwall and Cook, 1978). Inthis test, benzodiazepines are known to show high potency and efficacy in nonsedative doses. Therefore, the conflict model has been used to evaluate various neurochemical hypothesis for anxiolytic activity. Cook and Sepinwall (1975) reported that the GABA-T inhibitor, aminooxyacetic acid (AOAA), was without anti-conflict activity alone and did not potentiate the anticonflict effect of diazepam. Tye et al. (1979) also reported that AOAA lacked anti-conflict effect. These data might suggest that alteration of brain GABA is not related to anti-anxiety action. However,

recent studies of Iadrola and Gale (1979,a,b) show that the neuroanatomical sites of action of this GABA-T inhibitor is not appropriate for anxiolytic activity. First of all this drug elevates brain GABA outside of nerve terminals. Increasing the concentrations of GABA at these sites may actually inhibit neuronal GABA release because of its action on presynaptic autoreceptors. In addition, the elevation of GABA by AOAA is more pronounced in extrapyramidal areas. The GABA-T inhibitor, valproic acid, on the other hand, increases brain GABA levels predominantly within nerve terminals and in brain sites in the limbic areas (Iadrola and Gale, 1979a,b). Therefore, the lack of anxiolytic action by some GABA-T inhibitors may be due to the inappropriate site of their action. Valproic acid's efficacy as an anxiolytic drug may be due to an action at more appropriate sites.

Sullivan et al. (1978) and Cook and Sepinwall (1979) systemically administered the GABA receptor agonists muscimol and 4,5,6,7-tetrahydroisoxazolo (5, 4-c) pyridin-3-ol (THIP) to examine their effect on conflict behavior. Whereas muscimol was weakly active, THIP was inactive. Neither muscimol nor THIP potentiated the anticonflict effect of benzodiazepines in this test. However, several investigators (Baraldi et al., 1979; Maggi and Enna, 1979; Enna et al., 1979) have reported that muscimol does not penetrate into the brain after systemic administration. This evidence may explain the lack of anticonflict activity of muscimol and

and its structural analogue, THIP. Recently, Guidotti (personal communication) injected muscimol and THIP intraventricularly and reported that both of these drugs produced anti-conflict activity in rats.

Earlier data which suggested that the anticonflict activity of benzodiazepines might be mediated by GABA were provided by Stein et al. (1975) and Zakusov et al. (1977). These investigators reported that the anti-conflict activity of benzodiazepines was reduced by the GABA antagonists picrotoxin, bicuculline or thiosemicarbizide.

Therefore, using the conflict model much data has been generated to suggest that the anxiolytic action of benzodiazepines may be mediated by enhancing GABA activity. It may be hypothesized then, that anxiety is due to a deficit in GABA neuronal function.

In order to test the hypothesis that decreased GABA neuronal activity was the neurochemical basis for the anxiogenic discriminative stimulus property of PTZ, generalization to the PTZ discriminative stimulus with GABA antagonistic drugs and antagonism of the PTZ discriminative stimulus with GABA minetic drugs was tested.

Picrotoxin and bicuculline were used as antagonists of the postsynaptic inhibitory action of GABA (Curtis and Johnson, 1974). Whereas bicuculline acts at the receptor sites (Curtis and Johnson, 1974), picrotoxin seems to act at chloride channels (Olsen et al., 1978a,b). Schecter and

Tranier (1977) found that GABA-transaminase inhibitors prolong the onset of seizure and death following picrotoxin but not following bicucuclline. Other differences between picrotoxin and bicuculline have been reported with respect to GABA-receptor sensitivity (Krnjevic, 1974), blockade of GABA binding to brain receptors (Zukin et al., 1974), stimulation of [^{3}H]-GABA release from brain tissue (Collins, 1973; Johnston and Mitchell, 1971), alteration of specific [^{3}H]-diazepam binding in cortical membranes (Tallman et al., 1978) and induction of epileptic spikes following topical application and systemic injection (Edmonds and Bellin, 1976).

Whereas, picrotoxin partially generalized to the PTZ discriminative stimulus, bicucuclline did not generalize. Therefore, these data do not conclusively support the hypothesis that the anxiogenic discriminative stimulus property of PTZ is related to decreased GABA neuronal activity.

3-Mercaptopropionic acid is á convulsant drug that acts by inhibiting glutamate decarboxylase and producing a subsequent decrease in the concentration of brain GABA (Lamar, 1970). Like picrotoxin, 3-mercaptopropionic acid partially generalized to the PTZ discriminative stimulus. Therefore these data although suggestive do not fully support the hypothesis that decreased GABA function is the basis of the anxiogenic discriminative stimulus property of PTZ.

RO-3663 is a convulsant benzodiazepine that selectively antagonizes the effect of GABA at spinal and peripheral neuronal sites (Schlosser and Franco, 1979). In addition, this

drug has been reported to inhibit the GABA enhancement of ^{3}H-diazepam binding (O'Brien and Spirt, 1980) and binding of the GABA receptor antagonist, alpha picrotoxin in Leeb-Lundberg et al. (1979). R05-3663 dose-dependently generalized to the PTZ discriminative stimulus. Furthermore, the discriminative stimulus strength of R05-3663 was attenuated by diazepam. Although the exact neurochemical basis through which R05-3663 produces its discriminative stimulus cannot be determined at present, these data support the hypothesis that decreased GABA neuronal activity is the neurochemical basis of the PTZ discriminative stimulus.

Based upon the differential antagonism of iontophoretically applied etomidate and GABA by bicuculline but not by strychnine, Evans and Hill (1978) proposed etomidate to be a central GABA-mimetic drug. Etomidate did not antagonize the PTZ discriminative stimulus. The inability of etomidate to antagonize the PTZ discriminative stimulus does not support the hypothesis that discriminative stimulus is related to a deficit in brain GABA function. It should also be noted, however, that whereas etomidate was ineffective in blocking the PTZ discriminative stimulus, other sedative hypnotics such as pentobarbital and phenobarbital which also possess anxiolytic activity were effective antagonists of the pentylenetetrazol discriminative stimulus.

At higher dose levels, gamma-hydroxybutyrate acts like a GABA agonist in the central nervous system (Anden and Stock, 1973; Roth and Nowycky, 1977). Pretreatment of the

animals with gamma-hydroxybutyrate did not antagonize the discriminative stimulus produced by PTZ also suggesting that the PTZ discriminative stimulus is not related to decreased GABA neuronal activity.

Gamma-acetylentic GABA (GAG) and gamma-vinyl-GABA (GAG) are inhibitors of GABA-T which increase the brain GABA levels of mice and rats (Jung et al., 1977a; Jung et al., 1977b) and protect animals against seizures induced by audiogenic stimulation, strychnine, isoniazid, thiosemicarbizide and electric shock (Schecter et al., 1977a,b), but not picrotoxin, bicuculline nor PTZ (Schecter et al., 1977a,b) antagonized the discriminative stimulus produced by PTZ. Thus these data do not support the hypothesis that the PTZ discriminative stimulus is related to a deficit of brain GABAergic functioning.

However, it may be premature to consider the above data as going against the hypothesis that the anxiogenic discriminative stimulus property of PTZ is related to decreased GABA neuronal activity. Recently, Iadrola and Gale (1979a,b) have shown that the GABA-T inhibitors GAG and GVG increase brain GABA primarily in nonsynaptosomal sites such as glia.

In order to overcome these shortcomings the indirectly acting GABA mimetic drug, valproic acid, was tested for antagonism of the PTZ discriminative stimulus. Although valproic acid inhibits GABA-T (Godin et al., 1969, Simler et al., 1973; Fowler et al., 1975), enzyme-kinetic studies show that it increases brain GABA levels primarily by causing an accumulation of succinic semialdehyde through inhibition of

succinic semialdehyde dehydrogenase (Van der Lann et al., 1979). The succinic semialdehyde in turn blocks the conversion of GABA to succinic semialdehyde through product inhibition. These actions are preferentially localized within the nerve terminal (Van der Lann et al., 1979; Bruinvels et al., 1979). In addition, whereas other GABA-T inhibitors cause GABA elevation primarily in the compartments outside of nerve terminals, GABA elevation produced by valproic acid is primarily located in nerve terminals (Iadrola and Gale, 1979; Sarhan and Seilar, 1979). Valproic acid has also been reported to inhibit the reuptake of GABA into nerve terminals (Harvey, 1976). Valproic acid dose-dependently antagonized the anxiogenic discriminative stimulus produced by PTZ. These data support the hypothesis that a deficit in GABA neuronal activity is the neurochemical basis of the anxiogenic discriminative stimulus property of PTZ. These data also suggest that valproic acid may be a clinically effective anxiolytic drug and that deficits in GABA functioning are important for anxiety. Furthermore these data suggest that antagonism of the PTZ discriminative stimulus by benzodiazepines, and thus their anxiolytic action might be related to their property of enhancing GABA transmission.

2. Glycine

A significant correlation between the ability of a series of benzodiazepines to reduce ^{3}H-strychnine binding to glycine receptors and their clinical effects led to the

proposal that these drugs might produce their muscle relaxant and anti-anxiety action by mimicking glycine at its receptor sites (Young et al., 1974; Snyder and Enna, 1975). However, when Cook and Sepinwall (1975), using the data of Young et al. (1974), compared the ^{3}H-strychnine displacement potency of ten benzodiazepines with their anticonflict potency, they did not find a significant correlation. Because there was a significant correlation between anticonflict potency and potency in a human bioassay it was concluded that the affinity of benzodiazepines for the glycine receptor was not correlated with anxiolytic activity. Furthermore, Stein et al. (1975) reported that although strychnine reduced the anticonflict effect of oxazepam it also depressed unpunished responding suggesting that this effect was nonselective.

In order to test the hypothesis that decreased glycinergic activity was the neurochemical basis of the anxiogenic discriminative stimulus property of PTZ, the glycine antagonist (Curtis and Johnson, 1974) strychnine, was tested for generalization to the PTZ discriminative stimulus.

Although not completely strychnine generalized to the PTZ discriminative stimulus thus supporting the hypothesis that decreased glycinergic activity was the basis for the anxiogenic discriminative stimulus property of PTZ.

McDonald and Barker (1977) reported that whereas PTZ antagonized the post-synaptic inhibitory responses to GABA in a mammalian tissue culture system it did not affect glycine responses. Therefore, unless it is demonstrated that

PTZ antagonizes glycine the hypothesis that the neurochemical basis of the PTZ discriminative stimulus is due to decreased glycinergic activity might not be tenable. There was considerable evidence presented earlier that suggests that the discriminative stimulus produced by PTZ was related to decreased GABA Neuronal activity. Because both GABA and glycine are inhibitory neurotransmitters it may be suggested that interference with either of these inhibitory systems produces a discriminative stimulus that provides the basis for the common discriminative stimulus properties of these drugs.

3. Phosphodiesterase Inhibition

Based upon a significant correlation between the in vitro potency of several drugs in inhibiting phosphodiesterase and their potency in the thirsty rat conflict test, Beer et al. (1972) proposed that the anxiolytic activity of drugs may be related to their ability to inhibit this enzyme. In support of this hypothesis, Weinryb et al. (1975), found that a series of substituted pyrazol-(3,4b)-pyridines that were potent inhibitors of phosphodiesterase had significant anticonflict activity. However, Morrison (1969) and Cook and Sepinwall (1975) did not find significant anticonflict effect of phosphodiesterase inhibitors in a lever press conflict test. Furthermore, Collins et al. (1976) reported that the potent phosphodiesterase inhibitor, SQ 65,396 was without anxiolytic effect compared to diazepam and placebo in psychoneurotic patients.

In order to test the hypothesis that the ability of benzodiazepines to antagonize the discriminative stimulus property of PTZ was not related to their ability to inhibit phosphodiesterase, antagonism of the PTZ discriminative stimulus with the phosphodiesterase inhibitor, caffeine, was tested.

Caffeine did not antagonize the anxiogenic discriminative stimulus property of PTZ. This finding supports the hypothesis that the ability of benzodiazepines to antagonize the PTZ discriminative stimulus was not related to their ability to inhibit phosphodiesterase. These data also support previous work (Morrison 1969; Cook and Sepinwall, 1975; Collins et al., 1976) that suggests that the anxiolytic action of benzodiazepines is not related to their inhibition of phosphodiesterase.

4. Acetylcholine

There is no clear correlation between the effect of benzodiazepines on cholinergic function and their anti-anxiety action. Benzodiazepines were reported to increase rat brain acetylcholine levels (Consolo et al., 1975). The functional significance of this, however, is unclear as there was no correlation between the muscle relaxant or anti-PTZ of the benzodiazepines and their effect on acetylcholine even though PTZ has been reported to cause a decrease in rat brain acetylcholine (Giarman and Pepeu, 1962; Beani et al., 1969; Longoni et al., 1974; Consolo et al., 1975) by causing a massive release of this neurotransmitter. In one behavioral

study, Soubrie et al. (1976) reported that diazepam antagonized the hyperactivity elicited by anticholinergics in mice. This effect was blocked by picrotoxin implicating the involvement of GABA mechanism for this action of diazepam.

In conflict tests, cholinergic agonists (Morrison, 1969) or antagonists (Hanson et al., 1970; Miczek, 1973) were without significant anticonflict effect. In order to test the hypothesis that the anxiogenic discriminative stimulus property of PTZ was related to its effect on cholinergic function, generalization to and antagonism of the PTZ discriminative stimulus with cholinergic agonistic and antagonists was tested.

None of the cholinergic drugs tested either generalized to or antagonized the PTZ discriminative stimulus. These data do not support the hypothesis that the neurochemical basis of the anxiogenic discriminative stimulus property of PTZ was related to its action on cholinergic function. Furthermore, these data suggest that the ability of benzodiazepines to antagonize the PTZ discriminative stimulus was not related to their affect on cholinergic function.

5. Serotonin

There is considerable biochemical (Stein et al., 1975) and behavioral (for reviews see Sepinwall and Cook, 1978; Koe, 1979) evidence obtained from animal experiments that the benzodiazepines may exert their anti-anxiety action by decreasing serotonin activity.

Stein et al. (1975) reported that whereas tolerance developed to the depressant action and effect of benzodiazepines on decreasing norepinephrine turnover, tolerance did not develop to their anticonflict effect or their effect of decreasing serotonin turnover. Using conflict paradigms it has been demonstrated that reduction serotonin activity by inhibition serotonin synthesis (Robichaud and Sledge, 1969; Geller and Blum, 1970; Schoenfeld, 1976), blockade of serotonin receptors (Graeff and Schoenfeld, 1970; Graeff, 1974; Stein et al., 1973; Winter, 1972) or destruction of serotonin nerve terminals (Stein et al., 1975) produces significant anticonflict effects. However, there have been some reports (Winter, 1972; Blakely and Parker, 1973; Cook and Sepinwall, 1975a) that show a lack of anticonflict effect of serotonin antagonistic drugs. Whereas serotonin antagonists are usually reported to have anticonflict effects, serotonin agonists have been reported (Graeff and Schoenfeld, 1970; Winter, 1972; Geller et al., 1974) to significantly suppress conflict behavior.

In order to test the hypothesis that the anxiogenic discriminative stimulus property of PTZ was related to an agonistic effect of this drug on serotonin function, generalization to the PTZ discriminative stimulus with serotonin agonists and antagonism of the PTZ discriminative stimulus with serotonin antagonists was tested.

Neither of the serotonin agonists used in this study (5-HTP and fluoxetine) generalized to the PTZ discriminate

stimulus. Furthermore, the serotonin receptor antagonist, methysergide, did not antagonize the discriminative stimulus property of PTZ. These data do not support the hypothesis that the neurochemical basis of the anxiogenic discriminative stimulus property of PTZ was related to an agonistic effect on serotonin function. Furthermore, these data suggest that the ability of benzodiazepines to antagonize the PTZ discriminative stimulus is not related to their ability to decrease serotonin transmission.

It is interesting that serotonin antagonists are not clinically effective anxiolytics therefore reduction of serotonin function by these drugs might not be the neurochemical basis for their anxiolytic action as suggested by other animal studies.

6. Catecholamines

Benzodiazepines are known to decrease the turnover of both norepinephrine and dopamine (for review see Koe, 1979). There is no clear evidence, however, that this effect is related to the anxiolytic activity of these drugs. Fuxe et al. (1975) reported that diazepam antagonized increases in norepinephrine turnover produced by stress or injection of the anxiogenic drug yohimbine. Gray (1976) reported similar effects for chlordiazepoxide as well as norepinephrine synthesis inhibitors or lesions of the dorsal noradrenergic bundle against stress. Lader (1974) reviewed evidence for the involvement of catecholamines in anxiety and more recently

Redmond (1979) summarized considerable evidence for a role of norepinephrine in anxiety.

If activation of catecholaminergic system causes anxiety then drugs effective in decreasing catecholaminergic transmission should decrease anxiety. The beta-adrenergic receptor blockers, propranolol and oxprenolol have been reported to have anxiolytic activity (Jefferson, 1974; Krishman, 1976). However, whereas standard anxiolytics are effective against both the somatic and psychic components of anxiety, these drugs appear to be effective only against the somatic anxiety. In the conflict test, Sepinwall et al. (1973) reported propranolol to be without significant anticonflict effect.

Recently, Kruse et al. (1980) reported that the presynaptic alpha-adrenergic agonist, clonidine, produced significant anticonflict activity in the rat. Furthermore, this effect was antagonized by the pre-synaptic alpha-receptor blocker (Starke et al., 1975), yohimbine. Several investigators (Holmberg and Gershon, 1961; Ingram, 1962; Gershon and Lang, 1962; Garfield et al., 1967) had previously reported that yohimbine produced anxiety in humans.

To test the hypothesis that the anxiogenic discriminative stimulus property of PTZ was related to an action of increasing catecholaminergic transmission, catecholamine agonists were tested for generalization to the PTZ discriminative stimulus and catecholamine antagonists were tested for antagonism of the PTZ discriminative stimulus. Of the catecholamine agonists tested for generalization only cocaine

significantly generalized to the PTZ discriminative stimulus. The dopamine receptor agonist, apomorphine, as well as yohimbine partially generalized; however, d-amphetamine and methylphenidate did not. Propranolol that was previously reported to generalize to the discriminative stimulus property of cocaine (Silverman and Ho, 1977), did not generalize to the PTZ discriminative stimulus. These data leave it unclear whether the anxiogenic discriminative stimulus property of PTZ was related to an action of activating catecholaminergic mechanisms. However, when several catecholamine antagonists including clonidine, aceperone, propranolol, haloperidol or chlorpromazine were tested for antagonism of the PTZ discriminative stimulus they were found to be without effect. Therefore, these data do not conclusively support the hypothesis that the anxiogenic discriminative stimulus prospects of PTZ is related to an effect of increasing catecholaminergic activity.

7. Endorphins

The effect of benzodiazepines on endorphins is still being worked out. Therefore, the possible involvement of these newly discovered endogenous substances in mediating the anxiolytic activity of benzodiazepines on anxiety remains to be determined. Some of the behavioral effects of benzodiazepines, including their anticonflict effect, however, have been reported to be antagonized by the narcotic antagonist, naloxone (Billingsly and Kibena, 1978; Gylys et al., 1979). Thesedata might suggest that anxiolytic activity is mediated

by activation of endorphinergic systems.

To test the hypothesis that the anxiogenic discriminative stimulus property of PTZ was related to an antagonistic effect on endorphin systems, generalization to the PTZ discriminative stimulus with naloxone was tested as was antagonism of the PTZ discriminative stimulus with morphine. The failure of naloxone to generalize to the PTZ discriminative stimulus and the failure of morphine to antagonize this stimulus does not support the hypothesis that the anxiogenic discriminative stimulus property of PTZ is related to an antagonistic action on endorphin systems.

8. Endogenous Benzodiazepine Receptor (Ligand(s)

The demonstration of saturable, high-affinity, stereospecific benzodiazepine binding sites in the central nervous system (Squires and Braestrup, 1977; Mohler and Okada, 1977) suggested the presence of endogenous ligands capable of regulating the neurophysiological processes subserved by these receptors. Several such substances have been suggested as endogenous ligands for benzodiazepine receptors (Colello et al., 1978; Karobath et al., 1978; Marangos et al., 1978; Skolnick et al., 1978; Mohler et al., 1979; Nixon and Wolf, 1979; Skolnick et al., 1979), however, their potency is too weak to satisfy a neurotransmitter or neuromodulator role.

Although none of the candidates to date satisfy all the criteria for an endogenous ligand of the benzodiazepine

receptor, purines and nicotinamide do have benzodiazepine-like properties *in vivo*. Despite the relatively low affinity, Skolnick *et al*. (1979) reported that inosine produced a dose- and time-related antagonism of PTZ-induced convulsions and proposed that endogenously occurring purines such as inosine could be naturally occurring ligands for benzodiazepine receptors. Although the affinity of nicotinamide for the benzodiazepine receptor is approximately four to five fold lower than affinities reported for purines (Mohler *et al*., 1979), this naturally occurring substance has been reported to partially restore behavior suppressed by punishment in rats.

Marangos *et al*. (1979) reported that pentylenetetrazol inhibited ^{3}H-diazepam binding *in vitro* and suggested that PTZ may exert pharmacological actions by an interaction with the benzodiazepine receptor. Based upon the antagonism of the PTZ discriminative stimulus by benzodiazepines it is possible that the anxiogenic discriminative stimulus property of PTZ is related to its interaction with this receptor. It is possible that PTZ may exert its angiogenic action by inhibiting the binding of a naturally occurring anxiolytic ligand to benzodiazepine receptors or mimicking the action of a naturally occurring anxiety-inducing ligand.

R05-3663 is structurally related to diazepam, however, unlike diazepam it does not bind to benzodiazepine receptors (Speth *et al*., 1979; O'Brien and Spirt, 1980). Furthermore, whereas diazepam has GABAmimetic activity R05-3663 antagonizes the effect of GABA (Schlosser and Franco, 1979; O'Brien

and Spirt, 1980). O'Brien and Spirt (1980) speculated that if the anxiolytic action of benzodiazepines were considered to be mediated through an antagonistic action then a molecule structurally similar to R05-3663 could exist in brain to function as an endogenous anxiety-inducing ligand.

R05-3663 dose-dependently generalized to the PTZ discriminative stimulus. Furthermore, the discriminative strength of R05-3663 was attenuated by diazepam. These data provide behavioral evidence that R05-3663 may structurally resemble a naturally occurring anxiety-inducing ligand. If this is the case PTZ may act by a similar mechanism as this naturally occurring anxiety-inducing ligand.

SUMMARY AND CONCLUSIONS

In summary, it was demonstrated that laboratory rats can learn to discriminate between a subconvulsive dose (20 mg/kg) of PTZ and saline. A mean of 30 training sessions were required for the animals to reach the discrimination criterion. The discriminative stimulus produced by PTZ was dose- and time-dependent.

The anxiogenic stimulants cocaine, R05-3663 and strychnine generalized to the PTZ discriminative stimulus whereas yohimbine partially generalized. The discriminative stimulus produced by cocaine, R05-3663 and yohimbine was antagonized by diazepam. The discriminative stimulus produced by cocaine was not antagonized by haloperidol. The non-anxiogenic psychomotor stimulants d-amphetamine, methylphenidate and caffeine did not generalize to the PTZ discriminative stimulus. These data support the hypothesis that the discriminative stimulus produced by PTZ in the rat is related to its anxiogenic action in man.

Of the several convulsant drugs tested, only bemegride significnatly generalized to the PTZ discriminative stimulus whereas picrotoxin and 3-mercaptopropionic acid partially generalized. Bicuculline, nicotine or gamma-hydroxybutyrate did not generalize to the PTZ discriminative stimulus. These data suggest that the PTZ discriminative stimulus is probably

not related to a subconvulsant brain state likely to be produced by these drugs.

The PTZ discriminative stimulus was dose-dependently antagonized by benzodiazepine-type, barbiturate-type and propanediol carbamate-type anxiolytics as well as valproic acid. Because tolerance develops to the sedative but not anxiolytic benzodiazepines, lack of tolerance development to diazepam or chlordiazepoxide in antagonism of the PTZ discriminative stimulus suggests that this property was related to their anxiolytic action.

There was a significant correlation between the potency of the drugs effective in antagonizing the PTZ discriminative stimulus and their effective doses in a conflict test used to measure anxiolytic activity as well as their clinically effective doses.

The PTZ discriminative stimulus was not antagonized by the nonanxiolytic anticonvulsants ethosuximide, etomidate, trimethadone or diphenylhydantoin nor the nonanxiolytic central nervous system depressants morphine, ethanol, chlorpromazine, or haloperidol. These data suggest that the PTZ discriminative stimulus is not related to a subconvulsant brain state or nonspecific central nervous system stimulation.

The GABA antagonist, R05-3663, dose-dependently generalized to the PTZ discriminative stimulus, whereas picrotoxin and 3-mercaptopropionic acid partially generalized. The GABAmimetic drug, valproic acid, antagonized the PTZ dis-

criminative stimulus. These data suggest that the PTZ discriminative stimulus might be mediated through decreased GABA neuronal activity. Because of the known GABA mimetic property of benzodiazepines it was suggested that antagonism of the PTZ discriminative stimulus by these drugs may be related to their effect on GABA transmission.

The glycine antagonist, strychnine, generalized to the PTZ discriminative stimulus suggesting the possible involvement of decreased glycinergic activity for the discriminative stimulus property of PTZ. However, in view of the considerable evidence for the involvement of GABA and the lack of effect of PTZ on glycine in vitro it was suggested that general interference with inhibitory transmission may generate a discriminative stimulus like that produced by PTZ.

Drugs affecting acetylcholine or serotonin systems did not generalize to or antagonize the PTZ discriminative stimulus. These data suggested that the PTZ discriminative stimulus was not related to an effect on these neurotransmitter systems.

Cocaine generalized to the PTZ discriminative stimulus whereas yohimbine and apomorphine partially generalized. The PTZ discriminative stimulus was not blocked by drugs with anti-catecholaminergic activity. Therefore these data do not support the involvement of catecholamine mechanisms for the discriminative stimulus property of PTZ.

In view of the recent demonstration of benzodiazepine receptors in brain, it was suggested that PTZ may produce

its anxiogenic action by interacting with an unknown ligand for the benzodiazepine receptor. PTZ may either mimic a naturally occurring anxiety-inducing ligand or inhibit the action of a naturally occurring anxiolytic ligand.

In conclusion the data presented in this dissertation support the hypothesis that the discriminative stimulus property of PTZ in the rat is related to its anxiogenic action in man. It is suggested that the anxiogenic stimulus produced by PTZ is related to deficits in GABA neuronal activity.

Tedeschi (1969) suggested that for animal tests to have predictive value as models for a particular human condition, they must fulfill the following criteria:

1. Tests should be selective enough to differentiate false positives, and to distinguish side effects from therapeutic activity.

2. Tests should be sensitive enough to detect activity of reference agents within a reasonable dose range.

3. The relative potency of reference agents in the animal test should compare with their relative potency in man.

4. Tolerance should not develop to the measure presumed to reflect therapeutic efficacy.

The PTZ-saline discrimination paradigm described in this dissertation fulfills all of these criteria. Therefore it is suggested that this procedure may represent a new animal model of anxiety that can be used to detect anxiolytic activity of new compounds.

Currently, prevention of PTZ-induced seizures is used as a screening test for anxiolytic drugs. However, all of the drugs that prevent these seizures are not anxiolytics. To date there have been no false positives in the PTZ-saline discrimination procedure. All of the drugs that antagonize the PTZ discriminative stimulus are clinically effective anxiolytics. Currently, there is no animal model to detect anxiogenic side effects of drugs; the PTZ-saline discrimination may provide such a model.

In addition to these potential benefits for drug development, the PTZ-saline discrimination may be used to investigate the neurochemical mechanisms and behavioral factors related to anxiety as well as the mechanism of action of anxiolytic drugs. It is the author's hope that the work reported in this dissertation will contribute towards these ends.

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