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THE INTEGRATED RELATIONSHIPS OF PROTRIPTYLINE, THYROID STATUS AND NORADRENERGIC MECHANISMS IN RAT BRAIN

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THE INTEGRATED RELATIONSHIPS OF PROTRIPTYLINE, THYROID STATUS AND NORADRENERGIC MECHANISMS IN RAT BRAIN

BY

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ABSTRACT

Reasons for the observed lag-time for clinical efficacy of the tricyclic antidepressant drug, protriptyline, have been investigated. Our results demonstrate that chronic protriptyline administration results in several compensatory neural mechanisms: (1) changes in endogeneous cytoplasmic norepinephrine levels, (2) changes in norepinephrine turnover rate/recovery rate following the last dose in a chronic series of test drug administrations and (3) adaptations of the pre-synaptic receptor. The time course for these adaptive changes parallel the time required for the onset of their clinical effectiveness.

We propose that acutely the action of the tricyclics on norepinephrine re-uptake is largely negated by compensatory adjustments in turnover rate of the neurotransmitter; but with chronic administration, adaptations in pre-synaptic alpha-receptors occur. This phenomena in turn reduces the extent to which the norepinephrine neurons can offset or compensate for the blockade of norepinephrine re-uptake. Specifically, pre-synaptic alpha-receptors become hyposensitive, and receptor tolerance develops; not to the direct action of the tricyclic drug on neurotransmitter re-uptake, but to the compensatory neural adjustments which offset the action of the acute tricyclic administration and delay their clinical efficacy.

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DEDICATION

This thesis is dedicated --

to my father ED, for his inexhaustible ambition and unyielding integrity.

to my mother, NOELA, for her endless compassion and perpetual understanding.

AND MOST ESPECIALLY

to my wife, LAUREN, for her unrelenting support and never-ending affection throughout the entire course of this arduous, time-consuming endeavor.

PREFACE

In the last 25 years many observations have documented the suggestion that a relationship exists between central norepinephrine function and affective disorders. The catecholamine hypothesis of affective disorders is one theory advanced by researchers. It proposes that some, if not all depressions are associated with an absolute or relative deficiency of catecholamines, in particular norepinephrine (NE), at the functionally important adrenergic receptor sites in the brain. The tricyclic antidepressants (TCA), which normalize a depressed mood, block the active re-uptake of norepinephrine after release from the presynaptic nerve terminals. Thus both the time and the concentration of norepinephrine availability is increased in the synaptic cleft for post-synaptic attachment. It is likely, however, that the ability of the tricyclics to block norepinephrine re-uptake, though the primary mechanism of action, may not be the only mechanism involved. This is evident from the observation that re-uptake blockade of NE by the tricyclics occurs within minutes after their administration; however, clinical efficacy in patients is not obvious until these drugs have been administered chronically (10-20 days). This observed lag-time for clinical efficacy suggests the existence of an alternate mechanism of action for this class of antidepressants.

My research was designed to investigate three possible alternate mechanisms of action for the tricyclics, which might correlate better with the requirement for chronic administration.

Our results demonstrated that tricyclic antidepressant drugs, when administered chronically, do not alter monoamine oxidase activity *in vivo* in any of the three brain regions we examined (corpus striatum, forebrain, and hypothalamus). Similarly, we were not able to identify a noradrengeric relationship other than those that were both toxic and lethal when thyroid hormone was administered with the tricyclic antidepressant drug Protriptyline. We did, however, observe that in the hyperthyroid condition norepinephrine turnover is decreased in hypothalamus when compared to control animals.

The results we obtained from chronic protriptyline administration and its effects on norepinephrine levels and turnover, as well as the results we obtained when yohimbine was employed are enlightening. Chronic protriptyline administration produced decreased norepinephrine levels in hypothalamus, and decreased norepinephrine turnover, when compared to saline administered controls. The alpha-receptor antagonist (yohimbine) produced only minor effects on norepinephrine levels and turnover when experimental animals were pretreated chronically (18 days) with protriptyline.

These results suggest an adaptive response (hypo-sensitivity) of the presynaptic alpha-receptor (autoreceptor), and this phenomena also occurs at the post-synaptic alpha-receptor. These results, suggesting receptor adaptation, indicated the existence of another mechanism of action for the tricyclic antidepressants.

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CHAPTER I.

C H A P T E R I.

INTRODUCTION:

During the past 25 years many facts have been documented regarding the anatomical and biochemical phenomena which are correlated with mood. This evidence has implicated the involvement of brain neural systems which employ norepinephrine (NE) as their neurotransmitter (Glowinski and Iversen, 1966; Kobayashi, et al., 1974; Ross and Reis, 1974; and Malbon, 1979). From these studies and theories the "catecholamine hypothesis for affective disorders" has been derived (Bunney and Davis, 1965; Schildkraut and Kety, 1967; Segal, et al., 1974; Baldessarini, 1975; Leonard, 1975; de la Fuente, 1979; and Stern, et al., 1980).

This theory states that elevated mood is associated with an increased interaction of brain NE with its respective receptors; whereas, a depressed mood is associated with a decreased interaction between NE and respective receptors. In addition, it is also proposed that some, if not all, depressions are associated with an absolute or relative deficiency of catecholamines, in particular NE, at the functionally important adrenergic receptor sites in the brain (McClure, 1971; McClure, 1973; and Barchas, et al., 1978). Studies in man have also shown that drugs which cause depletion and inactivation of NE centrally produce sedation and/or depression, while drugs which increase or potentiate brain NE levels are associated with behavioral stimulation or excitation and generally exert an antidepressant effect.

An additional building block for this theory lies in the fact that tricyclic antidepressants (TCA's), which normalize a depressed mood,

block the active reuptake of NE after its release from the presynaptic nerve terminals (Rossi, 1976; Kant and Meyerhoff, 1977; and Sulser, et al., 1978). The reuptake process is believed to be the primary means for terminating the post-synaptic action of NE, and the tricyclics are able to increase the time for released NE to interact with respective target cell receptors (Wurtman, 1965; and Iversen, 1973).

Other evidence (Alpers and Himwich, 1972: Frazer, et al., 1978; and de Montigny and Aghajanian, 1978) suggests that the ability of the tricyclics to block NE reuptake, though the primary mechanism of action, may not be their only mechanism of action. Blockade of NE reuptake can be demonstrated after a single injection of a TCA drug (Callingham, 1967; Todrick and Tait, 1969; Schildkraut, et al., 1970; and Oswald, et al., 1972). Paradoxically, tricyclics do not exert a clinically observable antidepressant effect unless given chronically for two to three weeks. Therefore, blockade of the reuptake mechanism cannot be reconciled with the time course (lag-time) for the chronic clinical efficacy of these drugs.

As a further building block adding support to the idea that tricyclics act by another mechanism is the observation that TCA drugs inhibit monoamine oxidase (MAO) activity in vitro (Gabay and Valcourt, 1968; Halaris, et al., 1973; Roth and Gillis, 1974a; and Roth and Gillis, 1974b). MAO is a mitochondrial bound intracellular NE degrading enzyme (Johnston, 1968; Schnaitman and Greenawalt, 1968; and Greenawalt, 1972). If specific enzymatic parameters regarding NE degradation differ between normal individuals and patients prone to endogeneous depression, then

tricyclics may exert their antidepressant influences by altering or returning to normal certain enzyme imbalances. These imbalances may only be changed during long-term tricyclic treatment, i.e. MAO inhibition (Nies, et al., 1971; Youdim and Holzbauer, 1976; and Sullivan, et al., 1977).

Since NE availability at post-synaptic receptor sites is one of the fundamental correlates for mood changes (depression or anxiety) it has also been suggested that cellular NE levels may decrease in individuals prone to endogeneous depression, and chronic administration of tricyclics reduce or prevent these changes (Schildkraut, et al., 1970; Schildkraut, et al., 1971; and Leonard and Kafoe, 1976). Contrary to these findings however, Neff and Costa (1967) and Alpers and Himwich (1972) have reported that chronic TCA treatment with imipramine or protriptyline did not induce changes in cellular NE levels in rat brain.

The effect of chronic tricyclic administration on NE turnover has also been suggested as a possible mechanism or parameter which may be altered by chronically administered tricyclics (Schildkraut, et al., 1970; Schildkraut, et al., 1971; and Leonard and Kafoe, 1976); but this hypothesis is not supported by other studies (Neff and Costa, 1967; and Alpers and Himwich, 1972).

Another hypothesis this research investigated dealt with the effects and interrelationships between thyroid hormone administration and TCA drugs; and their individual and/or combined abilities to alleviate several depressive symptoms observed in clinically depressed

patients. Studies by Prange, et al., (1970), Wheatley, (1972) and Slusher, (1975), have reported confirming results that both patient mood and behavior improved when thyroid hormone was administered in conjunction with tricyclics, but the biochemical reasons for this improvement in mental health remains unresolved.

It has been documented that dysfunction of the thyroid gland has long been associated with mental illness (Gibson, 1962; Prange, et al., 1969; Breese, et al., 1974; Slusher, 1975; Singhal and Rastogi, 1978; and Bain and Walfish, 1978). Consequently, symptoms of depression are frequently associated with hypothyroidism; whereas, anxiety, fatigue and irritability are associated with hyperthyroidism. It may be proposed that endogeneous affective disorders are linked specifically to overall thyroid status. This hypothesis has been suggested and states that in hyperthyroidism there is increased receptor sensitivity (Emlen, et al., 1972; Engstrom, et al., 1974; Engstrom, et al., 1975; and Strombom, et al., 1977), which induces, through compensatory mechanisms, a reduced neuronal activity and decreased NE turnover, both of which result from a negative feedback mechanism (Strombom, et al., 1977; Oppenheimer, 1979; and Sterling, 1979). Conversely, the opposite would occur in hypothyroidism. Therefore, concurrent administration of thyroid analogs with tricyclics should hasten the biochemical mechanisms of action elicited by chronic tricyclic treatment. This would yield a shorter time interval for clinical efficacy for these drugs and would result in an elevated mood (Earle, 1970; Wilson, et al., 1970; and Wheatley, 1972).

This aspect of this research study was designed to investigate and identify any possible central nervous system (CNS) biochemical mechanism which might be altered following both concurrent thyroid hormone and tricyclic administration which may accelerate improvement in a depressed patient.

In summary, this study investigated several hypotheses which were suggested as alternative mechanisms of action associated with tricyclic administration, and possibly resolve the existing controversy over the occurrence of a lag-time, between drug administration and clinical efficacy. Also, this study was designed to identify the relationship between thyroid hormone administration, tricyclics and accelerated clinical improvement in depressed patients.

The several hypotheses this research attempts to test follow:

(1) that the requirement for chronic treatment with TCA drugs before clinical efficacy is observed is related to an action on mitochondrial MAO, and that the time course for this effect parallels the time course for clinical response.

(2) that simultaneous administration of thyroid hormone potentiates the action of TCA drugs through an effect on MAO activity, NE turnover or NE cellular levels.

(3) that the time course in clinical efficacy of TCA drugs parallels the time course for development of presynaptic receptor subsensitivity (and concommitant changes in neurotransmitter turnover).

Investigations into these possible alternative mechanisms of action of the tricyclics may shed some light on clinical TCA drug treatment, and the clinical efficacy of this class of drugs. These results may aid researchers and clinicians in reconciling the unresolved time-course or lag-time associated with these drugs and with their observable clinical actions.

CHAPTER II.

C H A P T E R II.

A. MONOAMINE OXIDASE: A Review of History, Current Research and Interrelationships with Depressive Illness.

The term 'monoamine oxidase' (MAO) (O_2 oxido-reductase, deaminating, E. C. 1.4.3.4) is used to designate a group of enzymes catalyzing the oxidative deamination of tyramine, tryptamine, serotonin, norepinephrine, dopamine and other monoamines. These enzymes are mainly bound to the outer mitochondrial membrane. They were first identified by Hare, (1928) as tyramine oxidases, and were further characterized by Blaschko, et al. (1937, 1954). Pugh and Quastel (1937) were the first researchers to study MAO activity in nerve tissue. MAO activity varies greatly in different regions of the brain, with highest activity being observed in the hypothalamus and basal ganglia (Collins, et al., 1970; Youdim, 1973A, Youdim, 1973B; and Hazama, et al., 1976). The first studies on the intracellular localization of MAO were performed by Hawkins (1952) and Blaschko (1957). These independent investigators observed that in adult rat liver homogenates 70-80% of the total MAO activity is found in the microsomal fraction of the subcellular fractionation preparation.

Evidence that MAO is localized in the outer mitochondrial membrane was first observed and reported by Schnaitman, et al. (1967), with additional support added by de Champlain, et al. (1969). This close association between MAO and the mitochondrial membrane has complicated the purification of the enzyme, and the study of its pure chemical nature

and characteristic enzymatic parameters can only be examined using subcellular mitochondrial preparations employing unsolubilized partially purified enzyme preparations.

There is substantial evidence available which states that MAO exists in more than one form (Gorkin, 1966; Sandler and Youdim, 1972; Houslay and Tipton, 1973; Neff and Yang, 1974; Neff, et al., 1974; and Youdim and Collins, 1975). Multiple forms of human brain mitochondrial MAO were first described by Collins, et al. (1970). The presence of different forms of MAO in tissue homogenates, mitochondrial preparations and in carefully prepared outer mitochondrial membrane preparations has been supported by the discovery of substrate selective inhibitors of MAO. The two drugs employed for the specific characterization of MAO forms were clorgyline and deprenil (Johnston, 1968; Knoll and Magyar, 1972; Houslay and Tipton, 1974; Neff, et al., 1974; and Bakhle and Youdim, 1975).

Johnston, (1968), proposed that clorgyline allows the investigator to distinguish with in vitro and in vivo methodologies between two forms of brain MAO; termed Type A and Type B enzyme. Type A enzyme is thus very sensitive to clorgyline and preferentially deaminates 5-hydroxytryptamine and norepinephrine. Type B enzyme is specifically inhibited by deprenil and is relatively insensitive to clorgyline (Knoll and Magyar, 1972). For the Type B form of MAO preferential substrates include benzylamine and phenylethylamine (Houslay and Tipton, 1974).

Dopamine, tyramine and tryptamine are substrates for both enzyme forms (Fuller, 1972; Squires, 1972; Yang and Neff, 1973; and Neff and Yang, 1974).

There is also significant evidence suggesting that intraneuronal MAO plays an important role in regulating the functionally active pools of NE in the nerve cell cytoplasm in the neurons of the CNS (Kopin, 1964; Bloom and Giarman, 1969; Green and Grahame-Smith, 1975; Youdim, 1975a; Youdim, 1975b; and Youdim, 1975c). The exact mechanism by which regulation for these homeostatic processes is accomplished by MAO has not yet been resolved. Intraneuronal MAO may metabolize neurotransmitter monoamines before and/or after reuptake into the presynaptic nerve ending, following stimulated release.

This degradative process appears to depend solely upon the activity of the MAO enzyme present in the nerve terminals and postsynaptic structures. The reuptake process also appears to be governed or regulated by the relative concentrations of free intra- and extraneuronal NE. MAO is apparently essential for keeping cytoplasmic NE in the neuron low, and within a specific steady-state equilibrium. Under conditions when MAO activity is decreased (MAO-inhibitor administration) the concentration of total cytoplasmic NE rises. It has also been suggested (Bloom, 1963; Eiduson, et al., 1964; Kordon and Glowinski, 1970; and Gripois, 1975) that NE which is present in the presynaptic neuron cytoplasm may react with the MAO enzyme in the cytoplasm and cause a change in the steric conformational structure of the MAO enzyme. This may or may not be reversible. This steric conformational

change in MAO results in an activity change (i.e. increasing or decreasing MAO activity in the cytoplasm) and has been further documented by Iversen, et al. (1975). This change in enzyme structure, which has been substrate induced, may alter the specific activity of MAO toward a specific substrate, and paradoxically not change the affinity for this substrate (NE).

Possibilities for these conformational changes in the enzyme molecule could occur by "folding" of MAO chains, since the enzyme exists as a monomer, dimer and tetramer (Collins and Southgate, 1970; Gorkin, 1972; Southgate, 1972; and Yasunobu and Oi, 1972). Each MAO chain has a molecular weight of 75,000. Folding of these chains could result in reducing the activity of the enzyme (i.e., less accessibility of the substrate (NE) to the enzyme's active sites); or folding may initiate a self-catalytic process, or enzyme inactivation process which may or may not be reversible. Similarly, it has been suggested (Eiduson, et al., 1964), that the MAO degrading mechanism consists of a three-point attachment to the enzyme surface by the catecholamine, which very likely involves the amine, one alpha hydrogen, and either the other alpha hydrogen or the beta-methylene, once again substantiating the complexity of this structure-activity relationship, and the possible steric variations for inactivity that are available.

It appears there is a self-regulatory mechanism in the nerve cell cytoplasm which can increase intraneuronal NE concentrations or decrease NE levels. This process is dependent upon signals which are increased or decreased, sequential to neural firing rates.

In most theories of brain dysfunction and function, MAO is of significant importance. Any change in the MAO activity usually alters the function of a specific CNS neurotransmitter, and in particular NE. A physiological role for MAO has been implicated in many processes: affective disorders, agressive behavior, psychosomatic stimulation, severe mood and behavioral changes leading to crimes of violence and suicide (Ashcroft, et al., 1966; Shaw, et al., 1967; Bourne, et al., 1968; and Nies, et al., 1971); drug induced seizures, sleep, environmental stress, hallicugen action (Glowinski, 1966; Garver, et al., 1975; Schildkraut, et al., 1976; Becker and Shaskan, 1977; Orsulak, et al., 1978; Baron, et al., 1980; Meltzer, et al., 1980); regulatory effects of adrenocortical steroids and of ovarian hormones, temperature regulation and eating (Glick and Greenberg, 1958; Avakian and Callingham, 1968; Southgate, et al., 1968; Carpenter and Bunney, 1971; Callingham and Della Corte, 1971; Holzbauer and Youdim, 1973; Ruhmann-Wennhold and Nelson, 1973; Garver, et al., 1975; Otten and Thoenen, 1976; and Rastogi and Singhal, 1978).

Since MAO exists in multiple forms (Johnston, 1968; Youdim, 1973; Houslay and Tipton, 1976; Green, et al., 1977; Jain, 1977; Student and Edwards, 1977; Trepel, et al., 1977; and Fowler and Callingham, 1978), and because it has a central role in the degradation of biogenic amines, the therapeutic benefit of drugs which inhibit this enzyme in clinical treatments deem it imperative that MAO be examined following both acute and chronic tricyclic administration.

Since the possibility exists that alterations in the metabolism of central monoamines cause several mental disorders, many investigators have studied MAO activities in the CNS (Leonard, 1975; Youdim and Holzbauer, 1976; Ananth and Luchins, 1977; and Barchas, et al., 1978). Ashcroft and Sharman (1960) were the first researchers to measure the metabolites of biogenic amines in the cerebral spinal fluid (CSF) of mentally ill patients. In certain types of endogeneous depression low CSF concentrations of 5-Hydroxy Indole Acetic Acid (5-HIAA) were observed (Dencker, et al., 1966; van Praag, et al., 1970; and Sjostrom, 1973). These findings also agree with the lower 5-hydroxytryptamine and 5-HIAA concentrations found in post-mortem brains of patients with depressive illness who committed suicide (Shaw, et al., 1967; and Bourne, et al., 1968).

Further support for a possible link between MAO and mental illness is documented by the beneficial effects achieved with drugs which inhibit MAO (Shaw and Hewland, 1973; Davidson, 1974; Neff, et al., 1974; Ananth and Lucins, 1977; and Campbell, et al., 1979). Since alterations in monoamine metabolism can occur during their degradation, variations in the function of MAO could ultimately result in a dysfunction of the neural pathways in which the respective monoamine (NE) plays a role as a specific transmitter substance. It is therefore important that the influence of tricyclics on MAO, when administered both acutely and chronically be examined (Roth and Gillis, 1975; Spiker and Pugh, 1976; Gabay and Achee, 1977; Honecker and Hill, 1977; Ponto, et al., 1977; Roth, 1978; and Achee and Gabay, 1978). These studies are

imperative if we are to understand the interrelationship between tricyclics and delayed clinical efficacy.

B. PROCEDURE AND METHODS

1. General experimental procedures.

Male albino rat (Charles River Laboratories, C.D. Strain, COBS) weighing 250-400 grams were used for the determination of MAO activity levels, NE pool size levels and NE turnover studies, as well as for all studies involving thyroid hormone interrelationships. All animals were housed in the animal care facility at Rhode Island Hospital and were maintained at the ambient temperature of 20-22° C. These animals were given Purina Rat Chow and water ad libitum and were subjected to a 14-hour lighting cycle (0500-1900 hours).

2. Drugs employed.

Radioactive substrates for monoamine oxidase included C^{14}-tryptamine bisuccinate and C^{14}-5-hydroxytryptamine. These chemical were purchased from New England Nuclear, Waltham, Mass. and are listed under the following catalogue numbers: NEC 259, tryptamine; NEC 225, 5-hydroxytryptamine.

The tricyclic antidepressant drug employed in these studies was protriptyline (Vivactil) manufactured by Merck Sharp & Dohme, Pennsylvania. Protriptyline was chosen as our experimental drug since it is a secondary amine tricyclic which preferentially blocks reuptake of the catecholamine NE (Ross and Renyi, 1975a; Ross and Renyi, 1975a; Ross and Renyi, 1975b). Desipramine, desmethylchlorimipramine and nortriptyline are other similar secondary tricyclics. In contrast,

imipramine, amitriptyline and doxepin are classified as tertiary amines, and preferentially block the reuptake of serotonin (Rossi, 1976).

3. Assay for monoamine oxidase.

MAO activity was measured by the method of Wurtman and Axelrod (1963). Rats were decapitated and brain tissues were surgically excised. The surgically removed tissues were weighed by flotation in 0.5 M potassium phosphate buffer (pH 7.4), 15 ml total volume. Tissues were then homogenized by a polytron cell disrupter (Model PT-10/20, with PT-10, ST generator, Brinkman, Switzerland) for two minutes at high speed (dial setting 10). All preparations were kept in an ice bath during cell disruption. Following cell disruption, 100 ul aliquots of each tissue sample was placed in assay tubes and reacted with labelled tryptamine bissuccinate or 5-hydroxytryptamine (New England Nuclear, NEC 259, and NEC 225), which were used as the substrates for the MAO enzyme.

The total assay volume was 1.2 ml (0.5 ml potassium buffer, pH 7.4; 0.5 ml distilled water; 0.1 ml tissue homogenate; and 0.1 ml substrate solution). Assay tubes were incubated for 20 minutes in a shaker water bath at 37° C. At the end of the incubation period, the reaction was quenched with 0.2 ml of 2.0 N HCL. This caused denaturing of MAO and prevented further substrate degradation (deamination). The deaminated radioactive products, 5-HIAA and Indole Acetic Acid (IAA), were then extracted by vigorous shaking with 6.0 ml of a toluene/ether solution (1:1). This procedure is a modification of the original Wurtman and Axelrod (1963) method, and allows a more efficient extraction of radioactive products (5-HIAA and IAA). Efficiency was

increased from 62± 2% to 82± 4% and was used in all subsequent MAO determinations (Kaiser, 1975A). Following a 10 minute centrifugation, (IEC International Centrifuge, Model PR-2) of this mixture (2000 rpm, 280 x g), a 4.0 ml aliquot of the organic phase was transferred to a liquid scintillation counting vial containing 10.0 ml phosphor [0.4 gram p-Bis-(2-5-phenyloxazolyl-benzene)] (POPOP), and 4.0 grams 2,5-diphenyloxazole (PPO) per liter of toluene (Bray, 1960). These samples were then counted in a liquid scintillation counter (spectrophotometer) (Searle Analytical, Mark III) for 10 minutes. A small amount of ^{14}C-tryptamine or ^{14}C-5-hydroxytryptamine (less than 0.3%) (Kaiser, 1975B) was extracted by this procedure, but it creates a negligible error in the extracted radioactive samples.

4. DNA methodology.

Homogenized tissue aliquots of 0.1 ml were taken from the cell preparations previously described (assay for MAO), placed in 15.0 ml graduated conical centrifuge tubes, and frozen until assayed. A standard DNA stock solution was prepared containing 200 mg of deoxyribonucleic acid sodium salt (highly polymerized, grade A, Cal-Biochem, La Jolla, CA.) per 100 ml of 1.0 N ammonium hydroxide (NH_4OH). This solution was stored at 4^{o} C.

Standards of 1.0, 2.0, 4.0, 8.0 and 12.0 ug DNA per sample were run with each assay in order to check their reproducibility and precision. All samples were then assayed by the method of Kissane and Robins (1958), which has been slightly modified to allow the use of larger tissue samples (Wunschel, personal communication). Modifications

involved the use of proportionately larger volumes of reagents for the extraction procedures. All samples were assayed in triplicate and averaged.

The calculations involved in determining the DNA standard Curve, the linear regression analysis to obtain the regression coefficient, and a graphical representation of the data can be found in the Appendix section, Figure

5. Scintillation counter efficiency adjustment and DNA calculations.

The calculations involved for the conversion of counts per minute (CPM) to nanomoles of IAA or 5-HIAA formed per hour per ug DNA follow:

I. For Efficiency Adjustment:

$$\text{Disintegrations per minute (DPM)} = \frac{\text{counts per minute (CPM)}}{\text{counting efficiency of counter}}$$

II. Conversion Equation (To convert DPM's to nanomoles of (5-HIAA or IAA formed per hour per ug DNA)

$$\frac{\text{DPM} \times \text{dilution factor}}{\text{counts of radioactive material used (total substrated added to samples)}} \times \text{nanomoles of radioactive product formed} \times \frac{3^*}{2} \times \frac{3^{**}}{1} =$$

nanomoles of IAA or 5-HIAA $\div$ ug of DNA per 100 ul sample formed per hour per gram

= nanomoles of product formed per hour per gram per ug DNA.

*This ratio refers to the volume of organic solution extracted in the separation procedure.

**This ratio refers to a reaction incubation time adjustment.

C. RESULTS

Initial experiments involving the determination of MAO activity levels in brain tissue were concerned with examining the enzymatic kinetic parameters of the enzyme. Classical velocity (V) versus time (T), and velocity (V) versus enzyme (E) concentration experiments were performed using homogenized tissue preparations from the three brain regions being examined (corpus striatum, forebrain and hypothalamus).

From these studies it was determined that a twenty minute incubation time, and a 100 ul aliquot of each homogenized tissue preparation would produce linearity, and neither time nor enzyme concentration would be limiting variables in future experiments.

Following these studies and employing both a twenty minute incubation time and the 100 ul aliquot of the homogenized brain tissue preparations, further experiments were conducted to determine both Km and Vmax values for the MAO enzyme in these three brain regions (see appendix section Figures 1-4; Tables 14-26.

After completing the enzymatic kinetic parameter studies, MAO activity levels were measured in the same three regions of rat brain in control animals. From these results it is apparent that in these brain regions there exists about 4 times more B-form MAO than A-form MAO, since C^{14}-serotonin is selective for only the A-form of MAO, whereas C^{14}-tryptamine is deaminated by both the A and B forms of MAO. These values are reported in Table 1. When C^{14}-tryptamine was employed as substrate for MAO, the total nmoles of product formed/hr/ug DNA is significantly greater ($p < 0.001$) than when C^{14}-serotonin was used as the

MAO enzyme substrate. Also, it is apparent that the forebrain preparation has significantly more MAO activity ($p < 0.01$) than the other two brain regions being examined, regardless of whether C^{14}-tryptamine or C^{14}-serotonin was employed as the MAO substrate.

TABLE 1 Monoamine oxidase activity levels in three regions of rat brain under control conditions.

TISSUE	C^{14}-serotonin	C^{14}-tryptamine
Corpus Striatum	0.4462 ± 0.032*(N=8)	2.2638 ± 0.161 (N=12)
Forebrain	0.5926 ± 0.032 (N=6)	3.1692 ± 0.139 (N=8)
Hypothalamus	0.3623 ± 0.015 (N=5)	2.1469 ± 0.061 (N=8)

*Values represent the Mean ± S.E.M. and are reported as nanomoles IAA or 5-HIAA formed/hr/ug DNA

Next, an initial set of experiments was undertaken to examine the effect of protriptyline on MAO activity in vitro (appendix section, Figures 5 and 6). Corpus striatum, forebrain and hypothalamus were dissected out of whole brain and homogenized in phosphate buffer (pH 7.4). Aliquots (100 ul) of these homogenized tissue preparations were then placed in reaction tubes and preincubated with varying molar concentrations of protriptyline (10^{-11} to 10^{-3} M) for thirty minutes at 20° C, prior to addition of labelled substrate as described in the Methods Section.

The results from these experiments report that when the concentration of protriptyline in the preincubation media approaches 10^{-6} M, MAO enzyme activity in the corpus striatum is inhibited when either C^{14}-tryptamine or C^{14}-serotonin are used as substrate. Similar results were observed in both forebrain and hypothalamus. We interpret these results to be a non-specific inhibitory effect. Numerical values and graphical illustrations are reported in the appendix section (Figures 5 and 6; Tables 27 and 28).

The next area this research investigated involved measuring the in vivo effects of protriptyline on MAO activity. Protriptyline was administered to experimental animals (male albino rats, Charles River, C.D. Strain) acutely (one daily injection), subchronically (3 or 6 daily injections) and chronically (18 daily injections). A dose of 10 mg/kg/day protriptyline hydrochloride dissolved in physiological saline was administered for this regiment of injections. This dose of protriptyline was selected since it appears extensively in the literature (Schildraut, et al., 1971; Leonard and Kafoe, 1976; Rossi, 1976). This dose of protriptyline is between 10 and 20 times the dose used in treating clinical depression (Long, 1977).

The results of these experiments are presented in the appendix section (Tables 3, 4, 5 and 6). Our observations from these studies indicated that in vivo protriptyline administration had no consistent effect upon MAO activity in the brain tissues we examined regardless of length of administration (1, 3, 6 or 18 consecutive injections) and regardless of whether MAO activity was examined 1, 3, 6, 12 or 24 hours after the last drug administration.

D. DISCUSSION

The activity of monoamine oxidase, one of the degrading enzymes in the metabolism of the neurotransmitter NE, has been measured under various experimental procedures in several regions of rat brain. This area of investigation was pursued, since the mechanism of action of the tricyclics remains controversial. Specific actions of the tricyclics on MAO activity may explain why chronic treatment is required to produce clinical efficacy.

We began our studies by initially developing and optimizing both our methodology and technique for isolating and quantitatively measuring tissue MAO. The numerical data we obtained for our enzymic kinetic parameters is supported by other investigators, and this adds support and validity to our procedures.

When examining the in vitro effects of protriptyline preincubation on MAO activity, we observed a decrease in enzyme activity at concentrations of 10^{-6} M and higher. Similar results for the tricyclics (i.e. MAO inhibition) have been reported by other investigators (Gabay and Valcourt, 1968A; Halaris, et al., 1973; Roth and Gillis, 1974A; Roth and Gillis, 1974B; Roth and Gillis, 1975; Roth, 1977).

Examining the effects of acute (one daily injection), subchronic (three or six daily injections) or chronic (eighteen daily injections) protriptyline administration in vivo on MAO activity, we found no consistently significant effect of protriptyline on MAO activity in the brain regions we examined.

Having extensively researched the first hypothesis of this study, and concluding that no specific *in vivo* effect for protriptyline on MAO activity could be substantiated, we proceeded to test our second hypothesis, "that thyroid hormone interacts with the tricyclics and may potentiate their effect on MAO activity, NE turnover, and on cellular NE levels."

C H A P T E R III

C H A P T E R III.

A. THE THYROID HORMONES: Thyroid status and its interrelationship with monoamine oxidase, norepinephrine biosynthesis and the tricyclic antidepressant drugs.

The mechanism of action of the thyroid hormones (T_3 and T_4) is of considerable interest because of the amazing diversity of their effects (Fleischmann, 1947; Fischer, et al., 1968; Prange, et al., 1971; Breese, et al., 1974; Strombom, et al., 1977; Singhal and Rastogi, 1978). These hormones exert profound effects on many enzymes and on almost all organ systems. They also play an important role in the complex biological processes involved in growth and cell differentiation (Oppenheimer, 1978). These hormones are thought to cause their actions by modulating or regulating the actions of other endogenous hormones or enzymes (Barchas, et al., 1978). This is accomplished by influencing thyroid hormone receptors associated with the nuclear chromatin (Sterling, 1979); and by altering mitochondrial function (Sterling, et al., 1978). The concept of an intracellular, nuclear receptor for thyroid hormone is based on earlier receptor models, which have been theorized for the action of steroid hormone action on respective target cells. In these models hormone action occurs via nuclear transcription (Samuels and Tsai, 1973; De Groot and Stausser, 1974; Samuels, et al., 1974; De Groot and Torresani, 1975; Mac Leod and Baxter, 1976; Chan and O'Malley, 1976). Other researchers (Gardner, 1975; Oppenheimer, 1975; Kurtz, et al., 1976; Surks and Oppenheimer, 1977), suggest that the

hormone penetrates the plasma membrane and is bound by a specific cytosol receptor. This hormone-receptor complex enters the nucleus, and increased transcription of a genetic message for increased mRNA occurs. This in turn results in mRNA directing increased synthesis of specific nuclear proteins.

Ample evidence is available to support the view that an association exists between mental disturbances and altered levels of hormones such as adrenal corticoids, thyroid hormones, androgens and the estrogens (Rubin and Mandell, 1966; Mandell and Mandell, 1967; Dewhurst, et al., 1969; Glass, et al., 1971; Wheatley, 1972). Only recently neuroendocrinologists have become interested in studying the effects of hormones on the functioning of the brain. Psychiatrists, however, have felt for many years that the solution to several etiological problems in psychiatry would become manifest only after a better understanding of neuroendocrinological mechanisms became apparent, and in particular those involving thyroid hormone mechanisms (Singhal and Rastogi, 1978).

Since the earliest descriptions of both hyperthyroidism and hypothyroidism, it has been suggested that certain psychiatric disorders may be manifestations of thyroid dysfunction. The clinically observable symptoms of thyroid dysfunction are sometimes of such severity that they bring the patient to the attention of the psychiatrist, and occasionally demand psychiatric hospitalization.

In clinical studies conducted (Jefferys, 1972; Thomson, et al., 1972; Mc Larty, et al., 1978; Nusynowitz and Young, 1979; Cohen and

Swigar, 1979), the prevalence of thyroid dysfunction in a psychiatric population (1320 patients) was 1.2% for males and 2.0% for females. It is doubtful whether these figures are significantly different from the prevalence of thyroid dysfunction in the general population (Whybrow, et al., 1969; Henschke and Pain, 1977; Tunbridge, et al., 1977). This suggests that the importance of thyroid disease and mental disturbances may not be of important clinical significance. Therefore, the value of thyroid function testing in psychiatric patients is still unclear. There are two criteria however, which must be discussed. First, thyroid dysfunction, (hypothyroidism or hyperthyroidism) may manifest itself as a psychiatric illness, and consequently some of the signs and symptoms of mental derangement mimic those observed in thyroid dysfunction (Singhal and Rastogi, 1978; de la Fuente, 1979). Thus, the diagnosis of these conditions on overt clinical parameters is sometimes very difficult. The occurrence or prevalence of thyroid dysfunction in a psychiatrically ill population is uncertain, and has been reported to be higher than the general population (Nicholson, et al., 1976; Weinberg and Katzell, 1977). It has also been reported to be the same as the general population (Bursten, 1961; Gibson, 1962; Clower, et al., 1969). It is also clear that the stress caused by various types of acute and chronic organic illness profoundly affect thyroid function tests (Johansson, et al., 1972; Mason, et al., 1973; Mason, 1975). Although psychiatric illness may produce similar alterations in thyroid status, the magnitude, duration, and frequency of these thyroid changes are unclear and require examination.

It was our intent to study the interrelationships between MAO activity in various brain regions following both in vitro pre-incubation with thyroid hormone (T_3 and T_4) and also following in vivo subcutaneous injection of thyroid hormone for varying time periods. Also, we studied the effects of thyroidectomy on MAO activity. The thyroid hormones are known to exert important influences on the central nervous system (CNS) (Fleischmann, 1947; Gibson, 1962; Harrison, 1964; Bain and Walfish, 1978; Oppenheimer, 1979; and Sterling, 1979), and in particular on both peripheral and CNS MAO activity (Novick, 1961; Fisher, et al., 1968; Breese, et al., 1974; Callingham and Lyles, 1974; Moonat, et al., 1975; Lyles and Callingham, 1976; Asaad and Clarke, 1978). It has been suggested by Novick, (1961) and Youdim and Holzbauer, (1976), that the thyroid hormones modify MAO activity, but the specific mechanisms by which these changes occur have not been resolved. There exists the possibility of modulating the biogenesis of mitochondria. This theory has been advanced by other researchers (Novick, 1961; Youdim and Holzbauer, 1976). Also, it has been suggested that the thyroid hormones can increase the synthesis of MAO itself (Fischer, et al., 1968). This theory has been documented for heart tissue (Coville and Telford, 1970; Lyles and Callingham, 1976). Also, it has been suggested that the thyroid hormones activate an inactive cytoplasmic MAO in brain tissue (Asaad and Clarke, 1976). Conversely, however, rat liver MAO activity was decreased following thyroid hormone administration (Moonat, et al., 1975). Thus, any apparent thyroid hormone induced influences

on MAO activity still remain unclear, and also tissue specific. Similarly, emotional or mental stress may also be related to possible MAO-thyroid relationships, and these relationships should be investigated.

With regard to norepinephrine levels and norepinephrine turnover following either T_3 or T_4 administration, once again controversies exist. Engstrom et al., (1974, 1975) as well as Schwark and Keesey (1975) have stated that T_4 pretreatment has no significant effect on brain concentrations of NE, dopamine or serotonin. However, Engstrom, (1974), Strombom et al., (1977) and Jacoby (1975) have reported increased turnover of brain catecholamines following T_4 pretreatment. Also, Kennedy et al., (1977) reported increased NE turnover in brown adipose tissue (BAT) pads of the rat with statistically significant increases in NE levels in this tissue with T_4 treatment. Contradictory to these studies however, Prange et al., (1970), has reported that thyroxine pretreatment caused a decrease in NE turnover and levels in the rat in both brain and heart tissue. Lastly, studies by Rastogi and Singhal (1974a, 1974b) have reported an increase in NE levels in brain and total animal body weight when T_3 was administered.

Since this controversy exists, regarding the effects of T_3 and T_4 administration on NE levels and turnover, it was imperative to include the effects of the thyroid hormones on these NE parameters in this study.

Also, since a similarity exists between the clinical manifestations of depression and those of hypothyroidism, the effects of thyroidectomy

were investigated in relation to NE levels and turnover. Evidence from many clinical and animal studies indicate that a number of metabolic and psychic disturbances are common to both thyroid deficiency and affective illness (Oppenheimer, 1979; Sterling, 1979).

Biochemical studies have demonstrated that both hypothyroidism and depression show a diminished response to infused NE (Schneckloth, et al., 1953; Prange, et al., 1967). Furthermore, psychological studies have suggested that the symptoms of myxedema, a severe form of hypothyroidism, makes an insidious appearance and is generally characterized by listlessness, lack of energy, slowness of speech, reduced sensory capacity, impairment of memory, social withdrawal and altered sleep patterns (Eayrs, 1960; Kales, et al., 1967). Several of these psychological symptoms are commonly observed in depressed patients; slowness of speech, reduced sensory capacity, lack of energy, social withdrawal, and altered sleep patterns (Libow and Durrell, 1965; Whybrow, et al., 1969; Davenport and Dorcey, 1972; Davenport, et al., 1976).

Even though there appears to be sufficient evidence to implicate thyroid dysfunction with associated depression, it is still impossible at present to implicate abnormal thyroid function as a result or a cause of affective disorders. In order to possibly clarify this hypothesis and gain insight into whether or not alterations in neurotransmitter mechanisms (i.e. pool size and/or turnover) cause suppressed behavior and learning deficiencies, which are viewed in both depressed and hypothyroid patients, we felt that it was imperative to investigate NE parameters during hypothyroidism.

Effective pharmacological treatments for mental illness have existed only during the last 30 years. This period has also witnessed a revolution regarding the care of psychiatric patients and has resulted in a decrease in the number of patients in both state and county mental facilities. This combination of both drug therapy and psychological and socioenvironmental treatment is responsible for the vastly improved prognosis for patients with mental illness (Barchas, et al., 1977; Berger, et al., 1977; Gold and Pottash, 1981; Spiker, 1981).

Since a high prevalence of mental dysfunction exists, and also a concommitant economic loss due to work disability and/or hospitalization, as well as the ever present danger of suicide, a persistent as well as compelling reason to search for a treatment for depression, that is both rapid, safe and convenient becomes necessary. Since treatment with thyroid hormones has been shown to enhance the pharmacological actions of several of the barbiturates (Conney and Garren, 1961; Prange, et al., 1966), and also since other clinical studies (Prange, et al., 1970A; Earle, 1970; Prange, et al., 1970B; Wheatley, 1972; Whybrow, et al., 1972; Slusher, 1975; and Schmidt, 1977) as well as some animal investigation (Prange and Lipton, 1962; Breese, et al., 1974) have documented an increase in drug efficacy, it has been theorized that the thyroid hormones may accelerate the antidepressant actions of the tricyclics or these other pharmacological agents (Cavalieri and Pitt-Rivers, 1981). Keeping in mind these observations we designed our experimental protocol to incorporate both protriptyline and T_3 administration. We planned to

investigate their possible synergistic interrelationships with regard to MAO activity, NE levels and NE turnover in three tissues of the rat brain.

The occurrence of psychiatric symptoms in patients suffering from hypothyroidism is well established (Asher, 1979; Rubin and Mandell, 1966; Oppenheimer, 1979; Sterling, 1979). Also, the similarity between the manifestations of anxiety states and hyperthyroidism is also well established (Dewhurst, et al., 1969; Singhal and Rastogi, 1978; Youdim and Holzbauer, 1976). Therefore, whether as a cause or an effect, there is evidence which implicates thyroid dysfunction with psychiatric illness.

Since this strong implication exists, between thyroid status and mental illness, it becomes more critical that effective, fast-acting pharmacological treatments for mental illness become available. These treatments will become available only after sufficient animal research has been conducted and only then will there be immense benefits for both patients and the general population.

B. PROCEDURES AND METHODS

1. General experimental procedures.

Pharmacological hyperthyroidism was induced by daily subcutaneous injection of L-thyroxine (either T_3 or T_4), 1.0 mg/kg/day, (10 mg/cc in normal saline with 2 drops of 1.0 N NaOH) for seven consecutive days (Barker, et al., 1949; Engstrom, et al., 1975; Kennedy, et al., 1977), and these animals were then sacrificed on the 8th day by decapitation. This dose of thyroxine was sufficient to maintain significantly elevated protein bound iodine (PBI) levels (Hammond, 1968; Engstrom, et al., 1975; Kennedy, et al., 1977).

Hypothyroidism was induced by surgical thyroidectomy at 7 weeks of age (approximately 175 grams body weight), a time at which thyroid function has stabilized to the adult level (Hammond, 1968; Kennedy, et al., 1977; Hamolsky, personal communication). Studies based on measurements of protein bound iodine (PBI) levels and basal metabolic rate have revealed that significant hypothyroidism does not develop in the rat until 7 to 8 weeks post-thyroidectomy (Hammond, 1968; Kennedy, et al., 1977). Therefore, no measurements other than body weight recordings were made until after this time period had passed.

Also, it is appropriate to note that an age difference existed among the experimental groups being examined, and this partially accounts for the observed differences in total body weight. The thyroidectomized rats were approximately 18 weeks of age when sacrificed, whereas, thyroxine treated animals were 12 weeks of age. In each instance corresponding euthyroid animals of equivalent age served as paired controls for each experimental group. Also, to adjust for varying total body weights and animal age differences, DNA determinations were conducted on all brain tissue fractions used in MAO activity level measurements.

2. Assay methodology for norepinephrine levels and turnover.

Norepinephrine (NE) in brain tissue was assayed by being extracted into 0.4 N perchloric acid (PCA) with subsequent purification over alumina columns. The NE was oxidized to its trihydroxyindole derivative and assayed fluorometrically on a spectrophotofluorometer (Aminco-Bowman) by the method of Lund (1950).

The method for NE determinations is as follows: brain tissue samples were homogenized in 0.4 N PCA (total volume, 10 ml) on a Brinkman polytron, high speed for 30 seconds. Following hemogenization another 25 ml of 0.4 N PCA was added to the homogenate (total volume - 35 ml). The homogenate was allowed to stand for one hour at 4° C, and shaken vigorously every 15 minutes until the hour had elapsed. The homogenate was then centrifuged (IEC-International Centrifuge, PR-2, 2000 rpm, 280 x g) for 20 minutes, and the supernatant was decanted. The pH of the supernatant was adjust between 8.3 to 8.5 with 0.5 N NH_4OH and 5.0 N NH_4OH and then poured on alumina columns. The alumina had been prepared by the method of Crout, (1961), (see 3. Alumina Preparation), and the NE was adsorbed onto the alumina beads. The NE was then eluded off these alumina beads with 0.3 N acetic acid (2.0 ml). The eluded NE fraction was oxidized with potassium ferricyanide (25%) and fluroescence was read on the spectrophotofluorometer with the excitation wavelength at 400 nmeters and emission wavelenght at 510 nmeters. An external standard with NE as well as an internal standard (H^3-DL-norepinephrine, New England Nuclear) was simultaneously assayed and recovery was 84.4 ± 5% (N=1834 determinations). All values were corrected for percentage recovery, (See Appendix Section, Figures 24 and 25).

3. Alumina preparation.

Powdered alumina (Fisher Scientific, chromatographic grade) is used for NE adsorption following the acidic activation of this alumina. For alumina activation 200-300 grams of neurtral alumina are boiled in

one liter of 2 N HCL for 30 minutes in a reflux apparatus. After boiling the cloudy supernatant is discarded, and one liter of distilled water is added to the acidified alumina while still in the reflux apparatus, and stirred briefly. After allowing the alumina to settle, the clear supernatant is decarded. These distilled water washings are repeated 12-15 times until the pH of the discarded supernatant attains a pH between 4.0-5.0. The alumina is then collected in a large suction funnel, and allowed to dry overnight at room temperature (20-22°C). The alumina is then heated in an oven at 100° C for 2 hours. The alumina is now activated and can be used for NE adsorption.

4. Alpha-methyl-para-tyrosine procedure.

Alpha-methyl-para-tyrosine (methyl ester, Sigma Chemical Co.) was used to measure NE turnover. This agent competitively inhibits tyrosine hydroxylase, the rate-limiting enzyme in the synthetic pathway for NE biosynthesis. At zero time, 400 mg/kg alpha-methyl-para-tyrosine (Brodie, et al., 1966) is injected interperitoneally (IP) into both experimental and control animals, and these animals are sacrificed by decapitation at varying time intervals after alpha-methyl-para-tyrosine administration. Depletion rates for all groups of animals were determined from 0 to 9 hours after alpha-methyl-para-tyrosine injection.

The validity of this method rests on several assumptions. First, NE is assumed to be maintained at a steady state level, existing in a single depletable compartment within the nerve ending. In this situation only, can efflux rate be said to be equal to synthesis rate.

Second, that tyrosine hydroxylase is and remains totally inhibited over the entire course of the turnover measurement. Third, that alpha-methyl-para-tyrosine acts only by blocking NE synthesis, does not interfere with tyrosine uptake (or required cofactors). Also, that the mechanisms for release or re-uptake of neurotransmitters are not altered. Fourth, that the neuronal pools of both DOPA and dopamine are very small, and thus cannot serve as a reservoir for NE synthesis after tyrosine hydroxylase has been inhibited. The fifth, and last criteria, is that alpha-methyl-para-tyrosine does not interfer with the experimental drug which is being studied. Similarly, the experimental drug cannot affect the enzyme inhibiting characteristics of alpha-methyl-para-tyrosine on tyrosine hydroxylase activity.

In addition, an alpha-methyl-para-tyrosine dose-response curve for tyrosine hydroxylase was performed, resulting in a dose for alpha-methyl-para-tyrosine which no longer produced an increase in the slope of the depletion curve for NE. It was at this dose (400 mg/kg) that all synthesis of NE by tyrosine hydroxylase had been inhibited (Figure 2),(Nagatsu, et al., 1964; Spector, et al., 1965; Volicer and Reid, 1969; Costa, 1971; Ostman-Smith, 1979).

C. RESULTS

1. Monoamine oxidase activity in three brain regions.

The effects of preincubation with tissue homogenate with triiodothyronine (T_3) on MAO activity in three rat brain regions (corpus striatum, forebrain and hypothalamus) has been measured using

in vitro techniques and employing either C^{14}-tryptamine or C^{14}-serotonin as substrate. In these studies concentrations of T_3 of 10^{-9} M and higher significantly reduced MAO activity in all brain regions studied. MAO activity was reduced by 20-40%, regardless of length of preincubation time with T_3 (0, 15, 30 or 60 minutes) (Appendix section, Figure 7 and Tables 29-34). In the hypothalamic preparation at least 30 minutes of preincubation with T_3 was required for MAO inhibition.

Similar results were obtained when preincubation times were 0, 15 or 30 minutes; and whether using C^{14}-tryptamine or C^{14}-serotonin as substrate.

At the time of sacrifice T_3 and T_4 administered animals were found to have a decreased body weight (24 $\pm$ 5 grams), whereas saline administered animals gained 54 $\pm$ 11 grams.

One to eight days of S.C. administration of T_3 (1 mg/kg/day) resulted in no consistent changes in MAO activity when the animals were sacrificed 24 hours after the last T_3 injection, when C^{14}-serotonin was used as substrate (Table 2). Similar results were obtained when C^{14}-tryptamine was used as substrate and when thyroxine (T_4) was employed instead of T_3 (Appendix section, Tables 35, 36, and 37).

At the conclusion of these *in vivo* T_3 and T_4 studies, we investigated the interactions of T_3 administration and subsequent protriptyline administration on MAO activity in corpus striatum, forebrain and hypothalamus. We chose to use only T_3 in these studies since it is the more active form of the thyroid hormones and is more potent, faster acting and more rapidly metabolized (Tata, 1964; Prange, *et al.*, 1970;

TABLE 2 The effect of triiodothyronine on monoamine oxidase activity in three brain regions.

Days of Injection	Corpus Striatum		Forebrain		Hypothalamus	
	Saline	T_3	Saline	T_3	Saline	T_3
No Injection	--------0.460 ± 0.03---------		------0.476 ± 0.04-------		------0.256 ± 0.02-------	
1	0.543 ± .05[1,2]	0.410 ± .06	0.729 ± .16	0.580 ± .10	0.254 ± .03	0.183 ± .02
2	0.468 ± .03	0.509 ± .06	0.499 ± .05	0.493 ± .03	0.264 ± .04	0.271 ± .03
3	0.666 ± .05	0.622 ± .11	0.761 ± .13	0.768 ± .13	0.385 ± .05	0.310 ± .04
4	0.570 ± .03	0.520 ± .06	0.601 ± .05	0.565 ± .03	0.295 ± .02	0.258 ± .02
5	0.563 ± .03	0.425 ± .03	0.670 ± .07	0.575 ± .09	0.273 ± .03	0.228 ± .02
6	0.465 ± .03	0.568 ± .12	0.640 ± .06	0.582 ± .04	0.279 ± .03	0.301 ± .03
7	0.603 ± .04	0.598 ± .08	0.683 ± .07	0.680 ± .09	0.299 ± .02	0.358 ± .03
8	0.521 ± .04	0.529 ± .10	0.719 ± .09	0.838 ± .12	0.315 ± .03	0.249 ± .03

N = 6 for each determination

[1]Mean ± S.E.M.; Nmoles IAA formed/hour/ug DNA; Serotonin as substrate

[2]No statistical significance was observed between any of the time pts. examined

Oppenheimer, 1975; Oppenheimer, 1979). No further data involving T_4 administration are presented.

The T_3 and protriptyline studies were performed since previous investigators (Prange, et al., 1969; Wilson, et al., 1970; Earle, 1970; Whybrow, et al., 1972) have documented that thyroid hormone, when administered with tricyclics, produces a quicker clinical improvement in depressed patients. In these studies animals were made hyperthyroid by seven consecutive S.C. injections of T_3 (1 mg/kg/day), a regiment previously shown to produce physiological changes analogous to hyperthyroidism (Hammond, 1968; Kennedy, et al., 1977). On the seventh day following their last T_3 injection, animals received one injection of protriptyline (10 mg/kg - S.C.); three hours after receiving protriptyline all animals had perished, convulsing prior to death (Table 3).

The next group of experiments involved simultaneous administration of T_3 (1 mg/kg - S.C.) and protriptyline (10 mg/kg - S.C.). Six hours after the second simultaneous injection of T_3 and protriptyline all animals perished, convulsing prior to death (Table 4).

These results (Tables 3 and 4) agree with those of other investigators, and suggest that T_3 pretreatment enhances the toxicity of many centrally acting drugs (Carrier and Buday, 1961; Conney and Garren, 1961; Prange and Lipton, 1962; Coville and Telford, 1970; Park, et al., 1972; Breese, et al., 1974).

Animals surviving simultaneous administration of protriptyline (5 and 1 mg/kg) and T_3 (0.1 mg/kg), resulted in MAO activity levels which

TABLE 3 *In vivo* effects of protriptyline administration on hyperthyroid animals.[1]

Protriptyline dosage	Results[2]
10.0 mg/kg	death within 3 hours
5.0 mg/kg	death within 3 hours
1.0 mg/kg	death within 6 hours

[1]Hyperthyroid animals had received 7 consecutive injections of T_3 (1 mg/kg/day) for 7 days, prior to protriptyline administration

[2]N = 5 for each group

TABLE 4 In vivo toxic effects of simultaneous administration of triiodothyronine and protriptyline on the rat.

	Protriptyline dosage	T_3 dosage (mg/kg/day - S.C.)	Results[1]
Group 1	10.0	1.0	death, 2nd day within 6 hours of injections
Group 2	5.0	1.0	death, 2nd day within 6 hours of injections
Group 3	1.0	1.0	death, 3rd day within 6 hours of injections
Group 4	10.0	0.5	death, 3rd day within 6 hours of injections
Group 5[2]	5.0	0.5	death in 3 of 5 animals within 6 hours after last injections
Group 6[2]	1.0	0.5	death in 2 of 5 animals within 6 hours after last injections
Group 7[2]	10.0	0.1	death in 4 of 5 animals within 6 hours after last injections
Group 8[3]	5.0	0.1	all animals survived 7 simultaneous injections
Group 9[3]	1.0	0.1	all animals survived 7 simultaneous injections

[1] N = 5 for each group of animals

[2] Groups 5, 6 and 7 MAO activity levels were extremely erratic when measured in the respective brain regions

[3] Groups 8 and 9 MAO activity levels were again erratic and inconsistent

were not reproducible, between animals of the same group, and were inconsistent when one group was compared to another group.

Our thyroidectomized (Tx) and sham-operated animals underwent surgery at seven weeks of age and eight weeks prior to sacrifice. During the eight week time interval, total body weight recordings of animal growth were maintained. During this interval Tx animals gained approximately 30 $\pm$ 8 grams; paired controls (sham-operated) gained 150 to 200 grams. This observation, that Tx animals exhibit deficient growth patterns, has been previously documented (Fleischmann, 1947; Barker, 1949; Eayrs and Taylor, 1951).

The data obtained for MAO activity levels in the three brain regions we examined are presented in Table 5. As illustrated in Table 5 the Tx group clearly exhibits a significant reduction in MAO activity levels regardless of substrate employed or brain region examined, when these values are compared to age-matched sham-operated controls.

Since Tx decreased MAO activity in the brain regions we examined, our next group of experiments investigated possible changes in MAO activity in both Tx and sham-operated animals following protriptyline administration (10 mg/kg/day-S.C.). Animals were administered protriptyline acutely (one injection) and subchronically (3 or 6 consecutive daily injections, S.C.). Animals were sacrificed at varying time intervals following the last administration of protriptyline. These results are presented in the Appendix section (Tables 11, 12 and 13).

TABLE 5 The effect of thyroidectomy on MAO activity in three brain regions

Substrate	Brain Region	Tx	Sham	
Tryptamine	Corpus Striatum	1.90 ± 0.14[1,2]	2.77 ± .19	$p < .01$
Tryptamine	Forebrain	2.34 ± 0.14	3.81 ± 0.36	$p < .01$
Tryptamine	Hypothalamus	1.68 ± 0.08	3.25 ± 0.40	$p < .02$
Serotonin	Corpus Striatum	0.33 ± 0.03	0.57 ± 0.05	$p < .01$
Serotonin	Forebrain	0.43 ± 0.04	0.73 ± .07	$p < .01$
Serotonin	Hypothalamus	0.24 ± 0.2	0.63 ± .09	$p < .01$

[1]Mean ± S.E.M., nmoles 5-HIAA or IAA formed/hr./ug DNA

[2]N = 12 or more animals for each pt.

Statistically significant differences between groups have been indicated; however, MAO activity levels are inconsistent, and no pattern for these changes in MAO activities exist.

2. Norepinephrine levels.

Prior to determining tissue levels of NE, NE concentrations were determined in known standard samples using fluorometric techniques. A standard curve was established and has been illustrated in the Appendix section (Figure 24). Following the determination of the NE external standard curve, labelled NE (Dl-H^3-norepinephrine) was added to

experimental samples to serve as an internal standard for our alumina column recovery. These data have been presented in the Appendix section (Figure 25).

Norepinephrine levels have been quantitatively determined in the hypothalamus of T_3 pretreated animals, Tx and sham-operated controls. These values are illustrated in Figure 1. Seven days of S.C. T_3 administration (1 mg/kg/day) significantly reduced the NE levels in hypothalamus ($p < 0.01$). On the other hand, neither Tx nor sham-operated control animals displayed significantly altered NE levels in hypothalamus. Numerical values for these data as well as for corpus striatum and forebrain are presented in the Appendix section (Table 38).

3. Norepinephrine turnover.

The rate and extent of depletion of NE after administration of alpha-methyl-para-tyrosine (MT) (Spector, et al., 1965; Brodie, et al., 1966; Spector, et al., 1967; Weiner, 1974; Ostman-Smith, 1979), has been studied as a measure for assessing NE turnover in control animals (saline administered), T_3 injected (1 mg/kg/day-7 days), Tx (7 weeks of age, 8 weeks prior to sacrifice), and in sham-operated Tx controls.

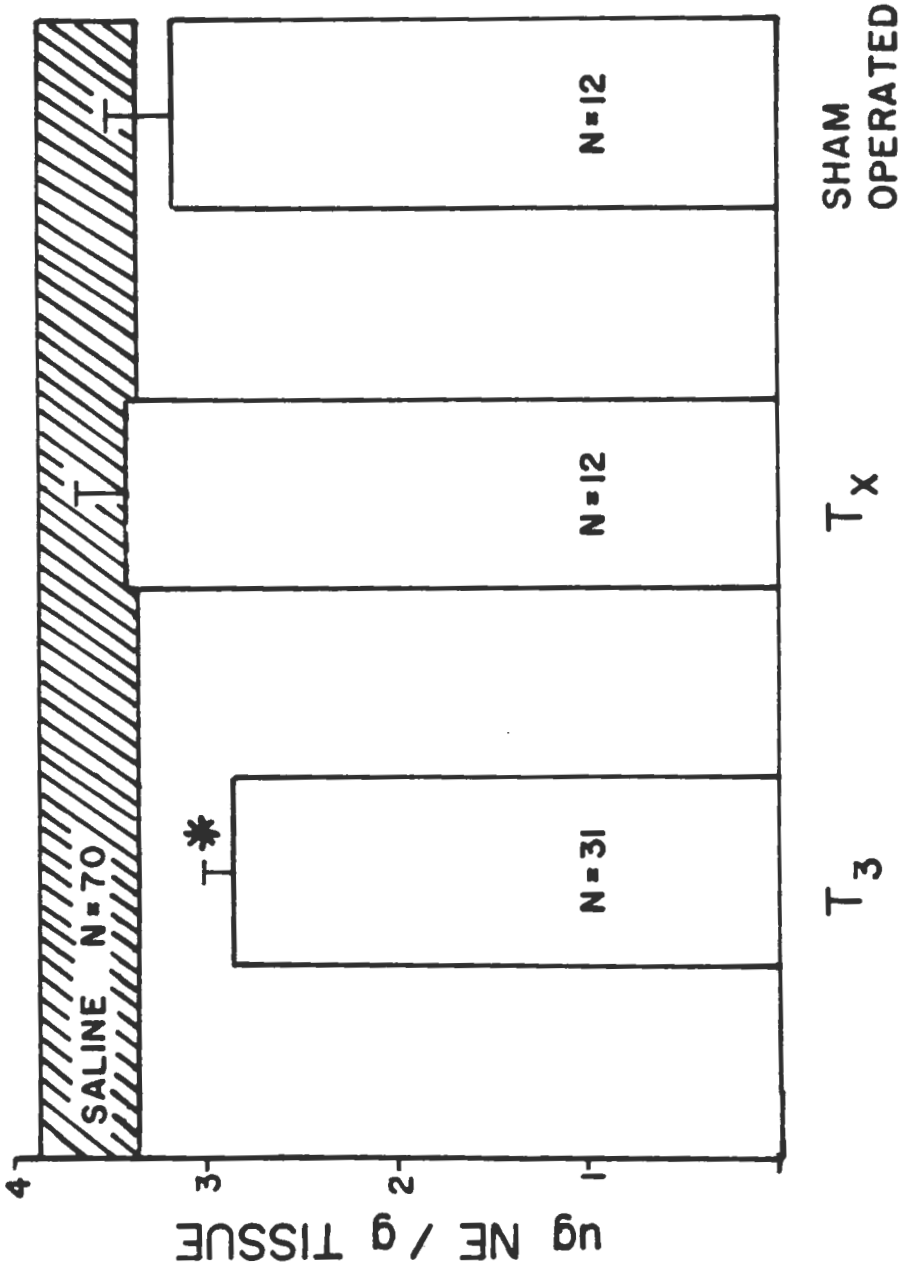
Prior to assessing NE turnover with MT a dose-response curve for tyrosine hydroxylase inhibition was conducted. Literature values (Brodie, et al., 1966; Spector, et al., 1967) for effective tyrosine hydroxylase (TH) inhibition indicate that 200 mg/kg MT (IP), is necessary with an additional booster administration every two hours to maintain inhibition. However, a single 400 mg/kg injection of MT will inhibit TH for 3.5 hours. The results of our dose-response curve are represented

FIGURE 1

TITLE: The effect of thyroidectomy and of administration of triiodothyronine on norepinephrine levels in the hypothalamus

LEGEND

Norepinephrine concentration per gram of tissue wet weight was measured in the hypothalamus in animals made hyperthyroid by 7 days of administration of triiodothyronine (1 mg/kg seven daily injections, S.C.), in animals thyroidectomized at 7 weeks of age, 8 weeks prior to measurement, and in sham operated controls, in comparison to saline injected controls. The cross-hatched band illustrates the mean $\pm$ S.E. for saline injected controls (n = 70). Vertical bars indicated the mean, S.E. and number of animals in each experimental group. The result for the T_3 treated group (*) is significantly less than control ($p < .01$, t-test).


ug NE / g TISSUE
4
3
2
1
SALINE N=70
*
N=31
N=12
N=12
T_3
T_X
SHAM OPERATED

in Figure 2. The numerical values for this plot can be found in the Appendix section (Table 39). Figure 2 illustrates NE levels in the hypothalamus 3 hours after a MT administration of 200, 400, 600 and 800 mg/kg - I.P.. We chose to use the 400 mg/kg dose in NE turnover studies. A similar MT dose-response curve was performed for T_3 administered animals (1 mg/kg/day - 7 days). Triiodothyronine administration increases tyrosine hydroxylase activity (Jacoby, 1975; Strombom, et al., 1977; Kennedy, et al., 1977), and this study was conducted to insure complete tyrosine hydroxylase inhibition at the 400 mg/kg dose. All data are presented in the Appendix section (Table 40) for corpus striatum, forebrain and hypothalamus. This dose was found to be effective, fully inhibiting tyrosine hydroxylase in pretreated hyperthyroid animals. No MT dose-response curve was performed for Tx animals.

Figure 3 reports NE depletion, when 400 mg/kg MT was administered to control animals that were sacrificed at varying time points after MT administration. The numerical data for each point, slope and regression coefficient in this plot, as well as for corpus striatum and forebrain (which are not presented graphically) can be found in the Appendix section (Table 41).

Figure 4 reports the results obtained for NE levels in hypothalamus after MT has been administered to hyperthyroid animals (1 mg/kg/day - 7 consecutive days). Numerical values for each point in this plot, as well as for corpus striatum and forebrain are listed in the appendix section (Table 42).

FIGURE 2

TITLE: NE levels in hypothalamus following MT administration: A dose-response curve.

LEGEND

NE levels were determined in hypothalamic tissue of the rat three hours after the administration of various doses of MT (200, 400, 600 and 800 mg/kg - I.P.). Each point represents the MEAN $\pm$ S.E. for three or more determinations. There was no statistically significant difference in NE levels in hypothalamic tissue following the administration of 400, 600 or 800 mg/kg MT.

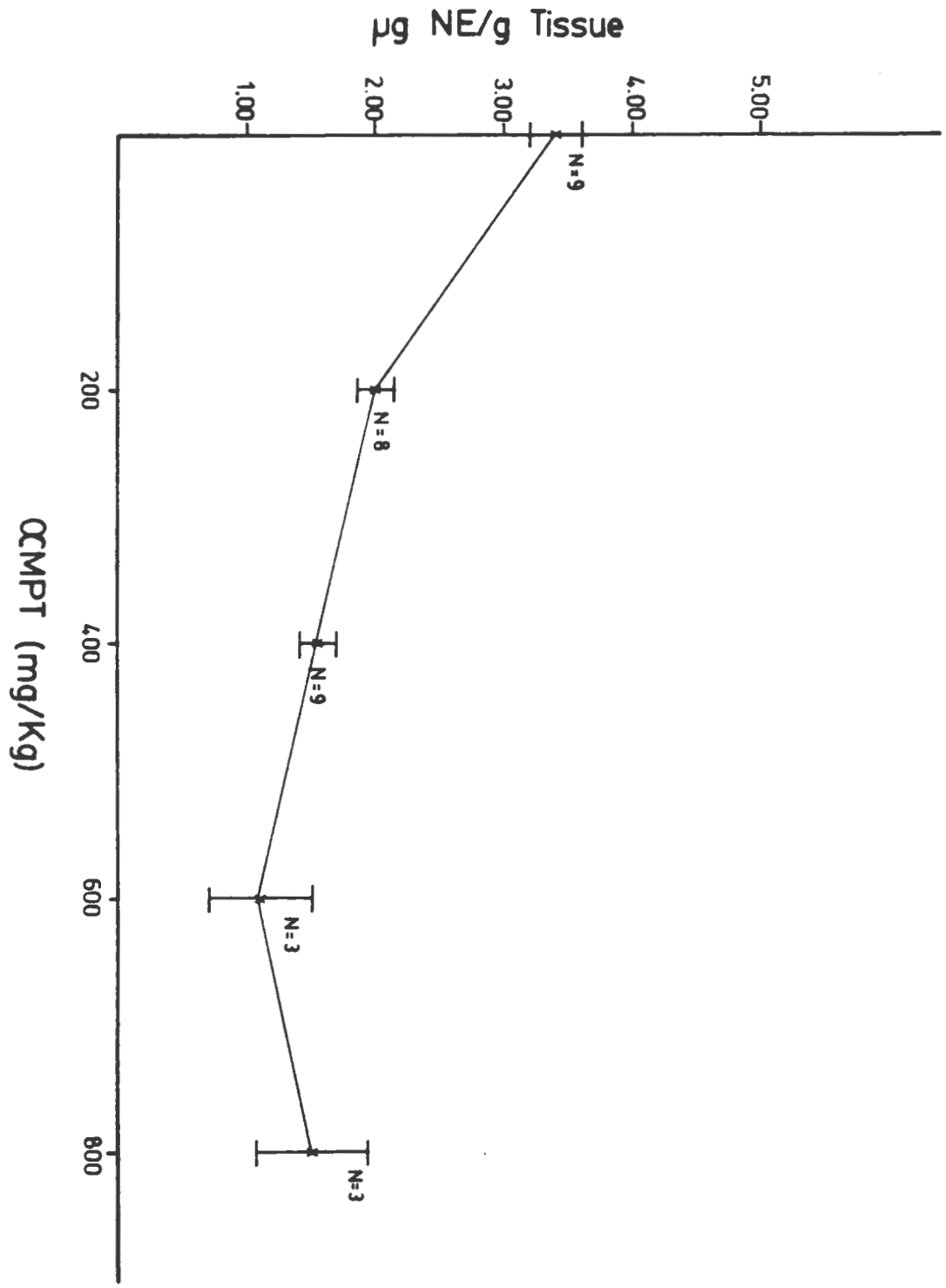

µg NE/g Tissue
1.00
2.00
3.00
4.00
5.00
N=9
N=8
N=9
N=3
N=3
200
400
600
800
αMPT (mg/Kg)

FIGURE 3

TITLE: NE depletion in hypothalamic tissue in control animals following administration of MT (400 mg/kg - I.P.).

LEGEND

NE levels have been determined in control animals at different time intervals following the administration of MT (400 mg/kg - I.P.). Each point represents the MEAN $\pm$ S.E. for five or more determinations. NE has been maximally depleted 4.5 hours after MT administration at the dosage of MT.

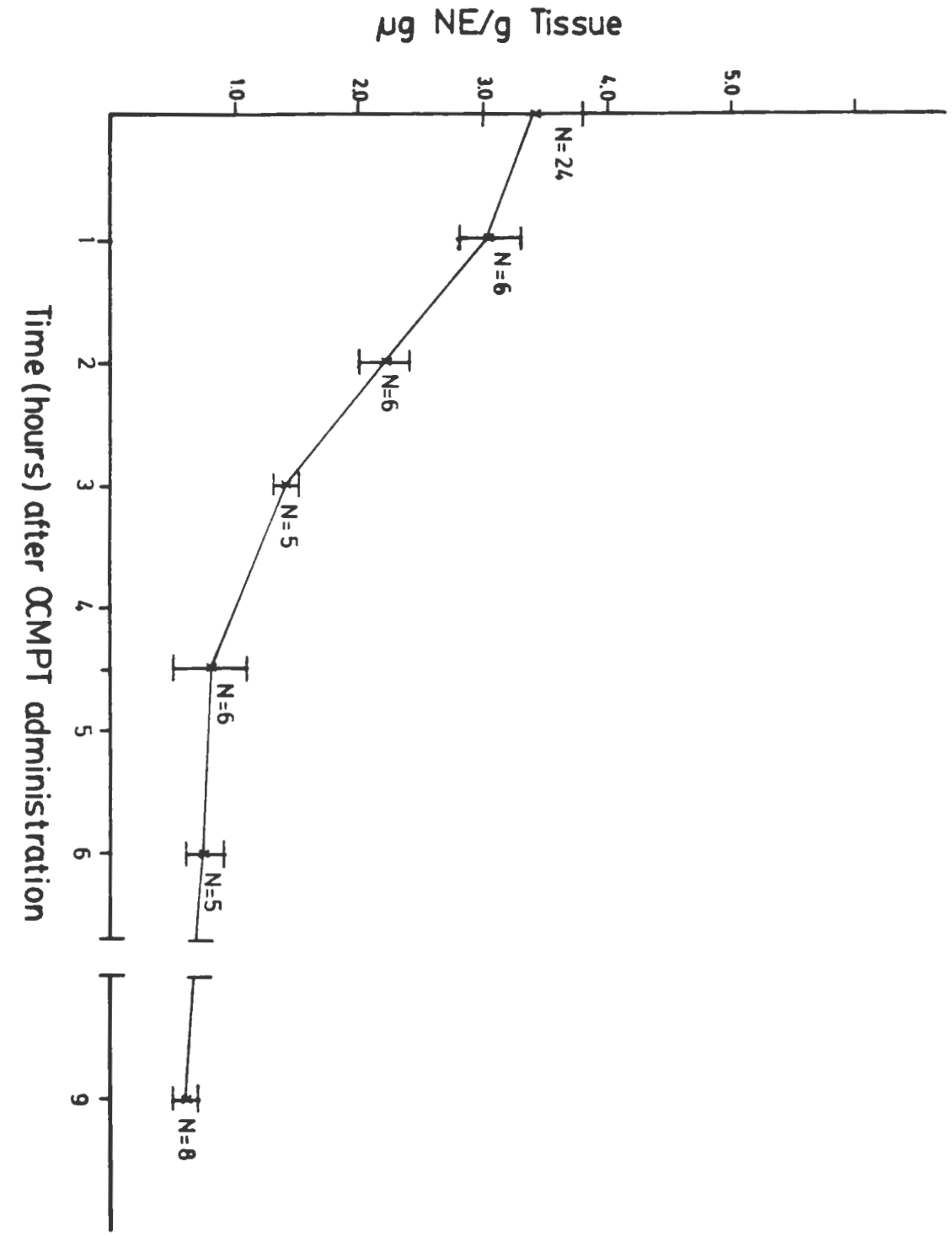

µg NE/g Tissue
1.0
2.0
3.0
4.0
5.0
N=24
N=6
N=6
N=5
N=6
N=5
N=8
1
2
3
4
5
6
9
Time (hours) after αMPT administration

FIGURE 4

TITLE: Turnover of norepinephrine in the hypothalamus of rats treated with triiodothyronine.

LEGEND

Animals were sacrificed 0, 1, 2, 3, 4, 5 or 6 hours after administration of a-MT (400 mg/kg, I.P.) and norepinephrine levels were measured in the hypothalamus. Each point represents the mean and standard error for a group of five animals except the zero time point which is derived from a group of 30 animals. Triiodothyronine-treated animals (-x-) were injected daily for seven days (1 mg/kg, S.C.).

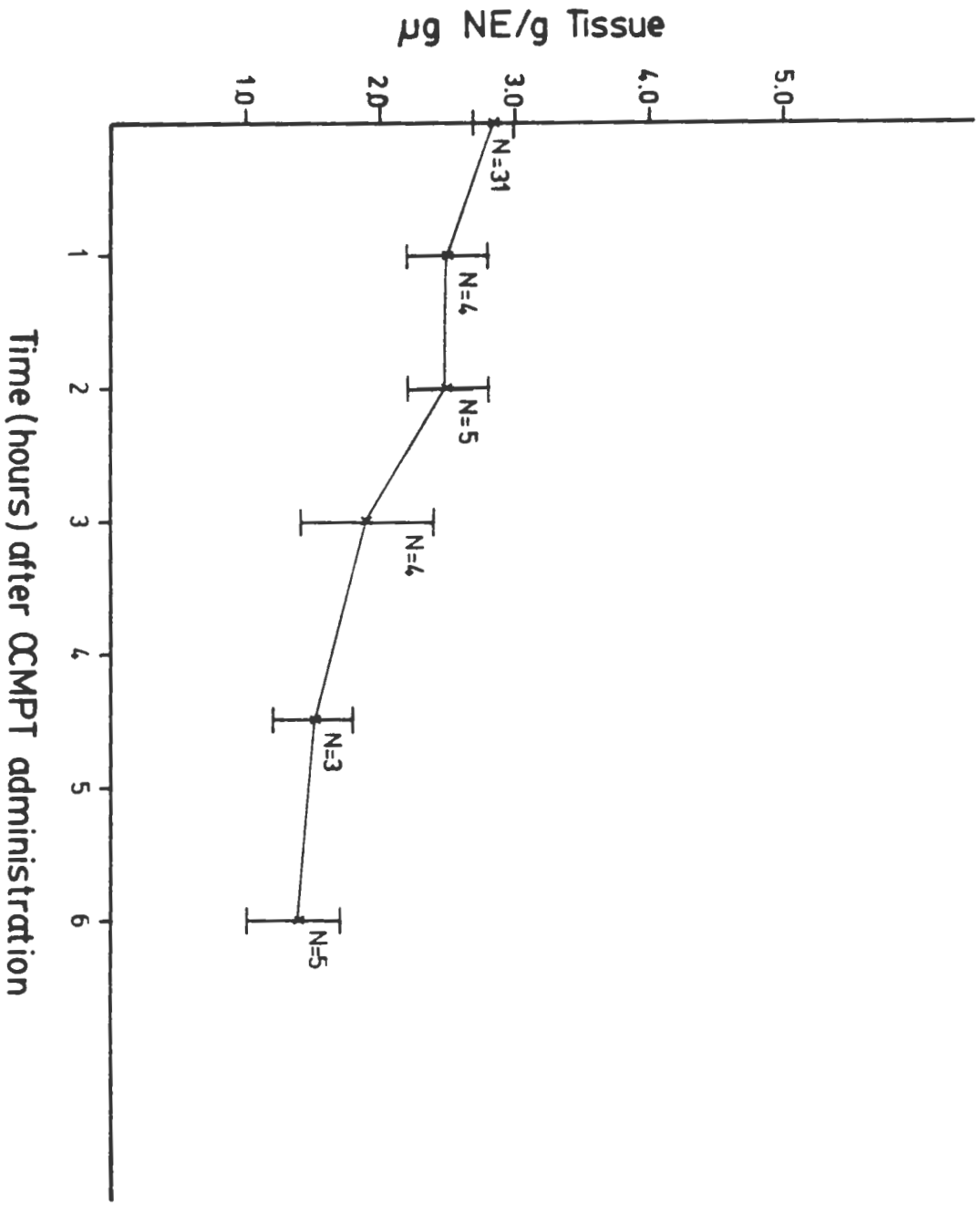

µg NE/g Tissue
1.0
2.0
3.0
4.0
5.0
N=31
N=4
N=5
N=4
N=3
N=5
1
2
3
4
5
6
Time (hours) after OCMPT administration

Figure 5 depicts the data from Figures 3 and 4 when these values are plotted as % initial concentration of NE.

The results of NE depletion following administration of MT (400 mg/kg - I.P.) in thyroidectomized (Tx) animals and age-matched, sham-operated controls are illustrated in Figure 6. Eight weeks after Tx, NE turnover is increased; however, repletion has also occurred more quickly. Numerical data for these points, as well as for corpus striatum and forebrain are listed in the Appendix section (Tables 43 and 44).

D. DISCUSSION

Our second hypothesis was that simultaneous administration of thyroid hormone potentiates the action of TCA drugs through an effect on MAO activity, NE turnover and/or NE cellular levels. Our data indicate that T_3 administration (1 mg/kg/day - 7 days) produces no inhibition of MAO activity *in vivo*, although MAO inhibition was observed *in vitro* in these brain tissues. Similar results have been reported by other investigators for rat heart and kidney (Callingham and Lyles, 1974; Lyles and Callingham, 1976; Asaad and Clark, 1978). Triiodothyronine administration produced a significant decrease ($p < 0.01$) in NE levels and NE depletion (turnover) in these brain regions when compared to saline administered animals.

Thyroidectomy, on the other hand, was associated with a decrease in MAO activity in these tissues. NE levels in these tissues were unchanged, however, an increase in both depletion and repletion rates was observed following MT administration.

FIGURE 5

TITLE: NE depletion in hypothalamus as a measure of % initial concentration three hours after MT administration (400 mg/kg - I.P.).

LEGEND

In control animals (-0-) NE has been depleted 58% three hours after MT administration. In hyperthyroid animals (-x-) NE has been depleted 35% three hours after MT administration (a 23% difference in NE turnover rate). A t-test for depletion rate resulted in a statistically significant difference between these two slopes ($p < 0.01$).

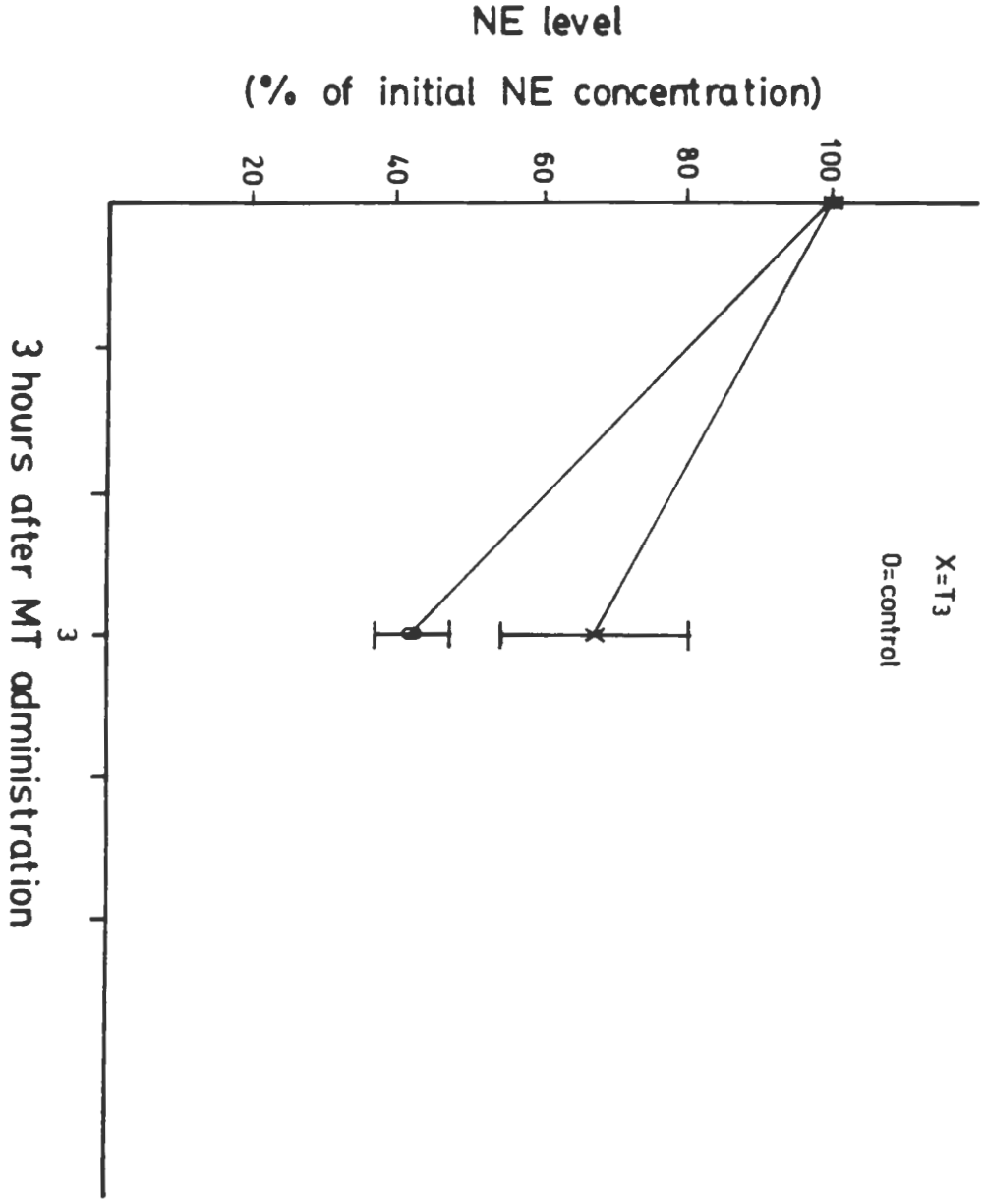
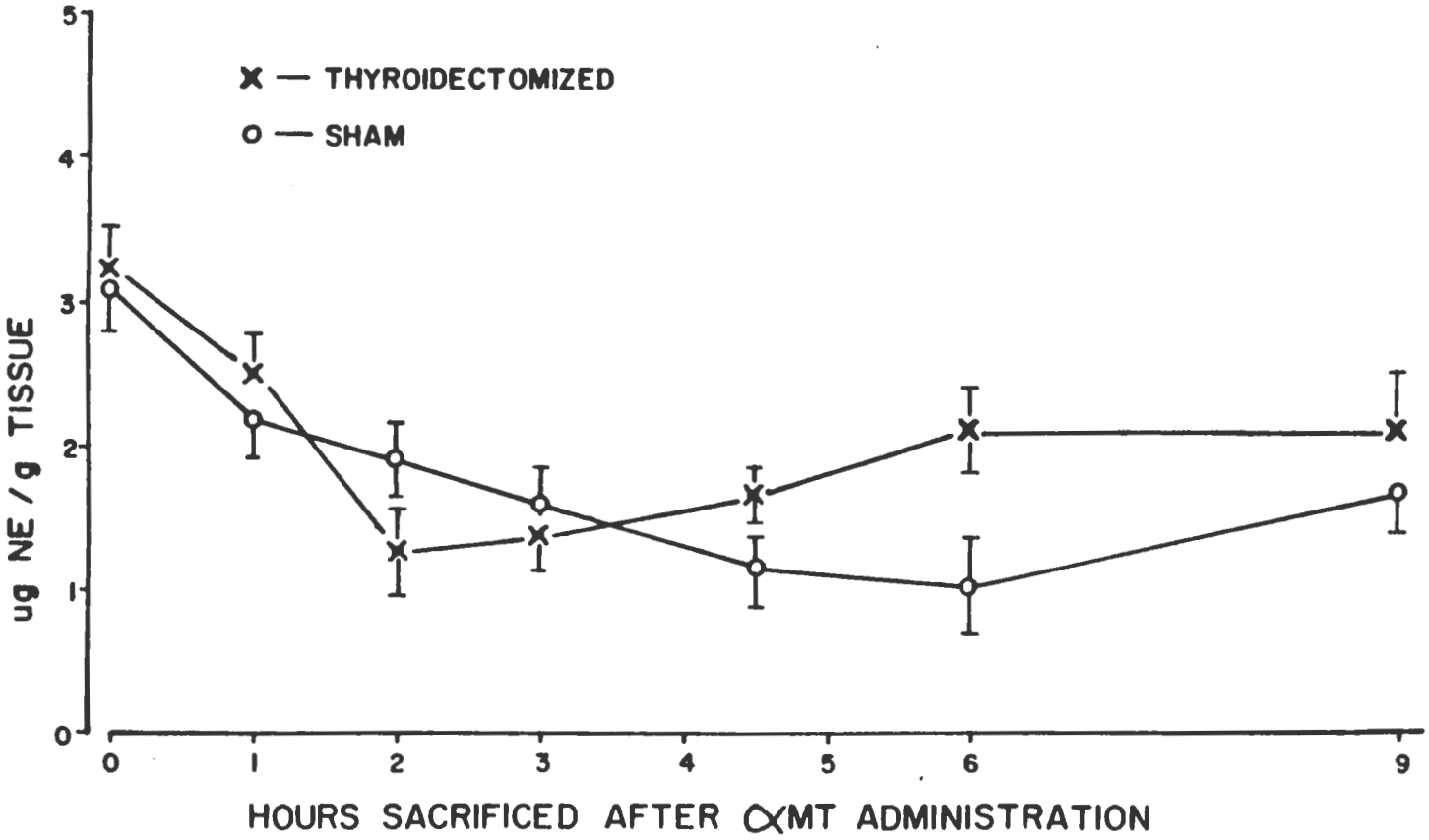

NE level
(% of initial NE concentration)
20
40
60
80
100
3 hours after MT administration
3
X=T3
O=control

FIGURE 6

TITLE: Turnover of norepinephrine in the hypothalamus of thyroidectomized rats.

LEGEND

Animals were sacrificed 0, 1, 2, 3, 4, 5, 6 or 9 hours after administration of a-MT (400 mg/kg, I.P.) and norepinephrine levels were measured in the hypothalamus. Thyroidectomized animals (-x-) and sham operated controls (-o-) were compared. Each point represents the mean and standard error for a group of six animals. The values for the thyroidectomized groups at 2, 4, 5 and 6 hours differ significantly from controls ($p < .05$ t-test). Regression analysis (t-test) of the slopes between 0 and 2 hours indicated a significant difference ($p < .05$).


X — THYROIDECTOMIZED
O — SHAM
ug NE / g TISSUE
0
1
2
3
4
5
0
1
2
3
4
5
6
9
HOURS SACRIFICED AFTER αMT ADMINISTRATION

Also it was observed that when rats are made hyperthyroid by T_3 administration and are then administered a single injection of protriptyline, the animals begin to convulse and die within six hours. Similar toxic results have been viewed by other researchers when employing imipramine (Prange and Lipton, 1962; Breese, et al., 1974). This observation remains unresolved. A possible suggestion for increased toxicity may be due to an increased interaction between T_3 and increased receptor sensitivity in heart tissue to catecholamines (Bax, et al., 1980; Chang and Kunos, 1981), thus accounting for the observed convulsions prior to death.

Reviewing our results, any interaction between thyroid hormone (T_3) and MAO activity in these brain regions is inconclusive, except for significantly decreased MAO levels following thyroidectomy. Zile, (1960), has reported that T_3 administration does not effect whole brain MAO activity; we are in agreement with these results. MAO activity in peripheral tissues (heart, liver and kidney) following T_3 administration however, has been shown to increase (Callingham and Lyles, 1974; Lyles and Callingham, 1974; Asaad and Clark, 1978), indicating organ specificity.

We feel our results indicate that T_3 does not effect brain MAO activity, and any alterations in MAO activity are non-specific. Thus, any interaction between the tricyclics and thyroid hormone is not related to MAO.

NE levels and turnover following T_3 administration or after thyroidectomy indicate a decrease in turnover in hyperthyroid rats

in the brain regions we examined. These results are in agreement with Prange, et al., (1970). We observed decreased NE levels in the brain areas of hyperthyroid animals and these results are in agreement with Engstrom (1974). Landsberg and Axelrod (1968) presented data in heart tissue which is in agreement with our data for brain tissue, that following thyroidectomy there is increased NE turnover. From these results it appears that our observations define situations in which adjustments in the parameters of functional norepinephrine activity occur contrary to what would be predicted by the NE hypothesis for affective disorders. We view these contrary changes in neurotransmitter parameters as adjustments that are compensatory in nature. Possibly a fundamental interaction occurs between thyroid hormone and brain NE mechanisms, which leads to an enhanced noradrenergic tone, predicted by the NE hypothesis for affective disorders; and that as a result of this yet-to-be-identified interaction, a compensatory decrease in NE turnover and NE levels result. Conversely, in thyroidectomized animals, the absence of this thyroid-hormone-induced facilitation of NE activity likewise triggers compensatory adjustments, i.e., decreased MAO activity and increased turnover.

CHAPTER IV.

C H A P T E R IV.

A. NOREPINEPHRINE: Norepinephrine and its functional relationship with the presynaptic receptor.

1. Norepinephrine levels and turnover.

The catecholamines (norepinephrine, epinephrine and dopamine) are low molecular weight substances that contain a catechol nucleus and an amine group. Norepinephrine (NE), one of these catecholamines, is synthesized and secreted by mamalian nerve tissue and serves an important function both in neural and endocrine integration.

The amino acid tyrosine (the substrate precursor to NE) is normally present in the circulation in levels between 10-15 mg/l (Spector, et al., 1963). Tyrosine is taken up from the bloodstream and concentrated within the brain and other neural tissues via an active transport mechanism (Chirigos, et al., 1960). Once inside the nerve cell ending or chromaffin cell, tyrosine undergoes a series of biochemical enzymatic transformations that ultimately lead to NE synthesis.

Norepinephrine is known by several different names: noradrenaline, levarternol and DL-arterenol all of which are trivial names for 3, 4-dihydroxy-phenyl-ethanolamine (Himms-Hagen, 1967).

Knowledge of catecholamine synthesis began in the late 1930's with the discovery by Holtz (1939) of decarboxylase and by Blaschko's (1939) hypothetical but correct, biosynthetic scheme starting from the dietary substrate tyrosine.

Norepinephrine is primarily stored in a bound form in nerve cells within chromaffin granules or dense-core vesicles (Kirshner, 1974).

This bound catecholamine further interacts with adenosine triphosphate (ATP) and forms a tetracatecholamine-ATP complex. This salt is further bound to soluble proteins, the chromgranins, which are located within the storage particle. The inability of labelled exosenuous catecholamines to enter these storage granules suggests that the endogenous ATP-amine complexes are still further combined with macromolecular components within the granules and form a still further stable complex (Weiner, 1970).

Results also suggest that NE is not located in a single pool, but that only a small percentage of neuronal NE is necessary for normal neural function (Glowinski, et al., 1966; Sulser and Sanders-Bush, 1970). NE thus exists in a large 'storage pool' and a much smaller 'functional pool' both of which are located within the nerve cell axoplasm.

Currently it is suggested that the functional pool contains newly synthesized NE, while the storage pool contains catecholamine which has been in the nerve cell for longer periods of time. This assumption is based upon the observation that newly synthesized NE is preferentially released during enhanced neural firing. Kopin, et al. (1968) has demonstrated that newly synthesized NE is preferentially released during stimulation of isolated cat spleen. In vivo studies have reported that utilization of newly synthesized NE is increased in response to certain stressful conditions; whereas, utilization of NE stored for longer time intervals was not affected (Thierry, et al. 1971; Glowinski, et al., 1972).

Evidence also exists to suggest that in addition to containing newly synthesized NE, the functional pool also contains NE which has been accumulated via the re-uptake process. Therefore, both newly synthesized NE as well as conserved NE are preferentially utilized through release in response to immediate stressful conditions (Potter, et al., 1962; Chidsey and Harrison, 1963).

The localization of NE in cell bodies, axons, and axon terminals is not random, but regulated by a specific set of chemical laws, which are still porrly understood. NE is present mainly in vesicles in nerve tissue. These vesicles are distributed along the axon and at the nerve endings. NE in these vesicles is in a state of equilibrium with unbound NE in the cellular fluids of the cytoplasm. Cytoplasmic NE is available for destruction by MAO and for limited leakage or diffusion out of the cell.

NE released presynaptically by stimulation is primarily transported back into the presynaptic neuron (80%) and is then once again bound in these presynaptic vesicles. This prevents degradation by MAO and preserves intracellular NE (Kopin, 1966; Titus and Dengler, 1966).

The re-uptake of NE by active transport and binding into the presynaptic vesicles protects NE from depletion and destruction by MAO. MAO, therefore plays an important role in the regulation of the "free" intracellular levels of NE. Very small quanta of the presynaptically released NE reaches the post-synaptic target tissue. Synaptic NE may be O-methylated and excreted, or can be excreted "free" or conjugated as glucuronides or sulfonates (Kopin, 1964; Axelrod, 1966).

Norepinephrine which has leaked from storage vesicles into the axoplasm is rapidly metabolized to physiologically inactive products (Kopin, 1964; Axelrod, 1966). Enzymatic destruction of NE by MAO appears to serve primarily as a means for the degradation of NE within the cell, thus limiting the concentration of free NE in the inactive cytoplasmic compartment and also controlling NE synthesis and the level of bound NE. Complete intraneuronal deamination of NE and other catecholamines by MAO readily occurs if uptake and storage into synaptic vesicles is blocked (i.e. reserpine, or cocaine administration) (Burn and Rand, 1968; Weiner, 1970).

Catechol-O-methyl-transferase (COMT), another enzyme responsible for the degradation of catecholamines, is apparently unimportant in the metabolization of intraneuronal NE. COMT is significantly involved in the catabolism of circulating catecholamines, which occurs chiefly in the liver and kidney.

The third hypothesis this research attempts to investigate relates changes in cellular NE levels and turnover to acute subchronic and chronic tricyclic administration, and these changes parallel their delayed clinical efficacy. As stated earlier the "catecholamine hypothesis for affective disorders" proposes that some, if not all depressions are associated with an absolute or relative deficiency of catecholamines, particularly NE, at the functionally important adrenergic receptor sites in the brain. Consequently, studies have shown that drugs which cause depletion and inactivation of NE in brain produce sedation or depression, whereas, drugs which increase or potentiate brain NE are

associated with behavioral stimulation or excitation and generally produce or exert an antidepressant effect in man.

Because a lag-time is evident between tricyclic administration and clinical efficacy (Schildkraut, et al., 1970; Schildkraut, et al., 1971; Sulser and Sanders-Bush, 1971; Leonard, 1975; Leonard and Kafoe, 1976; and Berger, 1978), this aspect of this study will examine possible changes in intracellular NE and also turnover rates and attempts to observe if these changes parallel the time course for clinical efficacy for this class of antidepressant agents.

Because brain NE levels are maintained constant in the face of conditions which alter nerve firing rate, it has been suggested that NE which is utilized during enhanced neuronal firing is replaced by an increase in NE synthesis (Weiner, 1970; Costa and Meek, 1974; Bjorklund, et al., 1976; and Ostman-Smith, 1979). This compensatory mechanism has been termed "the theory of steady-state kinetics."

The mechanism by which increased noradrenergic nerve activity results in the stimulation of increased NE synthesis is not known. The most concrete explanation however, is that nerve stimulation releases NE from a nerve terminal, which consequently results in decreased intra-neuronal NE concentrations. Since NE competetively inhibits its own synthesis by interfering with the pteridine cofactor required by tyrosine hydroxylase (Udenfriend, et al., 1965; Gordon, et al., 1966; Kennedy, et al., 1977), nerve stimulation would result in a decrease in negative feedback inhibition of tyrosine hydroxylase. The converse also applies

(Spector, et al., 1965; Spector, 1966; Spector, et al., 1967; and Weiner, 1970). It is therefore appropriate for one to conclude that NE synthesis is controlled by a small, chemically undetectable pool of intraneuronal cytoplasmic NE.

The term "turnover" refers to a process of renewal of a substance in an organ. The concept of turnover implies that the substance being renewed exists at some steady-state level, which is balanced by identical rates of influx and efflux. In the case of brain NE the rate of NE influx equals the rate of NE syntehsis, since endogenous NE cannot enter the brain via the circulation due to the blood-brain barrier (Dobbing, 1961; Guroff and Udenfriend, 1962; Bertler, et al., 1966; and Oldendorf, 1974). The rate of efflux is determined by release of NE into the circulation and resultant metabolic destruction by MAO or COMT. Assuming that NE levels are maintained at some steady-state value, the NE influx rate (synthesis rate) would equal the NE efflux rate. Then this rate, which may be estimated by any method which can measure NE turnover, would give the investigator an indication of the rate at which brain NE is synthesized and utilized, which then, may be used as an indicator of central noradrenergic activity.

Many methods for measuring NE turnover exist and have been reviewed extensively by others (Costa and Neff, 1968; Costa, 1970; Costa and Neff, 1970; and Weiner, 1974). The method employed here is a measurement of the rate of decline of endogenous brain NE after inhibition of tyrosine hydroxylase by alpha-methyl-para-tyrosine. This method was first described by Brodie, et al., (1966).

Synthesis inhibition is accomplished by employing alpha-methyl-para-tyrosine, (MT), an in vivo reversible inhibitor of tyrosine hydroxylase (TH), the rate-limiting step in NE synthesis (Spector, et al., 1965; and Spector, 1968). After blockade of NE synthesis, brain NE levels decline in a monoexponential fashion (Brodie, et al., 1966), i.e. the concentration of NE declines at a rate that is proportional to the NE concentration remaining in the neuronal cytoplasm at any given time. This phenomena may be viewed as "the rate of NE utilization," and at the steady-state, "the rate of synthesis," which is proportional to cytoplasmic NE concentrations.

Therefore it was imperative that we investigate the noradrenergic effects caused by tricyclic administration during acute, subchronic and chronic treatment schedules, and thus possibly identify biochemical changes in NE levels and turnover rates. These data may then add support in identifying noradrenergic mechanisms necessary for latent clinical efficacy.

2. Presynaptic autoreceptor modulation of noradrenergic mechanisms.

Before introducing the concept "presynaptic alpha-receptors" (Norberg and Hamerger, 1964; Langer, 1974; Berthelsen and Pettinger, 1977; Malbon, 1979; U'Prichard and Synder, 1979), it is first necessary to examine the processes involved in NE release from presynaptic noradrenergic nerve terminals. NE is synthesized from the amino acid tyrosine, and is stored in vesicles in the neuroplasm. These vesicles in turn migrate to the nerve cell membrane, and release transmitter when an action potential arrives. It has also been demonstrated that an

influx of calcium into the neuroplasm causes these NE containing vesicles to migrate to the neuronal membrane (Rubin, 1970; Blaustein, et al., 1972; Phillis, 1974). The mechanism involved in this migration is still unclear. At the membrane, fusion occurs, and these vesicles discharge their contents into the synaptic cleft. These vesicles then re-form after discharge and are again filled with transmitter from the neuroplasm.

Brown and Gillespie (1957) first suggested that this release may be modulated by another mechanism, in addition to the arrival of an action potential. They also noted that the alpha-blocking agent phenoxybenzamine increased the overflow of NE in cat spleen caused by repeated nerve stimulation.

Other studies conducted by Starke, et al., (1971); and Enero, et al., (1972) using other alpha-receptor blocking agents also caused an increase in overflow of transmitter following repeated nerve stimulation. These observations led to the hypothesis that alpha-receptors are present in the outer surface of adr[illegible]nembranes of neurons and are involved in the regulation of NE release via a negative feedback mechanism. Thus, as NE is released into the synaptic cleft, it tends to limit its own release, by stimulating a presynaptic inhibitory alpha-receptor (Kirpekar and Puig, 1971; Starke, 1972; Starke and Schumann, 1972; Rochette, et al., 1976; Lorenz, et al., 1979). This phenomenon occurs in peripheral tissues as well as in the CNS (Bunney and Aghajanian, 1975; Carlsson, 1975; Roth, et al., 1975; Strombom, 1975; Langer and Dubocovich, 1977; Baraban and Aghajanian, 1980). When presynaptic alpha-receptors are stimulated by an appropriate agonist or blocked by an

appropriate antagonist (oxymetazoline, phenoxybenzamine, or phentolamine) (Kapur and Mottram, 1978; Kalsner and Chan, 1979; Wemer, et al., 1979; Baraban and Aghajanian, 1980) the negative feedback mechanism inhibiting or facilitating NE release is altered. The sequential release of NE resulting in transmitter overflow thus occurs "or" is prevented. It also appears that the presynaptic alpha-receptors modulate the release of NE by controlling the influx of calcium into the presynaptic nerve ending (Langer, et al., 1975). In summary, the presynaptic alpha-receptor mediates NE release, which is dependent upon synaptic neurotransmitter concentrations, and a functionally operative negative feedback mechanism. Activation of presynaptic alpha-receptors (autoreceptors) leads to a decrease in transmitter release, while blockade of these alpha-receptors results in an increase of NE release following nerve stimulation (overflow) (Langer, 1974; Langer, 1977; Starke, et al., 1977).

The physiological and biochemical events reulting from the activation or inactivation of alpha-receptors in the CNS remains unclear. Some evidence (Segal, et al., 1975; Greengard, et al., 1976; Skolnick and Daly, 1977; Kanof and Greengard, 1978; Hall and Ogren, 1981) in turn activates cyclic-AMP generating systems. Other studies (Glaubiger and Lefkowitz, 1977; Williams and Lefkowitz, 1977) indicate that the number of CNS alpha-receptors can be altered in a variety of brain areas by chronic treatment with specific drugs and hormones, which in turn results in altered CNS mechanisms. Still other studies suggest that drugs and hormones may sensitize or desensitize these pre- and post-

synaptic alpha-receptors (Comsa, 1950; Thibault, 1956; Potter, et al., 1962; Haggendal and Lindqvist, 1964; Lee, et al., 1967; Lipton, et al., 1968; Prange, et al., 1970).

It was our intent in this study to examine the effects of the alpha-receptor antagonist (yohimbine) on NE levels and turnover following chronic tricyclic administration.

Several studies have indicated that chronic administration of tricyclics cause changes in NE levels and NE turnover in brain and other tissues (Schildkraut, et al., 1970; Schildkraut, et al., 1971; Roffler-Tarlov, et al., 1973; Leonard and Kafoe, 1976; Anden and Grabowska, 1977; Frazier, et al., 1978; Svensson and Usdin, 1978). Since chronic tricyclic treatment is necessary for therapeutic results, it has been suggested that a slow developing adaptive process at the level of the autoreceptor may be occurring (Vetulani, et al., 1976; Crews and Smith, 1978; Sulser, et al., 1978; Bergstrom and Kellar, 1979; Collis and Shepherd, 1980).

We felt that if receptor adaptation occurs with chronic tricyclic treatment, employing yohimbine would allow us the opportunity to observe these changes in both NE levels and turnover rate. These results would then allow us to specifically test whether adaptive changes in presynaptic receptors might be an important component of the mechanism of chronic tricyclic action.

B. METHODS AND PROCEDURES

Initial experiments involving NE levels and turnover were performed in three areas of rat brain (corpus striatum, forebrain, and hypothalamus)

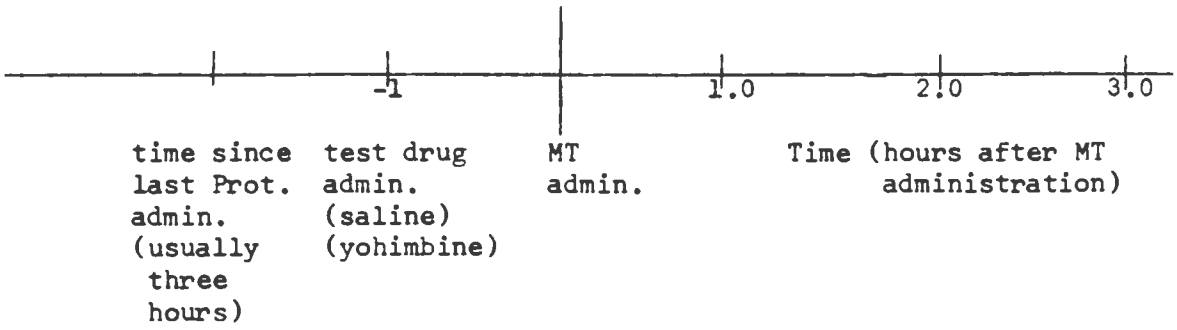
in control animals (saline injected), protriptyline administered (10 mg/kg/day for 1, 3, 6, and 18 days) and in thyroidectomized animals which also received protriptyline acutely, subchronically and chronically.

With the conclusion of these studies, the examination of both corpus striatum and forebrain was discontinued, since the primary neurotransmitter in the corpus striatum is dopamine (Hillarp, et al., 1966; Snyder and Coyle, 1969; Iversen, 1973; Hornykiewicz, 1973; Harris and Baldessarini, 1973), and 5-hydroxytryptamine (serotonin) for forebrain (Schwark and Keesey, 1975; Saavedra, et al., 1976; Bjorklund, et al., 1976). It was felt that further examination of these tissues would be unnecessary, and unrelated to the effects of chronic protriptyline administration on norepinephrine turnover. All studies involving yohimbine are performed only with hypothalamic tissue preparations. We chose to examine only the hypothalamus in our receptor-interaction studies because we considered the hypothalamus an important target tissue for the actions of protriptyline. This area of the brain is responsible for many neuroendocrine transmitter interactions (Stokes, et al., 1981) as well as regulating or modulating both physiological and emotional responses (Kordon and Glowinski, 1970; Dewhurst, et al., 1968; Kobayashi, et al., 1974, de la Fuente, 1979).

Yohimbine our presynaptic autoreceptor antagonist was obtained from Sigma Chemical Company, St. Louis, Missouri.

Our experimental protocol for drug administration involving our norepinephrine turnover studies is complicated and has been diagrammed

below for better understanding. This procedure allowed us to examine norepinephrine turnover when protriptyline and or our test drug, yohimbine, were at maximal plasma levels and optimally effective.



C. RESULTS

1. Protriptyline, NE levels and turnover.

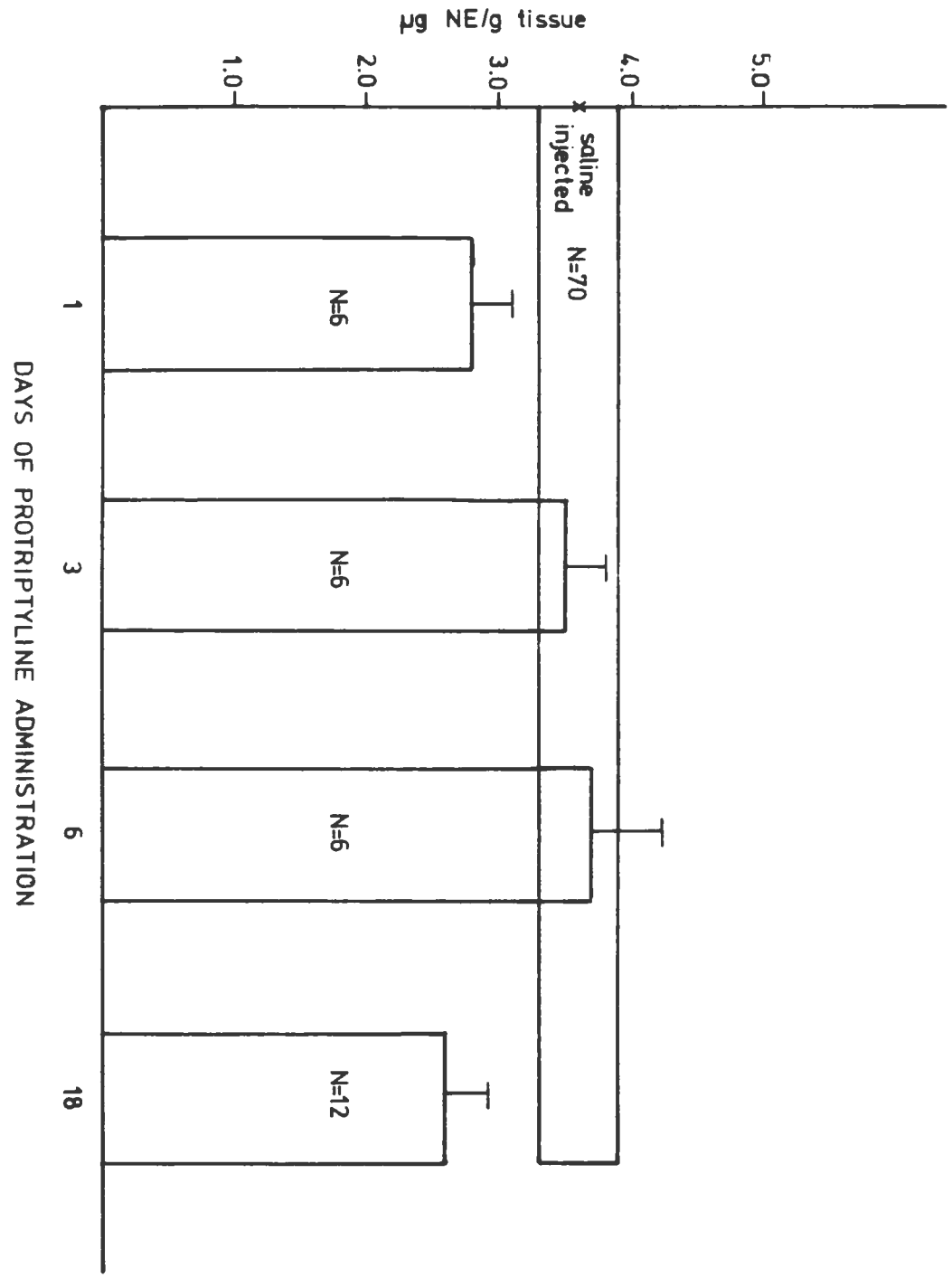
NE levels were determined in corpus striatum, forebrain and hypothalamus following 1, 3, 6 and 18 days of protriptyline administration (10 mg/kg/day - S.C.). The data obtained for these determinations are presented in Figure 7. The numerical values for NE determinations for corpus striatum and forebrain are presented in the appendix section (Tables 45 and 46). A statistically significant difference from control animals was recorded in NE levels in the hypothalamus when protriptyline treated animals were sacrificed 3 hours after the last test drug injection in both the acute and chronically administered protriptyline animals ($p < 0.01$). Chronic protriptyline administration also resulted in a statistically significant difference in NE levels in corpus striatum ($p < 0.01$). In the hypothalamus a 26% decrease in NE levels was recorded following acute drug administration and a corresponding 30% decrease in

FIGURE 7

TITLE: Norepinephrine levels in hypothalamic tissue following acute, subchronic and chronic protriptyline administration

LEGEND

Animals were administered protriptyline 1, 3, 6 and 18 consecutive days (10 mg/kg/day - S.C.). All animals were sacrificed 3 hours after their final drug administration. Acute and chronic protriptyline administration caused a statistically significant decrease in NE levels in hypothalamic tissue ($p < 0.01$) when compared to saline administered controls.


µg NE/g tissue
1.0
2.0
3.0
4.0
5.0
x saline injected N=70
N=6
N=6
N=6
N=12
1
3
6
18
DAYS OF PROTRIPTYLINE ADMINISTRATION

NE levels was observed following chronic administration. Similar results have been reported by others examining whole brain preparations (Schildkraut, et al., 1970; Schildkraut, et al., 1971; Roffler-Tarlov, et al., 1973).

Figure 8 presents data for NE depletion in hypothalamic tissue following acute protriptyline administration (10 mg/kg - S.C.), and followed by MT administration (400 mg/kg - I.P.). MT was administered three hours after protriptyline. These animals were sacrificed at various times after MT administration. A t-test for comparison of depletion rates between saline administered (Figure 3) and acute protriptyline administration resulted in a statistically significant difference in turnover rate ($p < 0.005$). This result is consistent with Leonard and Kafoe (1976). Numerical values for these data are listed in the appendix section (Table 47).

Figure 9 presents data for NE depletion in hypothalamic tissue following subchronic (6 consecutive daily injections) protriptyline administration (10 mg/kg/day - S.C.). MT was administered 6 hours after the last protriptyline injection. Animals were sacrificed at various times after MT administration. A t-test for the comparison of depletion rates between saline administered and protriptyline administered animals resulted in a statistically significant difference in depletion rate between groups ($p < 0.01$). Numerical values for each time point are listed in the appendix section (Table 48).

FIGURE 8

TITLE: Norepinephrine depletion in hypothalamic tissue following acute protriptyline administration

LEGEND

Animals were administered MT three hours after an acute administration of protriptyline. Animals were sacrificed 0, 1, 2, 3, 4.5, and 6 hours after subsequent MT administration. Each point represents the MEAN $\pm$ S.E. for 3 or more experiments.

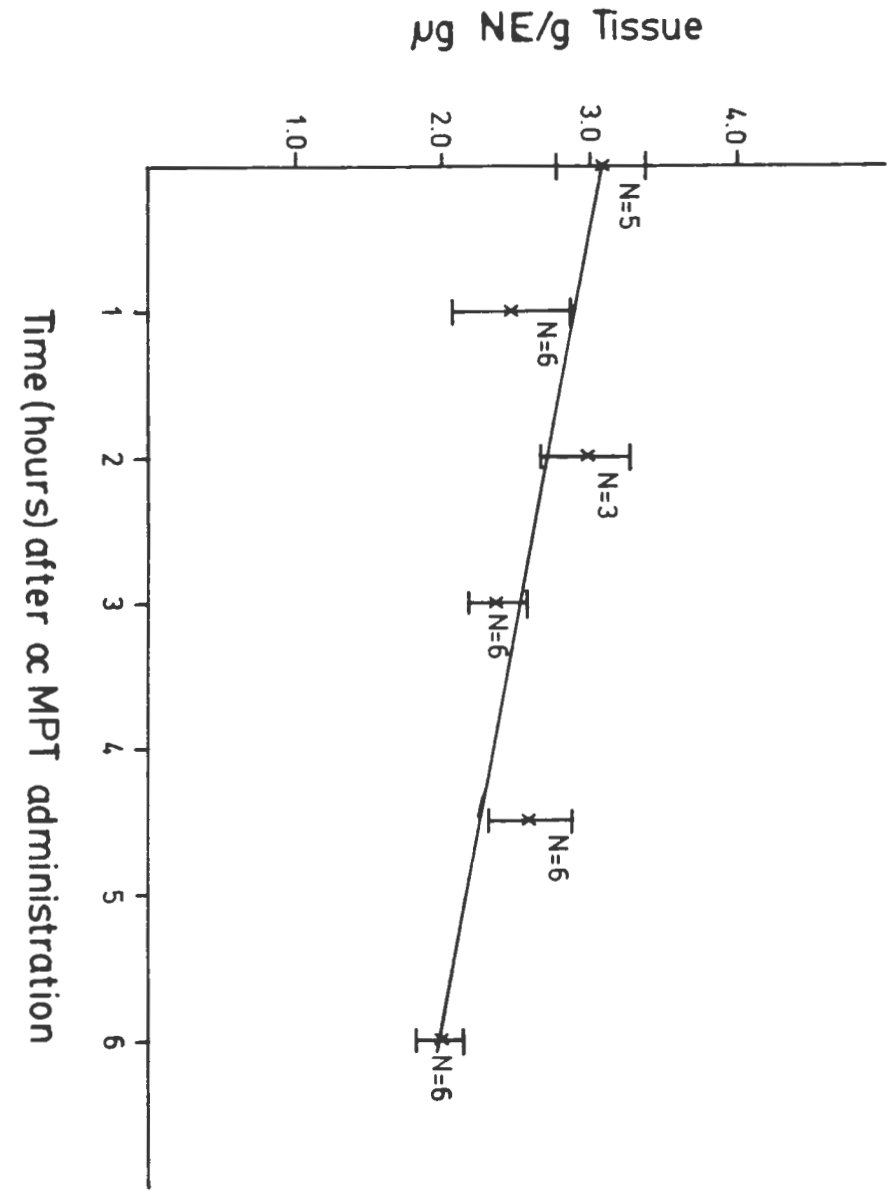
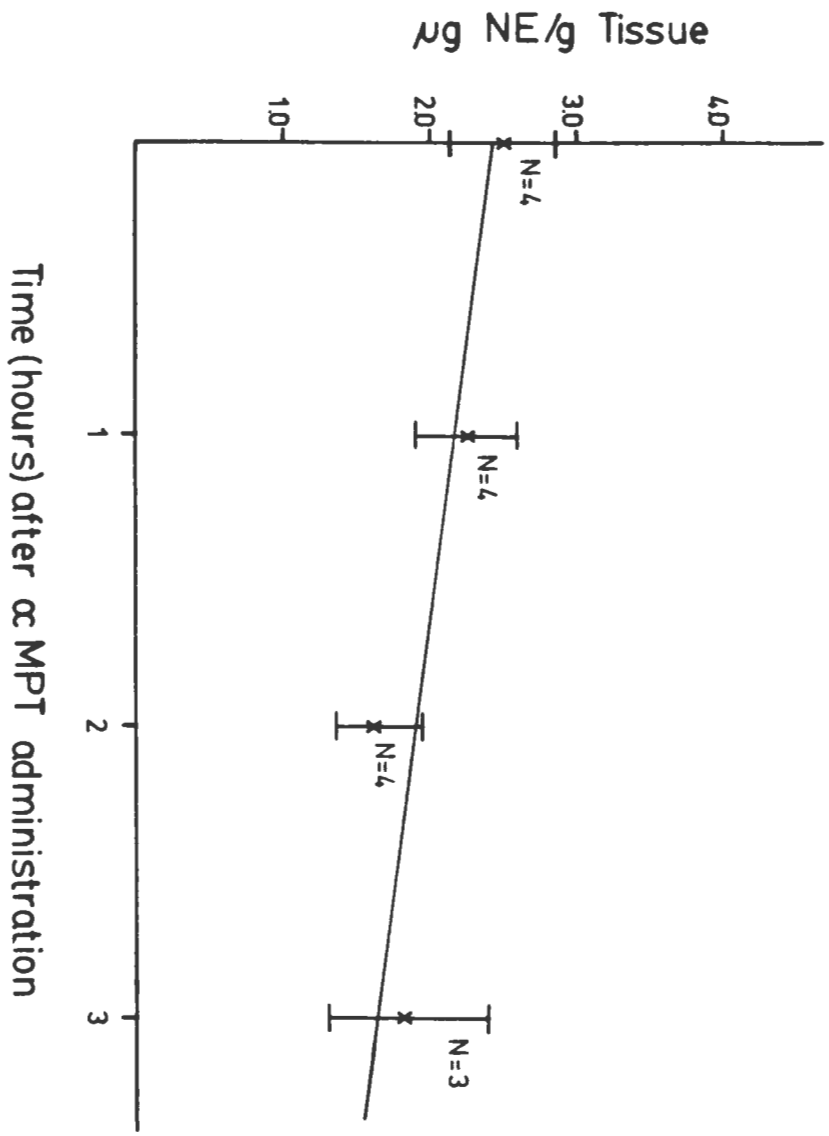

µg NE/g Tissue
1.0
2.0
3.0
4.0
N=5
N=6
N=3
N=6
N=6
N=6
1
2
3
4
5
6
Time (hours) after α MPT administration

FIGURE 9

TITLE: Norepinephrine depletion in hypothalamic tissue following subchronic protriptyline administration

LEGEND

Animals received six consecutive injections of protriptyline (10 mg/kg/day - S.C.). MT was administered six hours after the final protriptyline injection. Animals were sacrificed 0, 1, 2 and 3 hours after MT administration. All points represent the MEAN $\pm$ S.E. of at least 3 or more experiments.


μg NE/g Tissue
1.0
2.0
3.0
4.0
N=4
N=4
N=4
N=3
Time (hours) after α MPT administration
1
2
3

A MT dose-response curve was conducted on chronically treated protriptyline animals prior to examining NE turnover. Figure 10 presents our results for NE depletion in chronically treated protriptyline animals following the administration of various doses of MT (200, 400, 600 and 800 mg/kg). Numerical values for each data point in this figure are presented in the appendix section (Table 51). These data suggest that tyrosine hydroxylase (TH) is completely inhibited three hours after MT administration at any of the above doses. Thus, the 400 mg/kg dose was employed for our turnover studies in chronically administered animals.

Figure 11 represents NE depletion in hypothalamic tissue following 18 days of chronic protriptyline treatment. Numerical data for each time point are listed in the appendix section (Table 52).

Figure 12 is a composite of the data recorded in Figures 3 and 11. The decrease in NE depletion following chronic protriptyline administration becomes more evident. A t-test on the comparison of turnover rates between these two conditions between zero time after MT administration and three hours after MT administration indicate that a statistically significant difference exists between these two groups ($p < 0.01$). After the three hour time point values were not included in the depletion rate calculations.

When the data in Figure 12 are plotted as "percent of initial concentration of NE" (Figure 13), MT administration causes a 58% depletion in NE in saline administered animals when these animals are sacrificed three hours after MT administration. A 22% decrease in NE

FIGURE 10

TITLE: MT dose-response curve in animals administered protriptyline chronically

LEGEND

Animals received protriptyline chronically (10 mg/kg/day, 18 days) and were then administered various doses of MT three hours after the final protriptyline injection. Animals were sacrificed three hours after receiving MT (200, 400, 600 or 800 mg/kg - I.P.). Each data point represents the MEAN $\pm$ S.E. of at least 4 experimental determinations. No statistically significant difference in NE depletion was observed between any of the MT doses examined.

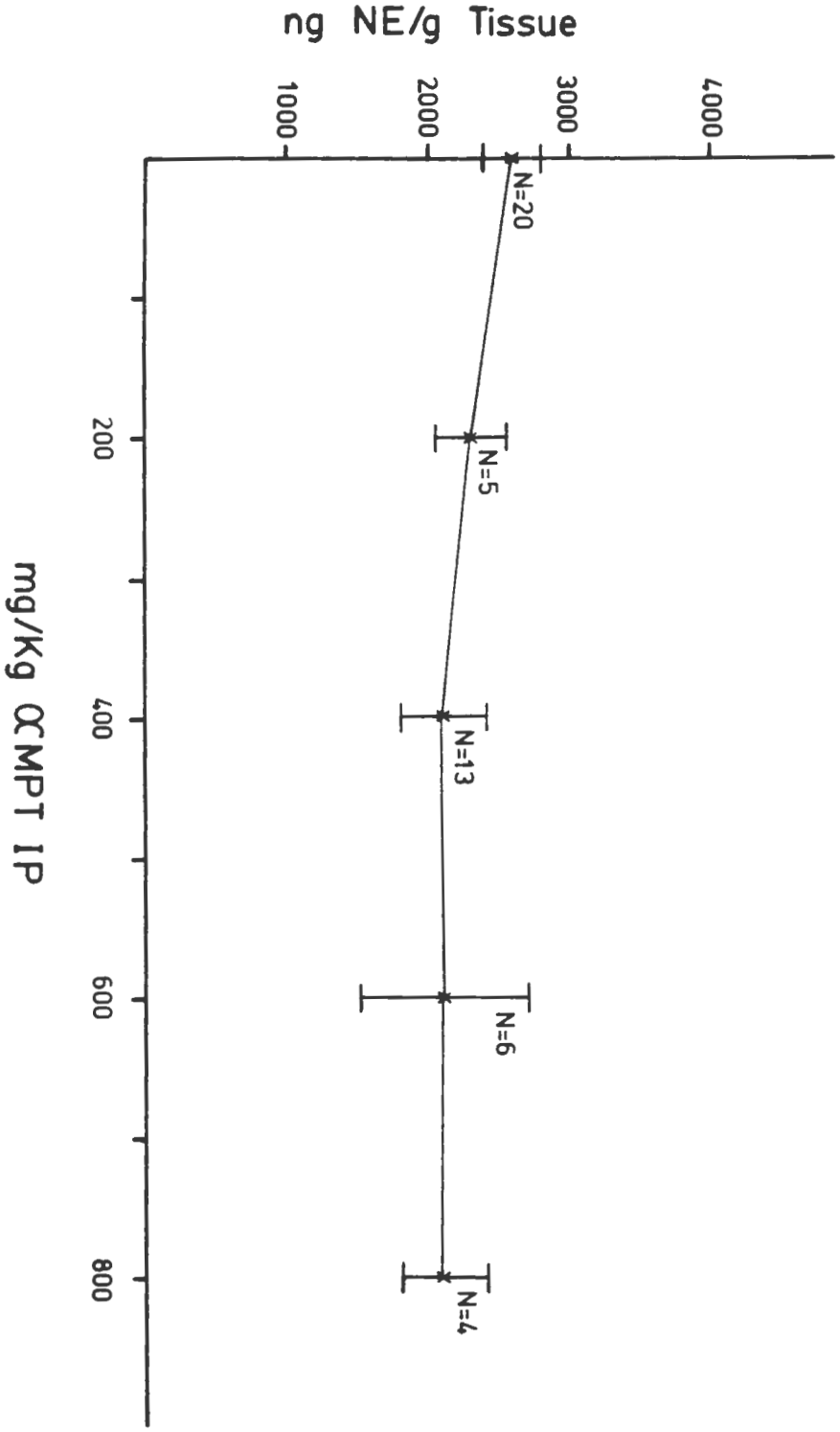

ng NE/g Tissue
1000
2000
3000
4000
N=20
N=5
N=13
N=6
N=4
200
400
600
800
mg/Kg OCMPT IP

FIGURE 11

TITLE: Norepinephrine depletion in hypothalamic tissue of animals receiving chronic protriptyline administration

LEGEND

Animals received protriptyline chronically (10 mg/kg/day, 18 days) and 3 hours after the final protriptyline injection, they were administered MT (400 mg/kg - I.P.). Animals were sacrificed at various times after MT administration (0, 1, 2, 3, 4.5, 6 and 9 hours). Each point represents the MEAN $\pm$ S.E. for at least 4 experimental determinations.

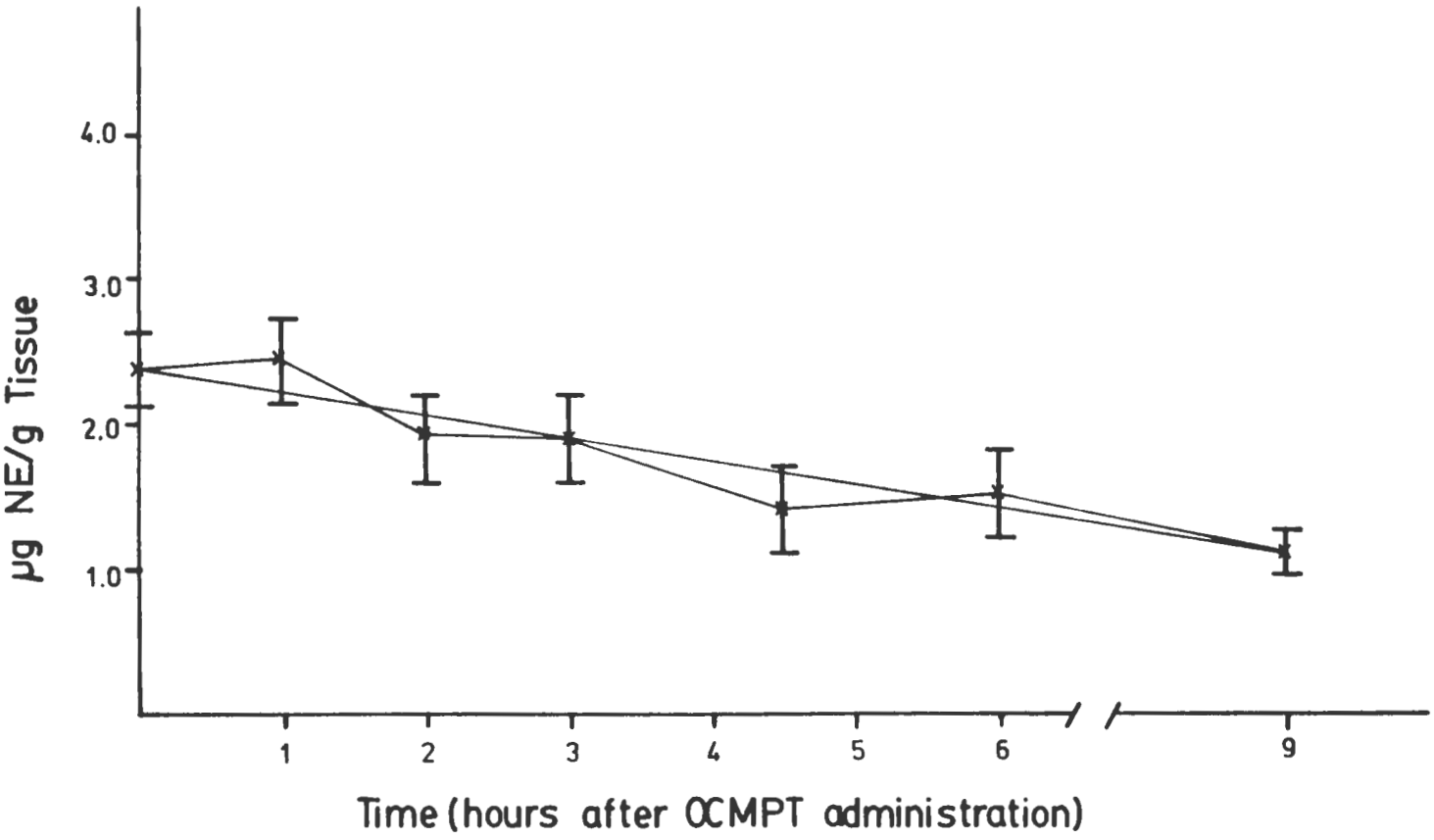
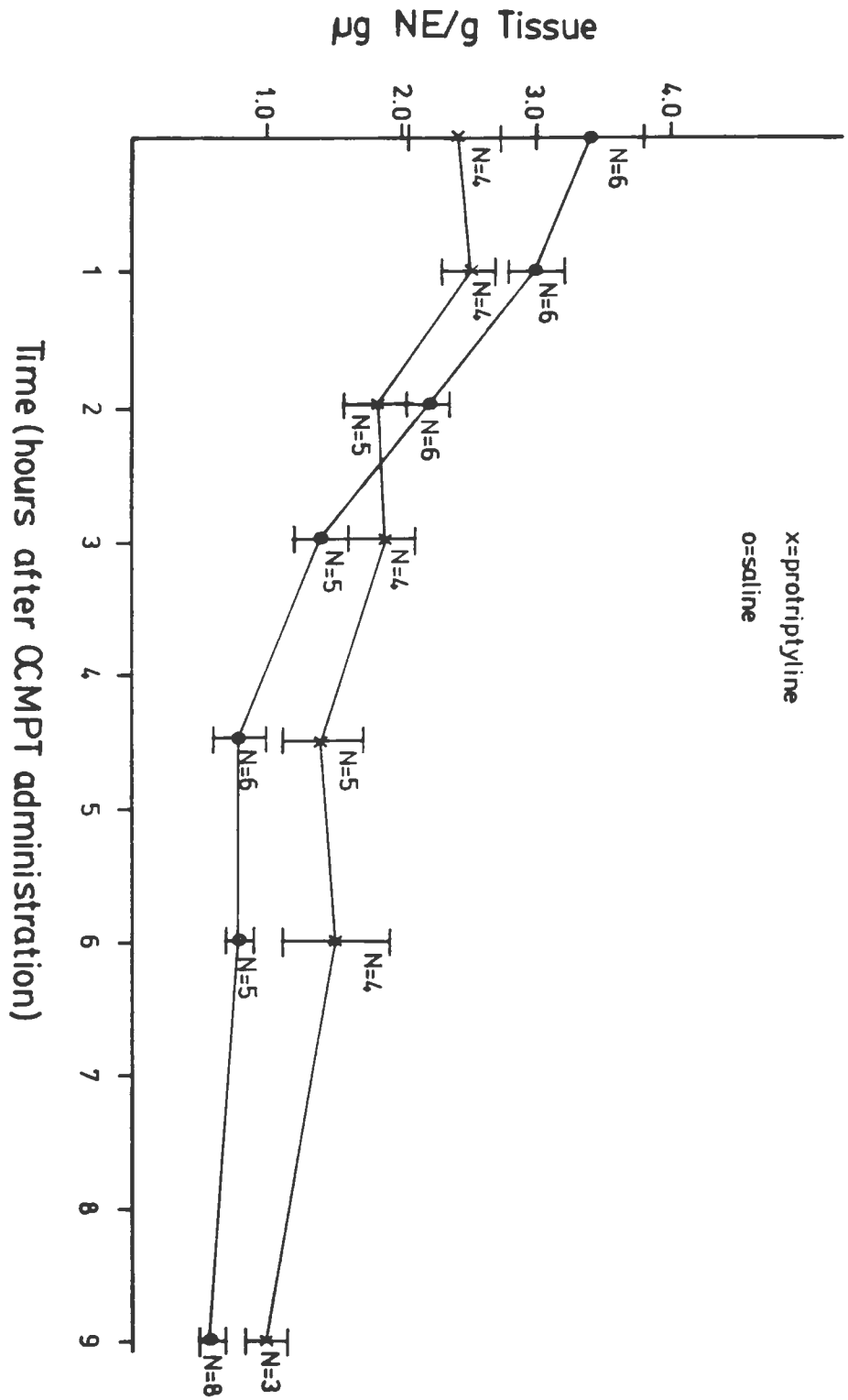

4.0
3.0
2.0
1.0
µg NE/g Tissue
1
2
3
4
5
6
9
Time (hours after OCMPT administration)

FIGURE 12

TITLE: A comparison of turnover rates in hypothalamic tissue in rats administered protriptyline or saline chronically

LEGEND

Animals were administered protriptyline (10 mg/kg/day, 18 days) (-x-) or saline (-0-). Three hours after the final injection of protriptyline or saline, animals were administered MT (400 mg/kg - I.P.). Animals were sacrificed at various times after MT administration (0, 1, 2, 3, 4.5, 6 and 9 hours). All values represent the MEAN $\pm$ S.E. for at least 3 experimental determinations. A t-test for comparison of depletion rates between 0 and 3 hours after MT administration indicates taht a statistically significant difference existed in depletion rate between these two groups ($p < 0.01$).


µg NE/g Tissue
4.0
3.0
2.0
1.0
x=protriptyline
o=saline
N=6
N=4
N=6
N=7
N=6
N=5
N=5
N=4
N=6
N=5
N=4
N=8
N=3
1
2
3
4
5
6
7
8
9
Time (hours after ∝MPT administration)

is observed in the hypothalamus when chronically treated protriptyline animals are sacrificed three hours after MT administration. A 36% difference in NE depletion is recorded between these treatment conditions.

Tables 6 and 7 list both the slope and regression coefficient for saline and protriptyline treated animals. Table 57 in the appendix section presents the statistical results when NE depletion was compared between these two groups.

Figure 14 is a turnover rate/recovery rate plot for the data presented in Tables 6 and 7. These data suggest that adaptations in NE turnover rate/recovery rate have resulted from chronic protriptyline administration.

A t-test for the comparison of turnover rate/recovery rate indicated that acute turnover rate/recovery rate is significantly different when compared to either subchronic or chronic turnover rate/recovery rate ($p < 0.001$). No statistically significant difference was observed when subchronic administration was compared to chronic administration for turnover rate/recovery rate.

2. Yohimbine studies and norepinephrine turnover.

Studies determining NE levels and turnover following yohimbine, protriptyline and MT administration were conducted. The first plot (Figure 15) employing yohimbine illustrates the effects of a single injection of yohimbine on NE levels in the hypothalamus of control animals. In Figure 15 yohimbine, using either a 2.0 mg/kg and 20.0 mg/kg dose, depletes hypothalamic tissue levels of NE. The 20. 0 mg/kg dose, however, depletes NE for a longer time interval. In Figure 15 a 56%

FIGURE 13

TITLE: NE depletion as a measure of % initial concentration in the hypothalamic tissue of chronically treated protriptyline or saline animals

LEGEND

Animals received protriptyline (10 mg/kg/day, 18 days) or saline, and were then administered MT 3 hours after the final protriptyline or saline injection. Animals were sacrificed three hours after MT administration. NE levels in protriptyline treated animals (-x-) was depleted 22% and in saline administered animals (-0-) a 58% depletion in NE level was recorded.

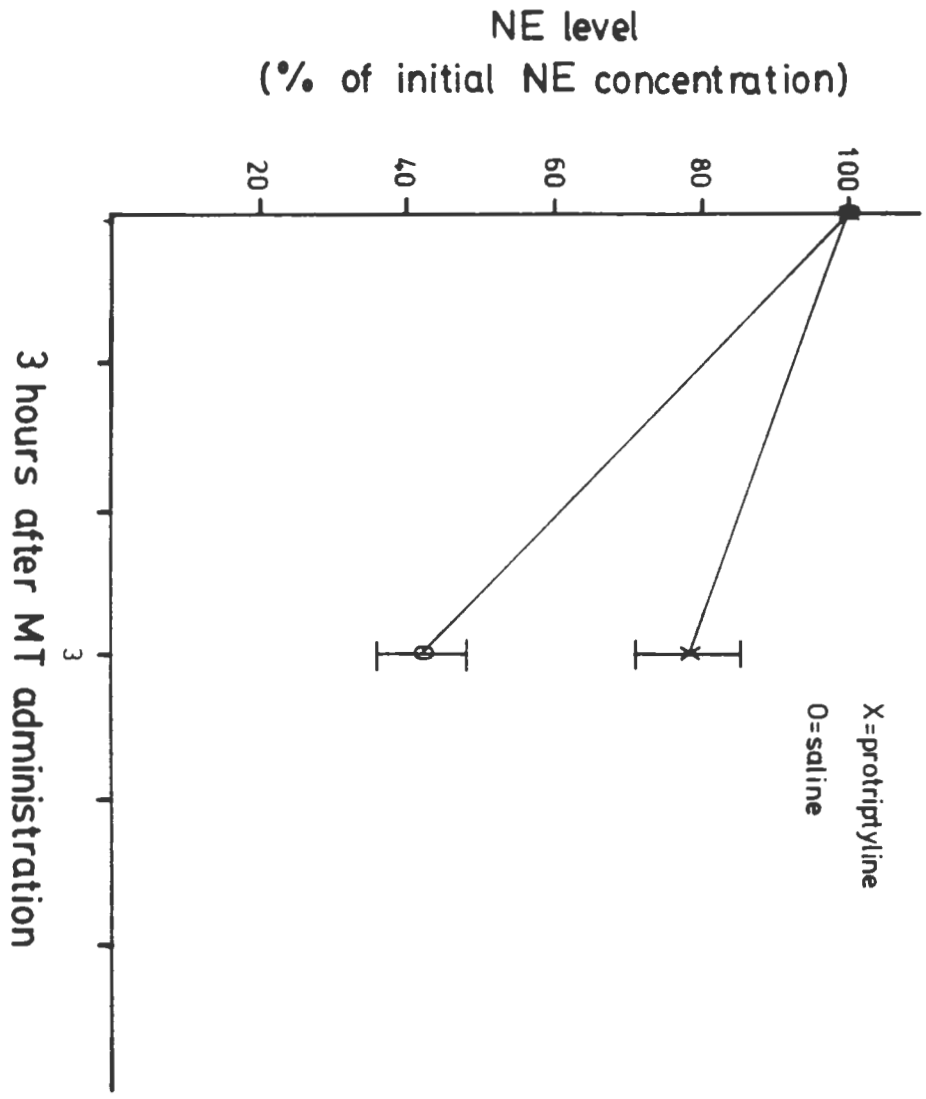

NE level
(% of initial NE concentration)
100
80
60
40
20
3
3 hours after MT administration
X=protriptyline
O=saline

TABLE 6 A comparison of slope and linear regression coefficients for saline treated animals following administration of MT (400 mg/kg - I.P.)

Saline	Slope*	(r^2)*
Acute (1 injection) hours after last prot. admin.		
3 hours	-0.6074	0.9825
6 hours	-0.6528	0.9575
Subchronic (6 injections) hours after last prot. admin.		
6 hours	-0.5212	0.9677
Chronic (18 injections) hours after last prot. admin.		
6 hours	-0.5912	0.9873
24 hours	-0.6543	0.9219
	MEAN -0.6054	
	S.D. 0.0546	
	S.E.M. 0.0244	

*These determinations were calculated from the MEAN and S.E. from the previous independent experiments

TABLE 7 A comparison of slope and linear regression coefficients for protriptyline treated animals following administration of MT (400 mg/kg - I.P.)

Protriptyline	Slope*	(r^2)*
Acute (1 injection) hours after last prot. admin.		
3 hours	-0.1475	0.6213
6 hours	-0.1336	0.8512
24 hours	-0.5241	0.9284
Subchronic (6 injections) hours after last prot. admin.		
2 hours	-0.2319	0.9100
6 hours	-0.2471	0.8449
24 hours	-0.3064	0.9313
Chronic (18 injections) hours after last prot. admin.		
3 hours	-0.1897	0.9331
6 hours	-0.2734	0.7809
24 hours	-0.2234	0.2790

*These determinations were calculated from the MEAN and S.E. from the previous independent experiments.

FIGURE 14

TITLE: Turnover rate/recovery rate for NE in rat hypothalamic tissue following acute, subchronic and chronic protriptyline administration

LEGEND

The turnover rate/recovery rate for NE in rat hypothalamic tissue following acute, subchronic and chronic protriptyline administration has been plotted and the slope for these 3 injection schedules has been determined. The acute turnover rate/recovery rate slope (-□-) is significantly different ($p < 0.001$) from either subchronic turnover rate/recovery rate (-x-) or chronic turnover rate/recovery rate (-o-).

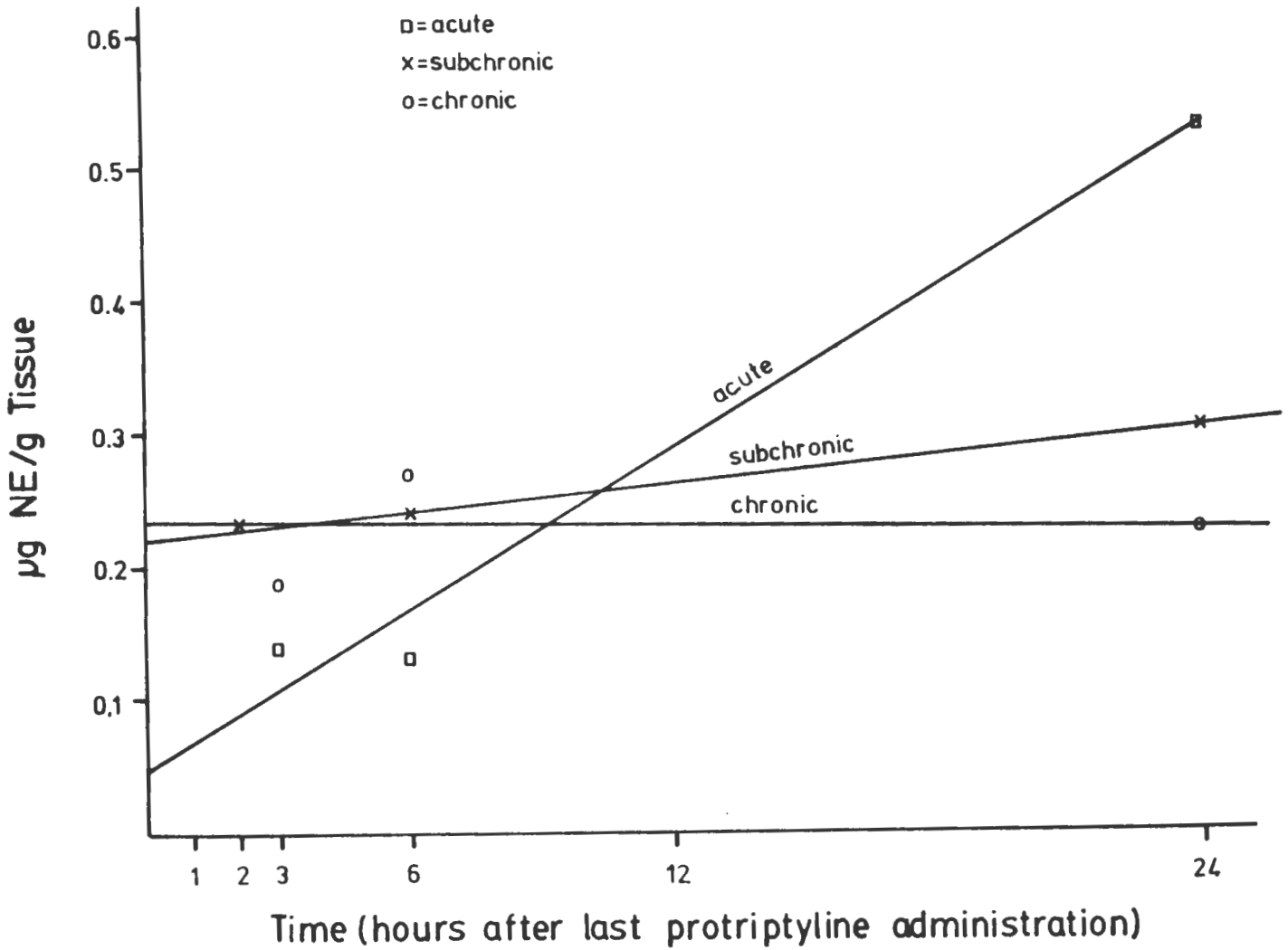
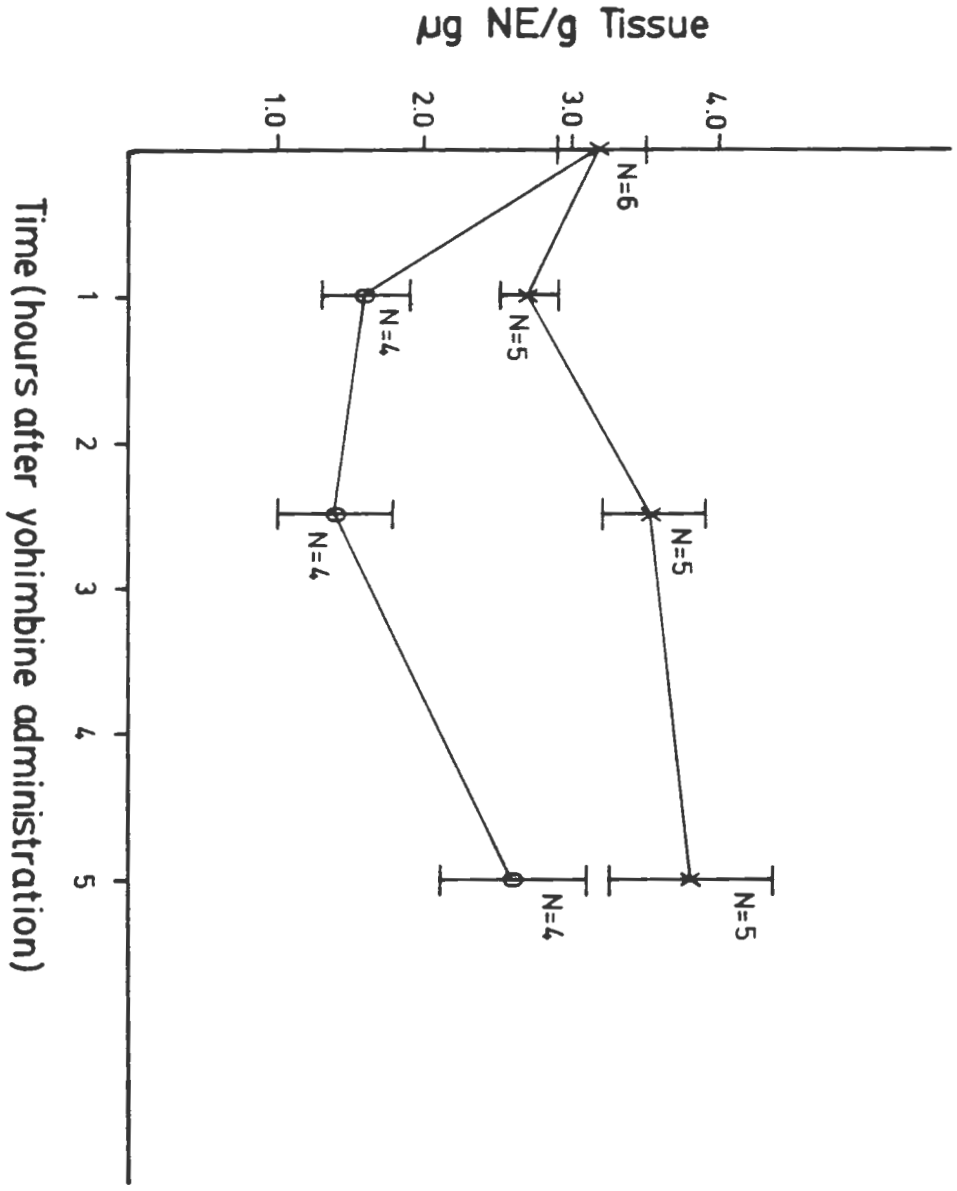

□=acute
x=subchronic
o=chronic
0.6
0.5
0.4
0.3
0.2
0,1
µg NE/g Tissue
acute
subchronic
chronic
1
2
3
6
12
24
Time (hours after last protriptyline administration)

FIGURE 15

TITLE: Norepinephrine depletion in hypothalamic tissue following yohimbine administration

LEGEND

Animals were administered yohimbine (2.0 mg/kg or 20.0 mg/kg) and were sacrificed 0, 1, 2.5 and 5 hours after this administration. All points represent the MEAN $\pm$ S.E. for at least 4 experimental determinations.


µg NE/g Tissue
1.0
2.0
3.0
4.0
N=6
N=4
N=5
N=4
N=5
N=4
N=5
1
2
3
4
5
Time (hours after yohimbine administration)

decrease in NE is reported following a single administration of yohimbine at 20.0 mg/kg. Similar results have been reported by Papeschi and Theiss (1975) in whole brain preparations. A 20.0 mg/kg dose was employed for our future studies, since it allowed a longer time interval for the measurement of turnover following MT administration. Numerical values for each time point are presented in the appendix section (Table 53).

Figure 16 illustrates the effects of yohimbine and MT administration on NE levels in control animals. NE levels in the hypothalamus are maximally depleted one hour after MT administration, and two hours after yohimbine administration. The 65% depletion in NE levels is not recorded in saline administered animals which received MT. Numerical values for these data are presented in the appendix section (Table 54).

Figure 17 reports the effects of a single administration of yohimbine (20.0 mg/kg) on animals which have been chronically administered protriptyline (10 mg/kg/day, 18 days). Animals were administered yohimbine three hours after the final protriptyline injection. This plot illustrates that three hours after yohimbine administration NE levels do not deplete in the hypothalamus. This result contrasts with our observation in Figure 15. In Figure 15, yohimbine administration resulted in a 56% decrease in NE levels. The numerical values for Figure 17 are presented in the appendix section (Table 55).

Figure 18 presents data illustrating the effects of both yohimbine and MT administration on NE levels following chronic

FIGURE 16

TITLE: NE turnover in hypothalamus of animals which have received yohimbine and sequentially MT

LEGEND

Animals were administered yohimbine (20 mg/kg - I.P.) and one hour later MT (400 mg/kg - I.P.). Animals were then sacrificed 0, 1, 2, 3, 4.5 and 6 hours after MT administration. Each data point represents the MEAN $\pm$ S.E. for at least 4 experimental determinations.

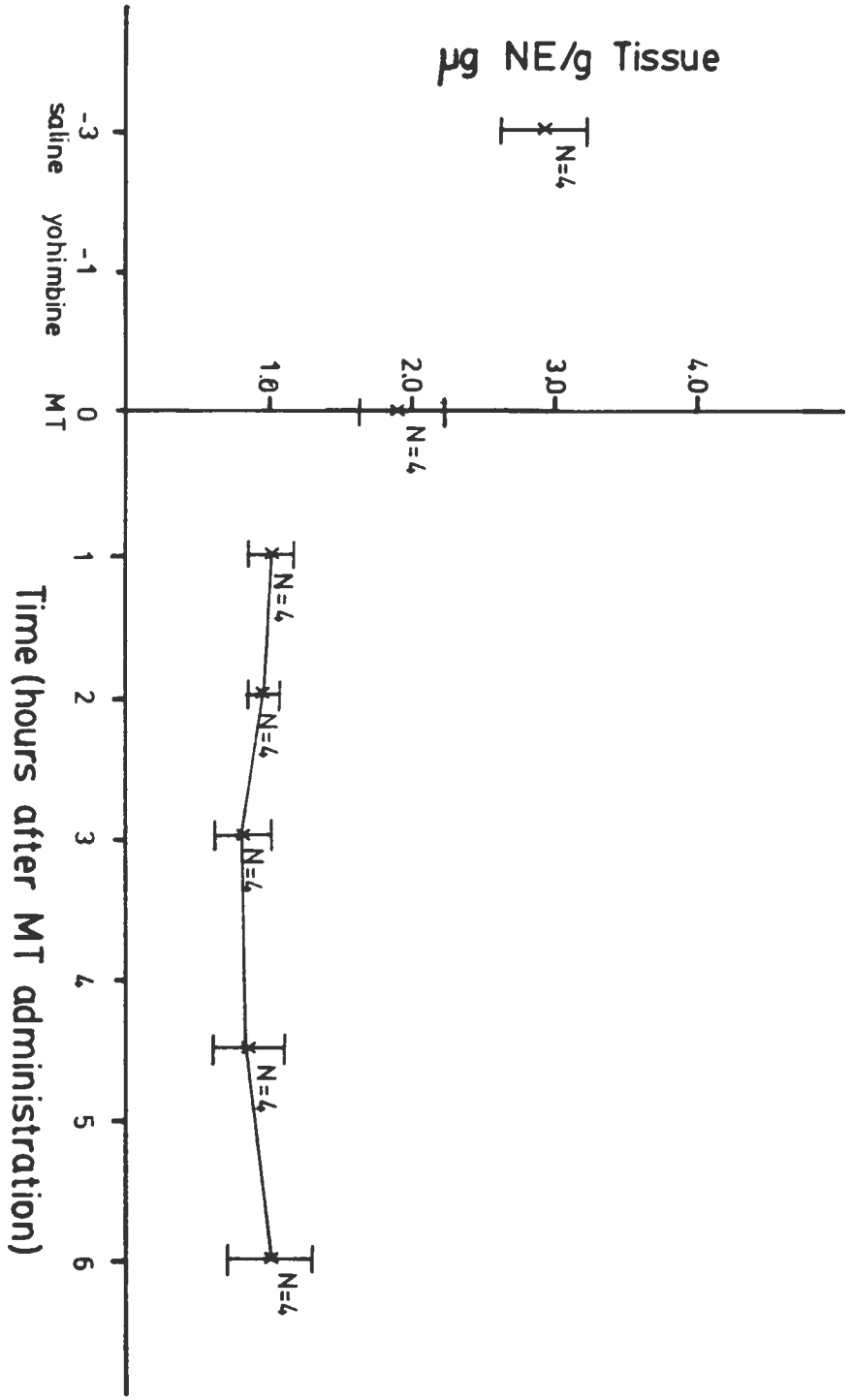

µg NE/g Tissue
4.0
3.0
2.0
1.0
N=4
N=4
N=4
N=4
N=4
N=4
N=4
-3
-1
0
1
2
3
4
5
6
Saline
yohimbine
MT
Time (hours after MT administration)

FIGURE 17

TITLE: NE depletion in the hypothalamus of animals receiving chronic protriptyline treatment and then administered yohimbine

LEGEND

Animals received protriptyline (10 mb/kg/day - 18 days S.C.) and three hours after their final protriptyline injection they received yohimbine (20 mg/kg, I.P.). These animals were sacrificed 0, 1, and 3 hours after yohimbine. Each data point represents the MEAN $\pm$ S.E. for at least 4 experimental determinations.

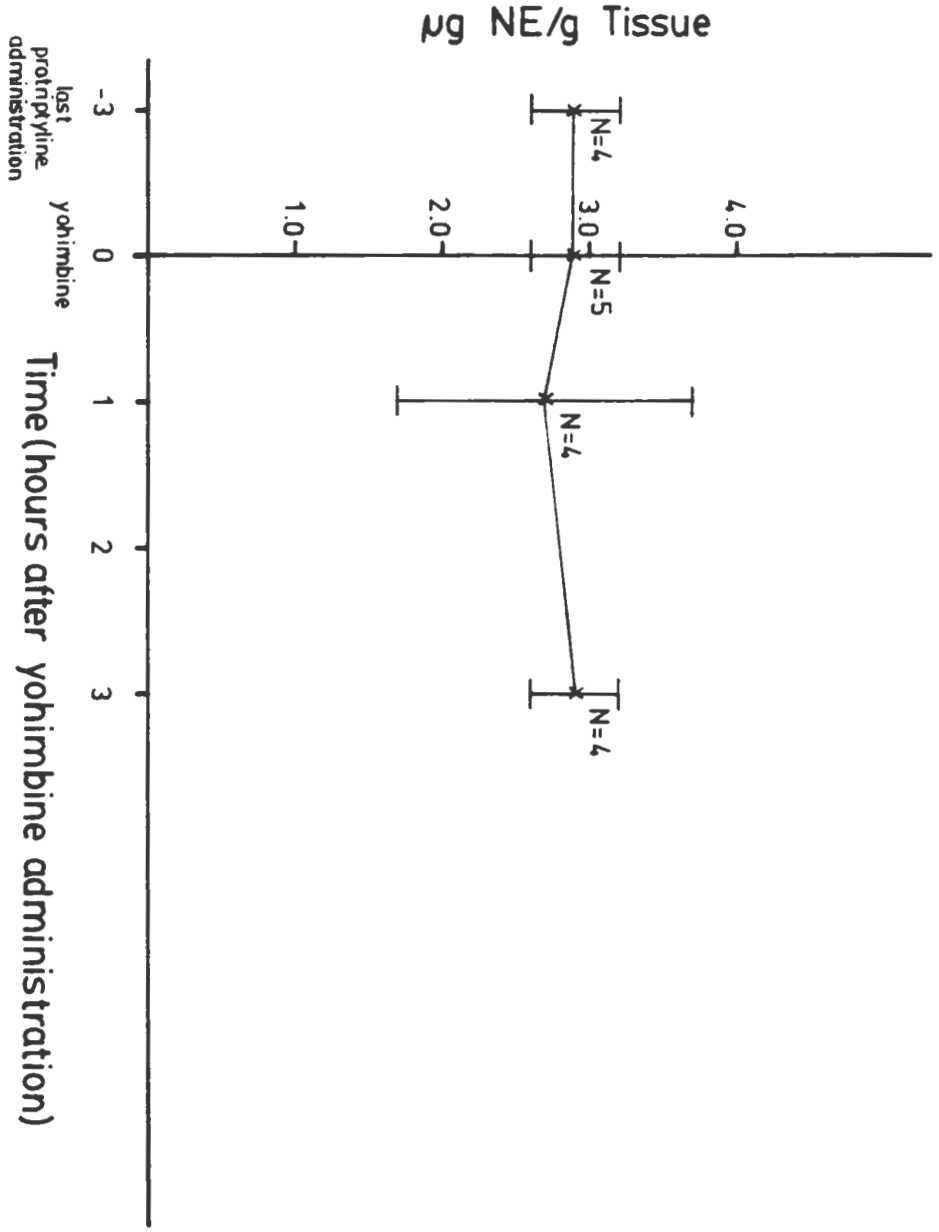
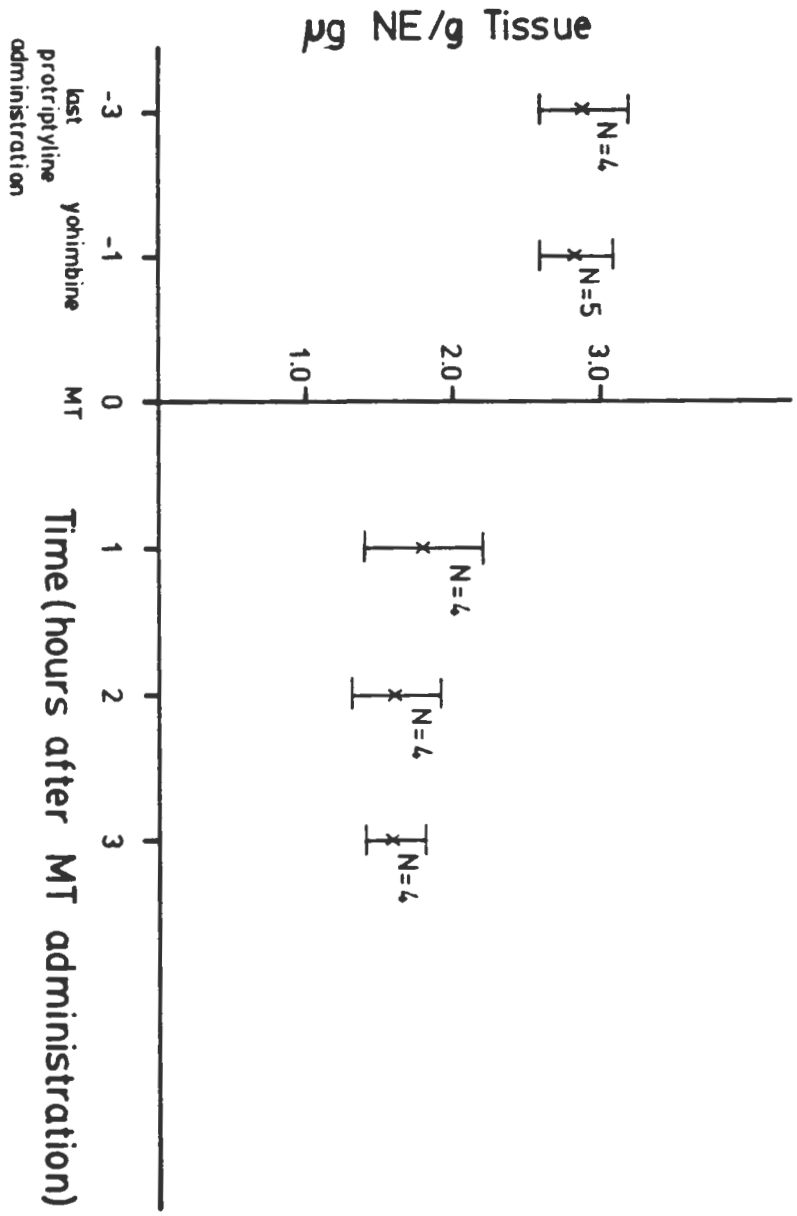

µg NE/g Tissue
4.0
3.0
2.0
1.0
N=4
N=5
N=4
N=4
last protriptyline administration
yohimbine
-3
0
1
2
3
Time (hours after yohimbine administration)

FIGURE 18

TITLE: NE depletion in hypothalamus following chronic protriptyline administration, yohimbine administration and MT administration

LEGEND

Animals were administered protriptyline chronically (10 mg/kg/day, 18 days - S.C.). Three hours after the final protriptyline administration animals were administered yohimbine (20 mg/kg) I.P.. One hour after yohimbine administration animals were administered MT (400 mg/kg - I.P.). Animals were then sacrificed 0, 1, 2, and 3 hours after MT administration. Each data point represents the MEAN $\pm$ S.E. for at least 4 experimental determinations.


µg NE/g Tissue
3.0
2.0
1.0
N=4
N=5
N=4
N=4
N=4
-3
-1
0
1
2
3
last protriptyline administration
yohimbine
MT
Time (hours after MT administration)

protriptyline administration (10 mg/kg/day, S.C. - 18 days). Three hours following MT administration, NE levels were not statistically different from the NE levels reported following chronic protriptyline administration and subsequent MT administration (see Figure 11). This study indicates that the effects of yohimbine appear nullified when protriptyline has been administered chronically. In control studies, it should be recalled that NE was depleted 56% and 70% three hours after yohimbine administration (Figures 15 and 16). Numerical values for each time point are listed in the appendix section (Table 56).

D. DISCUSSION

To test our third hypothesis "that chronic protriptyline administration may induce changes in specific NE parameters and the presynaptic autoreceptor" studies were designed to evaluate changes in NE levels and turnover, employing the alpha-receptor antagonist yohimbine after chronic protriptyline administration. The results of these studies strongly suggest that chronic administration of protriptyline alters presynaptic alpha-receptors. Chronic protriptyline administration decreases NE levels in the hypothalamus; a finding which at first seems contradictory to the "catecholamine hypothesis of affective disorders", which suggests that increased NE at post-synaptic receptor sites is required to alleviate depression. Increased neurotransmitter levels would indicate increased release following an action potential, and decreased NE levels would indicate decreased neurotransmitter release.

Secondly, chronically administered protriptyline decreases NE turnover when compared to control animals, and exhibits increased turnover when compared to acute protriptyline administration. This result also seems contrary to the "catecholamine hypothesis of affective disorders" since one would predict that increases in turnover (i.e. synthetic rate) are necessary (to alleviate depression) to maintain the constant efflux of NE to the post-synaptic receptor.

Thirdly, yohimbine administration (which in control animals illicited NE depletion), to chronically administered protriptyline animals has no effect or minimal effects on NE depletion (Figure 17). This suggests that chronic protriptyline administration may produce subsensitivity of alpha-2-(presynaptic)-receptors, which in turn modify NE release to the synapse.

These results suggest the existence of presynaptic alpha-2-receptors are undergoing adaptation when protriptyline is administered chronically to experimental animals. The implications of these adaptive mechanisms and their relationships to the catecholamine hypothesis of affective disorders will be discussed in Chapter V.

C H A P T E R V

C H A P T E R V.

A. SUMMARY AND CONCLUSIONS

Our studies attempted to identify possible alternate mechanisms of action in the CNS when the tricyclic, protriptyline, was administered chronically to rats. These studies also examined the effects of both protriptyline and thyroid hormone administration on monoamine oxidase (MAO) activity. The following results were observed:

(1) In vitro preincubation with protriptyline inhibits MAO activity in all three brain regions examined.

(2) In vivo administration of protriptyline has varying and inconsistant effects on MAO activity.

(3) Monoamine oxidase activity is inhibited in vitro by preincubation with triiodothyronine.

(4) Triiodothyronine administration has no in vivo effect on MAO activity in the three brain regions examined.

(5) Thyroidectomy decreases MAO activity in all three brain regions examined.

(6) The simultaneous administration of protriptyline and triiodothyronine have toxic lethal effects on animals when administered at the dose ranges we examined, and in those animals which survived simultaneous administration, monoamine oxidase data was inconsistent and inconclusive.

(7) Protriptyline administration to thyroidectomized animals results in variable and inconsistent effects on MAO activity.

Several of these observations (1, 3, 4) have been documented by others (Gabay and Valcourt, 1968A; Roth and Gillis, 1974A; Roth, 1977). In addition, other animal studies have documented toxic effects of concurrent triiodothyronine administration with the tricyclic imipramine, or with other pharmacological agents (Carrier and Buday,

1961; Conney and Garren, 1961; Prange and Lipton, 1962; Coville and Telford, 1970; Breese, et al., 1974). No documentation could be found identifying toxic interactions when protriptyline was administered with triiodothyronine.

At the completion of these studies it was concluded that any interaction between protriptyline and MAO activity was non-specific and our first hypothesis "that the requirement for chronic treatment with tricyclics before clinical efficacy is observed is related to an action on mitochondrial MAO, and that the time course for this effect parallels the time course for clinical response" was incorrect. Similarly, the toxic interactions of simultaneous triiodothyronine and protriptyline administration, though of interest, also remain unresolved.

Continuing, we next examined the effects of thyroidectomy and both triiodothyronine (T_3) and protriptyline administration on NE levels and turnover. We observed the following results:

(1) That seven days of triiodothyronine administration (1 mg/kg/day) results in decreased NE pool size in the brain areas examined.

(2) That thyroidectomy induces no change in NE pool size in these same brain regions.

(3) That both acute and chronic protriptyline administration (10 mg/kg/day) result in decreased NE pool size.

(4) That NE turnover is decreased in triiodothyronine treated animals when compared to saline administered control animals.

(5) That NE turnover in thyroidectomized animals is increased when compared to Sham-operated control animals.

(6) That NE turnover in the hypothalamus of both acutely and chronically administered protriptyline rats is decreased when compared to saline administered control animals.

The possible physiological significance of alterations in brain NE levels and turnover after triiodothyronine administration, and following acute and chronic protriptyline administration may be postulated to be related to compensatory and adaptive mechanisms, resulting from changes in noradrenergic alpha-receptor sensitivity. Decreases in both NE levels and turnover were observed after seven days of T_3 administration. Our observation of both a decrease in NE level and decreased turnover would be explicable if T_3 induces increased receptor sensitivity to NE. Increased receptor sensitivity would allow a decrease in the amount of NE released into the synapsis and still illicite a target cell response. Increased receptor sensitivity induced by T_3 has been documented (Engstrom, et al., 1974; Engstrom, et al., 1975; Strombom, et al., 1977).

Increased receptor sensitivity may also be responsible for the toxic side effects of protriptyline. Long (1977) has reported animal results in which heart palpitations and arrhythmias have occurred when T_3 was administered with other pharmacological agents. Possibly T_3 increases receptor sensitivity and sequential protriptyline administration results in an increase in toxic effects.

Both acute and chronic protriptyline administration induced changes in NE levels and turnover in hypothalamus. The acute effects of the tricyclics on brain noradrenergic parameters have been documented by many investigators (Schildkraut, et al., 1970; Schildkraut, et al.,

Roffler-Tarlov, et al., 1973; Leonard and Kafoe, 1976). Decreases in NE levels have been postulated to result from the negative feedback inhibition by NE on tyrosine hydroxylase (TH) activity, via the pteridine cofactor site. Decreased turnover also results from negative feedback inhibition. Alternations in both of these noradrenergic parameters result from the tricyclics primary mechanism of action (re-uptake blockage) and may be viewed as compensatory noradrenergic neural adjustments.

In our chronic protriptyline studies we observed decreased hypothalamic tissue levels of NE and also a decrease in NE turnover rate compared to chronic saline administered controls. These results suggest a decrease in NE synthetic rate. Other investigators have also demonstrated changes in other noradrenergic parameters which also suggest decreased synthetic rate. Mandel, et al., (1973) demonstrated that chronic imipramine treatment decreases midbrain tyrosine hydroxylase (TH) activity. Schildkraut (1965) and Schildkraut, et al., (1972, 1975) demonstrated that chronic treatment with amitriptyline or imipramine decreased the excretion rate of norepinephrine metabolites in the CSF. We postulate that the observed decrease in NE levels and turnover rate is an adaptive response resulting from increased efficiency of transmission as a consequence of re-uptake blockade.

Our studies suggest that adaptations in the presynaptic alpha-receptor in the hypothalamus have occurred following chronic protriptyline administration. In our final studies we employed yohimbine in an attempt

to further examine whether alpha-receptor adaptations have occurred and whether such changes correlate with the lag-time observed for clinical efficacy.

The following results were obtained when yohimbine was employed:

(1) Yohimbine administration (20.0 mg/kg, I.P.) produced a 56% decrease in total cytoplasmic NE levels in control animals three hours after test drug administration.

(2) Yohimbine administration (20.0 mg/kg, I.P.) to chronically treated protriptyline (10 mg/kg, 18 days, S.C.) animals produced NO decrease in total cytoplasmic NE levels three hours after test drug administration.

(3) Yohimbine administration (20.0 mg/kg, I.P.) followed by alpha-methyl-para-tyrosine (MT) administration (400 mg/kg, I.P.) to control animals resulted in a 72% decrease in total cytoplasmic NE levels three hours after MT administration.

(4) Yohimbine administration (20.0 mg/kg, I.P.) followed by MT administration (400 mg/kg, I.P.) to chronically treated protriptyline animals (10 mg/kg, 18 days, S.C.) produced a 40% decrease in total cytoplasmic NE levels three hours after MT administration.

These results can be explained in the following manner. Yohimbine, a pre-synaptic alpha-receptor antagonist, suppresses feedback inhibition of NE release. This phenomena results in depletion of cytoplasmic pre-synaptic NE levels. In chronically treated protriptyline animals constant exposure to NE in the synapse results in the evolution of a state of subsensitivity of both pre- and post-synaptic receptors. In other words, receptor tolerance develops as a result of constant NE exposure.

Constant exposure to elevated synaptic concentrations of NE produces receptor subsensitivity in a time course which parallels the observed lag-time for clinical efficacy for this class of antidepressant

agents. Post-synaptic receptor subsensitivity has already been documented in peripheral systems (Langer and Luchelli-Fortis, 1977).

B. SUMMARY

We have documented several compensatory neural mechanisms which occur during the chronic treatment period with tricyclics: (1) changes in endogeneous cytoplasmic total NE levels, (2) changes in NE turnover rate/recovery rate following the last dose in a chronic series of test drug administrations, and (3) adaptations in the pre-synaptic receptor.

1. Adaptations in total NE tissue levels.

Decreases in cytoplasmic NE levels following chronic protriptyline treatment (Figure 7) suggest a more efficient utilization of NE (less neurotransmitter is required to maintain normal physiologic function). Clinical studies support this postulate and report increases in H^3-NMN (a COMT metabolite) and decreases in H^3-DCM (MAO metabolies, 3, 4-dihydroxymandelic acid and 3, 4-dihydroxy-phenyl-glycol) following chronic tricyclic administration. These data indicate that more neurotransmitter is available to synaptic receptors (and COMT), despite an observed reduction in total endogeneous cytoplasmic NE levels.

2. Adaptations in NE turnover.

Acute protriptyline administration results in a decreased slope for NE turnover, three and six hours after tricyclic administration, when compared to saline treated animals. Twenty-four hours after protriptyline administration the slope for NE turnover has returned to control values (Tables 6 and 7). This observation has been observed by others when

measuring H^3-metabolites (H^3-NMN) following H^3-NE infusion, and employing acutely imipramine or DMI pretreatment. Since re-uptake blockade by tricyclics results in an accumulation of NE in the synapse, this accumulation would stimulate pre-synaptic alpha-receptors and in turn a negative feedback mechanism would become operative; thus resulting in a decrease in the release of pre-synaptic NE. This mechanism is responsible for the decrease in NE turnover following acute protriptyline administration.

With subchronic (6 day) protriptyline treatment, the slope for NE turnover appears to be elevated at both the three and six hour time points after the last protriptyline injection, when these slopes for turnover rate are compared to the turnover rates for acute protriptyline administration. Similarly, the slopes for turnover rate for chronically administered animals also suggest an increase in NE turnover rate, when compared to acute protriptyline administration. These data suggest the development of receptor adaptations following a chronic tricyclic treatment schedule.

Figure 18, a turnover rate/recovery rate plot, depicts this comparison of turnover slopes for acute, subchronic and chronic treatment groups. This figure explicitly demonstrates the modifications in neurotransmitter turnover rate/recovery rate in the three treatment schedules.

3. Adaptations in pre-synaptic receptor sensitivity.

Pre-synaptic alpha-receptor adaptation (hyposensitivity) following chronic protriptyline administration is suggested by our data. Since

yohimbine prevents pre-synaptic alpha-receptor stimulation, and allows NE overflow to the adrenergic synapse to occur, chronic protriptyline administration modifies this mechanism. Control of NE release by these receptors (which is dependent upon synaptic NE concentration) is altered, thus the negative feedback mechanism for facilitating or suppressing the controlled release of NE to the synapse becomes modified.

When animals were administered protriptyline chronically and subsequently administered yohimbine, a decrease in endogeneous NE levels was not observed in our studies (Figure 17). These results are contrary to earlier studies involving yohimbine administration to control animals (Figure 15). This observation suggests that chronic protriptyline administration induces pre-synaptic hyposensitiivity.

C. CONCLUSIONS

It appears that chronic protriptyline administration results in adaptive changes in central noradrenergic mechanisms. The time course for these adaptive changes parallel the time required for the onset of their clinical effectiveness. We propose that acutely the action of tricyclics on norepinephrine re-uptake is (largely) negated by compensatory adjustments in turnover rate of the neurotransmitter; but with chronic administration, adaptations in pre-synaptic alpha-receptors occur. This in turn reduces the extent to which the norepinephrine neurons can offset or compensate for the blockade of norepinephrine re-uptake. Specifically, pre-synaptic alpha-receptors become hyposensitive, and tolerance develops, not to the direct action of the tricyclic drug

on neurotransmitter re-uptake, but to the compensatory neural adjustments which offset the action of the acute tricyclic administration and delay their clinical efficacy.

C H A P T E R VI

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C H A P T E R VII

(Appendix Section)

TABLE 1 Kinetic parameters for monoamine oxidase activity in three brain regions

TISSUE	Km (x 10^{-6} M) 5-HT	(r^2)	tryptamine	(r^2)	Vmax (nmoles of 5-HIAA or IAA formed/hr.ug DNA) 5-HT	tryptamine
Corpus Striatum	28.0	0.9961	9.0	0.9988	0.2389	1.1362
Forebrain	45.0	0.9988	12.0	0.9994	0.7759	3.0816
Hypothalamus	27.0	0.9975	9.0	0.9989	0.3467	1.5766

N = 6
Km and Vmzx values were calculated following linear regression analysis.

TABLE 3.0 The effect of acute protriptyline administration (one injection) on MAO activity in three rat brain regions[1]

	Time sacrificed after drug administration (hours)	Protriptyline Group	Saline group[2]
C^{14}-tryptamine(IAA)			
Corpus Striatum	1	2.103 ± 0.366[3]	1.969 ± 0.345
	3	2.657 ± 0.276	2.702 ± 0.624
	6	2.800 ± 0.454	2.888 ± 0.201
	12	2.569 ± 0.147	2.262 ± 0.126
	24	2.275 ± 0.165	2.428 ± 0.145
Forebrain	1	3.002 ± 0.184	2.425 ± 0.370
	3	2.657 ± 0.276	2.702 ± 0.624
	6	4.166 ± 0.670	4.862 ± 0.831
	12	3.151 ± 0.246	2.837 ± 0.109
	24	3.365 ± 0.566	2.436 ± 0.110
Hypothalamus	1	2.029 ± 0.201	1.950 ± 0.331
	3	2.238 ± 0.309	2.330 ± 0.173
	6	2.196 ± 0.266	2.059 ± 0.268
	12	2.181 ± 0.189	1.927 ± 0.168
	24	1.401 ± 0.205	1.678 ± 0.152
C^{14}-serotonin(5-HIAA)			
Corpus Striatum	1	0.287 ± 0.041	0.273 ± 0.046
	3	0.539 ± 0.058	0.443 ± 0.068
	6	0.426 ± 0.052	0.465 ± 0.055
	12	0.370 ± 0.017	0.325 ± 0.027
	24	0.452 ± 0.041	0.455 ± 0.032
Forebrain	1	0.416 ± 0.029	0.409 ± 0.052
	3	0.535 ± 0.046	0.547 ± 0.058
	6	0.665 ± 0.135	0.850 ± 0.014
	12	0.459 ± 0.048	0.408 ± 0.014
	24	0.664 ± 0.098	0.457 ± 0.036
Hypothalamus	1	0.224 ± 0.040	0.228 ± 0.028
	3	0.423 ± 0.088	0.398 ± 0.063
	6	0.308 ± 0.030	0.284 ± 0.025
	12	0.264 ± 0.031	0.217 ± 0.021
	24	0.245 ± 0.026	0.306 ± 0.059

[1]Protriptyline administration (10 mg/kg/day) subcutaneous injection

[2]Equal volumes of saline were administered to controls

[3]N = 6 for all groups, values represent the Mean ± S.E.M. for MAO activity and are reported as nanomoles of 5-HIAA or IAA formed/hr./ug DNA

TABLE 4.0 The effect of subchronic protriptyline administration (three consecutive injections) on MAO activity in three rat brain regions[1]

	Time sacrificed after last drug administration (hours)	Protriptyline Group	Saline Group[2]
C^{14}-tryptamine			
Corpus Striatum	3	2.429 ± 0.235[3]	2.352 ± 0.112
	6	1.775 ± 0.150	1.711 ± 0.190
	12	1.861 ± 0.196	2.262 ± 0.146
	24	2.327 ± 0.227	2.027 ± 0.205
Forebrain	3	2.841 ± 0.209	2.647 ± 0.094
	6	2.824 ± 0.204	2.662 ± 0.151
	12	3.111 ± 0.203	3.337 ± 0.237
	24	3.261 ± 0.150	2.893 ± 0.283
Hypothalamus	3	2.487 ± 0.198	2.102 ± 0.298
	6	1.880 ± 0.125	1.493 ± 0.345
	12	2.025 ± 0.308	2.078 ± 0.348
	24	2.358 ± 0.182	1.937 ± 0.101
C^{14}-serotonin			
Corpus Striatum	3	0.568 ± 0.035	0.512 ± 0.027
	6	0.402 ± 0.026	0.385 ± 0.043
	12	0.402 ± 0.045	0.437 ± 0.021
	24	0.502 ± 0.031	0.495 ± 0.032
Forebrain	3	0.618 ± 0.053	0.507 ± 0.016
	6	0.622 ± 0.050	0.610 ± 0.027
	12	0.600 ± 0.033	0.674 ± 0.059
	24	0.681 ± 0.036	0.669 ± 0.058
Hypothalamus	3	0.598 ± 0.053	0.424 ± 0.038*
	6	0.428 ± 0.036	0.355 ± 0.060
	12	0.359 ± 0.047	0.573 ± 0.079*
	24	0.453 ± 0.022	0.442 ± 0.018

[1]Protriptyline administration (10 mg/kg) subcutaneous injection

[2]Equal volumes of saline were administered to controls

[3]N = 6 for all groups, values represent the Mean ± S.E.M. for MAO activity and are reported as nanomoles of 5-HIAA or IAA formed/hr./ug DNA

*$P < 0.05$ from protriptyline group

TABLE 5.0 The effect of subchronic protriptyline administration (six consecutive injections) on MAO activity in three rat brain regions[1]

	Time sacrificed after last drug administration (hours)	Protriptyline Group	Saline Group[2]
C^{14}-tryptamine			
Corpus Striatum	3	2.359 ± 0.175[3]	2.327 ± 0.240
	6	2.326 ± 0.170	2.173 ± 0.153
	12	1.995 ± 0.093	2.697 ± 0.218*
	24	2.132 ± 0.262	2.616 ± 0.288
Forebrain	3	3.304 ± 0.407	3.431 ± 0.264
	6	3.505 ± 0.261	2.923 ± 0.219
	12	4.808 ± 0.446	3.106 ± 0.147*
	24	2.411 ± 0.191	3.922 ± 0.743
Hypothalamus	3	1.791 ± 0.032	2.101 ± 0.145
	6	1.659 ± 0.101	1.839 ± 0.180
	12	2.798 ± 0.164	2.067 ± 0.256*
	24	1.903 ± 0.177	2.127 ± 0.211
C^{14}-serotonin			
Corpus Striatum	3	0.519 ± 0.059	0.512 ± 0.055
	6	0.589 ± 0.049	0.461 ± 0.049
	12	0.388 ± 0.029	0.426 ± 0.050*
	24	0.238 ± 0.039	0.374 ± 0.033*
Forebrain	3	0.669 ± 0.061	0.669 ± 0.048
	6	0.815 ± 0.073	0.599 ± 0.052*
	12	0.938 ± 0.080	0.672 ± 0.037*
	24	0.292 ± 0.036	0.619 ± 0.108*
Hypothalamus	3	0.395 ± 0.017	0.433 ± 0.026
	6	0.394 ± 0.025	0.429 ± 0.073
	12	0.478 ± 0.033	0.454 ± 0.059*
	24	0.207 ± 0.030	0.259 ± 0.040

[1]Protriptyline administration (10 mg/kg) subcutaneous injection

[2]Equal volumes of saline were administered to controls

[3]N = 6 or more animals for all groups examined; values represent the MEAN ± S.E.M. for MAO activity and are reported as nanomoles of 5-HIAA or IAA formed/hr./ug DNA

*$P < 0.05$ from protriptyline group

TABLE 6.0 The effect of chronic protriptyline administration (18 consecutive injections) on MAO activity in three rat brain regions[1]

	Time sacrificed after last drug administration (hours)	Protriptyline Group	Saline Group[2]
C^{14}-tryptamine			
Corpus Striatum	3	3.792 ± 0.330[3]	2.553 ± 0.262*
	6	2.965 ± 0.778	1.861 ± 0.094
	12	2.576 ± 0.212	2.743 ± 0.275
	24	1.792 ± 0.147	1.939 ± 0.131
Forebrain	3	3.338 ± 0.183	3.256 ± 0.167
	6	3.254 ± 0.174	3.373 ± 0.137
	12	3.339 ± 0.305	3.261 ± 0.181
	24	3.273 ± 0.226	2.894 ± 0.057
Hypothalamus	3	2.704 ± 0.390	2.452 ± 0.197
	6	2.441 ± 0.327	2.069 ± 0.154
	12	2.376 ± 0.118	2.319 ± 0.090
	24	2.183 ± 0.130	2.279 ± 0.142
C^{14}-serotonin			
Corpus Striatum	3	0.752 ± 0.077	0.512 ± 0.047*
	6	0.688 ± 0.192	0.447 ± 0.056
	12	0.631 ± 0.038	0.685 ± 0.045
	24	0.423 ± 0.044	0.453 ± 0.020
Forebrain	3	0.739 ± 0.042	0.826 ± 0.088
	6	0.709 ± 0.045	0.686 ± 0.037
	12	0.975 ± 0.074	0.851 ± 0.065
	24	0.720 ± 0.031	0.676 ± 0.028
Hypothalamus	3	0.495 ± 0.084	0.513 ± 0.058
	6	0.456 ± 0.054	0.416 ± 0.032
	12	0.551 ± 0.023	0.545 ± 0.030
	24	0.454 ± 0.047	0.355 ± 0.036

[1]Protriptyline administration (10 mg/kg) subcutaneous injection

[2]Equal volumes of saline were administered to saline rats to serve as controls

[3]N = 6 for all determinations; values represent the Mean ± S.E.M. for MAO activity and are reported as nanomoles of 5-HIAA or IAA formed/hr./ug DNA

*$P < 0.05$ from protriptyline group

FIGURE 1

TITLE: Velocity versus substrate concentration for MAO activity in the corpus striatum

LEGEND

Monoamine oxidase activity in the corpus striatum has been evaluated using Michalis-Menton kinetics and employing C^{14}-tryptamine as substrate. MAO enzyme activity begins to plateau at 20.0×10^{-6} M substrate concentration. Each point represents the MEAN of 6 experimental determinations. Similar results were obtained when forebrain or hypothalamus were examined and C^{14}-tryptamine was employed as substrate. Numerical values for all enzyme velocity versus substrate concentration are listed in the appendix section (Tables 19-26).

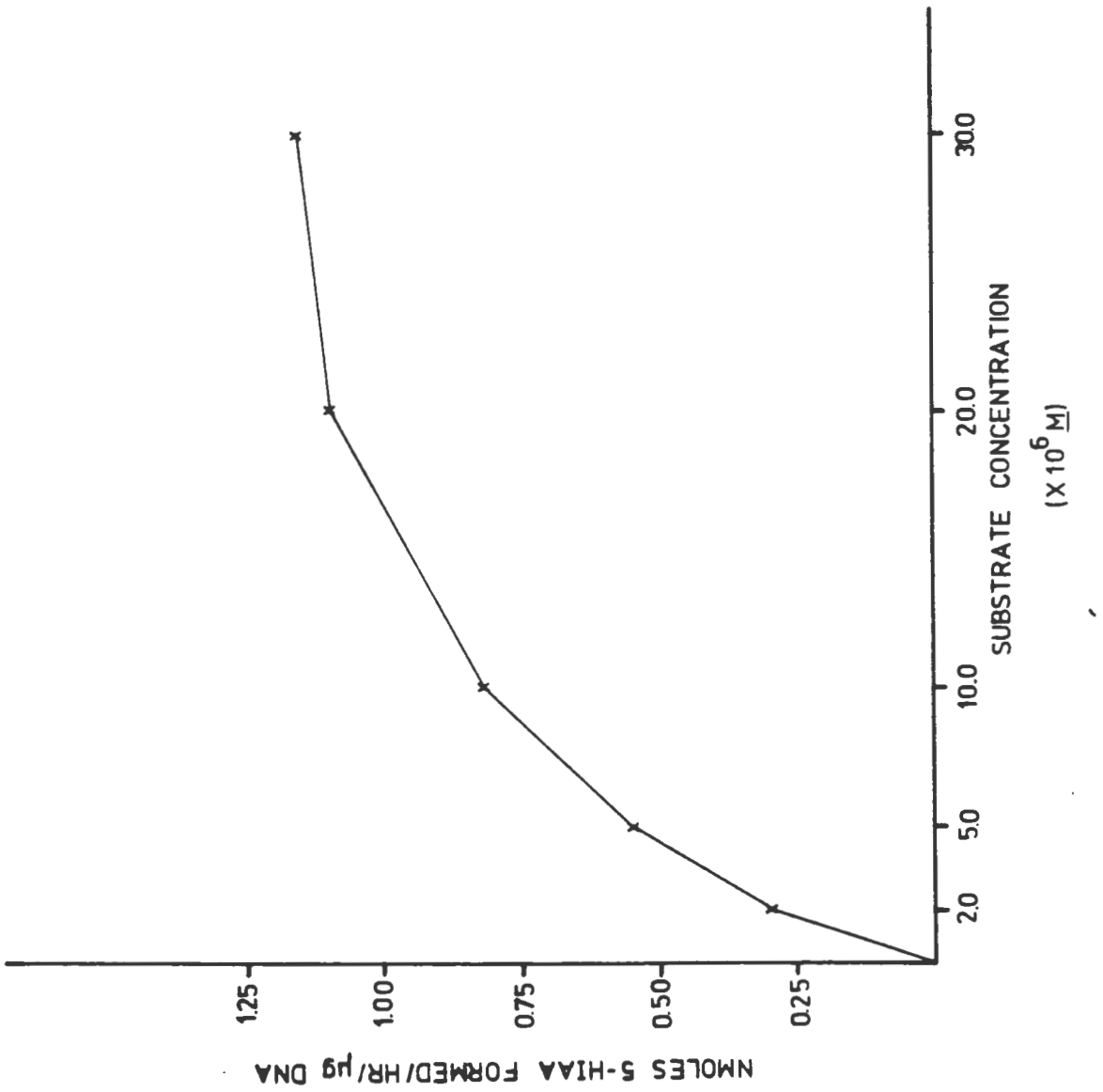

NMOLES 5-HIAA FORMED/HR/µg DNA
1.25
1.00
0.75
0.50
0.25
2.0
5.0
10.0
20.0
30.0
SUBSTRATE CONCENTRATION
(X 10⁶ M)

FIGURE 2

TITLE: Reciprocal plot for MAO enzyme velocity versus substrate concentration (Lineweaver-Burke)

LEGEND

This graph represents a reciprocal plot of the data obtained in Figure 1. The Km value for MAO is 9.0×10^{-6}M, which agrees with the values reported by Collins, (1972) and Tipton, (1972) in pig brain, rat liver and beef adrenal medulla. Similarly, the Vmax value obtained is 1.1362 nmoles of IAA formed/hr/ug DNA. The linear regression coefficient (r^2) is 0.9988.

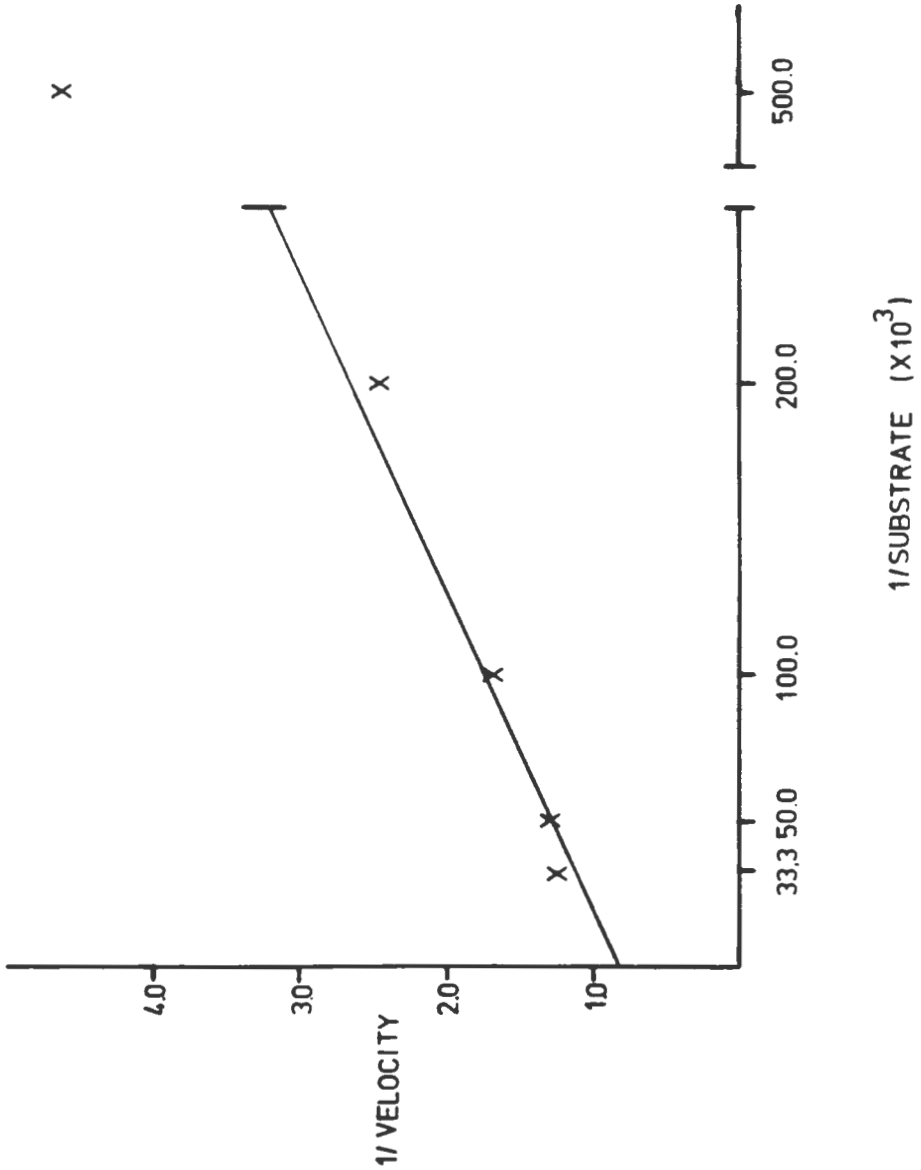

1/ VELOCITY
4.0
3.0
2.0
1.0
33.3 50.0
100.0
200.0
500.0
1/SUBSTRATE (X10^3)

FIGURE 3

TITLE: Velocity versus substrate concentration for MAO activity in corpus striatum.

LEGEND

Monoamine oxidase activity in the corpus striatum has been evaluated using Michalis-Menton kinetics and employing C^{14}-serotonin as substrate. MAO enzyme activity begins to plateau at 20.0×10^{-6} M substrate concentration. Each point represents the MEAN of 6 experimental determinations. Similar results were obtained when forebrain or hypothalamus were examined and C^{14}-serotonin was employed as substrate. Numerical values for all enzyme versus substrate concentration are listed in the appendix section (Tables 19-26).

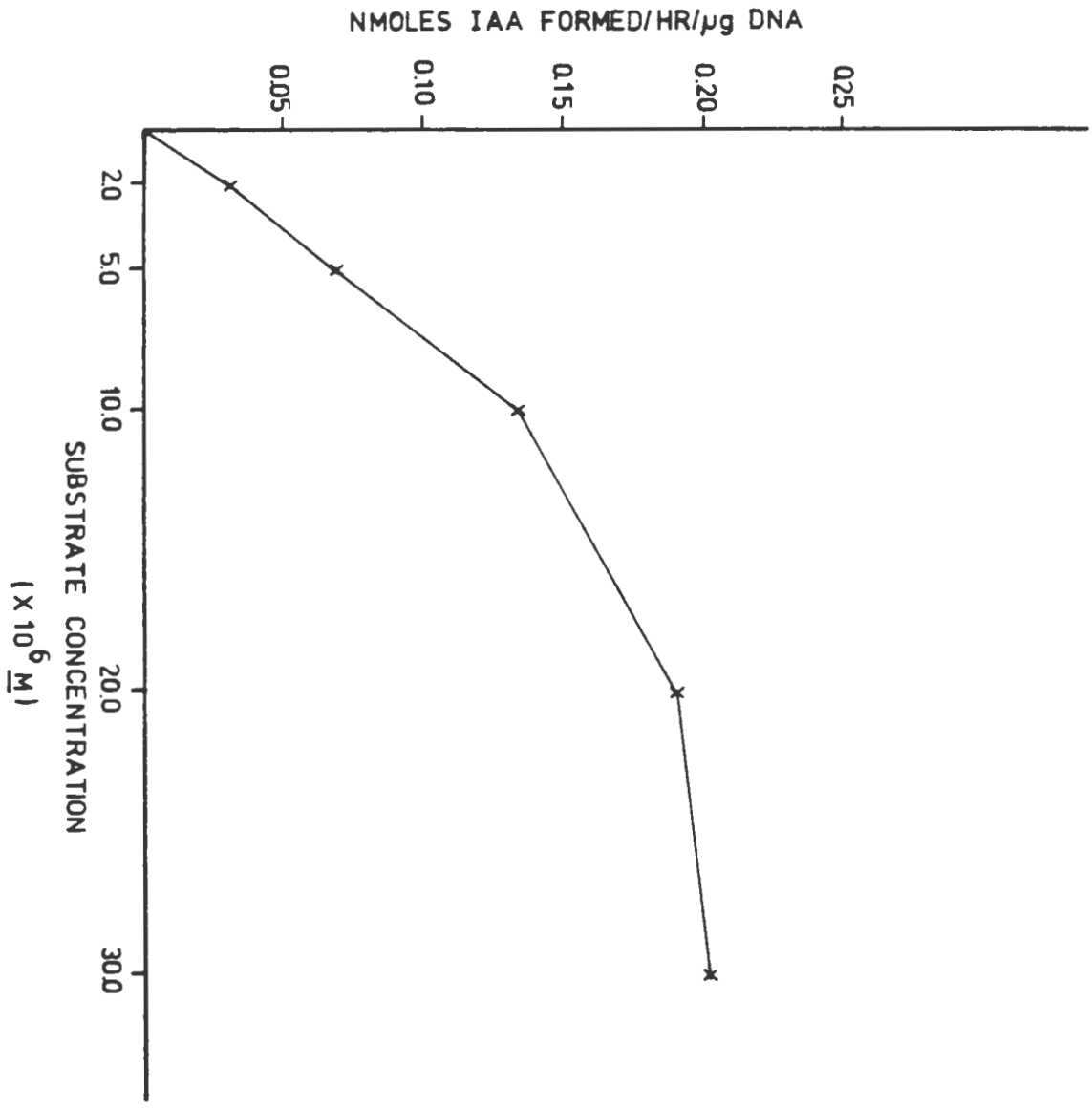

NMOLES IAA FORMED/HR/μg DNA
0.05
0.10
0.15
0.20
0.25
2.0
5.0
10.0
20.0
30.0
SUBSTRATE CONCENTRATION
(X 10⁶ M)

FIGURE 4

TITLE: Reciprocal plot for MAO enzyme velocity versus substrate concentration (Lineweaver-Burke)

LEGEND

This graph represents a reciprocal plot of the data obtained in Figure 3. The Km value for MAO, when C^{14}-serotonin has been employed as substrate, is 28.0×10^{-6}M. This value is in agreement with other investigators (Collins, 1972; Tipton, 1972; Fowler and Oreland, 1979; Suzuki, 1979). Similarly, the Vmax value obtained is 0.2389 nmoles of 5-HIAA formed/hr/ug DNA. The linear regression coefficient (r^2) is 0.9961.

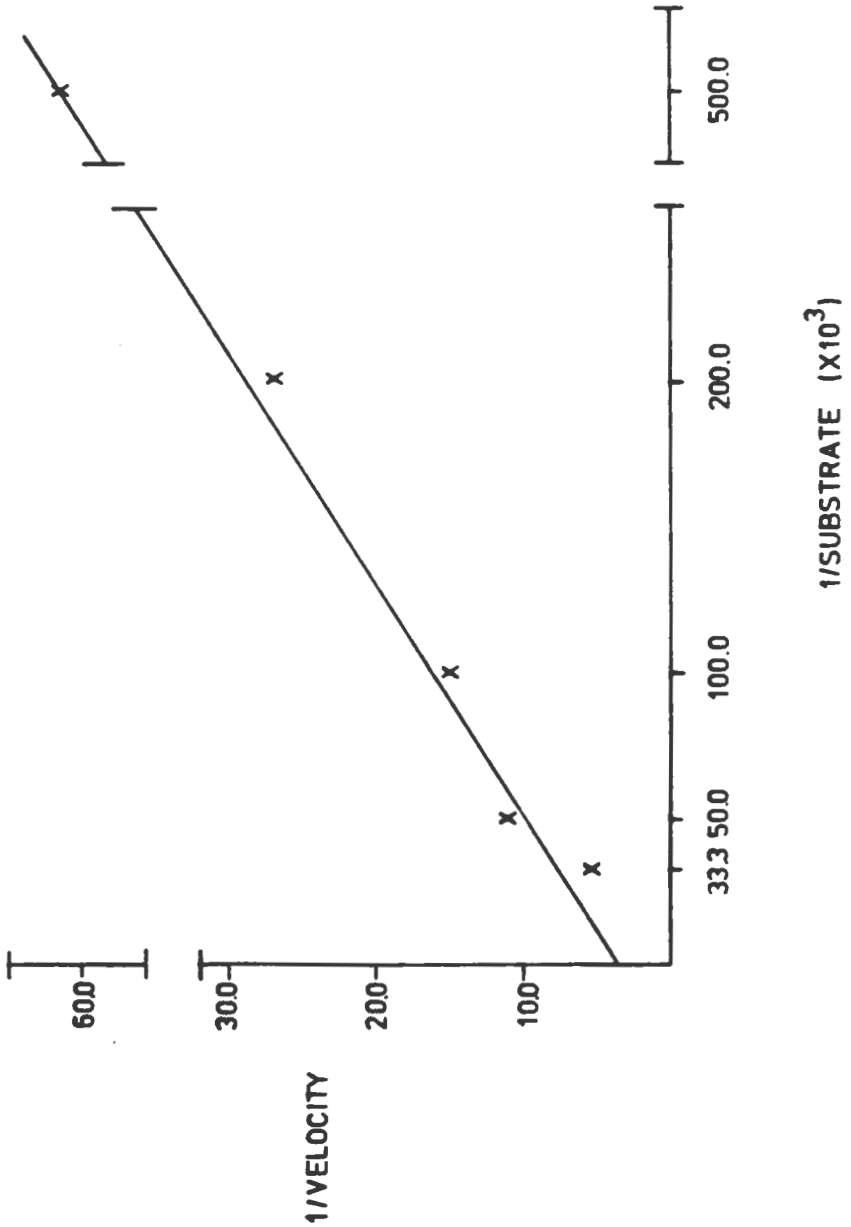

60.0
30.0
20.0
10.0
1/VELOCITY
33.3 50.0
100.0
200.0
500.0
1/SUBSTRATE (X10^3)

FIGURE 5

TITLE: Monoamine oxidase activity in corpus striatum following *in vitro* preincubation with varying molar concentrations of protriptyline.

LEGEND

MAO activity has been evaluated in the corpus striatum following preincubation with protriptyline. C^{14}-tryptamine has been used as the substrate. Each data point represents the MEAN $\pm$ S.E.M. for 6 experimental determinations. Inhibition of MAO activity begins at a molar concentration of protriptyline at 10^{-5} M.

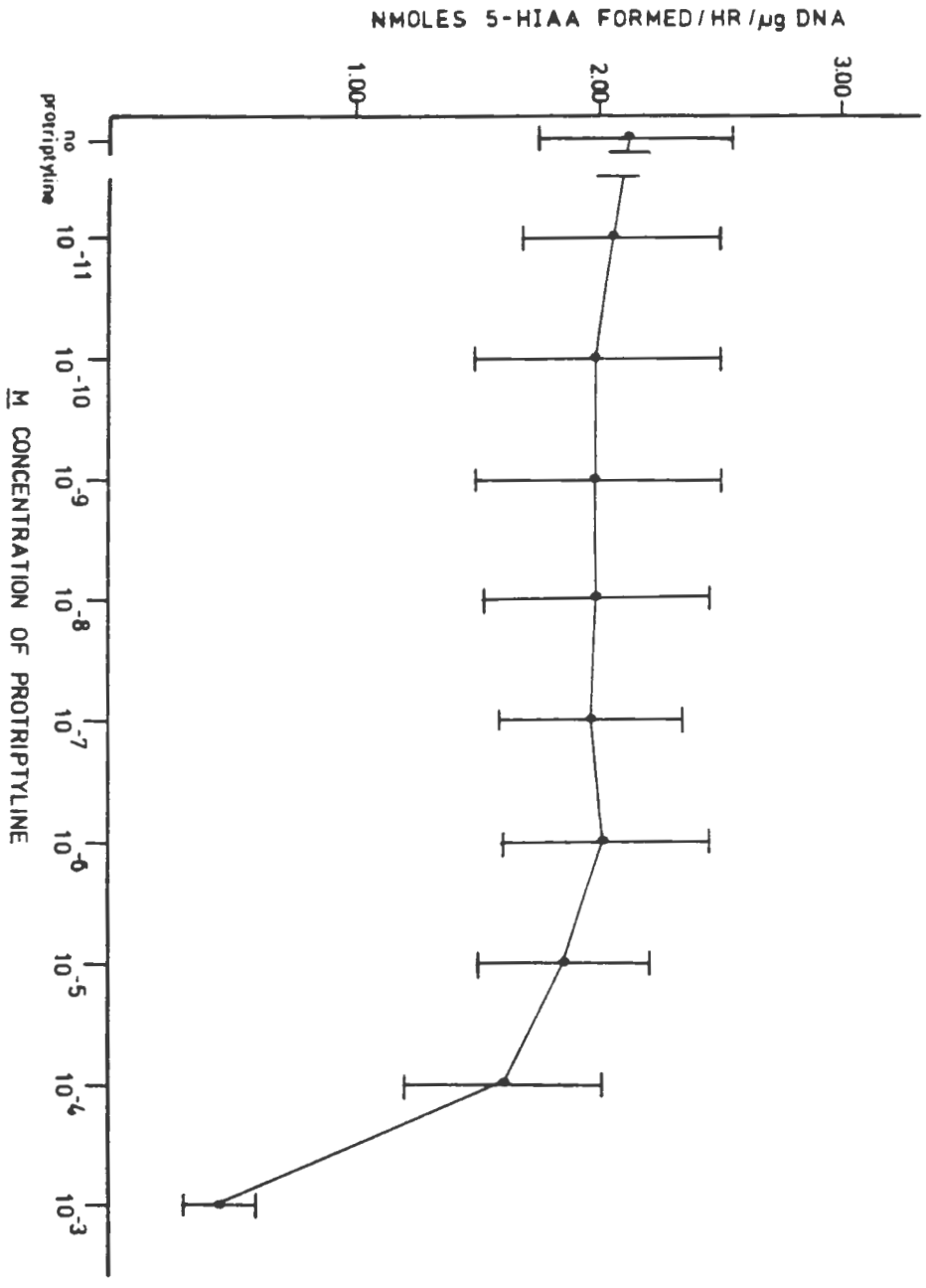

NMOLES 5-HIAA FORMED / HR / µg DNA
1.00
2.00
3.00
no protriptyline
10-11
10-10
10-9
10-8
10-7
10-6
10-5
10-4
10-3
M CONCENTRATION OF PROTRIPTYLINE

FIGURE 6

TITLE: Monoamine oxidase activity in corpus striatum following *in vitro* preincubation with varying molar concentrations of protriptyline.

LEGEND

MAO activity has been evaluated in the corpus striatum following preincubation with protriptyline. C^{14}-serotonin has been used as the substrate. Each data point represents the MEAN $\pm$ S.E.M. for 6 experimental determinations. Inhibition of MAO activity begins at a molar concentration of protriptyline at 10^{-5} M.

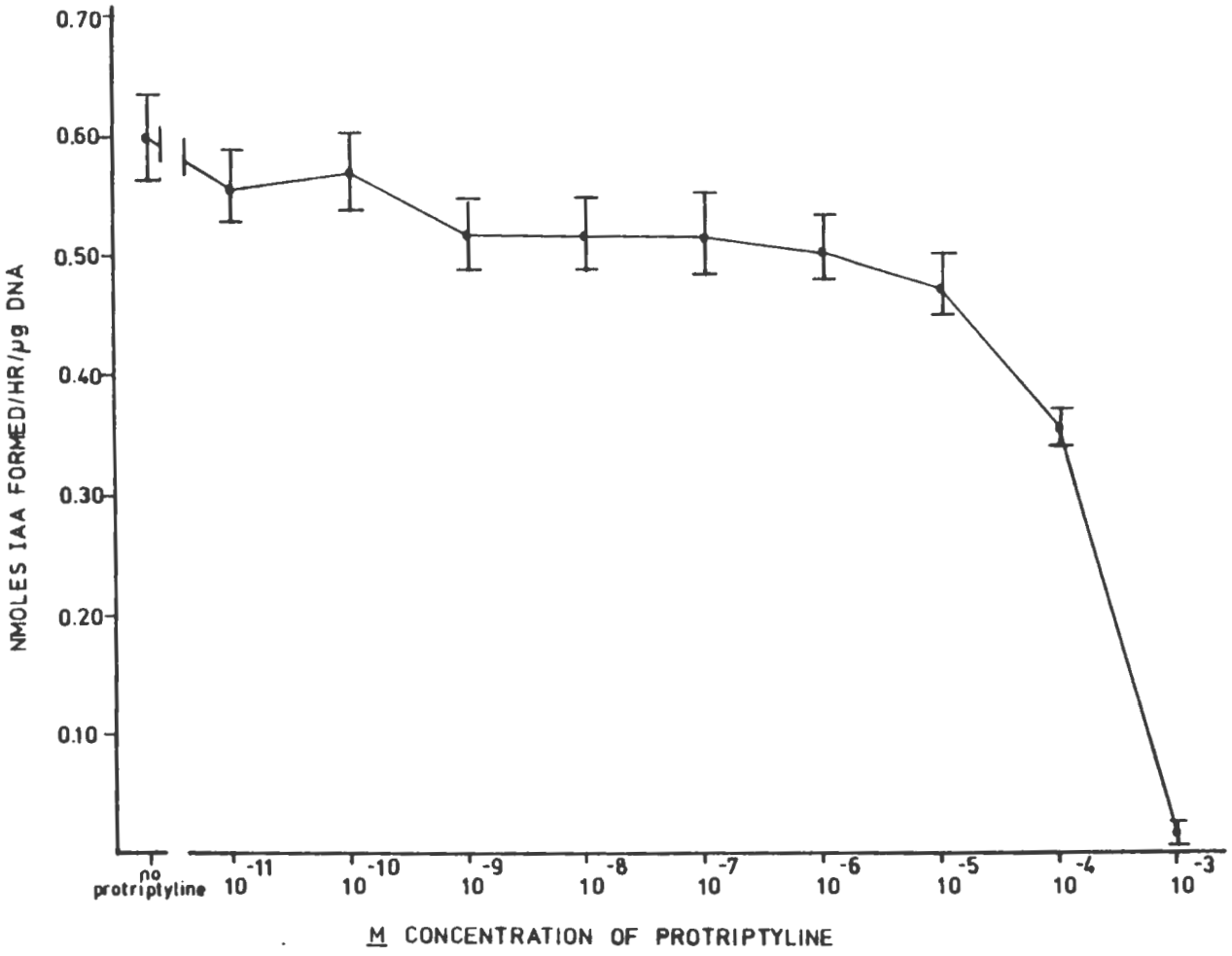

0.70
0.60
0.50
0.40
0.30
0.20
0.10
NMOLES IAA FORMED/HR/µg DNA
no protriptyline
10 -11
10 -10
10 -9
10 -8
10 -7
10 -6
10 -5
10 -4
10 -3
M CONCENTRATION OF PROTRIPTYLINE

TABLE 11 The effect of thyroidectomy and acute protriptyline on MAO activity in three brain regions.

	Time	Saline and Sham Control	Tx and Saline	Acute Protriptyline and Tx
Tryptamine				
CS	1	2.83 ± .17	3.30 ± .38	2.18 ± .31*
	3	2.75 ± .16	2.32 ± .25	2.10 ± .13+
	6	2.77 ± .19	2.98 ± .14	2.27 ± .13+
	12	3.13 ± .45	2.12 ± .24	3.24 ± .35
	24	2.72 ± .15	1.90 ± .08**	1.98 ± .08**
F	1	3.53 ± .21	3.75 ± .29	3.97 ± .40
	3	3.50 ± .20	3.08 ± .16*	2.84 ± .15
	6	3.85 ± .25	4.18 ± .30	3.19 ± .24
	12	3.11 ± .26	2.61 ± .14	3.08 ± .40
	24	3.87 ± .37	2.61 ± .14*	2.29 ± .12**
Hy	1	2.63 ± .39	3.04 ± .33	2.27 ± .22
	3	2.52 ± .36	2.60 ± .23	1.77 ± .16+
	6	2.04 ± .42	2.81 ± .19	3.03 ± .17
	12	5.35 ± .88	1.81 ± .12**	2.63 ± .15+*
	24	3.70 ± .72	1.81 ± .12*	1.97 ± .32*
Serotonin				
CS	1	0.67 ± .05	0.76 ± .11	0.61 ± .04
	3	0.64 ± .05	0.50 ± .05	0.48 ± .03
	6	0.81 ± .09	0.69 ± .03	0.56 ± .02++*
	12	0.83 ± .11	0.40 ± .03**	0.62 ± .08+*
	24	0.51 ± .03	0.32 ± .03**	0.34 ± .02**
F	1	0.76 ± .05	0.78 ± .08	0.91 ± .10
	3	0.75 ± .05	0.62 ± .04*	0.60 ± .04
	6	0.83 ± .04	0.82 ± .03	0.70 ± .05
	12	0.75 ± .07	0.53 ± .02*	0.59 ± .09
	24	0.71 ± .07	0.53 ± .02	0.41 ± .03+**
Hy	1	0.65 ± .06	0.64 ± .08	0.53 ± .05
	3	0.61 ± .05	0.48 ± .03	0.46 ± .02*
	6	0.50 ± .09	0.56 ± .04	0.69 ± .04
	12	1.17 ± .12	0.27 ± .02**	0.43 ± .03+**
	24	0.69 ± .15	0.27 ± .02*	0.27 ± .04*

N = 6 per group
* Significantly different than control $p < .05$
** Significantly different than control $p < .01$
\+ Significantly different than Tx and Saline $p < .05$
++ Significantly different than Tx and Saline $p < .01$

TABLE 12 The effect of thyroidectomy and three days protriptyline on MAO activity in three brain regions.

	Time	Sham Operated and Saline	Thyroidectomized and Saline	Thyroidectomized and Protriptyline
Tryptamine				
CS	3	1.71 ± .18	2.09 ± .18	2.48 ± .15**
	6	2.42 ± .15	2.38 ± .20	2.28 ± .22
	12	2.35 ± .12	3.20 ± .36*	2.30 ± .16
	24	2.38 ± .35	2.49 ± .20	2.14 ± .08
F	3	3.21 ± .23	2.95 ± .14	2.85 ± .16
	6	3.07 ± .25	4.06 ± .57	6.28 ± 1.29
	12	2.64 ± .10	3.09 ± .25	3.17 ± .09**
	24	2.96 ± .15	3.34 ± .38	2.98 ± .25
H	3	1.70 ± .10	2.19 ± .27	2.23 ± .21
	6	2.76 ± .32	2.12 ± .16	2.19 ± .13
	12	2.12 ± .29	3.32 ± .23**	2.38 ± .36++
	24	2.25 ± .25	2.75 ± .24*	2.02 ± .18++
Serotonin				
CS	3	0.51 ± .05	0.46 ± .04	0.54 ± .05
	6	0.65 ± .06	0.56 ± .07	0.57 ± .06
	12	0.52 ± .03	0.75 ± .09*	0.64 ± .04*
	24	0.62 ± .10	0.49 ± .06	0.46 ± .01
F	3	0.98 ± .06	0.62 ± .03**	0.58 ± .03**
	6	0.92 ± .07	1.09 ± .18	1.46 ± .36
	12	0.53 ± .02	0.68 ± .07*	0.69 ± .03**
	24	0.70 ± .08	0.73 ± .10	0.64 ± .05
H	3	0.50 ± .05	0.50 ± .06	0.40 ± .03
	6	0.79 ± .09	0.55 ± .09	0.47 ± .03*+
	12	0.43 ± .04	0.69 ± .04**	0.67 ± .11*
	24	0.54 ± .05	0.52 ± .04	0.40 ± .03*++

N = 6 per group
* Significantly different than control $p < .05$
** Significantly different than control $p < .01$
\+ Significantly different than thyroidectomized-saline $p < .05$
++ Significantly different than thyroidectomized-saline $p < .01$

TABLE 13 The effect of thyroidectomy and six days protriptyline on MAO activity in three brain regions.

	Time	Sham Operated and Saline	Thyroidectomized and Saline	Thyroidectomized and Protriptyline
Tryptamine				
CS	3	2.33 ± .23	2.08 ± .12	2.13 ± .19
	6	2.88 ± .15	1.68 ± .16**	1.77 ± .08**
	12	2.05 ± .14	2.41 ± .18	1.81 ± .24
	24	3.38 ± .37	2.59 ± .31	2.90 ± .18
F	3	3.43 ± .27	3.09 ± .16*	3.21 ± .18
	6	3.09 ± .09	2.19 ± .37	2.89 ± .17
	12	2.97 ± .16	2.62 ± .15	2.60 ± .16
	24	3.94 ± .44	5.05 ± .51	3.22 ± .23++
H	3	2.11 ± .15	1.89 ± .10	1.86 ± .22
	6	2.57 ± .31	1.67 ± .14*	2.26 ± .26
	12	2.25 ± .25	2.40 ± .15	1.64 ± .14*++
	24	2.37 ± .24	2.09 ± .25	2.09 ± .18
Serotonin				
CS	3	0.52 ± .05	0.52 ± .04	0.69 ± .04*++
	6	0.66 ± .05	0.57 ± .05	0.55 ± .03*
	12	0.54 ± .05	0.79 ± .05*	0.54 ± .06+
	24	0.60 ± .05	0.50 ± .05	0.50 ± .05
F	3	0.75 ± .06	0.68 ± .06*	0.83 ± .07
	6	0.70 ± .06	0.94 ± .14*	0.87 ± .07*
	12	0.70 ± .07	0.79 ± .04	0.69 ± .04
	24	1.03 ± .20	1.03 ± .10	0.58 ± .05++
H	3	0.44 ± .03	0.46 ± .04	0.58 ± .04**+
	6	0.59 ± .07	0.46 ± .07	0.61 ± .07
	12	0.53 ± .05	0.73 ± .04**	0.42 ± .04*++
	24	0.38 ± .04	0.33 ± .06	0.40 ± .04

N = 6 per group
* Significantly different than control $p < .05$
** Significantly different than control $p \geq .01$
\+ Significantly different than thyroidectomized-saline $p < .05$
++ Significantly different than thyroidectomized-saline $p < .01$

TABLE 14 Kinetic Parameters for Monoamine Oxidase Activity in Three Brain Regions

Corpus Striatum: using C^{14}-tryptamine as Substrate

1/Vmax	= 0.880116	1/Km	= 1.15749×10^{5}
Vmax	= 1.136213	Km	= 9.0×10^{-6} M
R^2	= 0.9988	slope	= 0.000008

: using C^{14}-serotonin as Substrate

1/Vmax	= 4.18507	1Km	= 3.607329×10^{4}
Vmax	= 0.23895	Km	= 28.0×10^{-6} M
R^2	= 0.9961	slope	= 0.000116

Forebrain: using C^{14}-tryptamine as Substrate

1/Vmax	= 0.324503	1/Km	= 8.18113×10^{4}
Vmax	= 3.08164	Km	= 12.0×10^{-6} M
R^2	= 0.9994	slope	= 0.000004

: using C^{14}-serotonin as Substrate

1/Vmax	= 1.2887	1/Km	= 2.2044×10^{4}
Vmax	= 0.7759	Km	= 24.0×10^{-6} M
R^2	= 0.9988	slope	= 0.0001

Hypothalamus: using C^{14}-tryptamine as Substrate

1/Vmax	= 0.63429	1/Km	= 1.1684×10^{5}
Vmax	= 1.576561	Km	= 9.0×10^{-6} M
R^2	= 0.99897	slope	= 0.00005

: using C^{14}-serotonin as Substrate

1/Vmax	= 2.88438	1/Km	= 3.6383×10^{4}
Vmax	= 0.346695	Km	= 27.0×10^{-6} M
R^2	= 0.99753	slope	= 0.000079

N=6 for all determinations using linear regression analysis

TABLE 15 Michalis-Menton Data for Corpus Striatum using C^{14}-tryptamine as Substrate (V vs S)

	2.0	5.0	10.0	20.0	30.0	Substrate Conc. (10^{-6} M) C^{14}-tryptamine
MEAN	0.290	0.543	0.811	1.124	1.163	
S.E.M.	0.07	0.12	0.17	0.23	0.24	

N=6 Values represent the Mean ± S.E.M. for nmoles of 5-HIAA formed/hr./ug DNA

TABLE 16 Lineweaver-Burke Data for Corpus Striatum using C^{14}-tryptamine as Substrate (1/V vs 1/S)

	33.3	50.0	100.0	200.0	500.0	(x 10^3)
MEAN	4.677	2.411	1.674	1.175	1.180	
S.E.M.	1.12	0.54	0.44	0.29	0.34	

30.0	20.0	10.0	5.0	2.0	(Substrate Conc. 10^{-6} M) C^{14}-tryptamine

N=6 Values represent the Mean ± S.E.M. for nanomoles of 5-HIAA formed/hr/ug DNA

TABLE 17 Michalis-Menton Data for Corpus Striatum using C^{14}-serotonin as Substrate (V vs S)

	2.0	5.0	10.0	20.0	30.0	Substrate Conc. (10^{-6}M) C^{14}-serotonin
MEAN	0.033	0.069	0.129	0.183	0.205	
S.E.M.	0.01	0.02	0.04	0.07	0.10	

N=6 Values represent the Mean ± S.E.M. for nmoles of IAA formed/hr./ug DNA

TABLE 18 Lineweaver-Burke Data for Corpus Striatum using C^{14}-serotonin as Substrate (1/V vs 1/S)

	33.3	50.0	100.0	200.0	500.0	(x 10^3)
MEAN	61.957	28.267	14.761	11.895	6.521	
S.E.M.	24.06	10.65	5.08	5.27	2.50	

30.0	20.0	10.0	5.0	2.0	(Substrate Conc. 10^{-6}M) C^{14}-serotonin

N=6 Values represent the Mean ± S.E.M. for nmoles of IAA formed/hr./ug DNA

TABLE 19 Michalis-Menton Data for Forebrain using C^{14}-tryptamine as Substrate (V vs S)

	2.0	5.0	10.0	20.0	30.0	Substrate Conc. (10^{-6}M) C^{14}-tryptamine
MEAN	0.466	0.936	1.417	2.057	2.333	
S.E.M.	0.06	0.12	0.12	0.23	0.22	

N=6 Values represent the Mean ± S.E.M. for nmoles of 5-HIAA formed/hr./ug DNA

TABLE 20 Lineweaver-Burked Data for Forebrain using C_{14}-tryptamine as Substrate (1/V vs 1/S)

	33.3	50.0	100.0	200.0	500.0	(x 10^3)
MEAN	2.297	1.148	0.726	0.510	0.445	
S.E.M.	0.25	0.13	0.05	0.05	0.04	
	30.0	20.0	10.0	5.0	2.0	(Substrate Conc. 10^{-6}M) C^{14}-tryptamine

N=6 Values represent the Mean ± for nanomoles of 5-HIAA formed/hr./ug DNA

TABLE 21 Michalis-Menton Data for Forebrain using C^{14}-serotonin as Substrate (V vs S)

	2.0	5.0	10.0	20.0	30.0	Substrate Conc. (10^{-6}M) C^{14}-serotonin
MEAN	0.445	0.109	0.189	0.288	0.365	
S.E.M.	0.01	0.02	0.04	0.04	0.04	

N=6 Values represent the Mean ± S.E.M. for nmoles of IAA formed/hr./ug DNA

TABLE 22 Lineweaver-Burke Data for Forebrain using C^{14}-serotonin as Substrate (1/V vs 1/S)

	33.3	50.0	100.0	200.0	500.0	(x 10^3)
MEAN	30.327	13.367	7.572	4.000	2.817	
S.E.M.	4.34	1.46	0.91	0.39	0.25	

30.0	20.0	10.0	5.0	2.0	(Substrate Conc. 10^{-6}M) C^{14}-serotonin

N=6 Values represent the Mean ± S.E.M. for nanomoles of IAA formed/hr. ug DNA

TABLE 23 Michalis-Menton Data for Hypothalamus using C^{14}-tryptamine as Substrate (V vs S)

	2.0	5.0	10.0	20.0	30.0	Substrate Conc. (10^{-6}M) C^{14}-tryptamine
MEAN	0.334	0.617	0.951	1.299	1.511	
S.E.M.	0.05	0.07	0.15	0.19	0.23	

N=6 Values represent the Mean ± S.E.M. for nanomoles of 5-HIAA formed/hr./ug DNA

TABLE 24 Lineweaver-Burke Data for Hypothalamus using C^{14}-tryptamine as Substrate (1/V vs 1/S)

	33.3	50.0	100.0	200.0	500.0	(x 10^3)
MEAN	3.329	1.770	1.196	0.878	0.793	
S.E.M.	0.45	0.25	0.19	0.16	0.18	

30.0	20.0	10.0	5.0	2.0	(Substrate Conc. 10^{-6}M) C^{14}-tryptamine

N=6 Values represent the Mean ± S.E.M. for nmoles of 5-HIAA formed/hr./ug DNA

TABLE 25 Michalis-Menton Data for Hypothalamus using C^{14}-serotonin as Substrate (V vs S)

	2.0	5.0	10.0	20.0	30.0	Substrate Conc. (10^{-6}M) C^{14}-serotonin
MEAN	0.027	0.060	0.107	0.171	0.185	
S.E.M.	0.01	0.02	0.02	0.04	0.02	

N=6 Values represent the Mean ± S.E.M. for nmoles of IAA formed/hr./ug DNA

TABLE 26 Lineweaver-Burke Data for Hypothalamus using C^{14}-serotonin as Substrate (1/V vs 1/S)

	33.3	50.0	100.0	200.0	500.0	(x 10^3)
MEAN	42.068	19.947	11.061	6.535	4.838	
S.E.M.	5.95	3.64	1.71	1.10	0.66	

30.0	20.0	10.0	5.0	2.0	(Substrate Conc. 10^{-6}M) C^{14}-serotonin

N=6 Values represent the Mean ± S.E.M. for nanomoles of IAA formed/hr./ug DNA

TABLE 27 In Vitro Monoamine Oxidase Activity following Preincubation with varying molar concentrations of Protriptyline (Preincubation - 30 minutes at 4° C)

Corpus Striatum	No Protriptyline	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	M.
MEAN	2.135	2.085	2.024	2.020	2.000	1.977	2.009	1.825	1.591	0.447	
S.D.	1.067	1.063	1.120	1.127	1.149	0.996	1.032	0.933	0.978	0.323	
S.E.M.	0.435	0.434	0.457	0.460	0.469	0.398	0.421	0.381	0.399	0.132	
Forebrain											
MEAN	13.005	11.955	11.902	11.412	11.478	11.548	11.281	11.026	10.370	3.423	
S.D.	2.931	2.538	2.563	2.395	2.184	2.245	2.139	2.211	2.120	0.704	
S.E.M.	1.197	1.032	1.046	0.978	0.891	0.917	0.874	0.903	0.866	0.288	
Hypothalamus											
MEAN	1.678	1.599	1.536	1.476	1.578	1.523	1.506	1.379	1.162	0.278	
S.D.	0.681	0.649	0.590	0.598	0.678	0.635	0.668	0.579	0.492	0.166	
S.E.M.	0.278	0.265	0.241	0.244	0.277	0.259	0.273	0.236	0.201	0.068	

N=6 for each determination

Values represent the nanomoles of 5-HIAA formed/hr./ug DNA

Monoamine Oxidase substrate used in this experiment was C^{14}-tryptamine

TABLE 28 In Vitro Monoamine Oxidase Activity following Preincubation with varying molar concentrations of Protriptyline (Preincubation - 30 minutes at 4° C)

Corpus Striatum	No Protriptyline	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3} M.
MEAN	0.591	0.556	0.562	0.514	0.516	0.518	0.503	0.478	0.352	0.017
S.D.	0.392	0.385	0.359	0.344	0.365	0.392	0.335	0.347	0.234	0.022
S.E.M.	0.160	0.157	0.146	0.141	0.149	0.160	0.137	0.142	0.095	0.009
Forebrain										
MEAN	3.530	3.291	3.291	3.289	3.230	3.280	3.200	2.943	2.214	0.221
S.D.	0.813	0.631	0.716	0.671	0.736	0.746	0.803	0.874	0.440	0.052
S.E.M.	0.332	0.258	0.292	0.274	0.300	0.304	0.328	0.357	0.180	0.021
Hypothalamus										
MEAN	0.434	0.390	0.371	0.400	0.411	0.382	0.367	0.347	0.210	0.009
S.D.	0.213	0.192	0.161	0.181	0.211	0.195	0.203	0.183	0.109	0.010
S.E.M.	0.087	0.978	0.066	0.974	0.086	0.080	0.083	0.075	0.045	0.004

N=6 for each determination

Values represent the nanomoles of IAA formed/hr./ug DNA

Monoamine Oxidase substrate used in this experiment was C^{14}-serotonin

TABLE 29 MAO Activity following preincubation with various molar concentrations of T_3 and for various preincubation time intervals

Corpus Striatum

Molar Concentration of T_3	Preincubation Times (Minutes)			
	No Preincubation	15 minutes	30 minutes	60 minutes
No T_3	0.480 ± 0.07	0.359 ± 0.04	0.403 ± 0.03	0.427 ± 0.04
10^{-9}M	0.320 ± 0.07	0.292 ± 0.07	0.302 ± 0.05	0.266 ± 0.07
10^{-7}M	0.334 ± 0.07	0.334 ± 0.13	0.322 ± 0.09	0.259 ± 0.09
10^{-5}M	0.263 ± 0.05	0.317 ± 0.12	0.324 ± 0.06	0.288 ± 0.09
10^{-4}M	0.194 ± 0.03	0.218 ± 0.03	0.167 ± 0.02	0.184 ± 0.03
10^{-3}M	0.084 ± 0.02	0.100 ± 0.02	0.069 ± 0.03	0.065 ± 0.01

N=3 for each determination

Values represent the Mean ± S.E.M., and are reported as nanomoles IAA formed/hr./ug DNA

Substrate employed was C^{14}-serotonin

TABLE 30 MAO Activity following preincubation with various molar concentrations of T_3 and for various preincubation time intervals

Forebrain

Molar Concentration of T_3	Preincubation Times (Minutes)			
	No Preincubation	15 minutes	30 minutes	60 minutes
No T_3	0.546 ± 0.05	0.551 ± 0.02	0.539 ± 0.03	0.523 ± 0.01
10^{-9}M	0.399 ± 0.01	0.397 ± 0.05	0.397 ± 0.05	0.384 ± 0.06
10^{-7}M	0.408 ± 0.02	0.419 ± 0.07	0.424 ± 0.07	0.405 ± 0.06
10^{-5}M	0.352 ± 0.04	0.404 ± 0.05	0.435 ± 0.04	0.376 ± 0.04
10^{-4}M	0.278 ± 0.01	0.302 ± 0.05	0.291 ± 0.03	0.254 ± 0.01
10^{-3}M	0.177 ± 0.04	0.145 ± 0.03	0.142 ± 0.03	0.125 ± 0.03

N=3 for each determination

Values represent the Mean ± S.E.M., and are reported as nanomoles IAA formed/hr./ug DNA

Substrate employed was C^{14}-serotonin

TABLE 31 MAO Activity following preincubation with various molar concentrations of T_3 and for various preincubation time intervals

Hypothalamus

Molar Concentration of T_3	Preincubation Times (Minutes)			
	No Preincubation	15 minutes	30 minutes	60 minutes
No T_3	0.224 ± 0.02	0.207 ± 0.04	0.203 ± 0.03	0.192 ± 0.04
10^{-9}M	0.257 ± 0.02	0.201 ± 0.09	0.156 ± 0.02	0.142 ± 0.07
10^{-7}M	0.194 ± 0.05	0.215 ± 0.07	0.215 ± 0.08	0.151 ± 0.07
10^{-5}M	0.149 ± 0.03	0.204 ± 0.08	0.183 ± 0.06	0.112 ± 0.07
10^{-4}M	0.068 ± 0.02	0.094 ± 0.05	0.095 ± 0.03	0.047 ± 0.02
10^{-3}M	0.029 ± 0.01	0.044 ± 0.03	0.032 ± 0.02	0.009 ± 0.01

N=3 for each determination

Values represent the MEAN ± S.E.M., and are reported as nanomoles IAA formed/hr./ug DNA

Substrate employed was C^{14}-serotonin

TABLE 32 MAO Activity following preincubation with various molar concentrations of T_3 and for various preincubation time intervals.

Corpus Striatum

Molar Concentration of T_3	Preincubation Times (Minutes)			
	No Preincubation	15 minutes	30 minutes	60 minutes
No T_3	1.121 ± 0.18	1.243 ± 0.18	1.384 ± 0.19	1.313 ± 0.19
10^{-9}M	1.208 ± 0.18	1.318 ± 0.19	1.398 ± 0.19	1.450 ± 0.12
10^{-7}M	1.281 ± 0.17	1.463 ± 0.27	1.441 ± 0.26	1.377 ± 0.17
10^{-5}M	1.274 ± 0.18	1.347 ± 0.16	1.253 ± 0.16	1.318 ± 0.18
10^{-4}M	1.005 ± 0.17	0.932 ± 0.11	0.977 ± 0.15	1.019 ± 0.31
10^{-3}M	0.466 ± 0.08	0.489 ± 0.06	0.426 ± 0.06	0.622 ± 0.17

N=3 for each determination

Values represent the MEAN ± S.E.M., and are reported as nanomoles 5-HIAA formed/hr./ug DNA

Substrate employed was C^{14}-tryptamine

TABLE 33 MAO Activity following preincubation with various molar concentrations of T_3 and for various preincubation time intervals

Forebrain

Molar Concentration of T_3	Preincubation Times (Minutes)			
	No Preincubation	15 minutes	30 minutes	60 minutes
No T_3	3.220 ± 0.53	3.653 ± 0.59	3.617 ± 0.45	3.641 ± 0.38
10^{-9}M	3.609 ± 0.36	3.758 ± 0.47	3.945 ± 0.45	3.886 ± 0.37
10^{-7}M	3.616 ± 0.36	3.701 ± 0.51	3.819 ± 0.54	3.813 ± 0.42
10^{-5}M	3.331 ± 0.34	3.532 ± 0.42	3.506 ± 0.43	3.547 ± 0.39
10^{-4}M	2.906 ± 0.31	2.803 ± 0.49	2.994 ± 0.43	3.174 ± 0.38
10^{-3}M	1.779 ± 0.24	1.847 ± 0.22	1.724 ± 0.20	2.314 ± 0.49

N=3 for each determination

Values represent the MEAN ± S.E.M., and are reported as nanomoles 5-HIAA formed/hr./ug DNA

Substrate employed was C^{14}-tryptamine

TABLE 34 MAO Activity following preincubation with various molar concentrations of T_3 and for various preincubation time intervals

Hypothalamus

Molar Concentration of T_3	Preincubation Times (Minutes)			
	No Preincubation	15 minutes	30 minutes	60 minutes
No T_3	1.156 ± 0.26	1.605 ± 0.33	1.727 ± 0.25	1.615 ± 0.37
10^{-9}M	1.229 ± 0.26	1.653 ± 0.31	1.737 ± 0.34	1.559 ± 0.25
10^{-7}M	1.235 ± 0.26	1.815 ± 0.40	1.767 ± 0.39	1.554 ± 0.28
10^{-5}M	1.257 ± 0.23	1.488 ± 0.30	1.581 ± 0.29	1.555 ± 0.23
10^{-4}M	0.931 ± 0.19	1.122 ± 0.22	1.114 ± 0.22	1.173 ± 0.21
10^{-3}M	0.473 ± 0.13	0.534 ± 0.11	0.497 ± 0.10	0.701 ± 0.24

N=3 for each determination

Values represent the MEAN ± S.E.M.,and are reported as nanomoles 5-HIAA formed/hr./ug DNA

Substrate employed was C^{14}-tryptamine

FIGURE 7

TITLE: The *in vitro* effect of various molar concentrations of T_3 on activity of monoamine oxidase from three brain regions

LEGEND

Brain homogenates were preincubated with concentrations of triiodothyronine (T_3) from 10^{-3} to 10^{-9} M, or without T_3, for 60 minutes (similar results, not depicted here, were obtained when the preincubation period was 0, 15 or 30 minutes). Monoamine oxidase activity was determined by measurement of concentrations of indole acetic acid (IAA) formed per hour per microgram of tissue DNA following addition of ^{14}C-serotonin to the homogenates. Each point illustrated is the mean of three determinations with the standard error depicted.

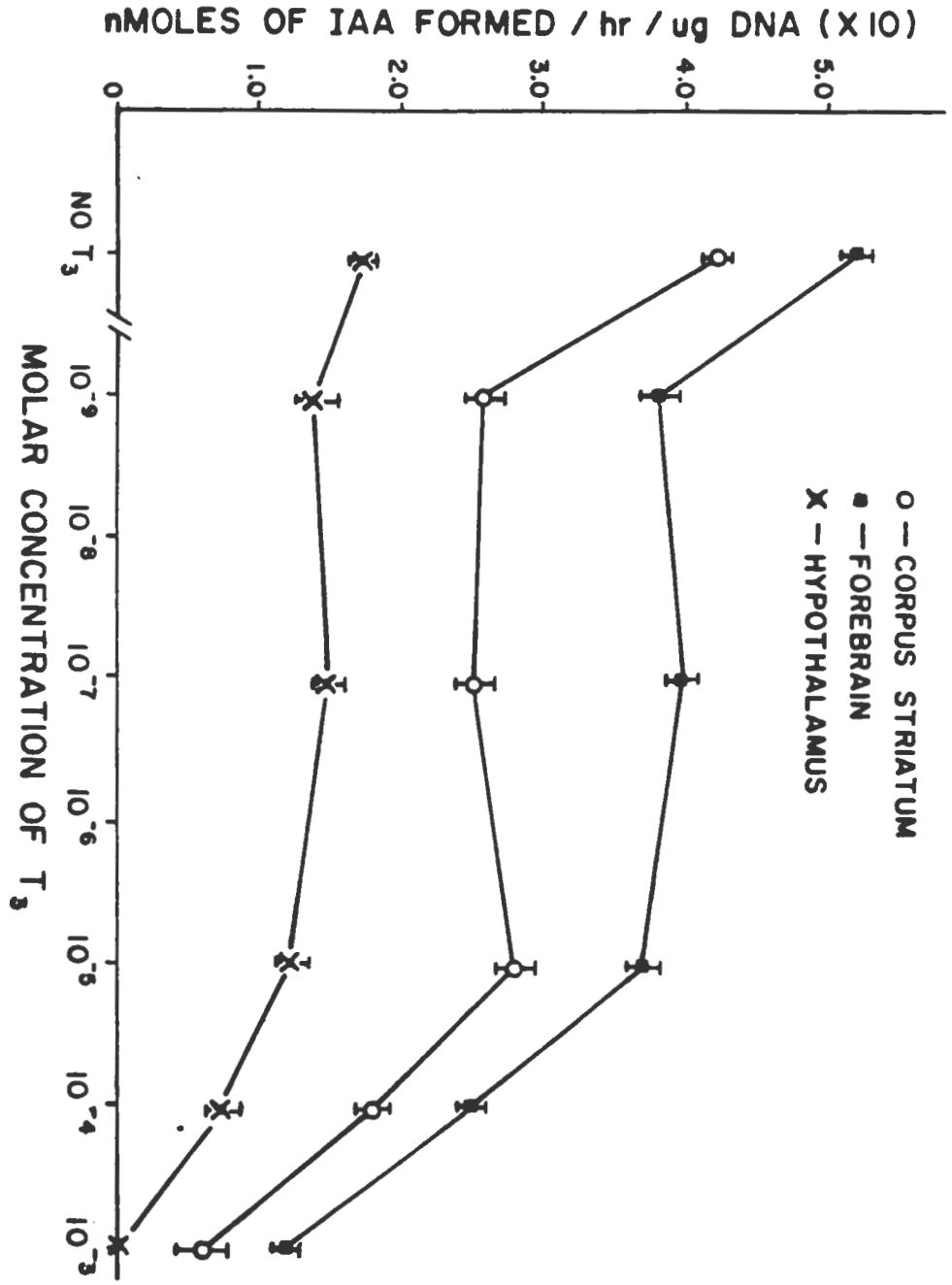

nMOLES OF IAA FORMED / hr / ug DNA (X 10)
0
1.0
2.0
3.0
4.0
5.0
NO T_3
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
MOLAR CONCENTRATION OF T_3
O — CORPUS STRIATUM
● — FOREBRAIN
X — HYPOTHALAMUS

TABLE 35 Effect of Trilodothyronine on MAO Activity in Three Brain Regions

	Corpus Striatum		Forebrain		Hypothalamus	
	Saline	T_3	Saline	T_3	Saline	T_3
Days of Injection						
No Injection	2.151 ± 0.26		2.968 ± 0.18		2.147 ± 0.18	
1	3.313 ± 0.21	2.647 ± 0.29	3.741 ± 0.56	3.716 ± 0.71	1.810 ± 0.15	1.711 ± 0.05
2	2.782 ± 0.16	3.317 ± 0.33	3.077 ± 0.25	3.259 ± 0.29	1.952 ± 0.21	1.981 ± 0.18
3	3.669 ± 0.37	4.339 ± 0.61	4.217 + 0.68	4.843 ± 0.54	2.795 ± 0.20	2.607 ± 0.43
4	3.808 ± 0.23	3.296 ± 0.42	3.932 ± 0.53	3.510 ± 0.29	2.703 ± 0.27	2.111 ± 0.31
5	3.550 ± 0.27	3.255 ± 0.17	4.093 ± 0.49	3.353 ± 0.36	2.590 ± 0.29	2.276 ± 0.13
6	2.835 ± 0.16	3.702 ± 0.55	3.593 ± 0.30	3.817 ± 0.15	2.092 ± 0.13	2.697 ± 0.17
7	3.406 ± 0.24	3.425 ± 0.40	4.050 ± 0.45	3.884 ± 0.44	2.640 ± 0.29	2.422 ± 0.14
8	3.012 ± 0.22	2.709 ± 0.47	4.888 ± 0.82	4.750 ± 0.69	2.038 ± 0.21	2.109 ± 0.27

N=6 for each determination, no statistical significance was observed

Values represent the MEAN ± S.E.M., and are reported as nanomoles 5-HIAA formed/hr./ug DNA

Substrate employed was C^{14}-tryptamine

All animals were sacrificed 24 hours after the last T_3 injection

TABLE 36 Effect of Thyroxine (T_4) on MAO Activity in Three Brain Regions

	Corpus Striatum		Forebrain		Hypothalamus	
	Saline	T_4	Saline	T_4	Saline	T_4
Days of Injection						
No Injection	0.345 ± 0.05		2.968 ± 0.18		2.147 ± 0.18	
1	0.933 ± 0.21	0.969 ± 0.29	0.871 ± 0.14	0.889 ± 0.23	0.675 ± 0.11	0.531 ± 0.11
2	0.420 + 0.06	0.548 + 0.07	0.454 + 0.04	0.613 ± 0.10	0.274 + 0.04	0.346 + 0.02
3	0.425 + 0.02	0.342 ± 0.06	0.529 ± 0.05	0.561 ± 0.05	0.255 ± 0.02	0.293 ± 0.04
4	0.464 ± 0.03	0.374 ± 0.04	0.589 ± 0.11	0.433 ± 0.03	0.259 ± 0.03	0.233 ± 0.01
5	0.359 ± 0.10	0.292 ± 0.07	0.506 ± 0.03	0.625 ± 0.11	-.262 ± 0.04	0.284 ± 0.04
6	0.354 ± 0.11	0.330 ± 0.05	0.401 ± 0.08	0.387 ± 0.05	0.244 ± 0.05	0.251 ± 0.06
7	0.618 + 0.04	0.518 ± 0.05	0.641 ± 0.05	0.738 ± 0.13	0.535 ± 0.11	0.375 ± 0.03
8	0.345 ± 0.06	0.295 ± 0.06	0.585 ± 0.13	0.494 ± 0.17	0.271 ± 0.06	0.209 ± 0.06

N=6 for each determination

Mean ± S.E.M.; Nmoles IAA formed/hr./ug DNA; serotonin as substrate

All animals were sacrificed 24 hours after the last T_4 injection

No statistical significance was observed

TABLE 37 Effect of Thyroxine (T_4) on MAO Activity in Three Brain Regions

	Corpus Striatum		Forebrain		Hypothalamus	
	Saline	T_4	Saline	T_4	Saline	T_4
Days of Injection						
No Injection	2.151 ± 0.26		2.716 ± 0.20		1.926 ± 0.17	
1	2.161 ± 0.19	2.241 ± 0.21	2.101 ± 0.15	2.184 ± 0.17	1.554 ± 0.15	1.373 ± 0.21
2	2.043 ± 0.34	2.724 ± 0.52	4.894 ± 1.27	3.202 ± 0.59	2.128 ± 0.36	2.048 ± 0.20
3	2.886 ± 0.09	2.415 ± 0.35	3.468 ± 0.38	3.477 ± 0.41	2.194 ± 0.25	2.263 ± 0.22
4	2.954 ± 0.22	2.758 ± 0.24	3.969 ± 0.65	3.452 ± 0.35	2.253 ± 0.28	2.162 ± 0.17
5	2.197 ± 0.53	1.691 ± 0.25	3.394 ± 0.28	3.474 ± 0.22	1.900 ± 0.39	1.938 ± 0.20
6	1.792 ± 0.28	1.557 ± 0.11	2.321 ± 0.23	2.089 ± 0.08	1.619 ± 0.14	1.256 ± 0.19
7	1.967 ± 0.13	1.365 ± 0.18	2.190 ± 0.16	2.847 ± 0.33	1.450 ± 0.24	1.095 ± 0.11
8	1.725 ± 0.18	1.482 ± 0.13	2.966 ± 0.25	2.460 ± 0.51	1.518 ± 0.14	1.274 ± 0.28

N=6 for each determination

MEAN ± S.E.M.; Nmoles 5-HIAA formed/hr./ug DNA; C^{14}-tryptamine as substrate

All animals were sacrificed 24 hours after the last T_4 injection

No statistical significance was observed

TABLE 38 Norepinephrine Pool Size Levels

Condition	Corpus Striatum	Forebrain	Hypothalamus
Controls (saline)	0.954 ± 0.04 (N=12)	0.459 ± 0.03 (N=12)	3.573 ± 0.23 (N=70)
T_3 - treated (1mg/kg/day - 7 days)	0.827 ± 0.11*,** (N=12)	0.421 ± 0.04*** (N=12)	2.828 ± 0.135* (N=31)
Thyroidectomized (at 7 wks., 8 wks. prior to sacrificed)	1.379 ± 0.13 (N=12)	0.719 ± 0.03**,*** (N=12)	3.396 ± 0.27 (N=12)
Sham Operated	1.221 ± 0.13 (N=12)	0.521 ± 0.03* (N=12)	3.139 ± 0.34 (N=12)

* $p < 0.01$ from Tx

** $p < 0.01$ from Sham

*** $p < 0.01$ from controls

TABLE 39 Numerical values for NE levels in hypothalamus following alpha-methyl-para-tyrosine administration and sacrificed three hours after the administration of alpha-methyl-para-tryosine. Data shown graphically in Figure 9.

Dosage of MT	NE Level		Number of Animals
No MT	Mean	3.3759	N=9
	S.D.	0.4112	
	S.E.M.	0.2444	
200 mg/kg	Mean	1.9865	N=8
	S.D.	0.2679	
	S.E.M.	0.0947	
400 mg/kg	Mean	1.5639	N=9
	S.D.	0.3326	
	S.E.M.	0.1109	
600 mg/kg	Mean	1.1204	N=3
	S.D.	0.3554	
	S.E.M.	0.2052	
800 mg/kg	Mean	1.5911	N=3
	S.D.	0.3798	
	S.E.M.	0.2193	

TABLE 40 Alpha-methyl-para-tyrosine dose response curve for tri-iodothyronine treated animals.

		Corpus Striatum	Forebrain	Hypothalamus
0 Time	MEAN	0.5821	0.2421	2.4926
No MT	S.D.	0.1580	0.0648	0.3632
	S.E.M.	0.0912	0.0374	0.2097
		N=3	N=3	N=3
200 mg/kg		0.3017	0.2390	1.8375
3 hr. pt.		0.1047	0.1084	0.2274
		0.0524	0.0542	0.1137
		N=4	N=4	N=4
400 mg/kg		0.3255	0.2090	1.2367
3 hr. pt.		0.2242	0.1211	0.5095
		0.1294	0.0699	0.2942
		N=3	N=3	N=3
600 mg/kg		0.3140	0.1668	1.4371
3 hr. pt.		0.1126	0.0768	0.8366
		0.0650	0.0443	0.4830
		N=3	N=3	N=3
800 mg/kg		0.2405	0.3303	1.7169
3 hr. pt.		0.1811	0.1075	1.2052
		0.1102	0.0621	0.6959
		N=3	N=3	N=3

TABLE 41 Turnover of Norepinephrine in the hypothalamus, corpus striatum and forebrain for control rats following administration of 400 mg/kg of alpha-methyl-para-tyrosine

TISSUE		TIME (hours after MT administration)						
		No MT	1.0	2.0	3.0	4.5	6.0	9.0
Corpus Striatum								
	MEAN	0.6978	0.5883	0.5781	0.4709	0.4292	0.3238	0.3080
	S.D.	0.1767	0.1400	0.0870	0.2087	0.1086	0.0882	0.1241
	S.E.M.	0.0721	0.0572	0.0389	0.0852	0.0486	0.0360	0.0439
Forebrain								
	MEAN	0.4536	0.2644	0.1940	0.1419	0.1780	0.1998	0.1592
	S.D.	0.0747	0.0458	0.0972	0.0377	0.0482	0.0887	0.0479
	S.E.M.	0.0305	0.0187	0.0436	0.0169	0.0197	0.0362	0.0169
Hypothalamus								
	MEAN	3.4047	2.9928	2.1869	1.4213	0.8212	0.8001	0.6264
	S.D.	0.9982	0.6084	0.4625	0.3837	0.5195	0.2608	0.1573
	S.E.M.	0.4075	0.2721	0.1888	0.1269	0.2121	0.1166	0.0556

N=6 for each point

slope for hypothalamus = -0.6074 linear regression coefficient (r^2)= 0.9825

slope for corpus striatum = -0.0587 = 0.9680

slope for forebrain =-0.1298 = 0.9347

TABLE 42 Turnover of Norepinephrine in hypothalamus, corpus striatum and forebrain for rats treated with triiodothyronine (T_3) (1mg/kg/day - 7 days). MT - 400 mg/kg

TISSUE	TIME (hours after MT administration)					
	NO MT	1.0	2.0	3.0	4.5	6.0
Corpus Striatum						
MEAN	0.8971	0.8703	0.6821	0.4447	0.6505	0.6938
S.D.	0.0966	0.3074	0.2066	0.1375	0.1440	0.2251
S.E.M.	0.0394	0.1375	0.0924	0.0687	0.0644	0.1007
Forebrain						
MEAN	0.4408	0.3222	0.2767	0.2307	0.2086	0.1717
S.D.	0.1320	0.0606	0.0778	0.0340	0.0510	0.0742
S.E.M.	0.0539	0.0271	0.0345	0.0170	0.0255	0.0332
Hypothalamus						
MEAN	2.8280	2.4068	2.4474	1.8941	1.6823	1.2003
S.D.	0.7495	0.5586	0.4825	1.0193	0.8231	0.7216
S.E.M.	0.1346	0.2793	0.2158	0.5067	0.3766	0.3227

N=4 to 6 determinations for each point

slope for hypothalamus = -0.2615 linear regression coefficient (r^2) = 0.9625

slope for corpus striatum = -0.1545 = 0.9106

slope for forebrain = -0.0676 = 0.9350

FIGURE 23

TITLE: DNA standard curve

LEGEND

Known concentrations of deoxyribonucleic acid sodium salt (1.0, 2.0, 4.0, 8.0 and 12.0 μg) were added to assay tubes and reacted with potassium ferrous cyanide. DNA fluorescence was then read on a spectrophotofluorometric with excitation at 510 μ meters and emission at 400 μ meters.

The calculations involved in determining the DNA standard Curve are listed below. A linear regression was used to determine the regression coefficient.

a_o = 2.9774 = intercept (or blank) = 2.98 $\pm$ 0.32

a_1 = 7.3235 = slope (relative fluorescence units/ug DNA) = 7.32 $\pm$ 0.05

r^2 = 0.999803 = regression coefficient

Hg DNA	calculated fluorescence
0	2.9774
1	10.3010
2	17.6245
4	32.2716
8	61.5657
12	90.8598

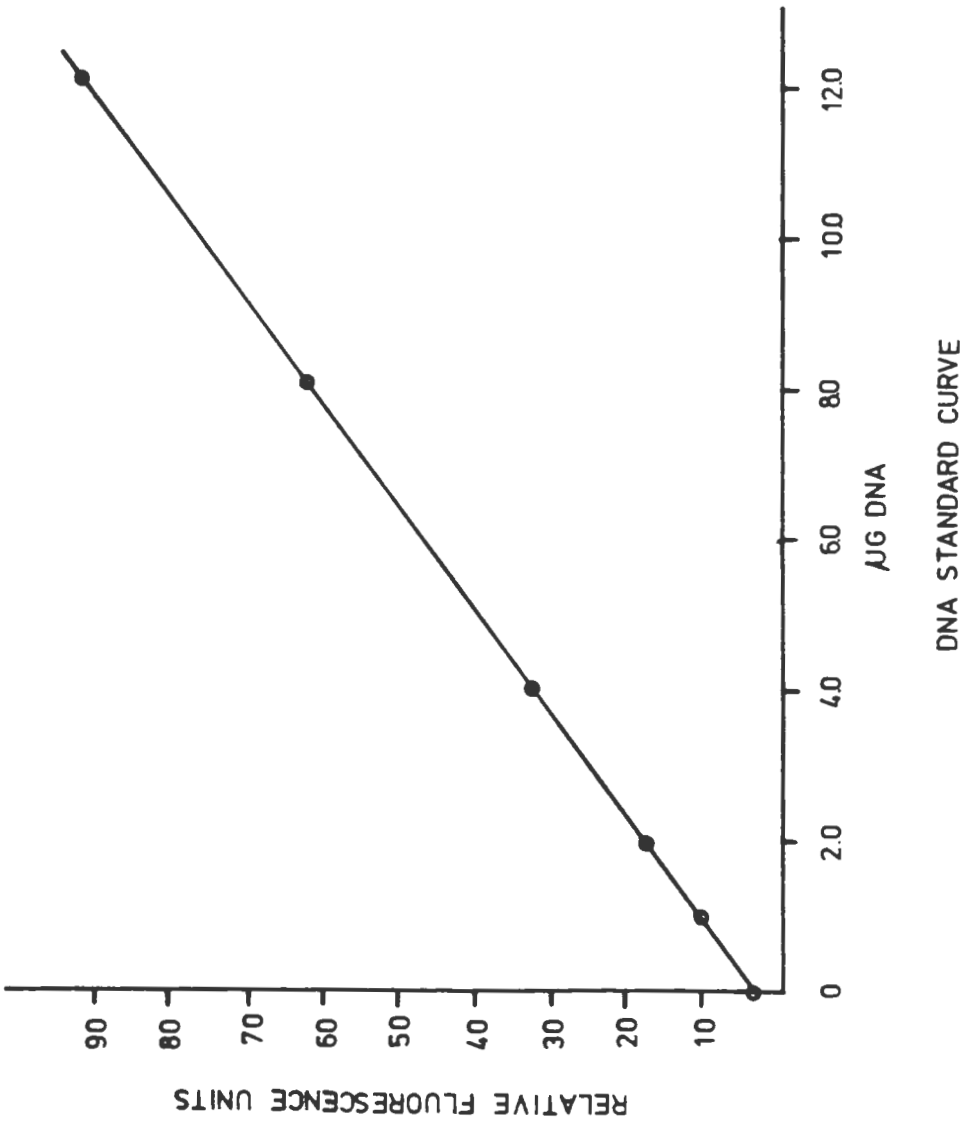
The standard error of a_o = 0.3174

The standard error of a_1 = 0.0514

Actual fluorescence units plotted

	0 (Bk)	1	2	4	8	12
X	3.20	9.92	17.28	32.53	62.29	90.38
s.d.	0.995	1.45	2.12	2.69	2.98	2.93
S.E.M.	0.22	0.33	0.47	0.60	0.67	0.66

At least 12 determinations were performed to calculate the MEAN $\pm$ S.E.M. (N=12).



DNA STANDARD CURVE

FIGURE 24

TITLE: Norepinephrine standard curve for an external norepinephrine standard

LEGEND

Varying concentrations of NE were added to assay tubes (0.1, 0.2, 0.3, 0.4 and 0.5 mg). Fluorescence was then recorded on a spectrophotometer. Each data point represents the MEAN ± S.E. for at least 3 experimental determinations. The linear regression coefficient was 0.9805.

Data used in Figure 24

Standards:	Relative Fluorescence Units	
0.1 ug NE	MEAN	12.4
	S.D.	1.9
	S.E.M.	1.1
0.2 ug NE		20.3
		2.3
		1.3
0.3 ug NE		27.4
		4.8
		2.8
0.4 ug NE		34.0
		1.4
		1.0
0.5 ug NE		47.3
		1.8
		1.3

N = 3 for all determinations

y-intercept = 3.23

slope = 83.5 relative fluorescence units per 1 ug NE standard

(r^2) = 0.9805

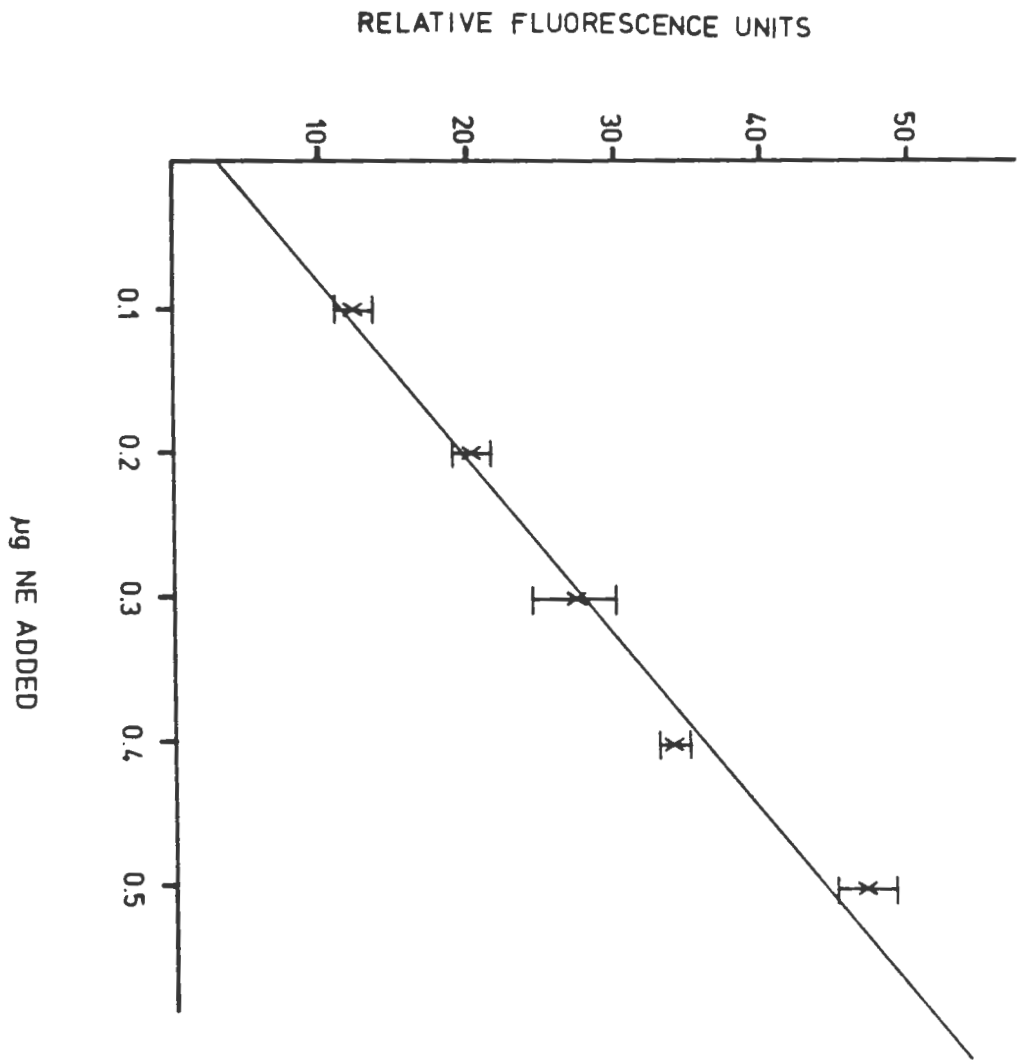

RELATIVE FLUORESCENCE UNITS
10
20
30
40
50
0.1
0.2
0.3
0.4
0.5
µg NE ADDED

FIGURE 25

TITLE: Norepinephrine standard curve for the internal H^3-norepinephrine standard

LEGEND

Varying concentrations of labelled H^3-NE were added to assay tubes and allowed to pass over prepared alumina columns and H^3-NE recovery was recorded. Each data point represents the MEAN $\pm$ S.E. for at least 3 experimental determinations. The linear regression coefficient is 0.9955.

Data used in Figure 25

Standards:	CPM
0.1 ug H^3-NE	MEAN 411.4
	S.D. 52.2
	S.E.M. 30.1
0.2 ug H^3-NE	739.2
	99.5
	57.5
0.3 ug H^3-NE	979.3
	126.5
	73.1
0.4 ug H^3-NE	1234.3
	201.9
	116.5
0.5 ug H^3-NE	1585.0
	466.1
	269.1

N = 3 for all determinations

y-intercept = 137.2

slope = 2842.3 = relative CPM per 1 ug H^3-NE

(r^2) = 0.9955

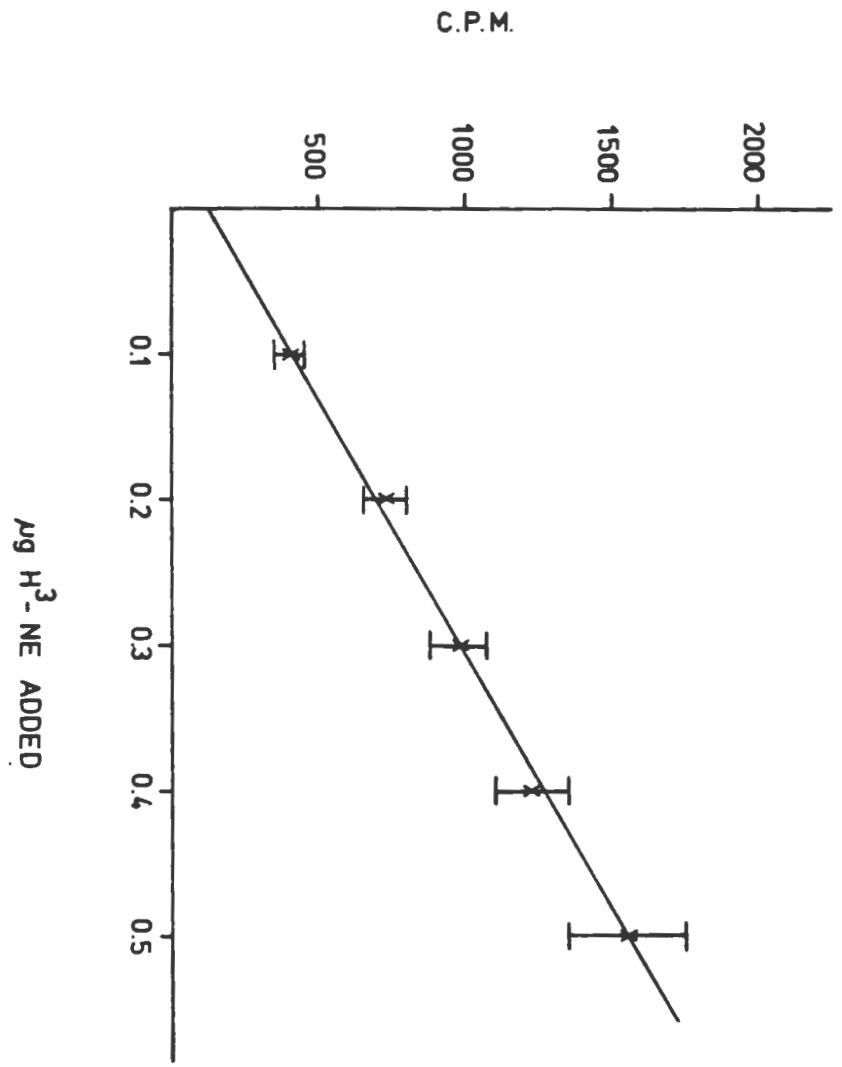

C.P.M.
500
1000
1500
2000
0.1
0.2
0.3
0.4
0.5
µg H³-NE ADDED

FIGURE 26

TITLE: Turnover of norepinephrine in the hypothalamus of rats treated with triiodothyronine (1 mg/kg/day - 7 days) and their respective controls

LEGEND

Animals were administered alpha-methyl-para-tyrosine and were sacrificed 0, 1, 2, 3, 4.5 and 6 hours after this MT administration. Turnover rates between these two groups, T_3 administered (-x-) and saline administered (-o-) were significantly different ($p < 0.01$) when turnover was measured between 0 time and 3 hours post MT administration. Triiodothyronine administered animals exhibited decreased NE turnover when compared to respective saline administered animals. Each point represents the MEAN $\pm$ S.E. for at least 5 experimental determinations.

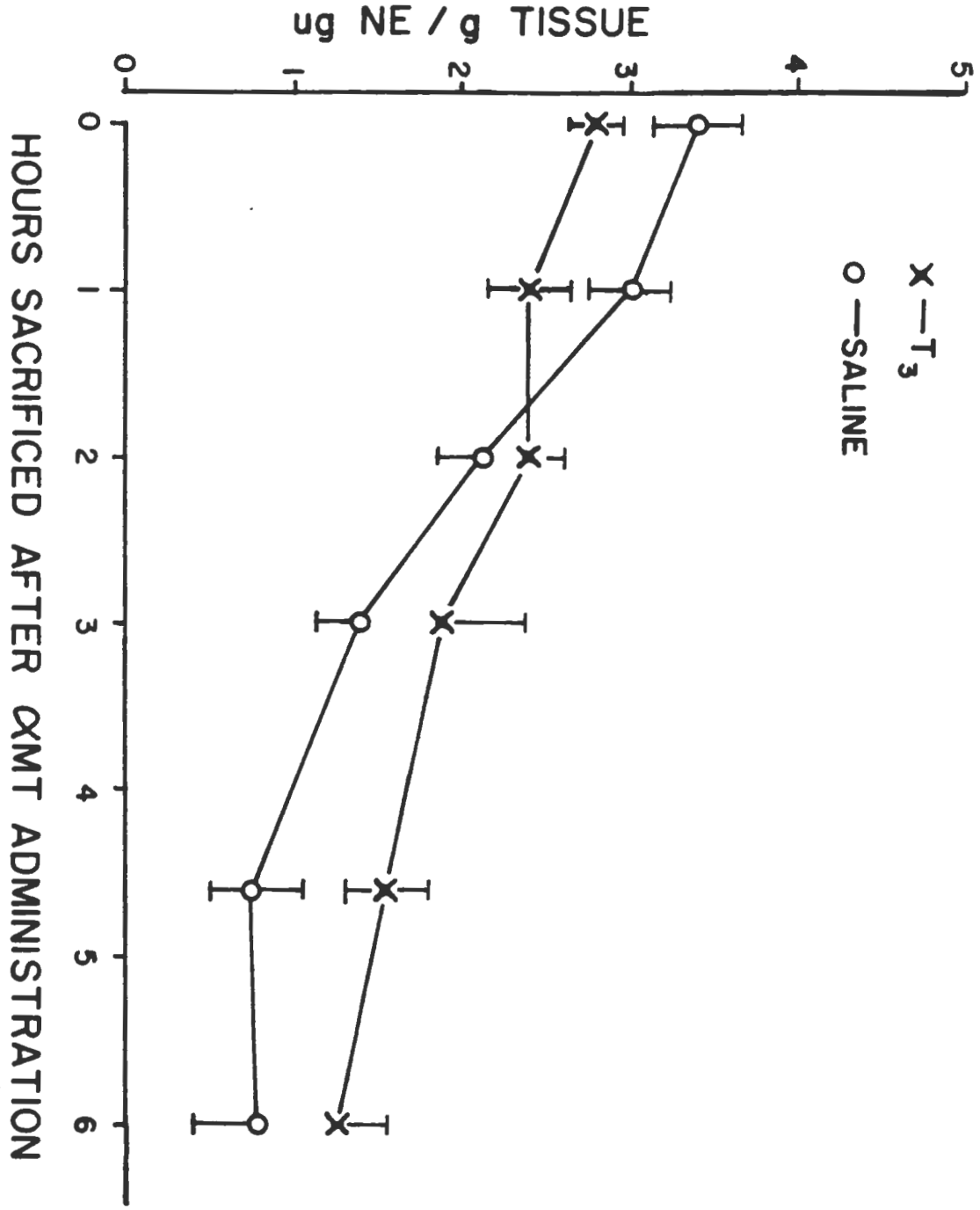

ug NE / g TISSUE
0
1
2
3
4
5
X — T_3
O — SALINE
HOURS SACRIFICED AFTER αMT ADMINISTRATION
0
1
2
3
4
5
6

TABLE 43 Norepinephrine turnover in thyroidectomized rats following 400 mg/kg MT administered I.P.

TISSUE		TIME (hours after MT administration)						
		No MT	1.0	2.0	3.0	4.5	6.0	9.0
Corpus Striatum								
	MEAN	1.2092	0.8718	0.7349	0.3185	0.5460	0.4164	0.6189
	S.D.	0.4222	0.4804	0.3520	0.1924	0.1294	0.1789	0.2966
	S.E.M.	0.1493	0.2149	0.1574	0.0860	0.0528	0.0730	0.1212
Forebrain								
	MEAN	0.6734	0.3794	0.2831	0.2469	0.2310	0.2957	0.2648
	S.D.	0.1123	0.0945	0.0783	0.0347	0.0562	0.2000	0.0774
	S.E.M.	0.0397	0.0423	0.0350	0.0155	0.0230	0.0817	0.0316
Hypothalamus								
	MEAN	3.2560	2.5190	1.2864	1.3817	1.6773	2.0973	2.0818
	S.D.	0.7005	0.5630	0.6429	0.5279	0.5145	0.6984	0.9936
	S.E.M.	0.2477	0.2518	0.2875	0.2361	0.2100	0.2851	0.4057

N=5 to 8 animals for each determination

slope for hypothalamus = -0.9848 linear regression coefficient (r^2) = 0.9793

slope for corpus striatum = -0.2809 = 0.9679

slope for forebrain = -0.1952 = 0.9212

TABLE 44 Norepinephrine turnover in SHAM operated animals following 400 mg/kg MT administered I.P.

TISSUE		TIME (hours after MT administration)						
		No MT	1.0	2.0	3.0	4.5	6.0	9.0
Corpus Striatum								
	MEAN	1.0213	0.7702	0.6463	0.5988	0.6497	0.3955	0.6377
	S.D.	0.2135	0.3099	0.1514	0.1367	0.1940	0.3604	0.3000
	S.E.M.	0.0755	0.1265	0.0677	0.0611	0.0788	0.1471	0.1225
Forebrain								
	MEAN	0.5364	0.3206	0.4189	0.5397	0.2106	0.2903	0.2442
	S.D.	0.1211	0.0401	0.0565	0.2082	0.0780	0.1577	0.1145
	S.E.M.	0.0428	0.0164	0.0253	0.0931	0.0318	0.0644	0.0467
Hypothalamus								
	MEAN	3.0530	2.2246	1.8903	1.9670	1.1626	1.0477	1.7161
	S.D.	0.6856	0.8266	0.5722	0.5490	0.5518	0.7040	0.3216
	S.E.M.	0.2424	0.3375	0.2336	0.2241	0.2253	0.2874	0.1313

N=6 for each determination

slope for hypothalamus = -0.3678 linear regression coefficient (r^2) = 0.8891

slope for corpus striatum = -0.1875 = 0.9631

slope for forebrain = -0.0728 = 0.9909

TABLE 45 Norepinephrine levels in three brain regions following a single acute administration of protriptyline or following 3 consecutive administrations of protriptyline. All animals were sacrificed 3 hours after the final protriptyline injection.

Brain Region		Protriptyline Admin. animals	Saline Admin. animals
One Admin.(acute)			
Corpus Striatum	MEAN	0.6682	0.6862
	S.D.	0.2106	0.1803
	S.E.M.	0.0860	0.0901
		N=6	N=5
Forebrain		0.3812	0.3779
		0.0458	0.1168
		0.0187	0.0477
		N=6	N=6
Hypothalamus		2.8048*	2.8606
		0.0401	0.7853
		0.0232	0.3512
		N=3	N=5
Three Admin.(subchronic)			
Corpus Striatum	MEAN	1.0008	0.9781
	S.D.	0.3702	0.2845
	S.E.M.	0.1511	0.1272
		N=6	N=5
Forebrain		0.4126	0.6490
		0.3056	0.2320
		0.1367	0.0947
		N=5	N=6
Hypothalamus		3.3400	4.0169
		0.8197	1.5751
		0.3347	0.6430
		N=6	N=6

*Significantly lower than controls when N=70, however, the difference is not significantly lower than its paired saline control. Therefore this difference may be due to injection stress, and may not really be a difference.

TABLE 46 Norepinephrine levels in three brain regions following six consecutive injections of protriptyline or following 18 consecutive injections of protriptyline. All animals were sacrificed 3 hours after the final protriptyline administration

Brain Region		Protriptyline Admin. animals	Saline Admin. animals
Six Admin.(subchronic)			
Corpus Striatum	MEAN	0.9880	0.8761
	S.D.	0.2138	0.3205
	S.E.M.	0.0571	0.0966
		N=14	N=11
Forebrain		0.5288	0.4523
		0.1479	0.1484
		0.0410	0.0397
		N=13	N=14
Hypothalamus		3.3694	3.7511
		1.2736	1.1922
		0.3404	0.4215
		N=14	N=8
Eighteen Admin.(chronic)			
Corpus Striatum	MEAN	0.7129	1.0129*
	S.D.	0.3278	0.4671
	S.E.M.	0.1037	0.1408
		N=10	N=11
Forebrain		0.3722	0.4114
		0.1022	0.1515
		0.0295	0.0437
		N=12	N=12
Hypothalamus		2.5661	3.5061*
		0.7011	1.0071
		0.2114	0.3560
		N=11	N=8

*Difference from protriptyline administered ($p < 0.01$)

TABLE 47 Norepinephrine depletion in acutely administered protriptyline animals

		HYPOTHALAMUS
No MT Admin.		
	MEAN	3.1740
	S.D.	0.6192
	S.E.M.	0.2769
		N=5
Hours after MT Administration		
1.0	MEAN	2.5383
	S.D.	0.8538
	S.E.M.	0.3486
		N=6
2.0		3.0440
		0.5972
		0.2671
		N=5
3.0		2.3917
		0.3514
		0.1435
		N=6
4.5		2.5867
		0.4275
		0.1745
		N=6
6.0		2.0500
		0.2868
		0.1283
		N=5

$(r^2) = 0.6213$

Slope = -0.1475

TABLE 48 Norepinephrine depletion in protriptyline treated animals (10 mg/kg/day-6 days-S.C.) and then administered MT 6 hours after their final protriptyline injection. These animals were then sacrificed at various time intervals after MT administration.

TIME after MT administration		NE levels
NO alpha-M-P-tyrosine	MEAN	2.5106
	S.D.	0.7771
	S.E.M.	0.3886
	N=4	
1 hour		2.2380
		0.5791
		0.2855
	N=4	
2 hours		1.6192
		0.4771
		0.2386
	N=4	
3 hours		1.8607
		0.7946
		0.4588
	N=3	

slope = -0.2471

(r^2) = 0.8449

TABLE 49 Norepinephrine levels in three brain areas of thyroidectomized rats and Sham-operated controls following 3 and 6 consecutive days of protriptyline administration. Animals were sacrificed 12 hours after the final protriptyline administration

Three days of Prot. Admin.		Corpus Striatum	Forebrain	Hypothalamus
Thyroidectomized Rats	MEAN	0.8411	0.5842	3.3131
	S.D.	0.5734	0.2318	0.4941
	S.E.M.	0.2341	0.0946	0.2017
		N=8	N=6	N=6
Sham-operated Controls		0.0659	0.4851	3.2305
		0.5222	0.1296	0.7209
		0.2132	0.0529	0.2943
		N=5	N=6	N=6
Six days of Prot. Admin.				
Thyroidectomized Rats	MEAN	0.7979	0.5909	2.6163
	S.D.	0.3550	0.1020	0.6866
	S.E.M.	0.1449	0.0456	0.2803
		N=6	N=6	N=6
Sham-operated Controls		0.5798	0.4740	2.8174
		0.2277	0.1230	1.2590
		0.1018	0.0502	0.5140
		N=6	N=6	N=6

TABLE 50 **Norepinephrine levels in three brain areas of thyroidectomized rats and Sham-operated controls following 12 and 18 consecutive days of protriptyline administration. Animals were sacrificed 12 hours after the final protriptyline injection**

Twelve days of Prot. Admin.		Corpus Striatum	Forebrain	Hypothalamus
Thyroidectomized Rats	MEAN	0.9537*	0.4708	3.0393
	S.D.	0.3722	0.1085	0.3806
	S.E.M.	0.1520	0.0485	0.1702
		N=6	N=6	N=6
Sham-operated Controls		1.4290	0.4608	3.8033
		0.3715	0.0779	0.8986
		0.1661	0.0332	0.3668
		N=8	N=6	N=5
Eighteen days of Prot. Admin.	MEAN	0.4887*	0.6672	2.9943
	S.D.	0.1717	0.2064	1.1603
	S.E.M.	0.0701	0.0843	0.4737
		N=5	N=6	N=6
Sham-operated Controls		0.7275	0.5591	2.9770
		0.1988	0.1538	0.2848
		0.0811	0.0626	0.1163
		N=6	N=6	N=6

*Statistically different from Control $p < 0.01$

TABLE 51 Alpha-methyl-para-tyrosine dose response curve in animals which have received protriptyline chronically (18 days, 10mg/kg/day) in the hypothalamus

Dose of MT		Hypothalamus
No MT	MEAN	2.5590
	S.D.	1.0676
	S.E.M.	0.2387
		N=20
200 mg/kg	MEAN	2.2408
	S.D.	0.6440
	S.E.M.	0.2880
		N=5
400 mg/kg	MEAN	2.0801
	S.D.	0.9180
	S.E.M.	0.2546
		N=13
600 mg/kg	MEAN	2.1090
	S.D.	1.5431
	S.E.M.	0.6299
		N=6
800 mg/kg	MEAN	2.1039
	S.D.	0.6098
	S.E.M.	0.3049
		N=4

TABLE 52 Alpha-methyl-para-tyrosine depletion curve in animals receiving 18 days chronic protriptyline administration (10 mg/kg/day)

TISSUE		TIME (hours after alpha-M-para-tyrosine administration)						
		0 Time No MT	1.0	2.0	3.0	4.5	6.0	9.0
Corpus Striatum								
	MEAN	0.6521	0.6606	0.7868	0.7830	0.4603	0.4534	0.2960
	S.D.	0.1699	0.1659	0.2212	0.3246	0.1563	0.1112	0.0483
	S.E.M.	0.0850	0.0742	0.1106	0.1452	0.0699	0.0699	0.0216
		N=4	N=5	N=4	N=5	N=5	N=5	N=5
Forebrain								
	MEAN	0.2741	0.4021	0.3351	0.2564	0.2250	0.2375	0.2849
	S.D.	0.0291	0.1545	0.0785	0.0606	0.0648	0.0297	0.1015
	S.E.M.	0.0168	0.0691	0.0351	0.0271	0.0324	0.0171	0.0454
		N=3	N=5	N=5	N=5	N=4	N=3	N=5
Hypothalamus								
	MEAN	2.4008	2.4572	1.9041	1.8610	1.4058	1.5247	1.0260
	S.D.	0.6864	0.4616	0.5598	0.5120	0.4701	0.6426	0.2227
	S.E.M.	0.3432	0.2665	0.2503	0.2560	0.2102	0.3213	0.1286
		N=4	N=3	N=5	N=4	N=5	N=4	N=3

*Alpha-methyl-para-tyrosine was administered 3 hours after the final protriptyline injection.
Corpus Striatum slope = -0.0464 linear regression coefficient (r^2) = 0.9384
Forebrain slope = -0.0113 = 0.8666
Hypothalamus slope = -0.1897 = 0.9331

TABLE 53 Norepinephrine depletion in hypothalamus following administration of yohimbine, in control (saline administered) animals

Dosage of Yohimbine		TIME (hours after Yohimbine administration)			
		0 Time (No Yonimbine)	1 hour	2.5 hours	5.0 hours
2.0 mg/kg					
	MEAN	3.3099	2.6988	3.5365	3.8163
	S.D.	0.5918	0.4708	0.8431	1.8294
	S.E.M.	0.2416	0.2106	0.3770	0.8181
		N=6	N=5	N=5	N=5
20.0 mg/kg					
	MEAN	3.3099	1.6829	1.4616	2.6973
	S.D.	0.5918	0.5312	0.7125	1.1293
	S.E.M.	0.2416	0.2656	0.4114	0.5646
		N=6	N=4	N=4	N=4

TABLE 54 Norepinephrine turnover in animals receiving saline, followed by yohimbine (20 mg/kg) followed by alpha-methyl-para-tyrosine (400 mg/kg)

		Hypothalamus
Controls (saline only)	MEAN	2.9968
	S.D.	0.5840
	S.E.M.	0.2920
		N=4
1 hour after Yohimbine admin.		1.9212
		0.4589
		0.2294
		N=4
1 hour after MT, 2 hours after Yohimbine		1.0348
		0.1929
		0.0965
		N=4
2 hours after MT, 3 hours after Yohimbine		0.9750
		0.1249
		0.0559
		N=4
3 hours after MT, 4 hours after Yohimbine		0.8724
		0.2391
		0.1069
		N=4
4.5 hours after MT, 5.5 hours after Yohimbine		0.9404
		0.2593
		0.1297
		N=4
6 hours after MT, 7 hours after Yohimbine		1.0261
		0.3847
		0.1924
		N=4

TABLE 55 Norepinephrine depletion in hypothalamus of chronically protriptyline administered animals following yohimbine administration (20 mg/kg). Animals were administered yohimbine 3 hours after the final protriptyline injection

		Hypothalamus
18 days Prot. admin., no Yohimbine	MEAN	2.9131
	S.D.	0.5240
	S.E.M.	0.2620
		N=4
18 days Prot., sacrificed 5 minutes after Yohimbine		2.8602
		0.4089
		0.1828
		N=4
18 days Prot., sacrificed 1 hour after Yohimbine		2.6571
		2.0500
		1.0250
		N=4
18 days Prot., sacrificed 3 hours after Yohimbine		2.9131
		0.5240
		0.2620
		N=4

TABLE 56 Norepinephrine turnover in hypothalamus of chronic protriptyline administered rats, followed by yohimbine administration (20 mg/kg) followed by alpha-M-p-tyrosine administration (400 mg/kg). Animals received yohimbine 3 hours after their protriptyline injection, and received alpha-M-p-tyrosine, 1 hour after the yohimbine injection

		Hypothalamus
18 days Prot. admin.	MEAN	2.9131
	S.D.	0.5240
	S.E.M.	0.2620
		N=4
18 days Prot., 5 minutes after Yohimbine		2.8602
		0.4089
		0.1828
		N=5
18 days Prot., 1 hour after Yohimbine, 5 minutes after MT		2.6571
		2.0500
		1.0250
		N=4
18 days Prot., 2 hours after Yohimbine, 1 hour after MT		1.7741
		0.7365
		0.4252
		N=4
18 days Prot. 3 hours after Yohimbine, 2 hours after MT		1.6614
		0.3737
		0.1868
		N=4
18 days Prot., 4 hours after Yohimbine, 3 hours after MT		1.6025
		0.3163
		0.1581
		N=4

TABLE 57 Comparison of turnover rates, level of statistical significance, slope and linear regression coefficients in both saline and protriptyline treated animals.

COMPARISON	slope	(r^2)	Probability	Group with decreased turnover
acute prot. (6') vs.	-0.1130	0.1183	0.6411 N.S.	----------
6 day prot. (6')	-0.2740	0.2003		
acute prot. (6') vs.	-0.1130	0.1183	0.7363 N.S.	----------
18 day prot. (6')	-0.2903	0.3397		
6 day prot. (6') vs.	-0.2740	0.2003	0.0549 N.S.	----------
18 day prot. (6')	-0.2903	0.3397		
acute prot. (24') vs.	-0.5423	0.4513	0.9234 N.S.	----------
18 day prot. (24')	-0.1834	0.2035		
acute prot. (24') vs.	-0.5423	0.4513	0.8405 N.S.	----------
6 day prot. (24')	-0.2791	0.8219		
acute prot. (3') vs.	-0.1402	0.1992	0.6061 N.S.	----------
18 day prot. (3')	-0.2344	0.3427		
acute saline (6') vs.	-0.6776	0.6881	$P < 0.01$	Acute Prot.
acute prot. (6')	-0.1130	0.1132		
6 day saline (6') vs.	-0.5324	0.7393	0.7219 N.S.	----------
6 day prot. (6')	-0.2740	0.2003		
18 day prot. (6') vs.	-0.2903	0.3397	0.6862 N.S.	----------
18 day saline (6')	-0.6138	0.3163		
acute saline (3') vs.	-0.6023	0.7283	$P < 0.005$	Acute Prot.
acute prot. (3')	-0.1402	0.1992		
acute saline (3') vs.	-0.6023	0.7283	$P < 0.05$	Chronic Prot.
18 day prot. (3')	-0.2344	0.3427		
18 day saline (24') vs.	-0.6243	0.6699	$P < 0.05$	Chronic Prot.
18 day prot. (24')	-0.1834	0.2035		
6 day prot. (24') vs.	-0.2791	0.8219	0.6095 N.S.	----------
18 day prot. (24')	-0.1834	0.2035		

TABLE 58 A statistical comparison (t-test) for slope of NE depletion curves following alpha-methyl-para-tyrosine administration

Comparison	Statistical Results	These groups have decreased turnover
saline controls vs. acute protriptyline	$p < 0.005$	acute prot.
saline controls vs. T_3 admin. (7 days)	$p < 0.025$	T_3
saline controls vs. chronic Prot. (18 days)	$p < 0.005$	Prot.
thyroidectomized vs. Sham-operated controls	$p < 0.05$	Sham
thyroidectomized vs. T_3 admin. (7 days)	$p < 0.01$	T_3
thyroidectomized vs. acute Prot.	$p < 0.005$	Prot.
thyroidectomized vs. chronic Prot.	$p < 0.005$	Prot.