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The Effect of Adrenalectomy and Stress on Autoanalgesia in the Rat

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THE EFFECT OF ADRENALECTOMY
AND STRESS ON AUTOANALGESIA
IN THE RAT
BY
PATRICIA A. SEYMOUR

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
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Abstract

A literature review showed that since the discovery of the endogenous opioid peptide, Beta-endorphin, and the fact that it is secreted along with ACTH as part of the stress response, much investigation has been directed toward determination of the role of this substance in autoanalgesia which is known to result from a variety of stressors. ACTH (and presumably, Beta-endorphin) are known to be secreted in very high amounts following stress in 24-hour adrenalectomized. The present study, therefore, predicted that cold water swim (CWS) stress would induce analgesia, as measured by the tail-withdrawal analgesia test, in adrenalectomized and control rats, 24 hours and 120 hours after the CWS stress occurred. A 3X3X4 analysis of variance with four repeated measures indicated that this hypothesis was not supported, although in general, stressed animals exhibited significantly longer tail-withdrawal latencies immediately after stress than stressed animals receiving naltrexone and non-stressed animals. It was concluded that further investigation is necessary in order to elucidate the physiological basis of autoanalgesia, as well as the biological role of Beta-endorphin in response to stress.

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Introduction

Much interest and investigation into the area of endogenous opioid peptides have been engendered by the biochemical demonstration of the existence of opiate receptors by Pert and Snyder (1973). Using tritiated naloxone, an opiate antagonist, morphine binding sites were localized in the central nervous system and guinea pig intestine. Since it would be unlikely that there would be a receptor without there being an endogenous ligand, this marked the beginning of the search for an endogenous opiate-like substance.

Hughes (1975) first isolated a factor from mammalian brain extracts which interacted with the opiate receptor and inhibited neurally evoked activity in the mouse vas deferens and the guinea pig myenteric plexus. Subsequently, Hughes et al (1975) isolated two pentapeptide enkephalins from brain with morphinomimetic properties, met-enkephalin and leu-enkephalin, and characterized them.

Beta-endorphin was first identified in the pituitary by Cox et al (1975) and was isolated from camel pituitary extracts and characterized by Li and Chung (1976). This peptide proved to be identical to sequence 61-91 (the C-fragment) of the ovine pituitary hormone Beta-lipotropin. Two other related peptides, alpha and lambda-endorphins, were also isolated and characterized (Ling and Guillemin, 1976).

Immunocytofluorescence techniques with well-characterized antisera to Beta-endorphin located the peptide in cells of the pars intermedia and the pars distalis of the rat hypophysis, but not in the neurohypophysis (Bloom et al, 1977). Much smaller amounts of Beta-lipotropin and Beta-endorphin have been found in the hypothalamus, substantia nigra, thalamus, periaqueductal gray, and the arcuate nucleus of the median

eminence (Watson et al, 1977).

Beta-lipotropin is believed to be the prohormone precursor from which Beta-endorphin and the other endogenous opiates are cleaved, since Beta-lipotropin itself possesses no opioid activity but does generate fragments with morphinomimetic activity when incubated (Lazarus et al, 1976). Evidence to support this belief was the finding that ovine pituitary contains an enzyme that cleaves the bond between amino acids 60-61 of Beta-lipotropin (Bradbury et al, 1976).

The presence of endorphins within fiber tracts of the central nervous system known to be associated with the transmission of pain impulses led to the speculation that Beta-endorphin might act as an endogenous opioid to modulate the perception of pain. Receptor binding assays indicate that Beta-endorphin is more potent than the exogenous opiates (Li and Chung, 1976; Bradbury et al, 1976) and the enkephalins (Bradbury et al, 1976) in displacing tritiated opiate agonists and antagonists from the receptor, thereby indicating a high affinity of this endorphin for the opiate receptor.

Beta-endorphin has been shown to possess analgesic properties when administered through several routes to several species. Dose-dependent elevation of response latencies in tail-flick and hot plate procedures was produced when it was administered to mice intracerebrally and intravenously (Tseng et al, 1976), and to rats when administered intraventricularly (Bloom et al, 1976). These effects, with the exception of intravenous injection, were shown to be 18 to 33 times more potent than the effects of morphine, and were immediately reversed by the administration of naloxone (Loh et al, 1976). Dependency to Beta-endorphin and cross-tolerance with morphine have also been demonstrated

(Tseng et al, 1977).

Since the early studies of Selye in the 1950's it has been recognized that adrenocorticotrophic hormone (ACTH) is the primary pituitary hormone secreted in response to acute stress. ACTH is also thought to be originally formed as part of the Beta-lipotropin molecule since it is identical to sequence 1-39 of Beta-lipotropin. More recently it has been demonstrated that the endogenous opiate-like peptide, Beta-endorphin, is also secreted as part of the stress response (Guillemin et al, 1977). It was demonstrated that plasma and pituitary concentrations of both ACTH and Beta-endorphin were found to vary concomitantly in all situations investigated, including response to traumatic stress, response to purified corticotropin-releasing factor in vitro, and in response to adrenalectomy. In addition, ACTH and Beta-endorphin secretion is inhibited by pretreatment with dexamethasone, a synthetic glucocorticoid (French et al, 1977). Guillemin et al (1977) hypothesize that a holistic response of the organism to stress involves the secretion of pituitary hormones, some (such as ACTH and growth hormone) of which are involved in somatotropic (metabolic) adaptive reactions, whereas others (such as Beta-endorphin) are involved in neurotropic or psychotropic adaptive reactions.

As a result of these findings, there has been a vast amount of research attempting to relate Beta-endorphin to the occurrence of non-pharmacologically-induced analgesia (autoanalgesia), which has been demonstrated in man and other species after exposure to a wide variety of natural and experimentally-induced stressful situations. Beecher (1957) first noted that soldiers injured on the battlefield rarely complained of pain from even the most serious wounds. Conditioned fear

(Chance et al, 1978), inescapable footshock (FS) (Akil et al, 1976), hypertension-induction (Zamir and Segal, 1979), centrifugal rotation (Hayes et al, 1978), 2 deoxy-D-glucose (2-D-G) injections (Spiaggia et al, 1979), and cold water swim (CWS) (Lal, Spaulding and Fielding, 1978) have all been shown to produce analgesia in rats. In addition, it has been shown that FS stress in rats promotes a five to sixfold increase in both Beta-endorphin and ACTH plasma levels (Rossier et al, 1977).

Although much evidence seems to suggest that autoanalgesia is mediated through the activation of central endorphin systems, there is also evidence to suggest that endorphins do not mediate stress-induced analgesia. Naloxone, which antagonizes endorphin analgesia (Tseng et al, 1976) only partially reverses footshock (Akil et al, 1976) and CWS (Lal, Spaulding and Fielding, 1978) analgesias. Normal animals pretreated with corticosterone were found to exhibit analgesia after CWS, an unexpected result if the stress were mediated by Beta-endorphin (Bodnar et al, 1979). In addition, it was shown that exposure to brief ether anesthesia or horizontal oscillation, both of which have been reported to increase ACTH secretion, did not produce analgesia in rats as measured by the tail-flick test (Hayes et al, 1978). Therefore, the mechanism(s) underlying autoanalgesia remain undetermined, thereby necessitating further research.

Ruhmann-Wennhold and Nelson (1977) demonstrated that, in adrenalectomized rats, ACTH secretion reaches a peak about two hours after surgery and drops down to normal levels within 24 hours as the animal presumably recovers from stress. Since there is no corticosteroid feedback present, however, there is then a slow, steady rise in

ACTH secretion, even in the absence of additional stress. In rats that were stressed 24 hours after surgery, ACTH secretion was greatly increased, and by one week later reached levels approximately four times greater than in non-stressed adrenalectomized rats.

Since high ACTH levels had been shown to be accompanied by high Beta-endorphin levels (Guillemin et al, 1977), Holaday et al (1979) tested for analgesia in adrenalectomized animals. They found that adrenalectomy alone, i.e. in non-stressed animals, did not produce analgesia as measured by the tail-flick test, since no differences between latencies were observed in adrenalectomized animals and sham-adrenalectomized controls, one week after surgery. They concluded that either the apparent adrenalectomy-induced increases in circulating endorphin concentrations do not change analgesic thresholds, or that a functional tolerance may have occurred. However, since they did not stress their animals as did Ruhmann-Wennhold and Nelson (1977), perhaps Beta-endorphin levels did not reach levels high enough to induce analgesia.

The present study, therefore, proposed to investigate the effect of adrenalectomy combined with a well-established analgesia-inducing stressor, CWS, on analgesia in rats. Adrenalectomized, sham-adrenalectomized and control rats were subjected to one of three experimental treatments: Stress (CWS), stress and naltrexone, or non-stress, in a 3X3X4 factorial design with four repeated measures over a period of seven days after surgical treatment.

Four analgesia tests were administered to each animal: A pre-test to establish a baseline measurement of tail-withdrawal latencies before the stress treatment, a second test (post-test I) immediately after the CWS, a third test (post-test II) 24 hours after the CWS, and

a fourth test (post-test III) 120 hours after the post-test II.

Since Ruhmann- Wennhold and Nelson (1977) demonstrated that between two and 24 hours following adrenalectomy, ACTH secretion was in the normal range, in correspondence to the recovery of the animal from the stress of adrenalectomy, it was hypothesized that 24-hour adrenalectomized animals before exposure to CWS stress (pre-test) would exhibit the same tail-withdrawal latencies as sham-adrenalectomized and control rats.

It has also been demonstrated that the immediate ACTH response of 24-hour adrenalectomized animals to stress was very similar to the response of intact animals. Mean plasma ACTH stress responses of 5.6 mU/100 ml. and 6.1 mU/100 ml. were demonstrated for intact and 24-hour adrenalectomized animals respectively (Ruhmann-Wennhold and Nelson, 1977). Therefore, it was hypothesized that adrenalectomized-stressed rats, immediately after exposure to CWS stress (post-test I) would exhibit the same tail-withdrawal latencies as sham-adrenalectomized-stressed rats and control-stressed rats.

Ruhmann-Wennhold and Nelson (1977) also showed that ACTH secretion following stress was maintained for one hour in intact animals, but continued to increase in adrenalectomized animals. By seven days plasma ACTH levels increased to 57 mU/100 ml., an increase of approximately ten times the normal level. Therefore, it was hypothesized that adrenalectomized-stressed rats would not exhibit the same tail-withdrawal latencies as sham-adrenalectomized and control-stressed rats, 24 hours and 120 hours after exposure to CWS stress. It was predicted that adrenalectomized-stressed rats would exhibit significantly longer latencies than sham-adrenalectomized-stressed rats and control-stressed

rats on post-tests II and III.

It was also predicted that all stressed animals (not receiving naltrexone) would exhibit significantly longer tail-withdrawal latencies than non-stressed controls at the post-test I. In addition, it was predicted that all stressed animals (not receiving naltrexone) would exhibit longer tail-withdrawal latencies at post-test I than at the pre-test. Finally, it was predicted that stressed animals that received naltrexone would exhibit shorter tail-withdrawal latencies than stressed animals that did not receive naltrexone.

Method

Subjects

Ninety Sprague-Dawley male albino rats obtained from the Charles River Breeding Laboratories served as subjects. Animals weighed approximately 225 to 325 grams and were housed individually in a temperature and humidity-controlled room with a normal diurnal (12 hour light/12 hour dark) lighting cycle. Food and tap water were available to all animals ad libitum, and .4 molar saline was available to the adrenalectomized animals.

Apparatus

The apparatus for the CWS was a 95 cm. high cylindrical pail, with a diameter of 31 cm., filled to a height of 18 cm. with water and crushed ice in order to maintain a water temperature of 2-4°C. Standard rat restrainers were used to restrain rats during analgesia testing.

Procedure

Animals were randomly assigned to one of nine groups as follows:

- 1) adrenalectomy + stress, 2) adrenalectomy + stress + naltrexone, 3)

adrenalectomy + nonstress, 4) sham-adrenalectomy + stress, 5) sham-adrenalectomy + stress + naltrexone, 6) sham-adrenalectomy + nonstress, 7) control + stress, 8) control + stress + naltrexone, and 9) control + nonstress (see Figure 1). Therefore, there were three surgical treatments (adrenalectomy, sham-adrenalectomy, and control) and three stress treatments (stress, stress + naltrexone, and non-stress).

On each day, surgery was performed between 12 p.m. and 6 p.m. Adrenalectomies were performed by bilateral dorsal incisions under pentobarbital (10 mg./kg. body weight, IP) anesthesia. At the time of surgery, adrenal glands were inspected in order to insure that they were intact. Animals from which non-intact adrenals were removed were discarded from the study. The successful completion of surgery was also confirmed by the measurement of saline intake for two weeks following surgery. Sham-adrenalectomies were performed in the same manner except that adrenal glands were exposed but not removed.

Three adrenalectomies (one from each of the three adrenalectomy groups) and three sham-adrenalectomies (one from each of the three sham-adrenalectomy groups) were performed on any given day. During this time, the three corresponding control animals (animals receiving no surgical treatment) remained in home cages. This day was designated Day 1 for these animals.

On Day 2, the various stress treatments were administered. The stress treatment (CWS) consisted of gently placing an animal into a pail of iced water ($2-4^{\circ}\text{C}$) for 3.5 minutes, a procedure which has been shown to reliably induce analgesia (Bodnar et al, 1979). Animals in the nonstress groups remained in restrainers during this time.

Animals in the stress + naltrexone groups received naltrexone

Figure 1

3X3X4 Factorial Design with

Four Repeated Measures

TREATMENT

	PRE-TEST	POST-I	POST-II	POST-III
ADRENALECTOMY	S			
	S+N			
	NS			
SHAM-ADRENALECTOMY	S			
	S+N			
	NS			
CONTROL	S			
	S+N			
	NS			

S=STRESS
 NS=NONSTRESS
 S+N=STRESS + NALTREXONE

(10 mg./kg. body weight, IP) in a solution of 10 mg. naltrexone per 1 ml. distilled. Injections were given 20 minutes before the animal's first analgesia test (pre-test), and again every 24 hours thereafter for the remainder of the testing period, to insure continuous blockade of opiate receptors after the initial 24 hour surgery recovery period. Tests on these groups enabled the assessment of analgesia without the presence of Beta-endorphin activity.

Analgesia was tested by the tail-withdrawal method as described by Miksic and Lal (1977). Animals were placed in restrainers with their tails hanging freely. Tails were then immersed 5 cm. in 55° C water, in order to induce a pain response (tail-withdrawal). Latency to tail-withdrawal for each animal was recorded by an electronic timer to the nearest .01 second. During the analgesia testing the experimenter was blind as to animal group membership.

Pre-test. On Day 2, approximately 24 hours after surgery, animals were placed into restrainers. After ten minutes of habituation each animal was tested for analgesia. This measurement was taken in order to establish a pre-stress baseline for each animal, and to compare initial latencies among groups. Immediately after the pre-test, animals in the stress groups were stressed while animals in the nonstress groups remained in their restrainers.

Post-test I. After CWS, each animal was returned to its restrainer. After a ten minute habituation period, all animals were tested for analgesia. This measurement was used to assess analgesia resulting from the stress treatment, and to compare latencies among all groups. Each animal was then returned to its home cage.

Post-test II. On Day 3, approximately 24 hours after the post-

test I, animals were placed into restrainers. After a ten minute habituation period each animal was tested for analgesia. This measurement was used to assess differences in the time-course of analgesia among groups. Each animal was then returned to its home cage.

Post-test III. On Day 8, approximately 120 hours after the post-test II, animals were placed into restrainers. After a ten minute habituation period, each animal was again tested for analgesia. This measurement was used to further assess differences in the time-course of analgesia among groups. Each animal was then returned to its home cage.

Results

Means and standard deviations for all nine experimental groups across four repeated measures are presented in Table 1. The data of one animal from the adrenalectomy + stress group was discarded since the animal died before the completion of the experiment. Examination of the data suggested that cell variances were heterogeneous. An $F_{\max}$ test indicated that violation of the assumption of homogeneity of variance was present, $F_{\max} (36,9) = 47.84, p < .01$. A $\log (X + 1)$ transformation was executed and was successful in removing the heterogeneity of variance, $F_{\max} (36,9) = 8.02, p > .05$. Means and standard deviations for the transformed data are presented in Table 2.

A 3X3X4 analysis of variance with four repeated measures was performed on these transformed data using the BMD P2V statistical package. An analysis of variance summary table is presented in Table 3. The analysis indicated that the surgical treatment produced no significant differences among groups, $F (2,71) = 1.93, p > .05$. The stress treatment, however, did produce a significant difference among groups, F

TABLE 1

Means and Standard Deviations of Latency^a to Tail-
Withdrawal for Each Experimental Group Across Four
Repeated Measures

Treatment Group	<u>n</u>	Pre-test		Post-test I		Post-test II		Post-test III	
		<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>
AX+S	8	3.50	2.16	7.39	2.72	3.63	2.19	2.60	.33
AX+S+N	9	3.27	1.92	5.26	1.82	3.02	1.45	3.73	.61
AX+NS	9	2.81	.48	4.54	.90	3.32	.78	2.86	.50
SH+S	9	2.50	.55	7.70	3.32	3.46	.70	3.57	.42
SH+S+N	9	3.16	1.16	6.54	2.09	3.41	1.28	3.47	.32
SH+NS	9	2.81	1.22	4.37	1.40	2.78	1.30	2.78	.28
C+S	9	2.64	.97	5.87	1.24	3.56	1.57	3.10	.36
S+S+N	9	2.36	.86	5.54	1.04	2.72	.77	3.23	.56
C+NS	9	2.04	.71	3.55	1.89	2.90	.81	2.94	.46

a = latency in seconds

n = number of subjects per cell

AX = adrenalectomy

SH = sham-adrenalectomy

C = control

S = stress

N = naltrexone

NS = nonstress

TABLE 2
Means and Standard Deviations
of Transformed Data

Treatment Group	Pre-test		Post-test I		Post-test II		Post-test III	
	<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>
AX+S	.63260	.16•	.90474	.14	.62574	.20	.54968	.11
AX+S+N	.60012	.16	.78028	.13	.57999	.15	.64705	.17
AX+NS	.57806	.06	.73812	.07	.61782	.09	.55249	.19
SH+S	.53900	.07	.90908	.18	.64438	.07	.64341	.13
SH+S+N	.60409	.12	.86333	.12	.62875	.13	.64188	.09
SH+NS	.56287	.13	.71336	.14	.55384	.15	.56793	.09
C+S	.54614	.13	.83034	.08	.63466	.15	.59936	.11
C+S+N	.51304	.12	.80948	.08	.56203	.09	.60550	.13
C+NS	.47354	.09	.62599	.17	.58250	.09	.56917	.16

AX = adrenalectomy

SH = sham-adrenalectomy

C = control

S = stress

N = naltrexone

NS = nonstress

TABLE 3
Summary Table for 3X3X4 Analysis of
Variance with Four Repeated Measures

Source of variation	SS	df	MS	F
A (Surgery treatment)	.11994	2	.05997	1.93
B (Stress treatment)	.33022	2	.16511	5.30*
AB	.03622	4	.00906	.29
Error	2.21155	71	.03115	-----
C (Trials)	2.73231	3	.91077	78.19**
AC	.08878	6	.01480	1.27
BC	.27971	6	.04662	4.00**
ABC	.12289	12	.01024	.88
Error	2.48108	213	.01165	-----

* $p < .01$

** $p < .001$

(2,71) = 5.30, p .01. An Eta squared indicated that this significant treatment effect accounted for approximately 4% of the total variance ($\eta^2 = .0393$). Results also indicated that no significant surgery X stress interaction was found, $F(4,71) = .29$, $p > .05$.

For the repeated measures within subjects it was found that a significant difference among trials occurred, $F(3,213) = 78.19$, $p < .001$. An Eta squared indicated that this factor accounted for approximately 32.5% of the total variance ($\eta^2 = .3252$). The stress X repeated measures interaction was also found to be significant, $F(6,213) = 4.00$, $p < .001$, while the surgery X repeated measures and the surgery X stress X repeated measures interactions were not, $F(6,213) = 1.27$, $p > .05$, and $F(12,213) = .88$, $p > .05$ respectively. An Eta squared indicated that approximately 3% of the total variance was accounted for by the significant stress X repeated measures interaction ($\eta^2 = .0333$).

To further investigate the significant stress X repeated measures interaction, simple effects tests were performed. Variation due to the simple effects was computed from the stress X repeated measures summary table which is presented in Table 4.

The profiles of means corresponding to the cell totals of the summary table are plotted in Figure 2. The profiles indicate that all three stress groups showed an increase in latency to tail-withdrawal at post-test I, which occurred immediately after the CWS stress for the stress and the stress + naltrexone groups. It is interesting to note that the non-stress group also showed an increase in tail-withdrawal latency at this point in time. At post-tests II and III the tail-withdrawal latencies for all three groups generally decreased.

An analysis of variance for the simple effects of the stress

TABLE 4

Stress X Repeated Measures Summary Table^a

	Pre-test	Post-test I	Post-test II	Post-test III	Totals
S	15.159	23.471	16.908	15.911	71.4996
S+N	15.243	21.775	15.719	16.816	69.5536
NS	14.331	18.441	15.660	14.998	63.4303
TOTALS	44.7336	63.6878	48.2868	47.7253	204.4335

a = numbers in table represent totals for each stress group
collapsed across surgery groups

S = stress (e.g. Adrenalectomy + S, Sham-Adrenalectomy + S,
and Control + Stress groups combined)

N = naltrexone

NS = nonstressed

FIGURE 2

Mean Latencies to Tail-withdrawal for Each Stress

Group Across Four Trials

— STRESS
--- STRESS+DRUG
- - - NONSTRESS

factor indicated that there was a significant difference among the three stress groups at post-test I, $F(2,57) = 14.88$, $p < .01$, but that no significant differences among the groups occurred at pre-test, $F(2,57) = .58$, $p > .05$, or post-tests II and III, $F(2,57) = 1.13$, $p > .05$, and $F(2,57) = 1.88$, $p > .05$, respectively. An analysis of variance summary table is presented in Table 5.

To further investigate the significant difference among stress groups that occurred at post-test I, a Newman-Keuls analysis was performed. It was found that all three groups were significantly different from each other. Figure 2 shows that the stress group exhibited significantly longer tail-withdrawal latencies than the stress + naltrexone group ($p < .01$) and the non-stress group ($p < .01$), and the stress + naltrexone group exhibited significantly longer tail-withdrawal latencies than the non-stress group ($p < .05$). A summary of the Newman-Keuls test is presented in Table 6.

An analysis of variance for the simple effects of the repeated measures factor indicated that there were significant differences among the four trials for the stress group, $F(3,213) = 46.73$, $p < .01$, for the stress + naltrexone group, $F(3,213) = 28.97$, $p < .01$, and the non-stress group, $F(3,213) = 10.51$, $p < .01$. An analysis of variance summary table is presented in Table 7. Three additional Newman-Keuls analyses were performed to investigate these significant differences. Summaries of these Newman-Keuls tests are presented in Tables 8, 9, and 10.

The Newman-Keuls analysis of the differences among the four trials for the stress group indicated that post-test I tail-withdrawal latencies were significantly longer than tail-withdrawal latencies at all

TABLE 5
 Analysis of Variance Summary Table for
 Simple Effects of Stress

Source of variation	SS	df	MS	F
Stress at pre-test	.0191	2	.00955	.5779
Stress at post-test I	.4919	2	.24595	14.8835*
Stress at post-test II	.0373	2	.01865	1.1286
Stress at post-test III	.0621	2	.03105	1.8789
Error	-----	57	.01540	-----

* $p < .01$

TABLE 6
Newman-Keuls Summary Table for
Stress at Post-test I^a

		Nonstress	Stress + Naltrexone	Stress
		.6924	.8177	.8814
Nonstress	.6925	-----	**	**
Stress + N	.8177		-----	*
Stress	.8814			-----
r =			2	3

a) numbers in table represent means for each stress group
for post-test I.

** $p < .01$

* $p < .05$

df = (r,71)

TABLE 7
 Analysis of Variance Summary Table for
 Simple Effects of Trials

Source of variation	SS	df	MS	F
Trials for stress	1.633	3	.5444	46.7296*
Trials for stress + drug	1.0125	3	.3375	28.9699*
Trials for non-stress	.3673	3	.1224	10.5064*
Error	2.48108	213	.01165	-----

* $p < .01$

TABLE 8
Newman-Keuls Summary Table for
Trials at Stress Group^a

		Pre-test .56924	Post-test III .59748	Post-test II .63493	Post-test I .88139
Pre	.56924	-----	n.s.	**	**
III	.59748		-----	*	**
II	.63493			-----	**
I	.88139				-----
r =			2	3	4

a) numbers in table represent means for each trial for the
stress group

** $p < .01$

* $p < .05$

n.s. = not significant

df = (r, 213)

TABLE 9
Newman-Keuls Summary Table for
Trials at Stress + Naltrexone Group^a

	Pre-test	Post-test II	Post-test III	Post-test I
	.57241	.59026	.63148	.81770
Pre .57241	-----	n.s.	**	**
II .59026		-----	*	**
III .63148			-----	**
I .81770				-----
r =		2	3	4

a) numbers in table represent means for each trial for the
stress + naltrexone group

** $p < .01$

* $p < .05$

n.s. = not significant

df = (r, 213)

TABLE 10

Newman-Keuls Summary Table for

Trials at Nonstress^a

	Pre-test	Post-test III	Post-test II	Post-test I
	.53816	.56320	.58806	.69249
Pre .53816	-----	n.s.	*	**
III .56320		-----	n.s.	**
II .58806			-----	**
I .69249				-----
r =		2	3	4

a) numbers in table represent means for each trial for the
non-stress group

** $p < .01$

* $p < .05$

n.s. = not significant

df = (r, 213)

other tests ($p < .01$). The post-test II latencies were significantly longer than the pre-test ($p < .01$) and post-test III latencies ($p < .05$).

The Newman-Keuls analysis of the differences among the four trials for the stress + naltrexone groups indicated that post-test I latencies were significantly longer than latencies at all other tests ($p < .01$), and that the post-test III latencies were significantly longer than the pre-test ($p < .01$) and post-test II ($p < .05$) latencies.

The Newman-Keuls analysis of the differences among the four trials for the non-stress groups indicated that post-test I latencies were significantly longer than latencies at all other tests ($p < .01$), and that the post-test II latencies were significantly higher than the pre-test latencies ($p < .05$).

On the basis of the above analyses it was concluded that the significant stress X repeated measures interaction can be accounted for by the occurrence of significant differences in latencies among the three stress groups at the post-test I only and significant differences in latencies within each stress group over time.

Although the overall F test for the main effect of surgery was found to be non-significant, specific individual comparisons among certain group means were made on the basis of a priori predicted outcomes.

One such comparison was performed to investigate whether a significant difference occurred at the pre-test, between the adrenalectomy + stress group, and the sham-adrenalectomy + stress and control + stress groups combined. It was found that no significant difference occurred, $F(1,71) = 1.22$, $p > .05$.

A second comparison was performed to investigate whether a signif-

icant difference occurred at the post-test I, between the adrenalectomy + stress group, and the sham-adrenalectomy + stress and control + stress groups combined. It was found that no significant difference occurred, $F(1,71) = .23, p > .05$.

A third comparison was performed to investigate whether a significant difference occurred at the post-test II, between the adrenalectomy + stress group, and the sham-adrenalectomy + stress and control + stress groups combined. It was found that no significant difference occurred, $F(1,71) = .04, p > .05$.

A final comparison was performed to investigate whether a significant difference occurred at the post-test III, between the adrenalectomy + stress group and the sham-adrenalectomy + stress and control + stress groups combined. It was found that no significant difference occurred, $F(1,71) = .98, p > .05$.

Discussion

In summary, the results indicated that no significant difference occurred among the three surgical treatment groups, contrary to expectation. However, as predicted, it was found that CWS stress induced significantly longer tail-withdrawal latencies in all stressed animals.

As indicated by the lack of significance of the main effect for surgery, no significant difference was found among the three surgical treatment groups. An additional analysis by an individual comparison of means indicated that the adrenalectomy + stress group exhibited the same pre-test latencies as the sham-adrenalectomy + stress and the control + stress groups, as hypothesized. Another individual comparison of means found no significant difference in post-test I latencies be-

tween the adrenalectomy + stress group and the sham-adrenalectomy + stress and control + stress groups, also as hypothesized. Two additional individual comparisons of means indicated that, contrary to the hypothesis, no significant difference in post-test II or post-test III latencies occurred between the adrenalectomy + stress group, and the sham-adrenalectomy + stress and control + stress groups.

Essentially, adrenalectomized animals exhibited the same tail-withdrawal latencies as sham-adrenalectomized animals and non-operated controls across all four trials. Three explanations of these results are possible. First, Beta-endorphin may not be secreted concomitantly with ACTH over such a long period of time. This is doubtful in light of the remarkable parallelism exhibited between the two peptides in all other experimental situations, but could easily be investigated by the collection and analysis of blood samples by means of permanent catheter implantations at the time of surgery. Second, tolerance to Beta-endorphin may have developed over the time course of the experiment. This could be investigated by administration of naltrexone to animals, as in this study, and then withdrawing the drug and testing for analgesia. Presumably, naltrexone administration would block opiate receptors and prevent the occurrence of tolerance. Third, Beta-endorphin may be secreted in increasing amounts following stress in adrenalectomized animals, but may not be the substance/mechanism responsible for the analgesia observed after CWS stress. Clearly, further research is necessary to make any conclusions regarding these results.

As was expected, it was found that all stressed animals that did not receive naltrexone, regardless of surgical treatment, exhibited significantly longer tail-withdrawal latencies than the non-stressed

controls at post-test I. It was concluded therefore, that CWS stress did induce significant analgesia in these animals. Similarly, all stressed animals that did not receive naltrexone exhibited significantly longer tail-withdrawal latencies after stress (at post-test I) than before stress (at pre-test), as predicted. These findings are well-documented with CWS as the stressor (Lal, Spaulding, and Fielding, 1978; Bodnar et al, 1979).

In contrast to the findings of other researchers (Lal, Spaulding, and Fielding, 1978) it was found that non-stressed animals also exhibited significantly longer latencies at the post-test I than on the pre-test. An important difference in the design of the present experiment seems to account for this finding. Non-stressed animals in the present study were restrained for ten minutes, were tested for analgesia (pre-test), and remained in restrainers for the 3.5 minute stress period and for an additional 10 minutes before the post-test I. Non-stressed animals in the previous study remained in restrainers for five minutes, were tested for analgesia, and then were tested again five minutes later. Perhaps restraint and/or immersion of the tail in hot water induced the significantly longer latencies for the non-stress group. It is possible that this showed up in the present experiment since animals were retested 13.5 minutes later, while in the previous study they were tested only five minutes later.

The prediction that animals in the stress + naltrexone group would exhibit shorter tail-withdrawal latencies than stressed animals that did not receive naltrexone was not supported since the naltrexone group exhibited significantly shorter latencies only at the post-test I. No significant differences occurred at either post-tests II or III. In

addition, the naltrexone group displayed significantly longer latencies than the non-stress group at post-test I, an unexpected finding if Beta-endorphin is to be implicated in stress-induced analgesia, since reversibility of effects by naloxone is a universally accepted test of opioid peptide activity (Grevert and Goldstein, 1977).

Although quite unexpected, this finding is well-documented in the literature. In fact, this is a very controversial point in that some studies report that naloxone partially reverses stress-induced auto-analgesia, while others report no reversal at all. The matter is further complicated by the fact that some researchers call partial reversal, what others call lack of antagonism.

Lal, Spaulding, and Fielding (1978) concluded that analgesia induced by CWS stress was not antagonized by naloxone in either rats nor mice as measured by tail-withdrawal, tail-flick and phynylquinone-writing analgesia tests. Their data appear to reflect what others (Akil et al, 1976) call partial reversal, since latencies of groups receiving naloxone fell between the stress groups and the control groups. Akil et al (1976) reported partial reversal by naloxone of analgesia induced by acute footshock (FS) stress. Chance and Rosecrans (1979) found that large doses of naloxone failed to "modify" analgesia elicited by acute FS or conditioned fear in rats. They also found that naloxone failed to "modify" analgesia following FS in mice. Their data appear to reflect the occurrence of complete failure to reverse analgesia since the latencies of naloxone-treated animals were equal to the latencies of stressed animals. Similarly, El-Sobky, Dostrovsky, and Wall (1976) reported that the perception of pain induced by electric

shock to the forearm, in human subjects, was not altered by administration of naloxone. These researchers found no significant differences in analgesia between groups receiving naloxone and controls.

Contrary to the findings that report naloxone's partial reversal or non-reversal of autoanalgesia, Lewis, Cannon, and Liebeskind (1980) demonstrated that inescapable FS in rats caused profound analgesia that was completely antagonized by naloxone when shock was delivered intermittently for 30 minutes. In contrast, when shock was delivered continuously for three minutes, it was found that naloxone failed to antagonize the analgesic response to the stressor.

Additional evidence for the involvement of Beta-endorphin in alterations of pain threshold is accumulating. Dehen et al (1977) studied the effect of naloxone on one patient with congenital insensitivity to pain. No significant variation in pain threshold was observed in normal controls after naloxone or placebo. For the patient, the pain threshold fell by 67% within ten minutes of naloxone injection, while no significant effect was observed with a placebo. It was postulated that congenital insensitivity to pain may be related to a tonic hyperactivity of an endogenous opioid peptide system.

Hosobuchi et al (1979) found that stimulation of the periaqueductal gray area produced significant analgesia in human patients with chronic pain of peripheral origin. In addition, this stimulation resulted in significant increases (50-300%) in the concentration of ventricular immunoreactive Beta-endorphin, and its effects were reversed by naloxone.

In sum, evidence both for and against the role of Beta-endorphin in non-pharmacologically induced analgesia continues to accumulate.

Many different explanations have been offered in the attempt to resolve the issue.

One such proposal is that perhaps another endorphin that is not antagonized by naloxone mediates stress-induced analgesia, since the occurrence of a naloxone-insensitive endorphin in the CNS is not unprecedented (Lal, Spaulding, and Fielding, 1978).

Lewis, Cannon and Liebeskind (1980) proposed that there are two substrates involved in stress-induced analgesia. As described above, FS stress, depending only on its temporal characteristics, appears to activate either an opioid or non-opioid analgesia mechanism, each with physiologic inputs that are activated by certain stress conditions.

Similarly, Spiaggia et al (1979) propose the existence of at least two independent pain-inhibitory branches of a pain-modulatory system, one with opiate-like characteristics which is activated by acute exposure to morphine, and one with non-opiate characteristics, which is activated by acute exposure to such stressors as CWS. They demonstrated that full and reciprocal cross-tolerance develops to the analgesic effects of two qualitatively different stressors, CWS and 2-deoxy-D-glucose (2-D-G) injections, but that these two stressors differed in their respective interactions with opiate analgesia. Analgesia induced by CWS and that induced by morphine were found to be independent of each other since cross-tolerance failed to develop between them. Animals made tolerant to morphine, however, failed to exhibit 2-D-G analgesia, indicating that cross-tolerance between these two agents did occur.

In addition, Hayes et al (1978) demonstrated that bilateral lesions of the dorsolateral funiculus of the rat spinal cord reduced morphine analgesia by 73% but had no effect on shock-produced analgesia in the

same rats. These results suggested that narcotic and non-narcotic modulation of nociceptive input at the spinal level involves supraspinal mechanisms descending via separate pathways in the spinal cord.

Thus, it appears that the results of the present study and those reported by others, may be possibly explained by an as yet undetermined non-opiate mechanism. It is known that a myriad of responses are evoked in an organism in response to stress; any one of these may be implicated in the mediation of autoanalgesia. Clearly, further investigations utilizing neurochemical, neurophysiological and behavioral assessments are necessary in order to fully elucidate the physiological basis of stress-induced analgesia, as well as the biological role of Beta-endorphin in response to stressful situations.

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