Pharmacology Biochemistry & Behavior, Vol. 22, pp. 769–779, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Naltrexone Antagonizes the Biobehavioral Adaptation to Cold Water Stress in Rats

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Received 21 March 1984

GIRARDOT, M.-N. AND F. A. HOLLOWAY. *Naltrexone antagonizes the biobehavioral adaptation to cold water stress in rats.* PHARMACOL BIOCHEM BEHAV 22(5) 769–779, 1985.—The reported studies on the development of tolerance to the analgesic effects of stress have been mostly concerned with the involvement of opioid or non-opioid substances in stress-induced analgesia (SIA). To further investigate the processes involved in SIA tolerance, rats were exposed to forced intermittent cold water swim (ICWS, 18 exposures, 3/min, 10 sec each) on 16 consecutive days. On days 15 and 16, they were injected prior to swim with saline and naltrexone (10 mg/kg), respectively. During swim, three types of readily identifiable behaviors were observed. They may be characterized by immobility and horizontal floating (Type I), intensive activity and escape attempts (Type II), and passivity and "behavioral despair" (Type III). In the acute condition, only Type II and Type III appear in sequence. In the chronic condition, the sequence of behaviors is: Type I, Type II, and Type III. Thirty minutes after swim, analgesia, core temperature, and degree of inactivity were measured. With chronic exposure, tolerance developed to the analgesia, core hypothermia and hypoactivity induced by the ICWS. Type I behavior appeared on day 3 or 4 and persisted throughout the chronic treatment. Type II behavior did not adapt. Naltrexone (10 mg/kg) antagonized the adaptive aspect of all those variables where adaptation or tolerance were found (analgesia, hypoactivity, core hypothermia, and Type I behavior) but had no effect on Type II behavior where no adaptation was observed. It is suggested that the endorphins have a functional role in the behavioral and physiological adaptation to aversive stressful environmental situations.

Stress-induced analgesia Cold water swims Stress tolerance Naltrexone

THE studies by Selye [35] on the physiological responses to stress demonstrated that aversive situations are met with a massive generalized adaptive response which includes autonomic adjustments such as alterations in heart rate and respiration, muscle vasodilation, skin vasoconstriction and glucose mobilization, as well as a neuroendocrine response of the pituitary-adrenal and sympatho-medullary axis. More recently, it was found that intense and aversive stressful situations such as acute exposure to inescapable shock, rotation, and intraperitoneal injections of hypertonic saline also induce a decreased responsiveness to nociceptive stimuli [17] which is now referred to as stress-induced analgesia (SIA). These initial findings have since been extended to a large variety of stressors including cold water swim, food deprivation, and acute administration of 2-deoxy-D-glucose (for review, see [1, 7, 31]).

In most cases, tolerance develops to SIA when the animals are repeatedly exposed to the stressor. This phenomenon of SIA-adaptation has largely been studied and evaluated merely as a criterion to support or reject the possible involvement of endorphins in the analgesia induced by acute stress [6, 14, 24, 30, 40, 41]. A study on how this adaptation to chronic stress develops, on its robustness, and on what endogenous mechanisms are involved in these adaptive processes would contribute to the understanding of stress-tolerance and of CNS plasticity. The present investigation was designed to study these aspects of adaptation to chronic stress.

The model selected is chronic exposure to intermittent cold water swim (ICWS, 18 exposures, 3 per min, 10 sec each). Number, duration, and pattern of exposures were shown to specifically activate different types of cold water stress analgesia-inducing systems [13,14]. When exposed acutely to this particular stressor, rats display an analgesia involving an opioid-mediated system. This assumption is based on findings that the opiate antagonist naltrexone significantly antagonizes the decreased responsiveness to the tail-flick procedure following exposure [13], and that ICWS-analgesia is cross-tolerant to morphine [14]. This stressor also induces strong skin and core hypothermia as well as hypoactivity [13]. Further, a very characteristic and readily identifiable new sequence of behaviors progressively appears in rats exposed to chronic ICWS. In the acute condition, only two types of behavior are present. They have been described elsewhere [34]. Upon first contact with the cold water, the animals display behaviors characteristic of escape from painful and aversive situations (Type II) with high levels of activity, intense vocalization, and rapid swimming and jumping movements. After approximately half of the 18 10-second exposures, the animals display a

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passive behavior. At this stage, the body is oriented vertically, the function of the rare movements seems to be to keep the head out of the water (Type III). This behavior is referred to as "behavioral despair." A new type of readily identifiable behavior progressively appears in the chronic condition (Type I). After 3 to 4 days of the ICWS treatment, as soon as the animals are in contact with the cold water, their bodies shape into a straight horizontal line from head to the tip of the tail. The rats then float, lying sidewise and parallel to the surface of the water with a tendency to cross the hindlegs. This Type I behavior lasts for approximately 6 daily exposures of the ICWS and is then followed, in irreversible sequence, by Type II ("escape") and Type III ("behavioral despair") behaviors. Therefore, in addition to adaptation to the analgesic effect of ICWS, alterations in the various effects of ICWS and behavioral processes which develop throughout the course of chronic exposure will be evaluated to elucidate their possible contribution to chronic SIA tolerance, and to attempt to define mechanisms commonly involved in the general phenomenon of adaptation to chronic stress.

A role in the adaptive analgesic response to noxious stress has often been ascribed to the endorphins [9,19]; the endogenous opiates also seem to function as thermoregulators in stressful conditions by either increasing or decreasing body temperature, depending on the initial effect of stress [35]. Based on these various findings, it was suggested that the endorphins contribute to, or induce, adaptation to aversive acute stressful events. They may also be mobilized to allow adaptation to chronic stress. Results provided in the present study by the use of the opiate antagonist naltrexone in rats chronically exposed to ICWS may confirm this hypothesis. Theoretical considerations concerning a global functional role for the endorphins in the adaptive response to stress in general will be formulated.

GENERAL METHOD

Male albino Sprague-Dawley rats (Sasco, Inc., NE), weighing 300–350 g, were the experimental subjects. They were housed three to a cage. Water and food were available ad lib. The experiments were performed between 0900 and 1200 during the light part of a 12:12 dark/light automatically regulated cycle, the lights being switched on at 0845. Room temperature was kept constant at 22–23°C. Room air flow was constant.

Analgesia was tested using the tail-flick test which consists of measuring the latency between the onset of a high intensity heat beam (5 amperes) focused on the tail and the occurrence of a withdrawal reflex. The maximum latency possible was set at 15 seconds with the trial being then terminated if no tail flick occurred in order to prevent tissue damage to the tail. Each reported measure represents the mean of 3 consecutive trials at 40-second intervals. For the three successive trials, the heat beam was sequentially applied at 5, 8, and 11 cm from the proximal end of the tail. The animals were not restrained but were allowed to enter a dark glove placed on the platform of the tail-flick apparatus. This procedure presented a considerable advantage over more conventional restraining devices in that stress resulting from tail-flick measurements was minimal. Stress due to handling was avoided by conditioning the animals to the tail-flick procedure for 7 days prior to the beginning of the experiment. Rectal body temperature was measured with the probe of a telethermometer (YSI, Model 4B TUC). Inactivity was tested by using the same procedure as the tail-flick test without applying the heat beam on the tail. This method has been used previously to demonstrate that ICWS-analgesia was not due to hypoactivity [13] (as measured by the tail-flick test). The latency between the onset of the trial and the first movement of the tail represented the level of inactivity. The cut-off point was arbitrarily set at 40 seconds. In experiments where all measurements were performed, the following sequence was adopted: tail-flick latencies, and, during 40-sec intervals, inactivity and body temperature. Thirty minutes after stress, we also systematically submitted the rats to the writhing reflex; it was normal in all cases.

The intermittent cold water stress procedure consists of inescapable swims in 2°C water at a frequency of 3 per min with each exposure lasting 10 sec and the total swimming time being 3 min. After the swim, the animals were blotted dry with paper towels and returned to their respective home cages.

Data Analysis

Throughout the course of the present and additional experiments on tolerance to the analgesic effect of ICWS, we noticed a large variability between groups of the baseline tail-flick latencies (TFL). This variability could not be accounted for by factors such as age, sex, or species since they were identical in all groups. Neither could they be due to the intensity or placement of the light beam since they were similar for all groups. In this study, a variable had to be found that best represents stress-induced analgesia. This variable should provide measurements of SIA equal in different groups and should not be dependent on baseline latencies. We submitted 2 groups of animals (N=6) of the same age, weight, species, and source to the chronic ICWS procedure for 14 days. The baseline TFL were different between the two groups (4–5 sec in all the animals of one group and 5–6 sec in the animals of the other group). Tail-flick latencies were measured prior to and 30 minutes after exposure to stress. Based on the results obtained, we then derived the analgesia index, which is commonly used in studies on analgesia, and is equal to:

$$\text{A.I.} = \frac{\text{TFL post-stress–baseline TFL}}{\text{15 (cut-off point)–baseline TFL}}$$

and the ΔTFL which is equal to TFL post stress—baseline TFL. These calculations were done for the results obtained on day 1 and day 14 of the chronic exposure. We used the *t*-test to compare the post-stress tail-flick latencies, the A.I. and the ΔTFL in both groups on days 1 and 14, independently. We also performed correlation studies (Pearson product moment correlation) between each of the three measures (A. I., ΔTFL, and post-stress TFL) and the baseline TFL. The results are shown in Table 1.

It is clear from the results obtained that only the ΔTFL were not different in the two groups of rats which consistently displayed significantly different baselines, and that only the ΔTFL was independent of the baseline tail-flick latencies. For these reasons, ΔTFL was selected as the variable for the measure of stress-induced analgesia in the present study.

Three aspects of the development of tolerance to all the variables were statistically evaluated. The extent of development of tolerance was ascertained by means of a linear regression analysis and the analysis of variance (ANOVA). Best fitting curves were derived from polynomial regression analysis. The rate of development and the time required for

TABLE 1

CORRELATION BETWEEN BASELINE LATENCIES AND THREE MEASURES OF SIA DERIVED FROM POST-STRESS TAIL-FLICK LATENCIES

	Analgesia Index		Post-stress Tail-flick Latencies		Tail-flick Latencies	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
t test*	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.01$	N.S.	N.S.
Baseline Tail-flick Latencies						
r	0.60	0.31	0.66	0.78	0.17	0.00
Significance	$p<0.05$	N.S.†	$p<0.05$	$p<0.01$	N.S.†	N.S.†
Analgesia Index						
r			0.99	0.82	0.88	0.94
Significance			$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$
Post-stress Tail-Flick Latency						
r					0.85	0.62
Significance					$p<0.01$	$p<0.01$

*Comparing the mean between the two groups.
†N.S.=Not significant, $p<0.1$.

asymptotic acquisition of tolerance were determined by applying the Duncan method for comparing the variables on individual days of exposure. For the correlation studies, the Pearsons' product moment correlation method was used. The effect of naltrexone on the variables studied was ascertained using the ANOVA with repeated measures method and the Duncan test for individual comparisons between the control, saline, and naltrexone conditions. Significance levels were set at $p<0.05$ unless otherwise specified.

EXPERIMENT 1: ANALGESIC, THERMAL, ACTIVITY AND BEHAVIORAL ADAPTATION TO CHRONIC ICWS

Method

Baseline tail-flick latencies, core temperature, and activity were measured in six rats prior to exposure to ICWS. All variables were again measured 30 minutes after exposure to ICWS. Behavior was quantified during the swimming sessions. These procedures were repeated on 14 consecutive days for all steps except baseline core and skin temperature and activity levels which were only measured again on day 14 to ascertain stability. On days 15 and 16, the rats were injected with saline and naltrexone, respectively (refer to Experiment 3). They were then exposed to ICWS at only 14-day intervals. On day 114 (day 1 being the first day of exposure of chronic ICWS), baseline tail-flick latencies were measured and the rats were exposed to ICWS during which behaviors were quantified. Thirty minutes later, tail-flick latencies, core temperature and activity levels were measured. Six additional rats were used to evaluate the effect of repeated exposures to the analgesimetric test, and served as controls for the evaluation of the effect of chronic stress on tail-flick latencies. They were submitted to the same tail-flick test procedure as the experimental animals, but were not stressed.

Results

Figure 1 represents the effects of chronic ICWS on tail-flick latencies, core temperature and activity during the 14 days of exposure. Also shown are the results obtained on day 114, after the rats were allowed a resting period of 98 days with limited exposures to ICWS at 14 day intervals. Marked differences were found in the various behavioral and physiological responses to ICWS throughout the course of the chronic exposure. While adaptation occurred to the analgesia, core hypothermia, hypoactivity and some of the behavioral responses to ICWS, there was no change in one of the monitored behaviors. The variables were independently analyzed for evaluation of more precise differences between the 14 days of exposure.

Adaptation to the analgesic effect of ICWS is represented in Fig. 1A. The ΔTFL are compared to those in the control non-stressed group. In the stressed animals, the tail-flick latencies (TFL) increased significantly from baseline levels when tested after the first exposure. This increase was tempered with repeated exposure; the linear regression analysis revealed that the slope of ΔTFL from day 1 to day 14 was significant, $F(1,82)=75.5$, $p<0.001$, and could not be accounted for by variations in baseline TFL due to repeated tail-flick tests since these remained unchanged throughout the 14 days of exposure ($F<1$) and since the slope of the linear regression in the control animals was not significant ($F<1$) when the ΔTFLs were compared from day 1 to day 14. Further, the ΔTFLs were significantly different in the stressed and control animals, $F(1,10)=190.8$, $p<0.001$, and there was a significant interaction between the two groups and the days of exposure, $F(13,130)=6.9$, $p<0.001$. The major features of the pattern of tolerance development to the analgesic effect of ICWS are: (1) tolerance developed rapidly during the first 5 days of exposure; the ΔTFLs were different when cross-compared on days 1, 2, 3, and 4 ($p<0.01$); only

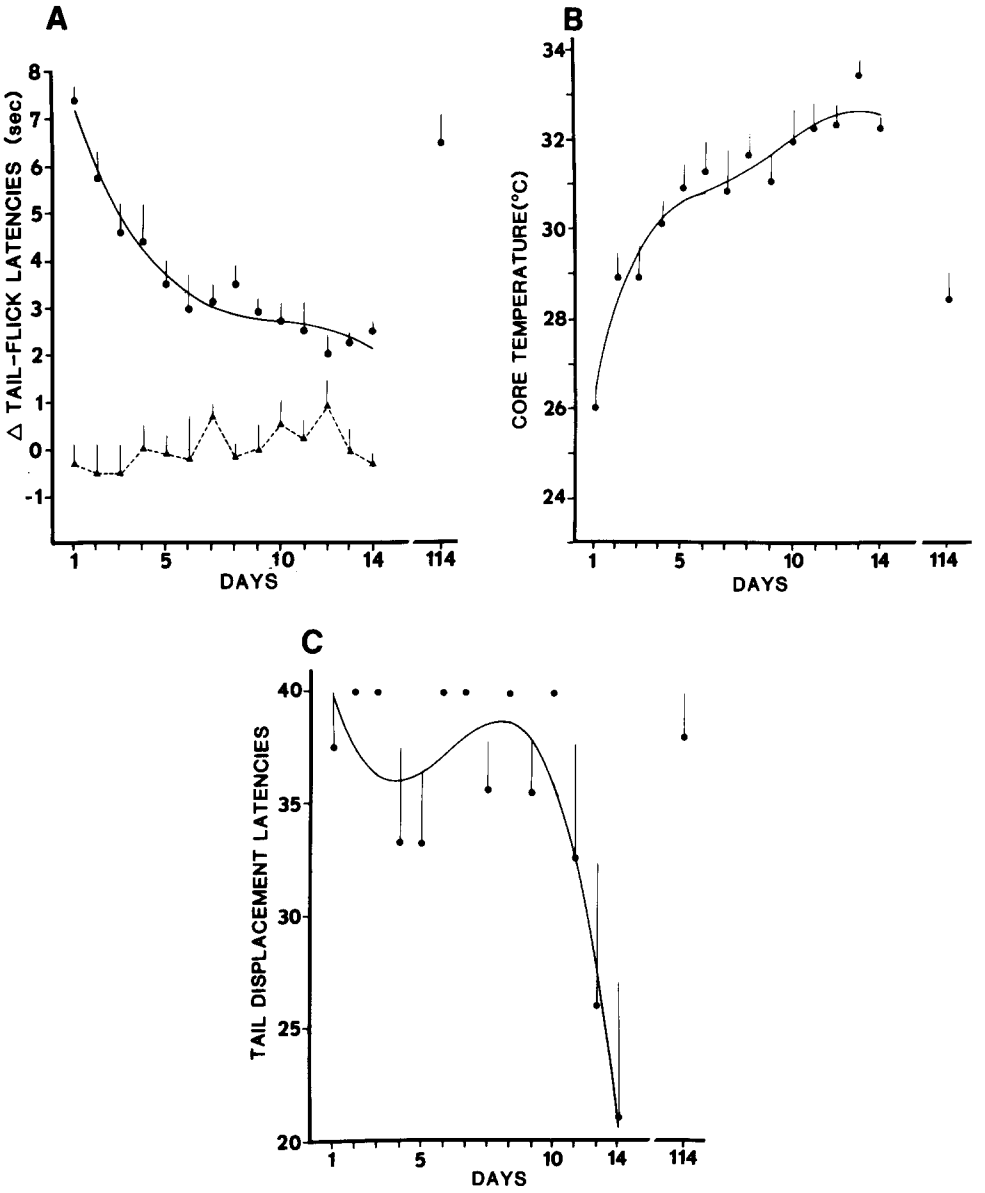

FIG. 1. ΔTail-flick latencies, core temperature and index of hypoactivity in rats submitted to ICWS on 14 consecutive days and again on day 114 measured 30 minutes after ICWS. Figure 1A represents the best fitting curve passing through the daily mean ΔTFL in the stressed rats (full line) determined by polynominal regression analysis, $F(3,80)=40.95$, $p<0.001$. The interrupted line represents the daily mean ΔTFL in the control, unstressed animals (n=6). Figure 1B represents the best fitting curve for core temperature, $F(4,78)=30.66$, $p<0.001$. Figure 1C represents the best fitting curve passing through the daily mean indices of hypoactivity, $F(3,80)=10.03$, $p<0.001$. Full circles or triangles and vertical lines are the means±S.E.M.

on days 2 and 3 were they not different; (2) tolerance was acquired on day 5 and remained unchanged through day 14; from day 5 to day 14, the ΔTFLs were not significantly different but on each of these 10 days they differed from the ΔTFLs on days 1–4; (3) tolerance, however, was not complete since the ΔTFLs remained statistically different from those in the control non-stressed group ($p<0.001$ on all days but days 10 and 11 where $p<0.025$, and day 13 where the two groups did not display significantly different ΔTFLs).

Adaptation to the hypothermic effect of ICWS is illustrated in Fig. 1B. A linear regression analysis showed a significant slope for the increase in body temperature with repeated exposure, $F(1,81)=85.4$, $p<0.001$. This tolerance development was not due to differences in baseline core temperature (day 1: 38.4±0.16; day 14: 38.7±0.21). The features of this development of tolerance are: (1) core hypothermia tolerance developed rapidly during the first 5 days. The ANOVA and Duncan tests provided evidence that: (2)

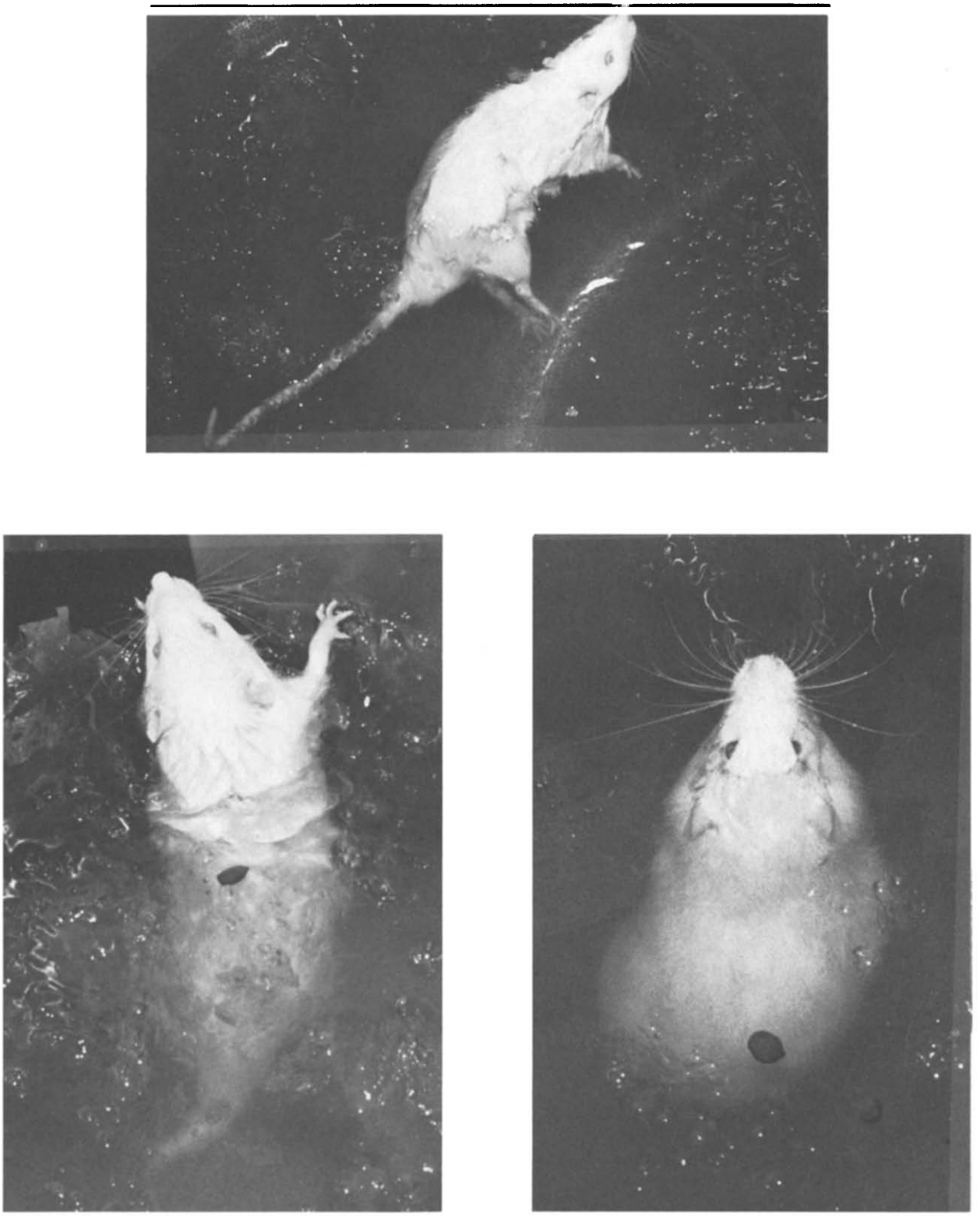

FIG. 2. Examples of Type I (top), Type II (bottom, left) and Type III (bottom, right) behaviors.

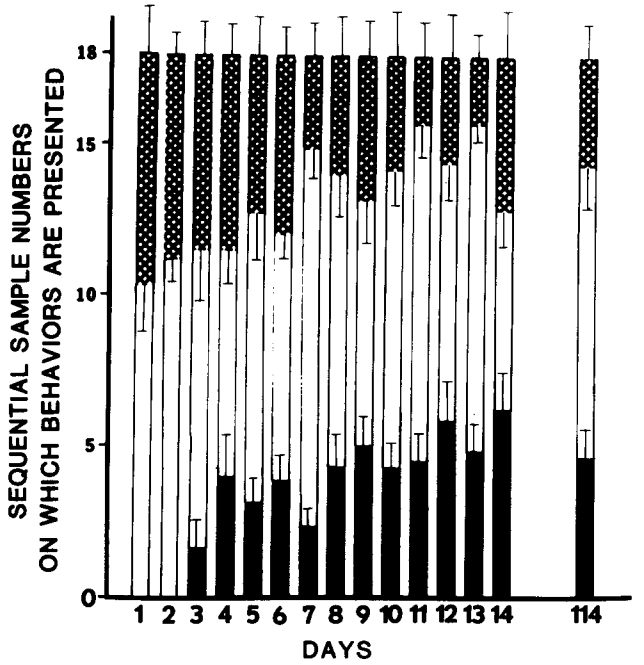

FIG. 3. Type I (full bars), II (open), and III (diamonds) behaviors during exposure to ICWS on 14 consecutive days and on day 114. The 3 types of behavior were quantified by adding, for each of them, the number of 10 sec exposures during which they were present for more than 5 sec. The three types of behavior were displayed according to the following sequence: Type I (if present), Type II, and Type III.

TABLE 2
LONG-TERM EFFECTS OF ADAPTATION TO CHRONIC ICWS

	Day 1	Day 14	Day 114
ΔTail-Flick Latencies			
Mean ± S.E.M. (sec)	7.4 ± 0.3	2.5 ± 0.2	6.6 ± 0.5
vs. Day 1 (Significance)	—	$p<0.01$	N.S.
vs. Day 14 (Significance)	—	—	$p<0.01$
Core Temperature			
Mean ± S.E.M. (°C)	26.2 ± 0.6	32.1 ± 0.3	28.3 ± 0.6
vs. Day 1 (Significance)	—	$p<0.01$	$p<0.01$
vs. Day 14 (Significance)	—	—	$p<0.01$
Index of Inactivity			
Mean ± S.E.M. (sec)	37.5 ± 2.5	21.0 ± 6.3	38.0 ± 2.0
vs. Day 1 (Significance)	—	$p<0.01$	N.S.
vs. Day 14 (Significance)	—	—	$p<0.01$
Behavior Type I			
Mean ± S.E.M. (%)	0	34.5 ± 7.0	25.8 ± 5.7
vs. Day 1 (Significance)	—	$p<0.01$	$p<0.01$
vs. Day 14 (Significance)	—	—	N.S.

tolerance was acquired on day 5 and reached an asymptotic level which remained stable during the last 9 days of the experiment, and (3) tolerance was not complete since, on day 14, the baseline core temperatures were consistently higher than the core temperature following exposure to ICWS.

We also measured skin temperature. However, due to experimental time constraints, we used the rectal probe (taped on the proximal end of the tail) instead of the skin thermal probe. Although use of a rectal probe for skin temperature measurement may be questionable, the results thus obtained showed that no adaptation developed to the skin hypothermic effect of ICWS as shown by a lack of significant difference for post-stress skin temperatures when they were compared on day 1 and day 14 (day 1: 26.8±0.6; day 14: 25.6±0.5), while the baseline skin temperatures remained constant (day 1: 32.2±0.6; day 14: 31.9±0.6).

Tolerance developed to the hypoactivity produced by ICWS (Fig. 1C). Indeed, the slope for the linear regression from day 1 to day 14 was significant, $F(1,82)=11.35$, $p<0.001$. The levels of activity remained stable from day 1 to day 11 and the adaptive result of chronic exposure was seen only starting on day 12. It is possible that the lack of tolerance development during the first days of chronic exposure may result from the experimental procedure used whereby a maximum of 40 seconds is arbitrarily set for determination of hypoactivity. Baseline activity levels were not statistically different when compared on days 1 and 14 (mean±S.E.M.=8.8±4.7 and 6.5±1.8 on days 1 and 14, respectively).

Examples of the three types of behavior seen which were displayed in sequence during ICWS in the rats chronically stressed are shown in Fig. 2 and were quantified by adding, for each type of behavior, the number of 10 second exposures during which it was present for more than 5 seconds. The results are shown in Fig. 3.

While changes in the measures of Type I and Type III behaviors appeared through the course of chronic exposure (slope of the linear regression analysis: $F(1,82)=46.9$, $p<0.001$ and $F(1,88)=18.6$, $p<0.001$, respectively), there were no changes in Type II behavior (slope for linear regression: $F<1$). It should be again noted that the incidence of Type I behavior (if present at all) always will occur prior to the appearance of Type II or Type III behavior. On the contrary, the incidence of Type III behavior always occurs after all incidences of Type I and Type II behaviors. Thus, the changes in Type III behavior may merely reflect artifactual consequences of the experiment. Since the total number of exposures during a single session is limited (18), the relative number of exposures during which Type III behavior (the last one to occur) is present varies as a function of the relative contribution of Type I and Type II behaviors. For this

reason, all subsequent references to Type III behavior in this study will be descriptive only.

The pattern of Type I behavior adaptation with chronic exposure suggests that this behavior appears on day 3–4 of chronic ICWS, that it progressively increases from day 3 to day 8, and then reaches an asymptotic level which remains stable throughout the next 6 days of the experiment, where its relative contribution reaches approximately 1/3 of the total time of exposure.

The results on loss or preservation of tolerance after 98 days of limited exposure (1/14 day) in the animals first chronically submitted to ICWS on 14 consecutive days are reported in Table 2. (The results on the testing day (day 114) can be seen in Fig. 1.) In Table 2, the effect of ICWS on day 114 on all variables where tolerance or adaptation to chronic ICWS were observed are compared to the effect of ICWS when the animals were acutely and chronically stressed (days 1 and 14, respectively). Adaptation to both the analgesic and hypoactivity effects of chronic stress was lost when tested on day 114; indeed, the TFLs and activity levels 30 minutes after stress returned to their initial levels after the first exposure ($F<1$, comparing ΔTFL and index of hypoactivity on day 1 and 114). Tolerance to the core hypothermic effect of ICWS was lost ($p<0.01$) when post-stress core temperatures were compared on day 14 and 114; however, this loss was only partial since the post-stress core temperatures were statistically different between day 1 and 114 ($p<0.01$). The most robust aspect of adaptation to ICWS was behavioral. Indeed, even after the animals were allowed a resting period of 98 days with exposures at 14-day intervals, Type I behavior was still present on day 114 at a level not significantly different from day 14 ($F<1$).

To briefly summarize the results of Experiment 1, it was found that tolerance developed to the analgesia, core hypothermia, and hypoactivity effect of ICWS, and that Type I behavior adapted, with chronic ICWS exposure, while skin hypothermia and Type II behavior did not. Of the four variables which did adapt, the behavioral aspect was the most robust since it was still present following a resting period of 98 days; tolerance to the core hypothermic effect of ICWS was partially lost, and adaptation to the analgesic effects of, and the hypoactivity resulting from exposure to ICWS, were completely lost. This experiment established that some effects of acute ICWS are tempered with chronic exposure while others are not. It might be interesting to find out whether or not a single endogenous mechanism accounts for the adaptation of two or more of the variables studied. Correlation studies were thus performed. Keeping in mind that correlation does not equate with causality, a significant correlation between two variables will nevertheless provide a clue for possible common mechanisms; non-significant results will tend to rule out the possibility that some variables do adapt because of a causal relationship between them.

EXPERIMENT 2: CORRELATION BETWEEN TYPE I BEHAVIOR, CORE HYPOTHERMIA, TAIL-FLICK LATENCIES, AND ACTIVITY LEVELS IN RATS SUBMITTED TO CHRONIC ICWS

This experiment was designed to seek possible relationships between the variables studied for which adaptation and tolerance developed with chronic exposure. Significant findings will provide a clue for the evidence of a central mechanism that allows adaptation to chronic aversive stress. This experiment may also provide evidence for a possible functional role for Type I behavior. The testing hypothesis was that this readily identifiable behavior has a functional role in energy conservation which is of survival value during exposure to the particular stressor used. This assumption was based on the specific attributes of this behavior which are: (1) immobility, thus lack of energy expenditure, and (2) floating, with a large part of the body being kept out of the cold water and exposed to the warmer ambient temperature. Based on this hypothesis, it was inferred that post-stress core temperature and/or activity levels may be positively related to the relative presence of Type I behavior.

Method

Correlation studies were performed using 12 rats weighing 300–350 g. The animals were submitted to chronic ICWS on 14 consecutive days. On day 14, baseline tail-flick latencies were measured. The rats then were submitted to the ICWS condition during which the three types of behavior were quantified. Tail-flick latencies, core and skin temperature, and levels of activity were measured 30 minutes after exposure to stress.

Results

The results of Experiment 2 are reported in Table 3 and indicate that Type I behavior and post-stress core temperature are positively, but only weakly, related ($r=0.53$, $0.1>p>0.05$); both variables are negatively related to tail-flick latencies ($r=-.69$, $p<0.05$ and $r=-.58$, $p<0.05$, respectively). It is of interest that the direction of the correlation (positive or negative) is similar to the effects of chronic ICWS on these variables. Type I behavior and core temperature, which are positively related, both increase with chronic exposure, while TFL, which is negatively related to the other two variables, decreases. This suggests that adaptation of these three variables may be attributable to a common central mechanism. This possibility is further tested in Experiment 3. Activity was not related to any of the other variables studied.

While a positive relationship was found between Type I behavior and post-stress core temperature, this relationship is too weak ($0.1>p>0.05$) to ascertain that the functional role of this behavior is energy conservation.

EXPERIMENT 3: EFFECT OF NALTREXONE ON PAIN RESPONSIVENESS, CORE AND SKIN TEMPERATURE, ACTIVITY, AND BEHAVIOR IN CHRONICALLY STRESSED ANIMALS

Method

The six animals used for Experiment 1 were submitted to the following experimental procedure. On days 15 and 16, after measuring the baseline tail-flick latencies, they were injected with saline and naltrexone hydrochloride (10 mg/kg, IP), respectively. Thirty minutes after injection, they were submitted to the ICWS procedure during which the three types of behavior were quantified. Tail-flick latencies, core and skin temperature, and activity levels were measured 30 minutes following ICWS.

Results

The results of this experiment are illustrated in Fig. 4. Reported are the effects of ICWS on days 13 and 14, day 15 (saline), and 16 (naltrexone) of the chronic ICWS treatment. ANOVA and Duncan tests were used to compare the variables on day 14 (controls), 15 (pre-injection with saline), and day 16 (pre-injection with naltrexone). The general finding is

TABLE 3

CORRELATION BETWEEN THE EFFECTS OF CHRONIC ICWS ON TAIL-FLICK LATENCY, CORE TEMPERATURE, INACTIVITY INDEX, AND TYPE I BEHAVIOR

	Tail-Flick Latencies	Core Temperature	Index of Inactivity	Type I Behavior
Mean ± S.E.M.	10.3 ± 0.53 (sec)	31.6 ± 0.44 (°C)	20.8 ± 4.0 (sec)	28.3 ± 3.7 (%)
Correlation Coefficient* (r)				
Core Temperature	−0.69	—	—	—
Significance	$p<0.05$	—	—	—
Inactivity Index	+0.09	−0.06	—	—
Significance	N.S.	N.S.		
Type I Behavior	−0.58	0.53	−0.04	—
Significance	$p<0.05$	$0.1>p>0.05$	N.S.	

*Pearson product moment correlation.

that all those variables to which adaptation or tolerance developed were significantly affected by naltrexone which antagonized the adaptive processes, while those variables for which no tolerance was observed were not affected by naltrexone. Specific analysis of the effects of naltrexone provided evidence for a significant increase of ΔTFL in the naltrexone-pretreated condition as compared to the saline ($p<0.01$) and control condition ($p<0.05$), while the ΔTFL in saline and control conditions did not significantly differ. Core temperature was significantly lower in the naltrexone than in the saline ($p<0.05$) or control ($p<0.05$) conditions; there was no significant difference in the saline vs. control condition. Skin temperature was not significantly different in the three conditions. The rats were significantly less active 30 minutes after ICWS in the naltrexone than in the control or saline conditions ($p<0.05$ in both cases); saline and control conditions were not statistically different. Type I behavior was significantly reduced in the naltrexone condition when compared to the control or saline conditions ($p<0.01$ in both cases), while they were not significantly different in the control vs. saline conditions. Type II behavior was not significantly different when comparisons were made between the naltrexone and saline or between the saline and control condition. However, a significant difference was seen in the naltrexone vs. control condition ($p<0.05$). This may merely be due to highly variable findings of Type II behavior measurements throughout the chronic experiment. This assumption is confirmed by a lack of significant difference between the naltrexone and control conditions on day 13 (no drug) where Type II behavior was not quantitatively different. Type III behavior was not significantly different in the three conditions.

SUMMARY AND DISCUSSION

The dose of 10 mg/kg of naltrexone used in this study may be considered too high to irrefutably implicate an endorphin-dependent phenomenon since much lower doses significantly antagonize the effect of morphine [2], and since, at doses higher than those required to antagonize the effect of morphine, naltrexone acts on non-opiate systems [10]. However, it is well documented that the effect of endorphins acting on the Δ and κ opiate receptors (enkephalins and dynorphins) are less readily antagonized by the opiate antagonists than the effect produced by morphine or β-endorphin (e.g., [25,36]), and it has been suggested that a dose 10 times as high as that required to antagonize morphine's effect may still be considered in the range of opiate effect [9]. The dose used in the present study is in this accepted range. Further, since naltrexone's effect seems to be produced by means of opiate receptor blockade [5], it will be assumed that the pharmacological effects seen in the present study result from the antagonism of physiologically-released endorphins.

The major findings are that tolerance to the decreased responsiveness to nociceptive stimuli, core hypothermia, and hypoactivity induced by acute ICWS developed with repeated exposure to the stressor. Further, a newly described behavior unseen in the acute condition (Type I) was acquired with repeated exposures. Of all the variables studied, this behavior was the most robust. Naltrexone antagonized all these adaptive processes. In the chronically stressed animals, Type I behavior, core hypothermia, and analgesia seem to be related. However, the experiments seeking a functional role for Type I behavior provided only marginal results. There is no evidence for adaptation of the "escape" or Type II behavior or of the development of tolerance to the peripheral hypothermal effect of ICWS (as measured in this study). Naltrexone did not significantly affect these two variables in the animals submitted to chronic ICWS.

Based on these findings, it is suggested that an encompassing role for the endorphins may be adaptation to aversive stress. This theory implies two assumptions. The first one is that the tonic release of endorphins is insufficient to induce any of the effects observed upon their administration, and that they are released only when the organism is confronted with aversive environmental stimuli. The second assumption is that the effect of endorphins will vary depending on the survival or well-being value of the processes underlying adaptation to stress. Many reports in the literature seem to validate these assumptions. In support of the first as-

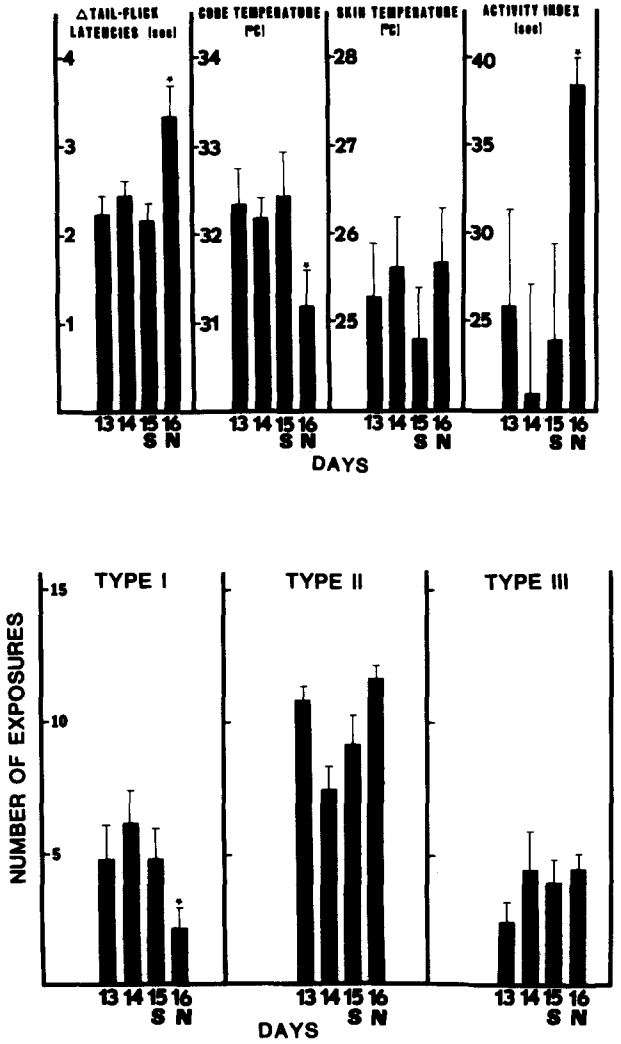

FIG. 4. ΔTFL, core and skin temperatures, activity index and behaviors I, II, and III in chronic ICWS-submitted rats on days 13 and 14 (no drug, controls), day 15 (saline) and day 16 (naltrexone, 10 mg/kg). The behaviors were measured during ICWS; the other variables were measured 30 min after ICWS. Saline and naltrexone were injected IP 30 min before ICWS. *The levels are significantly different from both the control (day 14) and saline (day 15) conditions.

sumption, the demonstration of tolerance to the analgesic effect of endorphins in the tail-flick test in rats [38] suggests that endorphin receptors are not continuously occupied by endorphins. Further, if the endorphins were tonically released, the opiate antagonists should produce effects opposed to those normally induced by the endorphins. Studies on the effects of naloxone and naltrexone have provided conflicting results. It has been suggested that the discrepancies are due to the stress attributable to the pretreatment of the rats tested [9]. For example, while no consistent effect is seen in water non-deprived (thus non-stressed) rats, the opiate antagonists consistently decreased fluid consumption in thirsty rats [27]. Studies on the effect of β-endorphin and enkephalins on the release of vasopressin also have provided conflicting results. The suggestion was made that the discrepancies [3,39] were due to the presence or absence of prior water loading [9] which by itself represents a stressful manipulation that demands a physiological process to overcome its effect. The prior treatment of the animals also may be important in determining the presence or absence of opioid effects on hormone release in *in vitro* studies [9]. In *in vivo* studies of the effect of opiate antagonists on pain responsiveness, the search for a sensitizing effect also met with a lack of consistency. Here again, it is possible that the discrepancies may be due to the level of stress applied by the procedure used to test pain responsiveness. For example, the differences in findings may be due to the analgesimetric test used and the relative latency for the response to occur. In mice, naloxone only consistently reduced the response in the hot plate test [11, 12, 16, 20, 21] when an escape jump was taken as the end point and the temperature of the hot plate adjusted so that the latency to jump was prolonged to 1 minute or more. Naloxone had no effect on the much shorter pawlick latency [12]. Although latency responses that short are associated with standard errors large enough to obscure true differences, it is also possible that when the response has a short latency, the elapsed time is insufficient for mobilization of endorphins to occur, but that longer latency responses allow time for release of endorphins which further prolong the latency [20]. This assumption is supported by the studies on stress-induced analgesia. It is now generally accepted that aversive stress induces an opioid and a non-opioid type of analgesia. SIA may in addition be hormonal or neural (for review, [39]). One of the factors determining the induction of the hormonal endorphin-analgesia is the duration of exposure. With short duration, electric shock or CWS induce a hormonal analgesia which is not antagonized by the opiate antagonists [8, 13, 23]; when the stressor is of longer duration, thus allowing endorphin release from the pituitary or the adrenal glands, the antagonists readily reverse the analgesia induced by the stressor. At last, while the studies on the effect of opiate antagonists on body temperature were at first conflicting [4, 15, 18], it was later suggested that the endorphins have a thermoregulatory role in cool and hot environments. For example, naloxone and naltrexone caused core temperature to be significantly lower than controls when exposed to cold (4°C) and to be higher than controls upon exposure to hot temperatures (38°) [37]. All of these studies support the first assumption on the basis of the possibility that the endorphins are not constantly released, but that their function may be to overcome the effects of aversive stimuli encountered in the environment.

As stated previously, this theory also assumes that the effect of endorphins will vary depending on the survival or well-being value of the processes underlying adaptation to stress. This implies that the endorphins may produce different and eventually opposite effects. The examples already cited of the effect of naloxone or naltrexone on temperature in rats exposed to either hot or cold temperatures are representative of this bipolar effect. Indeed, endorphins either increase or decrease core temperature depending on whether the environment induces a decrease or increase of core temperature [37]. This supports the suggestion made by others for a regulatory role for endorphins [15]. This specific theorized function ascribed to the endorphins does not, however, explain other accepted roles such as endorphin-related stress-induced analgesia. The broader concept now suggested of adaptation to aversive stress encompasses both the previously suggested regulatory role based on studies of hormonal release [9] and temperature control, and the adaptive role based on pain and stress-induced analgesia studies.

Regulatory role is included in the general function of adaptation, whereby physiological variables, if disturbed by environmental insults, should return to normal set levels.

Stress-induced analgesia with its decline with chronic exposure supports the general concept suggested here for the role of endorphins and also explains why naltrexone produces opposite effects when rats are submitted to either acute but relatively short lasting stressors, or long lasting acute and chronic stress [13]. Acute stress-induced analgesia has already been explained in terms of adaptation [9,19]. This concept implies that the first exposure to a noxious insult should be met with an intact perception of pain; at this stage, an attenuation of pain perception would be of doubtful survival value to the animal, since it is important that potentially damaging painful stimuli be rapidly and reliably detected so that avoiding action can be taken. Accordingly, naltrexone does not affect pain responsiveness following certain types of short-acting stressors [8, 13, 23, 28], suggesting that, at this stage, the endorphins are not released. However, once the appropriate action has been taken, severe prolonged pain can become incapacitating. In this situation, a system attenuating pain perception that is activated after the initial response to the noxious stimulus, and gives prolonged relief, might be useful. Naltrexone does antagonize the analgesia induced by relatively longer lasting stressors [8,13], suggesting that endorphins are released. When the duration of stress is still increased or when it is repeatedly encountered, it is essential that the organism regains its primary function to react against possible newly encountered noxious stimuli. Therefore, if some internal mechanism still induces analgesia, another mechanism should be existent which would function to antagonize any remaining analgesic effect of stress. Naltrexone increased the tail-flick latencies in rats submitted to chronic ICWS and in rats submitted to 60 inescapable ICWS exposures [13] when tested 30 minutes after exposure (if not submitted to ICWS, the chronically stressed rats do not display increased tail-flick latencies after administration of naltrexone, in preparation). This suggests that the endorphins have now acquired an analgesia-inhibitory role.

It would be interesting to determine what neural mechanisms are involved in the various adaptive responses to stress. Particularly intriguing is the shift in the role for endorphins from analgesia-induction (acute stress condition) to analgesia-inhibition (chronic condition). One possibility is that the analgesia-inducing opioid system has collateral branches which impinge upon and inhibit (in the chronic ICWS condition) the analgesia-inducing non-opioid system. Such a mechanism has already been hypothesized and documented [22], but its existence remains to be ascertained. Another possibility is that chronic exposure to stress progressively activates a new type of endorphinergic neurons and receptors, thereby producing hyperalgesia. A possible candidate for this opioid system is the dynorphins which specifically act on κ receptors. It has been shown that their endogenous level is reduced in rats submitted to acute painful stimuli, but are largely increased in rats with chronic arthritis [32,33]. The paradoxical shift seen in the present and previous experiments from an opioid analgesia induction (acute stress) to an opioid analgesia-inhibition (chronic stress) may thus be explained in terms of a balance in the relative release and/or receptor sensitivity of various types of opioid systems. In the acute stress condition, the opioid-analgesia is probably induced through the activation of μ or Δ-enkephalinergic receptors. Indeed, rats submitted to acute electric shock displayed a parallel increase of analgesia and levels of brain enkephalins [26]. In the chronic stress condition adaptation to SIA may be due to: (1) a decrease of the stress-induced release of enkephalins (cf. [26,29]) resulting in a progressive decline of SIA with repeated exposure, and (2) increases of the level of dynorphins and/or κ-receptor sensitivity, which may be actively involved in SIA-adaptation by producing hyperalgesia. This explanatory model which integrates the present findings that naltrexone decreases ICWS-analgesia in the acute condition but enhances it in the chronic condition remains hypothetical, but may nevertheless account for findings that (1) acute shock increases the level of enkephalins in the brain [26] and decreases the level of dynorphins [33]; (2) chronic stress decreases the level of brain enkephalins [26,29] but increases the level of dynorphins [32]; and (3) morphine lowers the response threshold to nociceptive stimuli in rats submitted to ICWS (Girardot and Holloway, to be submitted).

The anatomical distribution of the opioids in all areas related to noxious stress (hypothalamus, pituitary gland, adrenal medulla, pain-related areas) further supports our hypothesis that a major functional role of the various types of endorphins is adaptation to noxious stress.

In summary, anatomical, physiological, pharmacological and behavioral findings on endogenous opiates seem to indicate that a major functional role for the endorphins is adaptation to aversive stress. The present study supports this view. However, an extension of the variables studied in this investigation (analgesia, core temperature, activity and behavioral observations) to others such as the cardiovascular response to stress, further validation of skin temperature measures using the appropriate skin thermal device, and supportive studies at the biochemical or electrophysiological levels are required before this global role can undoubtedly be ascribed to the endorphins.

ACKNOWLEDGEMENTS

This research was supported financially by NIDA Grant 5 T32 DA07105-05 (R. S. Krug, P.I.) and a Minigrant from the Department of Psychiatry and Behavioral Sciences. We express our gratitude to NIDA for providing the naltrexone used in this study and to Lynn Montgomery for her skillful secretarial contribution.

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