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Cold Swim Stress-Induced Changes in the Levels of Opioid Peptides in the Rat CNS and Peripheral Tissues

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VASWANI, K. K., C. W. RICHARD, III AND G. A. TEJWANI. *Cold swim stress-induced changes in the levels of opioid peptides in the rat CNS and peripheral tissues.* PHARMACOL BIOCHEM BEHAV 29(1) 163–168, 1988.—Endogenous opioid peptides have been implicated in stress-induced analgesia and stress-induced feeding behavior. An earlier study from our laboratory showed that rats subjected to cold swim stress consumed significantly more food compared to controls [46]. The present study describes changes in the levels of various opioid peptides in the central nervous system and periphery due to cold swim stress. Male Sprague-Dawley rats were subjected to cold swim stress (1°C for 5 min), then sacrificed by decapitation; brain, pituitary, adrenals and plasma were collected. Tissue extracts were assayed for opioid peptides by RIA. Cold swim stress resulted in analgesia which could be blocked by prior administration of naloxone, as observed by a tail-flick latency test. Cold swim stress caused a 42% decrease in pituitary β-endorphin, but increased the level of this peptide in the hypothalamus and plasma by 36% and 337%, respectively. Dynorphin level decreased by 62% in the hypothalamus, but was not affected in the pituitary. Levels of Leu-enkephalin and Met-enkephalin decreased in the adrenal gland by 37% and 18%, respectively, but were not significantly affected in the CNS. These results indicate that cold swim stress has a differential effect on the level of CNS and peripheral opioid peptides, and that both central and peripheral opioid peptides may be important in stress-induced analgesia and feeding behavior.

β-Endorphin Dynorphin Leu-Enkephalin Met-Enkephalin Opioids Stress Analgesia

STRESSFUL stimuli result in profound behavioral and physiological responses in animals and humans, with one of their effects being analgesia [14, 19, 44]. Stress-induced analgesia can be resolved into an opioid and a non-opioid component, depending upon cross-tolerance to morphine, antagonism by naloxone, and demonstration of opioid peptide release in the brains of animals subjected to different forms of stress [14, 37, 44, 45, 49].

Endogenous opioid peptides have been implicated in physiological mediation of ingestive behavior [31, 32, 35]. For example, various types of stimulated feeding can be suppressed by opioid antagonists in a dose dependent and stereospecific manner [32,35]. Several opioid agonists, such as β-endorphin [16, 21, 25], dynorphin [31] and Met-enkephalin [25] can induce feeding, and this effect can be blocked by naloxone [24, 35, 46].

Various stress models such as immobilization, foot shock, cold exposure and tail pinch, which can cause opioid-dependent analgesia, also result in changes in opioid levels in the CNS and plasma [3, 22, 27, 36]. Our previous study showed an increase in food intake due to cold swim stress which was blocked by naloxone [46]. In the present study, we describe the changes in opioid peptide levels in CNS and plasma induced by cold swim stress, and discuss the possible physiological significance of this phenomenon. A preliminary report on this work has been presented [42].

METHOD

Chemicals

Met-enkephalin, Leu-enkephalin and dynorphin were purchased from Sigma Chemical Company (St. Louis, MO). Camel-β-endorphin was purchased from Peninsula Laboratories (Belmont, CA). Human β-endorphin was a generous gift of Dr. Manian (NIMH). Most of the other chemicals used in chromatographic procedures and radioimmunoassays were obtained from Sigma Chemical Company.

Antisera

Antiserum against β-endorphin was generated in our laboratory. Properties of this antiserum were described earlier

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TABLE 1
SCHEME FOR USE OF EXPERIMENTAL RAT GROUPS

Experimental Treatment	Control	Room Temp Swim	Cold Room Temperature Exposure	Cold Swim
8th day* (No drug, no saline) (Baseline tailflick latency)	I	II	III	IV
15th day (Saline, tailflick latency)	IV	I	II	III
22nd day (Naloxone, tailflick latency)	III	IV	I	II
29th day (Stressors and decapitation)	II	III	IV	I

*Animals were maintained for seven days on normal laboratory chow.

Groups are represented by I, II, III, and IV. Each group contained six animals.

[41,43]. Antisera for Met-enkephalin and dynorphin 1–13 were generous gifts of Drs. S. L. Sabol (NIH, Bethesda, MD) and A. Goldstein (Addiction Res. Fdn., Palo Alto, CA), respectively. Leu-enkephalin antiserum was purchased from Peninsula Laboratories (Belmont, CA).

RIA Assay Conditions

Radio-iodinated peptides were either prepared in our laboratory [47] or were purchased from Peninsula Laboratories (Belmont, CA). Conditions for the radioimmunoassay of various peptides were the same as described earlier [41, 43, 48].

Experimental Design

Twenty-four male Sprague-Dawley rats (200–240 g) were maintained as described previously [46,48]. Table 1 describes the experimental protocol used for subjecting the animals to various treatments. After a week of acclimatization on a normal laboratory rat chow, on 8th day rats were divided into four groups, each containing six animals. Animals in group I served as control, group II were subjected to five min swim at room temperature, group III were exposed to cold room for five min, and group IV were subjected to cold water swim at 1°C for five min. Base line analgesia was measured by determining tail flick latency and animals were given rest for seven days. On the 15th day, groups of animals were randomized with respect to particular stress. For example, animals in group IV served as control, group I was subjected to room temperature swimming and so on as shown in Table 1. Animals in all the groups were injected with saline before subjecting them to specific stressor and analgesia was determined by measuring tail flick

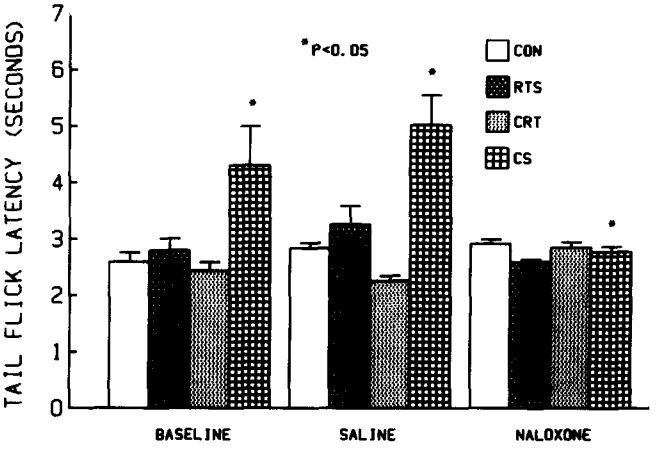

FIG. 1. Effect of naloxone on cold swim stress induced analgesia. Values are mean±SEM for n of 6 in each group. Values are compared by two way analysis of variance with repeated measures across the drugs. For treatments $F(3,20)=22.03$, $p<0.0001$; for drugs $F(2,15)=4.40$, $p<0.05$ and for treatments × drugs $F(6,40)=5.79$, $p<0.0005$. Multiple comparison of means was done by SNK test. *$p<0.05$. CON, control; RTS, room temperature swim; CRT, cold room temperature exposure; CS, cold swim.

latency. Animals were given rest for seven days and on the 22nd day, various rat groups were randomized once again as shown in Table 1. To see the effect of naloxone on stress induced analgesia (SIA), rats were injected with naloxone (1 mg/kg body weight), intraperitoneally, 20 min before subjecting them to various stressors and tail flick latency was noted. All the animals were given rest again for seven days, randomized on 29th day as shown in Table 1, and subjected to various stressors and then sacrificed by decapitation. Brain, adrenals, and pituitary were removed and plasma was collected. The brain was dissected into various parts (hypothalamus, hippocampus, striatum, cortex, medulla/pons and midbrain) by the method of Glowinski and Iverson [15]. Tissues were further processed as described previously [41,43].

Stress-Induced Analgesia (SIA) Measurements

Analgesia was measured by the method of D'Amour and Smith [9]. Rats were acclimatized to holders for a period of 20 min each day for at least 3 days to familiarize them with the environment and decrease the stressing effects that containment could have on them. After subjecting them to various treatments, a beam of light from a 650 W tungsten halogen lamp (Sylvania, DVY) was projected through a 5 mm diameter to the tail about 1.5 cm away from the tip of the tail. Light intensity was controlled by the lamp voltage rheostat, which was kept on 54 volts. The time until flicking of the tail (latency) was measured to the nearest tenth of a second by using a digital electronic timer (Cole-Parmer Instrument Co., Chicago, IL). The lamp was automatically shut off at 10 sec to avoid damage to the tail. The tail flick latency was recorded every 2 min over a 20 min period to obtain 8–10 measurements for every rat.

Statistics

To analyze the data on SIA, two way ANOVA (analysis of variance) was used, and multiple comparison of means was carried out by Student-Newman-Keuls (SNK) test

[20,39]. Data analysis of opioid peptide concentrations for the various tissues was done using one way ANOVA, and multiple comparison of means was carried out by Tukey's test [20,39].

RESULTS

Effect of Naloxone on Cold Swim Stress Induced Analgesia

Animals subjected to cold swim stress exhibited an increase in tail flick latency of 66% and 77% as compared to the controls given nothing or saline only, respectively. Both values were significantly different from that of the controls, but not from each other (Fig. 1). The other two groups (RTS and CRT) did not show any significant differences in tail-flick latency from their respective controls. Naloxone administration 20 min before subjecting the animals to stress abolished the increase in tail flick latency induced by cold swim stress, indicating the possible involvement of endogenous opioid peptides in SIA (Fig. 1).

Effect of Cold Swim Stress on Peripheral, Pituitary and Brain Opioid Peptide Levels

β-Endorphin. Rats subjected to CS and RTS showed an increase of 337% and 488% in plasma level of β-endorphin (Fig. 2a). This increase in the plasma β-endorphin level may be due to release of pituitary β-endorphin into the plasma, since pituitary β-endorphin level decreased by 42% and 33% in the CS and RTS groups, respectively (Fig. 2b). There were no significant changes in plasma or pituitary β-endorphin levels in rats subjected to CRT exposure.

The increase in plasma β-endorphin level in rats from both (CS and RTS) groups suggests that this is a result of physical exercise, that is swimming, and not due to the temperature differences of the water. The plasma and pituitary content of β-endorphin in the CS and RTS groups were not statistically significant from each other.

The hypothalamic content of β-endorphin increased by 45%, 49% and 86% in rats subjected to CRT, RTS and CS stress, respectively (Fig. 2c). The β-endorphin level of the cerebral cortex increased from 0.6 to 1 pmol/g wet wt of tissue in the CS group, and to 2.3 pmol/g wet wt of tissue in the CRT group (data not shown). There were no statistically significant differences in β-endorphin levels in the hippocampus, midbrain, medulla pons and striatum of the rats subjected to any of these stressors (data not shown).

Dynorphin. The pituitary showed no change in dynorphin concentration in rats subjected to any of the stressors (data not shown). Animals in the CS and CRT groups showed a decrease in the level of dynorphin in their hypothalamus by 63% and 48%, respectively (Fig. 3a). The striatum also showed a significant decrease in its dynorphin level of 34% and 21% respectively, in the CS and the CRT groups; however, rats subjected to RTS exposure showed an increase of 56% (Fig. 3b). No differences were observed in the remaining brain areas (data not shown). No detectable dynorphin immunoreactivity was found in the adrenals or plasma of these rats, under the conditions mentioned above [48].

Leu-Enkephalin. No significant changes in Leu-enkephalin levels were observed in the pituitary or any other brain area (data not shown). Adrenals showed a significant decrease in Leu-enkephalin concentration in all three groups of about 30–40% (Fig. 4). No detectable Leu-enkephalin immunoreactivity was observed in the plasma of animals in all three groups under the conditions used [48].

Met-Enkephalin. No statistically significant differences

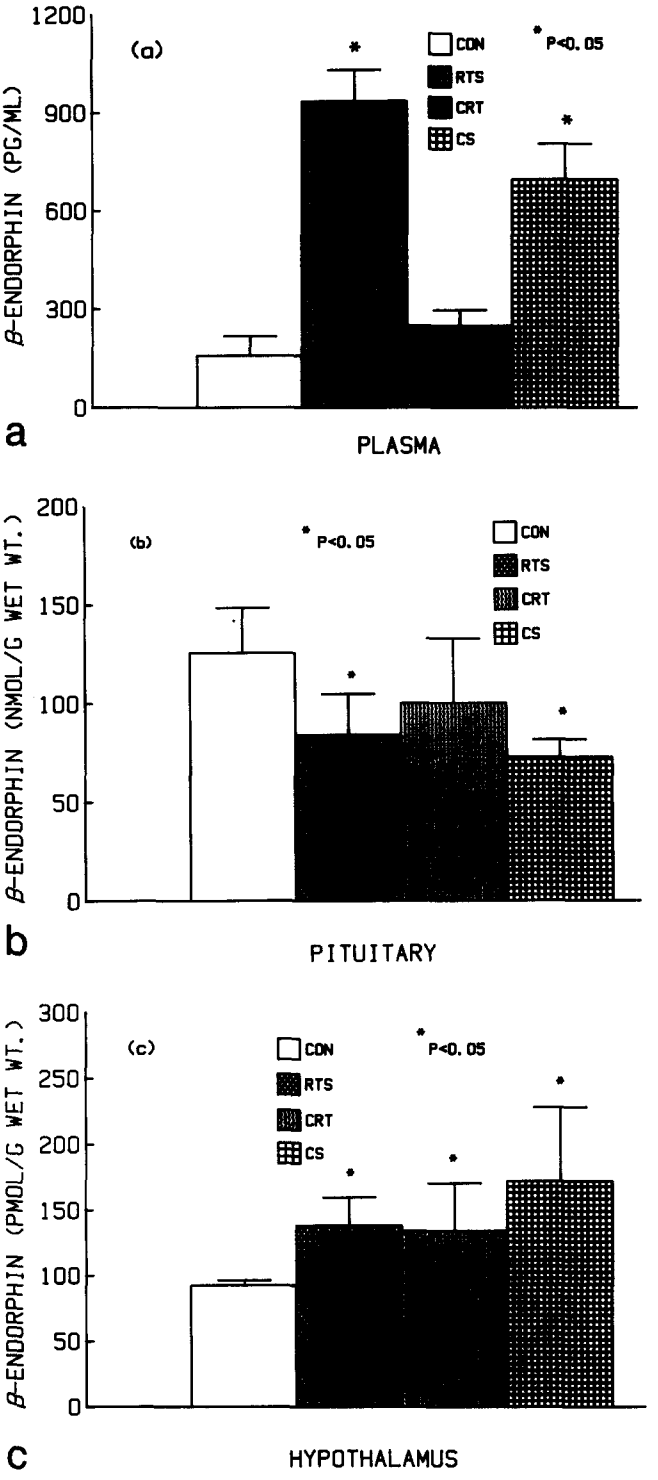

FIG. 2. Effect of cold swim stress on rat plasma, pituitary and hypothalamic β-endorphin. (a) Plasma. (b) Pituitary. (c) Hypothalamus. Values are mean±SEM for n of 6 in each group. For each tissue values were compared by one-way analysis of variance and then means were compared by Tukey's test. For pituitary $F(3,20)=3.02$, $p<0.05$; for plasma $F(3,20)=9.68$, $p<0.0005$ and for hypothalamus $F(3,20)=3.01$, $p<3.01$, $p<0.05$. *$p<0.05$. Values for pituitary are nmol/g wet wt., for plasma pg/ml and for hypothalamus pmol/g wet wt. CON, control; RTS, room temperature swim; CRT, cold room temperature exposure; CS, cold swim.

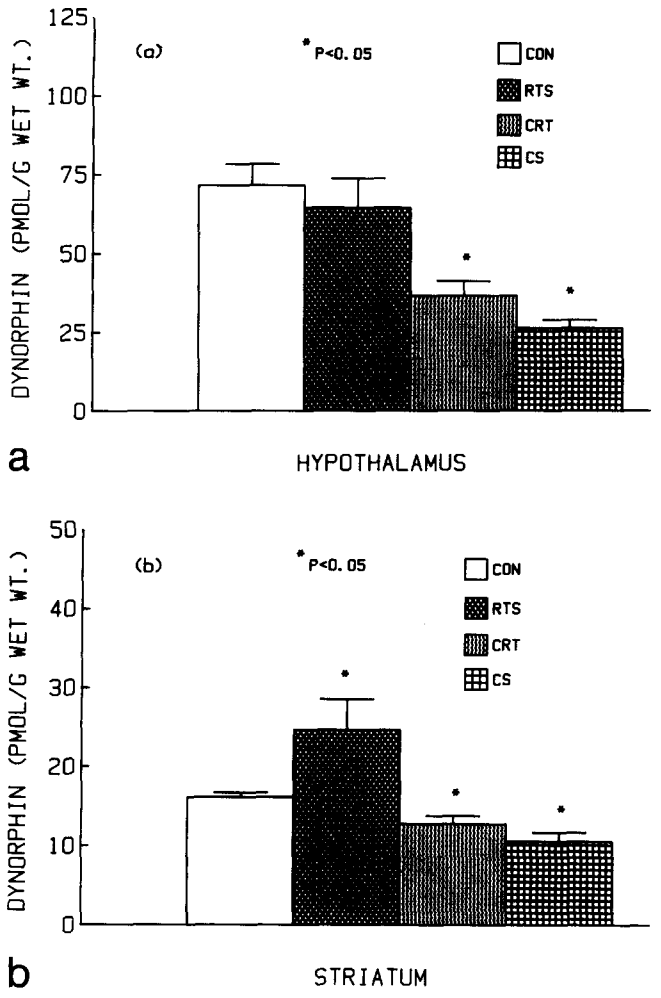

FIG. 3. Effect of cold swim stress on rat hypothalamic and striatal dynorphin. (a) Hypothalamus. (b) Striatum. Values are mean±SEM for n of 6 in each group. Values are compared for each tissue by one-way analysis of variance. Means are compared by Tukey's test. For hypothalamus $F(3,20)=15.05$, $p<0.0001$ and for striatum $F(3,20)=10.35$, $p<0.0005$. $^*p<0.05$. Values are pmol/g wet wt. CON, control; RTS, room temperature swim; CRT, cold room temperature exposure; CS, cold swim.

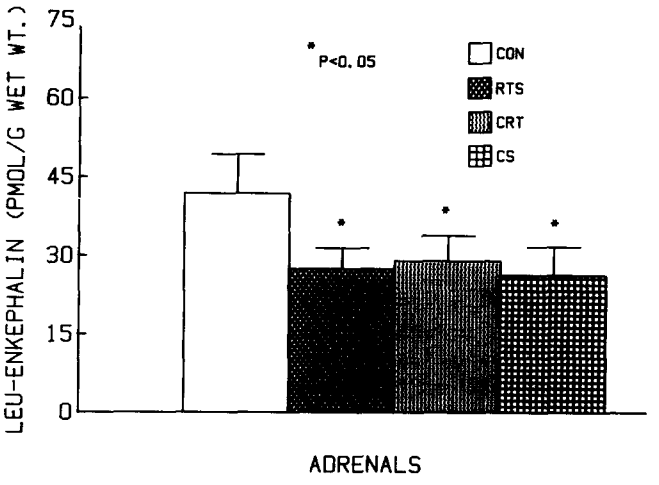

FIG. 4. Effect of cold swim stress on adrenal Leu-enkephalin. Values are mean±SEM for n of 6 in each group. Values are compared by one way analysis of variance and means are compared by Tukey's test. $F(3,20)=3.03$, $p<0.05$. $^*p<0.05$. Values are pmol/g wet wt. CON, control; RTS, room temperature swim; CRT, cold room temperature exposure; CS, cold swim.

were observed in Met-enkephalin levels in any of the brain regions, adrenals or pituitary with any of the treatments (data not shown).

In summary, in rats subjected to cold swim stress, there was a significant decrease in pituitary β-endorphin levels whereas the concentration of this opioid increased in plasma and hypothalamus. Dynorphin was significantly decreased both in hypothalamus and striatum. No changes were observed in Met-enkephalin and Leu-enkephalin levels, except in the adrenals where there was a decrease in the level of Leu-enkephalin in these animals.

DISCUSSION

Evidence from reported studies [49,50] indicates that analgesia can be classified into four categories: neural-opiate, hormonal-opiate, neural-non-opiate and hormonal non-opiate. The neural-opiate class includes analgesia produced by morphine, electrical stimulation of the brain, front paw shock and classical conditioning. The analgesia induced by these manipulations is not affected by the removal of the pituitary or the adrenals, but it is reduced or abolished by naloxone, morphine tolerance and lesions of the dorsolateral funiculus [49].

Several factors have been reported to induce analgesia due to stress. These include foot-shock induced analgesia [1,26], cold swim stress [4,6], food-deprivation [5,24], sexual behavior [8], conditioning [7,50] or learned helplessness [18]; all of these have been shown to produce analgesia that is antagonized by naloxone. Complexities are involved in stress-induced analgesia which are due to the activation of both opiate and non-opiate mechanisms.

Cold swim stress induced analgesia has been shown to be dependent on both the opiate and non-opiate mechanisms, since large doses of naloxone (20 mg/kg, SC) were not able to antagonize completely this analgesic effect under certain experimental conditions [6]. In the present study, we observed an increase in tail flick latency with cold swim stress which was reduced to normal control levels in animals given prior injection of naloxone, thus indicating an involvement of the opioid system (Fig. 1). This reversal of the increase in tail flick latency by naloxone is one of the criteria used to determine opioid dependency [10,11]. The cold swim stress has been shown to activate the pituitary-adrenal axis and deplete hypothalamic norepinephrine (see [46] for detailed references). The removal of either pituitary or adrenals does not have any effect on opioid-induced analgesia, but does seem to affect analgesia due to acupuncture, prolonged foot shock of all four paws, and immobilization induced analgesia [49].

Some workers have reported that opiate antagonists at doses as high as 20 mg/kg did not block the analgesia effect of cold swim stress [4,14]. However, it must be pointed out that in the study by Girardot and Holloway [14] instead of naloxone, naltrexone was used, which has delayed onset of action and much longer half-life compared to naloxone.

Bodnar *et al.* [4] observed that naloxone dose-dependently attenuated the cold-water swim-induced analgesia up to a maximum reduction of 50% at 20 mg/kg. In

contrast, naloxone had no effect upon normal flinch-jump thresholds. There are very important differences in our study and the one done by Bodnar *et al.* [4], who used older rats (350–500 g), different type of measurement of analgesia (flinch-jump thresholds) and most importantly, injected naloxone *subcutaneously immediately* before subjecting animals to cold swim stress. In our study, we injected naloxone intraperitoneally to rats, 20 min before subjecting them to cold swim stress, thus making sure that the drug had chance to equilibrate systemically.

Various stress models such as 3 hr immobilization, prolonged foot shock, or 2 hr cold-exposure-stress have been shown to result in decreased levels of β-endorphin in the anterior lobe of the pituitary and a concomitant increase in plasma β-endorphin levels [3, 22, 26, 36]. The latter observation has also been demonstrated in animals subjected to acute or chronic swimming [10,11]. This indicates a possible involvement of the pituitary in stress-induced analgesia. In the present study, cold swim stress caused a 42% decrease in pituitary β-endorphin levels and a 337% increase in plasma β-endorphin (Fig. 2). The increase in plasma β-endorphin may be due to release of β-endorphin from the anterior lobe of the pituitary. However, there are stressors which cause equally profound changes in plasma β-endorphin, but do not have any analgesic effects [22]. This is supported by the observations in the present study, where a group of rats subjected to room temperature swim showed decreased pituitary and increased plasma levels of β-endorphin, but did not exhibit analgesia. It has also been shown that acute stress results in an increase in the synthesis of pro-opiomelanocortin (POMC), and accelerates its conversion to alpha-MSH and β-endorphin [38]. In addition, tolerance can develop against stress-induced analgesia on chronic stimulation [2].

Milan *et al.* [28] have reported a decrease in β-endorphin levels in the hypothalamus upon foot shock-induced analgesia; this was also observed in the periventricular tissue (incorporating the periaqueductal gray area) which is highly populated by β-endorphinergic fibers and terminals projecting from the arcuate nucleus [23,40]. Similarly, in shuttle avoiding learning sessions, a decrease in β-endorphin was observed in the brain, except the hypothalamus [17]. In the present study, we observed an increase in the level of hypothalamic β-endorphin in animals subjected to cold swim stress. This may occur due to increased synthesis and/or decreased transport of β-endorphin from the hypothalamus to other brain areas, and could cause analgesia in the rats subjected to cold swim stress.

Two additional opioid families are present in the pituitary and other brain areas [33]. In the pituitary, enkephalins are present in the neural lobe, while dynorphin is localized in both the posterior and anterior lobe cells [2]. No changes in either enkephalins (Met-enk or Leu-enk) or dynorphin levels were observed with cold swim stress in the present study. Millan *et al.* [28] have shown that dynorphin-like immunoreactivity is decreased in the anterior lobe but remains unchanged in the posterior lobe and hypothalamus on acute foot-shock-stress. Various stress models used by Morley *et al.* [30] have revealed differential effects of stress on the levels of dynorphin in the cortex and hypothalamus. Restraint, tail pinch and cold swim (10–16°C for 10 min) stress did not have any effect on hypothalamic dynorphin, but cold exposure for 2 hr decreased the hypothalamic dynorphin levels. Cortical dynorphin was reduced only with tail pinch stress, and was not affected by any other kinds of stress [30]. In the present study, cold swim stress (1°C for 5 min) resulted in a decrease in the level of dynorphin in the hypothalamus (63%) and striatum (34%) (Fig. 3).

No changes were observed with respect to Met-enkephalin in any of the tissues (central or peripheral) studied (data not shown). A variety of foot shock schedules have been shown to be ineffective in modifying Met-enkephalin concentrations in various brain regions [13,28]. Stress due to intravenous injection of diazepam in rats has been shown to result in a decrease of Met-enkephalin level in the hypothalamus [12]. A significant decrease in Leu-enkephalin concentrations due to prolonged foot shock has been observed by Rossier *et al.* in the hypothalamus [34]. Cold swim stress did not affect Leu-enkephalin levels in the pituitary or in the brain (data not shown), but decreased it in the adrenals (Fig. 4).

In conclusion, it is clear that cold swim stress seems to affect various opioid peptides in a differential manner. The changes observed in the level of opioid peptides depend on the type of stressor used, which could affect the synthesis and processing of various opioid precursors differentially (i.e., POMC, preproenkephalin A and preprodynorphin). Both central and peripheral changes in β-endorphin levels, and central changes in dynorphin concentrations, may be important in stress-induced analgesia and feeding behavior, as suggested by Morley *et al.* [31] and Vaswani *et al.* [46].

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