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The Effect of Altered 5-Hydroxytryptamine Levels on β-Endorphin Content in Rat Brain (42326)

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Abstract. The purpose of the present study was to examine the effect of altering the concentration of 5-hydroxytryptamine (5-HT) on β-endorphin (β-Ep) content in the hypothalamus, thalamus, and periaqueductal gray (PAG)-rostral pons regions of the rat brain. The selective 5-HT reuptake inhibitor, fluoxetine (10 mg/kg), significantly lowered β-Ep content in the hypothalamus and the PAG. Parachlorophenylalanine, which inhibits 5-HT synthesis, significantly elevated β-Ep in all brain parts studied. Intracisternal injections of the neurotoxin, 5′,7′-dihydroxytryptamine, with desmethylimipramine pretreatment, significantly increased β-Ep content in the hypothalamus and the PAG. In adrenalectomized rats, fluoxetine significantly decreased β-Ep levels in the hypothalamus and increased the levels in the PAG. The results indicate that 5-HT may modulate the levels of brain β-Ep.

5-Hydroxytryptamine (5-HT) may be directly involved in the determination of pain sensitivity and/or reactivity, and in the antinociceptive action of morphine. Opiate and electrical stimulation induced analgesia is antagonized by pharmacologically mediated decreases in serotonergic neurotransmission (1–4). Fluoxetine, a specific 5-HT reuptake inhibitor, and quipazine, a putative 5-HT agonist, both produce analgesia, presumably by enhancing 5-HT effects at the postsynaptic receptors of 5-HT pathways (5–8).

Several studies have shown that acute doses of morphine alter 5-HT turnover (8–10). The precise mechanism by which morphine increases 5-HT turnover is unclear. Microinjections of morphine into the periaqueductal gray (PAG) result in increased 5-HT levels in spinal perfusates (11). β-Endorphin (β-Ep) administered intracisternally increases the concentration of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in the brainstem and the hypothalamus (12). These results suggest that the β-Ep and 5-HT systems are functionally related, and that they are part of a neurochemical circuit which mediates nociception. Drugs which alter the activity in one system would be expected to change the activity in the other. Therefore the aim of this study was to examine the effect of pharmacologically altering 5-HT neurotransmission on β-Ep content in discrete regions of the rat brain.

Materials and Methods. Sprague–Dawley rats purchased from Southern Animals Farms (Prattville, Ala.) and weighing 200–250 g were maintained for a minimum of 3 weeks, prior to any experimentation, in controlled light (12 L:12 D) and temperature (23 ± 1°C). Lights were on in the animal chamber from 0800 to 2000 hr. Animals were supplied with feed (Purina lab chow) and water *ad libitum*. Animals were sacrificed by means of a beam of microwave radiation (General Medical Corp., Peabody, Minn.) focused on their skull for 4.25 sec. The brain was immediately removed and frozen on dry ice. The level of β-Ep was determined in three brain regions: the hypothalamus, thalamus, and periaqueductal gray-rostral pons. The hypothalamus was dissected in coronal slices throughout its entire extent, from the preoptic areas to the caudal mammillary bodies. To dissect the thalamus, a horizontal cut was made at the anterior commissure. The atlas of Konig and Klippel (13) was used as a guide. The PAG-rostral pons region was dissected as a coronal slice extending from the caudal part of the medial geniculate nucleus to the inferior colliculi, and included the rostroentral part of the pons.

Brain regions were weighed and placed in 0.1 *N* HCl, preheated to 95°C, and boiled for 20 min. Samples were cooled on ice, homogenized, and centrifuged (20,000 rpm, 20 min). The supernatants were frozen overnight. Samples were thawed, neutralized to pH 7.5

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with 1 *M* Tris buffer solution, and recentrifuged (20,000 rpm, 20 min), yielding a clear supernatant. The samples were diluted with BSA–borate buffer, 0.1 *M*, pH 8.4. β-Endorphin-like immunoreactivity was measured by RIA (Immuno Nuclear Corp., Stillwater, Mich.). The cross-reactivity of this antibody was approximately 50% with β-lipotropin and less than 0.1% with α-endorphin (β-LPH 61–76), [D-Ala^2] β-Ep, dynorphin, α-neoendorphin, α-endorphin leucine enkephalin, methionine enkephalin, α-melanocyte-stimulating hormone (MSH), and β-MSH. Sample and antibody were incubated overnight. ^{125}I-β-Ep tracer was then added and the solutions were further incubated at 5°C for 48 hr. Phase separation was accomplished by the addition of goat anti-rabbit serum in the presence of carrier rabbit serum, with polyethylene glycol added as precipitating agent. The samples were centrifuged (2000 rpm, 20 min) and decanted, and both the decantates and precipitates were counted.

In this experiment all drugs were dissolved in 0.9% NaCl. Fluoxetine (Lilly 110140) and chlorimipramine (Ciba Geigy) were administered intraperitoneally at a dosage of 10 mg/kg, twice daily. Rats were sacrificed 12 hr after the final saline or drug injection. In one experiment, fluoxetine was administered to rats 8 days after adrenalectomy, on the same schedule as outlined above. After adrenalectomy, rats were offered 0.9% NaCl in their drinking water. *para*-Chlorophenylalanine (PCPA, Sigma)-treated rats were sacrificed 3 days after a single injection of 300 mg/kg at either 1200 or 2400 hr. Desmethylimipramine (DMI, USV laboratories, 25 mg/kg) was administered 1 hr prior to an intracisternal injection (200 μg) of 5,7-dihydroxytryptamine (5,7-DHT, Sigma) dissolved in sterile saline. Control rats received DMI pretreatment and an intracisternal injection of 20 μl of sterile saline. Data were subjected to a one-way analysis of variance and differences among means were analyzed using least significant difference (LSD) (14).

Results. Fluoxetine, a selective 5-HT reuptake inhibitor (15), significantly ($P < 0.01$) decreased the levels of β-Ep in the hypothalamus and PAG of rats sacrificed at 2400 hr during the dark phase hours (Fig. 1). Nine days after adrenalectomy, no change in β-Ep content was observed in the hypothalamus of adrenalectomized rats as compared to the control rats (Fig. 2). When fluoxetine was administered 8 days

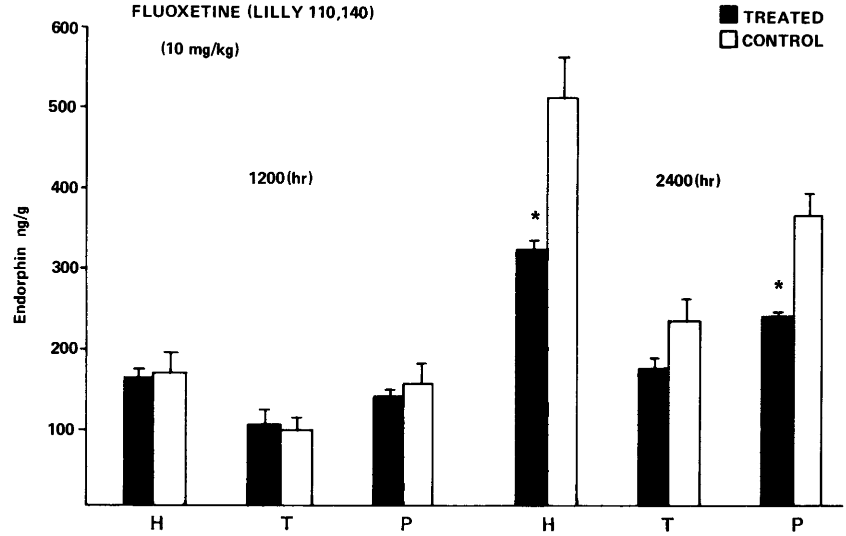

FIG. 1. Effect of fluoxetine (10 mg/kg) on β-Ep concentrations in specific regions of the rat brain. Values are expressed in nanograms per gram (wet weight) of brain tissue. Values represent the means + SEM of six animals. (H: hypothalamus, T: thalamus, P: PAG-rostral pons). Asterisk indicates that treated group was significantly ($P < 0.01$) different from control group.

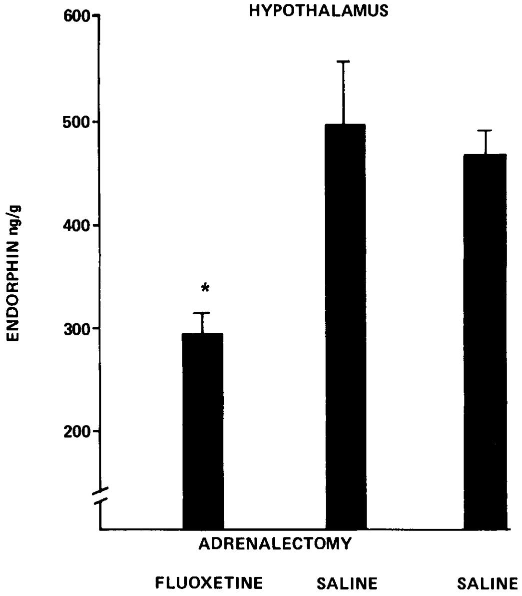

FIG. 2. Effect of fluoxetine on β-Ep concentrations in the hypothalamus of adrenalectomized rats. Values represent the means + SEM of six animals. Asterisk indicates that treated group was significantly ($P < 0.05$) different from saline–adrenalectomy group. There was no significant effect of adrenalectomy on β-Ep levels.

after adrenalectomy, significant ($P < 0.05$) reduction of β-Ep levels was observed in the hypothalamus (Fig. 2) and the PAG (Fig. 3).

Chlorimipramine, a tertiary amine tricyclic antidepressant that inhibits 5-HT reuptake (16), decreased β-Ep content significantly by 25% ($P < 0.05$) in the hypothalamus (treated 330.25 + 6 ng/g vs control 447.42 + 25 ng/g, $N = 6$, $P < 0.05$). No effect was observed in the thalamus or the PAG (data not shown).

Rats sacrificed 3 days after treatment with PCPA, an inhibitor of 5-HT synthesis (17, 18), had significant increases ($P < 0.01$) in β-Ep levels in the three brain regions examined in animals sacrificed 4 hr into the dark phase (Fig. 4). Intracisternal injections of the 5-HT neurotoxin 5,7-DHT, with DMI pretreatment (19), significantly ($P < 0.05$) elevated β-Ep in the hypothalamus and the PAG but not in the thalamus (Fig. 5).

Discussion. We have previously demonstrated the presence of diurnal variations in the levels of endogenous β-Ep in rat brain (21). β-Ep concentrations in the hypothalamus, thalamus, and the PAG of rats are elevated during the dark phase of the light/dark cycle (21). One would therefore expect that the time of day of animal sacrifice might alter the effects of 5-HT uptake inhibitors on β-Ep concentration. In this study, fluoxetine and chlorimipramine were ineffective in altering β-Ep content in animals sacrificed during the light phase. PCPA, which reduces 5-HT content, had no effect on the levels of β-Ep as compared to control rats sacrificed at 1200 hr. In contrast, animals sacrificed during the dark phase had significantly altered levels of β-Ep following the administration of drugs which alter 5-HT activity. The lack of responsiveness to 5-HT drugs during the light phase may be related to the low β-Ep level at this hour.

The results obtained here are in agreement with our previous findings (22). We have shown that treatments with PCPA or fluoxetine alter total brain β-Ep contents in a similar manner as observed in the present investigation (22). On the other hand, the results obtained here are in disagreement with other reported data (23, 24). The discrepancy among these results may be attributed to differences in the time of the day when animals were sac-

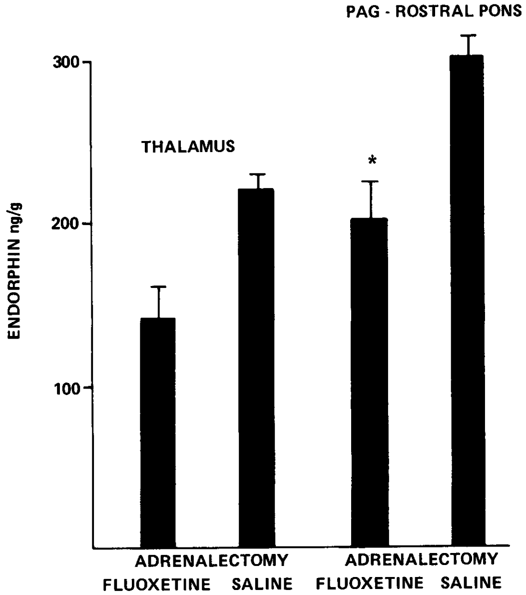

FIG. 3. Effect of fluoxetine on β-Ep content in the thalamus and the PAG-rostral pons of adrenalectomized rats. Values represent the means + SEM of six animals. Asterisk indicates that fluoxetine treatment results in significant ($P < 0.05$) effects on β-Ep level.

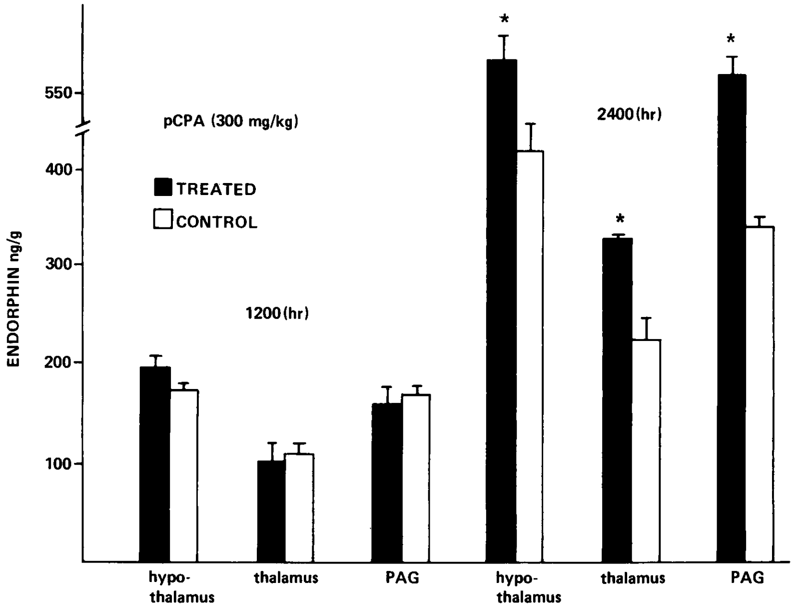

FIG. 4. Effect of PCPA (300 mg/kg ip) on β-Ep content in the hypothalamus, thalamus, and PAG-rostral pons. Values represent the means + SEM of six animals. Asterisk indicates that treated group was significantly ($P < 0.01$) different from control group.

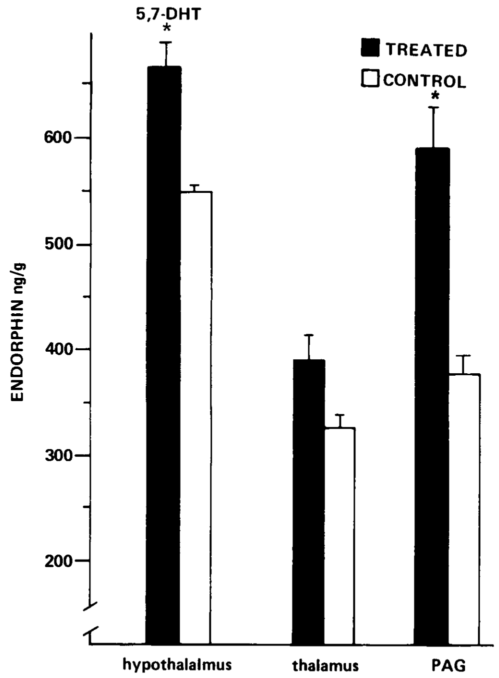

FIG. 5. Effect of an intracisternal injection of 5,7-dihydroxytryptamine (5,7-DHT) with DMI pretreatment on β-Ep content of the hypothalamus. Values are the means + SEM of six animals. Asterisk indicates that treated group was significantly ($P < 0.05$) different from control group.

rificed, differences in brain regions examined, the assay procedure, or the dose and frequency of the drug used.

Changes in the functional state of 5-HT by reuptake inhibitors such as chlorimipramine and fluoxetine would be expected to facilitate β-Ep analgesia (25). In this study, fluoxetine and chlorimipramine decreased β-Ep measured in the hypothalamus. Endorphinergic cell bodies located in the hypothalamus, in the arcuate nucleus, and in the neuronal process extend throughout several levels of the hypothalamus (26). The depressed levels of β-Ep in certain brain areas following enhanced 5-HT activity may be caused by stimulating the release and breakdown of the β-Ep. This effect might explain the potentiation of the analgesic effect using 5-HT reuptake inhibitors (25). Chronic morphine treatment has been shown to depress β-Ep levels in the intermediate lobe of the pituitary by reducing the activity of messenger ribonucleic acid coding for the β-Ep/ACTH precursor (27).

5-HT reuptake inhibitors were found to increase circulating corticosteroids (28). Dexamethasone, a potent glucocorticoid, reportedly decreases β-Ep levels in mouse pituitary cells in culture (29). A significant increase in β-Ep concentrations in discrete brain areas has been observed 1 month after adrenalectomy (30). In this study, no increase in β-Ep concentrations in the hypothalamus was observed 8 days after adrenalectomy. Meanwhile, fluoxetine significantly depressed β-Ep levels in adrenalectomized rats when compared to adrenalectomized controls. These results suggest that the fluoxetine-induced decreases in β-Ep levels in adrenalectomized animals do not result from a corticosteroid-mediated suppression of β-lipotropin synthesis.

Central 5,7-DHT administration produces a marked reduction in brain serotonin and tryptophan hydroxylase activity (19). Intracisternal injections of 200 μg of 5,7-DHT with DMI pretreatment selectively reduces 5-HT in the hypothalamus to less than 50% of control values, with comparable reductions in other brain areas (31). 5,7-DHT appears to provide an effective means of depleting central 5-HT. PCPA depletes both central and peripheral stores (32). In this study, intracisternal injections of 5,7-DHT and intraperitoneal PCPA injection both significantly elevated β-Ep levels, presumably due to an alteration of central 5-HT neuronal activity.

How 5-HT-containing circuits and endorphin-responding circuits interact to modify nociception is still unclear. Both the analgesic action of morphine and stimulation-induced analgesia (SPA) are mediated, at least in part, through the interaction of 5-HT bulbospinal neurons with ascending nociceptive pathways (33–36). The central gray and the medial thalamus have been implicated in the mediation of the animal's response to aversive stimuli (37). Although no consistent effect was observed in the rat thalamus in the present study, the PAG had significantly altered levels of β-Ep following pharmacological manipulation of 5-HT activity. Opiates with an action limited to the PAG have been shown to inhibit spinal nociceptive reflexes and to increase nociceptive threshold as measured by complex behavioral tasks (38, 39). Destruction of the nucleus raphe magnus blocks morphine analgesia and produces hyperalgesia, while stimulation of this nucleus causes analgesia (35). Intrathecal administration of methysergide, a 5-HT antagonist, reduces the antinociceptive effect of morphine (11). In SPA, the temporal properties of the analgesia, that is, the gradual onset and duration of the analgesia, which outlasts the period of stimulation, suggest that a neurochemical agent is released following stimulation (38). Morphine microinjected into the PAG results in elevated levels of 5-HT in the spinal perfusates (41). These data provide evidence of the existence of an endorphin–5-HT circuit in the mesencephalon which mediates descending control of afferent nociceptive stimuli. It was also suggested that certain ascending sensory systems may activate this circuit, providing a substrate for feedback control of spinal sensory processing of nociceptive information (41). It is interesting to note that those nuclei receiving endorphinergic projections also receive a substantial 5-HT input (39, 42, 43). Moreover, β-Ep may produce analgesia by an indirect mechanism, that is, by activating mesencephalic 5-HT neurons, which in turn inhibit pain transmission.

In another study (44), it was reported that long-term treatment with zimelidine, a selective 5-HT reuptake inhibitor, produced significant pain relief in chronic pain patients and significant reductions in the levels of β-Ep, and provided further evidence that β-Ep and 5-HT systems are functionally related. Additional research is needed to clarify the role of these endorphin–5-HT circuits in modulating nociception.

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