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A Role for Serotonin and Beta-Endorphin in the Analgesia Induced by Some Tricyclic Antidepressant Drugs

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SACERDOTE, P., A. BRINI, P. MANTEGAZZA AND A. E. PANERAI. *A role for serotonin and beta-endorphin in the analgesia induced by some tricyclic antidepressant drugs*. PHARMACOL BIOCHEM BEHAV **26**(1) 153–158, 1987.—The analgesic effect of acute or chronic nortriptyline, amitriptyline and clomipramine and their effects on morphine induced analgesia were evaluated in the rat. Clomipramine and amitriptyline, but not nortriptyline, induce analgesia, while all potentiate the effect of morphine when administered acutely. The analgesic effect of clomipramine is blunted by both the serotonin antagonist metergoline and the opiate receptor blocker naloxone, thus indicating an involvement of both the serotoninergic and endogenous opioid system. The involvement of the serotoninergic system is confirmed by the similar results obtained with the serotonin precursor 5-hydroxytryptophan administered alone or together with morphine. A relation between the serotoninergic and the endogenous opioid systems is also shown by the increase in hypothalamic beta-endorphin concentrations elicited by all the drugs used after acute or chronic treatment, with the only exception of nortriptyline, that has been shown to exert its effects mainly through the noradrenergic system. In conclusion, the analgesic effect of clomipramine and amitriptyline and their potentiation of morphine induced analgesia seems to be related to an activation of the endogenous opioid system mediated by serotonin.

Analgesia Tricyclic antidepressants Clomipramine Amitriptyline Nortriptyline Morphine 5-Hydroxytryptophan Serotonin Beta-endorphin

TRICYCLIC antidepressants are increasingly used in the treatment of pain [2, 3, 6, 9, 12, 20, 22]. We thought therefore it would be worthwhile to evaluate the effects of three typical tricyclic antidepressants: clomipramine (CIM), amitriptyline (AMI) and nortriptyline (NOR) on pain thresholds in the rat, alone or in combination with morphine under several experimental conditions.

In order to elucidate the mechanism(s) of action of these drugs in relieving pain, rats were treated acutely or chronically with either CIM, AMI, NOR, or the serotonin precursor 5-hydroxytryptophan (5-HTP). The effects of the serotonin antagonist metergoline (MCE) and of the opiate antagonist naloxone on CIM induced analgesia were also evaluated. Moreover, the effects of acute or chronic CIM, AMI or 5-HTP, and acute NOR on morphine induced analgesia were investigated.

With the aim of studying a possible role of beta-endorphin (BE) in the analgesic effects of the antidepressants we used, in a parallel study we measured BE concentrations in brain areas after acute or chronic treatments with either CIM, AMI, NOR or 5-HTP.

METHOD

In each experiment 6–8 Sprague Dawley CD rats, 150–200 g b.wt. (Charles River, Calco, Italy) were used. Rats were fed a dry pellet diet with water ad lib in an environment with a temperature of 20–22°C and a light:dark cycle of 14:10 hours as previously described [17]. Rats were treated and analgesia evaluated in the same environment, and all tests begun in the morning between 1000–1100 hr.

Analgesia was evaluated by the tail flick test, and was calculated as percentage of the maximal possible effect (% MPE) as described elsewhere [19]. In the tail flick test the latency between exposure of the tail to a focused radiant source and the time the rat flicked the tail, closing the circuit of a photoelectric cell connected to a timer, was measured. All experiments were conducted in blindness, in that the person performing the tail flick test (the operator was the same in all experiments) was not aware of the different treatments. Percent MPE was calculated according to the equation $(TL-BL)/(8.0-BL)\%$, where BL is the mean basal latency (3.5–4.5 sec), TL is the tail flick latency measured after treatment, 8.0 is the maximal latency accepted, chosen in order to avoid tissue damage to the tail.

Drugs were dissolved in water and administered intraperitoneally, with the exception of morphine that was administered subcutaneously. CIM (Anafranil, Ciba-Geigy, Origgio, Italy) was administered acutely at the dose of 20, 40 and 80 mg/kg, and 20 mg/kg twice daily for fifteen days in the

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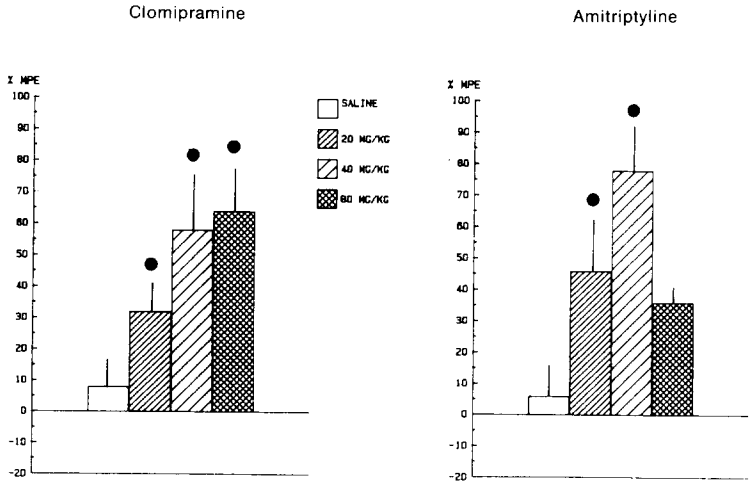

FIG. 1. Analgesic effect of graded doses of clomipramine or amitriptyline after acute administration. In this and all the following figures, each bar represents the mean±SD of the maximal effect observed with the treatment indicated. ●=$p<0.01$ vs. saline.

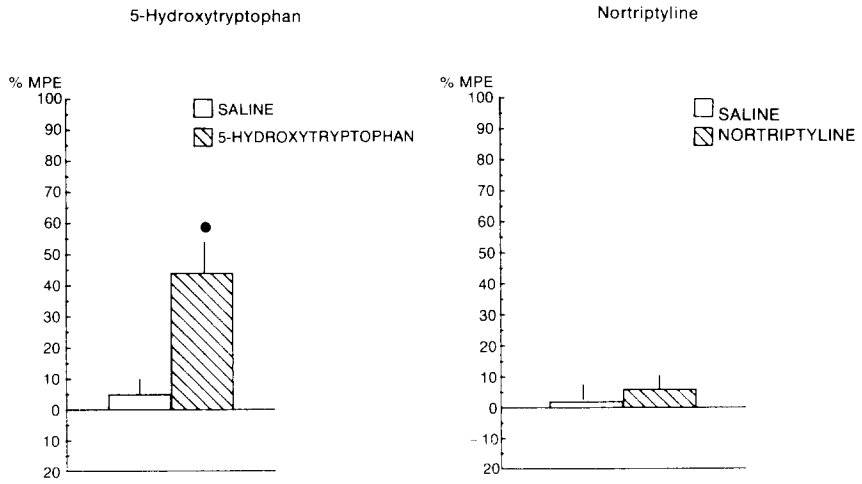

FIG. 2. Analgesic effect of 5-hydroxytryptophan or nortriptyline after acute treatment. ●=$p<0.01$ vs. saline.

chronic study, and 40 mg/kg when administered acutely with morphine and in the naloxone and metergoline antagonism studies. AMI (Laroxyl, Hoffmann-La Roche, Milano, Italy) was administered at the same doses of CIM; NOR (Noritren, Recordati, Milano) was administered only acutely or together with morphine at the dose of 40 mg/kg. 5-HTP (Sigma-Tau, Pomezia, Italy) was administered acutely at a dose of 50 mg/kg alone or together with morphine, while a dose of 30 mg/kg twice daily was used in the chronic studies.

In order to evidentiate a possible potentiation of the effect by the drugs administered concomitantly, morphine (Farmitalia-Carlo Erba, Milano) was administered at the dose of 1.25 mg/kg, a threshold dose, which, under our experimental condition, is the lowest dose capable of giving a significant increase in tail flick response.

Naloxone (Endo Labs, Garden City, NY) was administered 30 minutes after CIM at the dose of 10 mg/kg; the serotonin antagonist metergoline (Farmitalia-Carlo Erba, Milano, Italy) was administered at the dose of 7.5 mg/kg 30 minutes before the administration of CIM.

For the evaluation of BE concentrations, rats were killed 30 minutes after drug treatments by microwave irradiation in order to avoid any post-mortem enzymatic degradation of the peptides [15]. Brain areas were dissected according to Glowinsky [8] into the hypothalamus, midbrain, striatum, hindbrain, cortex and the pituitary.

Tissues were homogenized with a Polytron apparatus and BE was extracted in 1 N acetic acid as previously described [15]. The homogenate was centrifuged and the pellet frozen until protein determination by the method of Lowry [13].

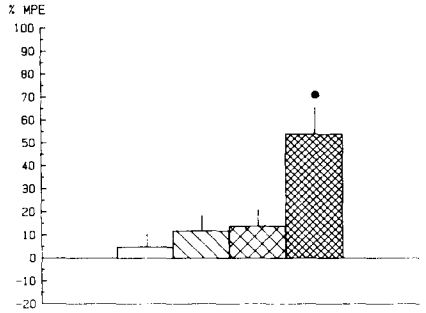

FIG. 3. Analgesic effect of amitriptyline, 5-hydroxytryptophan or clomipramine after chronic administration. Open column: saline; striped column: amitriptyline; wide hatched column: 5-hydroxytryptophan; narrow hatched column: clomipramine. ●=$p<0.01$ vs. saline.

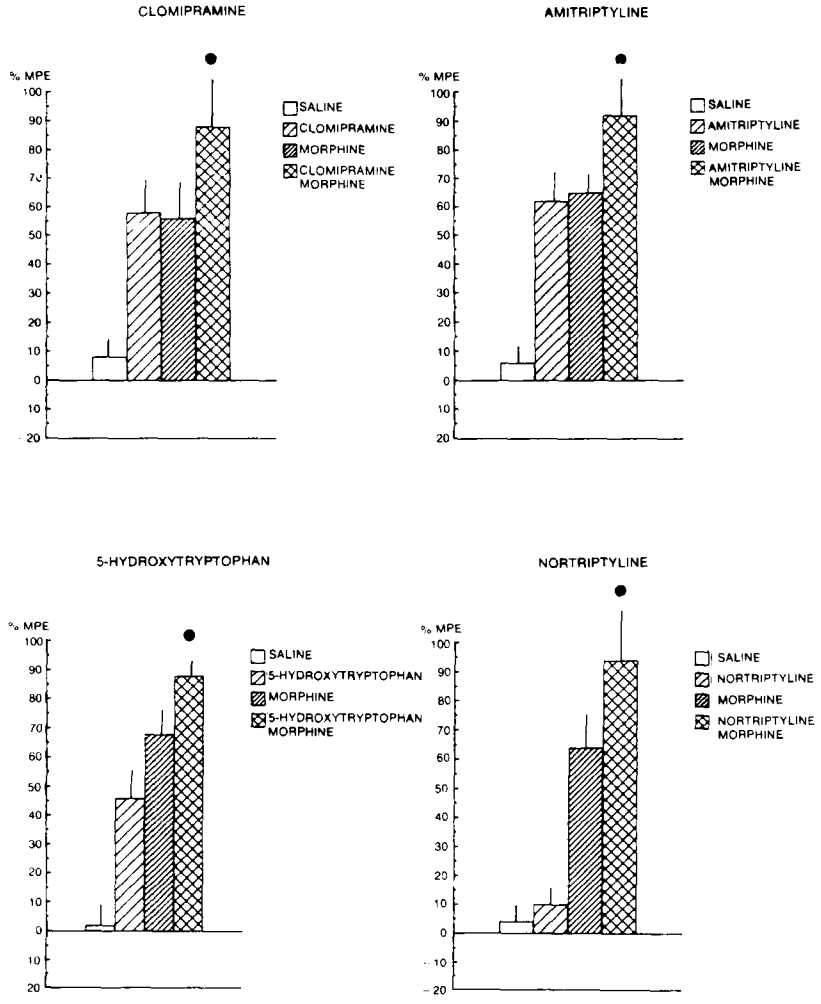

FIG. 4. Effect of the acute administration of either clomipramine, amitriptyline, 5-hydroxytryptophan or nortriptyline on the analgesia induced by morphine. ●=$p<0.05$ vs. morphine alone.

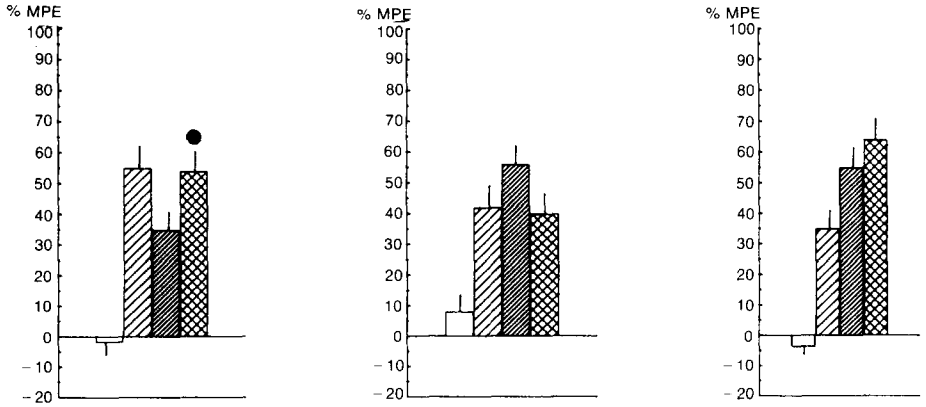

FIG. 5. Effect of the chronic administration of clomipramine (left panel), amitriptyline (central panel), or 5-hydroxytryptophan (right panel) on the analgesia induced by acute morphine. In each panel, open column: saline; wide striped column: drug alone; narrow striped column: morphine alone; hatched column: drug + morphine. ●=$p<0.05$ vs. morphine alone.

The supernatant was separated and frozen at −20°C till the time of BE evaluation by radioimmunoassay.

The radioimmunoassay we used has been fully validated and its characteristics previously reported [15]; in the same occasion and in a further study [19] we also showed that in brain areas over 97% of the BE immunoreactivity we find coelutes with BE on gel chromatography and HPLC, therefore we feel entitled to refer to the data as BE and not as BE-like immunoreactivity.

The doses of drugs in acute and chronic studies were the same as for the behavioral experiments, and rats were sacrificed thirty minutes after treatment. In two further experiments the serotonin receptor antagonist metergoline was administered either 30 minutes before CIM or chronically, concomitantly with the antidepressant as described above, and animals sacrificed thirty minutes after the administration of CIM.

Statistical analysis of results was obtained by Kruskal Wallis analysis of variance of ranks and the Student-Newman-Keuls tests. In the evaluation of peptides concentrations the test of Dunnett for mulitple comparisons was used [1].

RESULTS

In order to simplify the representation of data, in all figures the values reported refer to the maximal effect of the different treatments.

Figure 1 shows that both CIM and AMI elicit a statistically significant increase in analgesic thresholds after acute treatment. The observation that the highest doses of CIM did not yield a higher analgesic response than the 40 mg/kg and that for AMI the 80 mg/kg dose is even less effective than the lowest dose is consistent with a high toxicity of such doses for the rats. This hypothesis is confirmed by the observation of a high mortality (80%) in these rats during the day after the experiment. For CIM or AMI neither of the two treatments induced appreciable changes in behavior at the two lower doses (20 and 40 mg/kg), while rats could hardly move and appeared depressed after administration of the highest dose of the two drugs.

Similar to what was observed with CIM and AMI, two blockers of serotonin uptake, the serotonin precursor 5-HTP also increases the analgesic thresholds after acute administration, while NOR, a blocker of noradrenalin uptake, does not change the analgesic thresholds, as is shown in Fig. 2.

Basal analgesic thresholds were measured every other day during the chronic treatment with the antidepressants or the serotonin precursor 5-HTP and did not change throughout the time of the experiment (data not presented). Moreover, the data reported in Fig. 3 indicate that, different from what was observed after acute administration, only CIM still exerts a significant analgesic effect ($p<0.01$) after chronic administration, while AMI and 5-HTP are ineffective.

Figure 4 shows that when the same drugs were administered acutely together with morphine, CIM, AMI, 5-HTP, or NOR significantly potentiated the analgesic effect of a threshold dose of the opiate ($p<0.05$).

Similar to what was observed for the antidepressant drugs administered alone, only CIM potentiates the analgesic effect of morphine after chronic treatment ($p<0.05$), as is shown in Fig. 5.

The involvement of the serotoninergic and opioid systems in the analgesic effect of some of the antidepressant drugs we used is confirmed by the observation (Fig. 6) that the increase in analgesic thresholds elicited by CIM is significantly blunted by pretreatment with the serotonin receptor antagonist metergoline or the opiate receptor antagonist naloxone ($p<0.01$).

The concomitant involvement of the serotoninergic system and the endogenous opiates is confirmed by the measurement of the hypothalamic concentrations of BE in rats treated acutely or chronically with saline, CIM, AMI, NOR or 5-HTP (the concentrations for chronic NOR have not been determined), as is shown in the upper panel of Fig. 7. BE concentrations increase significantly after all treatments that induced an increase of the serotoninergic tone (5-HTP, AMI, CIM), while NOR, the blocker of the noradrenergic reuptake, was ineffective. The lower panel of the same figure shows that, consistent with the data obtained on analgesia, the increase in hypothalamic BE induced by the acute or chronic administration of CIM is blunted by the concomitant administration of the serotonin antagonist metergoline.

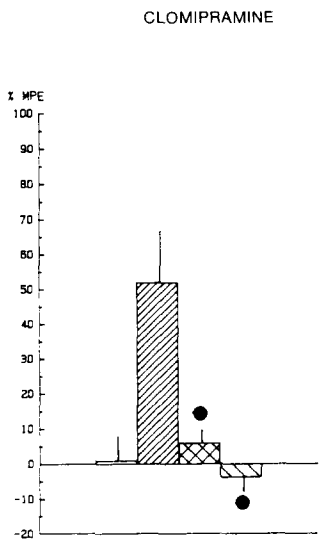

FIG. 6. Effect of the serotonin receptor antagonist metergoline or the opiate receptor antagonist naloxone on the analgesia induced by clomipramine. Open column: saline; narrow striped column: clomipramine; hatched column: clomipramine + metergoline; wide striped column: clomipramine + naloxone. ●=$p<0.01$ vs. clomipramine.

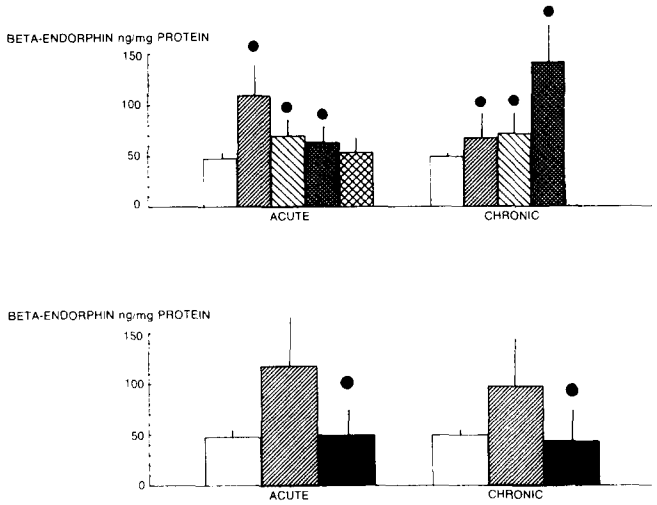

FIG. 7. Upper panel: effect of saline (open column); clomipramine (narrow striped column); amitriptyline (wide striped column); 5-hydroxytryptophan (narrow hatched column); or nortriptyline (wide hatched column) on the hypothalamic concentrations of beta-endorphin after acute (left panel) or chronic (right panel) administration. ●=$p<0.05$ vs. saline. Lower panel: effect of the serotonin antagonist metergoline (dark column) on the increase of the hypothalamic beta-endorphin induced by clomipramine (striped column) acutely (left panel) or chronically (right panel); open column is analgesic thresholds in saline treated animals. ●=$p<0.05$.

DISCUSSION

Our data confirm that CIM and AMI, two tricyclic antidepressants that inhibit the reuptake of serotonin and only in a lesser degree of noradrenalin, exert an analgesic effect of their own and potentiate the antinociceptive effect of morphine [5, 7, 10, 14, 16].

Moreover, they indicate that beyond the well known increase of met-enkephalin concentrations [6], these drugs induce an increase of BE that might account for their analgesic effect and the increase of the effect of morphine.

Consistent with the involvement of the serotoninergic system, the serotonin precursor 5-HTP affects analgesia, morphine antinociception and hypothalamic BE similarly to AMI and CIM. The concomitant involvement of both the serotoninergic and endogenous opioid systems is also confirmed by the observation that both the serotonin receptor antagonist metergoline and the opiate receptor antagonist naloxone inhibit the analgesic effect of CIM. Consistently, NOR, a tricyclic antidepressant with high selectivity for inhibiting noradrenalin, but not serotonin reuptake [21], does not elicit analgesia and does not induce an increase in the hypothalamic concentrations of BE. This observation might be important in order to explain the clinical observation that not all tricyclic antidepressants induce analgesia.

At difference with CIM, both AMI and 5-HTP completely or partially lose their effect when administered chronically. This result can be explained either by considering self regulatory changes of the nervous system due to receptor subsensitivity or autoreceptor activation, the development of tolerance, or to the observation that 5-HTP and AMI are less specific than CIM for the serotoninergic system. The first and second hypotheses seem to be not acceptable since CIM should also have behaved similarly. While this is not the case in our experimental setting, different results were obtained by other authors, who showed a decreased effect after chronic microinjection in the periaqueductal gray of rats [11], suggesting that their results might be interpreted as development of subsensitivity of the serotoninergic receptor.

As far as the site of action of the compounds we use is concerned, it seems to be mainly at a supraspinal level, since we evidentiate an involvement of BE, a peptide that is not present in the spinal cord.

Our results, although consistent with most literature [2, 3, 6, 9, 12, 20, 22], are in contrast with the results of Malseed [14] who did not observe analgesia after treatment with tricyclic antidepressants, however it has to be pointed out that this author conducted his studies in the cat, an animal that in many behavioral parameters differs from the rat.

As far as the potentiation of morphine induced analgesia by CIM and AMI is concerned, an effect of these drugs on morphine metabolism cannot be excluded, similar to what has been shown for desipramine [16]. On the other hand, tricyclic antidepressants might themselves bind the opiate receptor [3], while they do not seem to modify the affinity of morphine for its own receptor [10].

In conclusion, our results indicate that both BE and serotonin are involved in the analgesic effect of some antidepressants and suggest that their therapeutic effect is due to the activation of these two systems, both able to directly affect nociception.

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