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Analgesic effect of morphine: a role for β-endorphin

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Capsaicin, a neurotoxic agent that induces a decrease in hypothalamic β-endorphin, a specific antiserum and human β-endorphin fragment 6–31, a peptidergic β-endorphin antagonist have been used in the attempt of selectively affecting the function of β-endorphinergic system and of evaluating the possible role of this peptide in the analgesic effect of morphine. All 3 experimental approaches resulted in a decrease of the analgesia induced by morphine, thus suggesting that β-endorphin is involved in the effect of morphine.

Several pieces of evidence suggest that endogenous opioids such as the enkephalins or β-endorphin might be involved in the analgesic response, or in the development of tolerance to and/or dependence of morphine [3, 12]. The efforts to confirm this hypothesis have, however, always failed against the difficulty of getting an in vivo model in which the function of at least one endogenous opioid could be selectively inhibited in the central nervous system (CNS).

Consistently with the hypothesis of a role for β-endorphin in morphine-induced analgesia, it was recently shown that during rat development the analgesic responses to morphine parallel the increase in brain β-endorphin concentrations in the CNS [6].

In order to further investigate whether β-endorphin might participate in morphine-induced analgesia, we devised 3 experimental models in which the responses to this opioid were selectively impaired, as confirmed by the diminished autoanalgesia induced by stress or the blunting of the analgesic effect of exogenous β-endorphin.

The first experimental model consisted of animals bearing a 75% decrease in CNS β-endorphin obtained by the neonatal administration of capsaicin [9]. We previously showed in fact that capsaicin induces a decrease in β-endorphin concentrations in the hypothalamus and other brain areas of animals treated neonatally or as adults with the neurotoxic agent. In the same animals, brain concentrations of Met-enkephalin, somatostatin and substance P were not affected, although the treatment decreases somatostatin and substance P concentrations in primary afferent neurons [1].

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According to our previous report, rats were treated on days 1–5 of life with capsaicin 50, 100, 200, 400 mg/kg, and experiments were conducted 6 months later. After the end of the experiments, brain concentrations of β-endorphin were evaluated by radioimmunoassay as previously described [8], in order to confirm the decrease of the peptide (data not reported).

A second experimental model was represented by animals treated intracerebroventricularly (i.c.v.) with rabbit γ-globulins extracted either from preimmune serum, or anti-β-endorphin immune serum, obtained from the same rabbit after multiple immunizations with synthetic camel β-endorphin [8, 14]. Since this antiserum does not show any cross-reactivity with other opioid peptides such as the enkephalins or dynorphin, we can consider this a second model of selective blockade of the endogenous β-endorphin.

γ-Globulins were extracted and titrated for binding capacity as previously described with this same antiserum [14]. The antiserum was injected i.c.v. through a polyethylene cannula using a method described elsewhere [7]. The injection (10 μl) was performed 5 h before treatments, since in preliminary experiments we found that this time lag is necessary in order to observe a significant decrease in the basal anagesic thresholds.

In a third experimental setting, the effects of endogenous β-endorphin were antagonized by treatment with the endogenous β-endorphin fragment 6–31 (10 μg/rat i.c.v.) that was shown to behave as antagonist on β-endorphin behavioral and endocrine effects [5, 4, 10].

In every experiment at least 6 male rats (CD, Sprague–Dawley, Charles River, Calco, Italy) were used in each group, and all animals were used only once. The experiments were conducted in blindness and groups identified only at the end of each experimental session.

Morphine was administered s.c. at the dose of 5.0 mg/kg, while β-endorphin was administered i.c.v. at the dose of 2 μg/rat.

Analgesia was evaluated by the tail-flick test with modalities previously described [11]. Analgesic responses were measured as percentage of the maximal possible effect (%MPE) according to the equation

$$\%\text{MPE} = [(\text{test latency} - \text{mean basal latency})/(8.0 - \text{mean basal latency})] \times 100$$

where 8.0 was arbitrarily chosen as the maximal possible latency, in order to prevent damages to the tail that could affect subsequent measurements. Basal latencies were 3.5–4.0 s in all animals and only slightly increased (4.5–5.0 s) in capsaicin-treated rats.

The statistical evaluation of results was obtained by the Kruskal–Wallis analysis of variance of ranks, since the use of %MPE makes it difficult to use parametrical tests.

In order to verify the blockade of endogenous β-endorphin by capsaicin or the anti-β-endorphin immune serum, the rats receiving these treatments were also administered an intermittent footshock (2.5 mA for 1 s every 5 s over a period of 20 min). This kind of footshock was chosen since it had been previously shown to induce an analgesia that is reversible by the opiate antagonist naloxone and is mainly depen-

dent on the endogenous opioid β-endorphin [3].

Fig. 1 shows that animals treated neonatally with capsaicin respond significantly less than diluent treated controls to footshock-induced analgesia (left panel) and to morphine (right panel). The effect of the i.c.v. administration of anti-β-endorphin γ-globulins is shown in Fig. 2. The right panel of the figure indicates that basal analgesic thresholds are decreased in the animals treated with the immune serum and this decrease is sustained for at least 10 h. The upper right panel of the same figure shows that the analgesia induced by footshock is completely blunted in these animals similarly to what happens for the effect of morphine (right lower panel). The left panel of Fig. 3 shows that the β-endorphin antagonist fragment 6–31 induces an inhibition of the analgesic effect of morphine that is always statistically significant, although it is more evident in the first 30 min ($P<0.01$). The right panel of the figure shows that the inhibitory effect of fragment 6–31 is even more evident when analgesia is induced by β-endorphin.

The blunted analgesic responses to footshock by rats treated with capsaicin or the anti-β-endorphin immune serum, and the inhibition of the effect of exogenous β-endorphin by fragment 6–31 indicate that the 3 experimental models we chose are adequate for the objective of our study.

The data presented, in fact, seem to indicate the existence of a tonic β-endorphin effect on analgesic thresholds, on top of which morphine might exert its activity. This hypothesis is consistent with the lower pain thresholds we observed in rats treated with anti β-endorphin γ-globulins. We can only speculate on the mechanism of action of the immune serum in developing its effects. We conducted several studies on the guinea pig ileum and in vitro binding studies, but we were never able to find an inter-

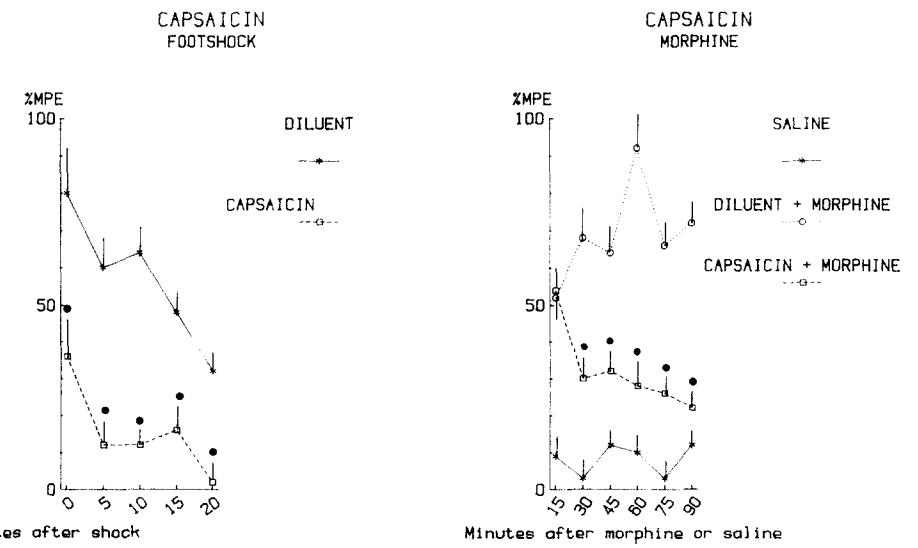

Fig. 1. Analgesic responses elicited by naloxone-reversible footshock (left panel), or morphine (right panel) in rats treated neonatally with capsaicin. Each point represents mean ± S.D. ●, $P<0.01$ diluent treated rats.

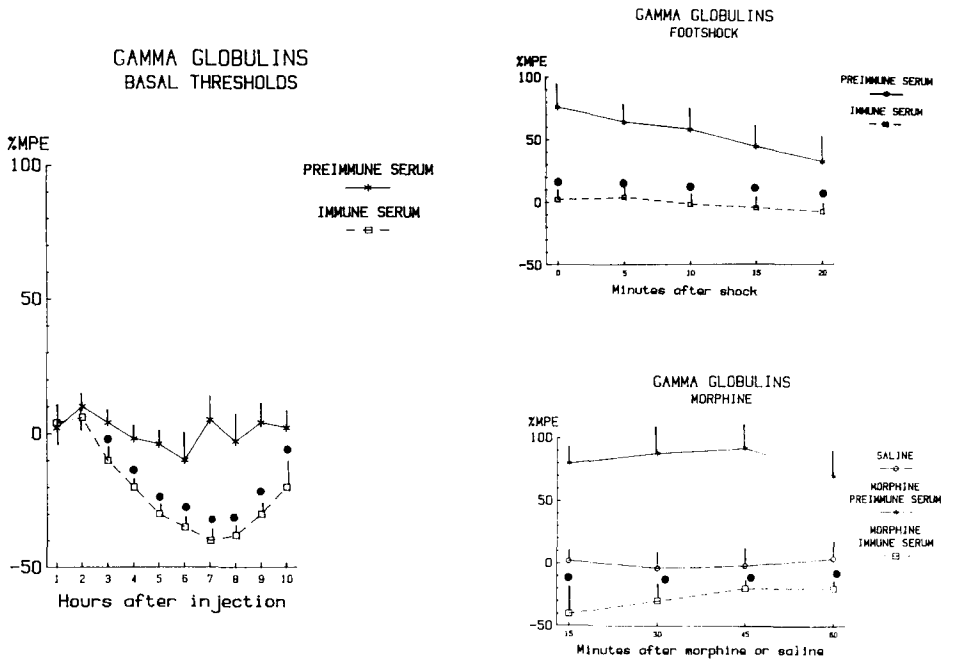

Fig. 2. The left panel shows the basal analgesic thresholds after treatment with preimmune serum or anti-β-endorphin γ-globulins. The right panel shows the effect of treatment with preimmune or immune serum on the analgesia induced by naloxone-reversible footshock (upper panel), or morphine (lower panel). ●, $P < 0.01$ vs preimmune serum treated rats.

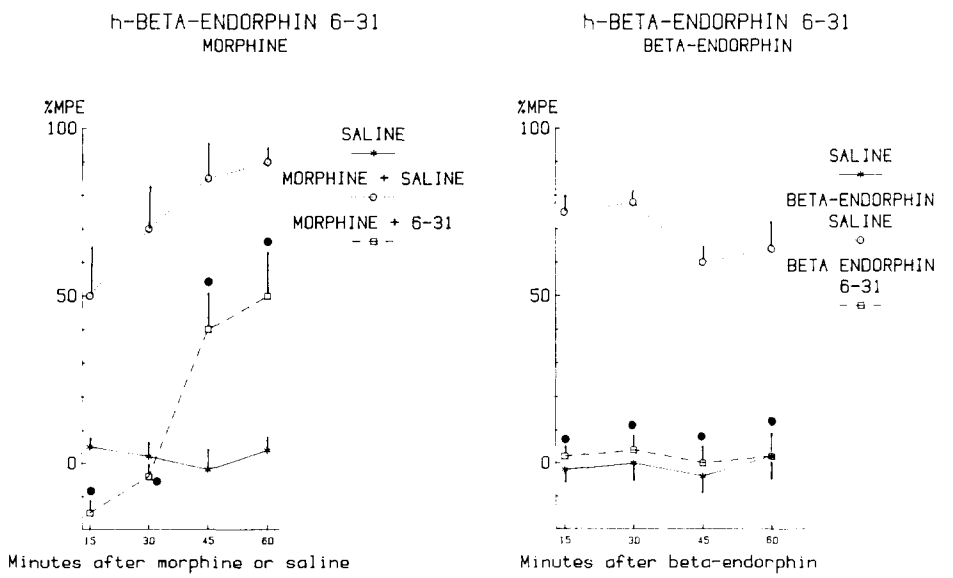

Fig. 3. Effect of i.c.v. human β-endorphin fragment 6–31 on the analgesia induced by morphine (left panel) or β-endorphin (right panel).

action of our serum with the opiate receptor. Alternatively it can be suggested that the antiserum blocks the β-endorphin tonically released or the β-endorphin that, according to some studies, is released following morphine administration.

Consistent with the hypothesis evaluated in this study, capsaicin, depleting hypothalamic β-endorphin, reduces the analgesia induced by footshock or morphine. Moreover, previous studies showed the interference of capsaicin with other effects of morphine such as the one on thermoregulation or on analgesia, evaluated by the hot-plate method [2, 13].

The data presented show that fragment 6–31 blunts the analgesic effects of β-endorphin and morphine, similarly to what was previously shown for the endocrine effects of the two opiates. However, its mechanism of action remains obscure, and a direct effect on the opiate receptor does not seem to be present [10].

In conclusion it seems possible to suggest a role for this peptide in the analgesia induced by morphine, by the use of 3 experimental approaches interfering through different mechanisms with the function of β-endorphin.

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