EFFECT OF SEAL SERUM PROTEINS ON THE ANALGESIC ACTION OF NARCOTIC DRUGS

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Endogenous opioid peptides (enkephalins, endorphins, dynorphins), formed from three high-molecular-weight protein precursors (containing 260-265 amino acid residues), namely pro-opiomelanocortin, proenkephalin A, and pro-enkephalin B [3, 6], are found in the central and peripheral nervous system, blood, etc., of man and animals. It can also be considered, however, that the body also contains other protein precursors, which are sources of endogenous polypeptides possessing morphinelike activity.

The aim of this investigation was to study the effect of a serum protein fraction from the harp seal (*Phoca groenlandica*) on the pain sensitivity of animals and also on the antinociceptive activity of narcotic analgesics.

EXPERIMENTAL METHOD

The serum protein fraction (SPF) was obtained by fractionation with ammonium sulfate from the blood serum of a harp seal. On PAG electrophoresis in the presence of SDS, the SPF was separated into seven components with molecular weights of between 60 and 90 kilodaltons (kD). Experiments were carried out on 320 male Wistar SPF on the general motor activity and emotional reactivity was assessed by the open field test [2]. The antinociceptive action of the substances was studied by three methods: by the "tail-flick" test of exposure to nociceptive thermal stimulation, with measurement of the latent period of the tail withdrawal reaction (LP TWR) [5]; by nociceptive chemical stimulation (intraperitoneal injection of a 2% solution of acetic acid), with counting the number of spasms in the course of 15 min [8]; by mociceptive electrodermal stimulation of the hind limbs, with recording of the vocalization reaction (VR) [1]. The narcotic analgesics morphine, trimeperidine, and fentanyl, their antagonists naloxone, nalorphine (N-allylmorphine), and the SPF were dissolved immediately before the experiment in isotonic sodium chloride solution and injected subcutaneously (sc), intraperitoneally (ip), or intravenously (iv), in a dose range of 1-10 mg/kg. Contraction of an isolated segment of the guinea pig ileum in response to electrical stimulation, under the influence of morphine (10^{-6} M), dalargin ($3 \cdot 10^{-8}$ M), naloxone (10^{-6} M), and SPF (5-25 μg/ml), and also under the influence of morphine and dalargin preceded by injection of SPF, was recorded in experiments in vitro. Fuller details of the technique were described previously [4].

EXPERIMENTAL RESULTS

SPF in doses of 1-5 mg/kg (ip) caused no change in either general motor activity of the animals or their emotional reactivity in the open field test.

In the acetic acid test on mice, SPF in doses of 2 and 5 mg/kg (sc, ip, iv) caused no significant change in the number of spasms (Fig. 1a).

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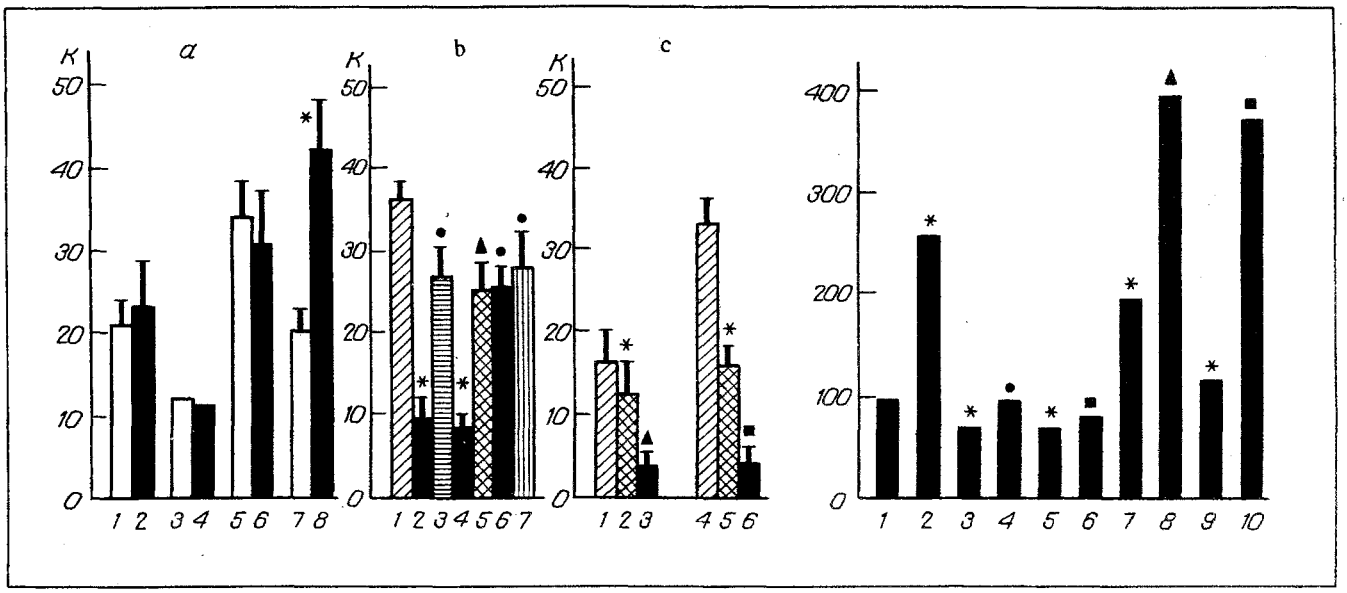

Fig. 1 Fig. 2

Fig. 1. Effect of SPF, nalorphine, morphine, trimeperidine, and fentanyl, and alfio of SPF with each of them in combination, on spasms in mice. a: 1) control (isotonic sodium chloride solution); 2) SPF (2 mg/kg, sc); 3) control (isotonic sodium chloride solution; 4) SPF (2 mg/kg, ip); 5) control (isotonic sodium chloride solution); 6) SPF (2 mg/kg, iv); 7) control (isotonic sodium chloride solution); 8) nalorphine (2 mg/kg, sc). b: 1) control (isotonic sodium chloride solution); 2) morphine (2.5 mg/kg, ip); 3) morphine (2.5 mg/kg, ip) + SPF (2 mg/kg, sc); 4) morphine (2.5 mg/kg, sc); 5) morphine 2.5 mg/kg, sc) + SPF (2 mg/kg, ip); 6) morphine (2.5 mg/kg, sc) + SPF (2 mg/kg, iv); 7) morphine (2.5 mg/kg, sc) + nalorphine (2 mg/kg, sc). c: 1) control (isotonic sodium chloride solution); 2) trimeperidine (10 mg/kg, sc); 3) trimeperidine (10 mg/kg, sc) + SPF (2 mg/kg, ip); 4) control (isotonic sodium chloride solution); 5) fentanyl (0.05 mg/kg, sc); 6) fentanyl (0.05 mg/kg, sc) + SPF (2 mg/kg, ip). Asterisk indicates significance of difference between parameters ($p < 0.001$) compared with control; compared with injection of trimeperidine (triangle), fentanyl (square), and morphine (circle). Ordinate, number of spasms.

Fig. 2. Effect of SPF, morphine, trimeperidine, fentanyl, and naloxone, and also of SPF with each of them in combination, on LP TWR in rats. 1) Control (isotonic sodium chloride solution) (100%); 2) morphine (5 mg/kg, ip); 3) SPF (5 mg/kg, ip); 4) morphine (5 mg/kg, ip) + SPF (5 mg/kg, ip); 5) naloxone (2 mg/kg, ip); 6) naloxone (2 mg/kg, ip) + morphine (5 mg/kg, ip); 7) trimeperidine (7 mg/kg, ip, 90 min); 8) trimeperidine (7 mg/kg, ip) + SPF (5 mg/kg, ip, 90 min); 9) fentanyl (0.05 mg/kg, ip) 45 min); 10) fentanyl (0.05 mg/kg, ip) + SPF (5 mg/k, ip, 45 min). Asterisk indicates significance of difference between parameters ($p < 0.001$) compared with control; compared with injection of morphine (circle), trimeperidine (triangle), and fentanyl (square). Ordinate, change in LP TWR (in %).

In rats tested by nociceptive thermal stimulation SPF in doses of 1-2 mg/kg (ip) did not change LP TWR, but with an increase in its dose to 5 and 10 mg/kg (ip) definite hyperalgesia was observed: shortening of LP TWR by 24 and 41% respectively. Similar results were obtained in these animals during nociceptive electrical stimulation: the threshold of VR was lowered by 21% after injection of SPF in doses of 1 and 2 mg/kg (ip), but reduced it by 33 and 41% respectively. Naloxone and nalorphine, classical antagonists of narcotic analgesics, had a similar action on rats and mice. For example nalorphine, in a dose of 2 mg/kg (sc), almost doubled the number of spasms in mice (Fig. 1a), and naloxone, in a dose of 2 mg/kg (ip) in rats reduced LP TWR by 24% (Fig. 2) and lowered the threshold of VR by 23%.

Consequently, like nalorphine and naloxone, SPF can induce hyperalgesia in rats.

In our experiments the narcotic analgesics (morphine, fentanyl, and trimeperidine), in the doses used, had a definite pain-relieving action: in rats LP TWR was increased two- to fourfold (Fig. 2) and the threshold of VR was raised by 20-40%; in mice the number of spasms was reduced under their influence by 2-3 times (Fig. 1b, c).

Combined administration of SPF with morphine resulted in virtually complete prevention and abolition of morphine analgesia by SPF (Figs. 1 and 2).

The study of interaction of SPF with narcotic analgesics of different chemical nature, namely the piperidine derivatives trimeperidine and fentanyl, showed that in this case SPF not only did not exhibit antagonism toward the antinociceptive action of the preparations, but, on the contrary, it potentiated their analgesic effect (Figs. 1 and 2). Under these circumstances the duration of action of these analgesics also was prolonged by 1.5-2 times under the influence of SPF (Fig. 2).

To elucidate the possible interaction of SPF with opioid receptors, experiments were carried out in vitro, using an isolated segment of the guinea pig ileum. The results showed that SPF in the concentrations studied had no appreciable effect on the amplitude of contractions of the segment of ileum evoked by electrical stimulation, and likewise did not change statistically significantly the inhibitory effect of morphine and dalargin. Naloxone completely prevented the action of opioids.

It follows from these results that SPF, in vitro, possesses neither opioid nor antiopioid activity, and it likewise had no modulating influence on acetylcholine release from presynaptic nerve endings through other neurochemical mechanisms.

In all probability the differences observed between results obtained in vivo and in vitro can be explained by the fact that SPF exerts its activity in experiments in vivo after interacting with proteolytic enzymes not present in an isolated system, i.e., that SPF may perhaps be the precursor of peptides which can modulate the analgesic effects of narcotic analgesics.

At the same time it is difficult to explain the fact that the character of interaction of SPF (or its metabolites) with narcotic analgefiica depends essentially on the chemical structure of the preparation, i.e., that SPF has an opposite action on the pain-relieving effects of morphine (a phenanthrene derivative), on the one hand, and of trimeperidine and fentanyl (piperidine derivatives), on the other hand.

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