PHARMACOLOGICAL REGULATION OF OPIOIDERGIC ANTINOCICEPTIVE MECHANISMS

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It is generally known that many neurotropic substances belonging to different classes, but unrelated to narcotic analgesics, can potentiate the antinociceptive (ANC) action of the latter. The list of these substances also includes some with no significant affinity for opioid receptors [3, 4, 6-8] and which do not exhibit any marked analgesic effect of their own. The mechanisms of this potentiation have not yet been adequately studied, despite the fact that the character of interaction we have examined is advantageous from the practical point of view, because the possible distinction between the points of application of narcotic analgesics and of substances potentiating their analgesic effect, suggests the simultaneous absence of potentiation of the side effects of the narcotic analgesics.

With these considerations in mind the aim of this investigation was to study the opioidergic contribution to the general pool of antinociception (AN), arising through the combined use of the classical opioid analgesic morphine (M) with different classes of neurotropic agents. To analyze the involvement of opioidergic processes in AN, we used the selective opioid antagonist naloxone.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice (males) weighing 18-20 g. Nociceptive responses were assessed on the basis of three tests, so that it was possible to judge to a certain degree the character of the effect of the substances predominantly at the spinal (test I — tail flick) and supraspinal levels (test II — hot plate — 55°C, and test III — tail pinching), and assuming different modalities of the nociceptive stimuli (thermal in tests I and II, mechanical in test III). Experiments were carried out on animals subjected to background testing, whose initial nociceptive thresholds (in the background) in test I did not exceed 5 sec, in test II — 15 sec, and in test III — 4 conventional units of the instrument scale. For test I we used the "Analgesia Test" system ("Hugo Sachs Elektronik," West Germany) with beam intensity corresponding to 9 on the instrument scale, directed on the mouse's tail 1 cm above its base, and for test III we used an analgesimeter ("Ugo Basile," Italy), with a weight of 280 g moving along a lever, by means of which the tail could be compressed at a distance of 0.6 cm from its base. Each animal was tested by tests II, I, and III in that order. The test substances (their names, affinity for receptors, doses, and methods of administration are indicated in Figs. 1-3) were injected 45 min, morphine in a dose of 3 mg/kg subcutaneously (sc) 30 min, and naloxone in a dose of 1 mg/kg — intraperitoneally (ip) 15 min before testing. In all experiments the solvent was injected into the control animals in the same volumes and at the same times. Each point corresponded to a group of not less than 6 animals. The test dose of M (3 mg/kg) was chosen on the grounds that it allows both strengthening and weakening of morphine AN under the influence of the test substances to be detected. The choice of dose for each test substance was made on the basis of data in the literature, taking account of the possibility that when a concrete substance was used, on the one hand, it could exert a specific action characteristic of the corresponding pharmacological class, but on the other hand it could not by itself exert an ANC action in the tests used.

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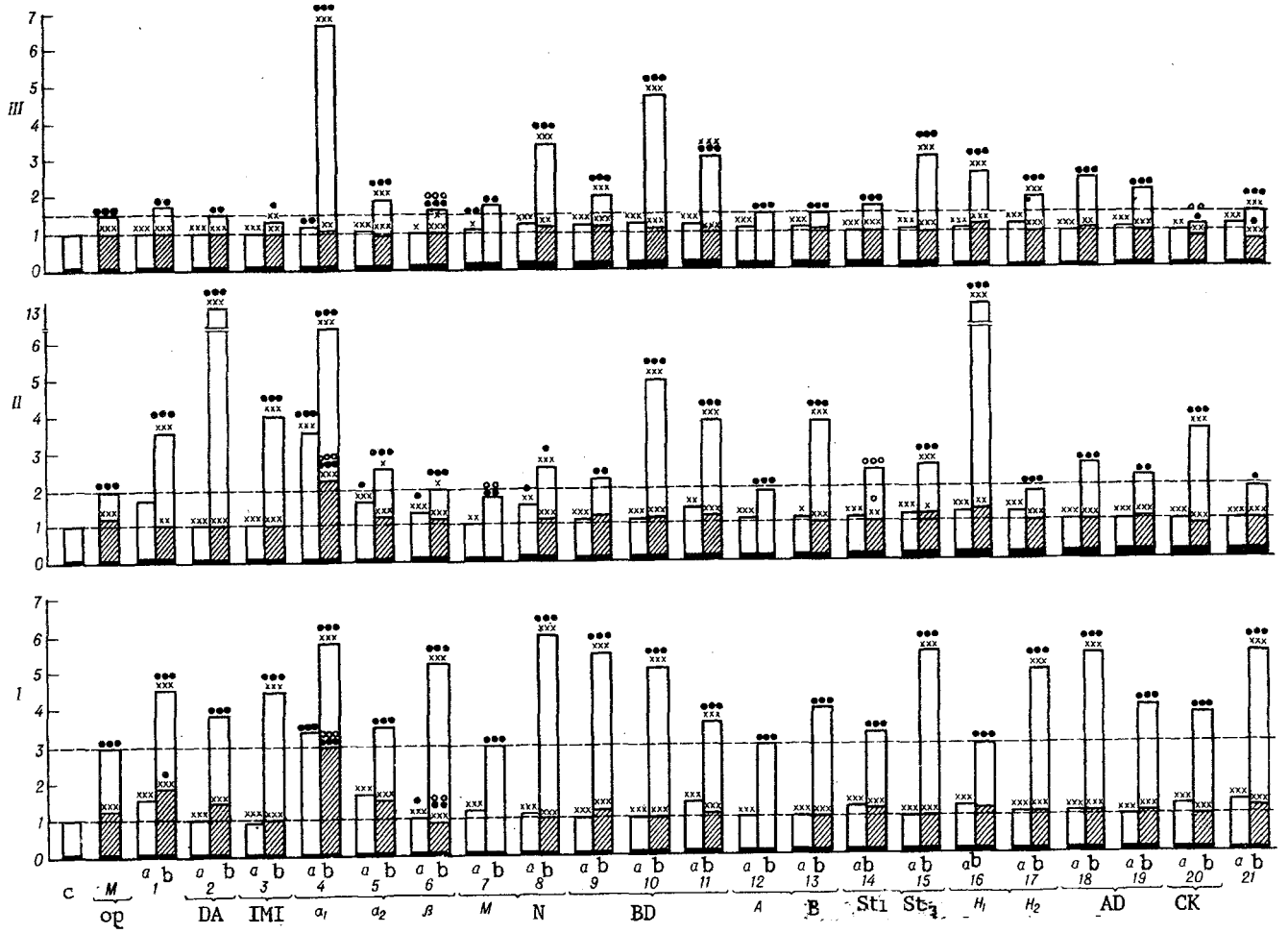

Fig. 1. Effect of neurotropic agents of different classes on ANC-action of M and effects of naloxone in mice. Tests: I) tail flick, II) hot plate, III) tail pinching. Ordinate, ratio of nociceptive response 45 min after injection of substances and initial (background) nociceptive response. C) control; M) morphine; ic (3 mg/kg); 1) chlorpromazine, sc (1 mg/kg); 2) haloperidol, ip (2 mg/kg); 3) imipramine, ip (30 mg/kg); 4) clofelin (clonidine) ip (0.5 mg/kg); 5) dihydroergotamine, ip (0.5 mg/kg); 6) propranolol, sc (0.5 mg/kg); 7) benactyzine, sc (5 mg/kg); 8) aprophen, sc (5 mg/kg); 9) diazepam, ip (0.5 mg/kg); 10) BCCEE, ip (1 mg/kg); 11) RO 151788, ip (1 mg/kg); 12) bicuculline, ip (3 mg/kg); 13) baclofen, sc (1.5 mg/kg); 14) cyproheptadine, ip (1 mg/kg); 15) ritanserine, ip (1 mg/kg); 16) pipolphen, sc (10 mg/kg); 17) ranitidine, ip (5 mg/kg); 18) caffeine, sc (75 mg/kg); 19) adenosine, ip (100 mg/kg); 20) proglumide, sc (200 mg/kg); 21) picamilon, sc (20 mg/kg). a) independent effect of substances, b) effect of substances against the background of morphine (3 mg/mg). Oblique shading — effect of naloxone (1 mg/kg) injected 15 min before testing, after preliminary combined injection of morphine (3 mg/kg) with the test substances. Statistical significance determined by Student's test: 1, 2, and 3 filled circles indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$ compared with control; 1, 2, and 3 crosses correspond to effect of morphine (3 mg/kg); 1, 2, and 3 empty circles correspond to effect of naloxone (1 mg/kg) preceded by morphine (3 mg/kg). Affinity of test substances for receptors: OP) opioid, DA) dopamine, IMI) imipramine, α_1, α_2, and β) for corresponding adrenoreceptors, M and N) acetylcholine receptors, BD) benzodiazepine, A and B) GABA receptors, St_1 and St_2) serotonin, H_1 and H_2) histamine, AD) adenosine, and CH) cholecystokinin receptors.

In experiments with central administration of M, it was injected intracisternally (ic) in a dose of 0.5 μg in a volume of 5 μl of physiological saline 30 min before testing. The respiratory function of the mice was assessed by comparing the original frequency with the frequency of respiratory movements 30 min after injection of the test substances. In experiments with

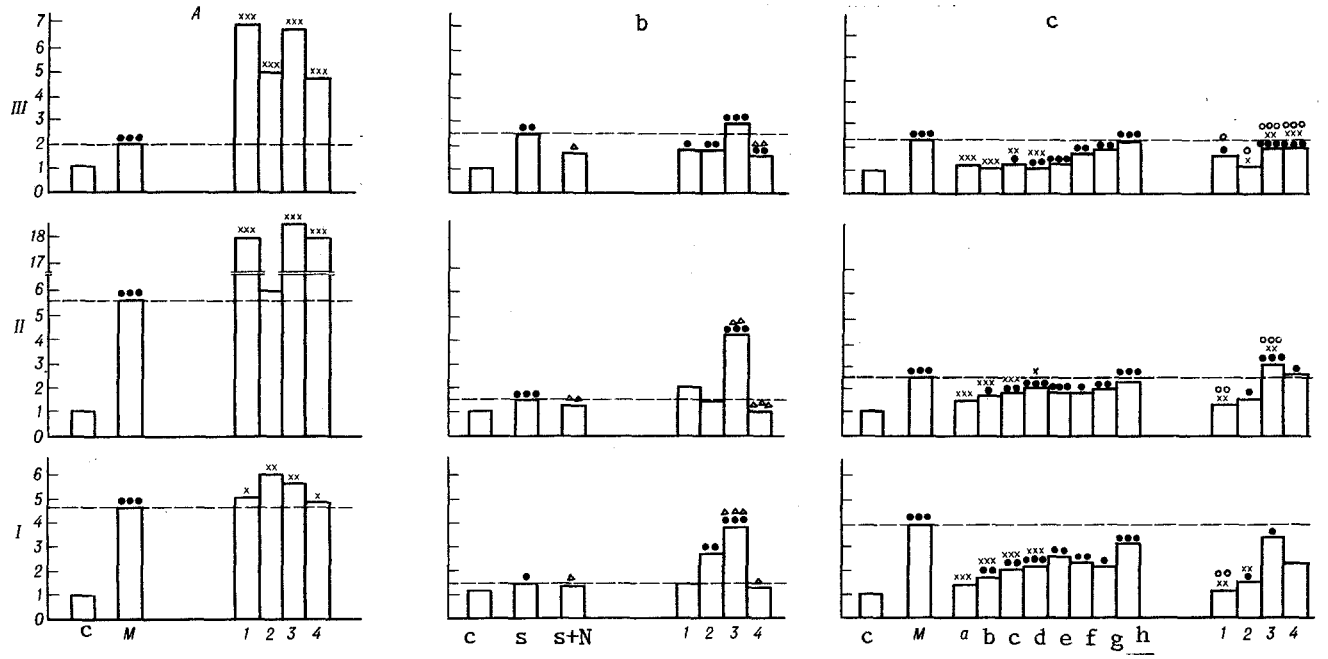

Fig. 2. Effect of BCCEE, RO 151788, clofelin, and ritanserine on central effect of M (a), stress-induced AN (b), and antagonistic properties of naloxone (c) in tests I (tail flick), II (hot plate), and III (tail pinching). C control; M) morphine, sc (3 mg/kg) and ic (0.5 μg — 5 μl); 1) BCCEE, ip (1 mg/kg); 2) RO 151788, ip (1 mg/kg); 3) clofelin, ip (0.5 mg/kg); 4) ritanserine, ip (1 mg/kg). a: effects of 1, 2, 3, and 4 preceded by intracisternal injection of M, b: preceded by stress-induced (S) naloxone-dependent (S + N) (naloxone, ip, 1 mg/kg) AH in combination with M (3 mg/kg); c) after injection of test dose of naloxone (0.05 mg/kg, ip); a, b, c, d, e, f, g, h) effect of naloxone in doses of 1, 0.25, 0.125, 0.05, 0.0125, 0.0078, 0.0039, and 0.0019 mg/kg respectively, preceded by subcutaneous injection of M (3 mg kg). Statistical significance determined by Student's t test: 1, 2, and 3 filled circles denote $p < 0.05$, $p < 0.01$, and $p < 0.001$ respectively compared with control; 1, 2, and 3 crosses denote compared with effect of morphine (3 mg/kg); 1, 2, and 3 empty circles — comparison with the test dose of naloxone (0.05 mg/kg); 1, 2, and 3 triangles — compared with stress-induced (S) AN.

naloxone-dependent stress-induced AN, the animals were subjected to stress (6 in a group) through the electrode floor by means of a stimulator with constant emission of square pulses of current with an amplitude of 1 mA for 30 min (stimulus duration 10 sec, intervals between stimuli 20 sec). The test substances were injected immediately after the end of stress, and analgesimetric tests were carried out 30 min after stress. The results obtained in the experimental and control groups of animals were compared by statistical analysis using Student's t test.

EXPERIMENTAL RESULTS

On examination of the experimental results (Fig. 1) the following features will be noted: first, among the substances studied there were some belonging to different classes which, in doses not inducing an independent ANC effect, could potentiate to various degrees the ANC-action of M. Some of these substances exhibited this property only in individual tests, whereas clofelin (clonidine), aprophen, β-carboline carboxyethyl ester (BCCEE), RO 151788, and ritanserine exhibited it in all the tests used. Second, an important principle could be detected, namely: morphine AN, besides its increase produced by the test substances, has a distinct naloxone-dependent character. This applies even to the nonopioid analgesic clofelin, especially in test III. Third, in some cases there was a powerful naloxone-dependent increase (potentiation) of the morphine AN (for example, under the influence of haloperidol, BCCEE, and pipolphen in test II, and also the influence of clofelin, aprophen, and BCCEE in test III), which was 1.5-6 times greater than the effect of M. Considering the results

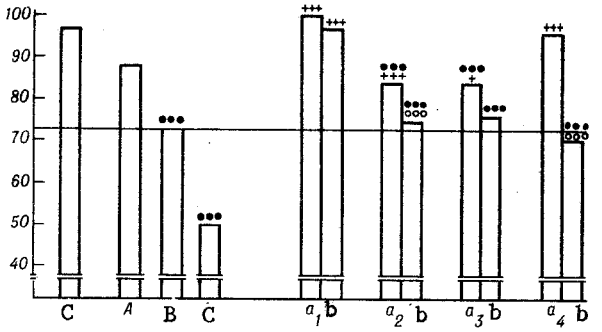

Fig. 3. Effect of BCCEE, RO 151788, clofelin, and ritanserine on respiratory function and its depression by M. Ordinate, frequency of respiratory movements per minute (in per cent). C) control, frequency of respiratory movement 30 min after subcutaneous injection of M in doses of: a) 3 mg/kg, b) 5 mg/kg, c) 10 mg/kg; 1) BCCEE, ip (1 mg/kg); 2) RO 151788, ip (1 mg/kg); 3) clofelin, ip (0.5 mg/kg); 4) ritanserine, ip (1 mg/kg); a) during independent injection of each drug, b) during injection combined with morphine (5 mg/kg). Statistical significance by Student's t test: 1, 2, and 3 filled circles denote $p < 0.05$, $p < 0.01$, and $p < 0.001$ compared with control; 1, 2, and 3 crosses — with effect of morphine (5 mg/kg); 1, 2, and 3 empty circles — significance for comparison of b and a.

showing the consistently naloxone-dependent potentiation of the ANC-effect of M by substances with different neurochemical mechanisms of action, we can postulate the nonspecific character of this phenomenon, which is connected, for example, with disturbance of the blood-brain barrier (BBB). This phenomenon could lead to increased penetration of systemically injected M into the brain, thereby causing an increase in AN. However, the investigation with central (intracisternal) injection of M combined with systemically injected substances (exhibiting their greatest activity in all the tests indicated above), shows that the potentiation we have examined is sufficiently independent of the function of BBB (Fig. 2a).

Since virtually any pharmacological action can be usually regarded as a unique kind of nonspecific stress factor, and since during stress endogenous opioid peptides may be released [9], it would be logical to suggest that the increase in AN during the combined use of M with the test substances was due to these same endogenous peptides.

However, this view is contradicted by the argument that the test substances, in the doses used, do not induce AN of their own accord, i.e., they are not sufficiently strong stress factors to provoke stress-induced AN. Meanwhile, a sufficiently powerful nonpharmacological stressor (Fig. 2b) does not necessarily lead to AN that is comparable in absolute magnitude with the effect of the test dose of M, whether injected centrally or systemically (except in test III). Consequently, the role of the endogenously released opioid peptides in the ANC reaction under the conditions studied is less important than the effect of exogenously injected morphine. Moreover, under the influence of substances capable of potentiating the ANC-action of M, no significant potentiation of stress-induced AN was found, whereas with ritanserine, a naloxonelike action actually was discovered. This suggests the unimportant role of endogenous opioid peptides in the potentiation of morphine AN.

Since the ANC action of morphine is dose-dependent in character, and in the experiments described above we used a test dose and obtained potentiation of ANC by means of substances which themselves did not exhibit an ANC action and did not interact directly with opioid receptors, it can be tentatively suggested that potentiation of the effect of M under these conditions was due to a probable increase in sensitivity of opioid receptors to it. With this in mind we studied the ability of BCCEE, ritanserine, RO 151788 and clonidine to potentiate the antagonistic effect of the competitive M antagonist naloxone. The results (Fig. 2c) showed that the possession of this property cannot be ruled out for BCCEE (tests I and II) and for RO 151788 (test III), but it is not a feature of clonidine or ritanserine, which do not potentiate the antagonistic effect of naloxone relative to morphine AN. In other words, clonidine and ritanserine probably cannot increase the sensitivity of opioid receptors to the corresponding specific ligands and, consequently, potentiation of the ANC effect of morphine in some cases may arise when sensitivity of opioid receptors to M is unchanged.

The character of marked potentiation of the basic (ANC) effect of M by the most active of the substances studied, thus revealed, necessitated a study of their effect on one of the principal side effects of M, namely depression of respiration. It was shown (Fig. 3), that under the conditions of potentiation examined above, the substances studied included some which not only do not change, but actually restore depression of respiration induced by morphine, such as, for example, BCCEE.

The results of these investigations thus suggest that the functional reserves of AN of the naloxone-dependent response, manifested as potentiation of the ANC effect of M, can be "released" through the action of substances which do not directly bind with opioid receptors, but only in the presence of M, which is essential for development of its independent and relatively weak ANC effect. The mechanisms of this potentiation, which are relatively independent of function of the BBB and of endogenous opioids, are indissolubly linked with a possible increase in sensitivity of the opioid receptors, and may also have a different, "nonreceptor" nature. Potentiation of the morphine-induced AN with simultaneous weakening of morphine-induced depression of respiratory function (for example, against the background of BCCEE), revealed by these experiments, indicate that, in principle, specific pharmacologic correction of the principal (analgesic) and side (respiratory) effects of narcotic analgesics can be corrected. Taking account of these data and also indications that the analgesic and positive reinforcing effects of narcotic analgesics [1, 5] on the ability of some neutrotropic agents to antagonise opiate-induce emotionally positive activation [2] can be dissociated, a real possibility arises for the creation of combined preparations with intensified primary and reduced side effects.

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