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EFFECT OF SYSTEMIC ADMINISTRATION OF BETA-CASOMORPHINE-7 ON NOCICEPTION IN RATS

V. A. Dubynin, A. S. Maklakova, V. N. Nezavibat'ko, L. A. Alfeeva, A. A Kamenskii, and I. P. Ashmarin

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The casomorphines were first isolated from commercial casein peptone [2, 3]. Tests of its various fractions showed that they contain a compound with high opioid activity, It was established that this compound is the hepta-peptide Tyr-Pro-Phe-Pro-Gly-Pro-Ile, and that it corresponds to the beta-casein fragment (60-66). Accordingly, this compound was called beta-casomorphine-7 (β-CM7). The presence of a large number of proline residues in its composition determines its high resistance to the action of proteolytic enzymes [6]. The ability of β-CM7 to be formed in the intestine [7], absorbed into the blood stream [10], and to act on peripheral mu-receptors [5], also has been demonstrated. Interest in compounds of this class has recently increased greatly in connection with the quest for new therapeutic substances of natural origin. Meanwhile, research with the casomorphines is still only on a small scale, and it is not mentioned in the USSR/CIS literature The analgesic properties of β-CM7 have been described mainly after intragastric injection or on various models (but never by systemic administration). Accordingly, the aim of the present investigation was to study dependence of the analgesic activity of β-CM7 on dose and on the initial pain sensitivity of experimental animals when injected intraperitoneally.

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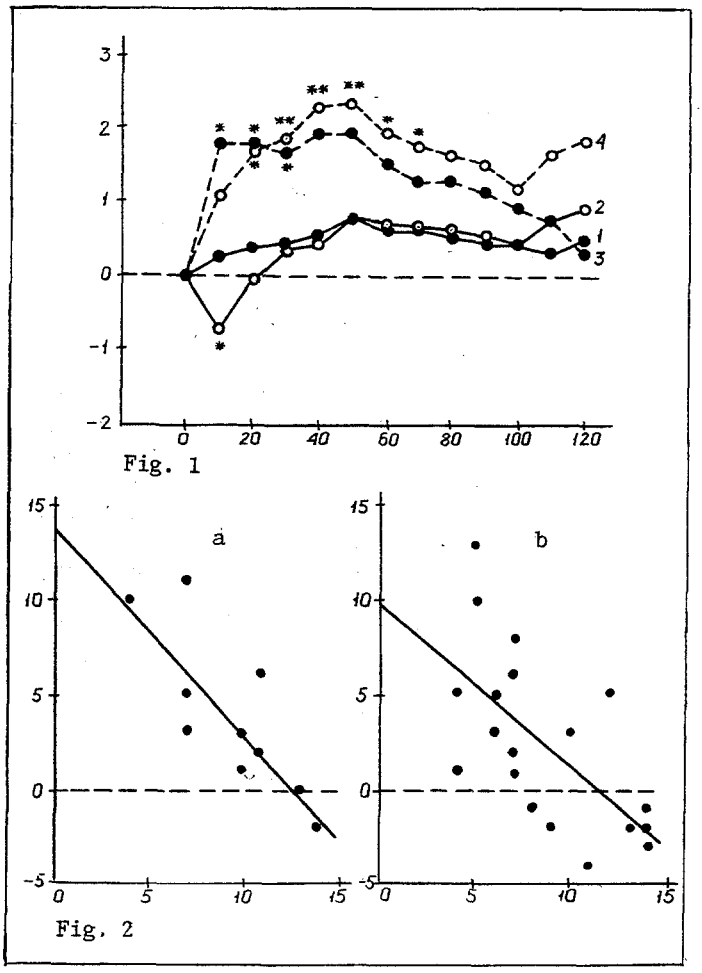

Fig. 1 Changes in duration of tail flick reaction in rats after intraperitoneal injection of different doses of β-CM7 Abscissa, time after injection (in min): zero point corresponds to measurement of nociceptive sensitivity beiore injection of peptide; ordinate, change in tail flick reaction time (in sec): current reaction time minus initial reaction time before injection of peptide. Level of initial nociceptive sensitivity (zero mark on ordinate) shown by broken line; 1) control (n = 49), 2) 5 mg/kg of β-CM7 (n = 22), 3) 10 mg/kg (n = 22), 4) 20 mg/kg (n = 11). Significant differences from changes in control *p < 0.05, **p < 0.01.

Fig. 2. Correlation between initial level of nociceptive sensitivity of rats and analgesic effect of injection of β-CM7 in doses of 20 (a) and 10 (b) mg/kg. Abscissa initial (beiore injection of peptide) tail flick reaction time (in sec); ordinate, change in reaction time (in sec) Initial level of nociceptive sensitivity (zero marker on ordinate) indicated by broken line, and each point corresponds to a concrete experimental animal, a: recording 20 min after injection of peptide (appearance of significant correlation), parameters of regression line (continuous line): $y = 14.0 - 1.10x$ ($p < 0.01$); b: recording made 30 min after injection of peptide (appearance of significant correlation), parameters of regression line (continuous line): $y = 9.86 - 0.86x$ ($p < 0.01$).

EXPERIMENTAL METHOD

Beta-casomorphine-7 was synthesized in the Regulatory Peptides Laboratory of the Institute of Molecular Genetics, Russian Academy of Sciences. Experiments were carried out on noninbred male albino rats weighing 150-250 g. An aqueous solution of the peptide was injected intraperitoneally into the rats in a volume of 0.2 ml. The β-CM7 was injected in three doses: 5 mg/kg (n = 22), 10 mg/kg (n = 22), and 20 mg/kg (n = 11). Equivalent volumes of distilled water (n = 49) were injected into control animals. The analgesic effects of the preparations were estimated in the tail flick test. Nociceptive stimulation was applied by immersing the tail in hot water at a temperature of 56 ± 1°C. The latent period of withdrawal (the time taken to avoid the painful stimulus) was measured before and in the course of 2 h after injection, at intervals of 10 min. The results were analyzed by standard statistical methods, using the "Statgraf" statistical program package (Stat. and Graph Corp., USA).

EXPERIMENTAL RESULTS

The initial reaction time for tail withdrawal by experimental animals was 9.1 ± 0.3 sec (expectation ± error of expectation, n = 104). After injection the reaction time was increased in groups receiving β-CM7 in doses of 10 and 20 mg/kg; in the group receiving the peptide in a dose of 5 mg/kg and in the control group, changes of this kind were absent (Fig. 1). In the first two cases a significant increase in reaction time was observed as early as 10-20 min after injection of the peptide, to 1.5-2.0 sec (16-22% of the initial level). This effect remained significant in the group receiving 10 mg/kg of the peptide during recording for 30-40 min ($p < 0.05$). In the group receiving 20 mg/kg of β-CM7 the analgesic effect was more prolonged (60-70 min) and its level was a little, but significantly, higher ($p < 0.01$-0.05); the greatest increase in reaction time for tail withdrawal was observed 40-50 min after injection. This suggests that in our experiments a direct dose–effect dependence was present. In fact, dispersion analysis (ANOVAR) showed that the effect of the factor of an increase in the injected dose of the peptide was significant during the first hour after injection ($p < 0.05$).

A more detailed examination revealed positive correlation between the intensity of the analgesic effects and the initial level of the animals' nociceptive sensitivity. This correlation was recorded in the group receiving 20 mg/kg of the peptide as early as 20 min after injection (Fig. 2a), whereas in the group receiving 10 mg/kg it appeared after 30 min (Fig. 2b). The correlation described above is characterized by a high level of significance ($p < 0.001$-0.05) and lasted throughout the 2 h of recording. Consequently, β-CM7 induces analgesia most strongly in animals with initially high sensitivity to pain. In fact, when subgroups of animals with initially shorter tail withdrawal time (4-8 sec) are examined, it is clear that in this case the peptide, injected in doses of 10 and 20 mg/kg, caused an increase of 30-50% in the reaction time; significant changes were recorded during 80-100 min after injection. The dose–effect relationship also was more significant ($p < 0.01$, ANOVAR method).

Judging by these results, doses of β-CM7 of 20 and, in particular, 10 mg/kg are close to the absolute minimal doses for manifestation of the analgesic properties of this peptide. Our results are in good agreement with those of analysis of the analgesic activity of other opioids. In fact, effective doses of beta-endorphin, when administered systemically, amount to 8-28 mg/kg, of morphine 1-10 mg/kg, and of dermorphine 5 mg/kg [1].

It may also be pointed out that in our case a dose of 5 mg/kg did not exhibit analgesic activity and, consequently, it lies below the threshold of sensitivity of the pain systems to β-CM7. On the contrary, this dose of the peptide in fact induced some degree of hyperalgesia immediately after injection (Fig. 1). This may perhaps be connected with the definite adaptogenic effect of β-CM7, for taking part in the tail flick test is itself a very powerful stressing factor. An additional series of experiments carried out on animals previously exposed to stress in the open field test showed that up to 50% of them had a tail flick reaction time which exceeded 14-15 sec. In this subgroup, β-CM7 in a dose of 5 mg/kg consistently caused the reaction time to fall (by 20-25%, $p < 0.02$-0.01, n = 16) during 1 h after injection.

There is as yet no general agreement in the literature on whether casomorphines can exist in the body long enough to exhibit their properties as physiologically active peptides. This is due, in particular, to the recent discovery of bipeptidyl-peptidase IV, an extracellular enzyme specialized for the degradation of proline-containing peptides [6]. Meanwhile other investigations have shown that beta-casomorphines, if injected directly into the stomach, have a direct effect on the secretion of insulin and somatostatin [8, 9] and on contractility of the gastroin-testinal tract [4].

Our findings in this respect, demonstrating that systemically injected β-CM7 has an analgesic action in doses close to effective doses of other opioids, appear to be highly encouraging. It may also be tentatively suggested that analgesically inactive doses of β-CM7 can induce certain neurotropic effects. These considerations make this peptide and its analogs promising objects for future research.

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