PHARMACOLOGY AND TOXICOLOGY

Effects of β-Casomorphin-7 on Different Types of Learning in White Rats

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The heptapeptide β-casomorphin-7, a casein fragment of cow's milk, accelerated the learning of a food-procuring habit in a T-maze by rats injected with this heptapeptide in a dose of 1 or 5 mg/kg body weight 5 min before the start of each learning session. This effect was blocked by pretreating rats with naloxone. On the other hand, β-casomorphin-7 in the indicated doses delayed learning of the active avoidance response involving the use of a painful reinforcing stimulus. The results of this study indicate that β-casomorphin-7 attenuates defense reactions by shifting the total balance of motivations toward food-procuring behavior.

Key Words: *learning; β-casomorphin-7 and analogs; opioids; naloxone*

It has been firmly established that opiates can play an important role in the genesis of mental disorders, the regulation of emotional states, and the modulation of learning processes. Opioid agonists most often hinder learning while opioid antagonists promote it, although, depending on the experimental conditions and doses used, this general rule has many exceptions [11], which explains to a large extent why the mechanisms underlying the effects of opiates remain unclear. Meanwhile, discovering how a newly discovered or synthesized opioid peptide influences behavior and, in particular, the learning of motor habits, is an essential stage of research into the behavioral effects of any such peptide.

Not long ago it was found that, in addition to several families of endogenous opioids, there are similar substances, so-called exorphins, which enter the body from the outside in food. A family of compounds belonging to this class comprises the casomorphins, which are casein derivatives [4]. One of its typical members, the heptapeptide Tyr-Pro-Phe-Pro-Gly-Pro-Ile, dubbed β-casomorphin-7 (β-C-7), has been shown capable of being absorbed into the blood [15] and acting on opiate μ- and δ-receptors [7]. It is logical to assume that β-C-7 and its derivatives, like other opioid peptides, not only influence pain sensitivity and locomotion [1,2], but can also be involved in regulating the elaboration of various conditioned responses. Accordingly, we undertook the present study to test β-C-7 and its analogs in various doses for their effects on the capacity of animals for learning.

MATERIALS AND METHODS

The heptapeptide β-C-7 and its des-Tyr analogs β-casomorphin-6 (Pro-Phe-Pro-Gly-Pro-Ile - β-C-6) and β-casomorphin-4 (Phe-Pro-Gly-Pro - β-C-4) used in this study were synthesized in the Laboratory of Regulatory Peptides of the Institute of

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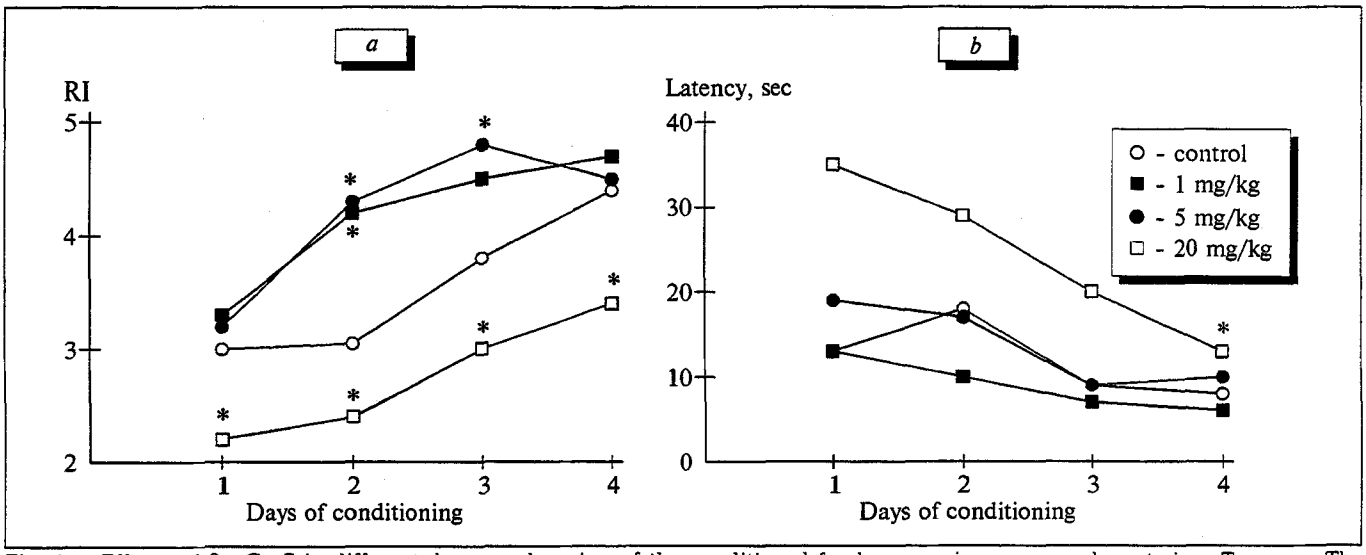

Fig. 1. Effects of β−C−7 in different doses on learning of the conditioned food−procuring response by rats in a T−maze. The peptide was injected daily 5 min before the rat was placed in the maze. Each group consisted of 20 animals. *$p<0.05$. RI = reaction incidence.

Molecular Genetics of the Russian Academy of Sciences. The study was conducted on 292 random-bred male white rats weighing 150-250 g. Aqueous solutions of the peptides were injected intraperitoneally (1 ml/kg body weight); the doses and times of injection are indicated below. One group of rats also received naloxone (Sigma) intraperitoneally, in a single dose of 1 mg/kg (1 ml/kg of aqueous solution) 10 min before being injected with β-C-7. Control rats were injected with equivalent volumes of distilled water by the same route.

A conditioned food-procuring response to a place (place learning) was elaborated in rats in a T-maze with 30×10 cm arms and a 10×10 cm starting chamber (the maze was made in workshops of Moscow State University). Rats were placed in the maze 5 times daily for not more than 3 min each time, using bread balls as the reinforcement. The following four variables were recorded: reaction incidence (RI), defined as the number of times the rat found the reinforcement during the 3-min period; latency of the rat's exit from the starting chamber; reaction time, defined as the time taken by the rat to reach the reinforcement-containing compartment of the maze; and error incidence, defined as the number of times the rat entered the compartment opposite to that containing the reinforcement.

The conditioned response of active avoidance was elaborated in a 30×22×35 cm chamber with an

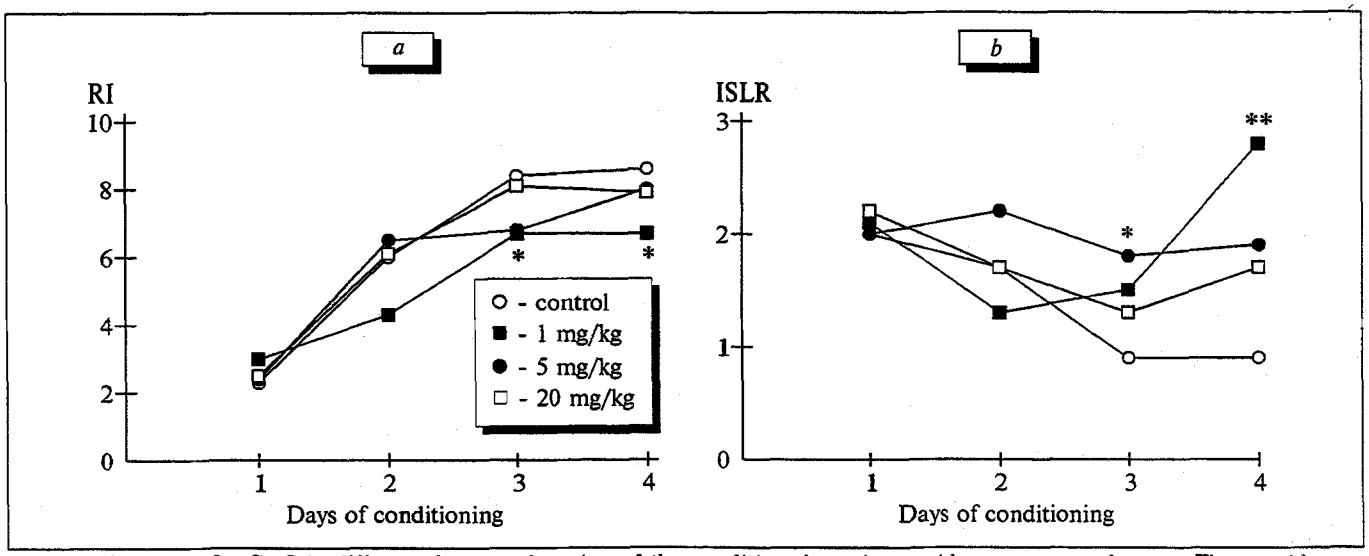

Fig. 2. Effects of β−C−7 in different doses on learning of the conditioned passive avoidance response by rats. The peptide was injected daily 5 min before the rat was placed in the test chamber. Each group consisted of 12 animals. *$p<0.05$, **$p<0.01$. RI = reaction incidence; ISLR = incidence of short latency (1−2 sec) reactions.

electrified floor (also made in Moscow University workshops). The conditioned stimulus was a bell and the unconditioned stimulus an electric shock, the voltage being selected on an individual basis in the 40-60 V range. The conditioned response was jumping to the shelf located 25 cm above the floor. Each rat was presented with 10 combinations of the conditioned and unconditioned stimuli daily for 4 successive days. The reaction incidence (number of jumps to the shelf in response to the conditioned signal) and the incidence of short-latency reactions (ISLR) (number of jumps to the shelf 1-2 sec after the electric shock) were recorded.

The results were subjected to statistical analysis by standard methods using Student's t test and the Mann-Whitney test for paired comparisons.

RESULTS

β-C-7 doses of 0.1, 1, and 5 mg/kg injected 5 min before placement in the T maze increased the RI on the 2nd day of learning by 16, 35, and 39%, respectively, as compared to the control group (Fig. 1), the increases after the 1 and 5 mg/kg doses being statistically significant ($p<0.05$). On the 3rd day, the corresponding increases were insignificant (8 and 15%) after the 0.1 and 1 mg/kg doses and significant (23%) at $p<0.05$ after the 5 mg/kg dose. No significant differences between the control and test groups in RI were recorded on the 3rd or 4th day, nor in the other three learning parameters (reaction latency, reaction time, and error incidence) on any day after the indicated doses.

A much higher dose of this heptapeptide (20 mg/kg) led to significant decreases in the RI to 73, 81, 77, and 80% ($p<0.05$) of the control values on days 1, 2, 3, and 4, respectively. The reaction latency was also higher in the test animals than in the controls, being significantly higher (by 82%; $p<0.05$) on day 4. The inhibitory action of this relatively high dose on the learning process was probably due to a reduction in the overall motor activity of the rats [2].

In an attempt to clarify the neurochemical basis of the observed effects, a group of rats was given the μ-opiate antagonist naloxone (1 mg/kg intraperitoneally) before being injected with β-C-7 at 5 mg/kg. These rats were found to perform in the T-maze much worse than those given β-C-7 alone in the same dose. Thus, the RI was 28-34% lower ($p<0.05$), while the reaction latency and time were 70-120% higher ($p<0.05$), which indicates that naloxone blocked the effects of β-C-7. However, the effects of casomorphins on learning are likely to involve δ-receptors as well, since, at any rate, these have been shown to mediate the influence of opioids on motor activity [3,10,13].

Although opioids deprived of the N-terminal tyrosine have been reported not to interact with the specific receptors [14], we detected neurotropic activity in the des-tyrosine analogs of β-C-7 (β-C-6 and β-C-4). Their neurotropic activity was somewhat weaker than that of the prototype, but produced similar effects. Thus, the rats injected with β-C-4 at 5 mg/kg made significantly fewer errors in the T-maze on day 4 of learning than did control animals (a 75% decrease; $p<0.05$), while those injected with β-C-6 in the same dose showed a 120% increase in RI over the control group ($p<0.01$) on day 1 and an 80% decrease in reaction time ($p<0.01$) on day 3. These results agree well with those reported from studies where antidepressant effects of des-tyrosine β-C-7 analogs were observed [6,8].

In an additional series of tests we found that food consumption by rats injected with β-C-7 at 5 or 20 mg/kg did not differ from that by control rats, indicating that the heptapeptide does not reduce feeding motivation.

The effects produced by the casomorphins in the tests using painful electric shock as the reinforcing stimulus differed substantially from those in the T-maze. The β-C-7 dose of 1 mg/kg administered to rats 5 min before the start of active avoidance conditioning reduced the RI by 20% on day 3 of learning ($p<0.05$) and by 22% on day 4 ($p<0.05$) and increased the ISLR by 211% ($p<0.01$) on the latter day (Fig. 2). The 5 mg/kg dose exerted similar but less marked effects, the only significant difference from the control group being a 100% increase in the ISLR ($p<0.05$). The inferior performance of the rats with regard to learning the active avoidance response cannot be accounted for by their reduced pain sensitivity since, as we found previously, the lowest analgesic dose of β-C-7 for rats is 10 mg/kg [1]. The dose of 20 mg/kg did not cause significant changes in any of the parameters we measured to evaluate how this response was learned.

The rate at which rats learned the conditioned response in the T-maze was presumably determined by the ratio between their food-procuring and defense motivations. In a separate (control) test, however, β-C-7 had no effect on the former motivation. This plus the ability of β-C-7 to eliminate the preeminent manifestations of a passive defense reaction in stressed animals [2] strongly suggest that the facilitated learning of the food-procuring habit in the T-maze by rats treated with β-C-7 can be attributed to a partial suppression of defensive behavior and the consequent predominance of behavior aimed at getting food.

Evidence associating the opioidergic system with perception has been reported [5], but in our experiments β-C-7 does not appear to have had any effect on perception, for, while improving learning in tests with the positive reinforcement, it impaired learning in those with the negative reinforcement.

In our view, β-C-7 mainly influenced the learning of the conditioned passive avoidance response in the same way as it did the learning of the food-procuring habit in the T-maze, i.e., by weakening defense motivation. During the learning of the conditioned avoidance response, however, in contrast to the learning of the conditioned feeding response, defense motivation predominates. It triggers the program of appropriate behavioral acts and its suppression slows the learning process and hinders the specialization of the habit being learned. Evidence that opioids have such effects on learning with negative reinforcement has been published in the literature [9,12].

In summary, β-C-7 facilitates learning involving positive reinforcement while having an opposite effect and, possibly, promoting the defensive function in a stressful situation. This pattern of behavioral activity exhibited by the peptide in low doses may have a direct bearing on the processes by which animals adapt to a changing environment, particularly during the early period of ontogeny, when their only food is mother's milk. In general, it may be concluded that the casomorphins, which are of special interest as "food opioids," merit further study with a view to exploring their therapeutic uses.

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