VOLTAGE-DEPENDENT ACTION OF THE OPIOID PEPTIDE DERMORPHIN

N. E. Babskaya

UDC 615.212.7.015.4.038

KEY WORDS: dermorphin; gastric musculature; acetylcholine; electrical stimulation; potassium contracture

In recent years the attention of many research workers has been drawn to the study of an extensive and steadily increasing class of peptide regulators [1]. Of particular interest in this respect are peptides which are endogenous regulators, whose functional role and mechanisms of action have not yet been finally elucidated. These regulatory peptides include the heptapeptide dermorphin, isolated for the first time in 1981 from the skin of South American frogs [12], and subsequently found in different parts of the central nervous system and stomach of various animals [11]. Dermorphin has been shown to possess central opioidlike antinociceptive activity, and in the degree of its analgesia it is superior to morphine [3, 5, 6, 13]. The peripheral effects of dermorphin are diverse. It affects the heart and respiration rate [14], modifies motor function and contractions of the gastrointestinal tract [4, 5, 7, 8], and inhibits cholinergic effects on the heart [2]. However, the direction in which these effects proceed depends on the mode of administration of dermorphin to the animals and it is evidently determined mainly by its effects on the central and autonomic nervous systems.

For a more detailed study of the mechanisms of action of dermorphin it is therefore essential to study its effect in simpler model experiments, using tissues in which the presence of a dermorphin system is assured.

The aim of this investigation was to study the possible mechanisms of action of dermorphin on a smooth-muscle strip of frog stomach.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (*Rana temporaria* L.). A transverse muscle strip 3 mm wide was excised from the stomach of the immobilized animals and placed in a horizontal chamber containing Ringer's solution of the following composition: NaCl 112.0; KCl 1.9; $CaCl_2$ 1.2; $NaHCO_3$ 1.1 mM. Mechanographic recording was carried out on a type N-338 automatic writer through a strain gauge amplifying system. The character of spontaneous activity of the musculature and evoked contractile responses to exogenous acetylcholine (ACh) in concentrations of $(1\text{-}5)\cdot10^{-6}$ M and to electrical stimulation was assessed. Direct electrical stimulation (10-15 V, 10-20 Hz, 1-3 msec) was applied to the preparation for 10-15 sec, through needle electrodes inserted into the muscle, from an ÉSL-1 stimulator. One series of experiments was carried out on a nerve-muscle preparation of the sartorius muscle, using single or repetitive indirect submaximal stimulation with a duration of 0.3-0.5 msec. Potassium contracture was evoked with KCl (40-110 mM), with an equivalent reduction in NaCl concentration in the solution. The synthetic preparation dermorphin was tested in concentrations of 10^{-5}-10^{-10} M.

EXPERIMENTAL RESULTS

The study of the action of dermorphin on a transverse smooth-muscle strip of the frog stomach showed that dermorphin, in a concentration as low as $1\cdot10^{-8}$ M completely blocks the effects of exogenous ACh (Fig. 1a).

Department of Physiology of Man and Animals, M. V. Lomonosov Moscow State University. (Presented by Academician of the Russian Academy of Medical Sciences I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 11, pp. 502-504, November, 1992. Original article submitted June 29, 1992.

0007-4888/92/0010-1649$12.50

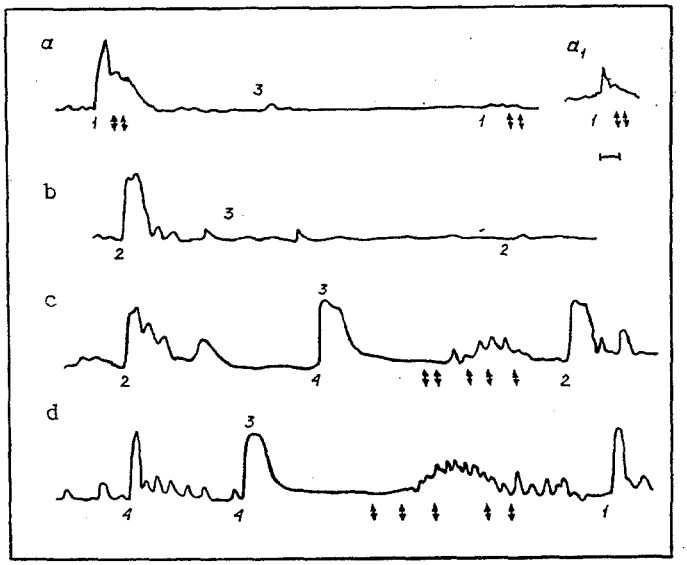

Fig. 1. Action of dermorphin (D) on transverse smooth-muscle strip of frog stomach: a) D ($1 \cdot 10^{-8}$ M) blocks effect of ACh ($1 \cdot 10^{-6}$ M); al) effect of ACh after rinsing preparation for 45 min; b) D ($1 \cdot 10^{-6}$ M) blocks effect of direct electrical stimulation (10 V, 20 Hz, 3 msec, duration 15 sec); c) absence of blocking effect of D ($1 \cdot 10^{-5}$ M) on effects of direct electrical stimulation; d) on effects of ACh when D injected against the background of K^+-contracture (KCl 100 mM); legend: 1) injection of ACh, 2) electrical stimulation, 3) injection of dermorphin, 4) injection of KCl; arrow indicates rinsing preparations with Ringer's solution; time marker 50 sec.

To determine the specificity of action of dermorphin a series of experiments was carried out using nerve-muscle preparations of skeletal muscles. It was found, however, that dermorphin in concentrations of up to 10^{-5} M caused no changes in contractile activity of the sartorius muscle during indirect stimulation, in single or repetitive modes. The absence of a blocking effect of dermorphin on neuromuscular transmission in skeletal muscles and the blocking of the effects of ACh on smooth muscle which we found, as well as the corresponding blockade of the effects of ACh on heart muscle [2] suggested that the action of dermorphin is associated with muscarinic cholinergic reception.

However, dermorphin not only blocked the effects of ACh on the gastric muscle strip. During its action against the background of slight relaxation, spontaneous activity of the preparations disappeared completely. In this connection it is worth noting the results obtained by several investigators, similar to our own observations, who showed that after intraperitoneal or subcutaneous injection of dermorphin emptying of the stomach in rats is delayed, and motor function of the small and large intestine is inhibited [4, 7, 9].

Meanwhile the disappearance of spontaneous activity against the background of relaxation which we observed could be evidence in support of an effect of dermorphin on the electrically excitable properties of muscle fibers. Accordingly, in the next series of experiments the action of dermorphin was tested against the background of direct electrical stimulation of a gastric muscle strip. It was found that dermorphin blocked the contractile activity of the preparations induced by direct electrical stimulation to the same degree as the effects of ACh (Fig. 1b).

The blocking action of dermorphin on all the above effects was reversible only with difficulty. Even when 60 min had elapsed after the beginning of rinsing of the preparations complete recovery of muscular activity was not observed. It must be pointed out that against this background of partial restoration, evoked contractile responses of low amplitude appeared a considerable time (sometimes 20-30 sec) after the beginning of stimulation. This significant increase in the latent period of development of the contractile response could evidently have been due to hyperpolarization of the muscle cell membrane, so that the critical level of depolarization was reached much more slowly. It was

therefore decided to study the effect of dermorphin against the background of artificial depolarization of the excitable membrane of the muscle fibers. Depolarization in this way was produced with KCl. With an increase in the K^+ concentration in the external solution to 100 mM (a concentration close to the intracellular concentration of this ion), maximal contracture developed. The K^+-contracture was unstable: the musculature quickly relaxed spontaneously. During rinsing with KCl a short-term increase in tone of the musculature and intensification of spontaneous contractions were observed, followed by restoration of the original level of activity. Testing of evoked responses before and after K^+-contracture revealed them to be completely identical.

Addition of dermorphin to the incubation medium at the peak of K^+-contracture did not change its character. Testing the effects of ACh and of direct electrical stimulation after short-term rinsing out of the K^+ ions revealed the complete absence of any blocking action of the peptide under these conditions although, as was pointed out above, the effects of dermorphin are reversible only with difficulty (Fig. 1c, d).

Depolarization of the electrical excitable membrane thus prevents the development of the blocking action of dermorphin on a gastric smooth-muscle strip. This suggests that dermorphin interacts with structures located in the region of the voltage-dependent ionic channels of the muscle cell membrane. These observations, and also the fact that we could find no blocking action of dermorphin on skeletal muscles, suggest that the action of the peptide is linked with the Ca^{2+}-channels of the membrane. In this connection it must be noted that many opioid peptides involved in the modulation of physiological processes have in the great majority of cases receptors that are coupled with voltage-dependent Ca^{2+}-channels [1, 10]. At the level of the nerve terminal activation of these receptors reduces Ca^{2+}-conductivity, as a result of which the quantity of mediatory released is reduced. At the effector cell level, however, the decrease in Ca^{2+}-conductivity creates hyperpolarization, which is the basis of modulation blockade. The mechanism of the blocking action of dermorphin on gastric smooth muscle is evidently similar in nature. It is important to point out in this connection that the effects of dermorphin develop only in the absence of active depolarization of the muscle fiber membrane.

When the results are assessed it can therefore be concluded that dermorphin, besides its well studied antinociceptive action, performs definite functions in the regulation of activity of the smooth muscle. These functions evidently consist of membrane stabilization and the suppression of the intrinsic automatism of the smooth-muscle cell; for this reason it becomes less receptive also for nervous stimulation.

REFERENCES

1. I. P. Ashmarin and M. A. Kamenskaya, Progress in Science and Technology. Series: Physiology of Man and Animals [in Russian], Vol. 34, Moscow (1988), pp. 3-180.
2. N. A. Sokolova, V. I. Degin, E. P. Yarova, and I. P. Ashmarin, Dokl. Akad. Nauk Rossii, **298**, No. 1, 254 (1988).
3. P. C. Braga, M. Tienge, and G. Biella, Neurosci. Lett., **52**, No. 1-2, 165 (1984).
4. M. Broccardo, V. Erspamer, and G. Falconieri, Brit. J. Pharmacol., **73**, 625 (1981).
5. M. Broccardo, Pharmacol. Res. Commun., **17**, No. 4, 345 (1985).
6. M. A. Cervini, A. C. Rossi, G. Perseo, and R. de Castiglione, Peptides, **6**, No. 3, 433 (1985).
7. K. Darlak, Z. Grronka, P. Jameki, et al., J. Med. Chem., **26**, 1445 (1983).
8. J. P. du C. Ferre, G. Soldani, and J. Ruckebusch, Regul. Peptid., **13**, No. 2, 109 (1986).
9. G. Giagnoni, D. Paralaro, L. Casiraghi, et al., Neuropeptides, **5**, No. 1-3, 157 (1984).
10. R. L. Macdonald and M. A. Werz, J. Physiol. (London), **377**, 237 (1986).
11. P. Melchiorri, G. Improta, L. Negri, and M. Broccardo, Peptide Hormones, Biomembranes and Cell Growth, Rome (1983), pp. 127-142.
12. P. C. Montecucchi, R. de Castiglione, and V. Erspamer, Inter. J. Peptide Protein Res., **17**, 316 (1981).
13. G. Sandrini, E. C. Degliuberti, S. Salvaderi, et al., Brain Res., **371**, No. 2, 364 (1986).
14. A. Tartara, M. Maurelli, and E. Marchioni, Farmacol. Ed. Sci., **41**, No. 3, 215 (1989).