MODULATION OF EXCITATION TRANSMISSION IN THE INTRACARDIAC GANGLIONIC SYSTEM BY THE OPIOID PEPTIDE DERMORPHIN

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KEY WORDS: intracardiac ganglia; opioid peptides; modulation of excitation.

It has recently been found that luteinizing hormone releasing hormone (LHRH), substance P, vasoactive intestinal polypeptide, and neurotensin modulate the transmission of excitation in the intracardiac ganglia [6, 8, 10]. This suggests that other regulatory peptides (RP) may act as modulators of intracardiac transmission: the most suitable candidates for this role are opioids, for their effect on excitation has been demonstrated in many autonomic ganglia [5].

The aim of this investigation was to study the effect of an opioid peptide (dermorphin), first discovered some years ago in a methanol extract of frog skin [3, 7], on the transmission of excitation in the intracardiac ganglia or amphibians. Dermorphin differs from other opioid peptides in its much higher activity and the longer duratin of its action [9]. This, and also the fact that dermorphin is an endogenous opioid of amphibians, served as the basis for its use in the present experiments.

EXPERIMENTAL METHODS

Experiments were carried out on a preparation of the isolated right atrium of the frog Rana temporaria L. with dissection of the intracardiac nerve, the two extracardiac vagosympathetic trunks, and the medulla. Besides other nerve fibers, the intracardiac nerves also include axons of intracardiac postganglionic parasympathetic neurons. Spike activity in these axons can be evoked by stimulation of preganglionic parasympathetic neurons located in the medulla; under these circumstances, however, the possibility of activation of central afferent fibers running in the composition of the intracardiac nerves without relaying (en passant) must also be taken into account. During the experiments the medulla was stimulated by single stimuli of above threshold strength and with a duration of 0.1 msec. Bipolar electrodes with an interelectrode distance of 1.2 mm, connected through a high-frequency attachment to the output of an ÉSU-2 stimulator, were used for stimulation. Spike activity was recorded frm the central end of the intracardiac nerve by means of bipolar silver electrodes with interelectrode distance of 0.3 mm, connected to the input of a UBP2-03 amplifier. The electroneurogram was recorded from the screen of an S1-18 oscilloscope by means of an FOR-2 camera; a parallel recording also was made on magnetic tape. These substances were applied to the endocardial surface of the right atrium by means of an automatic micropipet. The EMG was recorded for 15 min after the moment of application with an interval of 1-2 min.

The substances used in the experiments were: dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) in a concentration of 10^{-6} -10^{-12} M, synthesized as described previously [4]; naloxon [(-)-17-allyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one], in a concentration of 10^{-5} M (Du Pont); standard Ringer's solution for cold-blooded animals.

The experimental results were subjected to statistical analysis by nonparametric methods [1, 2].

EXPERIMENTAL RESULTS

Application of dermorphin in a concentration of 10^{-6}-10^{-12} M led to dose-dependent inhibition of spike activity in the intracardiac nerve, as shown by a decrease in the number

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TABLE 1. Changes in Spike Activity in Intracardiac Nerve during Action of Dermorphin (in concentration of 10^{-6}-10^{-12} M) and in Control on Application of Ringer's Solution

Dermorphin, M	Change in number of spikes, %								Change in amplitude, %							
	time after application, min															
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
10^{-6} (10)	11	23*	39**	55**	48**	55**	48**	48**	13	16*	30**	46**	51**	59**	59**	60**
10^{-8} (10)	4	12	27**	39**	50**	50**	46**	50**	5	8	17**	16**	50**	51**	50**	50**
10^{-10} (10)	7	10	13*	29**	33**	43**	41**	43**	3	9	9*	9*	18**	27**	27**	27**
10^{-11} (6)	0	+1	0	—3	—1	0	—2	0	—2	0	—2	—3	+1	0	0	—1
10^{-12} (10)	—2	—2	—4	—1	+1	0	—1	—1	—5	0	0	—1	0	0	0	—1
Control (30)	—4	3	—3	0	0	—2	0	—1	—6	—4	—1	—1	0	0	+1	—1

Legend. Changes in number of spikes and in maximal amplitude of volley given in percent of initial values and during 8 min after application. Asterisks indicate effects differing significantly from action of Ringer's solution at corresponding time after application: *$p < 0.01$, **$p < 0.001$. Magnitudes of effects given in Table 1 without (+) or (-) signs correspond to lowering of activity. Number of experiments in each series shown in parentheses.

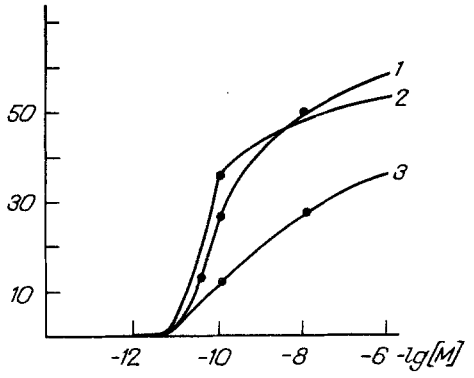

Fig. 1. Changes in basic parameters of spike activity in intracardiac nerve, expressed as a percentage, during the actin of dermorphin in a concentration of 10^{-6}-10^{-12} M. Action of each concentration assessed relative to maximal change in initial values. 1) Amplitude of volley; 2) number of spikes per volley; 3) duration of volley. Ordinate, maximal effect (in % of initial value).

of spikes per volley and in the maximal amplitude and duration of the volley (Fig. 1). The concentration of dermorphin inducing 50% inhibition of spike activity was 10^{-8} M. Analysis of the reduction in spike activity with time showed that under the influence of dermorphine in a concentratin of 10^{-6} M significant reduction of activity compared with the control occurred toward the 2nd minute after application, and the maximal effect was observed by the 4th minute; if lower concentrations were applied, the values of these parameters were increased. Dermorphin in a concentration of 10^{-11}-10^{-12} M had no significant effect on spike activity; however, considering the specific properties of the compound and the method of application of dermorphin it can be tentatively suggested that its concentration, if acting directly on the intracardiac ganglia, was lower than the minimal concentration determined experimentally (Table 1). Recording of the electroneurogram for 10 min or more after the maximal effect had been achieved did not reveal a gradual restoration of the discharge; recovery likewise was not observed even after the dermorphin had been rinsed out with Ringer's solution.

Inhibition of spike activity by dermorphin took place through activation of opiate receptors, for the effects of dermorphin were blocked by naloxone (10^{-5} M).

To discover any possible influence of dermorphin on the conduction of excitation in "en passant" fibers the electroneurogram of the intracardiac nerve was recorded during stimulation of two or three sympathetic ganglia; under these curcumstances postganglionic sympathetic axons running in the intracardiac nerve without relaying were activated. Dermorphin in a concentration of 10^{-6} M did not affect his "en passant" impulsation.

Our results thus show that the endogenous amphibian opioid peptide, dermorphin, can specifically inhibit intracardiac ganglionic transmission by acting through opioid receptors. The study of where these receptors are located – whether at the presynaptic or the postsynaptic level – will be a task for future experiments.

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ACTION OF BENZODIAZEPINES ON THE IMMUNE RESPONSE

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KEY WORDS: GABA-ergic system; benzodiazepine receptors; diazepam; oxazepam; immune response.

Isolated studies of the effect of GABA and GABA-positive substances on immunologic processes have been published in recent years. GABA, its cetyl ester, and sodium hydroxybutyrate and valproate have been shown to affect antibody formation in the spleen [4]. Injection of GABA and γ-hydroxybutyric acid stimulates immunogenesis, as shown by morphological and functional changes in the organs of immunity [1]. There are also data on the effect of GABA and γ-hydroxybutyric acid on the nonspecific factors of immunity: lysozyme activity and phagocytosis [6]. Changes in the GABA concentration in the tissues of the posterior lobe of the hypothalamus have been demonstrated under the influence of factors inhibiting function of the immune system [5]. The immunostimulating effect of muscimol, a direct agonist of the GABA receptor, and the suppressive effect of bicuculline, a competitive inhibitor of the GABA receptor, and also the depressive action of the chloride channel blocker picrotoxin on the immune response [2] have been established.

The benzodiazepines, widely used cytotropic agents, are known to modulate the GABA receptor, i.e., they increase pre- and postsynaptic GABA-mediated inhibition [10]. According to data in the literature [8], diazepam in doses of 1 and 2 mg/kg does not affect the delayed type hypersensitivity reaction, whereas in a dose of 8 mg/kg it inhibits this reaction strongly [7].

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