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EFFECTS OF SCORPION (*Buthus eupeus*) TOXINS ON CYCLIC AMP AND CYCLIC GMP LEVELS IN BRAIN AND HEART TISSUES

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KEY WORDS: scorpion toxins; cyclic nucleotides; concentration in brain and heart.

Toxic polypeptides isolated from venom of the scorpion *Buthus eupeus* [1, 2] interact selectively with the gating mechanism of the sodium channels of excitable membranes, slowing their inactivation [4]. This action of the toxins on the organ or organism leads to massive liberation of neuromediators from nerve endings [5, 6, 12]. Modification of excitable membranes by certain toxins (anemone toxin ATCP, MCD-peptide from bee venom) is known to stimulate cyclic nucleotide formation [8, 9].

It was therefore interesting to study the effect of the toxins of *Buthus eupeus* on cyclic AMP and cyclic GMP in brain and heart tissues.

EXPERIMENTAL METHOD

Native venom of the scorpion *Buthus eupeus*, obtained by electrical stimulation, and also the active polypeptide fraction (APF) isolated from the venom [2] were used. Isolated hearts of 45 guinea pigs were perfused and the cardiac contractions were then recorded [13]. Atropine, propranolol, and hexamethonium (10^{-4} g/ml) were injected into the perfusion fluid 10 min before injection of the venom or APF (10^{-7}-10^{-3} g/ml). The venom and APF were injected intraventricularly [10] into albino mice weighing 23-25 g (120 animals) in a volume of 5 µl in doses of 0.1-10 µg/20 g body weight. The latent period of the convulsive response was analyzed quantitatively. Atropine, tropacin, chlorpromazine, or haloperidol — all in doses of 1 mg/kg — was injected intraperitoneally into the mice 30 min before injection of the venom or APF. Arecoline (25 mg/kg) and apomorphine (10 mg/kg) were injected subcutaneously. Reserpine (5 mg/kg) was injected intraperitoneally into mice and guinea pigs 4 h before the beginning of the experiment. Sections through the forebrain and cerebellum of the mice were prepared by the method in [11]. The sections were preincubated for 40 min. Venom and APF (final concentration 10^{-6}-10^{-5} g/ml) or the incubation solution with an increased KCl concentration (100 mM) were added to the medium for 10 min. Cyclic AMP and cyclic GMP in the myocardium of the guinea pigs and in the mouse brain sections were determined quantitatively with the aid of commercial kits (from Becton and Dickinson, USA). Radioactivity of the samples was counted and the concentrations of cyclic nucleotides determined with an Ultrogamma automatic counter (from LKB, Sweden) coupled to a Data-Box 1222 minicomputer.

EXPERIMENTAL RESULTS

Intraventricular injection of venom or APF into the appearance of dose-dependent myoclonic contractions, reflex movements, and attacks of violent clonico-tonic convulsions. LD_{50} for the venom and APF was 4.7 and 0.66 µg/20 g respectively. Preliminary administration of blockers of catecholaminergic synapses (chlorpromazine, haloperidol, and reserpine) considerably accelerated the development of convulsions. A similar effect was produced by the central muscarinic and nicotinic blockers of acetylcholine synapses atropine and tropacin. Meanwhile specific stimulators of cholinergic and dopaminergic synapses arecoline and apo-

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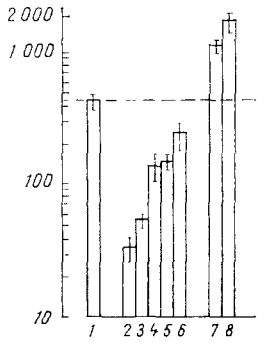

Fig. 1

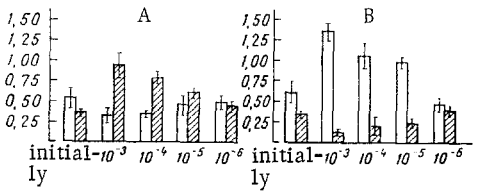

Fig. 2

Fig. 1. Effect of stimulators and blockers of catecholaminergic and cholinergic synapses on latent period of convulsive response evoked by intraventricular injection of polypeptide fraction of *Buthus eupeus* venom: 1) polypeptide fraction, 2-8) preliminary injection of drugs; chlorpromazine, reserpine, tropacin, haloperidol, atropine, apomorphine, and arecoline respectively. Ordinate, latent period (in sec).

Fig. 2. Effect of various concentrations of polypeptide fraction from *Buthus eupeus* venom on cyclic nucleotide concentrations in guinea pig myocardium. A) Phase of bradycardia, B) phase of tachycardia. Abscissa, concentration (in g/ml); ordinate, concentration (in pmoles/mg cyclic AMP (unshaded columns) and cyclic GMP (shaded columns).

TABLE 1. Effect of Polypeptide Fraction of *Buthus eupeus* Venom and KCl on Concentrations of Cyclic Nucleotides (in pmoles/mg) in Mouse Brain Sections (M ± m)

Experimental conditions	Forebrain		Cerebellum	
	cyclic AMP	cyclic GMP	cyclic AMP	cyclic GMP
control	0,33±0,026 (n=6)	0,036±0,0042 (n=6)	0,47±0,04 (n=6)	0,25±0,05 (n=6)
Polypeptide fraction† (10^{-6} g/ml)	0,55±0,11 (n=6)	0,036±0,009 (n=6)	0,88±0,04 (n=6)	0,98±0,1 (n=6)
P	<0,05	<0,05	<0,001	<0,001
KCl† (100 mM)	0,46±0,26 (n=6)	0,13±0,01 (n=6)	0,64±0,04 (n=6)	1,09±0,06 (n=6)
P	<0,001	<0,001	<0,05	<0,001

*Incubation for 40 min
†Preincubation for 40 min followed by addition of polypeptide fraction or KCl for 10 min.

morphine significantly lengthened the latent period (Fig. 1). It should be noted that neither the venom nor APF had any appreciable effect on the stereotyped behavior of the animals characteristic of arecoline and apomorphine.

Experiments on incubated mouse brain sections showed that the scorpion toxins, after an exposure of 10 min, considerably increased the cyclic GMP concentration in the cerebellum and also, to a lesser degree, in the forebrain. Changes in the cyclic AMP level were less marked after injection of both preparations. Potassium (100 mM KCl) depolarization of the membranes of the brain sections also led to elevation of the cyclic nucleotide levels (Table 1).

Experiments on the isolated perfused guinea pig heart showed that the venom and APF caused a clear biphasic effect: initial bradycardia followed by a phase of increased frequency and amplitude of cardiac contractions.

TABLE 2. Effect of Drugs on Changes in Frequency and Amplitude of Contractions of Isolated Guinea Pig Heart Due to Polypeptide Fraction of *Buthus eupeus* Venom (M ± m)

Experimental conditions	Heart rate, beats/min						Amplitude of cardiac contractions, mm					
	initially	n	after treatment				initially	n	after treatment			
			10–30 sec	P	30–60 sec	P			10–30 sec	P	30–60 sec	P
Polypeptide fraction (10^{-5} g/ml)	114,0±5,3	10	72,6±10,8	<0,01	139,6±5,1	<0,01	6,6±1,0	10	3,7±0,6	<0,05	11,1±1,3	<0,05
Hexamethonium (10^{-4} g/ml)	102,3±6,4	6	65,8±8,2	<0,05	126,1±9,3	<0,05	5,2±0,3	8	2,9±0,4	<0,05	10,4±1,1	<0,05
Propanolol (10^{-4} g/ml)	88,0±6,4	8	58,7±3,1	<0,01	81,0±7,0	>0,05	4,1±0,7	8	1,8±0,2	<0,05	3,5±0,8	>0,05
Atropine (10^{-4} g/ml)	102,0±13,4	6	101,7±12,6	>0,05	138,1±3,5	<0,05	4,1±0,4	6	3,6±0,3	>0,05	7,3±0,8	<0,02
Reserpine* (5 mg/kg)	87,7±5,9	15	58,6±3,8	<0,001	85,1±6,1	>0,05	5,9±0,8	15	4,2±0,6	<0,05	5,6±0,9	>0,05

*Reserpine was injected intraperitoneally into guinea pigs 4 h before experiment.

The first phase of action of the toxins was blocked by atropine, the second was prevented by propranolol and was absent in reserpinized animals. The ganglion-blocker hexamethonium did not change the character of action of the scorpion toxins on the isolated heart (Table 2).

The change in the cyclic AMP and cyclic GMP concentrations in the myocardium depended on the concentration of the toxins and the phase of their action on the isolated heart. During bradycardia the cyclic GMP level rose without any significant variations in the cyclic AMP concentration. Conversely, in the phase of tachycardia the cyclic AMP concentration rose sharply, whereas the cyclic GMP level fell (Fig. 2).

The results of the experiments on different objects thus indicate close correlation between the catecholaminergic and cholinergic effects of scorpion toxins and activation of the cyclase system. This relationship is manifested most clearly in experiments on the isolated guinea pig heart. Secretion of noradrenalin in the myocardium is known to be accompanied by an increase in the cyclic AMP concentration, and the inhibitory action of acetylcholine causes an increase in the cyclic GMP concentration [7]. When the action of scorpion toxins on the CNS is analyzed, both the direct depolarizing action of the neurotoxins on the neuron membrane and modulation of synaptic transmission under their influence must be taken into amount [3]. It isworth noting that Ahnert etal. [9], when explaining the link between activation of the sodium channel by anemone toxin and the predominant rise in the cyclic GMP level, consider that the latter effect may be used as an indirect indicator of the functional state of ionic channels modified by the toxins.

In the present writers' opinion scorpion neurotoxins can also provide important information on the role of cyclic nucleotides in the functioning of ionic chemically and electrically excitable membranes.

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CHARACTERISTICS OF FRONTAL-HIPPOCAMPAL INFLUENCES DURING THE FORMATION OF FOOD BEHAVIOR IN RABBITS AFTER BILATERAL DESTRUCTION OF THE LATERAL HYPOTHALAMUS

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According to the "pacemaker theory of motivation" [2, 4], the decisive role in the transformation of a biological need into a process of central nervous excitation is played by hypothalamic formations whose neurons, by virtue of the unique features of their metabolism and their exceptional informativeness about the state of the various blood constants, perform the functions of special pacemakers. However, starting with the 1960s, investigators have described behavioral reactions aimed at the satisfaction of the most important biological needs in animals with bilateral destruction of the principal motivation-generating hypothalamic formations [5, 6, 8-10]. In this connection it was interesting to analyze the role of certain brain structures and, in particular, of the dorsal hippocampus and frontal neocortex, in the development of the food behavior of rabbits with bilateral destruction in the region of the lateral hypothalamus.

EXPERIMENTAL METHOD

Experiments were carried out on 12 waking rabbits weighing 2.5-3 kg, taken from a total of 24 animals in which the lateral hypothalamus was destroyed bilaterally. The lateral region of the right and left hypothalamus was stimulated and subsequently coagulated through thin (0.1 mm) bipolar nichrome electrodes which, in accordance with Sawyer's atlas, were implanted into the previously scalped rabbit. The animals were fed and then tested for their food behavior in response to electrical stimulation of both the right and left lateral hypothalamus.

The hypothalamic structures were coagulated by a current of 1-2 mA acting for 20-40 sec.

After not less than 3 days the animals with bilateral destruction of the lateral hypothalamus, having successfully survived the recovery period, had electrodes implanted into their dorsal hippocampus and into the frontal region of their neocortex. Conditioning stimulation of these brain structures had the following parameters: 5-7 V, 50 Hz, pulse duration 1 msec.

To analyze the chemical mechanisms determining food behavior in animals with destructive lesions of the lateral hypothalamus and, in particular, the role of cholinergic and dopaminergic structures, atropine (1 mg/kg) and droperidol (0.3 mg/kg) were injected into the marginal vein of the rabbit's ear. The results were subjected to statistical analysis by Student's t-test. The location of the subcortical electrodes was verified histologically in brain sections cut to a thickness of 50-100 μ.

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