CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ACTIVATOR PEPTIDE: EFFECT ON CONTRACTILITY OF MUSCLES

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The writers previously found a new peptide in the calf myometrium which is an activator of several cyclic nucleotide phosphodiesterases (PDE) [1]. The peptide was isolated, its properties investigated, it was purified, and its molecular weight determined (1150 daltons). The specificity of the peptide for uterine tissue and its possible functional role were still unexplained. The investigation described below was undertaken to study these problems.

EXPERIMENTAL METHOD

Tissues (brain, liver, intestine, lungs, heart, uterus, skeletal muscles) of Wistar rats (females) aged 1-2 months and also the myometrium of calves, supplied from the slaughter house on ice 2-3 h after slaughter of the animals, were used.

The tissue extract containing the peptide was obtained as follows: The tissues were homogenized 1:5 in 100 mM Tris-HCl buffer, pH 7.5, containing 2 mM $MgSO_4$ and 1 mM β-MET,* and centrifuged for 1 h at 100,000g. The residues were resuspended in 5 mM Tris-HCl buffer, pH 7.5, containing 2 mM EDTA and 1 mM β-MET, centrifuged for 1 h at 100,000g, and the supernatant was used as the source of the peptide. For the physiological experiments extracts of calf uterus and of rat skeletal muscle and liver were partially purified by gel-filtration on a column (0.6 × 30 cm) with Sephadex G-25, equilibrated with 20 mM Tris-HCl buffer, pH 8.0, containing 1 mM $MgSO_4$ and 50 mM NaCl. The concentration of the peptide in the final preparation was 2 mg/ml.

*β-mercaptoethylamine.

TABLE 1. Effect of Peptide Fractions (100 μg) Obtained from Different Tissues on Activity of Standard PDE Preparation

Tissue	Change in PDE activity (in %) relative to control (without additives)
Rat brain	0 (6)
Liver	0 (12)
Lungs	0 (11)
Intestine	0 (11)
Heart	+150 ± 20 (13)
Skeletal muscle	+200 ± 35 (14)
Calf uterus	+300 ± 50 (15)

Legend. A standard preparation of calf uterus with specific activity of 8.1 nmoles cAMP/mg protein/min was used. Number of experiments given in parentheses.

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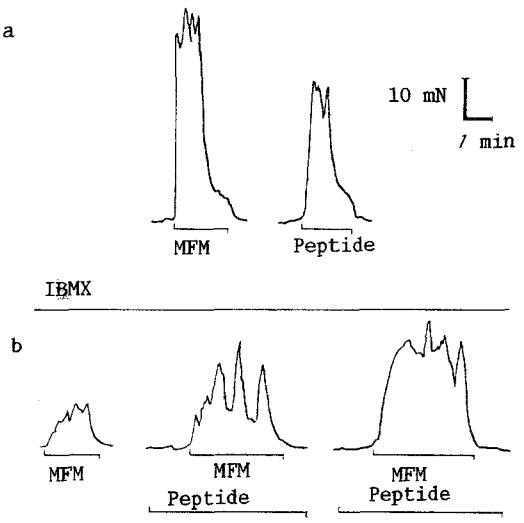

Fig. 1. Effect of PDE activator peptide on contractions of a longitudinal strip from the uterine cornu of an immature rat. a) Trace of muscle contractions evoked by methylfurmethide (MFM) and peptide; b) trace showing effect of peptide on uterine contractions evoked by MFM, on inhibition of PDE by 3-isobutylmethylxanthine (IBMX, 10^{-5} M). Concentrations: MFM 10^{-6} M, peptide: a) 8×10^{-5} g/ml, b) 8×10^{-5} and 1.6×10^{-4} g/ml.

The presence of the peptide in the extracts was shown by their ability to activate a standard preparation of PDE obtained from calf uterus by the method described previously [3]. Activity of PDE and adenylate cyclase (AC) and the content of inorganic phosphate and protein were determined as described in [4, 5].

Contractions of smooth muscles were recorded by the usual method [6]. All the muscles were kept in a bath with a capacity of 5 ml, containing Krebs' solution (pH 7.4, 37°C) and carbogen (95% O_2 + 5% CO_2). Spontaneous and evoked contractions were recorded under isometric conditions, using a transducer incorporating semiconductor strain gauge resistors, on a KSP-4 automatic recorder. The tension of the muscles was such that maximal contractions were observed (spontaneous and evoked). If a preparation of the rat phrenic nerve and diaphragm was used, the volume of the bath was 10 ml, the phrenic nerve was stimulated by suction electrodes and contractions were recorded on a high-speed N-373 automatic recorder.

EXPERIMENTAL RESULTS

The results of experiments to study the effect of peptide preparations obtained from various tissues on PDE activity of a standard preparation of the enzyme are summarized in Table 1. They show that only extracts obtained from muscle tissues (except intestinal tissue) had an activating effect on PDE. Preparations from brain, lungs, and liver caused virtually no change in activity of the enzyme. These findings suggest that the presence of PDE activator peptides in muscle tissues only may be of definite functional importance.

Some investigations have shown that the cyclic nucleotide level in the cell plays an important role in muscle contraction and relaxation [7, 11, 12]. It has also been suggested that certain muscle relaxants, which are PDE inhibitors (methylxanthines, in particular) exert their action by blocking activity of the enzyme, thereby raising the cyclic nucleotide levels in the muscle cell [8, 13]. It has also been stated that certain muscle tissues contain peptides which affect the contractile activity of particular muscles [9, 10].

Taking these data into consideration, in the next series of experiments the effect of the partially purified peptide from calf uterus was tested on contractions of smooth muscles. Experiments were carried out on longitudinal muscles of the uterine cornu of sexually mature and immature rats. In all cases the peptide induced contraction; concentrations of the peptide used in these experiments ranged from 400 μg to 2 mg per bath (Fig. 1). In some experiments the peptide caused an increase in contractions induced by methylfurmethide, which stimulates muscarinic acetylcholine receptors; the peptide itself in this case was added to the

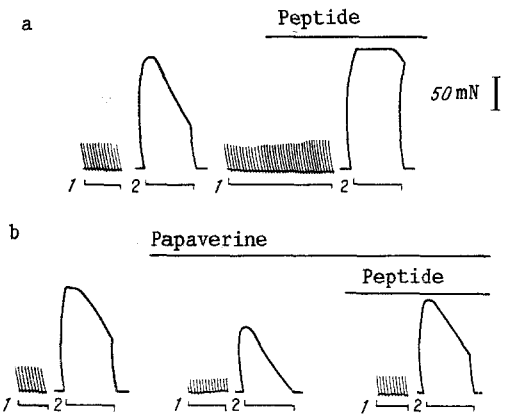

Fig. 2. Effect of PDE activator peptide on contractions of isolated rat diaphragm evoked by stimulation of phrenic nerve in the absence (a) and presence (b) of the PDE inhibitor, papaverine. 1) Single contractions (1 pulse, 10 sec); 2) tetanic contractions (100 Hz, 10 sec). Peptide: a) 10^{-4} g/ml, b) 2×10^{-4} g/ml, papaverine) 5×10^{-6} M.

bath in a minimal concentration (200 μg), which did not induce contractions. In the presence of a PDE inhibitor (3-isobutylmethylxanthine — IBMX, 10^{-5} M) the effect both of the peptide and of methylfurmethide was reduced, but preliminary addition of the peptide intensified the action of methylfurmethide in this case also (Fig. 1).

It must be pointed out that the peptide preparation from rat skeletal muscle also evoked contraction of the longitudinal muscles of the rat uterine cornu, whereas analogous fractions obtained from tissue not containing the peptide (rat liver) did not possess this property.

Considering that the PDE activator peptide was present not only in smooth muscles but also in skeletal muscles, the effect of the peptide was tested on contractions of muscles of this type (a preparation of the rat phrenic nerve and diaphragm was used). As the results of these experiments show (Fig. 2) the peptide intensified contractions of the diaphragm muscle evoked by stimulation of the phrenic nerve by an electric current (frequency of stimulation 100 Hz, amplitude 5 V, pulse duration 0.1 msec, duration of stimulation 10 sec), and the length of time the contractions were maintained at this frequency was increased. In the presence of the PDE inhibitor papaverine (5×10^{-6} M), which inhibits contractions, the peptide also had a potentiating action on contractions of the muscle.

The mechanism of the effect of the peptide on contractility of smooth and skeletal muscles is not clear. It can be tentatively suggested that its effect is associated with a change in the cyclic nucleotide level due to activation of PDE.

The effect of the peptide on another enzyme of cyclic nucleotide metabolism, namely AC, was studied in a special series of experiments. Unlike its action on PDE, the purified peptide (150-200 μg) was found not to change AC activity of the crude membrane fraction from rat uterus.

Changes in the cyclic nucleotide level under the influence of the peptide may thus take place as a result of activation of PDE. Meanwhile, when the polyfunctional nature of most peptides is taken into consideration [2], the possibility cannot be ruled out that the effects of the peptide which we observed are not connected with regulation of the cyclic nucleotide concentration in the muscle cell.

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LONG-TERM POTENTIATION IN SNAIL NEURONS

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In the investigation to be described below snail *(Helix pomatia)* neurons were used to study long-term plastic changes in the efficiency of synaptic connections. One model of these processes is long-term potentiation — a prolonged increase in the evoked response after application of short high-frequency stimulation. Long-term potentiation was first discovered in the hippocampus [8] and it was later found in other structures of the mammalian brain [10]. The possibility of obtaining such an effect in invertebrates was demonstrated in principle in investigations conducted on the crustacean neuromuscular synapse [7] and on neurons of the marine mollusk *Aplysia* [11, 12]. In investigations also conducted on mollusks it has been shown that an increase in the efficiency of synaptic connections can be brought about by serotonin (5-HT) [1, 5, 9]. The aims of the present investigation were: to study the possibility of formation of long-term (lasting more than 15 min) potentiation of EPSPs recorded in identified neurons of the snail CNS in response to stimulation of a nerve, and to study the effect of 5-HT and of intracellularly injected cAMP on these synaptic responses.

EXPERIMENTAL METHOD

Experiments were carried out on neurons on the dorsal surface of the subesophageal complex of ganglia of *H. pomatia*. The neurons were identified in accordance with Sakharov's classification [4]. A multibarreled microelectrode, one barrel of which served to record membrane potential, a second to pass a polarizing current through the membrane and measure the membrane resistance (both barrels were filled with 2M potassium citrate solution), whereas the remaining barrels were used for microiontophoretic injection of cAMP into the neuron [2], was inserted into the neuron. The resistance of the recording barrel was 10-15 MΩ. The membrane potential was shifted (by passage of a polarizing current) through 10-30 mV from the resting potential toward hyperpolarization, so that responses of the neurons to nerve stimulation and to mediators did not reach the level of action potential generation. The anal nerve was stimulated by tungsten electrodes. 5-HT (Serva) was added to the solution bathing the preparation in sufficient quantity to cause its final concentration to be 1×10^{-5} M. Washing out of the serotonin began 1 min after its application.

EXPERIMENTAL RESULTS

Experiments were carried out on neurons LPa2, LPa3, V1, V2, V4, and V6 and neurons of the F region of the visceral ganglion. In response to stimulation of the anal nerve an EPSP appeared in these neurons, its amplitude being constant during stimulation with a frequency of once every 5 min. After stimulation of the nerve with a frequency of 5 Hz for 30 sec the

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