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EFFECT OF PARATHYROID HORMONE ON $^{45}Ca^{++}$ AND ^{3}H-GABA TRANSPORT IN NERVE ENDINGS ISOLATED FROM THE RAT CEREBRAL CORTEX

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In hyperparathyroidism pathological changes in the activity of the nervous system arise [6], but their mechanisms are not sufficiently clear. In cells of the kidneys, bones, and intestine, parathyroid hormone (PTH) evokes several metabolic responses and, in particular, an increase in the Ca^{++} concentration [2, 4, 5, 10]. Assuming that PTH has a similar action on neurons, significant changes must be expected in their Ca^{++} transport and in activity of Ca^{++}-controlled processes. In connection with the possibility of influencing the integrative function of the nervous system, the study of the effect of PTH on the function of central synapses is particularly interesting. Ca^{++}, entering the cytoplasm through voltage-dependent channels, triggers neurotransmitter secretion in the nerve ending.

In view of the facts described above, it was decided to study the effect of PTH on $^{45}Ca^{++}$ transport in isolated nerve endings (synaptosomes) and Ca^{++}-mediated voltage-dependent release of the neurotransmitter — ^{3}H-GABA.

EXPERIMENTAL METHOD

Noninbred male rats weighing 180-200 g were used. Synaptosomes were isolated from the cerebral cortex as described above [1]. The resulting synaptosomes were suspended in incubation medium of the following composition (in mM): NaCl 145, KCl 5, CaCl 0.02, $MgCl_2$ 1.3, NaH_2PO_4 - 1.5, glucose 10, Tris-maleate 20 (pH 7.4, at 37°C). PTH (from Serva, West Germany) was added to the incubation medium in a dose of 138 U/mg.

Samples (50 µg synaptosomal protein [3] in 0.5 ml) were incubated at 37°C on a water bath with continuous shaking for 20 min, after which 0.5 ml of normal or depolarizing medium containing $^{45}Ca^{++}$ (1 µCi, from Amersham International, England) was added. After addition of the depolarizing medium the Na^{+} and K^{+} concentrations in the samples were 110 and 40 mM, respectively. Incorporation of $^{45}Ca^{++}$ into synaptosomes was stopped after 1 sec, 1 min, or 30 min by adding 2.5 ml of normal medium at 0-2°C, with its $CaCl_2$ concentration increased to 2 mM, and the synaptosomes were separated at once by filtration of the suspension through a GFC filter (Whatman, England) under negative pressure consisting of 2.5 ml of cold normal medium for displacement of $^{45}Ca^{++}$, bound with the outer membrane of the synaptosomes. The air-dried filters were transferred to flasks containing toluene scintillator for measurement of their radioactivity. The quantity of $^{45}Ca^{++}$ taken up by synaptosomes was expressed in nanomoles/mg protein.

Experiments to study the uptake and release of ^{3}H-GABA (D, L-2,3-^{3}H-γ-aminobutyric acid, 32 Ci/mmole, Izotop, USSR) were conducted on the P_2 fraction, most of which consists of synaptosomes. In this series of investigations the NaCl concentration in the normal medium was reduced to 132 mM and the Ca^{++} concentration was either reduced to zero or increased to 0.1 mM. The Na^{+}, K^{+}, and Ca^{++} concentrations in the solution used for depolarizing the synaptosomes were 107, 30, and 0.1 mM, respectively, and the concentrations of the remaining components were as indicated above. To tubes containing 0.6 ml of normal medium with or without

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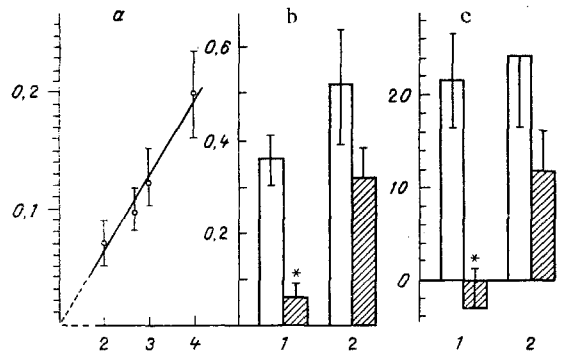

Fig. 1. Effect of PTH on $^{45}Ca^{++}$ transport and ^{3}H-GABA release by synaptosomes isolated from the rat cerebral cortex. a) Increase in $^{45}Ca^{++}$ accumulation by synaptosomes depending on PTH concentration in incubation medium: abscissa, logarithm of PTH concentration (in ng/ml); ordinate, excess of $^{45}Ca^{++}$ uptake over control (in nmoles/mg protein); b) increase in $^{45}Ca^{++}$ uptake in depolarizing medium 1 sec (1) and 60 sec (2) after beginning of depolarization: unshaded columns — control, shaded — 10 ng/ml of PTH; c) ^{3}H-GABA release by synaptosomes in Ca^{++}-containing medium evoked by depolarization (in % relative to release in normal medium): 1) synaptosomes were preincubated in medium without Ca^{++}; 2) the same, in the presence of 0.1 mM Ca^{++}.

0.1 mM Ca^{++}, 50 µl of a suspension of P_2 (50 µg protein in the sample) was added and the mixture incubated for 20 min at 37°C; 10 µl of incubation medium containing 2.5 µCi of ^{3}H-GABA and aminohydroxyacetic acid (from Sigma, USA) was added; the final concentration of the latter in the samples was 0.1 mM. After incubation for 2 min 0.5 ml of suspension was taken from each sample and transferred to a GF/C filter, and the incubation medium was separated by filtration under negative pressure. The residue of synpatosomes was washed 3 times (3 ml) with portions of incubation medium (37°C) not containing ^{3}H-GABA or Ca^{++}, after which the filter was transferred into another holder, connected to a peristaltic pump, and the residue of synaptosomes was superfused with 3 ml (30-40 sec) of normal or depolarizing medium (0.1 mM Ca^{++}) at 37°C. The filters were air-dried and transferred into flasks containing toluene scintillator of the following composition: PPO 5 g, POPOP 1 g, toluene up to 1 liter. Samples of 250 µl of medium were taken from the filtrates and transferred to flasks with scintillator containing, besides the components mentioned above, Triton X-100 (300 ml in 1 liter of scintillator). After measurement of radioactivity (using a counter from Intertechnique France) of the synaptosomes and filtrate the quantity of ^{3}H-GABA taken up by the synaptosomes, release of the transmitter into medium of normal composition (leakage) as a percentage of ^{3}H-GABA taken up, and voltage-dependent ^{3}H-GABA release in depolarizing medium compared with normal (in %) were calculated. The results were subjected to statistical anaysis by Student's t test and by the nonparametric tests of Wilcoxon (T) and Mann—Whitney (U).

EXPERIMENTAL RESULTS

PTH increased $^{45}Ca^{++}$ uptake by synaptosomes incubated in medium of normal composition ($p_t < 0.05$ for PTH in a concentration of 0.1 ng/ml and $p_t < 0.01$ for all other PTH concentrations). The action of PTH on $^{45}Ca^{++}$ uptake exhibited saturation; from 0.1 to 10 ng/ml (linearly between semilogarithmic coordinates (Fig. 1a)), and a further tenfold increase of the PTH concentration did not change the level of accumulated $^{45}Ca^{++}$: 0.84 ± 0.04 nmole Ca^{++}/mg protein with 10 ng PTH in 1 ml and 0.78 ± 0.04 nmole Ca^{++}/mg protein with 100 ng of PTH in 1 ml.

In the next series of experiments the time course of $^{45}Ca^{++}$ uptake by synaptosomes was investigated in normal and depolarizing medium. The highest rate of uptake was observed during 1 sec (Table 1), in agreement with data in the literature [9]. Depolarization of the synaptosomes (40 mM K^{+}) was accompanied by an increase in $^{45}Ca^{++}$ accumulation (for the 1st second $p_t < 0.02$). Incubation of the synaptosomes with 10 ng PTH in 1 ml changed the response of the synaptosomes to depolarization: during the 1st second the level of $^{45}Ca^{++}$ uptake in depolarizing medium remained virtually the same as in normal medium (Fig. 1b). By the 60th second the levels of $^{45}Ca^{++}$ accumulation in normal and depolarizing medium were approximately the same, and by the 30th minute the difference between the control and experiment had disappeared (Table 1).

TABLE 1. Effect of PTH (10 ng/ml) on $^{45}Ca^{++}$ Accumulation by Synaptosomes (M ± m)

Duration of incubation	K_0^+, mM	n	Uptake of $^{45}Ca^{++}$ by synaptosomes, nmoles/mg protein		
			control	PTH	
1 sec	5	10	0,63±0,01	0,83±0,04	$p_t<0,01$
	40	4	1,00±0,05	0,89±0,02	$p_u=0,05$
1 min	5	5	0,85±0,03	0,92±0,01	$p_T=0,05$
	40	4	1,33±0,11	1,25±0,06	—
30 min	5	5	1,02±0,04	1,18±0,07	$p_u<0,05$
	40	4	1,13±0,04	1,15±0,07	—

In the final series of experiments the effect of PTH on ^{3}H-GABA transport in synaptosomes (the P_2 fraction) was studied. No significant effect of PTH on transmitter uptake could be detected. Synaptosomes preincubated with PTH in medium without Ca^{++}, after transfer into normal Ca^{++}-containing medium (0.1 mM), released more ^{3}H-GABA than in the control, namely as much transmitter as the control synaptosomes in depolarizing medium (96.7 ± 4.7% of the ^{3}H-GABA release in depolarizing medium in the control).

The clearest difference in magnitude of the responses was obtained when ^{3}H-GABA release induced by depolarization was compared (Fig. 1c). Unlike in the control, synaptosomes preincubated with 10 ng of PTH in 1 ml in medium without Ca^{++} did not release ^{3}H-GABA in response to depolarization (Fig. 1c), $p_t < 0.02$. If the synaptosomes were preincubated with the same dose of PTH in Ca^{++}-containing medium, the difference in value of voltage-dependent release between the control and experiments was reduced (Fig. 1c).

The data given above are evidence that PTH can increase the inflow of Ca^{++} into resting nerve endings. Since PTH also has a similar effect on other targets [2, 4, 5, 10] we may be dealing with a certain common (ionophore [11], for example) mechanism of action of PTH on $^{45}Ca^{++}$ transport across the membrane.

Reduction of the fast, voltage-dependent uptake of $^{45}Ca^{++}$ into synaptosomes in the presence of PTH (Fig. 1c) may be due to inactivation of voltage-dependent Ca channels. PTH is known to induce depolarization of the membranes of certain cells [8], but preliminary depolarization of synaptosomal membranes inhibits the voltage-dependent inflow of $^{45}Ca^{++}$ during the 1st second irrespective of the nature of the depolarizer [9]. Depolarization does not affect the level of subsequent $^{45}Ca^{++}$ accumulation, which takes place more slowly [9]. It follows from the results that by the 30th minute synaptosomes incubated in a depolarizing medium accumulate about equal quantities of $^{45}Ca^{++}$ both in the control and in the experiment (Table 1). Under the influence of PTH the same compartment of the synaptosomes (cytoplasmic, probably) is filled with Ca^{++} under the influence of PTH as during membrane depolarization on nerve endings.

The results of the study of the effect of PTH on ^{3}H-GABA release agree with data on the effect of PTH on $^{45}Ca^{++}$ transport. Transmitter release is a function of the Ca^{++} concentration in the cytoplasm [7], and inflow of Ca^{++} into synaptosomes caused by PTH (Fig. 1a) leads to the more rapid release of ^{3}H-GABA into normal incubation medium. Meanwhile inhibition by PTH of ^{3}H-GABA release during depolarization of nerve endings (Fig. 1c) is most proabably due to a decrease in the rapid voltage-dependent inflow of $^{45}Ca^{++}$ into synaptosomes (Fig. 1b).

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EFFECT OF A SPIRAL PAIN SYNDROME ON REFLEX PAIN EVOKED BY NOCICEPTIVE THERMAL STIMULATION

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A difficult and neglected aspect of pain and analgesia is interaction between the nociceptive (NS) and antinociceptive (ANS) systems. It has now been shown that nociceptive impulsation at different levels of transmission is regulated through activation of ANS. Activation of the structures of ANS by electrical stimulation [5], by application of drugs to various zones of ANS [7], and by the creation of a generator of pathologically enhanced excitation (GPEE) in the key structures of this system [1] can depress pain of varied genesis. The mechanism of interaction between ANS and NS is not unidirectional. Although much research is currently in progress, mainly to study one aspect of this phenomenon, namely the regulatory effect of ANS on pain, there are isolated facts which indicate that pain of one type can contribute to manifestation of the antinociceptive effect against pain of another type. Cases when a pain syndrome has been abolished by additional nociceptive stimulation are known in clinical practice [4]. It has been shown that after nociceptive electrical stimulation of an animal's limbs the latent period of response tested by the hotplate method and the tail withdrawal test is increased [8]. These facts suggest that interaction between ANS and NS may lie at the basis of phenomenon such as activation or inactivation of ANS and regulation of its activity.

The aim of this investigation was to study the effect of a pain syndrome of spinal origin on reflex pain arising in rats to nociceptive thermal stimulation, and also to study neuronal activity of a key structure of ANS, the dorsal nucleus raphe, during development of the pain syndrome.

EXPERIMENTAL METHOD

Experiments were carried out on male and female Wister rats weighing 200-250 g. An experimental model of a spinal pain syndrome was created by forming a GPEE in the posterior horns of the lumbosacral segments of the spinal cord with the aid of penicillin [1]. An agar wafer measuring 8 × 3 × 1.5 mm, containing penicillin in a concentration of 25 U/mm^3, was applied to the dorsal surface of the lumbosacral segment of the spinal cord on the right side [2, 3]. The pain response to the thermal stimulation was studied by the hotplate test. The latent period (LP) of the complete pain response of the rat, placed on a hotplate (55 ± 0.5°C) was determined. Spontaneous unit activity in the dorsal nucleus raphe (AP 5.8-6.0 mm, LD 0.2-0.2 mm, H 5.5-6.5 mm according to the atlas [6]) was investigated extracellularly by means of glass microelectrodes filled with 2.5 M NaCl solution, before and during development of the pain syndrome. The results were subjected to statistical analysis by nonparametric tests and by Student's test.

EXPERIMENTAL RESULTS

Determination of LP of the animal's complete pain response to nociceptive thermal stimulation revealed a statistically significant increase in its duration both during development of the spinal pain syndrome induced by creation of the GPEE in the dorsal horns of the spinal

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