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MICROCHROMATOGRAPHIC STUDY OF HIPPOCAMPAL AREA CA3 PROTEINS DURING PROLONGED POST-TETANIC POTENTIATION IN SURVIVING SLICES

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The study of the molecular mechanisms of plasticity, the connection between electrical activity and metablism in neurons, and the dependence of protein synthesis in postsynaptic cells on the character of stimulation is one of the principal tasks in neurochemistry. The most adequate model for the analysis of these mechanisms is the phenomenon of prolonged post-tetanic potentiation (PPTP), developing in the hippocampus as a result of short high-frequency stimulation [2, 6, 7].

Most neurochemical studies of PPTP have been undertaken on whole hippocampal slices, consisting of a highly heterogeneous cell population. It is only by the use of micromethods that it has been possible to study α-motoneurons of the cat spinal cord under conditions of post-tetanic potentiation of monosynaptic reflexes, and to discover, by microdisk electrophoresis, changes in the spectrum of water-soluble proteins located in the zone of fast-migrating fractions, including brain-specific protein S-100 [1].

The study of synthesis of proteins and, in particular, of brain-specific proteins in a homogeneous population of postsynaptic cells during the development of PPTP is of undoubted interest. By using a system of synaptic connections (granule cells of the dentate gyrus — pyramidal cells of area CA3) incorporation of ^{3}H-leucine into water-soluble protein of this zone has been investigated during the development of PPTP (in surviving slices after stimulation of mossy fibers) [1].

EXPERMENTAL METHODS

Experiments were carried out by the method in [13] with certain modifications. Adult Wistar rats were decapitated under chloroform anesthesia, the hippocampus was removed, transverse slices 300-400 μ thick were cut, and placed in a thermostatically controlled chamber with a continuous flow of liquid (Yamamoto's medium), aerated with 95% O_2 and 5% CO_2.

Electrolytically sharpened bipolar tungsten electrodes with a distance of 50 to 300 between tips were used for stimulation. Tetanization was undertaken with a group of square pulses (50-300 Hz, duration 0.05-1 msec, 20-25 V) for 10-15 sec. Global activity was recorded by a glass electrode (diameter of tip 3-10 μ), filled with Yamamoto's medium.

After stimulation the slices were incubated for 1 h at 37°C in Yamamoto's salt solution containing 100 μCi/ml of [^{3}H]leucine (specific radioactivity 60 Ci/mole, from Amersham Corporation (England), and control "unpotentiated" slices were placed in a similar solution. At the end of incubation the slices were washed 3 times with ice-cold salt solution to remove extracellular label. Area CA3 was removed under a binocular loupe in cold extraction buffer (a 1% solution of Tween in Tris-phosphate buffer, pH 7.4), homogenized in the cold for 2 min with a mechanical tissue microhomogenizer at 2000 rpm, incubated for 12 h at 4°C, and centrifuged for 1 h at 1800g. The protein concentration in the supernatant was estimated from specific binding of protein with Coomassie blue G-250 [12].

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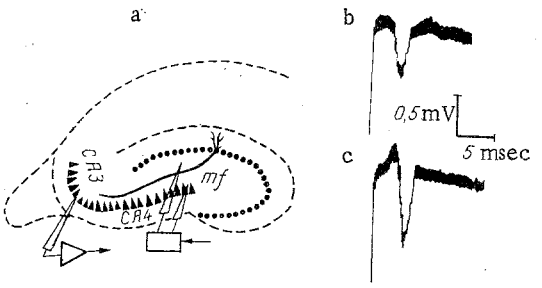

Fig. 1. Scheme for recording electrical activity from transverse slice through hippocampus (a), population spike of recording point before potentiation (b), and population spike 60 min after tetanization of mossy fibers (c).

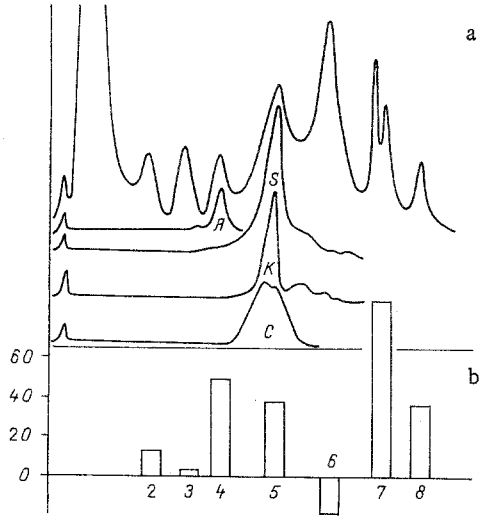

Fig. 2. Protein spectrum (chromatogram) of Tween extract of area CA3 (a). Abscissa: a) volume of eluate (in µl), b) of N-fraction; ordinate: a) optical density (in relative units), b) specific radioactivity (in %). Standard proteins: A) albumin, S) S-100, K) calmodulin, C) 14-3-2. Character of change in incorporation of ^{3}H-leucine during PPTP compared with control level in microchromatographic fraction (b).

Ion-exchange chromatography was carried out by the method in [10], modified by the writers for a microversion on DEAE-4I-cellulose (Whatman, England), described previously [4]. The protein mixture in an amount of 150-200 g was applied to the column. Elution began with "zero" Tris-phosphate buffer, pH 7.4, then by a stepwise NaCl gradient in 5 mM Tris-phosphate buffer: 0.025, 0.05, 0.075, 0.1, 0.2, 0.5, 0.75, 1.0, 1.5, and 2.0 M (150 µl of each dilution); the pH gradient from 7.4 to 4.0 began with the 0.2 M NaCl dilution. In the course of separation the optical density of the eluate was recorded continuously at 280 nm. Every 50 µl of elutate was collected in vials containing 10 ml of Bray's scintillation mixture (100 ml methanol, 60 g naphthalene, 4 g PPO, 0.2 g POPOP in 1 liter dioxan). Radioactivity was counted on an SL-30 scintillation counter (Intertechnique, France).

During statistical analysis of the results mean values of deviation of relative radioactivity compared with the control in each fraction was expressed as a percentage. The results were analyzed by Student's test, at the 95% level of significance.

EXPERIMENTAL RESULTS

During the first few minutes after tetanization of the mossy fibers initial depression of the response was observed in area CA3. Complete development of the potential to 1 h; under these circumstances an increase in amplitude of the second negative wave was observed, and in some cases the latent period of the population spike was shortened. The characteristic appearance of the response after development of PPTP is shown in Fig. 1.

In the course of analysis of the densitograms eight separate fractions, corresponding to a particular molarity of eluting buffer, were isolated. No significant changes in the relative content of each of them could be found during the development of PPTP. A typical densitogram of water- and Tween-soluble protein is shown in Fig. 2.

The rate of incorporation of labeled amino acid into protein, on the other hand, changed in a perfectly definite manner. Significant changes in the rate of incorporation compared with the control level were found in the acid part of the spectrum. Figure 2b shows that the rate of incorporation of label into the fraction eluted by from 0.1 to 0.2 M NaCl in Tris-phosphate buffer increased by 49% during potentiation. This fraction (Fig. 2b), as we know, consisted of proteins of albumin type (human serum albumin in eluted at the same molarity).

Incorportion of [^{3}H]leucine into proteins of fraction 5 (0.2-0.75 M NaCl) was increased by 37% compared with the control. Incidentally, with this molarity of NaCl low-molecular-weight acid brain-specific proteins were eluted in our microchromatographic system: S-100, calmodulin, and 14-3-2 (Fig. 2b). The results of neurochemical studies of these proteins showed that activation of their synthesis accompanied synaptic responses in the CNS [3, 5].

Some reduction (by 18%) in the level of incorporation of radioactivity compared with the control was observed in fraction 6 (0.75-1.0 M NaCl). The greatest increase in the rate of incorporation of precursor into proteins of Ca3 neurons in PPTP, by 87%, was found in fraction 7 (1.0-1.5 M NaCl), containing acid low-molecular-weight proteins and acid polypeptides (Fig. 2b).

Considerable changes in the rate of incorporation of [^{3}H]leucine into acid and, probably, brain-specific proteins and polypeptides were thus found, evidence that synthesis of precisely these proteins is activated in hippocampal pyramidal cells, during the development of PPTP. Potentiation of synapses in hippocampal slices is known to lead to an increase in activity of several enzymes and, correspondingly, to a long-term increase in phosphorylation of membrane proteins [9]. Calmodulin stimulates phosphorylation, whereas substances binding calmodulin inhibit the development of PPTP [8].

Together with data in the literature, the results of this investigation may indicate that activation of synthesis of acid tissue-specific proteins and neuropeptides, playing an active role in the regulation of Ca-dependent processes and connected with Ca-dependent phosphorylation, is an objective component of the development of post-tetanic potentiation in hippocampal neurons.

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