RAPID pH CHANGES ASSOCIATED WITH SYNAPTIC TRANSMISSION IN ISOLATED MAMMALIAN HIPPOCAMPAL SLICES

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When nerve cells of the mammalian CNS are in an active state changes take place in the ionic composition of their microenvironment. Data on changes in extracellular activity of K^+ and Ca^{++} ions [7, 8] have recently been supplemented by pH measurements. pH shifts caused by artificial stimulation of the cerebral cortex [7] and of hippocampal slices of rats [10] have been recorded by means of pH-sensitive microelectrodes. The temporal resolution of these measurements has been limited by the time constant of the pH-sensitive microelectrode, and amounted to several seconds.

However, it has recently been shown that the pH of the contents of synaptic vesicles isolated from the brain is strongly shifted toward the acid side [2, 3, 5]. It is therefore difficult to rule out that the functional activity of the brain, connected with synaptic transmission, may be accompanied by rapid pH changes over periods of time measured in milliseconds.

To test this hypothesis the writers have used an optical indicator for rapid recording of possible pH changes in hippocampal slices isolated from the brain of month-old rats.

METHODS

The technique of the work with slices was the same as that described previously [6]. The thickness of the slices was 200-400 μ. After isolation the slices were introduced into a working chamber in which a solution (pH 7.35), containing (in mM): NaCl 134, KCl 5, KH_2PO_4 1.25, $MgCl_2$ 2, $CaCl_2$ 1, $NaHCO_3$ 16, glucose 10, circulated continuously. The solution was saturated beforehand with a gas mixture containing 95% O_2 and 5% CO_2. The experiments were carried out at room temperature. A method of submerged slices, with their under surface in contact with the glass bottom of the working chamber, was used. Under those conditions the tissue received a satisfactory oxygen supply [6]. A pair of nichrome wires, 30 μ in diameter, glued together, tapering conically at their end, and insulated over their whole length except at the tip, was used for stimulation. Stimulating electrodes were introduced into the slice to a depth of about 200 μ. Electrical activity was recorded by means of an extracellular glass microelectrode filled with 0.25 M KCl.

Phenol red was used as the optical pH indicator and was added to the working solution in a concentration of 200 μM. In this concentration the dye had no toxic action on the slice. The nonprotonated form of phenol red has an absorption line with a maximum at 560 nm, whereas the protonated form has a line with a maximum at 440 nm. The transition interval lies between pH 6.4 and 8.2. Control experiments showed that the optical properties of the indicator remain virtually unchanged with an increase in the Ca^{++} concentration in the working solution to 10 mM, the Mg^{++} concentration to 10 mM, and the K^+ concentration to 50 mM. With the use of a monolayer culture of neuroblastoma Cl300 cells, intracellular penetration of the dye in the acting concentration was tested. This control test gave a negative result.

A halogen-filled 150 W incandescent lamp was used as the source of light. Light from the lamp passed through a heat filter, through an absorption filter with transmission maxi-

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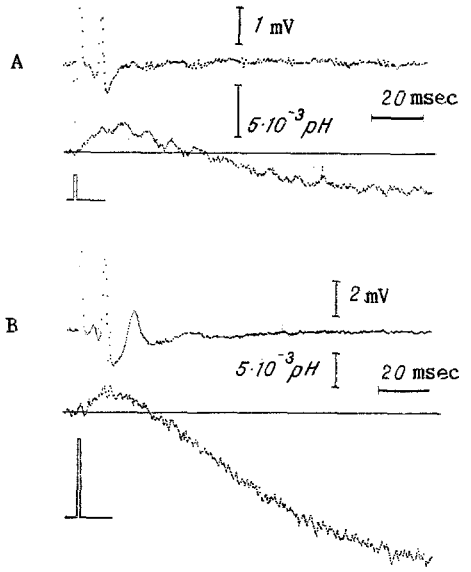

Fig. 1

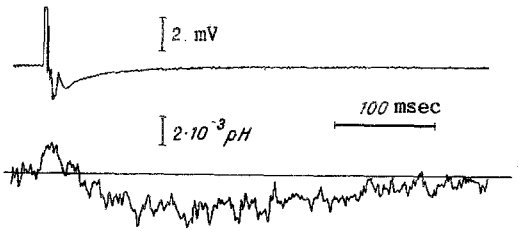

Fig. 2

Fig. 1. Rapid pH changes in brain slices during their electrical stimulation. A: top trace, electrical activity derived from neurons of dentate fascia during stimulation of perforant path (here and in Figs. 2 and 3 duration of stimulating pulse 200 μsec, period 2 sec); bottom trace, synchronized optical recording of pH changes in region of dendrites and bodies of granule cells, 500 cumulation cycles. Deviation of signal upward from zero line corresponds to pH shift toward acid side; B) the same, for hippocampal pyramidal neurons and stimulation of afferents in stratum radiatum.

Fig. 2. Development of second phage of pH change (alkalification) in a longer time cut (up to 1 sec). Above, electrical response of hippocampal pyramidal neurons; below, optical response.

mum at 560 nm, and was aimed at the chosen region by means of a short conical glass light guide (diameter of tip 200 μ), which pressed firmly against the upper surface of the slice. The light flux, after passing through the slice, was projected by means of a 100× objective of an inverted microscope (oil immersion, numerical aperture 1.3) on to the inlet aperture of a photoelectronic multiplier (PEM). To abolish the effect of slow drift of the signal from the output of the PEM a digital zero correction system was used. This system worked as follows. The signal from the PEM was led to the positive input of a differential amplifier, and a signal from the output of a digital-to-analog converter (DAC) was applied simultaneously to its negative input. The signal from the output of the differential amplifier was led to an analog-to-digital converter of the computer controlling the experiment. Before delivery of the stimulating factor a computer exhibited a signal at the output of the DAC equal to the signal at the output of the PEM. Under these circumstances the voltage at the output of the differential amplifier was zero. The computer then exhibited the stimulating factor and recorded the onset of the change in the optical signal. To increase the signal to noise ratio, the method of synchronized cumulation, with the number of cumulation ranging from 200 to 1000, was used. The values of the recorded pH changes were calibrated by the following procedure. After the end of the experiment the working chamber was filled consecutively with two solutions with pH 7.4 and 6.4. The composition of the solutions was similar to the composition of the working solution with the indicator, except that Tris-HCl (10 mM, pH 7.4) and MES (10 mM, pH 6.4) was used as the pH buffer instead of $NaHCO_3$—CO_2. After 10 min (necessary for diffusion of the solutions into the extracellular space of the slice) the change in light transmission by the slice was recorded in these two solutions. Changes in light transmission of the slide recorded in the experiment were subsequently divided by the calibration values. Thus optically recorded pH changes were reduced to pH units, averaged for the whole volume of the slice through which passed the light flux to be recorded.

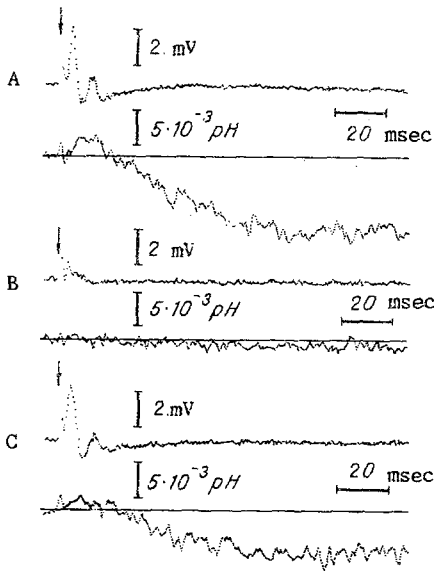

Fig. 3. Blocking of synaptic transmission in slice by Mg^{++} ions. A: top trace, electrical activity recorded from hippocampal pyramidal cells, bottom trace, corresponding pH change; B) synaptic transmission blocked by Mg^{++} ions (top trace), which led to depression of optical response (bottom trace); C) restoration of synaptic transmission (top trace) and pH changes (bottom trace) after 20 min of rinsing in normal solution containing dye.

In one series of experiments the perforant path of the hippocampus was stimulated, the electrical signal was recorded from the zone of the bodies of the granule cells of the dentate gyrus, whereas the optical signal was recorded from the zone of the bodies and dendrites of these neurons. In another series of experiments axons forming synapses on dendrites of the hippocampal pyramidal neurons were stimulated. The electrical signal was recorded from the zone of the bodies, the optical signal from the zone of the dendrites of the pyramidal neurons.

RESULTS

One trace of an electrical response from pyramidal neurons to a series of stimuli 200 μsec in duration, with a period of 2 sec, is shown in Fig. 1A. The negative change of potential corresponds to the extracellularly recorded population EPSP, the positive change to the population action potential of the pyramidal neurons evoked by synaptic transmission. The trace recorded during synchronized optical recording of the pH changes in the zone of the dendrites of these neurons is shown in Fig. 1B. Stimulation causes rapid acidification of the extracellular medium in the zone of the synapses by a pH value of about 0.002, averaged for the whole volume of the isolated zone, and this is followed by slower alkalification, with a pH value of 0.005-0.02 (Fig. 2). Similar pH changes were also recorded in the zone of the dendrites of neurons of the dentate gyrus. Blocking of synaptic transmission by addition of 10 mM Mg^{++} to the working solution was found to depress these changes completely and reversibly both in neurons of the dentate gyrus and in the pyramidal neurons (Fig. 3). Antidromically induced excitation of dentate gyrus neurons (stimulation of the axons of these neurons) likewise did not lead to any reliably recordable changes of pH, during up to 1000 cumulations. These facts suggest that the recorded pH changes are associated with synaptic transmission in hippocampal slices.

The rapid initial pH shift toward the acid side which we found develops in the course of a few milliseconds. It could not be found in investigations using pH-sensitive microelectrodes. As regards the subsequent, slower responses, in [7] artificial stimulation of the rat cerebral cortex with a frequency of 10-30 Hz led to a pH change of the extracellular

medium toward the alkaline side by about 0.05 pH unit in the course of a few seconds. This was followed by a longer pH shift toward the acid side by 0.2 unit. The longer pH changes which we recorded toward the alkaline side may be similar in nature to those described in [7].

It can be tentatively suggested that the rapidly developing pH changes toward the acid side cannot spread throughout the volume of the slice. In that case local pH changes toward the acid side are substantially above the average values for the whole volume. If such local pH changes are associated with release of the acid contents of the synaptic vesicles [2, 3], they ought to be strongly expressed above all in the synaptic spaces and ought to influence the work of the membrane mechanisms of synaptic transmission. The possibility cannot be ruled out that this is the cause of differences between synaptic and extrasynaptic chemoreceptors in relation to the same mediators [4, 9].

Recently a receptor of protons [1], activated at pH values below 7.0 and producing a depolarizing, desensitizes response, has been discovered in mammalian brain neurons. It has been shown that the degree of spread of proton sensitivity in the brain is very wide. It can be postulated that secretion of H^+ by some cells and reception of H^+ by other cells is yet another type of interneuronal communication.

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CHANGES IN GROUP BEHAVIOR IN CALLOSOTOMIZED RHESUS MONKEYS

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Extensive experimental data on various aspects of the study of the corpus callosum, the most important commissure of the brain, has now been obtained [1, 3-5]. In particular, morphologic and physiological investigations have demonstrated the complex structural and polyfunctional organization of the callosal system in higher mammals and man [6, 11, 13], and its essential role in sensory functions of the brain [9, 10, 13]. The results of behavioral experiments on carnivores and primates after division of the corpul callosum have demonstrated conclusively the role of this commissure in interhemispheric transmission of perceptual skills [7, 12, 14]. Clinical observations on callosotomized patients have revealed a disturbance of complex forms of behavior and changes in mental activity [8, 15]. This suggests that disturbance of communicative forms of behavior take place after division

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