Effects of Lowered pH of the Extracellular Solution on Non-Transsynaptic Component of Evoked Potentials in the Rat's in Vitro Cerebral Cortical Slices

YASUO TAKAHASHI and TETSURO OGAWA

Department of Physiology, Akita University School of Medicine, Akita 010

TAKAHASHI, Y. and OGAWA, T. *Effects of Lowered pH of the Extracellular Solution on Non-Transsynaptic Component of Evoked Potentials in the Rat's in Vitro Cerebral Cortical Slices.* Tohoku J. exp. Med., 1987, **152** (1), 63-65 — The effects of lowered extracellular pH on non-transsynaptic component of field potentials evoked by electric stimulation of the white matter which were recorded in layer IV of the occipital cerebral cortex were studied with the rat's in vitro brain slices. At a range of pH between 6.2 and 7.2, the amplitude of the non-transsynaptic potentials was decreased with a complete recovery to the initial normal level when the pH was restored to 7.4. With a decrease in amplitude, the latency of evoked potentials was also delayed a little in the acidic media. — extracellular pH; non-transsynaptic neuronal activity

It has been reported that a decrease in extracellular pH suppresses synaptic transmission in the mammalian central nervous system (Nachsen and Blaustein 1979), but its effects on non-transsynaptic activity have not been investigated yet. The present study was designed to approach this problem. Cerebral cortical slices, 0.5 mm thick, were prepared (Kato and Ogawa 1981) from rats (200-300 g) anesthetized with pentobarbital sodium (0.06 mg/g i.p.). They were preincubated for 1 hr in a standard medium (NaCl 124.0, KCl 5.0, NaH₂PO₄ 1.2, MgSO₄ 1.0, CaCl₂ 3.0, NaHCO₃ 25.0, glucose 10.0 in mM) at pH 7.4 and 25°C. During the recording session, one of slices was transferred to an experimental chamber which was continuously perfused with a medium whose pH and ionic composition were varied depending on experimental conditions. In the chamber, the slice was fixed at the bottom with a bipolar stimulating electrode pressed on the white matter. Evoked potentials were recorded in layer IV of the occipital cortex with a tip bared insulated needle electrode. In the present experiments, each medium was saturated with 95% O₂ and 5% CO₂, the pH of which was adjusted by changing the concentration of HCO₃⁻ through replacement with an equimolar sodium

Fig. 1. Effects of lowered extracellular pH on a non-transsynaptic component of evoked potentials. Top row: Evoked potentials in a standard medium (pH 7.4, 3.0 mM Ca^{2+}) consisting of non-transsynaptic (N1) and transsynaptic (N2-P2) components (left) were changed into a monophasic response of only N1 within 20 min when the standard bathing medium was replaced with a low Ca^{2+} and high Mg^{2+} solution. Middle row: The N1 component dwindled in an acidified medium (pH 6.2). Bottom row: In the standard medium all components were recovered.

isethionate. The pH of the medium in the chamber was occasionally measured with a fine tipped pH electrode (0.01 unit in accuracy, HORIBA 6029-10T, HORIBA, Kyoto) and its temperature was kept at 32°C.

Fig. 1 summarizes the results. In the standard medium, the evoked potential consisted of a fast negative spike (N1) and a subsequent slow negative-positive sequence (N2-P2) of potential. When the medium was replaced with a low Ca^{2+} (0.1 mM) and high Mg^{2+} (5.0 mM) medium at pH 7.4 to block synaptic transmission, the N2-P2 component was almost abolished in about 20 min, leaving N1 almost unaffected. Thus N1 proved to be a non-transsynaptic response. Then the pH of the medium was changed from 7.4 to 6.2 to examine the effects of lowered extracellular pH on N1 exclusively. In the acidic medium, the amplitude of N1 was gradually decreased, and in 20 min, it was reduced to 25% of the normal level. In addition, the latency of the negative peak of N1 was delayed from 2.5 to 3.0 msec. After 20 min, the medium was replaced again with the standard medium, in which the responses were almost restored to the normal in about 20 min. The depressive effects of an acidic medium at pH up to 6.2 were

reversible. Since the depressive effects were almost abolished when the pH was raised to 7.4 by adding NaHCO_3 to the bathing medium, it may be concluded that only a decrease in pH but not isethionate is responsible for the depressive effects.

In the mammalian central nervous system, little has been investigated about interaction between hydrogen ions and ionic channels of the non-synaptic neuronal membrane. In frog nerves, however, low external pH has been reported to reduce the permeability of neuronal membrane to sodium (Woodhull 1973), which seems to be relevant to the present findings, though further experiments will be required. The present result seems to be helpful for elucidation of the mechanisms of neural disorders associated with a decrease in pH of cerebrospinal fluid.

References

- 1) Kato, H. & Ogawa, T. (1981) A technique for preparing in vitro slices of cat's visual cortex for electrophysiological experiments. *J. Neurosci. Meth.*, **4**, 33-38.
  - 2) Nachsen, D.A. & Blaustein, M.P. (1979) Regulation of nerve terminal calcium selectivity by a weak acid site. *Biophys. J.*, **26**, 329-334.
  - 3) Woodhull, A.M. (1973) Ionic blockage of sodium channels in nerve. *J. gen. Physiol.*, **61**, 687-708.
-