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Excitatory amino acids and intracellular pH in motoneurons of the isolated frog spinal cord

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Double-barrelled pH-sensitive micro-electrodes were used to measure changes of intracellular and extracellular pH in and around motoneurons of the isolated frog spinal cord during application of excitatory amino acids. It was found that N-methyl-D-aspartate, quisqualate and kainate produced a concentration-dependent intracellular acidification. Extracellularly, triphasic pH changes (acid-alkaline-acid going pH transients) were observed during the action of these amino acids. The possible significance of such pH changes for the physiological and pathophysiological effects of excitatory amino acids are discussed.

Amongst the acidic amino acid receptors in the nervous system the N-methyl-D-aspartate (NMDA)-preferring subtype has obtained particular interest due to its possible involvement in phenomena such as long-term potentiation, epilepsy and neurodegeneration (see ref. 7 for a review). It has been described that the NMDA receptor-activated channel is highly permeable for Ca^{2+}, [5, 10, 12] in contrast to channels activated by quisqualate and kainate. Consequently, the rise in intracellular Ca^{2+} may serve as a mediator for secondary changes of neuronal excitability. Another possible factor, however, by which NMDA receptors could alter neuronal excitability may be a change in extra- and/or intracellular pH. To our knowledge, this possibility has not yet been explored. Therefore, we have investigated how acidic amino acids influence the extracellular (pH_e) and intracellular pH (pH_i) around and in motoneurons of the isolated frog spinal cord. Glutamate-induced changes of other intracellular ion activities in frog motoneurons have been already described [3].

Experiments were done on frog spinal cords [8, 9]. After decapitation, a ventral laminectomy was performed in cooled Ringer solution. The spinal cord, including dorsal and ventral roots of the lumbar segments, was removed and placed in a recording chamber (volume 1.5 ml), which was continuously superfused with Ringer solution by means of a roller pump (2.5 ml/min). The Ringer solution contained (mM): NaCl 98.0, KCl 3.6, $CaCl_2$ 2.0, $NaHCO_3$ 12.0, glucose 10. Note the absence of Mg^{2+} in the Ringer solution. Addition of 1 mM Mg^{2+}, in accordance with Ault

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et al. [2], strongly inhibited the action of NMDA. The pH of the solution was continuously monitored and adjusted to 7.2–7.5 by varying the proportion of O_2 and CO_2 in the gassing mixture. The temperature of the Ringer solution was adjusted to 18–21°C. Dorsal and ventral roots of the lumbar segments were drawn into a pair of glass suction electrodes which allowed stimulation and recording of compound potentials. All drugs were applied via the bathing solution (NMDA, quisqualic acid, and kainic acid; Sigma, Munich, F.R.G.; D-2-amino-5-phosphonovalerate (D-APV); Tocris Chemicals, Buckhurst Hill, U.K.). The general methods for the construction and calibration of double barrelled ion-sensitive micro-electrodes have been described previously [8, 9]. pH electrodes had a tip diameter of about 1 μm. The ion-sensitive barrel contained the pH-sensitive cocktail (Fluka 82500 [1]); the backfilling solution was composed of (mM): KH_2Po_4 40, NaOH 23, NaCl 15 (pH 7.0). The reference barrel was filled with 1 M magnesium acetate solution.

The results of this study are based on recordings from 15 motoneurons (11 spinal cord preparations). The recording periods usually lasted between 1 and 6 h. The mean membrane potential of these cells was -67.8 ± 6.7 mV (mean $\pm$ S.D.). Fig. 1 shows NMDA-induced changes of the extracellular and intracellular pH (pH_e, pH_i) in and around a motoneuron of the isolated frog spinal cord. In the extracellular space a triphasic pH change was observed (Fig. 1). During the first minute after the start of the NMDA superfusion a small acidification was seen. Later, an alkaline

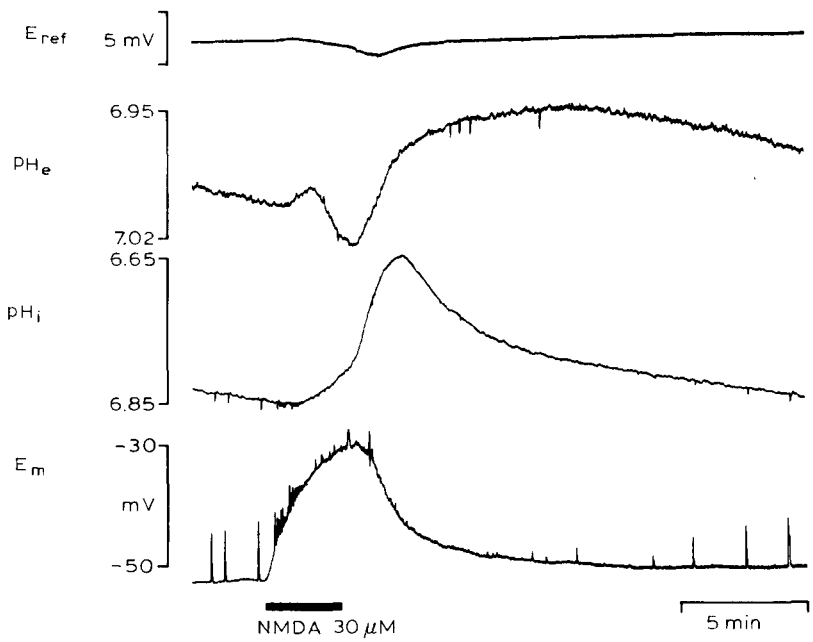

Fig. 1. Changes in pH_e and pH_i around and in a motoneuron of an isolated frog spinal cord during the application of NMDA into the bathing solution. In this experiment two double-barrelled pH-sensitive micro-electrodes were used. One of these electrodes recorded pH_e and the local potential (E_{ref}) in the ventral horn of the spinal cord; the other one had been impaled into a motoneuron to measure pH_i and the membrane potential (E_m) of the motoneuron.

going pH transient followed by a long lasting, final acidification was observed. Such an alkaline-acid sequence of activity-related pH changes has been previously described during stimulation of mammalian central [11, 17] and peripheral [6] nervous tissue. The initial acidification has also been observed in the mammalian nervous system. It required, however, extreme conditions like spreading depression and cerebral ischemia [13]. In general, NMDA-induced changes of extracellular pH were relatively small (0.04 ± 0.02 pH units acidification; $n=9$, mean $\pm$ S.D.). This is in contrast to the intracellular space, where considerable acidifications were seen. Fig. 1 illustrates an example of such experiments. Here, the pH_i was measured, parallel to the recording of the pH_e, as illustrated in the upper part of Fig. 1. One can see that a pure acidification is visible in a motoneuron during the action of NMDA. In this case, the membrane depolarization of about 20 mV was accompanied by an acidification of about 0.2 pH units. Statistically, a mean intracellular acidification of 0.21 ± 0.08 pH units was observed; mean $\pm$ S.D. These data are based on 15 motoneurons on which 30 μM NMDA were applied 22 times for 1.5–3 min. Repetitive stimulation (20 Hz, 20 s) on a dorsal root also produced triphasic extracellular pH changes and a pure intracellular acidification (not illustrated).

In a series of experiments we tried to define the mechanism underlying the intracel-

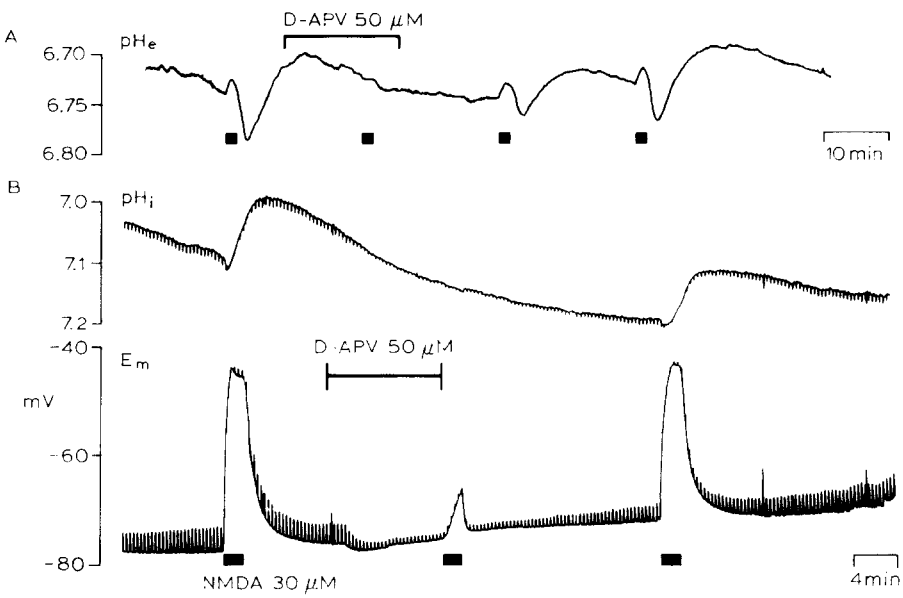

Fig. 2. Effects of D-APV on NMDA-induced changes of pH_e in the ventral horn and pH_i of a motoneuron. The uppermost trace shows an experiment with an extracellularly positioned pH-sensitive micro-electrode. After a control application, NMDA was applied in the presence of the NMDA receptor antagonist D-APV. The presence of this drug completely prevented the changes in pH_e usually seen during the action of NMDA. The two other traces show a similar experiment with an intracellularly positioned pH-sensitive micro-electrode, from a different spinal cord. Note that D-APV also completely inhibited the intracellular acidification usually seen during the action of NMDA. The vertical inflections on the E_m trace are postsynaptic potentials induced by regular, single stimuli on the dorsal roots. They are truncated by the frequency response of the pen recorder.

lular acidification during the action of acidic amino acids. First, we wanted to know whether the presence of the amino acids in the spinal cord itself can produce pH changes. Therefore, NMDA was applied during continuous superfusion of the spinal cord with D-APV, an NMDA receptor antagonist. Experiments such as the one illustrated in Fig. 2 clearly showed that D-APV blocked both the membrane depolarization and NMDA-induced changes of extra- and intracellular pH. This indicates that at least the binding of NMDA to the receptor, and not the presence of the acidic amino acids alone, is responsible for the pH transients. However, at the moment we do not know whether the changes in the H^+ activity are mainly a consequence of ion movements leading to alterations in the intra- and extracellular strong ion difference [16] or due to the metabolic production of acid. This question will be explored in future experiments.

There are differences in the physiological importance of the acidic amino receptor subtypes. For example, only the NMDA-preferring sub-type may participate in phenomena like long-term potentiation [4] and kindling-induced epilepsy [15]. However, both NMDA and kainic acid can produce neuronal cell necrosis [14]. In order to further analyze whether changes of pH might underly the above-mentioned changes in neuronal excitability, we have compared the changes of pH produced by these different glutamate receptor agonists. The results are illustrated in Fig. 3. We have found that NMDA, quisqualate, and also kainic acid produced similar changes of

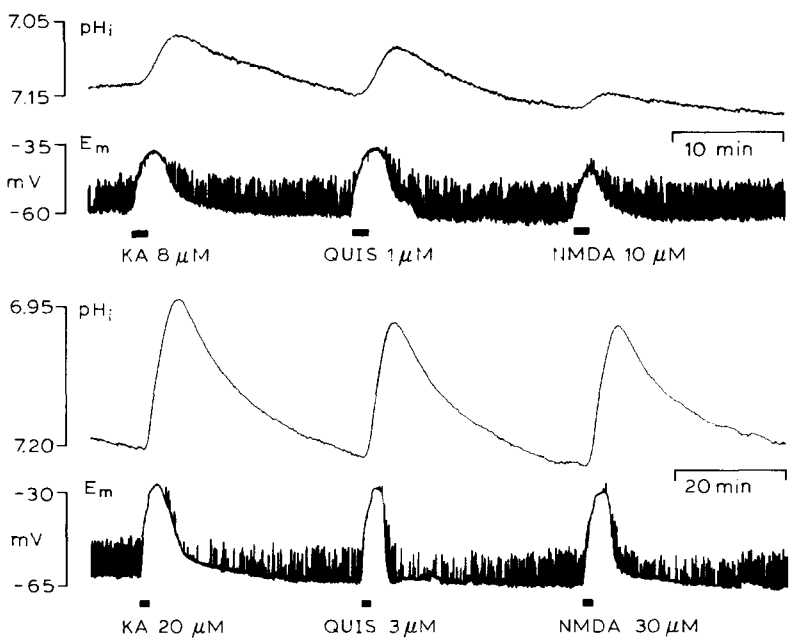

Fig. 3. Effects of different glutamate agonists on pH_i of a spinal motoneuron. The figure shows a continuous record with double-barrelled pH-sensitive microelectrode from a single motoneuron. The glutamate agonists NMDA, quisqualic (QUIS) and kainic acid (KA) were applied at two concentrations each (application time 90 s). The vertical inflections on the E_m trace are due to irregular, spontaneously appearing synaptic potentials.

intracellular pH. This conclusion is based on observations with relatively low concentrations of these amino acids (upper part of Fig. 3) as well as high concentrations (lower part of Fig. 3). This uniform effect of different glutamate receptor agonists on pH_i is different from their effects on intracellular Ca^{2+}. Kainic acid was much less effective in triggering increases in intracellular free Ca^{2+} than NMDA [12]. Therefore, our data do not support the idea that pH changes may contribute to LTP or kindling-induced epilepsy. However, intracellular acidosis may well be a factor contributing to the cytotoxic effects of these amino acids [14].

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