Life Sciences, Vol. 61, No. 5, pp. 523-535, 1997

0024-3205/97 $17.00 + .00

ELSEVIER

PII S0024-3205(97)00412-8

EFFECT OF ACIDOTIC CHALLENGES ON LOCAL DEPOLARIZATIONS EVOKED BY *N*-METHYL-D-ASPARTATE IN THE RAT STRIATUM

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(Received in final form May 2, 1997)

Summary

We have examined how various challenges to brain acid-base homeostasis, resulting in extracellular acidosis, alter *N*-methyl-D-aspartate (NMDA)-evoked depolarizations *in vivo*. Repeated stimuli were produced by perfusion of 200 μM NMDA for 2 min through a microdialysis probe implanted into the striatum of halothane anesthetized rats. Hypercapnia reduced NMDA-evoked responses in a concentration-dependent manner, with 7.5 and 15 % CO_2 in the breathing mixture reducing the depolarization amplitude to 74 % and 64 % of that of the initial stimuli, respectively. Application of 50 mM NH_4^+ progressively reduced dialysate pH, and a further acidification was observed when NH_4^+ was discontinued. Perfusion of NMDA after NH_4^+ application evoked smaller depolarizations (56 % of the corresponding control, 5 min after NH_4^+ removal), and this effect persisted for over 1 h. Perfusion of acidic ACSF did not alter the amplitude of NMDA-evoked depolarization, despite changes in dialysate pH confirming that exchange/buffering of acid equivalents took place between the perfusion medium and the surrounding tissue. This negative result probably reflected the remarkable capacity of the brain to buffer H^+. Together, these results demonstrate that extracellular acidosis, such as that associated with excessive neuronal activation or ischemia, inhibits NMDA-evoked responses *in vivo*.

Key Words: **NMDA receptors, acidosis, extracellular pH, hypercapnia, extracellular field (DC) potential, ischemia, microdialysis**

Electrophysiological studies with cultured mammalian neurons have shown that moderate acidity of the incubation medium reduces *N*-methyl-D-aspartate (NMDA) receptor-activated currents (17,32,51). Proton inhibition of NMDA receptor activation is voltage-insensitive and non-competitive with respect to the binding sites for NMDA/glutamate and glycine, and the site of H^+ action is near the external membrane face which is consistent with the predominant sensitivity of NMDA-evoked responses to changes in extracellular pH (pH_e). As the H^+ concentration producing half-maximal inhibition corresponds to near physiological pH (51), NMDA receptor function is likely to be altered by pH_e changes subsequent to both physiological acid-base regulation and pathological conditions (e.g. stroke or seizure activity).

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Analogies between the effects of alterations in tissue pH and dizocilpine (MK-801) on cellular Ca^{2+} uptake during complete cerebral ischaemia in rats have suggested that low pH may reduce Ca^{2+} influx via inhibition of NMDA receptor activation in this situation (23). However, there has so far been no demonstration of the effects of extracellular acidification on NMDA receptor function in the intact, functional brain. In addition, the effects of acidosis or pCO_2 changes on brain slices are opposite to those expected from the results obtained with cultured neurons outlined above: (i) In slices made anoxic by removal of O_2 supply or cyanide poisoning, low pH enhanced rather than attenuated the rise in the free cytosolic calcium concentration (41); and (ii) pharmacological blockade of NMDA receptor had no influence on the effects of CO_2 on the amplitude of field excitatory postsynaptic potentials (EPSPs) in rat hippocampal slices (26).

The purpose of this study was to examine how various challenges to brain acid-base homeostasis *in vivo*, resulting in extracellular acidosis, alter NMDA-evoked depolarization. Microdialysis probes incorporating a recording electrode (38,40) were used to induce repeated, local depolarizations by perfusion of NMDA through the probe. Challenges to the brain acid-base regulation were produced by hypercapnia, or by perfusion of acid media or NH_4^+ through the probe. Ammonium ions cause transient intracellular alkalinization subsequent to influx and protonation of NH_3, followed by marked acidosis when NH_4^+ perfusion is discontinued (49).

Methods

Animal preparation and intracerebral microdialysis

Male Sprague-Dawley rats (260-360 g; Bantin & Kingman, Grimson, Hull, U.K.) were used, with food and water available ad libitum. All animal procedures were in accordance with the British Home Office guidelines, and specifically licensed under the Animals (Scientific Procedures) Act 1986. Anaesthesia was induced and maintained with halothane (2 % and 1-1.5 %, respectively) in O_2:N_2O (30:70) (1:1 for hypercapnia group) through a mask or endotracheal tube. To minimize any possible interference of halothane anaesthesia with the processes under study (9,28), once the surgical procedure had been completed, the depth of anaesthesia was carefully controlled by monitoring electroencephalogram (EEG) and arterial blood pressure, and the concentration of halothane in the breathing mixture kept to a minimum (0.8 to 1.2 %). A femoral artery was catheterized for continuous monitoring of mean arterial blood pressure (MABP) and repeated measurements of blood gases and pH. A femoral vein was cannulated for administration of tubocurarine when animals were artificially ventilated (hypercapnia group), and rapid injection of 1 ml air to provoke cardiac arrest and terminate the experimental procedure. Body temperature was kept at 37-38°C throughout.

Concentric microdialysis probes incorporating a recording electrode in the inlet tube (microdialysis electrode ME-H2, Applied Neuroscience Ltd., London, U.K.) (38,40) were implanted in the dorsolateral striatum (coordinates: 0.8 mm anterior to bregma, 3 mm lateral and 6 mm deep from the cortical surface) (43). Unless otherwise stated, microdialysis probes were perfused with artificial cerebrospinal fluid (ACSF) (composition in mM: NaCl 125, KCl 2.5, $MgCl_2$ 1.18, $CaCl_2$ 1.26; pH 7.3 adjusted with 1 M NaOH, unbuffered) at 1 μl min^{-1} with a syringe pump (CMA/100, CMA/Microdialysis, Stockholm). A 120-min stabilisation/control period preceded the procedures described below.

Recording of extracellular direct current (DC) potential and EEG

The DC potential and EEG were derived from the potential between the electrode built into the probe and a chlorided silver reference electrode placed under the scalp (40). We had previously showed that the extracellular DC potential recorded with this electrode configuration is identical

to that recorded with a conventional glass capillary electrode whose tip is positionned immediately adjacent to the dialysis fibre (38). We had also demonstrated that perfusion of NMDA through the microdialysis probe evoked a concentration-dependent negative shift of the DC potential, which is blocked by NMDA antagonists (40). The recorded potential was first amplified (x 10) with a high-impedance input pre-amplifier (NL834, Neurolog System, Digitimer Ltd., Welwyn Garden City, U.K.). The alternating current component in the 1-30 Hz window, amplified 6,000 - 8,000 times, provided EEG, and the DC component, the DC potential. A dedicated application program written in ASYST (Keithley Asyst, Reading, U.K.) allowed these parameters to be continuously acquired, displayed, and stored on an IBM PC compatible computer equipped with an analogue/digital converter (DAS8, Metrabyte Corp., Taunton, MA) (36).

NMDA-induced depolarizations

To avoid NMDA-induced spreading depression and possible neurotoxic effects, brief, submaximal depolarizations were produced by switching the perfusion medium from ACSF to ACSF containing 200 μM NMDA for 2 min (40), using a liquid switch (CMA/110, CMA/Microdialysis). Unless otherwise stated, each NMDA-challenge was followed by 20 min of recovery. The first 1 or 2 NMDA-stimuli, always carried out under control conditions (i.e. physiological arterial blood gases; ACSF pH 7.3), were used as individual control.

Challenges to acid-base regulation of the brain

Three groups were subjected to different stimuli to challenge acid-base regulation:

(i) Hypercapnia. Animals were relaxed with tubocurarine (1 mg/kg i.v., repeated every 1-2 hour) and ventilated mechanically (75 cycle/min) with appropriate stroke volume to maintain normocapnia with N_2O:O_2 (1:1). Two control NMDA challenges were performed during normocapnia. Ten min after the 2nd NMDA-application 7.5 % CO_2 was added for 15 min to the control breathing mixture, followed by 29 min of recovery (normocapnia). NMDA-induced depolarizations were evoked 10 min into 7.5 % CO_2 hypercapnia, and 17 min into the following normocapnia (Fig. 1). This sequence was repeated with 15 % CO_2. Arterial blood gases and pH were measured immediately after each NMDA-application.

(ii) Perfusion of 10 mM phosphate-buffered acid media. After the stabilization/control period, the perfusion medium was switched to 10 mM phosphate-buffered ACSF (pH 7.3), and one NMDA-stimulus superimposed onto this medium (control NMDA-response) (Fig. 3). Ten minutes within the recovery period following this first NMDA-stimulus, the perfusion medium was switched to buffered ACSF pH 6.5, and after 10 min a second NMDA-stimulus applied (with NMDA dissolved in the same phosphate-buffered ACSF). This sequence was repeated with ACSF buffered at pH 5.8, and again at pH 7.3. Changes in dialysate pH were measured by recording the dialysate pH (pH_d), as it emerged from the implanted microdialysis probe. We used a needle type pH electrode (MEPH1, World Precision Instruments; Sarasota, FL) and a reference electrode (Type E255; 0.8 mm o.d.; Clark Electromedical Instruments, Reading, U.K.) inserted into a glass capillary filled with saline. The drift of the pH recording system did not allow us to obtain reliable absolute pH measurements, but changes in dialysate pH could be readily measured.

(iii) NH_4^+-induced local changes in brain tissue acid-base. Transient application of NH_4^+ through the microdialysis probe reproduced *in vivo* the classical method used to induce intracellular pH (pH_i) changes *in vitro* (7,49). Exposure of single cells to NH_4Cl causes transient intracellular alkalinization subsequent to influx and protonation of NH_3, whereas removal of NH_4Cl results in

marked intracellular acidosis due to rapid efflux of NH_3. Preliminary experiments, in which pH_d was recorded as described above, showed that application of 50 mM NH_4^+ through the microdialysis probe was appropriate to locally alter acid-base balance *in vivo*. This concentration is 5-fold that previously used *in vitro* (49) but microdialysis only delivers a fraction of the perfused chemicals to the surrounding tissue (5). Accordingly, immediately after the end of the 2nd control NMDA-evoked depolarization (Fig. 5), control ACSF was switched for 20 min to a medium containing 50 mM NH_4^+ with lowered Na^+ (75 mM) to preserve isotonicity. Five min after switching back to control ACSF (i.e., NH_4^+-removal induced acidosis), 4 additional NMDA-stimuli were produced, each of them followed by 20-min of recovery.

Two control groups ($n = 6$) were also examined, one for hypercapnia experiments (artificial ventilation), and another for acidotic media and NH_4^+ experiments (spontaneous ventilation). In control experiments, up to 10 NMDA-stimuli were superimposed onto control ACSF with normal conditions throughout.

Data presentation and statistical analysis

In order to conform to previous reports, in figures 1, 3 and 5 (i.e. representative recordings of NMDA-induced depolarizations), the polarity of the DC potential was reversed so that depolarization produces an upward deflection. NMDA-evoked depolarizations were quantified by measuring the peak amplitude. All values in Results are mean ± SEM. Statistical analysis of data at selected times point was by Student's *t* test, and overall significance determined by analysis of variance.

Results

NMDA-induced depolarizations in the rat striatum

Repeated 2-min perfusion of 200 μM NMDA through the microdialysis probe induced reproducible, brief depolarizations with a steep onset (Fig. 1, bottom trace). The mean amplitude of the first 2 depolarizations evoked by NMDA, averaged for each experiment, was 5.7 ± 0.2 mV, $n = 23$. The recovery was multiphasic, with an initial slow reduction of depolarization starting while NMDA was still being applied, a faster decrease immediately after switching back to normal ACSF, and a marked hyperpolarization followed by progressive normalisation. Repetitive application of NMDA under control ACSF resulted in a progressive, small increase in the amplitude of the NMDA-evoked responses (e.g. Figs. 2 and 6, hatched bars). The slope of the linear regression for amplitude of NMDA response versus number of NMDA application was 0.15 mV in the control group with artificial ventilation (control for hypercapnia; $p < 0.015$, analysis of variance) and 0.11 mV in the other (i.e. spontaneous breathing; $p < 0.001$, analysis of variance).

Effect of hypercapnia on NMDA-induced depolarizations

Arterial pCO_2 increased from 46.1 ± 2.8 mmHg to 95.0 ± 3.3 mmHg and 143.5 ± 5.8 mmHg after 15 min of ventilation with 7.5 % and 15 % CO_2, respectively ($n = 5$). The magnitude of the corresponding reductions in arterial blood pH was 0.26 ± 0.014 and 0.42 ± 0.02, respectively. The recovery period (i.e. 29 min without CO_2 in the breathing mixture) allowed arterial pCO_2 to return close to the initial values, but a small significant increase remained at the end of the recovery from exposure to 7.5 % CO_2 (51.3 ± 3.3 mmHg, $p < 0.01$). Hypercapnia

reduced NMDA-evoked responses in a concentration-dependent manner (Fig. 1 and 2), with 7.5 and 15 % CO_2 reducing the depolarization amplitude to 74 ± 4 % and 64 ± 2 % of that of the initial stimuli, respectively. This effect was rapidly reversed since the amplitude of responses evoked 17 min after hypercapnia was not significantly different than the corresponding one in the control group (Fig. 2). By itself, hypercapnia produced a concentration-dependent, positive shift of the DC potential (Fig. 1, top trace) (4.6 ± 0.3 mV, n = 6 and 6.9 ± 0.2 mV, n = 5 with 7.5 and 15% CO_2, respectively).

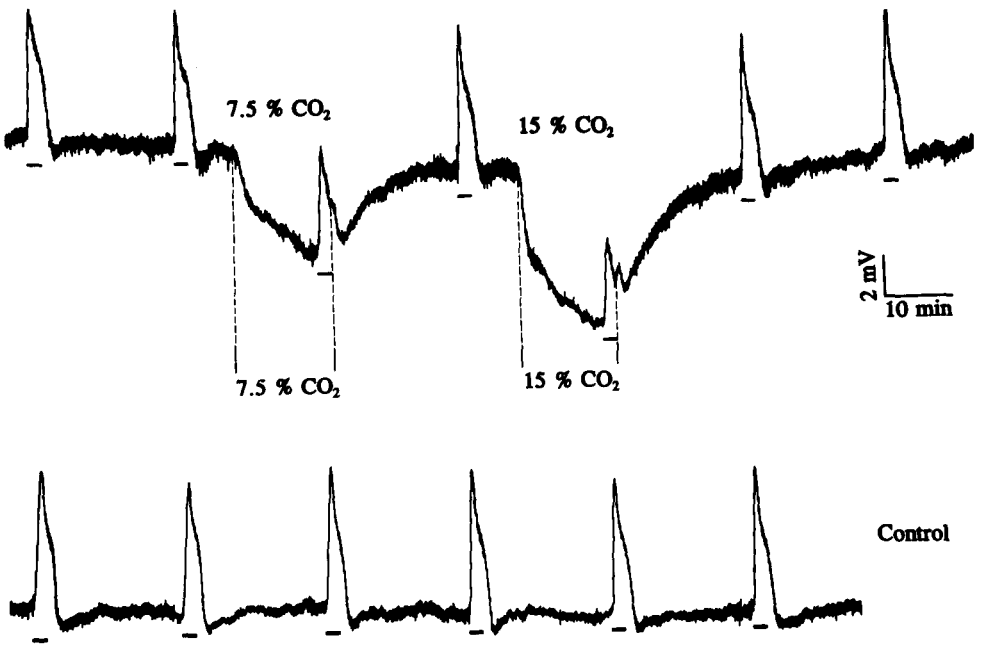

Fig. 1.
Representative recordings of the extracellular DC potential from a single hypercapnia experiment (top trace) and a corresponding control (bottom trace); Short horizontal bars (-), indicate that NMDA (200 μM) was perfused for 2 min through a microdialysis probe implanted in the rat striatum.

Effects of acid perfusion media on NMDA-induced depolarizations

Direct perfusion of acid media (10 mM phosphate-buffered, pH 6.5 or 5.8) for 10 min before co-application of NMDA did not alter significantly the amplitude of NMDA-evoked depolarization (Fig. 3 and 4). In contrast, pH_d increased by 0.31 ± 0.01 from ACSF pH 6.5, and 0.63 ± 0.02 from ACSF pH 5.8 (n = 5) (Fig. 4, right trace), confirming that exchange/buffering of acid equivalent took place between the perfusion medium and the surrounding tissue. Small reductions in pH_d were consistently associated with NMDA-induced depolarization; their average amplitude (0.048 ± 0.007 with phosphate-buffered ACSF at pH 7.3) was not altered by perfusion of ACSF pH 6.5, but increased significantly when ACSF was further acidified to pH 5.8 (0.074 ± 0.005).

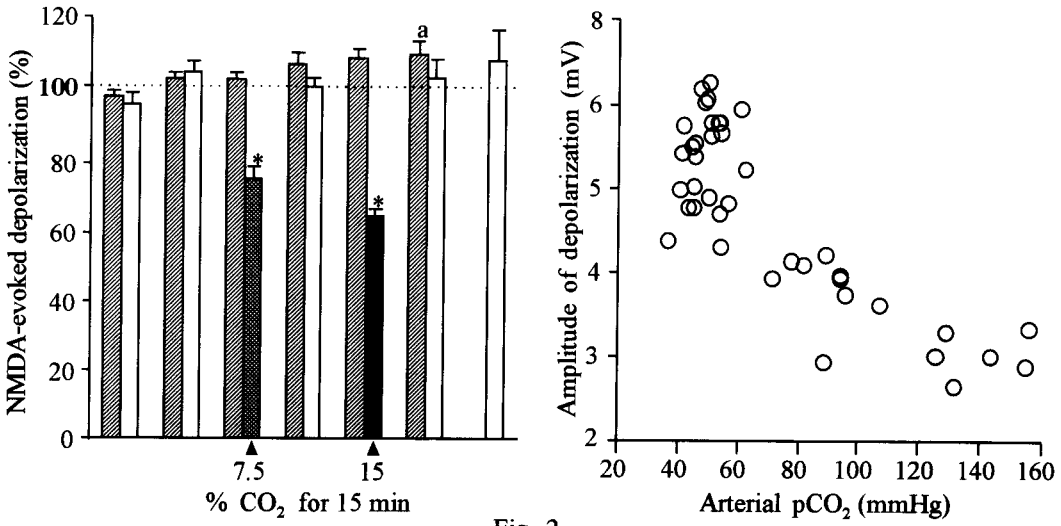

Fig. 2.

Effect of hypercapnia on depolarization evoked by application of NMDA to the rat striatum. Left panel: average changes (mean ± SEM) in the amplitude of NMDA responses in control (left, hatched bars; $n = 6$) and hypercapnia group (right bars; $n = 5$), expressed as percent of individual controls (i.e. mean of the first two NMDA responses computed for each animal); *, $P < 0.005$, comparison to 100 % by Student's t test; **a**, significant, progressive increase in the amplitude of the NMDA-evoked responses in control ($P < 0.015$, analysis of variance). Right panel: plot of amplitude of NMDA-evoked responses versus arterial pCO_2 ($P < 0.001$, analysis of variance).

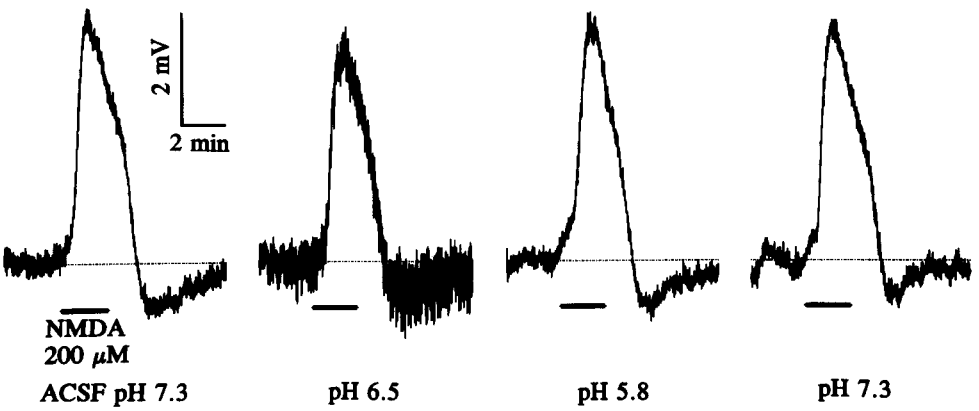

Fig. 3.

Effect of perfusion of phosphate-buffered acid media through the microdialysis probe on depolarization produced by co-application of NMDA for 2 min. These traces are representative depolarizations obtained in a single experiment; to facilitate comparison of the data, the DC potential sequences presented here were aligned by setting the 2-min period preceding changes in perfusion medium to 0 mV.

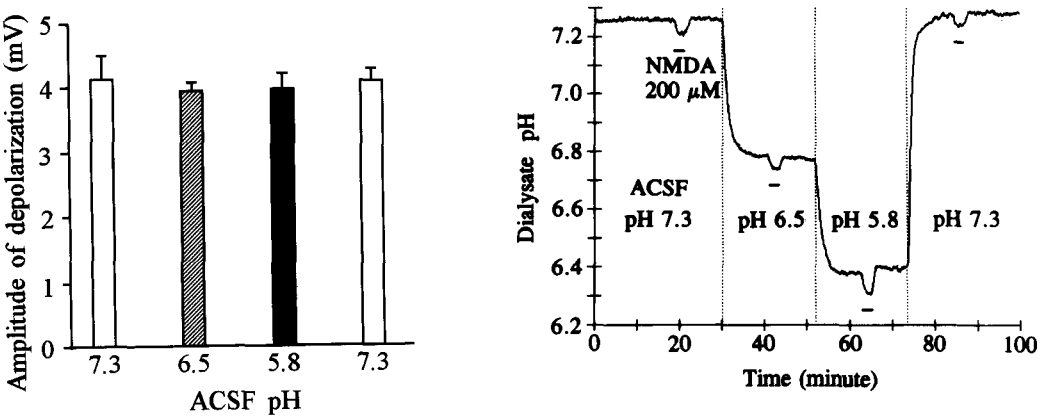

Fig. 4.

Effects of perfusion of phosphate-buffered acid media. Left panel: Average amplitude of NMDA-evoked depolarizations; bars are mean ± SEM (n = 5), no significant difference. Right panel: Representative changes in dialysate pH (pH_d) during perfusion with 10 mM phosphate-buffered acid ACSF; note the transient drop in pH_d during each NMDA-evoked depolarization (short horizontal lines).

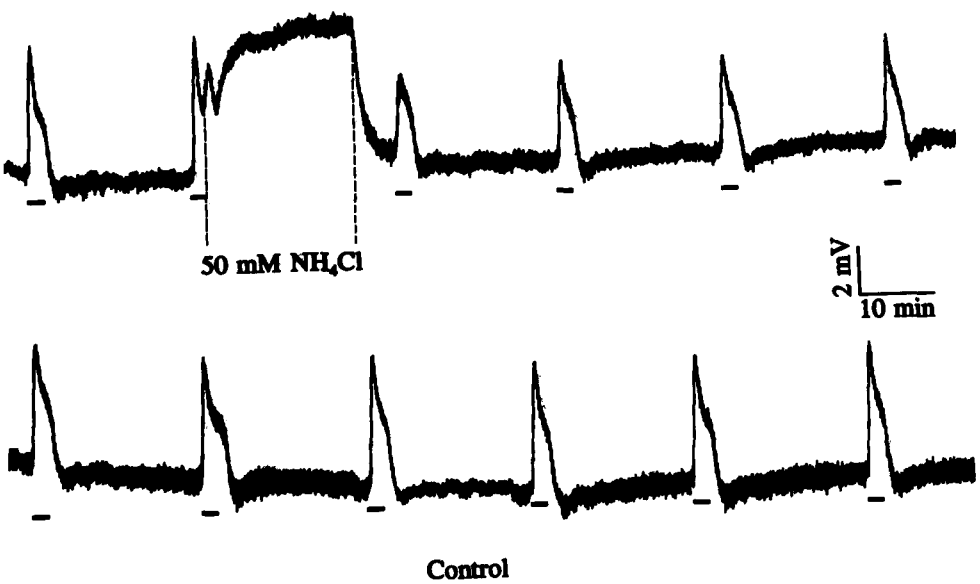

Fig. 5.

Effect of extracellular acidosis, subsequent to cessation of 50 mM NH_4Cl perfusion through the microdialysis probe, on depolarizations produced by application of NMDA for 2 min (horizontal bars). Representative recordings of the extracellular DC potential from a single experiment with NH_4Cl (top trace) and a corresponding control (i.e. normal ACSF perfused throughout, bottom trace).

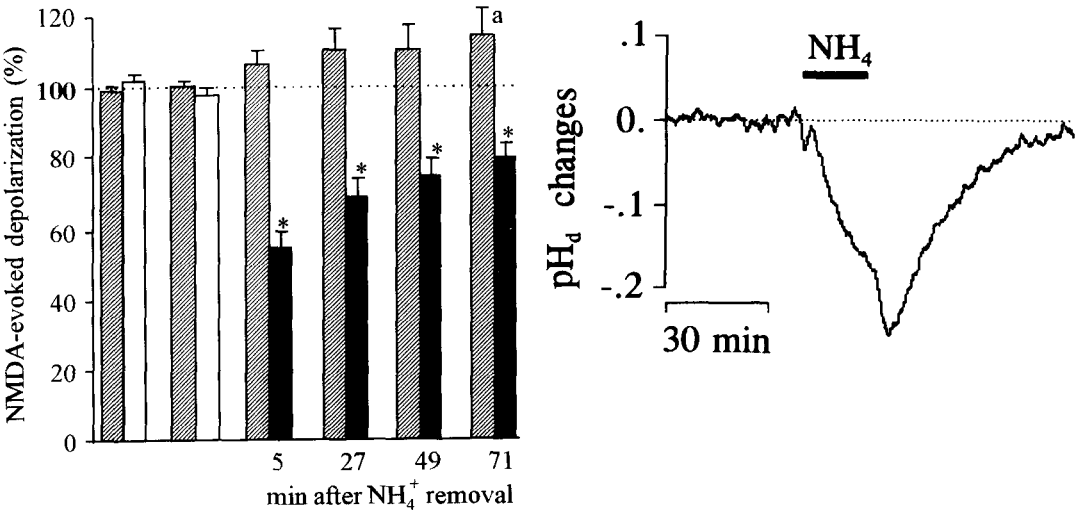

Fig. 6.
Effects of application of 50 mM NH_4Cl perfusion through the microdialysis probe. Left panel: Average changes (mean ± SEM, n = 6) in the amplitude of NMDA responses in control (left, hatched bars) and NH_4Cl group (right bars); changes are expressed as percent of individual controls (i.e. mean of the first two NMDA responses computed for each animal); **a** indicates a progressive increase of NMDA responses in the control group ($p < 0.001$, analysis of variance); * $p < 0.005$, comparison to 100 % by Student's t test. Right panel: Average changes in the pH of the dialysate (pH_d) produced by transient perfusion of NH_4Cl, computed from 6 separate experiments (scale = 1/10th of pH unit).

Effects of NH_4^+ on NMDA-induced depolarizations

Application of 50 mM NH_4^+ for 20 min progressively reduced pH_d (Fig. 6), and produced a step negative shift of the DC potential averaging 5.6 ± 0.2 mV (n = 7) (Fig. 5). When NH_4^+ perfusion was discontinued, a further acidification of the dialysate was observed, synchronous to rapid recovery of the DC potential, followed by progressive normalisation of pH_d. The amplitude of the pH_d reduction was 0.18 ± 0.02 (n = 7) immediately before NH_4Cl removal. NMDA applied through the microdialysis probe after removal of NH_4^+ from the perfusion medium evoked significantly smaller depolarizations (56 ± 2.6 % of the corresponding control, 5 min after NH_4^+ removal; $P < 0.001$, n = 6), and this effect persisted for > 1 h (Fig. 5 and 6).

Discussion

Methodological considerations

As hypercapnia and acidosis increase cerebral blood flow (18,34), these conditions could facilitate the elimination of NMDA from brain-to-blood and, consequently, reduce the local concentration of NMDA. However, such a potential interference would require significant diffusibility of NMDA across the blood-brain barrier, and there is no significant entry of NMDA in the brain and CSF under physiological conditions (1). This is exemplified by the weak convulsant effects of NMDA when it is administered systemically (2). Excitotoxic, intracerebral or intraventricular microinjections of NMDA increased the blood-brain barrier permeability to exogenous tracers

(13,31), but the consistent and reversible NMDA-evoked responses superimposed onto steady DC potential (Figs. 1 and 5, bottom traces) do not suggest that our NMDA applications were neurotoxic.

Vasodilation could also alter the size and tortuosity of the brain extracellular space and, therefore, the diffusion of NMDA applied by microdialysis (22,33). However, changes in cerebral blood flow did not affect *in vivo* recovery of extracellular lactate through microdialysis probe (25).

Repeated NMDA-evoked depolarizations in the rat striatum

The pattern of depolarizations produced by repeated perfusion of 200 μM NMDA was identical to that observed previously by application of gradually increasing concentrations of the agonist in the range 25-500 μM (40). The slight, progressive increase in depolarization amplitude which occurred throughout the experiment was a consistent feature (Figs. 2 and 6). This phenomenon may reflect progressive recovery of the recording tissue site from the injury produced by implantation of the microdialysis electrode. Both glucose phosphorylation and local cerebral blood flow were shown to be disturbed in the first hours after probe insertion (4). High resolution magnetic resonance imaging also suggested an altered extracellular volume (20), which could have influenced the diffusion of NMDA applied through the microdialysis fibre (33). Alternatively, the progressive increase in depolarization amplitude with repeated NMDA applications may reflect gradual potentiation of the responses, since NMDA-receptor activation plays an important role in persistent enhancement of synaptic efficiency such as long-term potentiation (6).

Effects of hypercapnia on NMDA-induced depolarizations

As CO_2 permeates freely across the blood-brain barrier, increasing arterial pCO_2 is a classical method of inducing brain acidosis *in vivo* (3,34,45). Niiro et al. (34) have demonstrated that changes in brain pH_e paralleled almost perfectly those in arterial blood pH, with brain pH_e decreasing by 0.10 and 0.31 when arterial blood pH was reduced by 0.12 and 0.35 during exposure to 5 and 10 % CO_2, respectively. Accordingly, as exposure to 7.5 and 15% hypercapnia reduced arterial blood pH by approximately 0.25 and 0.40 in our study, it can be reasonably assumed that brain pH_e reductions of a similar magnitude were produced (i.e. 6.9 to 7.05 pH_e). It is unlikely that perfusion of ACSF markedly interfered with pH_e changes produced by hypercapnia in the region surrounding the microdialysis probe because the perfusion flow rate was very slow, and the ACSF was not buffered in these experiments. This assumption is supported by the data obtained with direct perfusion of acid buffered media, a procedure which apparently failed to lower the surrounding pH_e (see next).

Hypercapnia concentration-dependently reduced NMDA-induced depolarization, thus confirming *in vivo* that moderate acidosis inhibits NMDA-receptor activated currents. However, as hypercapnia produces a marked positive shift of the DC potential which may reflect hyperpolarization (10), it is possible that a strengthening of the voltage-dependent Mg^{2+} block of NMDA-operated ion channels may contribute to inhibition of NMDA-evoked responses in this condition (29).

Microdialysis perfusion of acid media and NMDA-induced depolarizations

The lack of effect of direct perfusion of acid media through the microdialysis probe on NMDA-evoked depolarizations was unexpected. The most plausible explanation is that, even though pH_d measurements suggested that exchange/buffering of acid equivalents took place between the

perfusion medium and the surrounding tissue, perfusion of 10 mM phosphate-buffered ACSF at 1 μl/min was not capable of sufficiently reducing the pH_e of the surrounding tissue because of the remarkable capacity of the brain to buffer H^+ (11,46). This result exemplifies the difficulty of imposing local changes in the extracellular concentration of a given metabolite or ion, simply by changing its concentration in the perfusion medium flowing through a microdialysis probe. A better alternative is to use selective drugs (e.g. inhibitor of neurotransmitter uptake) or procedures (e.g. perfusion of NH_4^+, see next) which actively alter the homeostasis of the compound or ion under study.

Effects of NH_4^+ on NMDA-induced depolarizations

Transient exposure of isolated cells to NH_4^+ is a classical method of lowering their intracellular pH without utilisation of acid incubation medium (7,30,42,49). Upon application of NH_4^+ *in vitro*, an initial, rapid intracellular alkalinization results from permeation of uncharged NH_3 and its association with intracellular H^+. This alkalinisation is followed by gradual decrease of pH_i towards its initial resting value, due to the cation NH_4^+ also entering the cell driven by the membrane potential (7). When NH_4^+ is removed from the incubation medium at this point, pH_i falls immediately because of rapid efflux of NH_3, leaving behind H^+ which had been associated with NH_4^+ (42,49).

Our data show that NH_4^+ can be used also to locally challenge the brain acid-base homeostasis *in vivo*. However, under these conditions, application of NH_4^+ also results in a progressive acidification of extracellular pH (pH_e), which is then exacerbated when NH_4^+ perfusion is discontinued (Fig. 6). The progressive acidification of pH_e may be due to lactate efflux from the surrounding cells, reflecting anaerobic metabolism in response to high energy demand associated with NH_4^+-induced transmembrane ionic changes. Several elements support this hypothesis: (i) intracellular alkalinization directly stimulates glycolysis (14); (ii) application of NH_4^+ provokes an immediate negative shift of the DC potential suggesting membrane depolarization (Fig. 6); (iii) increase energy demand is known to be associated with increased production of lactate and its efflux to the extracellular space (16,24,48); and (iv) a separate study showed that dialysate levels of lactate are markedly increased when NH_4^+ is perfused through the microdialysis probe (M. Gotoh, S.E.C. Davis, and T.P. Obrenovitch, unpublished data). Extracellular acidification is not detectable when NH_4^+ is applied to isolated cells, presumably because the volume of the extracellular space (i.e. incubation medium) relative to the cells volume is enormous. As with isolated cells, the sudden additional drop of pH_e evoked by removal of NH_4^+ (Fig. 6) reflects intracellular acidosis and activation of Na^+/H^+ exchange because this event can be blocked by the selective blocker of Na^+/H^+ exchange, dimethylamiloride (M. Gotoh, S.E.C. Davis, and T.P. Obrenovitch, unpublished data).

The depolarizations evoked by NMDA after discontinuation of NH_4^+ perfusion were significantly reduced. With regard to the NMDA-evoked response recorded 5 min after NH_4Cl removal, its reduction could partly result from a direct action of NH_4^+ on NMDA-receptors (15,27) because extracellular levels of NH_4Cl could still be high in the tissue surrounding the probe. Such a direct action of NH_4^+ is unlikely with the subsequent NMDA-stimuli because they were applied when the DC potential had return to normal baseline, suggesting that high extracellular concentrations of NH_4Cl were no longer present (Fig. 5). Therefore, these results support that extracellular acidosis inhibits NMDA-receptor activated currents. As opposed to hypercapnia-induced inhibition of NMDA responses, that produced by NH_4^+-induced acidification cannot be attributed to strengthening of the NMDA channel Mg^{2+} block, because there was no indication of hyperpolarization in these conditions.

Implication to normal brain function

Whether or not the modulation of NMDA-receptor mediated responses by extracellular pH plays a role in normal brain function remains do be elucidated. Rae et al. (44) have recently reported a significant correlation between intracellular brain pH measured by ^{31}P magnetic resonance spectroscopy and samples of intelligent behaviour in young boys. Although better IQ correlating with higher pH_i does not imply a causal relation, since NMDA-receptors play a central role in brain development (8) and memory processes (6), it is tempting to propose that a contributing factor may be brain pH influencing NMDA receptor function.

Implication to excitotoxicity associated with cerebral ischaemia

Extracellular acidosis is often considered to decrease neuronal vulnerability to ischaemic damage (50) because it reduces NMDA activated currents, glutamate neurotoxicity, and oxygen-glucose deprivation injury in cultured neurones (17,21,47). Whether these data can be extrapolated to *in vivo* situations is uncertain however, because the pathophysiology of cerebral ischaemia is extremely complex. One must carefully examine when excessive cation influx through NMDA-operated channels and acidosis may co-exist during ischaemia. In addition, acidosis during ischaemia is likely to be associated with ATP depletion and, therefore, altered membrane potential which may result in deficient Mg^{2+} block of NMDA-operated ion channels.

It is generally assumed that the trigger of ischaemia-induced excitotoxicity is high extracellular glutamate, but this simplified hypothesis conflicts with key experimental evidence (37,39). For example, NMDA-receptor antagonists protect against delayed neuronal death, even when they are administered after a transient ischaemic insult, i.e. when extracellular glutamate levels are restored to normal levels. Alternative hypotheses compatible with the well-established protection obtained with NMDA-receptor antagonists include recurrent spreading depression in focal ischaemia (19,35) and potentiation of synaptic efficacy by transient ischaemia (anoxia-induced long-term potentiation, LTP) (12) processes which both require activation of NMDA receptors. With regard to recurrent spreading depression, it is relevant to note that this phenomenon apparently propagates through the penumbra, a region which features acidosis (35). Whether or not the induction of anoxia-induced LTP is inhibited by acidosis is not known.

Conclusion

These *in vivo* experiments confirm that extracellular acidosis, such as that associated with excessive neuronal activation or ischaemia, attenuates NMDA-evoked responses. Further studies with *in vivo* models are needed to determine the significance of this effect with regard to normal brain function and neuronal vulnerability in clinical situations.

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