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$GABA_A$ Membrane Currents are Insensitive to Extracellular Acidification in Cultured Sensory Neurons of the Frog

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Summary

The effects of decreasing extracellular pH from 7.4 to 6.0 or 5.8 on whole cell membrane currents induced by GABA (10–100 μM) were studied in dorsal root ganglion (DRG) neurons of the frog in short-term culture using the whole cell patch-clamp technique. In 45 of 50 cells the GABA currents were the same at both normal and reduced pH. In the remaining 5 cells, acidification increased the response. The reversal potential for the current, about +5 mV, was the same at reduced and normal pH. These results contrast with the effect of the same pH reduction which markedly reduces the current resulting from glutamate activation of receptors on central neurons (Traynelis and Cull-Candy 1990, Vyklický Jr. *et al.* 1990, Tang *et al.* 1990). These findings suggest that acidification under pathophysiological conditions plays a protective role in preventing excessive excitation not only by decreasing glutamate responses but also by leaving the inhibitory $GABA_A$ responses intact.

Key words

Sensory neurons – Short term culture – Frog – $GABA_A$ receptors – pH

Introduction

Extracellular acidification strongly decreases the effect of glutamate to open ion channels (Traynelis and Cull-Candy 1990, Vyklický Jr. *et al.* 1990, Tang *et al.* 1990). This effect was most pronounced on N-methyl-D-aspartate (NMDA) receptor-activated channels but significant inhibition of the responses to kainate and quisqualate was also observed. Decreasing pH also inhibits the opening of cationic voltage-gated channels for Na^+, K^+, and Ca^{2+} (Woodhull 1973, Ohmori and Yoshii 1977, Hagiwara *et al.* 1978, Prod'hom *et al.* 1987) and endplate acetylcholine-gated channels (Landau *et al.* 1981).

The data on GABA-gated chloride channels are much less conclusive. An increase in depolarization resulting from iontophoretic GABA application at low pH, without any change in the extrapolated reversal potential, was observed in dorsal root ganglion neurons of the cat (Gallagher *et al.* 1983). However, in mammalian neurons in culture, lowering pH led to a decrease in GABA-induced membrane currents (Gruol *et al.* 1980). This is in contrast with the study of Tang *et al.* (1990) who did not find any change of GABA-induced membrane currents in rat cultured hippocampal neurons when extracellular pH was decreased.

The aim of this study was to determine whether acidification can modify GABA-induced membrane currents in frog dorsal root ganglion neurons. Such information is of interest in trying to understand changes in nervous system functions under pathological conditions which lead to a fall in extracellular pH. Decreases by half pH unit have been found during epileptic seizures (Siesjö *et al.* 1985) and by more than one pH unit during brain ischemia (von Hanwehr *et al.* 1986).

Methods

Experiments were performed on dorsal root ganglion (DRG) neurons of adult frogs (Rana pipiens) which were isolated and cultured for 3 hours to 3 days

(Kuffler, in preparation). Briefly, DRG were removed, the ganglia cut into small pieces and bathed in a solution of collagenase-P (0.5 mg/l) and neutral dispase (2 mg/ml) for 1 hour in a silicon-coated glass dish and then gently triturated. The enzyme solution was then diluted with 2 ml of fresh Leibovitz 15 medium containing 0.25 % garamycin. The neurons were plated at low densities (30–50 per dish) in 35 mm Primaria culture dishes.

Whole-cell recordings were made at room temperature (22–24 °C). To minimize the problems with inadequate space clamping only neurons without obvious branching were used. Patch-clamp electrodes were pulled in two steps from borosilicate glass OD/ID 1.5/1.0 mm (Gardner KG 33). Voltage clamp was established using an Axoclamp 2000 amplifier. The electrodes had a resistance of 3–4 MΩ and the access resistance was typically 7 MΩ. Control and test solutions were applied by hydrostatic pressure employing a system (Vibratec Inc.) consisting of 5 tubes connected to a common thin plastic tube of 0.2 mm ID with the outlet facing the neuron at a distance of about 150 μm. A full exchange of the solutions around the neuron was reached in about 50 ms. The extracellular solution had the following composition (in mM): NaCl 130, KCl 3, $CaCl_2$ 2, $MgCl_2$ 1, PIPES 5, pH 7.4 of the control solution or lower pH as indicated in the text were adjusted with NaOH. Pipettes were filled with solution of following composition (in mM): CsCl 140, KCl 2.5, $MgCl_2$ 1, $CaCl_2$ 0.5, EGTA 5, HEPES 10, pH 7.2 adjusted with CsOH. All chemicals were supplied by Sigma (USA). When the effects of pH on responses to GABA were tested, a solution at the low pH was applied first and GABA in the same solution was tested afterwards.

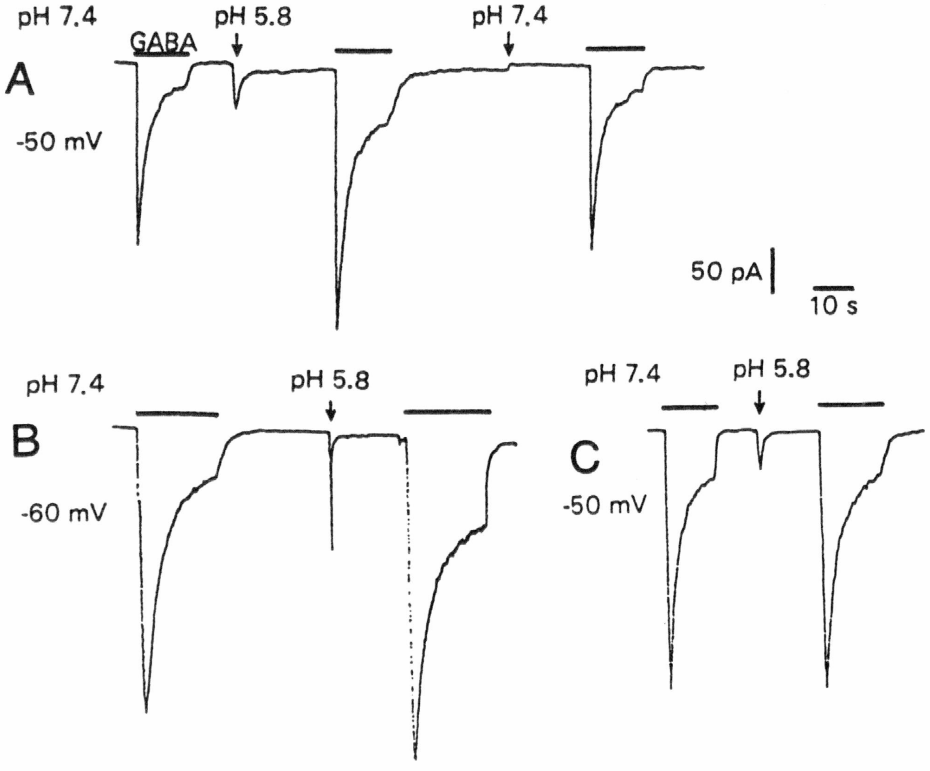

Fig. 1
Whole-cell membrane currents produced by GABA superfusion at normal (7.4) and reduced (5.8) pH in three different DRG neurons: **A,B,** and **C.** Clamped membrane potentials and pH values are indicated for each record. GABA concentration was 100 μM in A and C and 30 μM in B. In all three neurons reduced pH induced a fast inactivating inward current. Calibration applies to all records. The duration of GABA superfusion is indicated by horizontal bars; change of pH by an arrow. In records A and B the amplitude of the inward current response to GABA increased in acid solution.

Results

Frog DRG neurons maintained in short-term culture are 30–60 μm in diameter. As previously found, steady application of GABA (10–100 μM) produces an inward membrane current which decreases within 4–5 seconds to a new sustained level in all DRG neurons tested (Hattori *et al.* 1984, Akaike *et al.* 1986). Such responses are illustrated in Figs 1 and 2.

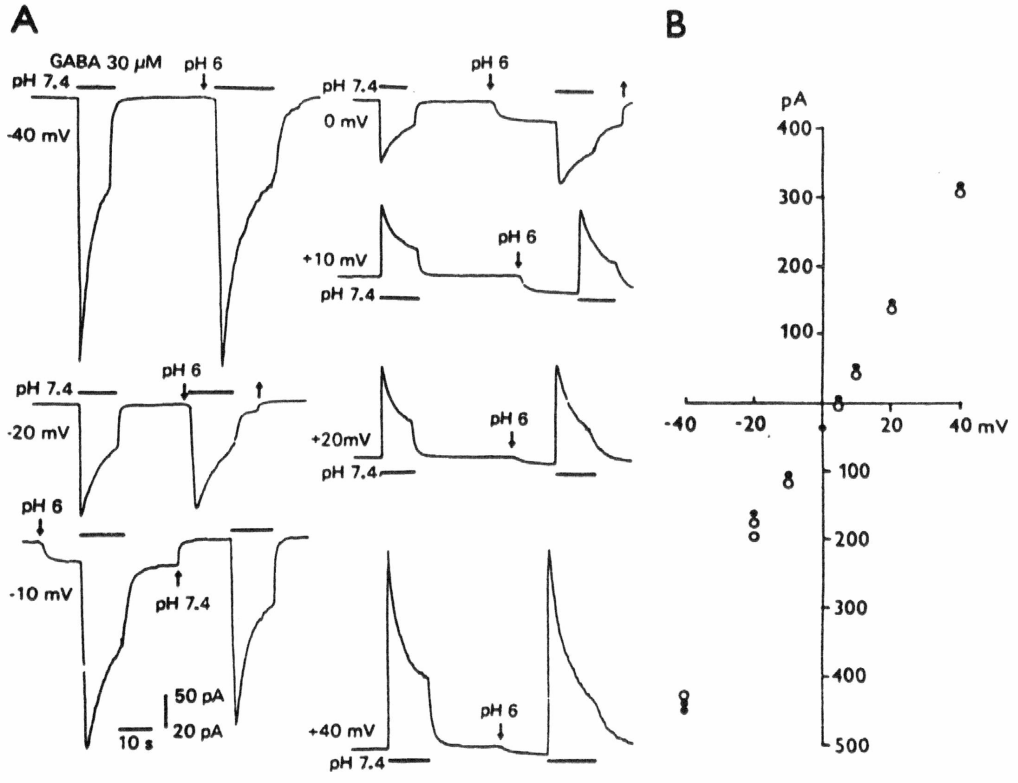

Fig. 2
Reversal potential for GABA current at pH 7.4 and pH 6. **A:** Responses to GABA (30 μM) at pH 7.4 and pH 6 at different membrane potentials. The duration of GABA superfusion is indicated by horizontal bars above or below the records. Calibration of 50 pA applies for records at -40 mV, -20 mV, +20 mV and +40 mV; calibration of 20 pA for -10 mV, 0 mV and +10 mV. **B:** Voltage-current relationship of the peak current induced by GABA from the records in A. Closed circles indicate responses at pH 7.4 and open circles at pH 6. The reversal potential, about +5 mV, is the same at pH 7.4 and pH 6.

A decrease in pH from 7.4 to 5.8 or 6 produced, by itself, a transient inward current (Fig. 1) in 20 % of the neurons (n=10). In contrast with the study of Akaike *et al.* (1990), who found the proton-induced fast inactivating current exclusively in small DRG neurons, we observed it in neurons of all sizes. The transient current was followed by a very small non-inactivating inward current usually less than 10 pA at membrane potentials of −50 or −60 mV. In the majority of neurons (n=40), however, the application of low pH solution resulted only in a small non-inactivating inward current without any preceding transient.

As illustrated in Figs 1C and 2, in 90 % of the neurons tested (n=45), decreasing the pH of the bathing solution from normal, 7.4, to 5.8 or 6 had no effect on the response to GABA. In the remaining 5 neurons, acidification to pH 5.8 led to an increase in the GABA response by 22.2±11.7 % (mean±SD) (Fig. 1A,B). In agreement with the results of Gallagher *et al.* (1983) and Gruol *et al.* (1980), the reversal potential for the GABA response was the same at normal and low pH (Fig. 2).

Discussion

Our results show that reducing extracellular pH does not result in significant changes of the whole cell membrane current produced by GABA in 90 % of the frog DRG neurons tested. In 10 % of the cells the response to GABA increased. This is in striking contrast with the inhibitory effects of acidification on glutamate-NMDA-receptor activated currents in neurons from the central nervous system (Traynelis and Cull-Candy 1990, Vyklický Jr. *et al.* 1990, Tang *et al.* 1990), end-plate acetylcholine-gated channels (Landau *et al.* 1981), voltage-gated cationic channels (Woodhull 1973, Ohmori and Yoshii 1977, Hagiwara *et al.* 1978, Prod'hom *et al.* 1987) as well as the chloride conductance in skeletal muscle (Hutter and Warner 1967).

Gallagher *et al.* (1983) found that lowering pH from 7.4 to 6.4 shifted the relation between GABA

electrophoretic current (dose) and membrane depolarization up and to the left in isolated cat DRG neurons without changing the reversal potential. They suggested that the pH effect is exerted on the Cl channel, the GABA uptake system or some other allosteric site rather than on the Cl pump or directly on the GABA-receptor recognition site. Conversely, Gruol *et al.* (1980) found that simultaneous iontophoretic application of H^+ with GABA (as well as glycine or glutamate) resulted in a reduction of the amino acid induced conductance increase in cultured embryonic mouse neurons. In the latter experiment the actual pH at the cell membrane produced by H^+ iontophoresis was not known. Neither of these studies is directly comparable to the present result because the method of recording, application of the GABA and H^+ as well as the species used are all different. The only exception represents the study of Tang *at al.* (1990) who demonstrated a decrease of GABA responses in rat cultured neurons when extracellular pH was increased and no change when pH was decreased. No quantitative data were given, apparently because the study was focused on the effects of pH on the NMDA receptor.

Although the response to GABA was the same at normal and reduced pH in 90 % of these frog DRG neurons, in 10 % of the cells there was a clear increase in the GABA response (e.g. Fig. 2A,B). We can not rule out that such an increase was an artifact due to improved recording conditions at low pH. If the leakage resistance decreased in low pH and thus improved the ratio of the membrane resistance to the pipette access resistance, the GABA current would appear to increase at low pH. However, it can not be excluded that the different results reflect substantial heterogeneity of $GABA_A$ receptor subunits (Barnard *et al.* 1987, Vicini 1991, Burt and Kamatchi 1991) with different sensitivity to H^+.

An extensive consideration of the possible mechanisms of proton modulation of ion channels and receptor interactions is beyond the scope of the present discussion (for a review see Hille 1992 p. 394). It is of interest, however, that the GABA-induced current is insensitive to acidification whereas the conductance of voltage-dependent cation channels (Woodhull 1973), channels activated by acetylcholine (Landau *et al.* 1981) or glutamate (Traynelis and Cull-Candy 1990, Vyklický Jr. *et al.* 1990, Tang *et al.* 1990) and even anion channels in skeletal muscle (Hutter and Warner 1967) are all reduced at low pH. Possibly all these channels share some common titratable feature, such as diffusely distributed negative charges at the mouth of the pore (Hille 1992), which is absent or inaccessible in the GABA-activated channel.

Reduced pH has been shown to protect cultured neurons from glutamate-induced and hypoxia-induced toxicity (Giffard *et al.* 1990) by attenuating the neurotoxic activation of NMDA receptors (Traynelis and Cull-Candy 1990, Vyklický Jr. *et al.* 1990). Our finding that reduced pH does not impair the activity of $GABA_A$ receptors, or may slightly increase it, is consistent with the view that extracellular acidification plays a physiological role in preventing the excessive excitability which could irreversibly damage neurons during hypoxia or epilepsy (von Hanwehr *et al.* 1986, Siesjö *et al.* 1985).

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