Short communication

An Inert Hydrogen Ion Buffer for Sodium Current Measurements in Ranvier Nodes

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The parameters of sodium currents in myelinated nerve fibres are shifted on the potential axis by any change in pH of the bathing medium (Hille 1968). Therefore, for standard measuring conditions the pH has to be kept at constant values by a buffer system 1. of sufficient buffering capacity in the physiological range, i.e. the pK_a should be near 7.2 for frog nerves, 2. of no measurable

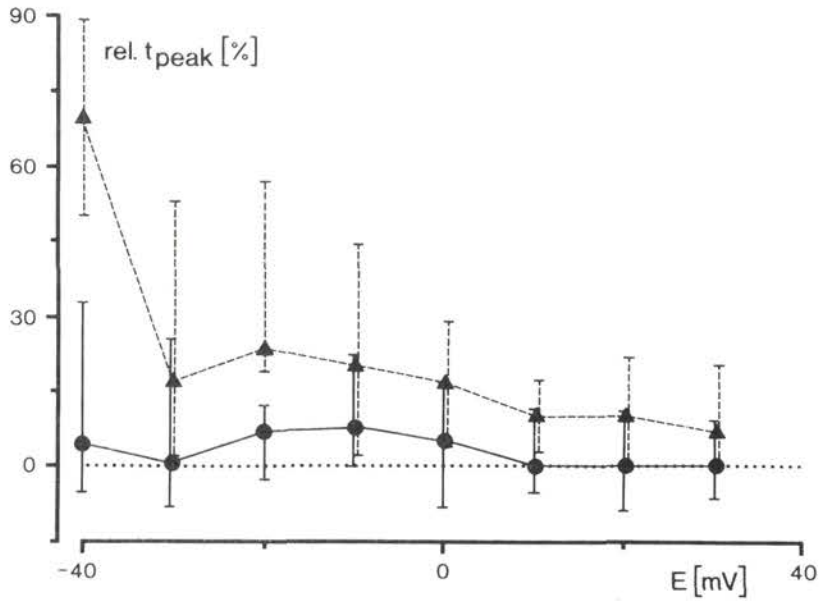

Fig. 1. Relative increase of the time to peak sodium currents under tris (triangels) and under BES (circles), respectively, as related to t_{peak} under bicarbonate buffer (dotted line), rel. t_{peak}, at different membrane potentials, E. Symbols: medians of seven experiments. Vertical bars: ranges of medians under tris (dashed) and under BES (continuous). Holding potential: $E_R = -70$ mV.

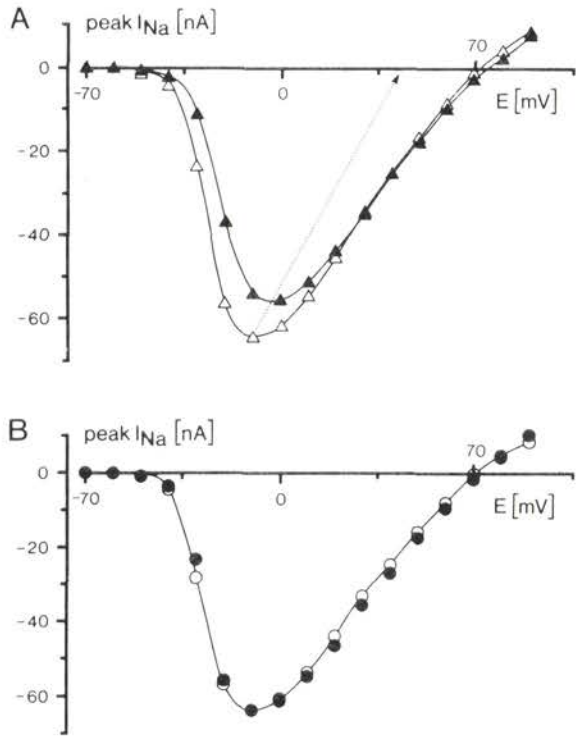

Fig. 2. Peak sodium current-voltage relations under bicarbonate buffer (open symbols), under tris (*A*, filled triangels), and under BES (*B*, filled circles). Sodium inactivation was removed by stepping to $E = -110$ mV 50 ms before application of test pulses. Holding potential: $E_R = -70$ mV. Continuous curves: spline interpolation (see Wiese and Koppenhöfer 1988). Dotted line: extrapolation of curve minima to peak $I_{Na} = 0$. Leakage currents were subtracted automatically assuming a potential independent leakage conductance.

pharmacological side effects, and 3. of sufficient stability against degradation (for further rules of merit, see Good et al. 1966).

The widely used tris(hydroxymethyl)-aminomethane/HCl buffer ("tris") has poor buffering capacity at physiological pH because of its pK_a-value of about 8.3. In this connexion N,N-Bis(2-hydroxyethyl)-2-aminomethanesulfonic acid/NaOH buffer ("BES") is preferable (Good et al. 1966). We tried to answer the question of undesired side effects by refering sodium currents as measured under tris and BES, respectively, to corresponding currents measured under bicarbonate buffer which does exist in the living animal (Lenfant and Johansen 1967).

Single myelinated nerve fibres of the frog (*Rana esculenta*) were conventionally potential clamped (Nonner 1969) and rinsed continuously by the specific bathing media at 10 °C. The dissection procedure used delivered clearly curled thick axons (median and range (Sachs 1984) of diameter: $19 \leq 20\,\mu m \leq 22$; $n = 5$), thus for this kind of experiments the series resistance artifact was assumed to be negligibly small (Koppenhöfer et al. 1987). We compared tris and BES, respectively, to $NaHCO_3/CO_2$ buffer (10.0 mmol/l each, at 112.0, 102.0, and 102.0 mmol/l NaCl, respectively; pH = 7.2 ± 0.1). For keeping the pH in the recording chamber at the desired level for the bicarbonate buffer the storage solution was gassed continuously with 95 % O_2: 5 % CO_2 ("carbogen"). Furthermore, the inevitable loss of CO_2 was minimized by gas-tight connecting glass fittings and by continuously washing the recording chamber with carbogen.

Positive test pulses were applied and both peak sodium currents peak I_{Na}, and the time to peak I_{Na}, t_{peak}, were measured. In Figure 1 the marked increase of t_{peak} by tris (triangels) as related to t_{peak} under bicarbonate buffer ($\alpha < 0.05$ as tested by the sign test; Dixon and Mood 1946) is shown. Under BES (circles), however, eventual changes of t_{peak} were of no significance.

Peak sodium current-voltage relations are shown in Figure 2. Under tris (filled triangels) the minimum of the curve was reversibly and significantly reduced by $4.7 \leq 9.0\,\% \leq 13.7$ (median and range; $n = 7$; $\alpha < 0.05$). Moreover, the minimum was shifted along the potential axis in positive direction by $1.6 \leq 4.1\,mV \leq 10.0$ ($n = 7$; $\alpha < 0.05$). Both effects could basically be due to the increase of t_{peak} shown in Figure 1 (triangels) and/or to a shift of the activation term, m, (Frankenhaeuser 1960) on the potential axis in positive direction. Note that extrapolation of the minima of the curves in Figure 2*A* (dotted line) to peak $I_{Na} = 0$ did not yield $E = 0$ as it is expected if a reduction of the nodal series resistance were responsible for the tris effects on peak sodium currents (see Wiese and Koppenhöfer 1988, Fig. 1). Under BES, however, eventual changes of I_{Na} peak were again of no significance (Fig. 2*B*). Therefore, we consider BES a reasonably inert buffer for sodium current measurements in Ranvier nodes.

References

Dixon W. J., Mood A. M. (1946): The statistical sign test. J. Amer. Statist. Assoc. **41**, 557—556

Frankenhaeuser B. (1960): A quantitative description of sodium currents in myelinated nerve fibres of *Xenopus laevis*. J. Physiol. (London) **151**, 491—501

Good N. E., Winget G. D., Winter W., Connolly Th. N., Izawa S., Singh R. M. M. (1966): Hydrogen ion buffers for biological research. Biochemistry **5**, 467—477

Hille B. (1968): Charges and potentials at the nerve surface. Divalent ions and pH. J. Gen. Physiol. **51**, 221—236

Koppenhöfer E., Sommer R.-G., Froese U. (1987): Effects of benzocaine and its isomers on sodium permeability and steady state inactivation in the myelinated nerve, obtained by an improved dissection technique. Gen. Physiol. Biophys. **6,** 209—222

Lenfant C., Johansen K. (1967): Respiratory adaptations in selected Amphibians. Respiration Physiol. **2,** 247—260

Nonner W. (1969): A new voltage clamp method for Ranvier nodes. Pflügers Arch. **309,** 176—192

Sachs L. (1984): Applied statistics — A handbook of techniques. Springer, New York

Wiese H., Koppenhöfer E. (1988): Minimizing the influence of the series resistance in potential clamped Ranvier nodes. Gen. Physiol. Biophys. **7,** 143—156

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