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CHEMICAL MODIFICATION OF POTASSIUM CHANNEL GATING IN FROG MYELINATED NERVE BY TRINITROBENZENE SULPHONIC ACID

BY MICHAEL D. CAHALAN AND PAMELA A. PAPPONE
From the Department of Physiology and Biophysics, University of California, Irvine, CA 92717, U.S.A.

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SUMMARY

1. We investigated the actions of externally applied trinitrobenzene sulphonic acid (TNBS) on the K currents of voltage-clamped frog myelinated nerve fibres. TNBS treatment irreversibly slowed the tail currents for K channel closing up to 10-fold. Time constants for the tail currents appear shifted 60–80 mV in the hyperpolarizing direction following TNBS treatment.

2. The time course of K channel opening was unaffected for depolarizing pulses to potentials positive to -20 mV. For smaller pulses to potentials between -80 and -30 mV the activation time course was slower following TNBS treatment.

3. TNBS had little or no effect on the steady-state conductance–voltage relation of K channels determined from tail current amplitude. The instantaneous current–voltage relation for K channels and potency of block by external TEA were unaffected by TNBS.

4. K tail currents showed fast and slow components both before and after TNBS treatment. Reaction with TNBS caused the fast component to decline in amplitude and the slow component to increase both in magnitude and time constant. The rate of reaction increased with increasing pH.

5. Full expression of altered K channel kinetics depends upon the ionic composition of the external solution. Tail currents were up to 10-fold slower when measured in 117·5 mM-K than when measured with 80 % of the external K replaced by Na.

6. The differential effects of TNBS on K channel closing and opening were modelled in a three-state kinetic scheme, with an increase in the energy barrier for closing an open channel following TNBS treatment.

INTRODUCTION

This paper describes irreversible alterations in K channel gating kinetics following treatment of the nerve membrane with an amino group-specific reagent, trinitrobenzene sulphonic acid (TNBS). Following TNBS treatment K channel closing rates are slowed up to 10-fold with only minor effects on opening rates. We have previously shown that TNBS shifts the steady-state voltage dependence of the Na channel inactivation process to more hyperpolarized potentials (Cahalan & Pappone, 1981).

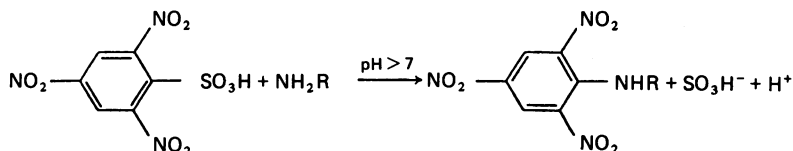
The rates of both development of and recovery from Na inactivation were shifted in the same direction, consistent with an increase in negative surface charge density following reaction with externally applied TNBS. This finding corresponded to the expected change in surface charge following TNBS reaction, since TNBS converts amino groups, positively charged at physiological pH, to neutral trinitrobenzene derivatives. However, the simple idea of altered surface charge cannot account for the modification of K channel gating that we have observed. Our results indicate that TNBS preferentially alters the rate constant for closing the K channel without altering the opening rate constant greatly.

Recently, there has been considerable interest in interactions between permeant ions and the gating process for both acetylcholine-activated channels (Gage & Van Helden, 1979; Marchais & Marty, 1979) and voltage-dependent K channels (Århem, 1980; Swenson & Armstrong, 1981). The idea has been put forward that occupancy of a site within the open channel by a permeant ion may inhibit the closing of the channel. We have found that the closing rates of K channels are profoundly influenced by the external K concentration only after TNBS treatment. TNBS thus may enhance the sensitivity of the K channel gating mechanism to occupancy by K ions. A preliminary report of these findings has appeared (Pappone & Cahalan, 1982). Our results suggest that a modifiable amino group at the outer membrane surface allows the K channel to close rapidly upon repolarization.

METHODS

Experiments were performed on single frog myelinated nerve fibres from the sciatic nerve of *Rana catesbiana* or *Rana pipiens*. Fibres were voltage clamped using the vaseline-gap method (Frankenhaeuser, 1957; Dodge & Frankenhaeuser, 1958; Hille, 1971). Unless otherwise stated, the fibre ends were cut in a solution of 120 mM-KF, 2 mM-HEPES, pH 7·4. The mounted fibres were allowed to equilibrate for 30 min before starting the experiment. The preparation was cooled to 6–8 °C. The temperature was kept constant to within 0·5 °C by a Peltier cooler.

For most of the experiments presented here, the fibre was bathed in a high-K Ringer solution, called K-Ringer, containing 117·5 mM-KCl, pH 7·4. The high external K concentration reduced the effects of ion accumulation (Dubois, 1981*a*) and permitted measurement of both inward and outward K currents. The composition of the solutions is shown in Table 1. Solutions used for reacting the fibre with TNBS had a pH of 9·5 to accelerate the reaction of TNBS with amino groups (Means & Feeny, 1971). Because the effects of TNBS on K currents are most evident at high external K concentrations (see Results), experiments examining the reaction of TNBS with K channels were done with K as the major cation in the solution. The K-TNBS solution was made by addition of 20 mM-K-TNBS to the high pH, K solution. This solution formed a precipitate on standing 10–20 min. In other experiments, the fibres were reacted with TNBS in high pH, 20 % K, Na solution to which 20 mM-Na-TNBS was added. This solution was stable indefinitely. TNBS was obtained from the Sigma Chemical Company, St. Louis, MO. The TNBS solutions were made up from crystalline TNBS immediately before use. The reaction of TNBS with amino groups is:



TNBS reacts specifically and covalently with primary amino groups under these conditions (Means & Feeny, 1971). The reaction of TNBS with amino groups liberates bisulphite ions which bind to the trinitrophenyl group of the reaction product, producing a negative modified group. The binding

of bisulphite is rapidly reversible (within 2–3 sec) (Means, Congdon & Bender, 1972) and bisulphite is therefore not present in our experiments after the TNBS solution is washed out. All the effects of TNBS presented in this paper are irreversible, and therefore are due to the conversion of amino groups to a neutral trinitrobenzene derivative.

Records of current were sampled at up to 100 kHz and stored on the disk of a Data General Nova 3 minicomputer. Current amplitude was calculated from the potential in the E pool assumimg an axoplasmic access resistance, R_{ED}, of 12·9 MΩ (Cahalan & Hall, 1982). Current records were usually filtered at 12·5 kHz. Errors in the voltage measurement due to the resistance in series with the membrane were electronically compensated using a filtered current signal, set assuming the series resistance had a value of 0·01 R_{ED} (Sigworth, 1980). A linear leak current proportional to the voltage-step amplitude was electronically subtracted from all current records. In some records, a P/4 pulse procedure (Armstrong & Bezanilla, 1974) was used to subtract the linear components from the membrane current records. The initial resting potential was assumed to be −80 mV. The accuracy of this assumption was determined by checking that the Na channel steady-state inactivation (h_{∞}) had a value near 0·8 at −90 mV at the beginning of the experiment, and that the final membrane potential at the end of the experiment was near 0 mV in K-Ringer.

TABLE 1. Solutions (mM)

Solution	[NaCl]	[KCl]	[RbCl]	[TMACl]	Buffer	pH
Na-Ringer	115	2·5	—	—	5 MOPS	7·4
K-Ringer	—	117·5	—	—	5 MOPS	7·4
Rb-Ringer	—	—	117·5	—	5 MOPS	7·4
20% K, Na	94	23·5	—	—	5 MOPS	7·4
20% K, TMA	—	23·5	—	94	5 MOPS	7·4
High pH, K	—	95	—	—	20 K-borate	9·5 or 10·5
High pH, 20% K, Na	76	19	—	—	20 Na-borate	9·5

All solutions contained 1·8 mM-$CaCl_2$. pH 7·4 solutions contained 100 nM-tetrodotoxin to block the current through Na channels. High pH solutions were titrated with KOH (high pH, K) or NaOH (high pH, 20% K, Na).

RESULTS

TNBS slows potassium channel gating

Reaction of the nerve membrane with TNBS alters the gating of K channels. The main effect of TNBS treatment is to slow the closing of K channels. In addition, TNBS slows the opening of K channels for small depolarizations. Fig. 1 shows membrane currents recorded with 117·5 mM-K in the external solution before and after exposure to TNBS, scaled to have the same peak amplitude. The upper set of records shows that for a moderate depolarization to −40 mV, both the opening and closing of K channels were slower following reaction with TNBS. Although both the opening and closing were altered, the effect on the rate of channel closing was much greater. The time for the current to reach half its maximum value increased 2·5-fold, from 24 to 59 msec, for the depolarization to −40 mV. The time constant for the rate of closing of K channels upon repolarization to the holding potential of −110 mV increased 6·1 fold, from 3·4 to 21 msec. This differential effect of TNBS on opening and closing rates is even more evident if one examines the time course of K channel activation at more depolarized potentials. The lower pair of records in Fig. 1 show that the time course of turn-on of K current during a depolarization to +40 mV was unaffected by TNBS treatment, while again the turn-off of current at the holding potential was dramatically slowed.

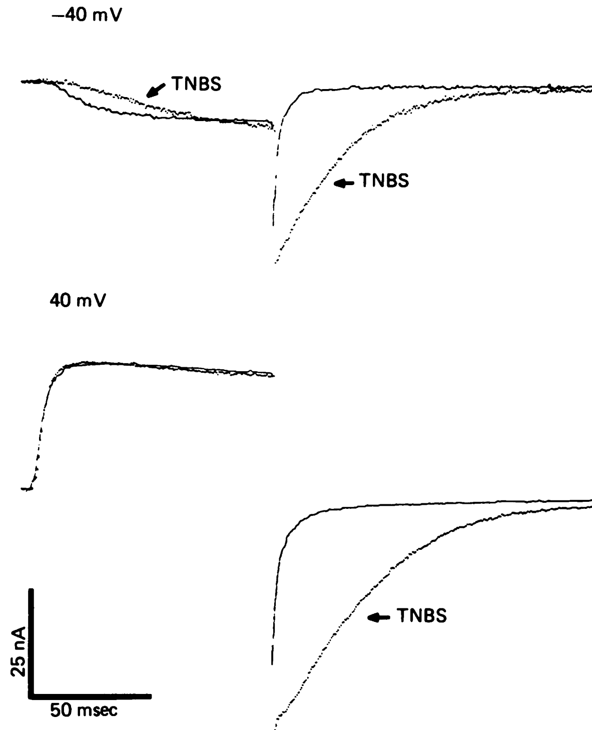


Fig. 1. Superimposed K currents recorded during depolarizations to −40 mV and +40 mV from the holding potential of −110 mV before (continuous traces) and after (dotted traces) exposure to TNBS. The TNBS records are scaled by a factor of 1·15 to have the same peak amplitude as the control at +40 mV. The fibre was treated with 20 mM-TNBS, pH 9·5 for 5 min. Control and TNBS K currents were recorded in K-Ringer, pH 7·4. *Rana pipiens*, 8 °C. Fibre 8–6.

Fig. 2 shows a method of estimating the extent and direction of a shift in the potential dependence of channel kinetics. Part *A* shows superimposed currents recorded during a depolarization to −50 mV before treatment and to −30 mV after reaction with TNBS, scaled to have the same peak amplitude. The rate of activation of K current is identical in the two records. This result suggests that the potential dependence of K channel activation was shifted by 20 mV to more depolarized potentials by TNBS modification. Part *B* shows a similar comparison of the turn-off of K currents before and after TNBS. The rate of decline of K current at −150 mV following TNBS is similar to those at −70 or −80 mV in the controls. These records suggest that TNBS shifted the voltage dependence of K channel closing by 70–80 mV towards more hyperpolarized potentials. Thus, describing the effects of TNBS on K current kinetics in terms of shifts in the voltage dependence would require both different magnitudes and different directions for the shifts in opening and closing kinetics. Such effects are clearly inconsistent with a simple change in the surface potential.

TNBS treatment slows the closing of K channels over the entire potential range from −150 to −80 mV as shown in Fig. 3*A*. The rate of K channel closing remained

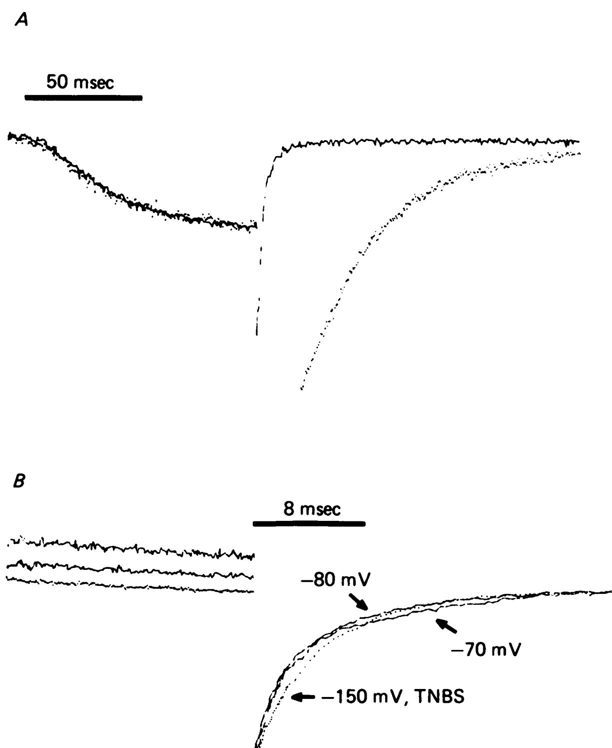


Fig. 2. Superimposed K currents recorded before (continuous traces) and after (dotted traces) TNBS treatment. All currents were recorded in K-Ringer, pH 7·4. *A*, the rate of activation of K current during a control depolarization to −50 mV compared with that during a pulse to −30 mV after TNBS treatment. The TNBS record was scaled by 1·07 to have the same peak amplitude as the control. Linear leak currents were digitally subtracted from the records by subtracting appropriately scaled currents recorded during a hyperpolarization to −150 mV from the holding potential of −110 mV. *B*, the rate of decline of K currents at −150 mV following TNBS treatment compared to control records at −80 and −70 mV. K current was activated by a 20 msec depolarization to 10 mV, and the fibre then repolarized to the potentials indicated. Control currents were scaled by 1·71 (−80 mV) or 2·26 (−70 mV) to have the same peak amplitude as the TNBS record. Same fibre as Fig. 1. P/4 pulse procedure.

highly voltage-dependent, but was up to 10-fold slower. TNBS treatment slowed the opening of K channels at moderately depolarized potentials, but had no effect on the opening kinetics at more positive potentials. Fig. 3*B* shows the time for the K current to reach half its maximum amplitude, $t_{\frac{1}{2}}$, during depolarization to the potentials shown. TNBS treatment increased $t_{\frac{1}{2}}$ for potentials negative to −20 mV, indicating that K channel-activation was slowed. TNBS increased $t_{\frac{1}{2}} \sim$ 4-fold for a depolarization to −50 mV. The effect was progressively less at more positive potentials. For depolarizations positive to −20 mV, TNBS had no effect on the kinetics of K current activation. This difference in effect of TNBS at moderate and strong depolarized potentials does not depend on the direction of K current flow. In Fig. 3*B* the K current was inward at −20 mV and outward at positive potentials, while at all these potentials the rate of current activation was unaffected by TNBS.

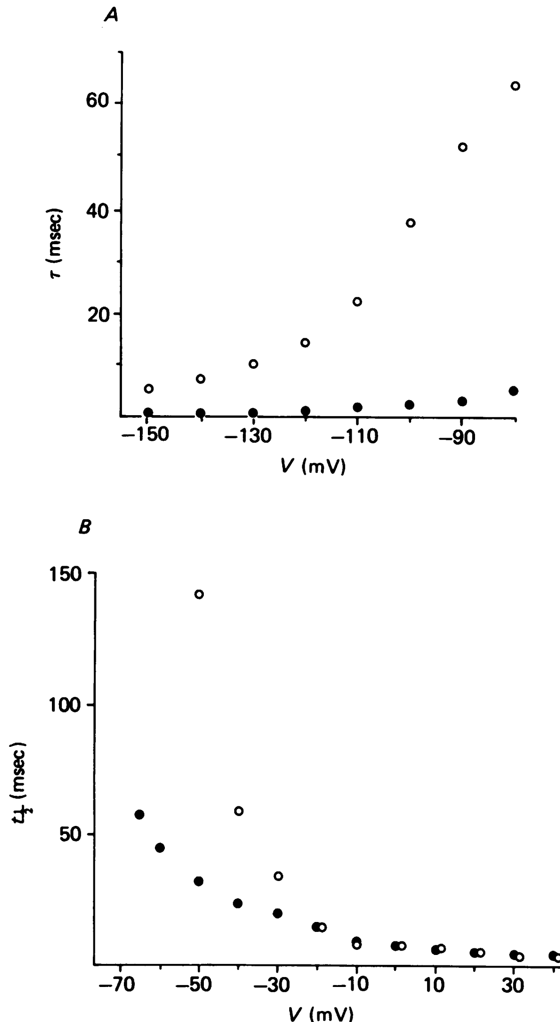


Fig. 3. *A*, time constants (τ) for the rate of decline of K current during repolarization to the potentials shown before (●) and after (○) TNBS treatment. K tail currents were fitted with a single exponential function with time constant τ by a least-squares method. K currents were activated by a 20 msec depolarization to −10 mV. All records in K-Ringer, pH 7·4. Same fibre as Figs. 1 and 2. P/4 pulse procedure. *B*, the time to reach half maximum amplitude, $t_{\frac{1}{2}}$, for K currents recorded before (●) and after (○) TNBS treatment. The fibre was depolarized from the resting potential of −110 mV to the potentials indicated. All records in K Ringer, pH 7·4. *Rana pipiens*, 8 °C. Fibre 8–5.

Steady-state activation and open channel properties

The steady-state conductance–voltage relation for K channels was little affected by TNBS treatment. Fig. 4*A* shows the conductance determined from tail current amplitude at the holding potential following depolarization to the potentials shown. In the range of potentials where few channels are activated, between −70 and −40 mV, there is a slight shift to more depolarized potentials, or a steepening of the relation. Aside from this minor effect, there was little change in the potential

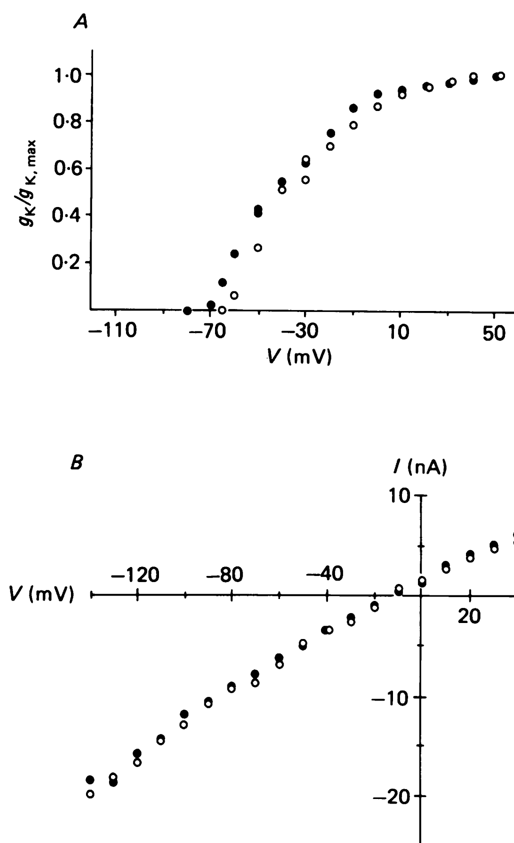


Fig. 4. *A*, normalized steady-state K conductance, $g_K/g_{K,max}$, as a function of potential before (●) and after (○) TNBS treatment. Fibre depolarized from the holding potential of −110 mV to the potentials indicated for 200–400 msec. Steady-state conductance was determined from the peak K current following repolarization to −110 mV. *Rana pipiens*, 6 °C. Fibre 1–10. *B*, instantaneous current–voltage relation before (●) and after (○) TNBS treatment. K current was activated by a 20 msec depolarization to −20 mV and the instantaneous current measured as the peak current following polarization to the potentials indicated for potentials positive to −80 mV. For more negative potentials, the instantaneous current was determined by fitting a single exponential function to the initial decline of the K current and extrapolating to the time of repolarization. Currents after TNBS are scaled by a factor of 0·91 to have the same value as the control at the holding potential, −110 mV. *Rana pipiens*, 8 °C. Fibre 8–5. P/4 pulse procedure.

dependence of K channel activation. The change in the conductance–voltage relation in this potential range was minimal, but was a consistent feature of the TNBS-treated fibres. In no experiment was the conductance–voltage relation shifted towards more hyperpolarized potentials.

Extremely long pulses (⩾ 400 msec) were necessary for the current to reach an apparent steady state for small depolarizations following TNBS. Because of the long depolarization the measurements may well be contaminated by other processes such as inactivation of the K channels or changes in the distribution of K. Either of these processes would tend to produce an apparent steepening of the conductance–voltage relation.

TNBS treatment did not affect the instantaneous current–voltage relation of K channels measured in K-Ringer, as shown in Fig. 4*B*. The current–voltage relation is linear in the control, as has been found previously in myelinated nerve fibres (Frankenhaeuser, 1962; Dubois & Bergman, 1977). TNBS treatment did not change the shape of the relation, or the reversal potential of the currents. There was no consistent effect of TNBS treatment on the maximum K conductance, and in seven randomly selected fibres the conductance was $112 \pm 11\%$ of control values. In

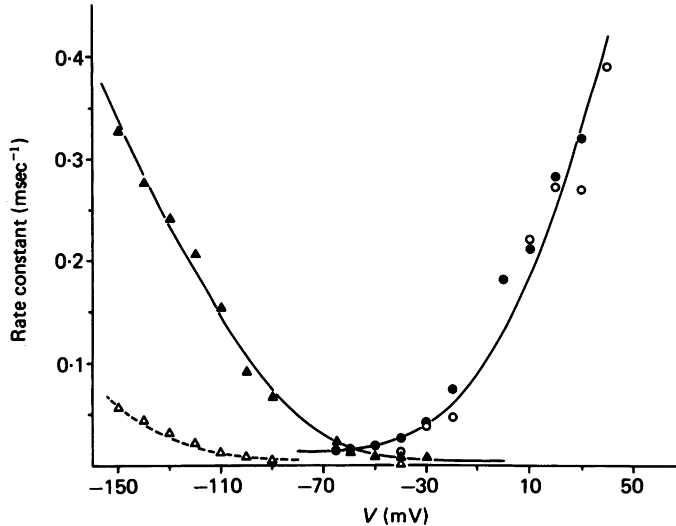


Fig. 5. Rate constants measured for the opening (circles) and closing (triangles) of K channels. Filled symbols are from control records, open symbols from measurements after TNBS treatment. Rate constants were determined by fitting the currents to a Hodgkin–Huxley type model. Continuous curves are calculated from eqns. (1) and (2) with $V_\beta = 83{\cdot}5$ mV and $k = 10$ mV. Dotted curve is calculated from eqn. (2) with $V_\beta = 148{\cdot}5$ mV and $k = 12{\cdot}2$ mV.

addition, the potency of block by TEA was unchanged by TNBS treatment. These results suggest that neither the open channel properties nor the number of functional K channels are altered by reaction of the nerve with TNBS.

TNBS affects channel closing more than channel opening

TNBS slows the closing K channels at hyperpolarized potentials and slows the opening of channels at moderately depolarized potentials, while leaving the opening rates at more positive potentials unaffected. In the light of these results, it seemed likely that the effects of TNBS could be explained as largely due to an alteration in the rate of K channel closing. Fig. 5 shows the rate constants for turn-on and turn-off of the K current determined by fitting an n^3, Hodgkin–Huxley model (Hodgkin & Huxley, 1952*b*), to the data. The points are data from a single fibre before and after exposure to TNBS. The activation rate constants, α_n, were minimally affected by the TNBS treatment. In contrast, TNBS greatly decreased the rate constants for closing of K channels, β_n. The continuous curves in Fig. 5 are empirical equations of the form

used by Dodge (1961, 1963) with parameters chosen to fit the control data. The equations used were

$$\alpha_n = \frac{0{\cdot}117\,(-V/12)}{\exp(-V/12)-1} + c, \tag{1}$$

$$\beta_n = \frac{0{\cdot}05\,[(V_\beta + V)/k]}{\exp[(V_\beta + V)/k]-1} + 0{\cdot}005, \tag{2}$$

where V is the membrane potential, $c = 0{\cdot}015$, $V_\beta = 83{\cdot}5$ mV and $k = 10$ mV. The dotted curve was calculated using eqn. (2) with $V_\beta = 148{\cdot}5$ mV and $k = 12{\cdot}2$ mV. This

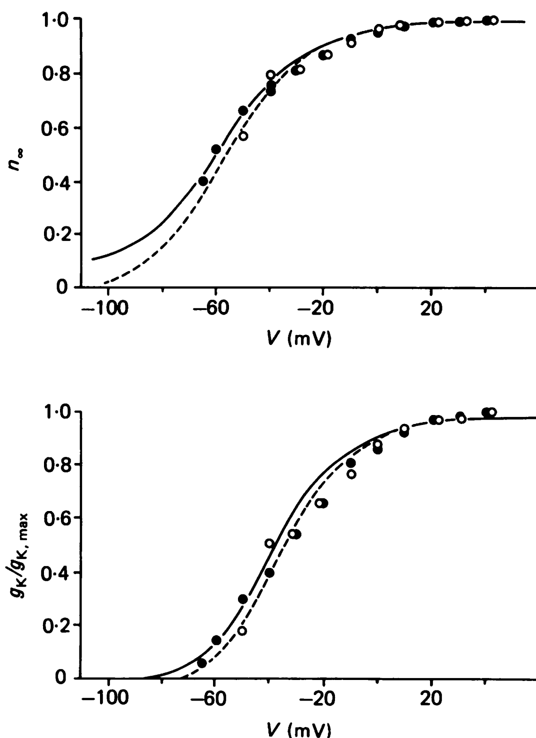


Fig. 6. Normalized peak K conductance, $g_K/g_{K,\max}$, and $n\infty$ measured before (●) and after (○) TNBS treatment. Continuous lines are calculated from eqns. (3) and (4) with $V_\beta = 83{\cdot}5$ mV, $k = 10$ mV, and $c = 0{\cdot}015$. Dotted lines are calculated with $V_\beta = 148{\cdot}5$ mV, $k = 12{\cdot}2$ mV and $c = 0$.

is equivalent to shifting the potential dependence of β_n by 65 mV to more hyperpolarized potentials. As shown in Fig. 5, altering β_n gives a good fit to the data obtained following TNBS treatment. The values of α_n following TNBS treatment could be fitted by reducing c in eqn. (1) to 0 (not shown). The magnitude of the apparent shift in β_n depends upon the extent of reaction with TNBS.

Fig. 6 shows the normalized maximum K conductance and n_∞ from the same fibre. The curves are calculated from the equations

$$n_\infty = \frac{\alpha_n}{\alpha_n + \beta_n}, \tag{3}$$

$$g_K/g_{K,\max} = n_\infty^3. \tag{4}$$

The continuous curves were calculated using parameters derived for the control data. The dotted curves were calculated with α_n and β_n altered to fit the TNBS data as discussed above. The equations predict only a minimal change in the steady-state conductance as is observed.

These changes in β_n and α_n are sufficient to reproduce the effects of TNBS on the time course of K currents. Fig. 7 shows currents measured in a fibre before and after TNBS, and calculated currents with α_n and β_n altered to simulate the effects of TNBS.

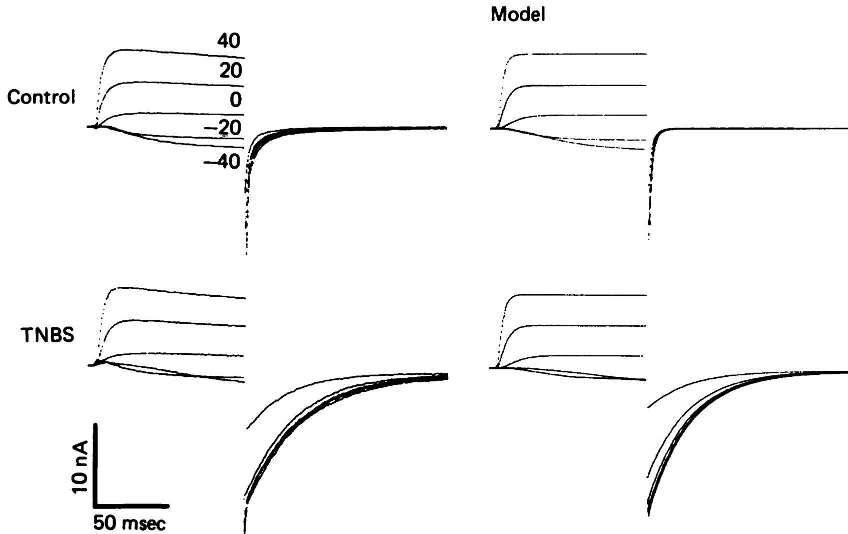
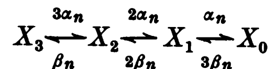


Fig. 7. Left: K currents recorded before and after exposure to 20 mM-TNBS, pH 9·5. Fibre was depolarized for 100 msec to the potentials indicated. Currents following TNBS treatment were scaled by a factor of 1·15 to have the same peak amplitude at +40 mV as the control. *Rana pipiens*, 8 °C. Fibre 8–6. Right: currents calculated from the four-state sequential model as described in the text.

The currents were calculated from a four-state sequential kinetic model equivalent to the Hodgkin–Huxley formalism:



$$X_3 \underset{\beta_n}{\overset{3\alpha_n}{\rightleftharpoons}} X_2 \underset{2\beta_n}{\overset{2\alpha_n}{\rightleftharpoons}} X_1 \underset{3\beta_n}{\overset{\alpha_n}{\rightleftharpoons}} X_0$$

X_3–X_1 represent closed states of the channel and X_0 represents the open, conducting state. As seen in Fig. 7, the -65 mV shift in β_n and the minor reduction in α_n reproduce all of the kinetic features of TNBS. Channel opening is slowed for moderate depolarizations, and upon repolarization channel closing is tremendously slowed. The major differences in the actual current records and the calculated currents are a decline in the current during large depolarizations and a fast component of the tail current following these depolarizations that are present in the real currents and not in the model currents. These effects may be the result of external accumulation of K, inactivation of K channels, and possibly a small population of unreacted channels. Thus, the kinetic effects of TNBS on K channels can be modelled by a large negative shift in the potential dependence of the closing rate constant.

The reaction of TNBS with K channels

TNBS treatment slows the closing kinetics of K channels in two ways: (1) by converting rapidly closing channels to more slowly closing channels and (2) by decreasing the rate of closing of the slowly closing channels. The time course of development of these effects on K tail currents is shown in Fig. 8. The top record shows the currents recorded in high pH, K solution before TNBS treatment. As has been previously reported, the K tail current recorded with a high external K

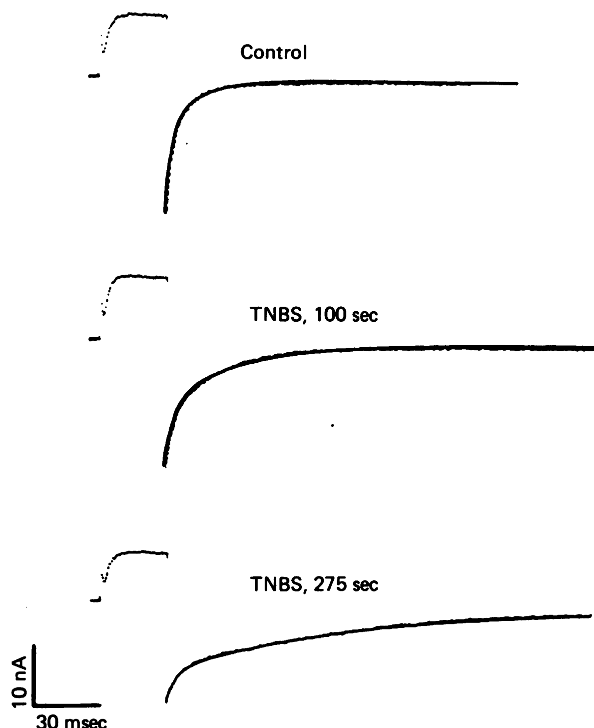


Fig. 8. Currents recorded during and after a 30 msec depolarization to +40 mV before and during exposure to 20 mM-TNBS at pH 9·5 for 100 sec and 275 sec. Dotted traces are actual current records. Continuous traces are plots of the sum of two exponential functions, least-squares fitted to the decline of K current. For the control record, the fast component had amplitude, A_f, of 8·8 nA and time constant, τ_f, of 4·3 msec. The slow component had an amplitude, A_s, of 1·6 nA, and a time constant τ_s, of 40 msec. 100 sec after exposure to TNBS $A_f = 5{\cdot}1$ nA, $\tau_f = 4{\cdot}4$ ms and $A_s = 4{\cdot}8$ nA, $\tau_s = 36$ msec. After 275 sec exposure, the values were $A_f = 2{\cdot}5$ nA, $\tau_f = 4{\cdot}2$ msec, $A_s = 5{\cdot}6$ nA, $\tau_s = 90$ msec. Holding potential −110 mV. *Rana catesbiana*, 6 °C. Fibre 9–6.

concentration shows at least two components (Dubois, 1981*b*). The magnitude of the slow component of the tail current was increased by raising the pH from 7·4 to 9·5 or 10. The control tail current could be fitted well by the sum of two exponentials, as shown in Fig. 8. In the control, 85% of the current decayed rapidly and 15% decayed slowly. Exposure of the fibre to TNBS at pH 9·5 resulted in a rapid decline in the magnitude of the fast component of the tail current and a concomitant increase in the magnitude of the slow component. After 275 sec exposure to TNBS, the fast

component of the tail current comprised only 30 % of the total amplitude while the slow component increased to 70 %.

High pH accelerates the TNBS reaction. Fig. 9*A* shows the fraction of the total tail current that decays rapidly during continued exposure to TNBS at pH 9·5 or pH 10·5. At both pHs, the fraction of channels closing rapidly, $A_f/(A_f+A_s)$, declines to a steady level of 30 %. Raising the pH from 9·5 to 10·5 decreased the time constant for the decline to $\frac{1}{3}$ that measured at pH 9·5. This strong pH dependence of the TNBS reaction indicates that TNBS reacts with an amino group having a high pK (Means *et al.* 1972). The TNBS treatment did not significantly affect the time constant of the remaining fast tail current, suggesting that the fast component represents current through unmodified channels.

TNBS treatment slows the time constant for decline of the slow tail current in addition to increasing its magnitude. Fig. 9*B* illustrates the further increase in τ_s as reaction with TNBS proceeds. In the fibre treated at pH 10·5 τ_s reaches a steady level. However, in the fibre treated at pH 9·5, τ_s continues to increase throughout the duration of the TNBS treatment. Again, the effects of TNBS were more rapid with treatment at pH 10·5 than for treatment at pH 9·5.

Multiple sites seem to be involved in the actions of TNBS. The time course of conversion of channels from fast to slow is more rapid than the time course of slowing of the channel closing (compare Figs. 9*A* and *B*). For the fibre treated at pH 10·5, both of these processes could be adequately fitted by a single exponential function. The time constant for conversion of channels from fast to slow was 27 sec, while that for changing the closing rate of the slow channels was 49 sec, suggesting that different sites are involved in these effects. Prolonged reactions with TNBS continues to slow the closing rates of modified channels after the magnitude of the fast component has declined to its steady-state level.

The effects of increasing external pH

The kinetic effects of TNBS on K channels cannot be mimicked by raising the external pH in the range from 7·4 to 10·5. Fig. 10 shows the effects of increasing the external pH on the voltage dependence of K channel activation and closing times and on steady-state activation. Increasing the pH from 7·4 to 9·5 reversibly shifted the potential dependence of the kinetic and steady-state parameters to more hyperpolarized potentials. The steady-state activation relation shows an inflexion suggesting that there are two populations of K channels differing in their potential dependence for activation (Dubois, 1981*b*). Increasing the pH appears to shift the potential dependence of the channels activating at more hyperpolarized potentials more than those activating at more positive voltages. Exposure of the nerve to pHs above 10·5 had effects qualitatively similar to those of TNBS (results not shown). However, the effects at very high pH were irreversible, and so seem to be due to chemical changes in the membrane beyond a simple deprotonation. These results suggest that if TNBS modifies K channel gating by neutralizing some charged group, this group has a pK greater than 10·5.

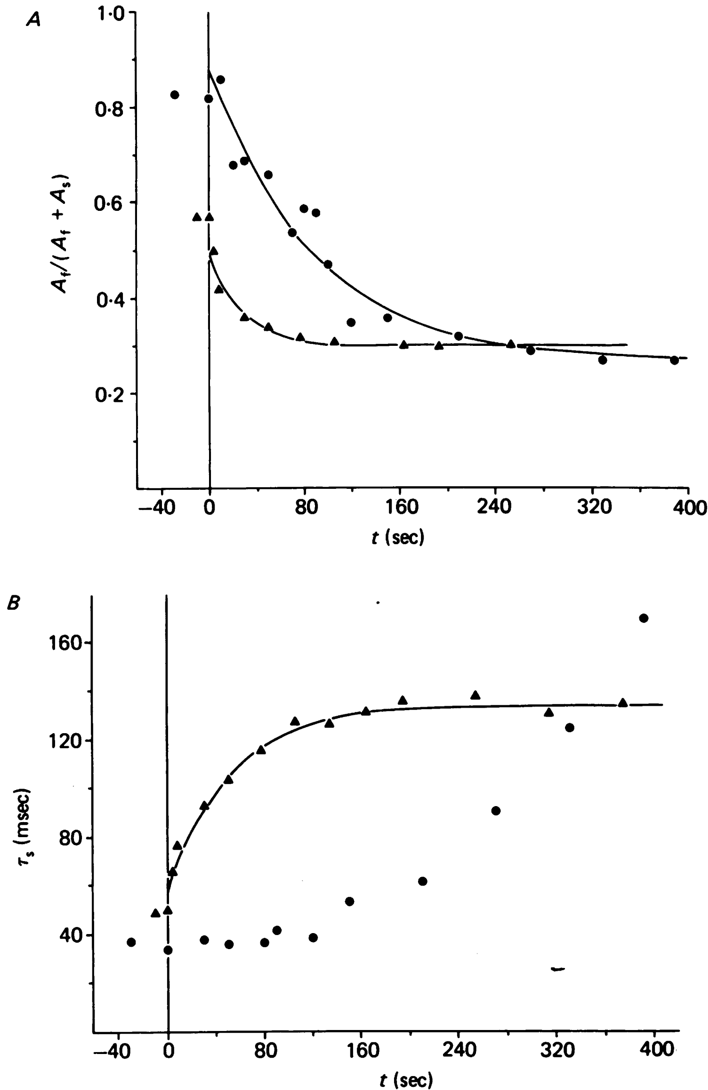


Fig. 9. *A*, change in the proportion of rapidly and slowly closing K channels with exposure to TNBS. Tail currents were fitted with the sum of two exponential functions as in Fig. 8. Ordinate is the fraction of the channels closing rapidly, $A_f/(A_f+A_s)$. Abscissa is the duration of exposure to TNBS. At time = 0, the solution was changed from pH 9·5 K-Ringer (●) or pH 10·5 K-Ringer (▲) to a solution of the same pH with 20 mM-TNBS added. Curves are a least-squares fit of an exponential function to the rate of decline of the proportion of rapidly closing channels. The time constant for the rate of decline was 85 sec at pH 9·5 and 27 sec at pH 10·5 (●) Fibre 9–6, (▲) Fibre 10–3. *B*, time constant of the slow component of K tail current τ_s, as a function of duration of exposure to TNBS at pH 9·5 (●) or pH 10·5 (▲). At time 0, the solution was changed from pH 9·5 or pH 10·5 K-Ringer to the same solution plus 20 mM-TNBS. Curve is an exponential function with time constant 49 sec which was least-squares fitted to the pH 10·5 data points. Same fibres as part *A*.

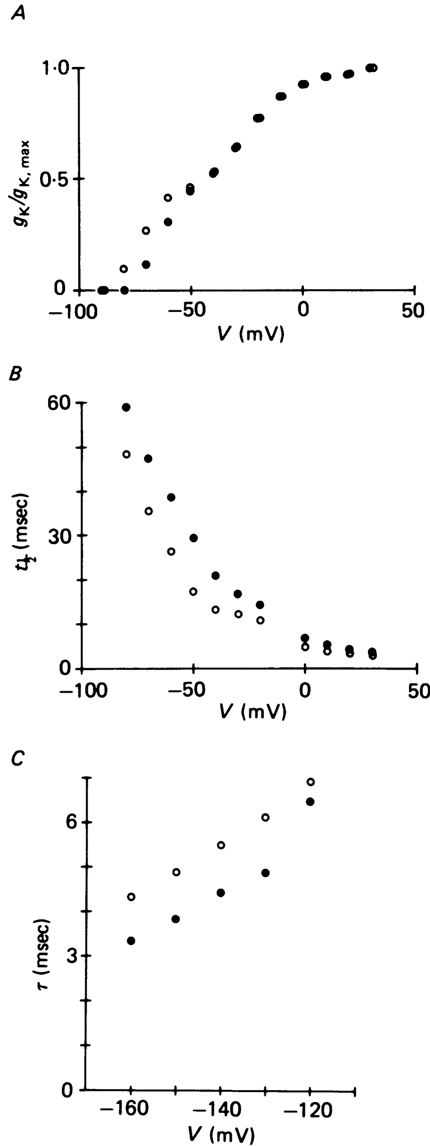


Fig. 10. Effects of increased pH on K currents measured at pH 7·4 (●) and pH 9·5 (○). External solutions were K-Ringer or high pH, K-Ringer as shown in Table 1. *Rana pipiens*, 6 °C, Fibre 9–5. *A*, normalized steady-state K conductance, $g_K/g_{K,max}$, as a function of potential. Fibre depolarized from the holding potential of −120 mV to the potentials indicated for 100 msec. Steady-state conductance was determined from the peak K current following repolarization to −120 mV. *B*, the time to reach half maximum amplitude, $t_{\frac{1}{2}}$. The fibre was depolarized from the holding potential of −120 mV to the potentials indicated. *C*, time constants for the rate of decline of K current during repolarization to the potentials shown. K tail currents were fitted with a single exponential function with time constant τ by a least squares method. K currents were activated by a 20 msec depolarization to −10 mV. P/4 pulse procedure.

Effects of external ions

Rubidium. The gating of K channels is influenced by the ionic composition of the solution bathing the fibre (Århem, 1980; Swenson & Armstrong, 1981). Fig. 11*A* compares tail currents recorded in K-Ringer and in Rb-Ringer in a normal nerve fibre. The K channels close 2 times slower in the Rb solution at −90 mV. Rb reversibly slows the closing of K channels over the entire potential range from −80 to −150 mV, as illustrated in Fig. 11*B*. The effect of Rb on the time constants for channel closing

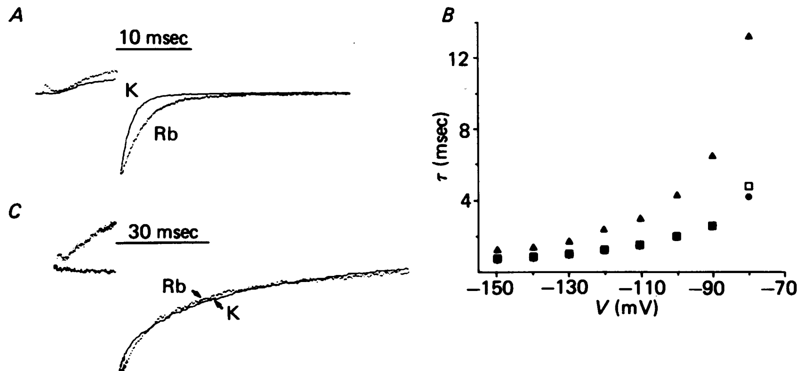


Fig. 11. *A*, currents in K-Ringer (continuous tracing) and with all of the K replaced by Rb (dotted trace) in an untreated fibre. Current was activated by a 10 msec depolarization to +30 mV, then the potential was returned to the holding potential of −90 mV. The Rb current record was scaled by 1·49 to have the same tail current amplitude as the control. *Rana catesbiana*, 7 °C. Fibre 5–7. *B*, time constant, τ, of a least-squares exponential fit to the decline in tail current as a function of potential in Rb-Ringer (▲) and in K-Ringer before (●) and after (□) the Rb measurements. Same fibre and pulse protocol as part *A*. *C*, currents in K-Ringer (continuous tracing) and Rb-Ringer (dotted trace) after treatment with TNBS. Current was activated by a 20 msec depolarization to +10 mV (Rb) or −10 mV (K). Tail currents recorded at the holding potential of −110 mV. Rb current record scaled by 1·53 to have the same tail current amplitude as the control. *Rana pipiens*, 8 °C. Fibre 8–6.

is greatest at more depolarized potentials. Rb did not alter the time course of opening K channels in the potential range from −50 to +50 mV. In Rb-Ringer, there was a small but reproducible shift in the steady-state conductance–voltage relation to more hyperpolarized potentials. The normalized conductance–voltage relation measured from tail currents was reversibly shifted by −11·4 mV ± 3·5 mV (mean ± S.E. of mean, $n = 5$) in Rb-Ringer compared to K-Ringer. The kinetic changes induced by Rb are consistent with a selective decrease in the closing rate constant for K channels, β_n.

Following TNBS treatment, Rb does not greatly affect the time course of K currents. Fig. 11*C* shows tail currents recorded in K-Ringer and Rb-Ringer after reaction of the fibre with TNBS. The closing of K channels was slowed by the TNBS treatment in both solutions. In contrast to the results in control fibres, there is no significant difference in the time course of K channel closing in Rb-Ringer and K-Ringer following reaction with TNBS. TNBS treatment also removed the effect

of Rb on the steady-state conductance–voltage relation; the potential dependence of K channel activation was the same in K- or Rb-Ringer. In summary, we find that external Rb slows the closing of K channels and shifts the potential dependence of activation in control fibres, and that these kinetic effects of Rb are absent after reaction with TNBS.

K concentration. Previous studies have shown that increasing the external K concentration slows the closing of K channels in myelinated nerve (Dubois, 1981*a*). In agreement with these results we find that replacing 80 % of the external K with Na or TMA resulted in a slight increase (0–30 %) in the rate of closing of K channels. Reduction of the external K concentration did not affect the rates of opening K channels when K was replaced by either Na or TMA. The observed changes in the shape of the steady-state conductance–voltage relation in the 20 % K solutions were all consistent with increased effects of ion accumulation and were not analysed further. Thus, in control fibres, reducing the K concentration has only minor effects on K channel kinetics.

Decreasing the external K concentration reduced the K permeability as has been previously reported (Dubois & Bergman, 1977). Replacing 80 % of the K with either Na or TMA reduced the permeability measured from inward currents to 20–35 % of the values measured in the high K solution. The permeability measured from outward currents was differentially affected by the two substitute cations. With TMA replacing K, the permeability was approximately 50 % of that measured in high K, while in the Na solution the permeability was 80 % of that in high K. These results suggest that both Na and TMA block inward current through K channels to a similar extent, but that TMA is more effective than Na in blocking outward current.

Reducing the external K concentration has large kinetic effects following TNBS treatment. Fig. 12*A* shows currents recorded in an untreated fibre in K-Ringer and in 20 % K, Na, The time course of channel closing is similar in the two solutions before TNBS treatment. Part *B* illustrates that following reaction of the fibre with TNBS channel closing is much slower when measured in 100 % K than when measured in 20 % K. Fig. 12*C* shows time constants for channel closing in a TNBS-treated fibre in 100 % K and 20 % K. TNBS increased the closing time constants 2–3 fold when measured in 20 % K solutions, in contrast to the up to 10-fold increases in closing time constant in 100 % K solutions. The channel opening kinetics following TNBS treatment were the same whether measured in 100 % K, 20 % K, Na, or 20 % K, TMA. Thus, TNBS treatment seems to increase the sensitivity of K channel closing rates to the external K concentration.

Reaction of the nerve membrane with TNBS does not change the shape of the instantaneous current–voltage relation for K channels in Rb or reduced K solutions. Fig. 13*A* shows the instantaneous current–voltage relation measured in K-Ringer and Rb-Ringer following TNBS treatment. As in the control fibres, the relation is linear in both solutions over the potential range from -140 mV to $+20$ mV. The change in reversal potential in going from K Ringer to Rb averaged $-4{\cdot}3$ mV in both control ($\pm 2{\cdot}9$ mV, $n = 3$) and treated ($\pm 3{\cdot}9$ mV, $n = 3$) fibres, corresponding to a permeability ratio, P_{Rb}/P_K, of 0·84. In addition, there is no change in the maximum conductance of K channels in Rb-Ringer after TNBS. Fig. 13*B* shows the instantaneous current–voltage relations measured in 20 % K, Na after TNBS. The relation is linear

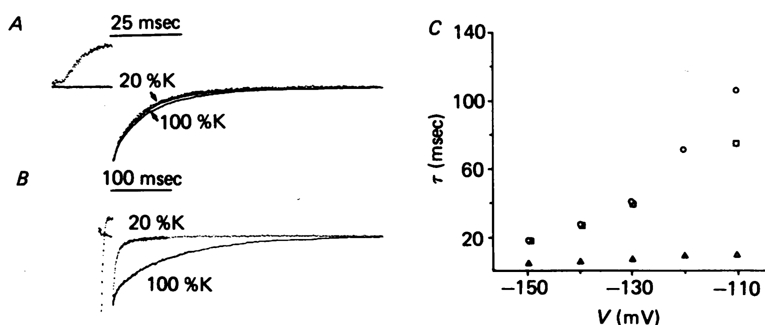


Fig. 12. *A*, currents in an untreated fibre in K-Ringer (100 % K: continuous tracings) and with 80 % of the K replaced by Na (20 % K, Na: dotted tracing). The two 100 % K records were taken before and after exposure to 20 % K. The second 100 % K record was scaled by 1·1 to have the same amplitude as the first. The current record in 20 % K was scaled by 4·28. Currents recorded during a 20 msec depolarization to −10 mV and repolarization to the holding potential of −110 mV. *Rana pipiens*, 6 °C. Fibre 10–8. *B*, currents in 100 % K (continuous tracing) and 20 % K (dotted tracing) after treatment with TNBS. Current record in 20 % K scaled by 1·32 to have the same tail amplitude as the control. Current recorded during a 20 msec pulse to −20 mV and repolarization to the holding potential of −110 mV. pH of all solutions was 9·5. *Rana catesbiana*, 6 °C. Fibre 9–6. *C*, time constants, τ, of a least-squares fit of a single exponential function to the tail currents in 100 % K (○, □) and 20 % K, Na (△) all after treatment with TNBS. The two sets of 100 % K data were taken before and after the measurements in 20 % K, Na. Time constants in 100 % K were fitted to the slow component of the tail current. Same fibre and pulse protocol as *B*.

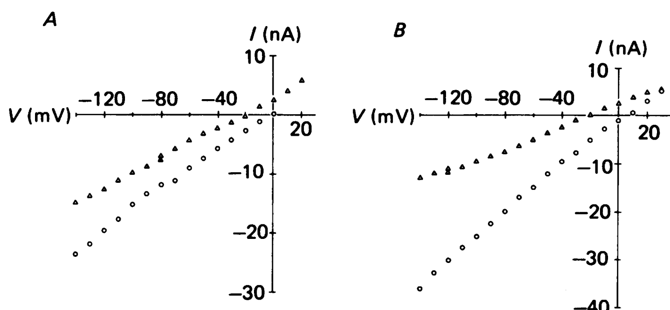


Fig. 13. Instantaneous current–voltage relations measured following reaction with TNBS. K current was activated by a 20 msec conditioning depolarization to the reversal potential. The instantaneous current upon polarization to the potentials shown was determined by back extrapolation to time zero of a single exponential function fitted to the decline of the current for potentials negative to −80 mV (*A*) or −110 mV (*B*). For more positive potentials, the current measurement was made 100 μsec following the pulse. All measurements made using the P/4 pulse procedure. *A*, current–voltage relation in K-Ringer (○) or with all the K replaced by Rb (△). *Rana pipiens*, 8 °C. Fibre 8–6. *B*, current–voltage relation in K-Ringer (○) or with 80 % of the K replaced by Na (△). *Rana pipiens*, 8 °C. Fibre 9–2.

at potentials from +20 to −80 mV, but the conductance decreases somewhat at more negative potentials. This effect is seen to a similar extent in control fibres and may reflect block of K channels by external Na at negative potentials. The K conductance is decreased in the low K solution after TNBS, as is also seen in untreated fibres (Dubois & Bergman, 1977). Thus, there is no evidence from our experiments that TNBS changes the permeability properties of the open channel.

As in control fibres a decrease in the external K concentration reduces the magnitude of the K current less than is predicted for independent movement of ions in the TNBS-treated fibres. This is shown in Table 2 in which the extent of block is calculated for various external K concentrations. Also shown in Table 2 is the degree of slowing of the closing rate of K channels in the various solutions. The amount of change in the channel closing rate parallels the extent of block of the channel conductance. This correspondence suggests that K channels may be prevented from closing by the presence of bound permeant ions.

TABLE 2. Current magnitude and closing time constant with different [K]

Relative [K]	20%	n	50%	n	80%	n
$I_{K,ind}/I_{K,exp}$	0·40 ± 0·03	12	0·78 ± 0·02	4	0·95 ± 0·04	3
$\tau_{x\%K}/\tau_{100\%K}$	0·37 ± 0·07	9	0·55 ± 0·12	4	0·80 ± 0·05	3

Data are from TNBS treated fibres. $I_{K,ind}$ is the current predicted from the independence principle (Hodgkin & Huxley, 1952*a*) from measurements in 117·5 mM-K-Ringer for the reduced K concentrations shown. $I_{K,exp}$ is the actual current measured in the same fibre in the indicated concentration of K. Peak inward currents were measured at the holding potential of −110 mV. $\tau_{100\%K}$ is the time constant of an exponential function fitted to the decline of K current at the holding potential in 117·5 mM-K-Ringer. $\tau_{x\%K}$ is the same parameter measured in the concentration of K shown. Both current and time constant measurements were made following a 20–100 msec depolarization to −20 to −10 mV to activate the K conductance. All measurements were made at pH 7·4. Na was used to replace K in the test solutions. All data given as the mean ± S.E. of the mean.

DISCUSSION

In this paper we have described an irreversible modification of K channel gating kinetics following treatment of the nerve fibre with externally applied TNBS. TNBS treatment slows K tail currents upon repolarization and also slows the rate of activation for moderate depolarizing potentials, but does not alter the time course of activation for large depolarizations. The changes in K channel gating produced by TNBS cannot be due to a simple change in the surface potential of the fibre. Changes in the surface potential might be expected, because TNBS reacts with primary amino groups which are normally positively charged and converts them to neutral derivatives, and because we have previously demonstrated that TNBS shifts the rates and steady-state values of Na channel inactivation, consistent with an increase in the negative surface potential seen by the Na channel inactivation gating process (Cahalan & Pappone, 1981). In contrast to the results for Na channels TNBS has differential effects on opening and closing kinetics of K channels in myelinated nerve fibres, and therefore some more direct action of TNBS upon the K channel gating mechanism is needed to account for our results. Our kinetic analysis suggests that TNBS alters the closing rate constant, β_n in the Hodgkin–Huxley model,

without greatly altering the opening rate constant. Since TNBS is membrane impermeant and specific for amino groups we conclude that an externally accessible charged amino group plays an important role in the closing of K channels.

A molecular interpretation of the kinetic effects of TNBS

Since the reaction of TNBS with amino groups changes the charge of the reacted group, the kinetic effects of TNBS could be due to the modification of mobile K channel gating charges. However, if reaction of the membrane with TNBS did alter the net gating charge of the channel, both opening and closing rates and the voltage dependence of the rate constants would change. TNBS did not affect the voltage dependence of the opening rate constants, so it is unlikely that TNBS acts by directly modifying K channel gating charge.

Alternatively, TNBS could act by modifying membrane groups which interact with channel gating charges. If part of the gating charge is positive, TNBS could act to stabilize the gating charge at the outside of the membrane, in the open state, by reducing the positive charge at the surface of the membrane. Fig. 14*A* shows a one barrier energy diagram representing this mechanism, analogous to those used to describe the effects of adrenaline, pH and divalent cations on K channel gating (Tsien, 1974; Shrager, 1974; Gilly & Armstrong, 1982). In this scheme TNBS treatment would reduce the energy of the open state, G_1, without changing the energy of the closed state, G_2, or the transition energy for moving from the closed to open state, $G_2 - G_{12}$. Stabilization of the open state would slow channel closing rates and opening rates, as seen following TNBS, but would also shift the steady-state conductance–voltage relation to more hyperpolarized potentials. No such shifts were found following TNBS treatment. Changing the barrier height, G_{12}, changes both opening and closing rate constants and therefore cannot reproduce the effects of TNBS. On electrostatic grounds a negative gating charge at the outside of the membrane, in the closed state, would be expected to be less stable following TNBS treatment, and channel opening would be speeded. We therefore conclude that the effects of TNBS cannot be explained by modification of a one barrier energy transition for channel gating.

In order to fit both the kinetic and the steady-state data it is necessary to postulate a more complicated energy profile for the movement of K channel gating charge. Fig. 14*B–D* shows a two barrier energy diagram which can reproduce the effects of TNBS on channel gating. Fig. 14*B* shows the shape of the barrier at the potential for which the open and closed states of the channel are equally probable before and after TNBS. The energy difference for the first transition in opening the channel, $G_3 - G_{23}$, is larger than that of the second transition, $G_2 - G_{12}$, to account for the fact that the last step in opening is not the slowest for K channels (Gilly & Armstrong, 1982; M. D. Cahalan & P. A. Pappone, unpublished observations). In this model TNBS raises G_{12}, the energy of the transition state for moving the channel from open to closed without affecting the energy of the open or closed states. Increasing G_{12} slows both opening and closing rates at this potential without changing the equilibrium distribution between the states. Parts *C* and *D* of Fig. 14 illustrate the effect of membrane potential on the shape of the energy barrier profile. At depolarized potentials the rate limiting transition is from G_3 to G_{23}. The energy change involved

in this transition is unaffected by TNBS, so there is no change in the rate of channel opening. At hyperpolarized potentials the rate limiting step for closing is the transition from state G_1 to G_{12}. With G_{12} higher following TNBS treatment channel closing is slowed. Thus, the two barrier model can qualitatively account for the kinetic effects of TNBS, as illustrated in Fig. 15*A*. The model predicts no change in the

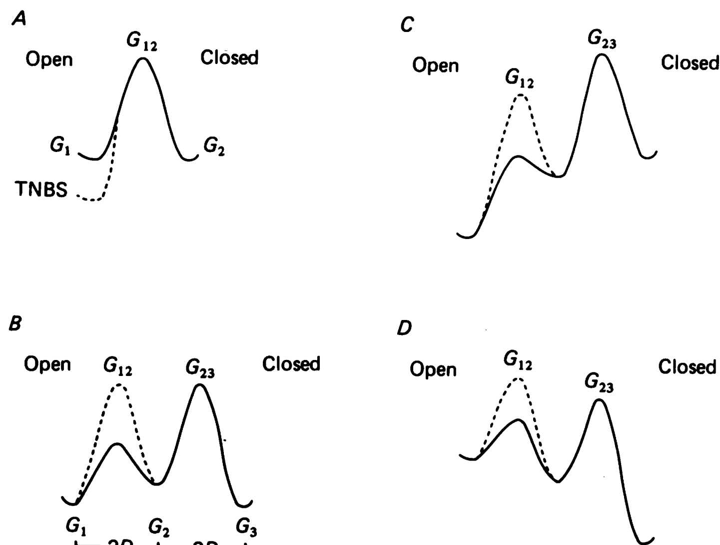


Fig. 14. Barrier models for K channel gating developed by analogy to similar models for ion permeation (Hille, 1975; Begenisich & Cahalan, 1980). Energies, G, of the barriers and wells are given in dimensionless units, multiplied by R, the gas constant, and T, the absolute temperature. The wells represent the energy of the open and closed states, and the barrier between them represents the energy of the transition between the states. The rates of movement from one state to the other are proportional to the difference in energy between the starting state and the transition state. For all calculations barriers were evenly spaced in the membrane field with D, the charge-distance product, equal to 20 V^{-1}. The resultant rate constants were scaled by a factor of 30 (analogous to the frequency factor, Hille, 1975), in order to approximate the time course of measured currents. *A*, one-barrier model for K channel gating. Continuous line represents control energy profile. G_1 is the open state and G_2 the closed state. G_{12} represents the energy of transition between the open and closed states. Dotted line shows change induced if TNBS reduces the energy of the open state. *B*, two-barrier model for K channel gating at -50 mV. G_1 represents the energy of the open state, G_2 and G_3 are closed states. G_{12} and G_{23} are transition energies between the states. Membrane potential is assumed to add linearly with distance from outside to inside the membrane. TNBS is postulated to raise the energy of transition state G_{12}, between the open state and the first closed state, as shown by the dotted line. *C*, two-barrier model at 0 mV. *D*, two-barrier model at -100 mV.

steady-state distribution of channels when G_{12} is increased, as was observed experimentally. Attempts to reproduce the effects of TNBS by altering the energies of G_1, G_2, G_{23} or G_1 and G_2 were unsuccessful (see for example Fig. 15*B*). Within the framework of this simple model, then, it seems likely that TNBS acts by increasing the energy of transition from the open state to the first closed state, without affecting the energy of the open or closed states.

Other kinetic models might equally well account for the observed currents, but

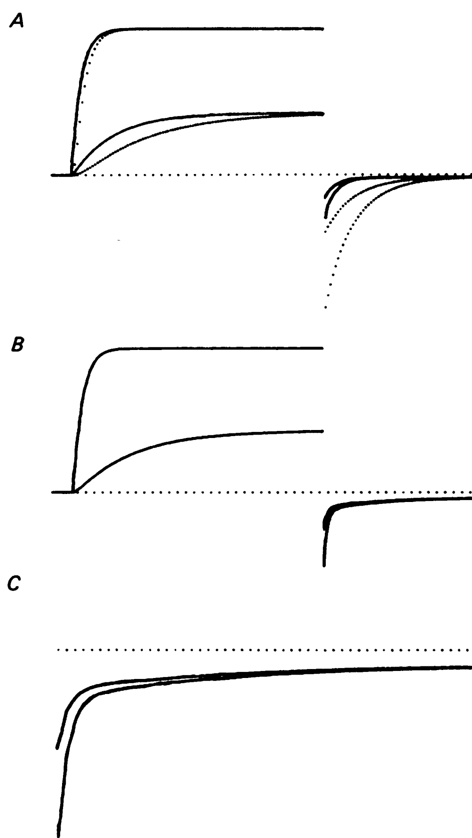


Fig. 15. *A*, calculated currents from the two-barrier model above. Currents shown by continuous lines: $G_1 = -4$, $G_{12} = 0$, $G_2 = -1$, $G_{23} = 5$, $G_3 = 0$. Currents shown by dotted lines: same parameters except G_{12} increased to 3. Calculations represent currents during pulses to −50 mV and +50 mV and during the return to a holding potential of −100 mV. *B*, calculated currents from the two-barrier model with $G_1 = -5$ and $G_2 = -3$ and other parameters same as for the currents shown by continuous lines in part *A*. *C*, Same tail currents as part *B* with the amplitude scaled by two and a 3·3-fold faster time base. Starting distribution of channels for the smaller tail current was 15·5 % in state G_3, 42·2 % in state G_2, and 42·2 % in state G_1. For the larger amplitude current 0 % were in state G_3, 7 % were in state G_2, and 93 % were in state G_1. 47 % of the smaller tail current deactivated rapidly and 70 % of the larger tail current deactivated rapidly.

some models for TNBS action can be ruled out. Consider for example, a model in which TNBS causes the K channel to have an additional open state, accessed in sequential fashion from the normal open state:

$$\text{closed} \rightleftharpoons \text{closed} \rightleftharpoons \text{closed} \rightleftharpoons \text{open} \rightleftharpoons \text{open}_{\text{TNBS}}.$$

This could result in a slowing of the tail currents but would not account for the observed slowing of activation for small depolarizing potentials.

The opening and closing of K channels are differentially altered by external protons in crayfish axon (Shrager, 1974) and external zinc and other divalent cations in squid axon (Gilly & Armstrong, 1982). These agents slow K channel opening, have little

effect on channel closing, and have an intermediate effect on the steady-state conductance. The proposal has been made that these cations bind to an external site and act electrostatically to slow the inward movement of negative gating charge during K channel opening. According to this view, channel closing is only slightly changed by protons, zinc, and other divalents, because open K channels have their negative gating charges located electrically remote from the proton-zinc site. The differential effects of TNBS on K channel opening and closing could similarly be due to the interaction of gating charges with TNBS-modified sites on the surface of the membrane. However, it would be necessary to postulate a component of positive gating charge moving outward during K channel opening, as well as a change in the energy of a transition state for the gating process, rather than a change in the energy of closed or open states.

There are several possible means by which TNBS modification of the membrane could change the energy of a transition state of the K channel. Quite probably the closing of K channels involves the reorientation within the membrane of both positively and negatively charged groups. Outward movement of negative charges may normally be made more energetically favourable by interaction with positively charged membrane components. These membrane components would then normally act as a catalyst for channel opening. TNBS could make the outward movement of negative charge more difficult through removal of the stabilizing positive charges. Reaction with TNBS puts a relatively large hydrophobic group on the modified amine. This could change the energy of the transition state through either steric or hydrophobic interactions occurring during the conformational changes of channel closing. Finally, TNBS could act by changing physical characteristics of the membrane, such as its fluidity or compressibility. This would raise the energy of a transition state that involved volume changes of the channel. Whatever mechanism is operating, the barrier model analysis suggests that the TNBS-modified transition state is rate limiting only at more hyperpolarized potentials.

Permeant ion effects on gating

Our results in normal fibres not treated with TNBS confirm and extend previous reports of permeant ion effects on K channel gating (Århem, 1980; Swenson & Armstrong, 1981; Matteson & Swenson, 1982). In Rb external solution we observe a hyperpolarizing shift in the steady-state conductance–voltage relation, as well as a slowing of tail currents, with minimal effects on rates of channel opening. These effects can be summarized within a single barrier model of the gating process by postulating that Rb reduces the energy of the open state relative to the energy of transition to the closed state. After TNBS treatment the effect of Rb upon channel gating are not manifest. Our modelling in the preceding section suggests that TNBS increases the height of the energy barrier for the open to closed channel transition. If Rb acts to lower the energy of the open state by the same amount following TNBS treatment, there would be a smaller fractional change in the open to transitional energy difference produced by Rb after TNBS. This could account for the lack of a Rb effect on channel kinetics.

Increasing the external K concentration produces minor kinetic effects in control fibres but greatly slows the closing of K channels after TNBS treatment. TNBS appears to enhance the effect of external K on the closing rate constant. In previous

studies of permeant ion effects upon gating, correlations have been made between channel closing rates and either instantaneous *I–V* shapes (Swenson & Armstrong, 1981) or acetylcholine-activated single channel conductance (Marchais & Marty, 1979; Gage & Van Helden, 1979; but see also Adams, Nonner, Dwyer & Hille, 1981 for exceptions to the above finding). These results have been interpreted as evidence that occupancy of open channel sites by permeant ions may inhibit the closing of the channel. In our experiments the enhanced K ion effect brought about by TNBS is probably not due to altered open channel properties leading to increased K occupancy, because: (a) comparable saturation effects were observed as external K was raised, both before and after TNBS treatment; (b) the Rb/K selectivity ratio was not altered by TNBS as measured by shifts in reversal potential when external K was replaced by Rb; (c) the instantaneous *I–V* relation was not altered by TNBS; and (d) block by external TEA was not altered by TNBS. However, the possibility remains that TNBS treatment enhances the interaction between ions in the open channel and the gating mechanism.

Functional subpopulations of K channels

Several investigators have suggested the existence of at least two populations of K channels in the node of Ranvier on the basis of kinetic evidence from tail currents (Ilyin, Katina, Lonskii, Makovsky & Polishchuk, 1977; Dubois, 1981*b*) and current fluctuations (Conti, Hille & Nonner 1982). We have also observed multiple exponential time constants in the tail currents (Figs. 8 and 9) and conductance–voltage curves that exhibit bumps or inflexion points suggestive of two populations of channels with slightly different voltage dependences (Fig. 10). However, we find that the two barrier model for K channel gating produces multi-exponential tail current kinetics, as shown in Fig. 15*C*. Variations in the initial distribution of channels among the three states changes the proportion of the tail current showing fast and slow closing kinetics. Complex tail current kinetics and apparent differences in the voltage dependence for activation of slowly and rapidly closing channels are therefore insufficient to demonstrate the presence of more than one type of K channel.

In our experiments with altered pH we noted that high pH (up to 10·5) reversibly increases the magnitude of the slow component of tail currents, suggesting perhaps that slow and fast channels might be interconvertible. The slow phase of K tail current is resistant to block by 4-aminopyridine (4AP) at concentrations that block the fast K channels in normal fibres (Dubois, 1981*b*). We tested for block of slow tail currents induced by TNBS and found that 4AP was still effective in reducing the K current, although with somewhat reduced potency compared to controls. It is intriguing to speculate that interconversion of K channels between slow and fast modes of operation might represent a physiological mechanism to regulate properties of neuronal firing in some systems. Krylov & Makovsky (1978) suggested that the slow channels may play a role in spike frequency adaptation, although Dubois (1981*b*) attributes some of the differences between spike firing properties of sensory and motor fibres to different proportions of two populations of fast K channels, rather than the slow channels. Our observations suggest that chemical interconversion of fast K channels to slow channels may involve modification of a lysine residue accessible to the external solution.

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REFERENCES

ADAMS, D. J., NONNER, W., DWYER, T. M. & HILLE, B. (1981). Block of endplate channels by permeant cations in frog skeletal muscle. *J. gen. Physiol.* **78**, 593–615.

ÅRHEM, P. (1980). Effects of rubidium, caesium, strontium, barium, and lanthanum on ionic currents in myelinated nerve fibres from *Xenopus laevis*. *Acta physiol. scand.* **108**, 7–16.

ARMSTRONG, C. M. & BEZANILLA, F. (1974). Charge movement associated with the opening and closing of the activation gates of the Na channels. *J. gen. Physiol.* **63**, 533–552.

BEGENISICH, T. & CAHALAN, M. D. (1980). Sodium channel permeation in squid axons I: reversal potential experiments. *J. Physiol.* **307**, 217–242.

CAHALAN, M. D. & HALL, J. (1982). Alamethicin channels incorporated into frog node of Ranvier: calcium-induced inactivation and membrane surface charges. *J. gen. Physiol.* **79**, 411–436.

CAHALAN, M. D. & PAPPONE, P. A. (1981). Chemical modification of sodium channel surface charges in frog skeletal muscle by trinitrobenzene sulphonic acid. *J. Physiol.* **321**, 127–139.

CONTI, F., HILLE, B. & NONNER, W. (1982). Properties of K current fluctuations in frog nerve. *Biophys. J.* **37**, 16a.

DODGE, F. A. (1961). Ionic permeability changes underlying nerve excitation. In *Biophysics of Physiological and Pharmacological Actions*. Washington, D.C.: AAAS, 119.

DODGE, F. A. (1963). A study of ionic permeability changes underlying excitation in myelinated nerve fibers of the frog. Thesis. The Rockefeller University. Univ. Microfilms, Ann Arbor, MI (no. 64–7333).

DODGE, F. A. & FRANKENHAEUSER, B. (1958). Membrane currents in isolated frog nerve fibre under voltage clamp conditions. *J. Physiol.* **143**, 76–90.

DUBOIS, J. M. (1981*a*). Simultaneous changes in the equilibrium potential and potassium conductance in voltage clamped Ranvier node in the frog. *J. Physiol.* **318**, 279–295.

DUBOIS, J. M. (1981*b*). Evidence for the existence of three types of potassium channels in the frog Ranvier node membrane. *J. Physiol.* **318**, 297–316.

DUBOIS, J. M. & BERGMAN, C. (1977). The steady-state potassium conductance of the Ranvier node at various external K concentrations. *Pflügers Arch.* **370**, 185–194.

FRANKENHAEUSER, B. (1957). A method for recording resting and action potentials in the isolated myelinated nerve fibre of the frog. *J. Physiol.* **135**, 550–559.

FRANKENHAEUSER, B. (1962). Potassium permeability in myelinated nerve fibres of *Xenopus laevis*. *J. Physiol.* **160**, 54–61.

GAGE, P. W. & VAN HELDEN, D. (1979). Effects of permeant monovalent cations on end-plate channels. *J. Physiol.* **288**, 509–528.

GILLY, W. F. & ARMSTRONG, C. M. (1982). Divalent cations and the activation kinetics of potassium channels in squid giant axons. *J. gen. Physiol.* **79**, 965–996.

HILLE, B. (1971). The permeability of the sodium channel to organic cations in myelinated nerve. *J. gen. Physiol.* **58**, 599–619.

HILLE, B. (1975). Ionic selectivity, saturation, and block in sodium channels: a four barrier model. *J. gen. Physiol.* **66**, 535–560.

HODGKIN, A. L. & HUXLEY, A. F. (1952*a*). Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J. Physiol.* **116**, 449–472.

HODGKIN, A. L. & HUXLEY, A. F. (1952*b*). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544.

ILYIN, V. I., KATINA, I. E., LONSKII, A. V., MAKOVSKY, V. S. & POLISHCHUK, E. V. (1977). Evidence of the existence of two independent components of potassium current in the Ranvier node of the frog *Rana ridibunda*. *Dokl. Akad. Nauk SSSR* **234**, 1467–1470.

KRYLOV, B. V. & MAKOVSKY, V. S. (1978). Spike frequency adaptation in amphibian sensory fibres is probably due to slow K channels. *Nature, Lond.* **275**, 549–551.

MARCHAIS, D. & MARTY, A. (1979). Interaction of permeant ions with channels activated by acetylcholine in *Aplysia* neurones. *J. Physiol.* **297**, 9–45.

Matteson, D. R. & Swenson, R. (1982). Permeant cations alter closing rates of K channels. *Biophys. J.* **37**, 17a.

Means, G. E. & Feeny, R. E. (1971). *Chemical Modification of Proteins*. San Francisco: Holden-Day, Inc.

Means, G. E., Congdon, W. I. & Bender, M. L. (1972). Reactions of 2,4,6-trinitrobenzenesulfonate ion with amines and hydroxide ion. *Biochemistry, N.Y.* **11**, 3564–3571.

Pappone, P. A. & Cahalan, M. D. (1982). Chemical modification of amino groups slows K channel closing in myelinated nerve. *Biophys. J.* **37**, 16a.

Shrager, P. (1974). Ionic conductance changes in voltage clamped crayfish axons at low pH. *J. gen. Physiol.* **64**, 666–690.

Sigworth, F. J. (1980). The variance of sodium current fluctuations at the node of Ranvier. *J. Physiol.* **307**, 97–129.

Swenson, R. P. & Armstrong, C. M. (1981). K^+ channels close more slowly in the presence of external K^+ and Rb^+. *Nature, Lond.* **291**, 427–429.

Tsien, R. W. (1974). Effects of epinephrine on the pacemaker potassium current in cardiac Purkinje fibers. *J. gen. Physiol.* **64**, 293–319.