Voltage-dependent action of valproate on potassium channels in frog node of Ranvier

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The influence of the anti-epileptic drug, valproate, on K conductance (gK) was investigated in voltage-clamped Ranvier nodes of Xenopus laevis. A double pulse method was used in order to eliminate the effect of accumulation of potassium ions in the perinodal space, thus enabling the determination of the 'true' magnitude of gK. Valproate (2.4 mM) had a voltage-dependent action on the magnitude of gK. With small step depolarizations more negative than about -50 mV, valproate increased gK (20 ms after the step) to approximately 12% of the maximal gK, an increase which disappeared due to a relatively rapid (< 200 ms) inactivation process. However, with step depolarizations more positive than about -50 mV, valproate markedly reduced gK (20 ms after the step) at greater depolarizations, with a maximum of about 40% of the maximal gK. Moreover, at these voltages gK was inactivated completely (< 10 s), whereas under control conditions the inactivation was only partial. Both the temporary increase and the steady state decrease of gK could contribute to an anti-epileptic effect by increasing the action potential threshold and by preventing excessive depolarizations of the nerve during epileptic seizures, respectively.

Anti-epileptic drugs, Valproate, K⁺ conductance, Voltage clamp, Node of Ranvier, (Voltage-dependent inhibition)

1. Introduction

During the last decade valproate (valproic acid, di-propylacetic acid) has become an important anti-epileptic drug because it shows strong anticonvulsant properties without inducing serious side effects. Its mechanism of action is not known, however, it has been postulated that valproate operates by increasing GABA-ergic inhibition in the brain. Evidence for such an action has been found in isolated nerves of the squid axon (Fohlmeister et al., 1984), the frog node of Ranvier (Van Erp and Van Dongen, 1984, 1987, Van Dongen et al., 1986) and in cultured mouse neurons (McLean and MacDonald, 1986).

Using the frog node of Ranvier, we have demonstrated that valproate increases the threshold for spike generation, decreases the peak of the action potential, and reduces the maximum forced firing frequency. In agreement with these observations, we have observed that voltage-dependent sodium and potassium currents, measured under voltage clamp conditions, are also reduced. Moreover, in the presence of valproate, the potassium current is more reduced at more positive membrane potentials (Van Dongen et al., 1986) Since the accumulation of K ions in the node during

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current flow may increase the outside K concentration about tenfold (De Bruin, 1982), a reduction in the K current may originate from either a decrease in driving force or a reduction in the conductance, or from both. We now investigate the action of valproate on the potassium conductance uncontaminated by changes in the driving force. Ion accumulation is usually prevented by the use of potassium rich external solutions, but this procedure may change the kinetics of the potassium conductance (De Bruin, 1984). Another way to obtain the 'true' potassium conductance is to analyse the instantaneous current jumps in response to voltage steps (De Bruin, 1982). We applied this procedure and conclude that valproate changed potassium conductance as a function of membrane voltage. Our results are consistent with the existence of three different types of potassium channels (Dubois, 1983, Jonas et al., 1989), which are influenced by valproate in a way that may contribute to its anti-epileptic effect.

2. Materials and methods

2.1 Voltage-clamp measurements

Single myelinated nerve fibres were dissected from the sciatic nerve of the clawed frog, *Xenopus laevis*. An isolated fibre was mounted on a macro-ion chamber containing six saline pools (cf. Van den Berg and Rijnsburger, 1980) connected with Ag-AgCl electrodes to the amplifier system. A modified Nonner clamp (Rijnsburger et al., 1985), which allows direct measurement of the current carrying internode necessary to calibrate the membrane currents, was used to voltage-clamp the Ranvier nodes. The holding potential was adjusted to a value at which the sodium inactivation parameter, $h_{\infty}$, was 0.7, which was assumed to be at $-70$ mV. When clamped at this holding potential, the fibres were accepted if they showed a stable holding current (i.e., changing less than 0.01 nA/min) and if depolarizing voltage steps elicited smoothly activating sodium and potassium currents. Linear leakage and capacity currents were compensated for with an analog subtractor, adjusted by visual inspection of the current traces during hyperpolarizing steps down to $-120$ mV. After low-pass filtering with a 4-pole Bessel filter with a (computer controlled) cut-off frequency of $<3.3 \times$ the sample frequency, the membrane currents were digitized by a transient recorder (Bio-mation 1010) and stored on hard disk by computer (PDP 11/34). Further analysis was performed on a VAX 11/750. Because of the non-exponential kinetics of the capacity current (Van Erp, 1988) and an incorrect adjustment of the analog subtractor, a small part of the capacity and leakage current was left, but this residual current could be eliminated digitally by subtracting a scaled response to a hyperpolarizing step from the current record.

A constant flow of Ringer solution (1 ml/min) was maintained through the central pool, in which the nodes were bathed. The Ringer solution contained in mM: 109 NaCl, 2.5 CaCl$_2$, 0.5 MgSO$_4$, 2.5 KCl, 5.0 Tris-HCl with a pH adjusted to 7.40. All experiments were performed at room temperature ($20 \pm 1^\circ$C). TTX was dissolved in the Ringer solution in a concentration of 400 nM to block sodium currents. Sodium valproate (Sanofi) was used at a concentration of 2.4 mM, a concentration at which valproate saturates the conductances of the node (Van Dongen et al., 1986). The internodes were cut in the end pools, which were filled with a solution composed of 118 mM KCl and 2 mM NaCl.

The effects of valproate developed very slowly and were poorly reversible (Van Dongen et al., 1986), therefore we could not perform experiments after washing out of the drug. We studied the effects of valproate about 15 min after its application in valproate Ringer solution.

2.2 Determination of the potassium conductance

Potassium currents were elicited in the presence of TTX by step depolarizations, preceded by a 2 s lasting prepulse to $-90$ mV to remove resting potassium inactivation. The potassium current $I_K(E,t)$ at the clamped membrane voltage $E$ can be written by

$$I_K(E,t) = g_K(E,t)(E - E_K(t))$$  \hspace{1cm} (1)

where $g_K(E,t)$ is the time-dependent potassium conductance.
conductance at E, and Ei(t) the K equilibrium potential, which may vary as function of time due to an increase in the K concentration in the perinodal space (up to 30 mmol) during an outward flowing potassium current. To eliminate Ei(t) from the determination of gK(E,t), the following double pulse method was used (De Bruin, 1982). A voltage step with amplitude E1, activating gK, was followed after a certain time interval, to, by a second deactivating voltage step to level E2, resulting in the so-called tail current, an instantaneous change of the potassium current at t0 followed by relaxation to a new steady state value (fig 1). At t0 before relaxation of the potassium conductance to a new steady state, gK(E2,t0) = gK(E1,t0). From eq (1) it follows that gK(E1,t0) is given by

\[ g_{K}(E_1,t_0) = \Delta I/(E_1 - E_2) \quad (2) \]

where \( \Delta I = \{ I_{K}(E_1,t_0) - I_{K}(E_2,t_0) \} \), is the difference between the two current levels at the moment of the second step. The fast tail current was measured by changing the sample interval to 20 \( \mu \)s, 1 ms before t0 (fig 1). The bandwidth was chosen between 5 to 15 kHz to reduce high-frequency noise, which limited the resolution of the measurement of \( \Delta I \) at small depolarizations. In our experiments, E1 ranged from -60 to +10 mV with increments of 10 mV, while E2 was either -70 or -120 mV. By varying the duration, t0, of the first voltage step, it was possible to reconstruct gK as a function of time. The interval time, t0, was usually 20 and 200 ms, while intervals of 10 s were used to study the inactivation of gK in the three pulse protocol. From our data an activation or inactivation time constant could not be estimated. Therefore, in four nodes, where the variation of Ei(t) was small as judged by visual inspection of the tail current, we used the K current directly to investigate the effects of valproate on the kinetics of the K conductance.

The relation between the normalized potassium conductances and the membrane voltage was fitted using a non-linear, least squares method with a Boltzmann function of the form

\[ g_{K}/g_{K_{max}} = 1/[1 + e^{(E-E_0)/E_c}] \quad (3) \]

where \( g_{K_{max}} \) is the maximum K conductance at large depolarizations, E0 the midpoint potential at which \( g_{K}/g_{K_{max}} = 0.5 \) and Ec a constant, indicating the steepness of the curve.

3. Results

3.1 Valproate and the activation of gK

In order to obtain gK, potassium currents were elicited at depolarizing voltages E1 and after an interval, t0, the membrane voltage was stepped to E2 (holding potential or prepulse level). An example of the double pulse method is given in fig 1. Potassium currents in response to a series of increasing voltage steps (E1) in Ringer solution are depicted in fig 1A, while the effect of valproate on these currents is shown in fig 1B. After 40 ms, when in most cases peak conductance was reached, the membrane voltage was stepped back to the holding potential of -70 mV. The K current in response to this step is the tail current, which we need to estimate gK(E1,t0) from eq (2). It can be seen that the tail current is inward ('undershoot'), relaxing after its initial value to approximately zero. If K ions had not accumulated, the amplitude of the tail current at -70 mV (\( \approx E_K \)) would have been approximately zero.

The inward direction of the tail current reflects that EK has become more positive than -70 mV due to an increase in K concentration at the outside of the node. The K currents in the presence of valproate were always smaller than the control currents, which is in agreement with previous findings (Van Dongen et al, 1986). From the reduction of the tail current at 40 ms during large depolarizations we can conclude that valproate reduced the maximal potassium conductance, \( g_{K_{max}} \), to 59 ± 5.5% (mean ± standard error, S E , n = 11) of its original value in Ringer solution. Except for small changes (< 10%) in the adjustment of the leakage conductance, \( g_{L} \), and the leakage equilibrium potential, E_L, valproate did not require a readjustment of the subtractor, indicating that valproate does not influence the leak, as we reported earlier (Van Dongen et al., 1986).

An example of the dependence of the potassium conductance, obtained from the tail currents, on membrane voltage in the absence and presence
of valproate is shown in fig 2A The magnitude of the K conductances at \( t_0 = 20 \) ms in seven nodes was investigated over the voltage range \(-60\) to \(-10\) mV The results are given in table 1, the relative conductance change \( \Delta g_r(E) \) was calculated according to

\[
\Delta g_r(E) = \frac{g_{K,\text{control}}(E) - g_{K,\text{valproate}}(E)}{g_{K,\text{control}}(-10)} \tag{4}
\]

At large depolarizations, \( g_K \) was reduced in valproate Ringer solution The differences between \( g_{K,\text{control}} \) and \( g_{K,\text{valproate}} \) became smaller at smaller depolarizations. It is remarkable that at small depolarizations the K conductance in the presence of valproate even exceeded that in control solution (five out of seven nodes) Regression analysis performed on the mean relative K conductance change \( \Delta g_r(E) \) (nodes one to six, table 1) with

\[
\Delta g_r(E) = aE + b \tag{5}
\]
yielded \((\pm \text{S E})\) \( a = 0.0100 \pm 0.0003 \ \text{mV}^{-1}, \ b = 0.53 \pm 0.01 \) and a correlation coefficient \( r = 0.9986 \) Thus there was an excellent correlation between the mean relative K conductance change, \( \Delta g_r \), and the membrane voltage From regression analysis it followed that the membrane voltage at which \( \Delta g_r(E) = 0 \) e.g. the voltage at which \( g_{K,\text{valproate}} = g_{K,\text{control}} \), amounted to \(-53\) mV (with \(-57\) mV and \(-50\) mV as the lower and upper 95% confidence limits, respectively) The linear relation given by eq (5) applies to each of the nodes investigated The slopes, \( a \), and the correlation coefficients, \( r \), of the estimated regression lines of the seven nodes (table 1) showed only a small variation The mean values of \( a \) and \( r \) \((\pm \text{S E})\) obtained from node one to seven were 0.011 \((\pm 0.0005 \ \text{mV}^{-1})\) and 0.985 \((\pm 0.007)\), respectively. Thus all nodes showed the same pattern in their voltage dependence The variation in the effect of valproate on the magnitude of \( g_K \) between different nodes is reflected in the spread of voltages at which \( \Delta g_r = 0 \) (the intersection point of the regression line of a single node with the E-axis), which amounted to \(-49 \pm 6\) mV \((\text{S E})\) and in the variation of parameters \( b \) (eq 5) \( 0.55 \pm 0.07 \ \text{(S E)} \). The greater the inhibition at \( E = -10 \) mV, the smaller the increase in the K conductance in the presence of valproate at \( E = -60 \) mV In node 5, e.g. where the K conductance was strongly reduced at \( E = -10 \) mV, the voltage at which \( \Delta g_r = 0 \) could not be reached The scatter

Fig. 1 Family of potassium currents, elicited by step depolarizations between \(-70\) and \(+90\) mV with intervals of \(10\) mV and preceded by a conditioning pulse to \(-90\) mV to remove resting inactivation The bandwidth of the measurement was \(5\) kHz Leakage and capacity currents were subtracted (A) K currents in control Ringer solution After \(40\) ms the voltage was changed to \(-70\) mV, resulting in a fast tail current The maximal instantaneous current change \((\Delta I)\) exceeded \(10\) nA, which gave rise to saturation in this plot (B) The effect of valproate on the K currents and tail currents after an incubation time of about \(15\) min In the inset of the figure a fast recording of the tail current from a different node (node 1 of the table) is shown, corresponding to a step from \(0\) to \(-70\) mV \((\Delta I = 4.0\) nA\) in a bandwidth of \(10\) kHz.
in the relative conductances in the presence of valproate may be due to differences between nerve fibres.

Another way to compare the effects of valproate on the K conductance is to normalize the 'true' conductances obtained both in control Ringer solution and in Ringer solution containing valproate and to fit the $g_K$–E relation with eq (3). The normalized conductances were plotted as a function of membrane voltage (fig 2B), resulting in the well-known sigmoid relation (Frankenhaeuser, 1963) Fitting eq (3) to this relationship yielded the midpoint voltage, $E_0$, and slope factor, $E_c$ ($\pm SE$). In control Ringer solution, $E_0 = -30.2 \pm 4.6$ mV ($n = 7$), while in the presence of valproate the curve was significantly ($P = 0.001$, paired t-test) shifted in the hyperpolarizing direction with $E_0 = -37.5 \pm 4.3$ mV ($n = 7$). The steepness, $E_c$, did not change. In control solution, $E_c$ amounted to $-14.4 \pm 2.2$ mV and in the presence of valproate it was $-14.7 \pm 1.8$ mV ($n = 7$). To obtain information on the effect of valproate on the activation kinetics, we used the K current directly. Using the Hodgkin-Huxley formula (Hodgkin and Huxley, 1952), the next function was fitted to the potassium current, using a nonlinear least squares method

$$I_K(t) = I_{K,\infty}[1 - e^{-t/\tau_n}]^4$$

where $I_{K,\infty}$ is the (pseudo) steady state potassium current at the voltage step studied and $\tau_n$ the time constant of the activation process. In fig 2C the activation time constant, $\tau_n$, is depicted as a function of membrane voltage. The value of $\tau_n$ was smaller in Ringer solution containing valproate than in control Ringer solution ($n = 4$). Since K accumulation tends to decrease $\tau_n$ (cf. De Bruijn, 1982) and K accumulation in valproate-treated nodes will be smaller, the actual difference between control and valproate time constants will be larger. We observed that, for a wide range of membrane voltages, the reduction of $\tau_n$ in the presence of valproate was similar to the shift in the $\tau_n$–E relation in the hyperpolarizing direction.

In fig 2D the conductance of the same node as in fig 2A is shown but now at $t_0 = 200$ ms. At all membrane potentials the conductances in the presence of valproate at 200 ms after the step were smaller than in control Ringer solution, thus also at the levels of $-60$ mV, when the K conductance in the presence of valproate was enhanced at $t_0 = 20$ ms. This was found for all nodes of Ranvier compared at these two time intervals ($n = 5$). This implies that the early increase in the potassium conductance in the presence of valproate was inactivated within a time span of 200 ms. Comparing the K conductances in control solution at 20 and at 200 ms, we noted that the degree of inactivation was small. However, a similar comparison showed that an appreciable amount of inactivation had already occurred in the presence of valproate (cf. fig 2A, D).

3.2 Valproate and the inactivation of $g_K$

The inactivation of $g_K$ in control Ringer solution was slow and incomplete. In the presence of valproate inactivation developed earlier than in control solution and to a greater extent. Because of the pronounced inactivation, the amplitudes of the tail currents in the presence of valproate became very small and $g_K$ could not be determined with acceptable accuracy. We therefore studied the inactivation process with the following three pulse protocol to increase the magnitude of $\Delta I$ in eq (2). Conditioning voltage pulses ($E_3$) with a duration of 10 s were applied to inactivate the K conductance. At the end of $E_3$ the test pulse to $+50$ mV ($E_1$) was given, followed 12 ms later by the pulse $E_2$ back to $-70$ mV (fig 3). Assuming that no recovery from the slow inactivation took place during the short pulse $E_1$, the voltage dependence of the inactivation of the K conductance could be reconstructed using eq (2). The magnitude of the K conductance at $+50$ mV is plotted as function of the conditioning potential in fig 3A. We obtained $g_K$ from the K conductance determined with hyperpolarized conditioning voltages. In agreement with our results in 3.1, valproate reduced $g_K$ to $61 \pm 12\%$ ($SE$, $n = 4$) of its control value. At conditioning potentials more positive than $-50$ mV, the ratio $g_K/\bar{g}_K$, which equals the inactivation parameter $k_{oo}$, amounted to $23 \pm 4\%$ ($SE$, $n = 4$) in control solution. This level of partial steady state inactivation is usually found in the node (cf. Schwarz and Vogel, 1971).
Fig. 2 (A) The potassium conductance of node 4 (table 1), determined according to eq (2), at $t_0 = 20$ ms in control Ringer solution, 
(C, open symbols) and in Ringer solution containing valproate (V, closed symbols) as function of membrane voltage. The level of $E_1$ was increased from $-80$ to $-10$ mV with increments of 5 mV (thus increment only in 3 nodes), and $E_2 = -120$ mV. The K conductance in the presence of valproate was higher than in control Ringer solution for potentials around $-60$ mV, but lower at more depolarized voltages. The accuracy of the determination of $g_K$ was limited by the high-frequency noise current, which was $\leq 0.1$ nA (rms). This corresponded to a standard deviation of $\leq 3.5$ nS at $-80$ mV, which defined with depolarization to $\leq 1.3$ nS at $-10$ mV. For most voltages this error is smaller than the diameter of the data points (the largest error at $-80$ mV is about $1.5 \times$ diameter data point). For example, the amplitude of $\Delta I$, measured in a bandwidth of 10 kHz, in response to the step from $-60$ to $-120$ mV in control Ringer solution was 0.4 nA, and doubled in the presence of valproate. At more negative potentials the tail currents could not be detected above the background noise. (B) Normalized values of $g_K$ (node 3), obtained 20 ms after the step, as function of voltage, corresponding to $n_{in}^A$. Normalization of control values (open circles) and valproate values (closed circles) with their respective estimates of $g_K$. The solid line represents the fit of eq (3) to the relative conductances. From the fit the following parameters $\pm$ standard deviation (S.D.) were obtained for the control situation: $E_0 = -37.7 \pm 0.7$ mV, $E_c = -9.4 \pm 0.5$ mV, and in the presence of valproate $E_0 = -46.7 \pm 0.7$ mV and $E_c = -10.7 \pm 0.7$ mV. Note that the experimental data obtained in the presence of valproate deviate from eq (3) and exhibit a bend at about $-40$ mV. (C) The activation time constant, $\tau_a$, obtained from fitting eq (6) directly to the K currents of a single node, plotted as function of membrane voltage. According to the Akaike test criterion, an index for fit quality (Akaike, 1981), an exponent of 4 yielded better fittings of eq (6) to our data than did an exponent of 2, which is used more often for *Xenopus* nodes (Frankenhaeuser, 1963). Time constants obtained in control Ringer solution are represented by the upper curve (C), and in the presence of valproate by the lower curve (V). The bars represent the S.D. estimated from fitting with eq (6). The valproate data seem to be shifted along the voltage axis, as compared with the control data. (D) The magnitude of the K conductance of node 4 (fig 2A), but now 200 ms after the step. At all membrane potentials the K conductance in the presence of valproate was smaller than in control Ringer solution. Note, e.g., that at $-60$ mV $g_K$ in control Ringer solution was more than 2 times larger than at 20 ms due to a large activation time constant (cf. De Bruin, 1982), and that in the presence of valproate $g_K$ was nearly half its value at 20 ms.
TABLE 1
Dependence of the relative potassium conductance on membrane voltage in control Ringer (C) and in Ringer containing valproate (V). The numbers correspond to the different nerve fibres. Conductances were calculated from depolarizing pulses of 20 ms by means of eq (2) and normalized on the control $g_{K}$ at $-10$ mV. For node 7, the K conductance was not measured at $-60$ mV. The relative conductance change $\Delta g_{r}$ (eq 4) is also shown. Its mean value is indicated by $\bar{\Delta}g_{r}$, and lcl and ucl are the lower and upper 95% confidence limits, respectively.

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</tbody>
</table>

However, in the presence of valproate, the magnitude of the inactivation parameter at conditioning depolarizations more positive than $-50$ mV was only $2 \pm 4\%$ (S.E., n = 4), i.e., inactivation was complete after 10 s. In fig 3B the relative conductances are plotted as function of conditioning membrane potential. Fitting eq (3) to these data showed that valproate shifted the inactivation curve in a hyperpolarizing direction. In the control situation the fit yielded (± S.E.) $E_{0} = -62.4 \pm 1.4$ mV (n = 4), where in the presence of valproate $E_{0} = -74.1 \pm 1.9$ mV (n = 4). The shift amounted to $11.7 \pm 0.7$ mV, which was significant ($P = 0.0006$). The steepness, $E_{c}$, was $5.9 \pm 0.7$ mV and $7.1 \pm 0.6$ mV in the absence and presence of valproate, respectively, and was not significantly different.

To study the time course of inactivation and to compare our results of complete inactivation with the inactivation of the K current, we measured K currents elicited by long-lasting (20 s) depolarizing steps around 0 mV ($-10$, $0$, and $+10$ mV, fig 4). The currents were directly fitted according to (Schwarz and Vogel, 1971)

$$I_{K}(t) = A_{1}e^{-t/\tau_{1}} + A_{2}e^{-t/\tau_{2}} + A_{3}$$

(7)

where $A_{1}$, $A_{2}$, and $A_{3}$ are the amplitudes of the different components of the potassium current.
4. Discussion

From tail current analysis we conclude that valproate has a dual action on the potassium conductance in the Ranvier node (i) a voltage-dependent reduction of the potassium conductance at depolarizations more positive than about −50 mV, which was inactivated completely within 10 s, (ii) a conductance increase at voltages more negative than about −50 mV, which was inactivated

and $\tau_{k,1}$ and $\tau_{k,2}$ the time constants of the inactivation processes We calculated $A_i/I_{K,peak}$ (contribution of component $i = 1, 2, 3$ relative to the control peak current) in four nodes of Ranvier and pooled the data obtained at −10, 0 and +10 mV In control Ringer solution, $A_1/I_{K,peak}$ ($\pm$ S.E) was $35.7 \pm 4.2\%$, $A_2/I_{K,peak}$ $56.8 \pm 3.0\%$ and $A_3/I_{K,peak}$ $72.8 \pm 3.5\%$ (n = 12) In the valproate Ringer solution these ratios were $40.6 \pm 9.7$, $46.0 \pm 6.0$ and $-0.73 \pm 4.6\%$ (n = 12) Only the reduction of $A_3/I_{K,peak}$, the inactivation parameter $k_{\infty}$, to zero in the presence of valproate was significant (P = 0.0281), consistent with results obtained from tail current analysis The value of 73.3% estimated for $A_3/I_{K,peak}$ in control Ringer solution was low compared to the value of 23% from the tail currents, illustrating once more the importance of determining the true $g_K$ in the node The time constants of the current components $A_1$ ($\tau_{k,1}$) and $A_2$ ($\tau_{k,2}$) were not influenced by valproate The values of $\tau_{k,1}$, amounting to $0.21 \pm 0.08$ and $0.27 \pm 0.17$ s in control- and valproate-containing solutions respectively, were not significantly different.

The magnitude of $\tau_{k,2}$ in control- and valproate-containing solutions was estimated 5.01 ± 1.18 and 4.65 ± 1.26 s, respectively The differences were not significant $K$ accumulation could have masked an existing difference between the time constants of inactivation in the presence and absence of valproate

Fig 3 (A) The potassium conductance measured at +50 mV as a function of conditioning membrane potential in control Ringer solution (C, open circles) and in Ringer solution containing valproate (V, closed circles) Conditioning voltages from −100 to −30 mV, with intervals of 10 mV and with a duration of 10 s, were followed by a short test pulse to +50 mV To estimate the conductance from the tail current, a voltage step to −70 mV was given at the end of the test pulse (arrow in top figure, where the pulse protocol is indicated) The solid lines represent the fit with eq (3) multiplied by $g_{K_{\text{control}}}$ and $g_{K_{\text{valproate}}}$, respectively Because of the fixed step of 120 mV for the measurement of the tail current, the error in the data was 1.2 nS, which is about equal to the diameter of the points (B) The relative conductances, representing the inactivation parameter $k_{\infty}$, as function of membrane voltage Solid line is the fit of eq (3) to the data yielding (±S.D) for the control curve $E_0 = -64.2 \pm 0.2$ mV and $E_c = 4.8 \pm 0.2$ mV and for the valproate curve $E_0 = -77.8 \pm 0.7$ mV and $E_c = 5.3 \pm 0.6$ mV

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within 200 ms. These phenomena are probably related to shifts of conductance-voltage relations along the voltage axis in the hyperpolarizing direction. In the node of Ranvier, the potassium conductance kinetics are complex because of the existence of several types of channels (Ilyin et al., 1980; Dubois, 1983) or a multi-state process (Palti et al., 1976; De Bruin et al., 1984; Conti et al., 1984). Recently Jonas et al. (1989) demonstrated the existence of three types of single K channels in Xenopus nodes, thereby supporting the Dubois hypothesis. Our results can be explained qualitatively in terms of this hypothesis. Dubois (1983) hypothesized, on the basis of the current components \( A_1 \) to \( A_3 \) (eq 7), that there are three types of potassium channels in the frog node of Ranvier. Two types of fast (ms) activating channels, i.e., \( I_{K,1} \), which are inactivated slowly (tens of seconds), and \( I_{K,2} \), which are inactivated rapidly, (about a second), and one type of relatively slow (tens of ms) activating channels \( I_{K,s} \), which are not inactivated. According to this view, the \( g_K - E \) relation in Fig. 2A is composed mainly of \( I_{K,1} \) and \( I_{K,2} \), since the contribution of \( I_{K,s} \) was small only 20 ms after the voltage step. The observed shift along the voltage axis must then be related to a shift of one type or both types of fast channels. Since the resulting increase in the K conductance around \(-60\) mV only lasted for less than 200 ms, at least the rapidly inactivating population, \( I_{K,2} \), will be shifted to hyperpolarizing voltages. Thus valproate inhibited either \( I_{K,1} \) or \( I_{K,2} \) or both and completely blocked the slow K current \( I_{K,s} \). To our knowledge, valproate is the first agent which blocks the slow channels completely. It is, however, not as selective as, for example, 4-aminopyridine (4-AP), which blocks only fast channels. The shift of fast channels may also explain a faster time course of the activation process.

Although our results at least qualitatively support the Dubois hypothesis, we did not find any indication of a bend in the \( g_K \)-voltage relationships occurring around \(-40\) mV as reported by Dubois (1983) in potassium rich solutions at \(12^\circ\)C. This bend was used as evidence for the existence of two fast conductances. It is possible that this bend is less pronounced at room temperatures. Such a bend could be present in the presence of valproate (cf. Fig. 2B), as judged from the residual values obtained from fitting eq (3) to our ‘true’ conductance-voltage relations, supporting the multi-population hypothesis.

The voltage-dependent inhibition of \( g_K \) at voltages more positive than \(-50\) mV is in agreement with our previous results of the reduction of steady state K current (Van Dongen et al., 1986). In those experiments, the measured decrease in the K current in the presence of valproate was thus not completely due to K accumulation. In the earlier experimental results, the maximal reduction of \( g_K \) by valproate was 25%, instead of the present value of 40%. Furthermore, the transient increase in the K conductance in the presence of valproate was completely masked by the effect of K accumulation, reducing the driving force. It would be interesting to investigate \( g_K \) at earlier times to avoid fast inactivation and at lower valproate concentrations, since it seemed that the nodes with a small reduction of \( g_K \) by valproate exhibited a large conductance increase. Moreover, the concentration...
of sodium valproate used (2.4 mM) is high compared to therapeutic concentrations.

A different effect of valproate on the K conductance (obtained from the K current) was demonstrated in the giant axon of the squid (Fohlmeister et al., 1984). At the high concentration of 20 mM valproate, the K activation kinetics were slowed down, whereas the steady state conductance was only slightly affected, if at all. In Aplysia neurons such high concentrations of valproate (5-30 mM) increased the K conductance about –50 mV, but a voltage- or time-dependent behaviour of the drug was not investigated (Slater and Johnston, 1978, Johnston, 1984). Valproate decreased the K conductance in giant axons of the crayfish (Nosek, 1981a, b), a result which is in line with our observations at depolarizations more positive than –50 mV.

Valproate reduces sodium conductance in the Ranvier node (Van Dongen et al., 1986), an effect that is known for a number of anti-epileptic drugs like diphenylhydantoin and ethosuximide (Neuman and Frank, 1976, Schwarz and Vogel, 1977, MacDonald and McLean, 1982, Courtney and Etter, 1983). In the presence of valproate the h∞ curve was also shifted along the voltage axis in the hyperpolarizing direction (Van Dongen, 1988), analogous to the shifts in the conductance-voltage relations for potassium. The observed shifts along the voltage axis were much larger than the predicted changes in the voltage drop across the series resistance of the node (<1 mV), and can be ascribed to a screening effect of surface charges on the nerve membrane, as is known for many charged substances (Hille et al., 1975a, b).

How can the dual effect of valproate at a concentration of 2.4 mM contribute to an anti-epileptic action? At the resting potential the potassium conductance in the presence of valproate was small compared to the leakage conductance. However, in response to a sudden (step-like) change in voltage, the fast K channels (e.g. I\textsubscript{K,2}) were activated at a lower depolarization level and faster, resulting in a fast outward K current, which disappeared within 200 ms. Because of this transitory increase in the potassium conductance, more current is necessary to depolarize the nodal membrane in the presence of valproate, i.e., a temporary increase in the firing threshold. A second anti-epileptic action may originate from the reduction of the steady state potassium conductance by valproate. Increases in the extracellular potassium concentration during epileptic seizures are claimed to be responsible for the continuation of spike trains (Sykova, 1983) or epileptic discharges (Moody et al., 1973, Dichter and Ayala, 1987). During epileptic discharges, valproate will decrease the potassium conductance, resulting in a smaller release of potassium ions into the extracellular space, thereby preventing a continuation of spikes. Thus in axonal membranes, valproate may exert its anti-epileptic action not only by reducing the sodium conductance but also by at least two actions on the potassium conductance. The relative contribution of the different mechanisms probably depends on the type of neuron.

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