test solution 8 mM sodium deoxycholate was added. The pH of this solution was 8.0.

Results. The 'resting' discharge rate recorded while perfusing the segment with the modified Krebs-Henseleit solution was increased when perfusing the segment with the solution containing bile salt as illustrated in the figure summarizing 8 technically successful experiments. Rate of discharge (spike counter) increased throughout the experiments lasting for more than 100 min. In the figure the rate of nervous firing is expressed as a percentage of the control rate, since 'resting' rate of discharge varied between experiments.

Sustained increases of nerve fiber activity were poorly correlated with changes in intraluminal pressure as recorded by the pressure transducer located at the proximal end of the intestinal segment. However, transient intestinal contractions were accompanied by small transient increases of rate of signalling. Administration of atropine (0.25 mg/kg b.wt) did not influence the sustained rate of discharge. This dose of atropine has earlier been shown to inhibit the vagally induced decreases of heart rate in rats[6]. In control experiments with a solution devoid of bile salt no change of discharge rate was observed (fig.).

In all experiments the recorded nerve fiber activity decreased markedly after application of lidocaine hydrochloride onto the intestinal serosa and onto the mesentery.

Discussion. In a rather extensive series of experiments by Karlström and collaborators[2–5] evidence was provided for the view that the net fluid secretion from the small intestine evoked by sodium deoxycholate was in part produced by the activation of the enteric nervous system. This conclusion may indirectly be supported in the present series of experiments, which showed that the afferent nervous activity recorded from periarterial nerves was significantly increased when the intestinal mucosa was exposed to a solution containing 8 mM sodium deoxycholate.

It is not known whether the increased nervous activity is secondary to a direct action of the bile salt on nerve endings in the small intestinal mucosa, or whether sodium deoxycholate acts via some other endogenous compound. Karlström et al were unable to block the effects of bile salt on net fluid transport by indomethacin or histamin-1 blocking agents. They proposed that the bile salt itself might acts as a calcium ionophore on sensory nerve endings[7].

The sensory modality reflected in the increased afferent firing in the periarterial nerves can of course not be determined from the present study. However, it has been reported that perfusing the small intestine with a 10 mM bile salt solution causes abdominal pains in humans[8].

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Selective blockade of components of potassium activation in *Myxicola* axons

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Summary. The K^+ conductance in *Myxicola* giant axons activates in two phases which are pharmacologically separable. The fast phase of K^+ activation is specifically inhibited by 4-aminopyridine and by the substitution of D_2O for H_2O. We suggest *Myxicola* giant axons, like the amphibian node of Ranvier, may possess more than one variety of K^+ channel.

Key words. Potassium conductance; *Myxicola* axons; neuronal cell bodies; multiple K^+ channels.

A variety of K^+ channels has been characterized in axons and neuronal cell bodies. In addition to the classical delayed rectifier K^+ channels first described by Hodgkin and Huxley[1] in squid giant axons other K^+ channels include an anomalous rectifier[2], a Ca^{++}-dependent K^+ channel[3], a rapidly-inactivating K^+ channel[4], and a second-messenger-dependent K^+ channel[5]. Although many of these channels are only found in neuronal cell bodies, multiple K^+ channel types have also been identified in nodes of Ranvier[6–8]. The present study shows that K^+ activation in *Myxicola* axons occurs in two pharmacologically separate phases, suggesting that *Myxicola* may have more than one type of K^+ channel.

Methods. Myxicola axons were dialyzed and voltage-clamped by methods which included series resistance compensation and leak and capacity current subtraction[9]. The internal solution contained 450 mM K^+ glutamate; 50 mM KF; 30 mM K_2PO_4; pH = 7.30 ± 0.05. The external solution was Na^+ free and contained 10^{-6} M tetrodotoxin; 10 mM KCl; 10 mM $CaCl_2$; and 50 mM $MgCl_2$. The external substitute for Na^+ was Tris, also adjusted to pH 7.30 ± 0.05, and the temperature was 5.0 ± 0.5 °C. The time course of the K^+ conductance is distorted by K^+ accumulation in the Frankenhauser-Hodgkin space[8,10,11] but can be minimized either by using high K^+ solutions, or by calculating the K^+ conductance from simultaneous measurements of membrane current and the driving force, $(V\text{-}E_K)$. Both methods gave comparable results, but most of our experiments employed the second approach. Axons were held at −80 mV. Membrane currents were first recorded during 25-ms depolarizations to potentials between −60 mV and +100 mV (10-mV steps). Next, two-pulse protocols were applied in which axons were depolarized to a voltage V_1 for a time t_1; and then repolarized to a voltage V_2. For a given V_1 and t_1, V_2 was varied in steps (1-2 mV) small enough for determination of the reversal potential, $E_K(V_1, t_1)$. For each test voltage V_1, t_1 was increased from 0 to 25 ms and $E_K(V_1, t_1)$ redetermined for the same repolarization potentials V_2. Another test voltage V_1 was selected and the entire procedure repeated. We determined instantaneous I(V) curves for values of t_1 of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0 and 25.0 ms to accurately resolve changes in E_K. We monitored the K^+ current at +100 mV. Any deterioration caused us to terminate an experiment.

In all experiments the instantaneous $I_K(V)$ relationships were

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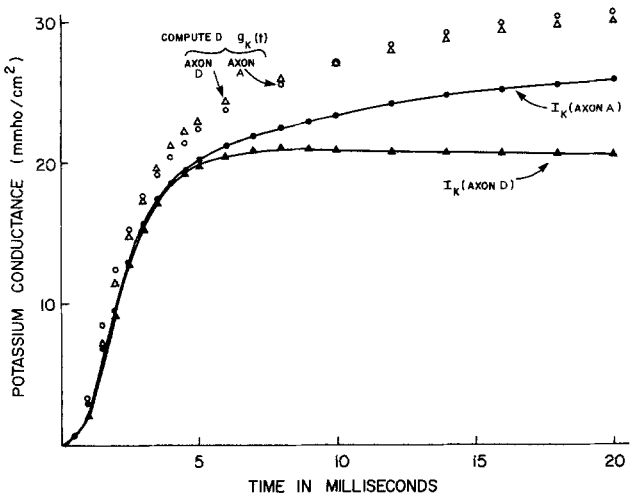

Figure 1. The time course of the K^+ conductance for two axons with different degrees of K^+ accumulation. The test depolarization was +100 mV. The filled triangles (axon D) and circles (axon A) are the measured outward currents at the indicated times which were used with simultaneous experimental $I_K(t)$ measurements to calculate the K^+ conductances for axons D (open triangles) and A (open circles). The absolute values of outward membrane current at 20 ms were 4.3 mA/cm^2 (axon D) and 5.4 mA/cm^2 (axon A).

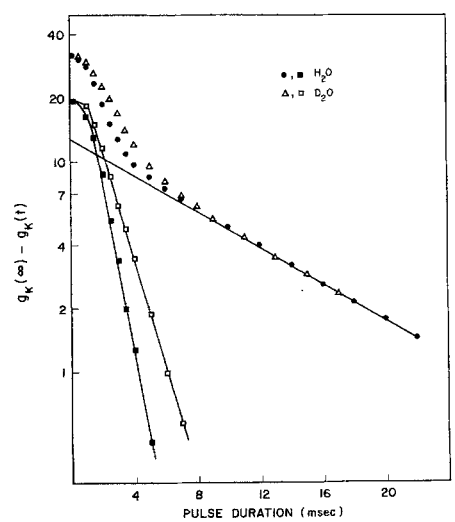

Figure 2. Semilogarithmic plots of the K^+ conductance as a function of time for a depolarization to +80 mV. The filled circles represent differences between the calculated $g_K(t)$ data and the final steady-state K^+ conductance ($g_K[\infty]$) in H_2O. The solid-line is the best least squares fit to these $[g_K(\infty)-g_K(t)]$ data for times in excess of 10 ms. The time constant of the slow component was 10.4 ms. The filled squares represent the fast component of K^+ activation in H_2O calculated from the differences between the filled circles and the extrapolation of the slow component to zero time. After an initial delay, the time constant of the fast phase of K^+. After an initial delay, the time constant of the fast phase of K^+ activation in H_2O was 1.07 ms. The K^+ conductance was also determined after D_2O substitution (open triangles). Here the raw $g_K(\infty)-g_K(t)$ data were multiplied by 1.22 to account for the decrease in $\bar{g}_K$ seen in D_2O. The solid line that was the best fit to the slow component in H_2O also provides an adequate fit to the data in D_2O. The open squares represent the fast component of g_K activation in D_2O determined from the differences between the open triangles and the extrapolated slow component.

linear so that we could use the measured $E_K(V_1, t_1)$ to convert membrane currents to conductances:

$$g_K(V_1, t_1) = I_K(V_1, t_1)/[V_1 - E_K(V_1, t_1)] \quad (1)$$

Whenever the solutions were changed, all of the $E_K(V_1, t_1)$ determinations were repeated using the same series of test voltages. As noted earlier, we performed some experiments in high K^+ solutions, but observed the same biphasic kinetics and pharmacological specificity seen at 10 mM K^+.

Results. The time course of the potassium conductance. Figure 1 shows raw current records and the calculated values of $g_K(V, t)$ for two axons. It is apparent in axon A that K^+ activation is biphasic and only small inward currents were seen following repolarization. Axon D seemed to have a single rapid phase of K^+ activation. However, this behavior arises from a decrease in the driving force for K^+ during the test depolarization because, on repolarization, there were large inward K^+ currents. The calculated conductance-time curves were identical for these axons after normalization, even though the time-dependent correction factors needed to convert K^+ currents into conductances were quite different. Similar results in all experiments suggest our protocol provides accurate data for the time course of g_K. The presence of two components of K^+ activation is obvious in semilogarithmic plots (fig. 2). The filled circles are the difference between the calculated K^+ conductance at time t and the steady-state K^+ conductance at +100 mV in H_2O. After an initial delay, the K+ conductance increases rapidly and then much more slowly. The time constant of the slow component of g_K activation was 10.4 ms. When the slow component was removed by plotting the differences between the calculated conductances and the extrapolated value of the slow component, we obtained a time constant for the rapid phase of K+ activation of 1.07 ms. Data similar to that in figure 2 yielded two resolveable time constants for voltages of +20 mV or greater. For smaller potentials the time constants were difficult to define unambiguously. However, the major question of interest to us was whether the components of K^+ activation were separable by the use of pharmacological agents.

Pharmacological sensitivity of conductance components. In the first set of experiments we applied 4-aminopyridine externally

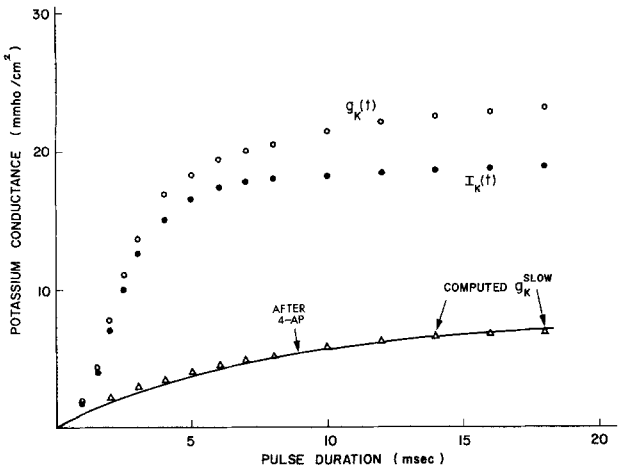

Figure 3. Selective block of the fast component of K^+ activation by 10 mM 4-AP. The filled circles are experimental values of the outward K^+ currents (scale not shown) obtained at the indicated times, while the open circles are the corresponding $g_K(t)$ values after correction for K^+ accumulation. The open triangles represent the slow component of g_K activation derived by the procedure in figure 2. The solid line is the calculated K^+ conductance after the addition of 4-aminopyridine to the external bathing solution.

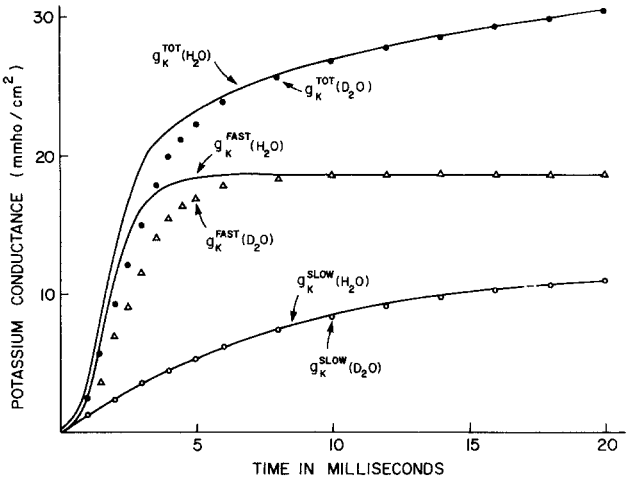

Figure 4. Effects of D_2O substitution on the activation of the K^+ conductance of *Myxicola*. The three solid lines represent (top to bottom) the time course of the total K^+ conductance and its derived fast and slow components in H_2O for a step to +80 mV. The filled circles are the total K^+ conductance as a function of time following D_2O substitution (scaled by a factor of 1.22 to account for the solvent effect on $\bar{g}_K$), while the open triangles and circles are the derived fast and slow components respectively.

(4AP). In an untreated axon the K^+ conductance (pen circles in fig. 3) increases biphasically. We extracted the slow component of K^+ activation by the curve fitting procedure illustrated in figure 2, giving the open triangles. We then added 10 mM 4-AP to the external solution, repeated the instantaneous $E_K(t)$ measurements (even though with 4AP there is no significant accumulation), and then converted the currents to conductances (solid line in fig. 3). The calculated and experimentally measured slow K^+ conductances superimposed without normalization. That is, the measured K^+ activation kinetics in the presence of 4AP were precisely the same as seen for the slow component in untreated axons over the entire range of voltages in 4 axons. We conclude that 4AP selectively eliminates the fast component of g_K activation in *Myxicola*.

We were able to produce a similar separation using D_2O substitution[12]. The upper solid curve and filled circles in figure 4 show K^+ activation at +80 mV in H_2O and D_2O respectively (instantaneous values of $E_K(t)$ measured separately with each solvent were used to calculate $g_K(t)$ from the raw currents). Since D_2O substitution decreased the maximum conductance by 20–30%[10], the D_2O data had to be normalized. While the derived slow components in H_2O and D_2O show the same kinetics (fig. 2), the residual fast component in D_2O is retarded by 40–50% as measured from half-activation times. Thus, D_2O selectively slows activation of the fast component of the K^+ conductance. Similar results were obtained in 3 other test voltages in this axon and in 4 others. D_2O substitutions were also performed after blocking the slow component of g_K activation with 4-aminopyridine, but there was no further effect.

Hyperpolarization delays the activation of the K^+ conductance[13,14]. In three experiments we found that hyperpolarizations increased the time delay for the fast component of K^+ activation, but did not affect the time constants of either the fast or slow components.

We were unsuccessful in several attempts to selectively manipulate the slow component. Extracellular tetraethylammonium (TEA^+) suppresses both the fast and slow components. Internal dialysis of *Myxicola* axons with EGTA-Ca^{++} buffer solutions containing less than 10^{-8} M free [Ca^{++}] completely eliminated the slow component of g_K activation, but the fast component was also slowed and the total K^+ conductance was decreased to 50% of control values. Thus we could not determine if the slow component of g_K activation in *Myxicola* was Ca^{++}-activated.

Discussion. We have presented experimental evidence which demonstrates that the K^+ conductance in *Myxicola* activates biphasically and that these two phases are pharmacologically separable. The fast phase is specifically slowed by both 4-aminopyridine and D_2O. One possibility is that there are two K^+ channels. The alternative explanation would involve a complex activation sequence differentially affected by 4-aminopyridine and D_2O. While multiple phases of K^+ activation are not seen in squid giant axons, there is ample evidence for their presence in nodes of Ranvier[8,15]. However, the K^+ channel population in *Myxicola* seems distinct because the 4-AP insensitive slow phase of K^+ activation has time constants (corrected for temperature) two orders of magnitude smaller than slow activation in frog node[8]. *Myxicola* giant axons do not fire repetitively, even in solutions with reduced extracellular [Ca^{++}]. The presence of a slow K^+ channel would tend to prevent repetitive firing and could account for such behavior. In any case, the possibility that two K^+ channel populations may exist must be kept in mind in interpreting any single channel data obtained from this preparation.

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