

*Lack of Effect of Internal Fluoride Ions on Potassium Channels in Squid Axons*

Dear Sir:

A few years ago Adams and Oxford (1983) reported a significant, reversible reduction of potassium ion current,  $I_K$ , in squid axons after internal perfusion with solutions containing fluoride ions. This result is important not only because of the intrinsic interest in the effects of fluoride on ionic currents, but also because fluoride has often been used in studies of ionic blockade of  $I_K$  by internal cations, most notably  $\text{Na}^+$  and  $\text{Cs}^+$ , which have usually been added to the internal perfusate in the form of  $\text{NaF}$  or  $\text{CsF}$ , respectively (Adelman, 1971; Bezanilla and Armstrong, 1972; French and Wells, 1977; French and Shoukimas, 1985; Clay, 1985). Clearly, the interpretation of cationic blockade of the  $I_K$  channel in these experiments would be complicated by an effect of  $\text{F}^-$  on  $I_K$ . Moreover, as noted by Adams and Oxford (1983), the observations by Almers and Armstrong (1980) of a loss of  $I_K$  after removal of permanent cations from both the internal and external solutions may have been compromised by the presence of fluoride in the internal perfusate in their experiments. These questions appear to be moot, based on my results, because I have been unable to reproduce the fluoride effect reported by Adams and Oxford (1983). I have not observed any effect of fluoride on  $I_K$  even in experiments lasting 1 h or longer, whereas I have observed a significant reduction of  $I_K$  by internal chloride ions, as originally reported by Adelman, Dyro, and Senft (1966) and by Adams and Oxford (1983).

Experiments were performed on internally perfused squid axons using methods that have been previously described (French and Wells, 1977; Clay and Shlesinger, 1983). The temperature was in the 6–9°C range. In any single experiment it was maintained constant to within  $\pm 0.1^\circ\text{C}$ . The internal perfusate contained 300 mM  $\text{K}^+$ , 25 mM  $\text{HPO}_4^{-2}$ , 505 mM sucrose, and either 250 mM glutamate,  $\text{F}^-$ , or  $\text{Cl}^-$ . The axons were superfused, externally, with artificial seawater (ASW) containing 0.5  $\mu\text{M}$  tetrodotoxin, 10 mM  $\text{CaCl}_2$ , 10 mM Tris-HCl, 50 mM  $\text{MgCl}_2$ , 10 mM KCl, and 430 mM NaCl. Liquid junction potentials were  $\leq 3$  mV. The voltages given below represent nominal values which have not been corrected for these relatively small voltage offsets. The only apparent significant, difference between the techniques used here and in Adams and Oxford (1983) concerns the method by which the axoplasm was removed. In these experiments it was removed by suction applied to a cannula which was passed through the axon one or more times. In Adams and Oxford (1983) the axoplasm was squeezed out from the axon with a small rubber roller.

My observations concerning  $\text{F}^-$  and  $\text{Cl}^-$  are illustrated in Fig. 1. The records in the upper left hand panel are superimposed measurements of  $I_K$  with voltage steps to  $-40$ ,  $-20$ ,  $0$ , . . .  $+80$  mV with glutamate as the major anion. These results were

obtained just before a change of the internal solution to the perfusate containing fluoride as the major anion. I did not observe a change of  $I_K$  with the latter conditions even 45 min after switching to the fluoride containing solution, as illustrated by the records in the upper right hand panel of Fig. 1. Similar results were obtained in three other axons with exposure times to  $\text{F}^-$  ranging between 20 and 100 min. In two other experiments I perfused initially with the fluoride solution for 15 min followed by a change to the glutamate solution. I did not observe a change in  $I_K$  in these experiments after the solution change. Finally, in two other experiments I observed a relatively rapid reduction of  $I_K$  and a significant increase in leakage current after a change to the chloride containing solution, as originally demonstrated by Adelman, Dyro, and Senft (1966) and by Adams and Oxford (1983). The reduction of  $I_K$  under these conditions is illustrated in the lower two panels of Fig. 1.

The reasons for the differences between my results and the results in Adams and Oxford (1983) are not readily apparent. The fluoride solution in these experiments consisted of 250 mM KF, 25 mM  $\text{K}_2\text{HPO}_4$ , and 505 mM sucrose, as compared with 320 mM KF, 15 mM  $\text{K}_2\text{HPO}_4$ , and 370 mM sucrose in their work. I do not believe that the difference in results could be attributable to the relatively minor differences in these solutions. Undoubtedly, the change of internal perfusate from the glutamate to the fluoride solution does not result in complete exchange of the major anion. However, Adams and Oxford (1983) reported a significant reduction of  $I_K$  with 50 mM  $\text{F}^-$ , so even if the exchange of  $\text{F}^-$  for glutamate were incomplete in these experiments, a significant reduction of  $I_K$  should have occurred, according to their work. Moreover, the difference in results does not appear to be attributable to perfusion technique, because I did observe an effect of  $\text{Cl}^-$  on  $I_K$ , as illustrated in Fig. 1.

The lack of effect of  $\text{F}^-$  suggests that the studies of cationic blockade noted above may not have been complicated by a reduction of  $I_K$  by fluoride. In particular, the addition of  $\text{CsF}$  to the internal perfusate does not affect inward current through the  $I_K$  channel (Clay, 1985), which is consistent with the results reported here. Cesium ions block outward current through the  $I_K$  channel. Inward current is unaffected by the internal application of  $\text{Cs}^+$ . Consequently, an effect of  $\text{F}^-$  on  $I_K$  in these experiments would have been evidenced by a reduction of inward  $I_K$  current, which I did not observe. Similarly, French and Wells (1977) found that inward current through the  $I_K$  channel was unchanged by the addition of  $\text{NaF}$  to the internal perfusate.

Reports in the literature concerning the effects of anions on membrane currents are surprisingly few in number, as noted by Adams and Oxford (1984). The only voltage-clamp study on squid axons other than theirs and this report is the original work of Adelman, Dyro, and Senft (1966), which was concerned primarily with the effect of chloride. Specifically, they used fluoride as the major anion in their control results followed by a change to chloride as the major anion, which produced a significant reduction in both  $I_K$  and the sodium ion current,  $I_{\text{Na}}$ , as well

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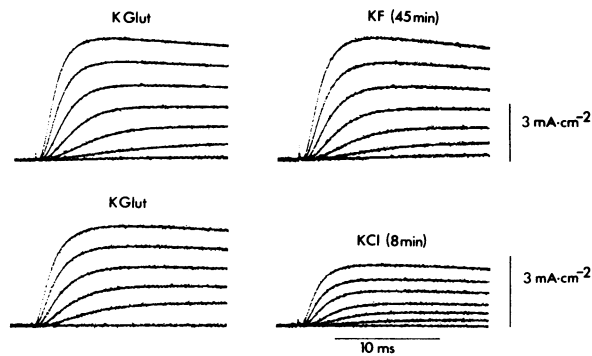


FIGURE 1 *Top panels:* Lack of effect of internal fluoride on  $I_K$ . Potassium current records on the left for depolarizations to  $-40$ ,  $-20$ ,  $+80$  mV from a squid axon internally perfused with 250 K glutamate. Records on the right from the same axon 45 min after internal perfusion with 250 KF. *Bottom panels:* Effect of internal chloride on  $I_K$ . Potassium current records on the left for depolarizations to  $-40$ ,  $0$ ,  $+20$ ;  $+80$  mV from a squid axon internally perfused with 250 K glutamate. Records on the right for depolarizations to  $-40$ ,  $-20$ ;  $+80$  mV from the same axon 8 min after internal perfusion with 250 KCl. Holding potential for all results was  $-80$  mV.

as a significant increase in leakage current. The  $I_K$  component after return to control conditions was reduced relative to their original control results. Adams and Oxford (1983) attributed this reduction to fluoride. Alternatively, it could be attributed to an irreversible reduction of  $I_K$  by chloride. In any case, the results in Adelman, Dyro, and Senft (1966) do not provide a direct test of the effects of fluoride on  $I_K$ . The work on membrane excitability by Tasaki, Singer, and Takenaka (1965) is suggestive of a lack of effect of  $F^-$  on  $I_K$ , although they did not measure ionic currents directly. Nevertheless, if fluoride suppresses  $I_K$  as significantly as Adams and Oxford (1983) indicate, it is surprising that Tasaki, et al. (1965) did not report an effect of 400 mM KF in the internal perfusate on action potential parameters.

The rather marked discrepancy in experimental results noted here is disquieting. Whatever the source of the discrepancy, these results demonstrate that fluoride ions probably do not interact directly with the  $I_K$  channel.