Block of Outward Current in Cardiac Purkinje Fibers by Injection of Quaternary Ammonium Ions

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ABSTRACT We have studied the effects of iontophoretic injection of the quaternary ammonium compounds tetroethylammonium (TEA) and tetrabutylammonium (TBA) in cardiac Purkinje fibers. We find that TBA+ is a more effective blocker than TEA+, but injection of either compound reduces the time-dependent outward plateau currents, transient outward current (Ito), and the delayed rectifier (Ikr). Our findings provide evidence that these outward cardiac currents are carried by channels that in some respects are pharmacologically similar to squid axon potassium channels. We demonstrate that this procedure is a new tool that can be useful in the analysis of membrane currents in the heart.

INTRODUCTION
Pharmacological probes can be powerful tools for studying ionic channels in excitable cells because they provide a method for dissecting individual current components from total current records. In squid giant axons and in myelinated amphibian nerve fibers, this approach has provided strong experimental evidence for the existence of separate pathways for Na and K ions, as well as key information about the structure of these ionic channels (for reviews see Hille, 1970; Armstrong, 1975). However, in the heart, the pharmacological approach has been less successful.

The plateau phase of the cardiac Purkinje fiber action potential is a period of electrical activity that separates the initial rapid upstroke of the action potential from the final phase of repolarization. It generally lasts from 250 to 750 ms and occurs over a range of voltages between -20 and +20 mV. There are three distinct voltage- and time-dependent ionic currents that activate over this voltage range and time course. These include two outward currents, the transient outward current (Ito) and the delayed rectifier (Ikr). Although the ionic basis of these currents is not completely known, experimental evidence suggests that they are carried at least in part by potassium ions (Kenyon and...
Gibbons, 1979a and b; Siegelbaum et al., 1977; Siegelbaum and Tsien, 1980; Noble and Tsien, 1969). In addition to these outward currents, an inward calcium current ($I_{\text{in}}$) also activates, and at least partially inactivates, in this potential range (see Reuter, 1979, for review).

Attempts to dissect $I_{\text{in}}$ from total current records using calcium current blocking agents have only been partially successful. Compounds such as D600, which block the calcium channel, have also been shown to reduce the transient outward current (Siegelbaum et al., 1977; Siegelbaum and Tsien, 1980) as well as the delayed rectifier (Kass and Tsien, 1975). Thus drug-sensitive current records obtained by subtracting current recorded in the presence of a blocking agent from drug-free current records include outward current components in addition to $I_{\text{in}}$.

An alternative approach to this problem is to find compounds that block the outward currents $I_{\text{to}}$ and $I_{\text{r}}$ without affecting $I_{\text{in}}$. Several reported observations have suggested the use of known potassium channel blocking agents for this purpose. Kenyon and Gibbons (1979a) found that tetraethylammonium (TEA) applied externally at relatively high concentrations (40 mM) reduces but does not completely abolish $I_{\text{to}}$ after long periods of exposure. These authors also found that $I_{\text{to}}$ was sensitive to 4-aminopyridine (Kenyon and Gibbons, 1979b), although these effects are often accompanied by increased contractile activity (Yanagisawa and Taira, 1979), which complicates the use of this compound in cardiac preparations. More recently, Marban (1981) and Marban and Tsien (1982) have shown that $I_{\text{to}}$ and $I_{\text{r}}$ can be greatly reduced by replacement of intracellular potassium ions with cesium ions using the ionophore nystatin.

In the squid axon, the tetraethylammonium ion and many of its derivatives block potassium channels when added to the axoplasm, but are relatively ineffective when applied to the outside of the axon membrane (Tasaki and Hagiwara, 1957; Armstrong and Binstock, 1965; Armstrong, 1971, 1975). TEA is also a weak blocker when added to the external solution bathing Purkinje fibers (Kenyon and Gibbons, 1979a; Haldimann, 1963), but prolongs the action potential when applied intracellularly (Ito and Surawicz, 1981). We were thus interested in examining the effects of intracellular application of this and other quaternary ammonium compounds on membrane currents in this cardiac preparation.

In this paper we report results of experiments in which we determined the effects of iontophoretic injection of tetraethylammonium ion (TEA$^+$) and tetrabutylammonium ion (TBA$^+$) on Purkinje fiber membrane currents. We find that TBA$^+$ may be a more effective blocker than TEA$^+$, but iontophoretic injection of either compound blocks the outward plateau currents $I_{\text{to}}$ and $I_{\text{r}}$. This finding suggests that these outward currents in the heart are carried by channels that resemble potassium channels in the squid axon in their sensitivity to quaternary ammonium compounds. In addition, we show that this straightforward technique provides a useful new tool for analyzing time-dependent outward Purkinje fiber plateau currents and the slow inward current that they overlap. Some preliminary aspects of this work were reported to the Biophysical Society (Kass et al., 1981).
Most of the experiments in this study were carried out in shortened (1–2 mm) Purkinje fiber bundles (100–200 μm Diam) obtained from either ventricle of calf hearts. A small number of experiments were performed in Purkinje fibers from canine or sheep hearts, and no significant species-related differences were observed in the response to quaternary ammonium compounds. All figures of experimental results shown in this paper are from calf fibers. Membrane current was measured with a previously described two-microelectrode voltage-clamp arrangement (Siegelbaum and Tsien, 1980). Microelectrodes were pulled from thin-walled omega-dot glass (1.2 mm outer Diam × 0.9 mm inner Diam; Glass Co. of America, Bargaintown, NJ) and bevelled using a modified “spin-bar” beveller (Corson et al., 1979).

In most control experiments in this study, current-passing micropipettes were filled with 1.5 M K-citrate and bevelled to tip resistances on the order of 15 MΩ. Iontophoresis was carried out by replacing the K-citrate electrode with an electrode filled with either 1.5 M TEA-Cl or 1.5 M TBA-Br (or TBA-Cl). These electrodes were also bevelled, but the apparent tip resistances, after beveling, were considerably higher than the tip resistances of comparably prepared K-citrate electrodes. The average tip resistance of a bevelled quaternary ammonium-filled micropipette was between 30 and 40 MΩ. The higher tip resistances of these electrodes resulted in slightly slower voltage-clamp response times. The quaternary ammonium blocking compounds were injected under conditions of either voltage or current clamp. In both cases, the amount injected was estimated by measuring the total outward charge passed during the injection period.

In some experiments, recordings were obtained with a simpler arrangement of a continuous TEA or TBA electrode impalement. In this case, control records were obtained before significant iontophoresis of the blocking compound could take place (see Figs. 1 and 2). These results were the same as those obtained with the more complicated multiple-impalement procedure.

The standard modified Tyrode solution contained 150 mM NaCl, 4 mM KCl, 5.4 mM CaCl₂, 0.5 mM MgCl₂, 5 mM glucose, and 10 mM Tris-HCl buffer, pH 7.4. All solutions were pre-gassed with 100% O₂. Temperature was regulated with a thermoelectric device (Cambion, Cambridge, MA) over a temperature range of 34–37°C, with temperature held constant to within 0.5°C during a given experiment. Fiber volume was estimated by measuring the apparent dimensions of the cell bundle, which is covered with connective tissue and is observed under the dissecting microscope.

Contractile activation was monitored with an optical technique that is described in a previous paper (Kass, 1981). All signals were recorded simultaneously on a Brush 2400 chart recorder (Gould, Instruments Division, Cleveland, OH) and on a PDP-11/03 computer (Digital Equipment Corp., Maynard, MA). Current records were filtered at 1 kHz and the optical signal was filtered at 50 Hz.

D600 was kindly supplied by Knoll, Ludwigshafen am Rhein, Federal Republic of Germany. TEA was obtained as a chloride salt, and TBA was obtained both as chloride and bromide salts from Eastman Organic Chemicals, Rochester, NY. The supplier specified the limits of purity of TEA-Cl and TBA-Br as ≥98%, whereas TBA-Cl was specified to be ≥90%. Because we found no difference in the actions of the chloride and bromide salts of TBA, we used the bromide salt in most of our experiments.

Voltage-Clamp Protocols

Holding potentials were chosen to inactivate the excitatory sodium current (Colatsky, 1980), but not the calcium current \( I_{Ca} \) (Reuter, 1979) or the transient outward current...
We used the approach of Noble and Tsien (1969) to study the effects of TBA⁺ or TEA⁺ iontophoresis on \( I_x \). In this approach, currents are measured at the holding potential after the break of long voltage pulses, which are imposed to activate \( I_x \). The kinetics of this channel are sufficiently slow to allow resolution of tails of decaying outward current that accompany termination of these voltage pulses. The slow current tails reflect the decay of \( I_x \) to its original state at the holding potential. The initial magnitudes of the current tails are thus proportional to \( I_x \) turned on during the preceding voltage pulses. \( I_x \) has been found to be separable into two first-order mechanisms with distinguishable kinetics. The terms \( I_{x1} \) and \( I_{x2} \) have been introduced to describe the fast and slow components, respectively, of \( I_x \) (Noble and Tsien, 1969). In the present set of experiments in which 1-s voltage steps were used to activate this current, \( I_x \) consists predominantly of its faster component \( I_{x1} \). Finally, for purposes of comparison only, the method of Siegelbaum et al. (1977) was used to estimate the transient outward current in Table I.

**RESULTS**

Plateau Currents: TEA⁺ and TBA⁺ Block the Transient Outward Current

Fig. 1 illustrates a voltage-clamp protocol used to investigate Purkinje fiber plateau currents as well as the results of an experiment that examines the effects of intracellular tetraethylammonium ions (TEA⁺) on them. The figure shows membrane current evoked by 250-ms voltage depolarizations from the \(-52\)-mV holding potential before and after iontophoretic injection of TEA⁺. In the control record, the current waveform is complex, reflecting the overlap of the three time-dependent plateau currents.

At the onset of the voltage step the control current is dominated by a rapid outward transient that subsides within the first 80 ms of the depolarization. This current component is referred to as the transient outward current, \( I_{to} \) (Siegelbaum and Tsien, 1980; for other nomenclature see also Kenyon and Gibbons, 1979a and b; Fozzard and Hiraoka, 1973; Dudel et al., 1967). Its time course obscures other membrane currents during early phases of depolarizing voltage steps.

The time-dependent changes in membrane current evident after \( I_{to} \) subsides are caused by both the activation of the delayed rectifier \( I_r \) and the inactivation of the slow inward current \( I_{si} \). Thus slower changes in membrane current are complicated by the interaction of these two currents. The relative contribution of each of these components to total membrane current varies considerably between preparations. Nevertheless, it is clear that analysis of any one of these membrane currents is restricted without selective pharmacological inhibition of one or both of the other current components.

The records in Fig. 1 were obtained using a current-passing micropipette filled with 1.5 M TEA-Cl solution. After the control measurements, the experimental conditions were changed from voltage to current clamp to permit controlled injection of TEA⁺. The compound was injected by passing depolarizing current pulses 33 nA in magnitude and 500 ms in duration, at a rate of 1 Hz. After an injection period of 11 min, a total outward charge of 10 \( \mu \)C had been passed, voltage clamp was re-imposed, and the second record in
Fig. 1 was obtained. After this injection, there was a small outward shift in holding current and a slight change in tail current upon repolarization (arrow), but the most striking effect of this procedure is the marked reduction of the transient outward current. We will thus first discuss the block of this current component and address other effects of quaternary ammonium iontophoresis in subsequent sections.

TEA⁺ injection sharply reduces, but does not completely block the transient outward current, as is evidenced by the small "hump" in the post-TEA⁺ current trace in Fig. 1. This record typifies the extent of $I_{to}$ block we observed in six similar experiments with TEA⁺ iontophoresis. Thus, although the reduction of $I_{to}$ was encouraging, we looked for a compound that blocks this current more completely than TEA⁺.

Because these experiments are carried out in multicellular preparations (see Discussion), a suitable blocker must be able to move freely from cell to cell in addition to being more potent than TEA⁺. Armstrong (1969, 1971), and more recently, Swenson (1981) and French and Shoukimas (1981) have characterized squid axon potassium channel block by a diverse group of quaternary ammonium ions. The potency of block generally increases with tail group length for asymmetric compounds and with head size for symmetric ions. We chose to investigate the effectiveness of tetrabutylammonium ion, as this symmetric compound was found to be a more potent potassium channel blocker than TEA⁺ (French and Shoukimas, 1981), but should still be small enough to move from cell to cell in a Purkinje fiber bundle.

The effects of iontophoretic injection of TBA⁺ on Purkinje fiber plateau currents are shown in Fig. 2. A similar voltage protocol to that in Fig. 1 was followed in this experiment, and the control currents (a) are characterized by
a similar waveform. Following the capacity transient, the current is dominated by the transient outward current. As in the previous experiments, the current-passing micropipette was filled with a concentrated salt solution of the blocking agent (1.5 M TBA-Br). However, in this case, TBA+ was injected under voltage-clamp conditions. The amount injected can be estimated by measuring total outward current in response to each voltage step.

This procedure provides a monitor of the onset of $I_{\text{to}}$ block as the intracellular TBA+ concentration increases on a pulse-by-pulse basis. With prolonged passage of charge, the outward transient is progressively reduced ($b-d$). In contrast to the records in Fig. 1, the currents recorded after injection of appreciable amounts of TBA+ are not characterized by any obvious TBA-insensitive component of $I_{\text{to}}$ ($d$).

A more complete example of the effects of TBA+ iontophoresis on membrane current is given in Fig. 3, which shows currents in response to a series of voltage steps before and after injection of TBA+. The control records in this figure were obtained with a K-citrate current passing electrode to ensure TBA-free records. This electrode was replaced by a TBA-Br-filled micropipette for iontophoretic injection of TBA. The new impalement site was kept to within 30 μm of the original site. Changing the current microelectrode did not result in any distortion or injury-related changes in membrane current recorded under voltage clamp. As can be seen in the figure, the holding current was unaffected by this procedure and remained constant for the duration of this experiment. This indicates that seals around each of these electrodes were equally effective.

TBA ions were injected under current clamp, and superposition of control and TBA-containing records indicates that substantial block of $I_{\text{to}}$ occurred at each voltage tested (panel A). As in Fig. 2, the reduction of $I_{\text{to}}$ by TBA+ appears more complete than in the comparable experiment with TEA+ (Fig. 1). The transient outward current peaks ~20 ms after the application of depolarizing voltage pulses (see Boyett, 1981). Thus measurement of current
20 ms after imposing test voltages before and after injection of TBA not only determines the effects of this blocker on the early current-voltage relation, but also emphasizes changes caused by reduction of $I_{to}$. The most prominent effect of TBA injection is the appearance of net inward current at voltages positive to $-40$ mV (Fig. 4). This is caused by the disclosure of calcium current that had been obscured by $I_{to}$ before injection.

Fig. 3 also shows that TBA iontophoresis can be used to dissect the transient outward current from total current records. The records in panel B
were obtained by subtracting the current that remains in the presence of TBA\(^+\) (labeled TBA in panel A) from the current that was recorded before TBA\(^+\) had been injected (control traces in A). The difference current that results from this procedure directly shows the transient outward current in response to the indicated test voltages. This pharmacological approach is more accurate than previous measurements of the transient outward current (cf. Siegelbaum et al., 1977, Fig. 1), and will provide a means for direct measurement of the voltage- and time-dependent properties of this current as well as its ionic sensitivity. The component of TBA-sensitive current apparent at later times during voltage depolarizations is considered in the section on delayed rectification.

The data from our experiments designed to test the effects of TEA and TBA injection on \(I_\text{to}\) are summarized in Table I. Both agents block this current, but since TBA emerged as a more potent blocker, we focused the remainder of this study on its effects on Purkinje fiber membrane currents.

**Effect of TBA\(^+\) Iontophoresis on Contractile Activation**

The transient outward current, carried through channels regulated by calcium-sensitive conductances, has been shown to be well correlated with contractile activation in the Purkinje fiber (Siegelbaum et al., 1977; Siegelbaum and Tsien, 1980). If iontophoresis of TEA\(^+\) and TBA\(^+\) inhibits calcium release from the sarcoplasmic reticulum in these preparations, then it is possible that the observed reduction in \(I_\text{to}\) is caused not by a direct block of the \(I_\text{to}\) channel, but by an indirect action on the internal store.

We tested for this possibility by using contractile activation as a rough
bioassay for the release process. Fig. 5 shows an experiment in which contractile activation was monitored in addition to membrane current. A light beam was focused on a small region of the preparation under the voltage-measuring microelectrode and changes in the emerging light were measured as an index of tension development (Kass, 1981).

### Table I

| Experiment | Species | $V_{hold}$ | $\Delta I_{hold}$ | Total charge injected | Percent block | Apparent core volume $mm^3$ |
|------------|---------|------------|------------------|-----------------------|---------------|---------------------------|
| 147-2 Calf | -52     | -1         | 10               | 75                    | 0.028         |                           |
| 148-1 Calf | -40     | 0          | Not measured     | 38                    | 0.024         |                           |
| 148-2 Calf | -52     | -10        | 9.75             | 50                    | Not measured  |                           |
| 148-3 Calf | -40     | 0          | 14               | 72                    | Not measured  |                           |
| 172-4 Calf | -35     | 0          | 15               | 80                    | 0.039         |                           |
| 151-3 Sheep| -52     | +1         | 5                | 87                    | 0.015         |                           |
| 151-4 Sheep| -40     | -1         | 12               | 67                    | 0.014         |                           |

**TBA**

| Experiment | Species | $V_{hold}$ | $\Delta I_{hold}$ | Total charge injected | Percent block | Apparent core volume $mm^3$ |
|------------|---------|------------|------------------|-----------------------|---------------|---------------------------|
| 153-4 Calf | -52     | +1         | 3                | 72                    | 0.012         |                           |
| 153-2 Calf | -54     | +2         | 6                | 80                    | 0.027         |                           |
| 169-2 Calf | -48     | 0          | 5.2              | 100                   | 0.015         |                           |
| 174-2 Calf | -40     | -2         | 5.4              | 85                    | Not measured  |                           |
| 176-1 Calf | -40     | -1         | 4.6              | 68                    | 0.015         |                           |
| 177-1 Calf | -40     | -4         | 5.7              | 100                   | 0.035         |                           |
| 178-2 Calf | -40     | -2.5       | 3.8              | 100                   | 0.033         |                           |
| 182-1 Calf | -40     | +8         | 8.75             | 93                    | 0.03          |                           |
| 187-2 Calf | -50     | 0          | 4.4              | 100                   | 0.033         |                           |
| 191-1 Calf | -40     | -4         | 6.1              | 100                   | 0.01          |                           |
| 192-2 Calf | -40     | 0          | 2.8              | 100                   | Not measured  |                           |
| 194-1 Calf | -52     | +2         | 3.2              | 93                    | 0.0175        |                           |
| 197-1 Calf | -43     | -2.5       | 3.53             | 100                   | 0.019         |                           |
| 200-1 Calf | -40     | -5         | 0.9              | 100                   | 0.0143        |                           |
| 217-1 Calf | -52     | 0          | 3                | 100                   | 0.015         |                           |
| 221-1 Calf | -40     | 0          | 1.5              | 100                   | 0.005         |                           |
| T331 Calf  | -40     | 0          | 7.8              | 87.5                  | 0.031         |                           |
| T207 Calf  | -40     | +1         | 0.63             | 100                   | Not measured  |                           |
| T209 Calf  | -40     | 0          | 4.6              | 95                    | Not measured  |                           |
| 196-1 Dog  | -52     | +1.5       | 2.2              | 100                   | 0.005         |                           |
| 212-2 Dog  | -53     | +5         | 2.7              | 80                    | 0.006         |                           |
| 214-4 Dog  | -56     | +1         | 5.7              | 100                   | 0.01          |                           |
| 216-1 Dog  | -48     | +5         | 2.8              | 78                    | 0.006         |                           |
| T612 Dog   | -40     | -8         | 2.6              | 100                   | Not measured  |                           |

Panel A shows the effects of injection of TBA$^+$ on membrane current and contractile activity evoked by the voltage step shown. These records were obtained during the same experiment that was shown in Fig. 3, and the transient outward current is apparent in the control current record (left column). TBA$^+$ was injected under current clamp and voltage clamp was then re-imposed. In the presence of TBA$^+$, current evoked by the same voltage step...
FIGURE 5. Effect of TBA injection on membrane current and contractile activation. (A) Chart records of membrane current and an optical monitor of the twitch before (left) and after injection of TBA⁺ (right). TBA⁺ (4.4 μC outward charge) was injected under current clamp. (B) Peak contractile response was measured from the optical signal and plotted against test voltage before (○) and after (●) TBA⁺ injection. Curve is hand-drawn. Preparation 187-2.
no longer shows an outward transient (right column), but the twitch is unaffected.

Panel B shows the influence of TBA injection on the peak contractile activity vs. voltage relation in this fiber. The data were obtained by repeating measurement of the optical signal for a series of membrane voltages before and after injection of TBA. At voltages between −40 and +5 mV, there is little effect of TBA on this relation despite the marked block of $I_{to}$ that occurred at these voltages (Fig. 4). Thus over these voltages, it is unlikely that inhibition of intracellular calcium release is responsible for the observed reduction in $I_{to}$.

Nevertheless, at voltages more positive than +5 mV, contractile activity is reduced by as much as a factor of three in this experiment. This reduction at positive potentials when sufficient TBA is injected to substantially block $I_{to}$ varies from fiber to fiber (see Fig. 6). However, continued injection of this compound was eventually accompanied by a significant and uniform reduction in contractile activation over all voltages (Fig. 6).

Effects of TBA on Membrane Current in the Absence of $I_{to}$

Although these experiments provide evidence that the block of $I_{to}$ by TBA is not likely to be a consequence of interference with intracellular calcium release, they do raise the possibility that at these concentrations the blocker affects calcium entry via $I_{di}$. It is difficult to investigate the effects of TBA on $I_{di}$ when $I_{to}$ is prominent in the control records, as any changes in calcium current will be masked by the marked reduction in $I_{to}$. However, it is possible to study these effects when $I_{to}$ is very small or absent in control records. We have studied the effects of injection of TBA on membrane current under two different experimental conditions in which $I_{to}$ was minimized to test for an action of this blocker on $I_{di}$.

In ~10–15% of the preparations we study, control records display little or no evidence of the transient outward current. These preparations thus provide us with a small, naturally occurring population suitable for investigating the effects of TBA on $I_{di}$. Current records from one of these preparations are shown in Fig. 7 before and after injection of TBA. In contrast to earlier figures, the control trace is not dominated by $I_{to}$, and there is little change in the peak inward current after injection. Over the course of this study, we came across a total of five similar $I_{to}$-free preparations, and, in each case, TBA injection had little effect on peak inward currents evoked by depolarizing test pulses.

A more systematic approach to this problem is provided by ionic substitution experiments. Siegelbaum and Tsien (1980) have shown that replacement of extracellular calcium by Sr or Ba strongly suppresses the transient outward current. We chose to follow this procedure and replaced Ca by Sr to produce conditions suitable for testing for an action of TBA on $I_{to}$. The results of one of these experiments are presented in Fig. 8, which shows that under these
FIGURE 6. Effect of progressive TBA injection on contractile activation. Peak tension was measured and plotted against test voltage before (○) and after two successive periods of TBA injection: 3 μC (■) and 7 μC (■). A smooth curve was fit to the TBA-free data and then scaled down by a factor of 0.68 to determine the curve through the 7-μC TBA data. The inset shows tension records in response to test pulses to -23, -11, 0, and +23 mV for each run. I_{to} was completely suppressed after injection of 3 μC TBA. Preparation 217-1.

FIGURE 7. Effect of TBA injection in a preparation containing little I_{to}. Membrane current in response to test pulses from -40 to -5 mV before and after injection of 3 μC TBA. Control record shows little evidence of I_{to}. Inward shift in late current suggests a reduction in I_{x} by TBA, but early peak inward current is not affected. Preparation TS421. Apparent core volume was 0.03 mm^{3}.

conditions TBA injection has little effect on the magnitude or on the time course of the slow inward current.

From the dimensions of this preparation (0.0037 mm^{3}) and the amount of charge injected (1.5 μC), intracellular TBA concentration is estimated to be 4.2 mM (see Discussion). This is more than twice the average TBA concentra-
tion effective for $I_{to}$ block in similar preparations (see Table III and Discussion). Thus this result, confirmed in four other experiments, shows that at these concentrations TBA has little effect on $I_{a1}$ in contrast to its potent effects on $I_{to}$. As is the case for tension, prolonged injection of TBA ultimately results in a reduction in $I_{a1}$ in these experiments. However, because $I_{a1}$ is much less sensitive than $I_{to}$ to injection of this compound, it is possible to perform experiments for prolonged periods after block of the transient outward current by TBA with little change in $I_{a1}$. By avoiding excessive TBA injection, we have been able to carry out experiments for periods in excess of 2 h after injection of the quaternary ammonium compound.

**Iontophoresis of TBA$^+$ Blocks Delayed Rectification**

The results presented so far have emphasized the effects of quaternary ammonium compounds on currents that respond within the first 100 ms of
voltage depolarizations. However, both TEA⁺ and TBA⁺ injection consistently reduced outward current over the entire duration of the test pulses that we imposed (cf. Fig. 1, Fig. 3). The delayed rectifier \( I_\kappa \) activates slowly during voltage steps positive to \(-30\) mV and contributes time-dependent outward current to the later portions of the current records in these experiments. Therefore we investigated the effects of these compounds on \( I_\kappa \) to determine whether reduction in this current contributes to the late shifts in current we observed.

The records in Fig. 9 show the effects of TBA⁺ injection on \( I_\kappa \) tail currents measured as described in Methods. The currents were measured at a \(-34\)-mV holding potential after 1-s steps to the voltages indicated in the figure. The control records (A) show \( I_\kappa \) current tails whose magnitudes increase as the prior voltage step is made more positive. After these recordings, TBA ions were injected under current-clamp conditions, and the same voltage steps were re-imposed. In the presence of TBA⁺ the subsequent \( I_\kappa \) tails are suppressed after each voltage tested.

Fig. 10 shows a more complete analysis of the effects of TBA⁺ iontophoresis on \( I_\kappa \). In this experiment, the \( I_\kappa \) tail magnitudes were recorded at a \(-34\)-mV holding potential after a series of 1-s voltage depolarizations. These tail magnitudes are plotted vs. test pulse voltage to determine the 1-s isochronal activation curve for \( I_\kappa \) (see Methods). We monitored the effects of progressive iontophoresis of TBA⁺ on the activation of \( I_\kappa \) by following the control measurements with two sequential periods in which the blocker was injected under current clamp. The progressive suppression of \( I_\kappa \) in this experiment is evidence that TBA ions block this current in a dose-dependent manner.
We were able to obtain a rough estimate of the relative potencies of TEA and TBA on \( I_a \) in one experiment (Fig. 11), in which we first injected TEA\(^+\) ions until additional injection produced no further reduction in \( I_a \). This was then followed by iontophoresis of TBA ions. The additional reduction of \( I_a \) produced by TBA\(^+\) is strong evidence that this compound is, in fact, more effective than TEA\(^+\) at blocking this current.

Results from these, and all of our additional experiments on inhibition of \( I_a \) by TEA and TBA injection, are summarized in Table II.

**Characteristics of Plateau Current Measured in the Presence of TBA\(^+\)**

Our results indicate that TBA\(^+\) can be introduced into the interior of Purkinje fiber cells at sufficiently high concentrations to markedly block the two time-dependent outward plateau currents \( I_a \) and \( I_w \) with minimal effects on the time-dependent inward current \( I_i \). But what are the characteristics of the remaining time-dependent current? In the experiments shown in Figs. 1–4, membrane current measured in response to voltage depolarizations after injection of either TEA or TBA ions is characterized by an initial inward surge that then inactivates. As \( I_w \) and \( I_i \), the other time-dependent plateau currents, have been blocked, this current should be carried for the most part by the calcium current \( I_a \).

The calcium sensitivity of the membrane current that remains after TBA\(^+\) loading is shown in Fig. 12. In this experiment, 6.1 \( \mu \)C of TBA\(^+\) had been injected as a pretreatment. After this injection the current evoked by the
depolarization shown had no sign of an outward transient, although before TBA treatment this current component had been prominent. The calcium concentration in the external solution was reduced from 5.4 to 1.8 mM while the same voltage pulse was imposed once every 4 s. Reduction of \( \text{Ca}_0 \) rapidly reduced the inward current evoked by this voltage pulse, and this effect was fully reversible. This observation is expected if the time-dependent current recorded after TBA injection is primarily \( I_{\text{ad}} \).

We also investigated the effects of the organic calcium channel blocker D600 on membrane current recorded after TBA loading. These results are of particular interest because \( I_0 \) and \( I_s \) are both reduced by calcium antagonists such as D600 (Siegelbaum et al., 1977; Siegelbaum and Tsien, 1980; Kass and Tsien, 1975). Consequently, if these currents are not first blocked by a procedure such as TBA injection, they will contribute an outward component to D600-sensitive current records and preclude pharmacological dissection of the calcium current \( I_{\text{ad}} \).

Fig. 13A shows the effects of D600 on membrane current in a preparation that had been pre-loaded with 3.72 \( \mu \text{C} \) of TBA\(^+ \). As in the previous experiment, the current recorded before TBA injection contained a sizeable outward transient that was blocked by the injection procedure. D600 (10 \( \mu \text{M} \)) completely blocks the time-dependent current evoked on depolarization, but does not affect the holding current. The current that is blocked by D600 is seen
more directly in the lower current trace that was obtained by subtracting the current recorded in the presence of D600 from the D600-free signals. The difference current shows no sign of $I_{\text{to}}$, in contrast to difference records obtained in untreated fibers (see Siegelbaum and Tsien, 1980; Siegelbaum, 1978).

Panel B shows the voltage-dependence of the D600-sensitive current after TBA injection. We determined this current-voltage relation by repeating the D600 subtraction procedure at a series of voltage steps. Peak inward current was measured from the difference currents, which were obtained as in A, and then peak current was plotted against test potential. Currents measured in

**TABLE II**

| Experiment | Species | Total charge injected | Percent block | Apparent core volume | mm$^3$ |
|------------|---------|-----------------------|---------------|---------------------|-------|
| TEA        |         |                       |               |                     |       |
| 170-4      | Calf    | 19.5                  | 90            | 0.012               |       |
| 172-2      | Calf    | 7                     | 74            | 0.039               |       |
| 172-4      | Calf    | 15                    | 39            | 0.079               |       |
| TBA        |         |                       |               |                     |       |
| 169-2      | Calf    | 5.2                   | 88            | 0.014               |       |
| 172-4      | Calf    | 6.0                   | 96            | 0.079               |       |
| 176-1      | Calf    | 4.6                   | 94            | 0.010               |       |
| 176-2      | Calf    | 3.3                   | 92            | 0.030               |       |
| 177-1      | Calf    | 5.7                   | 100           | 0.023               |       |
| 178-2      | Calf    | 6.0                   | 84            | 0.033               |       |
| 182-1      | Calf    | 5.0                   | 100           | 0.03                |       |
| 183-1      | Calf    | 6.7                   | 84            | 0.017               |       |
| 200-1      | Calf    | 0.9                   | 98            | 0.014               |       |
| 201-1      | Dog     | 0.5                   | 100           | 0.004               |       |
| 202-1      | Dog     | 0.7                   | 78            | 0.004               |       |
| 212-2      | Dog     | 2.7                   | 100           | 0.005               |       |
| 212-4      | Dog     | 5.7                   | 100           | 0.010               |       |
| 216-3      | Dog     | 4.5                   | 95            | 0.015               |       |

response to voltages negative to $-35 \text{ mV}$ displayed little or no time dependence, and were thus measured 10 ms after imposition of the test steps. The current-voltage relation obtained by this method (B) agrees reasonably well with data for $I_{\text{to}}$ obtained in other preparations in which outward current overlap is less severe (cf. Reuter and Scholtz, 1977).

**DISCUSSION**

*Iontophoresis of TEA$^+$ and TBA$^+$: a Pharmacological Tool*

This study was designed to investigate new pharmacological procedures that can be used to simplify the analysis of cardiac membrane currents. Our results
(summarized in Table III) demonstrate that in Purkinje fibers, iontophoresis of the quaternary ammonium compounds TEA⁺ and TBA⁺ can block the outward transient \( I_{ow} \) and the delayed rectifier \( I_v \). Several additional interesting findings are apparent in Table III. In calf, \( I_v \) and \( I_{ow} \) are equally sensitive to TBA injection, and both currents are somewhat less sensitive to injection of TEA. Also, although most of our experiments were carried out in calf Purkinje fibers, the data from our experiments in dog and sheep preparations demonstrate consistent effects of TEA and TBA in these preparations as well. Although our results were obtained in Purkinje fibers, it is reasonable to expect that this procedure may be just as effective in other cardiac preparations.

![Figure 12](image_url)

**FIGURE 12.** Effect of extracellular calcium on membrane current after injection of TBA⁺. Records obtained after injection of TBA⁺ (total outward charge = 6.1 \( \mu \)C). Membrane currents evoked by the indicated voltage step were recorded in 5.4 mM \( \text{Ca}_o \) (a) and 8 min after lowering \( \text{Ca}_o \) to 1.8 mM (b). Trace c was obtained 7 min after returning to 5.4 mM \( \text{Ca}_o \). Preparation 191-1.
Comparison with Studies in Nerve

The block of these Purkinje fiber channels by quaternary ammonium compounds in some ways resembles block of squid axon potassium channels by these compounds. As in squid axon, but not in myelinated nerve (Hille, 1970; Armstrong and Hille, 1972; Armstrong, 1975), TEA+ is more effective when it is applied to the inside surface of the Purkinje fiber sarcolemmal membrane (cf. Kenyon and Gibbons, 1979a). Although we did not systematically compare

![Figure 13. D600-sensitive current after injection of TBA+. Records were obtained after injection of TBA+ (3.53 μC total outward charge). (A) Membrane current recorded in the absence (a) and presence of D600 (10 μM) in response to 200-ms voltage depolarizations to −2 from a −43 mV holding potential. Lower trace: D600-sensitive current obtained by subtracting trace b from trace a. (B) Peak inward D600-sensitive current ( O) plotted against pulse voltage. Smooth curve of the form \( I = (1 + \exp[-(V + 7.8)/6.1])^{-1} \). Preparation 197-1.](image)
the two compounds, our observation that TBA$^+$ is more effective than TEA$^+$ as a blocker of Purkinje fiber outward currents also agrees with the finding in squid axons that K channel blocking potency of symmetric tetraalkylammonium compounds generally increases with increasing alkyl chain length (French and Shoukimas, 1981).

Our results are not sufficiently quantitative to provide detailed characteristics about the structure of these cardiac channels. However, these similarities suggest that $I_{to}$ and $I_x$ are carried by channels that resemble K channels in the squid axon in their sensitivity to intracellular quaternary ammonium ions. Thus these currents are likely to be carried, at least in part, by potassium ions. This finding, based on pharmacological evidence, is consistent with previous results implicating potassium ions as the principal $I_{to}$ and $I_x$ charge carrier (Kenyon and Gibbons, 1979a and b; Isenberg, 1978; Siegelbaum and Tsien, 1980; Noble and Tsien, 1968, 1969).

In the squid giant axon, internal perfusion of high levels of TEA and its derivatives results in rather nonspecific block of ionic current. For example,

### Table III
**EFFECTS OF TEA/TBA ON OUTWARD PLATEAU CURRENTS: MEAN DATA**

| Current | Species | Blocker | Number of experiments | Mean percent blocked | Mean charge injected | Mean core volume |
|---------|---------|---------|-----------------------|----------------------|---------------------|------------------|
| $I_t$   | Calf    | TBA     | 9                     | 93                   | 5.04                | 0.028            |
| $I_t$   | Calf    | TEA     | 3                     | 68                   | 13.83               | 0.043            |
| $I_x$   | Dog     | TBA     | 5                     | 94.6                 | 2.82                | 0.008            |
| $I_x$   | Calf    | TEA     | 5                     | 63                   | 12.2                | 0.030            |
| $I_o$   | Calf    | TBA     | 18                    | 93                   | 4.3                 | 0.021            |
| $I_o$   | Dog     | TBA     | 5                     | 91.6                 | 3.2                 | 0.007            |
| $I_o$   | Sheep   | TEA     | 2                     | 77                   | 8.5                 | 0.0145           |

Rojas and Rudy (1976) have shown that these compounds block sodium currents as well as potassium currents when they are perfused at high intracellular concentrations. Similarly, we find that in Purkinje fibers, high intracellular TBA concentrations brought on by prolonged injection are also accompanied by nonspecific actions. Under these conditions TBA reduces $I_{si}$ and contractile activity in addition to reducing $I_{to}$ and $I_x$. However, it must be pointed out that significant nonspecific effects are observed at TBA concentrations roughly two to three times higher than those concentrations that produce effective block of outward currents.

### Intracellular Blocker Concentrations

What intracellular TEA$^+$ or TBA$^+$ concentrations are needed to produce the amount of block observed in our experiments? We cannot compute exact concentrations for our data; however, reasonable estimates can be made with the following assumptions.

Purkinje fibers are multicellular preparations, and we must first assume
that both TEA and TBA ions pass readily from cell to cell. In our experiments
preparation lengths varied from 1 to 2 mm, and the blocking compounds were
injected at impalement sites located midway between the ends of these fibers.
This creates longitudinal diffusion pathways of 0.5 to 1 mm from the current-
passing microelectrode. Because individual cells of Purkinje fiber bundles are
on the order of 100 μm in length, in order to be effective, the blocking
compounds must be distributed throughout several cells.

Weingart (1974) has shown that TEA ions move freely across the nexus
membrane that separates adjacent cells in calf ventricular muscle. Because
the Corey-Pauling-Kolten model gives an approximate diameter of 9 Å
(French and Shoukimas, 1981) for the sphere circumscribing the tetrahedral
TBA⁺ cation (compare with 8–8.4 Å for TEA⁺), it is reasonable to assume
that this molecule does not markedly differ from TEA⁺ in cell-to-cell-move-
ment, and that both cations are distributed throughout the cell columns in
these experiments.

Assuming a transfer number of 1 for movement of TBA⁺ out of the current
electrode and using the number of moles injected per coulomb of outward
charge passed (1.04 × 10⁻⁵ M/C) along with the data in Table III, we can
compute upper limits for intracellular blocking agent concentrations. For
example, in calf, this approximation yields an average intracellular TBA
concentration of 1.9 mM in the experiments on Iₜ, and a 2.13-mM TBA
concentration in the Iₒ-blocking experiments. This concentration range is of
the same order of magnitude as found in the block of potassium channels in
squid axons (French and Shoukimas, 1981).

Time Dependence of the Block

TEA ion and its derivatives produce a time-dependent block of potassium
currents in squid axons. This time dependence, sensitive to membrane poten-
tial as well as blocker concentrations, is caused by diffusion of the blocking
molecule into the open potassium channel (Armstrong, 1969, 1971). French
and Shoukimas (1981) have compared the time course of block of TEA⁺ and
TBA⁺ over several concentrations and voltages. They found the blocking time
constants varied from 1.9 to 0.8 ms for both compounds over a blocker
concentration range of 0.3–3 mM.

In a preliminary set of experiments, we have found no change in TBA block
of membrane current recorded during test pulses applied from different
holding potentials (−50 to −80 mV) or preceded by hyperpolarizing condition-
ing steps (20 ms to 5 s). This insensitivity to voltage could, in part, be
caused by the kinetics of the quaternary-ammonium-sensitive currents blocked
during the test pulses. These currents in the Purkinje fiber are 1.5–2 orders of
magnitude slower than the blocking time constants reported for quaternary
ammonium ion block of potassium channels in nerve. On the other hand, we
have not yet systematically studied the blocker-sensitive current that follows
the break of depolarizing voltage steps to determine whether voltage-depen-
dent changes in block might alter the time course of these current tails, as has
been described in squid axon (Armstrong, 1969, 1971).
In summary, we find that iontophoretic injection of quaternary ammonium compounds provides a technique for blocking time-dependent outward currents in the cardiac Purkinje fiber. This procedure should be useful in the analysis of plateau currents in this and other cardiac preparations and contribute to the general understanding of ionic currents in the heart.

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