The Effects of Acetylcholine on the Electrical Activity of Canine Cardiac Purkinje Fibers

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SUMMARY We studied the effects of acetylcholine (ACh) on small bundles of canine cardiac Purkinje fibers exposed to normal, or low-chloride (isethionate) Tyrode’s solution in a rapid superfusion system. In superfusate containing 4 mM K+, the resting potential of Purkinje fibers may be either “low,” near —40 mV, or “high,” near —90 mV. ACh, at $10^{-8}$ to $10^{-4}$ M, increased the membrane potential from both the low and high resting levels and, in low-Cl solution, often induced a maintained shift in potential from the low to the high level. The increase in membrane potential caused by ACh was greater at the low than at the high level. ACh, at $10^{-8}$ to $10^{-4}$ M, reduced action potential duration in both normal and low-Cl Tyrode’s solution, the effect being more marked in the latter. These effects of ACh were reversibly abolished by atropine ($5 \times 10^{-5}$ M), indicating that they were mediated via muscarinic ACh receptors, and they probably result from an increase in membrane K⁺ conductance since $10^{-3}$ M ACh reversibly reduced, by 13% on the average, the amplitudes of the steady changes in membrane potential evoked by applying small current pulses ($-5$ to $-25$ nA, 200 msec). ACh ($10^{-3}$ M) also diminished the rate of, or stopped, spontaneous activity arising from either level of membrane potential. The cessation of spontaneous slow response activity, arising from the low level, sometimes was accompanied by a maintained shift of the membrane potential to the high resting level. It is concluded that the action of ACh on Purkinje fibers is qualitatively similar to its action on sinoatrial nodal and atrial cells.

IT IS well known that stimulation of the vagus nerve or the direct application of acetylcholine (ACh) reduces the slope of spontaneous pacemaker depolarization, lowers the frequency of spontaneous activity, and may increase the maximum diastolic potential in fibers of the frog sinus venosus and in fibers of the mammalian sinoatrial node. The same maneuvers lead to an increase in the resting potential and to a marked decrease in action potential duration in atrial fibers. All of these effects are inhibited by atropine. During the action of ACh, the permeability of the membrane to potassium ions is increased and this increase provides, at least in part, an explanation for the results described above, although recently it has been suggested that a reduction in the slow inward current also might contribute to some of those effects. Early studies of the effects of ACh on the membrane potential of Purkinje fibers yielded equivocal results, and it has, therefore, often been assumed that these fibers are insensitive to ACh. More recently, however, ACh has been shown to lower the frequency of spontaneous activity in canine Purkinje fibers. In the present study, use was made of a fast perfusion system which permitted several brief applications of ACh to be made during a single impalement. This greatly facilitated the demonstration of the usually small, but reproducible, effects of ACh on Purkinje fibers.

Under appropriate conditions, over a limited range of external potassium concentrations ([K⁺]o), the resting potential of canine cardiac Purkinje fibers may have either of two values. Thus at 4 mM [K⁺]o, the low level is near —40 mV and the high level is near —90 mV. We find that ACh can: (1) cause an increase in membrane potential from either the low or the high resting level, and may thereby cause partially depolarized fibers to fully repolarize (cf. Gadsby and Cranefield); (2) slow, or even abolish, spontaneous activity arising from either level of resting potential; and (3) reduce the duration of action potentials arising from the “higher” level of resting potential. All of these effects are readily demonstrated in fibers exposed to solutions virtually free of Cl ions but also may be seen in the presence of normal, i.e., Cl-containing, Tyrode’s solution. Our results unequivocally demonstrate that Purkinje fibers are sensitive to ACh, and further suggest that the action of ACh on these fibers is qualitatively similar to its well known action on sinoatrial nodal and atrial cells.

Methods

Small, unbranched bundles of free-running Purkinje fibers (about 0.1–0.3 mm wide and 1.5–5 mm long) were dissected from right ventricles of dog hearts and suspended between two insect pins in the narrow channel of a modified Hodgkin-Horowicz flow system. The perfusion and recording systems have been described in detail elsewhere. Throughout the present experiments, a constant flow of 5 ml/min was maintained, at which rate the composition of the solution bathing the preparation could be changed with a half-time of approximately 0.5 second. Conventional 3 M KCl-filled microelectrodes were used for potential recording and for current injection.

The composition of the normal Tyrode’s solution was: NaCl, 137 mM; KCl, 4 mM; NaHCO₃, 12 mM; NaH₂PO₄,
1.8 mM; MgCl₂, 0.5 mM; CaCl₂, 2.7 mM; and dextrose, 5.5 mM. The equivalent "low-Cl" solution contained: Na-isethionate (Koch-Light), 141 mM; KHCO₃, 4 mM; NaHCO₃, 8 mM; Na₂HPO₄, 1.8 mM; MgCl₂, 0.5 mM; Ca-methanesulfonate (made from methanesulfonic acid; Eastman Kodak), 2.7 mM; and dextrose, 5.5 mM. The potassium concentration was varied between 0 and 12 mM by substituting KHCO₃ for NaHCO₃, or vice versa.

The following drugs were added, as required, from refrigerated, concentrated stock solutions: acetylcholine chloride (Sigma); atropine sulfate (Amend); hexamethonium bromide (Sigma); α-bungarotoxin (gift from Professor Edward A. Reich). All solutions were equilibrated with 95% O₂ and 5% CO₂. The temperature of the perfusate was continuously monitored close to the preparation with a small thermistor bead and was kept between 35°C and 37°C.

Action potential duration was measured from the upstroke to the intersection of the tangent fitted to the final phase of rapid repolarization with a line at the level of the resting potential. Changes in the duration of the action potential, or in the magnitude of resting, or electrotonic, membrane potentials are given as the mean ± SD.

Results

Resting Potential

As mentioned above, at a [K⁺], of 4 mM, the resting potential may be either near —40 mV or close to —90 mV. ACh increased the resting potential at both these levels. As illustrated in Figure 1, a and b, the brief (10- to 15-second) application of 10⁻⁶ M ACh to a fiber with a resting potential initially near —40 mV in 4 mM K⁺, low-Cl solution, caused a rapid and reversible hyperpolarization of about 6 mV. A similar brief application of 10⁻⁵ M ACh caused a greater hyperpolarization and often resulted in a maintained shift in resting potential from the "lower" to the "higher" level (Fig. 1, a and b). The same concentrations of ACh consistently gave rise to much smaller hyperpolarizations (typically less than 2 mV for [K⁺] > 4 mM) when reapplied at the higher level of resting potential (Fig. 1, a–c). Thus in a preparation different from that represented in Figure 1, exposed to 5.4 mM K⁺, low-Cl solution, in which the resting potential was —87 mV, the average hyperpolarization measured during repeated 20-second applications of 10⁻⁵ M ACh was 1.6 ± 0.2 mV (five measurements).

At either level of resting potential, a greater hyperpolarization resulted from the application of 10⁻⁵ M ACh than from 10⁻⁶ M ACh, as seen in Figure 1, a–c.

Duration of the Action Potential

The application of sufficiently high concentrations of ACh to Purkinje fiber bundles stimulated at a constant rate via external bipolar electrodes caused a rapid and reversible decline in the duration of the action potential in both normal and low-Cl solutions. ACh was usually applied for 30-60 seconds, and action potentials recorded toward the end of these test periods were compared with control action potentials recorded just before the application of ACh, and again after 1–3 minutes of washing out the drug.

These effects of ACh are illustrated in Figure 2 which shows action potentials recorded during a single maintained impalement of a typical preparation. In Figure 2a, the shortened action potential recorded after 40 seconds of superfusion with 10⁻⁵ M ACh is shown superimposed on the control action potential recorded some 45 seconds earlier in 4 mM K⁺, normal Tyrode’s solution. The effect of ACh is to cause an earlier onset of the final phase of rapid repolarization; the resulting shortening of the action potential in this instance amounted to 8.3%. The shortening obtained in this preparation after a similar exposure to 10⁻⁵ M ACh was more marked (10.3%), as shown in

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** Effects of ACh on the resting potential. Traces a, b, and c are chart recordings of the membrane potential, Vₘ. The length of the horizontal bars above the potential trace indicates the duration of each brief exposure to ACh. The numbers over those bars give the applied concentration of ACh (in mM) during each exposure. The horizontal bar (10s) in the lower right corner represents 10 seconds (4 mM K⁺, low-Cl solution).

![Figure 2](http://circres.ahajournals.org/)

**Figure 2** Effects of 10⁻⁶ m and 10⁻⁵ m ACh on the Purkinje fiber action potential in normal Tyrode’s solution (a and b, labeled CT) and in low-Cl solution in which the major anion was isethionate (c and d, labeled Ise⁻). Both solutions contained 4 mM K⁺. The stimulation rate was 1 Hz. In each panel the shortened action potential, recorded after 30–40 seconds of exposure to ACh, and the control action potential, recorded immediately before the drug application, were superimposed by using a storage oscilloscope whose display was then photographed. The upper ends of the 100 mV calibration bars indicate the zero potential level and the horizontal bars (0.5s) represent 0.5 second.
The action potentials illustrated in Figure 2 were obtained at a constant stimulation frequency of 1 Hz, at which rate the average reduction in action potential duration resulting from the addition of $10^{-5}$ M ACh to normal Tyrode's solution was 8.2 ± 3.6% (five measurements in three preparations). When the stimulation frequency was lowered to 0.5 Hz, the duration of the control action potential was increased (by 5–30% in the present experiments) and, under these conditions, the shortening during exposure to $10^{-5}$ M ACh averaged 9.2 ± 4.7% (eight measurements in five preparations).

Conductance

The changes in resting and action potential observed during exposure to ACh (Figs. 1 and 2) may be explained if acetylcholine causes the net membrane current to become more positive at voltages between the high level of resting potential and the "plateau" potential. Such a change might result either from an increase in an outward component of membrane current or from a decrease in an inward component. The direction of any change in membrane conductance which accompanies the action of ACh should help to distinguish between these two possibilities. For this reason the fiber input conductance near the resting potential was monitored in 11 short, thin Purkinje fibers of average length 2.3 mm (range, 1.5–2.7 mm) during brief test exposures to ACh. Small rectangular current pulses were repetitively applied via a second intracellular microelectrode, inserted in the midregion of the fiber within 100 μm of the potential recording electrode, and the resulting small electrotonic potential changes were continuously recorded. These experiments were carried out in 12 mM K*, low-Cl solution in an attempt to reduce any complications arising from voltage-dependent changes in membrane conductance, since preliminary results had indicated that the ACh-induced hyperpolarization became smaller at higher external K* concentrations. Thus the average hyperpolarization during brief applications of $10^{-5}$ M ACh in this solution was 0.73 mV (SD ± 0.70 mV; 55 measurements in 12 preparations): this hyperpolarization was maintained throughout ACh exposures lasting for more than 1 minute.

To avoid regenerative depolarizations, inward (hyperpolarizing) current pulses, 5–25 nA in amplitude, were used for the conductance measurements, although similar results were obtained with sufficiently small outward current pulses. The pulses were 200 msec long to allow the membrane potential changes to reach a steady state (Fig. 3, b and c). As shown in Figure 3, the amplitude of the resulting steady potential changes, which ranged from −3.4 to −6.3 mV in the absence of ACh, declined rapidly during exposure to ACh but returned to the control value shortly after washout of the drug. The average ratio of the peak amplitudes of the electrotonic potentials measured in the absence of ACh, and after 15 seconds of exposure to $10^{-5}$ M ACh, was 1.13 (SD ± 0.14; 31 measurements on 11 preparations). Figure 3 illustrates two further points: (1) the small increase in input conductance during the action of ACh is accompanied by only a small reduction in membrane time constant, as expected for preparations which are short in comparison to the fiber space constant (compare Fig. 3b with Fig. 3c), and (2) the time courses of the reversible increases in conductance and membrane potential are closely similar.

The large standard deviation of these results reflects the considerable variability between preparations in their response to ACh; indeed, one of the 11 preparations studied in these conductance experiments showed an increase in neither membrane potential nor input conductance during exposure to ACh in 12 mM K* solution. In general, a positive correlation was found between the magnitude of the hyperpolarization and the increase in input conductance. Thus four fibers showed negligibly small average conductance increases, ranging from 0 to 3%, and similarly small average increases in membrane potential, ranging from 0 to 0.2 mV; in the remaining seven fibers these changes were 5 to 44% and 0.5 to 2.6 mV, respectively. This variability in the response to ACh also may be seen in the combined results from experiments in which $10^{-5}$ M ACh was briefly applied at the lower level of resting potential in 4 mM K* solution. Excluding trials in which this application resulted in a "regenerative" hyperpolarization to the higher level of resting potential, the hyperpolarization averaged 7 mV (SD ± 4 mV; range, 0–15 mV) in 36 preparations from 17 hearts. However, it is important to note that the application of ACh never caused a depolarization.
The results just described in connection with Figure 3 establish that the hyperpolarization observed during ACh action is associated with an increase in membrane conductance. If the hyperpolarization is caused by the conductance increase, then that conductance must be selective for an ion on which the driving force is outwardly directed at both the high resting and plateau levels of membrane potential. Since ACh hyperpolarizes the membrane and shortens the action potential in both normal Tyrode’s and low-Cl solutions, the simplest explanation for these effects is that ACh causes an increase in the permeability of the membrane to potassium ions in Purkinje fibers, just as it does in preparations of frog sinus venosus, dog atrium, and guinea pig sinus node. An alternative interpretation, discussed later, is that ACh reduces an inward current and thereby causes a hyperpolarization which, because of the presence of inward-going rectification, leads to an increase in membrane conductance.

The ACh Effects are Mediated by Muscarinic Receptors

The ACh-induced increase in membrane potential and the shortening of the action potential described above were reversibly abolished in the presence of high concentrations of atropine. Since the ACh-induced hyperpolarization is much larger at the lower than at the higher level of resting potential (Fig. 1), this effect of atropine is most readily demonstrated at that lower level of resting potential.

Figure 4 shows the increase in membrane potential associated with a 15-second exposure to 10^{-5} M ACh in 4 mM K+, normal Tyrode’s solution, and the subsequent decline in membrane potential during the washout of ACh. The steady resting potential of this fiber was —46 mV and the magnitude of the initial hyperpolarization induced by ACh was approximately 11 mV. Figure 4b shows that after 2.5 minutes in the presence of 5 × 10^{-5} M atropine sulfate, the application of ACh did not increase the membrane potential. Similar results were obtained in all six preparations exposed to atropine. As seen in Figure 4c, however, after 50 minutes of exposure to atropine-free superfusate, the test application of ACh again caused a hyperpolarization of the membrane, this time of about 9 mV. On the other hand, the ACh-induced hyperpolarization was not altered after equilibration of the preparation with up to 1 mM hexamethonium bromide or with α-bungarotoxin, 100 μg/liter (two experiments each, data not shown), both of which are rather specific blockers of nicotinic ACh-receptors (see, e.g., Goodman and Gilman and Chang and Lee). These results strongly suggest that the predominant effects of ACh on Purkinje fibers are mediated via muscarinic ACh receptors.

Effects of ACh on Spontaneous Activity

Recent investigations have shown that the application of ACh to Purkinje fibers reduces the slope of pacemaker depolarization and thereby lowers the rate at which spontaneous action potentials arise from the high level of resting potential; that decline in spontaneous rate is greater during the application of higher concentrations of ACh. In the present experiments, spontaneous activity was even found to be abolished by sufficiently high concentrations of ACh, as the record in Figure 5a illustrates. This preparation was spontaneously active in normal, Cl-containing Tyrode’s solution at a frequency of approximately 0.2 Hz but became quiescent during the 30-second exposure to 10^{-5} M ACh. Within a few seconds of removing the ACh, however, spontaneous activity reappeared and, in this instance, the frequency temporarily increased to about 0.3 Hz before returning to the control level.

ACh may also abolish spontaneous “slow response” action potentials arising from the lower level of resting potential (Fig. 5, b and c). In the experiment shown in Figure 5b, spontaneous activity was suppressed during the 15-second exposure to 10^{-5} M ACh but reappeared almost immediately when the ACh was washed out. In some preparations the frequency of those spontaneous slow response action potentials temporarily increased following the removal of the ACh (see below). As shown in Figure 5c, the cessation of slow response activity in

![Figure 4](http://circres.ahajournals.org/)  
**Figure 4** The effect of 5 × 10^{-5} M atropine sulfate on the ACh-induced hyperpolarization at the lower level of resting potential. The three records of membrane potential were obtained just before (a), during (b), and 50 minutes after (c) a 7-minute exposure to atropine (Atr). At each of these times, 10^{-5} M ACh was applied for the periods between the broken lines, as indicated by the upper horizontal bar (4 mM K+, low-Cl solution).

![Figure 5](http://circres.ahajournals.org/)  
**Figure 5** The effects of ACh on spontaneous activity arising from either the higher (a), or the lower (b and c) resting potential level in three different Purkinje fiber preparations. The potential records are chart recordings. In each case the vertical bar represents 100 mV and its upper end indicates the zero potential level; the lower horizontal bars each represent 10 seconds. ACh, 10^{-5} M, was applied for the period indicated by the horizontal bar over each record: a: 4 mM K+, normal Tyrode’s solution; b and c: 4 mM K+, low-Cl solution.
seen that the ACh-induced hyperpolarization is similarly of ACh is similar in both cases: thus in Figure 2 it can be on action potentials were studied equally in high-Cl and conditions (cf. ref. 16). On the other hand, the effects of ACh in low-Cl solutions. The studies on resting potential were studied in detail at the lower resting potential level, the ACh-induced hyperpolarization was sometimes sufficient to initiate one, or several, or even a prolonged train of slow response action potentials as illustrated in Figure 6b. It seems likely that the post-ACh acceleration of slow response activity, which was mentioned in connection with Figure 5b, is closely related to the post-ACh initiation of slow responses (Fig. 6b) and the post-ACh membrane depolarization (Fig. 6a). Whatever the mechanism for these effects, it is unlikely that they result from the activation of nicotinic ACh receptors, since we have found that they persist in the presence of 1 mM hexamethonium bromide or 100 µg of α-bungarotoxin per liter (two experiments each, data not shown).

Discussion

The effects of ACh on Purkinje fibers reported above, namely, an increase in resting potential, a shortening of the action potential, and a slowing or stopping of spontaneous activity arising from either level of resting potential, could all be obtained in either low-Cl or normal Tyrode’s solutions. The studies on resting potential were generally carried out in low-Cl solutions, however, since both levels of resting potential may usually be obtained in the absence of spontaneous activity under these conditions (cf. ref. 16). On the other hand, the effects of ACh on action potentials were studied equally in high-Cl and in low-Cl solutions, and the results suggest that the action of ACh is similar in both cases: thus in Figure 2 it can be seen that the ACh-induced hyperpolarization is similarly very small in both chloride and isethionate solutions. Although the percentage shortening of the action potential is greater in the low-Cl solution, the duration of the action potential in the absence of ACh is also greater in this solution. It seems likely that the apparent increase in the effect of ACh in low-Cl solutions is secondary to the increase in the duration of the control action potential, since a reduction in the frequency of stimulation, another maneuver which results in a prolongation of the control action potential, also tends to increase the degree of ACh-induced shortening (see Results, above).

In the present experiments, the shortening of the action potential was found to increase with the concentration of ACh applied and was abolished by atropine. Similar, although larger, effects were previously described for atrial fibers from cat hearts and dog hearts. A 10% reduction in the duration of the action potential of canine cardiac Purkinje fibers in the presence of a high ACh concentration (10−4 g/ml, i.e., approximately 5.5 x 10−4 M) was reported by Schmidt and is quite consistent with the present results. Since the principal effect of ACh on the Purkinje fiber action potential appears to be a reduction in the duration of the plateau phase without changing the voltage level at which it occurs (Fig. 2), there seems to be little need, at present, to invoke a decline in the peak amplitude of the slow inward current to explain this effect (cf. other studies).

The fiber input conductance was found to increase rapidly and reversibly in the presence of ACh (Fig. 3) and with a time course closely similar to that of the ACh-induced increase in resting potential. Assuming that the internal resistance of the fiber did not change appreciably during the 15 seconds of exposure to ACh, this result indicates that the membrane conductance was increased during that time. Since short, thin preparations (average length, 2.3 mm) were used for this study, it is probable that the membrane potential at the end of the 200-msec. current pulses was approximately uniform throughout the fiber in the 12 mM K+ solution. In that case the measured ratio of the steady electrotonic potential deflections in the absence and presence of ACh, respectively, should approximate the inverse ratio of the membrane conductances in these two conditions. The former ratio will underestimate the latter if the steady membrane voltage is not spatially uniform, but in that case a further small correction for the expected change in space constant also should be applied and this will tend to reduce the discrepancy between the two ratios. We may therefore conclude that the average increase in membrane conductance in 12 mM K+, low-Cl solution, resulting from the application of 10−4 M ACh, was approximately 13% in the 11 preparations examined. It is also reasonable to assume that this change represents an increase in the membrane conductance to K+ since chloride ions were largely absent and a similar increase in Na+ or Ca2+ conductance would be expected to cause membrane depolarization, not hyperpolarization. The possibility should be considered, however, that the increase in K+ conductance is the result of the hyperpolarization, and not its cause, due to the presence of inward-going rectification. ACh might induce the hyperpolarization, for example, by reducing a steady...
state Na⁺ or Ca²⁺ membrane current (the conductance to this current need be only small since the driving force is expected to be quite large). Indeed, several studies have indicated that ACh reduces the magnitude of the slow inward current,⁶⁻¹⁰ and other results suggest that a significant fraction of this current does not inactivate with time.²³ It seems reasonable to postulate, therefore, that ACh might diminish steady state, slow inward current and thereby hyperpolarize the membrane. However, the slow inward current is thought to be activated only at voltages positive to −40 mV (see, e.g., Kass et al.²¹ and Reuter²⁵). Therefore, although such an effect could, in theory, contribute to the hyperpolarization observed in 4 mM K⁺ solution at the lower level of resting potential (e.g., Fig. 1a and b), it is much less likely to do so in 12 mM K⁺ solution in which the resting potentials were more negative than −60 mV. Clearly, the small hyperpolarization caused by ACh at the higher level of resting potential (Fig. 1, a−c) cannot be explained in this way, although a further effect of ACh on steady state, “background” inward current cannot, of course, be excluded. On the other hand, our results are consistent with the hypothesis that ACh causes a specific increase in the potassium permeability of the Purkinje fiber cell membrane via an interaction with muscarinic receptors, as has previously been shown for atrial and sinus preparations (see ref. 9 for review). The 2.6% average increase in tracer-potassium uptake caused by 1.2 × 10⁻⁶ M ACh in canine cardiac Purkinje fiber preparations recently reported by Musso and Vassalle²⁶ provides more direct evidence for an increase in potassium permeability in this tissue and supports the present findings.

There have been several reports of a diminished rate of spontaneous activity in isolated canine cardiac Purkinje fiber preparations in the presence of ACh concentrations in the range 10⁻⁷ to 10⁻⁴ g/ml.¹³⁻¹⁵,²⁶ In their preliminary communication, Danilo et al.¹⁵ noted that the rate of spontaneous activity declined significantly in response to ACh concentrations greater than 10⁻⁷ M and that this effect was abolished in the presence of 2 × 10⁻⁶ M atropine. Similar results were obtained in the present study, and relatively high concentrations of ACh (e.g., 10⁻⁵ M) were often found to induce quiescence in fibers showing spontaneous activity of either the slow response (slow upstroke) type (Fig. 5a) or the “normal” (rapid upstroke) type (Fig. 5b). The suppression of spontaneous slow response activity, particularly if accompanied by a shift of the membrane potential to the high resting level (Fig. 5c), is an effect which is potentially antiarrhythmic.¹⁶ Thirty-five a possibly related observation is that of Coraboeuf et al.¹⁵ who found that the addition of 10⁻⁴ M ACh to Purkinje fibers previously depolarized by exposure to Tyrode’s solution saturated with 20% CO₂ resulted in “immediate repolarization.” Since the concentration of ACh reaching the membrane of Purkinje fibers in intact hearts is not known, however, the importance of ACh in the termination of ventricular arrhythmias cannot, at present, be assessed.

The transient increase in the frequency of occurrence of spontaneous, rapid upstroke, action potentials arising from the higher resting potential level which sometimes followed the exposure to ACh (Fig. 5a) was not investigated further. Its mechanism is unknown but it may be related to the post-vagal, or post-ACh, sinus node tachycardia studied by Loeb and Vassalle,²⁶ who suggested that that tachycardia was caused by catecholamines released as the result of an interaction between ACh and nicotinic ACh receptors. It seems unlikely, however, that a similar mechanism can explain the post-ACh depolarization (Fig. 6a) or the post-ACh initiation (Fig. 6b), or increase in frequency, of slow response action potentials arising from the lower level of membrane potential, since these effects persist in the presence of high concentrations of hexamethonium or α-bungarotoxin, which are both good antagonists of nicotinic ACh receptors. That the post-ACh effects in partially depolarized fibers were generally most marked when the ACh-induced hyperpolarization was also marked suggests a possible alternative explanation: namely, that these post-ACh effects result from a very small net increase in inward current which slowly decays and which itself is caused in some way by the preceding hyperpolarization. This suggestion is supported by the observation that, at the lower level of resting potential, a small, prolonged afterdepolarization often follows the gradual decline of moderate hyperpolarizations caused by the application of linear, slowly rising and then falling, “double ramps” of inward current (D.C. Gaday and P.F. Cranefield, unpublished observations).

A final point relates to the observed variability of the effects of ACh from one preparation to another, mentioned earlier. Some difference was found even between the responses of otherwise apparently similar fiber bundles dissected from the same ventricle. The source of this variability is unknown, but one possibility is that it reflects differences in the density of muscarinic ACh-receptors from one Purkinje fiber bundle to another.

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References

32. Reuter H: Divalent cations as charge carriers in excitable membranes. Prog Biophys Mol Biol 26: 3-43, 1973
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