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THE ACTION OF ACETYLCHOLINE ON BACKGROUND CONDUCTANCE IN FROG ATRIAL TRABECULAE

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SUMMARY

The action of acetylcholine (ACh) on membrane potential and currents in frog atrial muscle has been studied with a double sucrose gap technique. The results show the following.

1. ACh induces the development of an extra current, outward at the resting potential, which is dependent on the ACh concentration.

2. The preparation does not show any sign of desensitization.

3. The reversal potential of the current induced by ACh is between 0 and 20 mV more negative than the resting potential and behaves as a K electrode.

4. The mechanism of ACh-induced K conductance presents inward going rectification properties.

5. The delayed outward current is not affected by ACh. However the evolution of its tail current seems to indicate a process of K accumulation related to the ACh-induced current.

INTRODUCTION

The electrophysiological action of ACh on cardiac atrial tissue results in a substantial shortening of the action potential duration, the overshoot being lowered or unaffected (Burgen & Terroux, 1953; Hoffman & Suckling, 1953; Hutter & Trautwein, 1956; Ware & Graham, 1967), an increase in the membrane conductance as shown by measurements of time and space constants (Trautwein & Dudel, 1958) and an increase in K permeability (Harris & Hutter, 1956; Hutter, 1961; Trautwein, 1963). These results support the hypothesis that ACh induces an increase in a specific K permeability of the cardiac cell membrane.

The application of ACh also provokes a decrease in the mechanical response. This effect was previously considered as a consequence of the shortening of the action potential duration resulting from an increase in K permeability (Burgen & Terrroux, 1953; Antoni & Rothmann, 1968). More recently Prokopczuk, Lewartowsky & Czarnecka (1973), Giles & Tsien (1975); Ikemoto & Goto (1975), Garnier, Goupil, Nargeot & Ojeda (1976*a*), Giles & Noble (1976) reported evidence that the negative inotropic effect could also be mediated by a decrease of the slow inward current.

The aim of the present paper is to present more direct measurement of the K conductance changes produced by ACh in frog atrial trabeculae using a voltage clamp technique. Preliminary reports have already been published (Garnier & Rougier, 1969; Garnier, Goupil, Nargeot, Ojeda & Rougier, 1976*b*; Garnier, Goupil, Nargeot, Ojeda & Rougier, 1976*c*).

METHODS

The current and voltage clamp experiments were performed at room temperature using a double sucrose-gap technique previously described by Rougier, Vassort & Stämpfli (1968).

(*a*) *The preparation.* The preparation consisted of thin trabeculae (50–100 μm in diameter, 3–4 mm in length), isolated from the atrium, mostly from *Rana esculenta*, but also some from *R. catesbeiana* (Figs. 3*C–D*, 9 and 10).

(*b*) *The bath.* The test compartment of our apparatus was 200 μm wide. But to this must be added the Vaseline seals width (about 100 μm each). However if we take into account the diffusion of sucrose from the sucrose gap regions, and if we consider the effective test compartment as the region bathed by more than 80% Ringer, we have to reduce the 'total test compartment' by about one fibre diameter at each edge (Kleber, 1973; Attwell & Cohen, 1977). In consequence the effective width of the test compartment is about 200 μm. In other words, the Vaseline seals can be neglected as done in our original description of the technique (Rougier *et al.* 1968). In these conditions, the transgap leakage currents and the cable non-uniformity errors are relatively small (De Hemptinne, 1973; MacGuigan, 1974; Giles & Noble, 1976; Brown, Noble & Noble, 1976). Nevertheless, our results have to be considered as only semi-quantitative, because the true membrane current is contaminated by an unknown proportion of leakage current from the region bathed by abnormal Ringer. Even the kinetic analysis has to be taken as a semi-quantitative reflexion of the real membrane current.

The sucrose-gap width is about 300 μm; adding the Vaseline seals leads to a width of 500 μm; taking account of the diffusion of Ringer from the Ringer pools leads to an effective width of about 300 μm.

(*c*) *Solutions.* The composition of the Ringer solution used was NaCl 110·5 mM; KCl 2·5 mM; $NaHCO_3$ 2·4 mM; $CaCl_2$ 1·8 mM; pH 8·2. This solution differs from the frog plasma most notably in the absence of the magnesium and sulphate ions and in the presence of bicarbonate in a much lower concentration. The K^+-rich and K^+-depleted solutions were made by substituting NaCl for KCl and vice versa in equimolar quantity.

To the isotonic sucrose (214 mM), $CaCl_2$ was added to prevent cell uncoupling (Kleber, 1973), the final resistivity being 0·8 μs.

In some experiments, permeability inhibitors were used: $MnCl_2$ 4 mM in order to block the slow inward current, and tetrodotoxin (TTX) $5 \cdot 10^{-7}$ g/ml. in order to block the fast initial current (Rougier, Vassort, Garnier, Gargouil & Coraboeuf, 1969). In a recent paper Connor, Barr & Jackobsson (1975) have reported that this concentration of TTX fails to abolish the fast inward current completely in frog atrial traceculae. Therefore the first 10 msec of the pulses might be affected by spatial non-uniformity. Nevertheless this artifact does not greatly affect the present study in which the currents are measured more than 20 msec after the onset of the depolarizing pulses.

ACh and carbachol were used as chloride salts at concentrations between 10^{-8} and 10^{-5} M, these two compounds having comparable effects (carbachol differs in being hydrolysed more slowly). Atropine 10^{-6} M was used as cholinergic antagonist.

The test compartment was perfused at a constant speed (1 ml./min) using a peristaltic pump.

(*d*) *Nomenclature.* Holding potential (H.P.) is the potential at which the fibre is maintained H.P. = 0 corresponds to the resting potential which is the potential at which no net current is flowing (zero current potential). V = variations of potential from the H.P.; the depolarizing pulses are positive. I = membrane currents; outward currents are positive. I_B, $I_{K,1}$, $I_{x,1}$ = respectively unspecific background current ($I_B = I_{Na,\infty} + I_{(Ca,Na)\infty} + I_{x,\infty} + I_p$ (pump current) (see MacAllister, Noble & Tsien, 1975), K time independent current, time and voltage dependent current of the delayed rectifier. I_{ACh} = extra-current induced by the addition of ACh or carbachol. $E_{K,1}$, $E_{x,1}$, E_{ACh} = respectively reversal potential for $I_{K,1}$, $I_{x,1}$ and I_{ACh} currents.

RESULTS

1 *Effects of acetylcholine on atrial action potential and membrane resistance*

The addition of ACh 10^{-7} M to the Ringer solution, the preparation being pulsed at a constrat rate (0·5 Hz), induces within only a few seconds a significant shortening of the action potential (Fig. 1*A*). The membrane potential is not or only very slightly modified (hyperpolarization of 1–3 mV). The fast initial depolarization of the action potential does not seem to be affected (the dV/dt measurement obtained is around 30 V/sec and not changed with ACh); but the slow depolarization and the

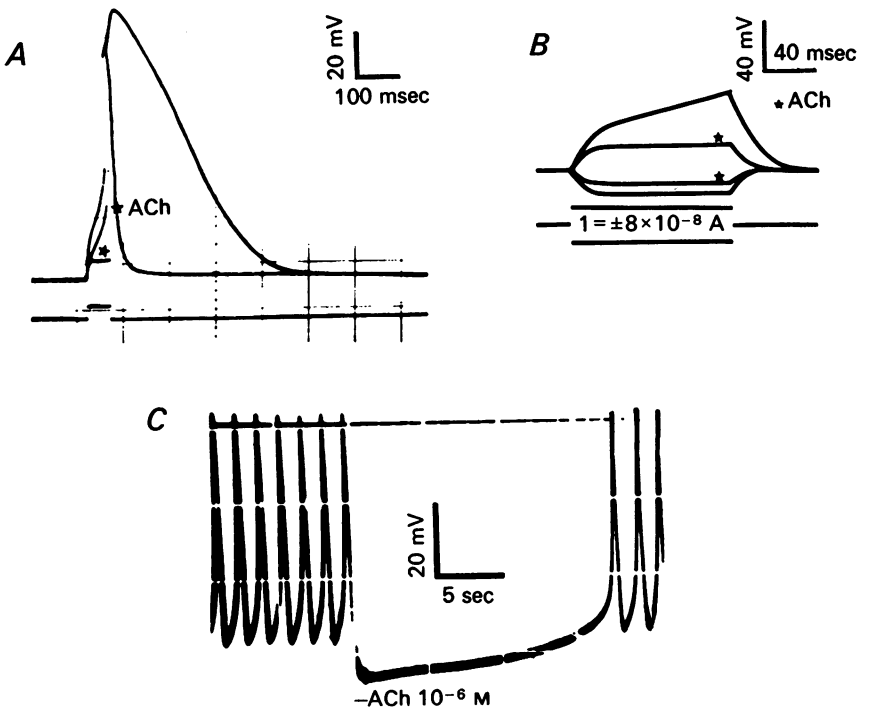

Fig. 1. *A*, effect of ACh (10^{-7} M) on the action potential of frog auricular fibres. Note the increase in current threshold. *B*, action of ACh on the membrane resistance. Experiment performed in a Ringer TTX solution. *C*, effect of a short application (1 sec) of 10^{-6} M ACh on the pace-making activity induced by a constant depolarizing current ($5 \cdot 10^{-8}$ A). Note the large hyperpolarization following the introduction of ACh and the return of the pace-making activity during the washout of ACh.

plateau are almost abolished. Furthermore a substantial increase in the current threshold is noted and is consistent with a marked fall in the membrane resistance with ACh (Fig. 1*B*). The pace-making activity induced by a constant depolarizing current is stopped when ACh is added and a marked repolarization due to the decrease in the membrane resistance is observed (Fig. 1*C*). All these effects are abolished by the addition of atropine (10^{-5} M).

2 *Measurements of the current induced by ACh:* (I_{ACh} *extra current*)

The I_{ACh} extra current is estimated by measuring the variation of current induced by the application of ACh in the test compartment for only a few seconds. The experimental protocol is as follows: the membrane is held at a given potential and when the current needed to maintain this potential has reached a steady value, ACh is introduced. Fig. 2 gives an example of such an experiment: the membrane is depolarized

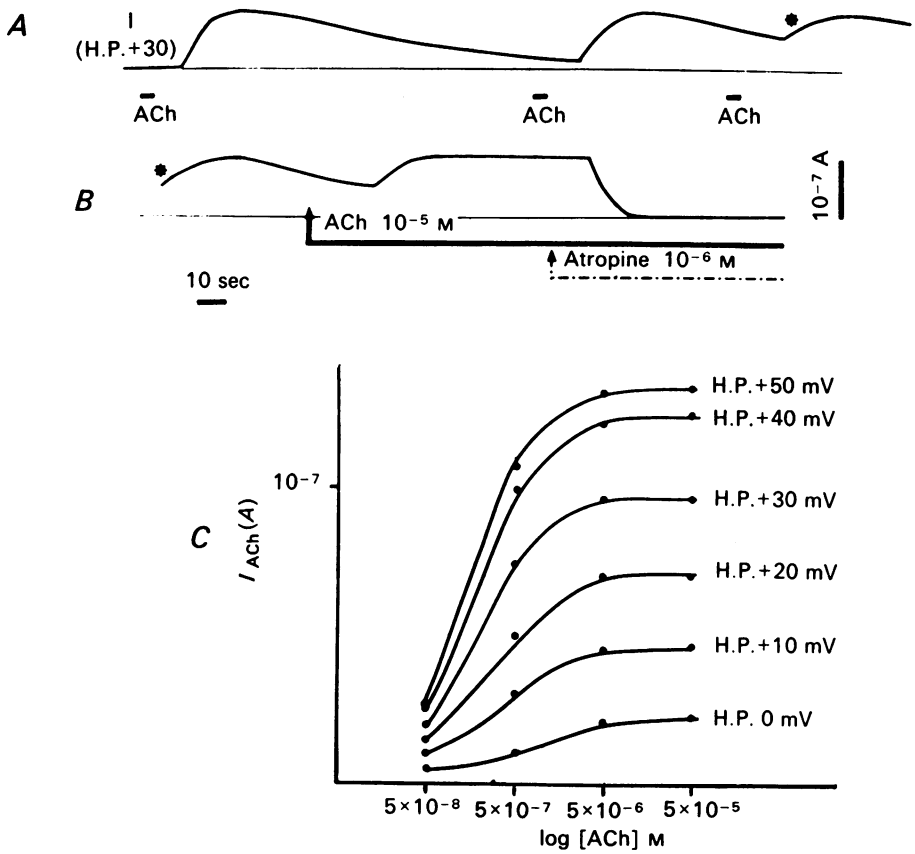

Fig. 2. *A* and *B*, effect of successive applications of 10^{-5} M-ACh on the background current, holding potential +30 mV.

Note that (1) for each short application (5 sec) of ACh the current reaches the same maximum value and remains steady for a long lasting application; (2) the current induced by acetylcholine is inhibited by atropine. *C*, development of the ACh induced current (I_{ACh} extra current) as a function of the ACh concentration (logarithmic scale) for various holding potentials. Note that the dose–response curves have a sigmoid shape and saturate at 5×10^{-6} M-ACh extra current has been measured at the steady-state value for a longer ACh application. Results are taken from Fig. 3*C–D*.

by +30 mV from its resting potential (H.P. = +30); the perfusion with ACh (10^{-5} M) during 5 sec induces, after a delay, an outward current which reaches a maximum value and then declines toward zero in an approximately exponential manner. If ACh is reintroduced a similar current develops, reaching the same maximum value and decaying in the same way (Fig. 2*A*). Furthermore if the duration

of perfusion with ACh is lengthened the current induced reaches a steady value (Fig. 2*B*). These observations are important because they indicate that this preparation does not show any sign of desensitization. If atropine is added this extra current is abolished very rapidly (Fig. 2*B*).

However, the onset of this ACh extra current cannot be considered as simply reflecting the binding of ACh to the cholinergic receptors but must also represent the diffusion of the drug into the bundle. In consequence, the steady-state level of the ACh extra current is assumed to be an indication that the concentration at the centre of the bundle also reaches a steady-state value.

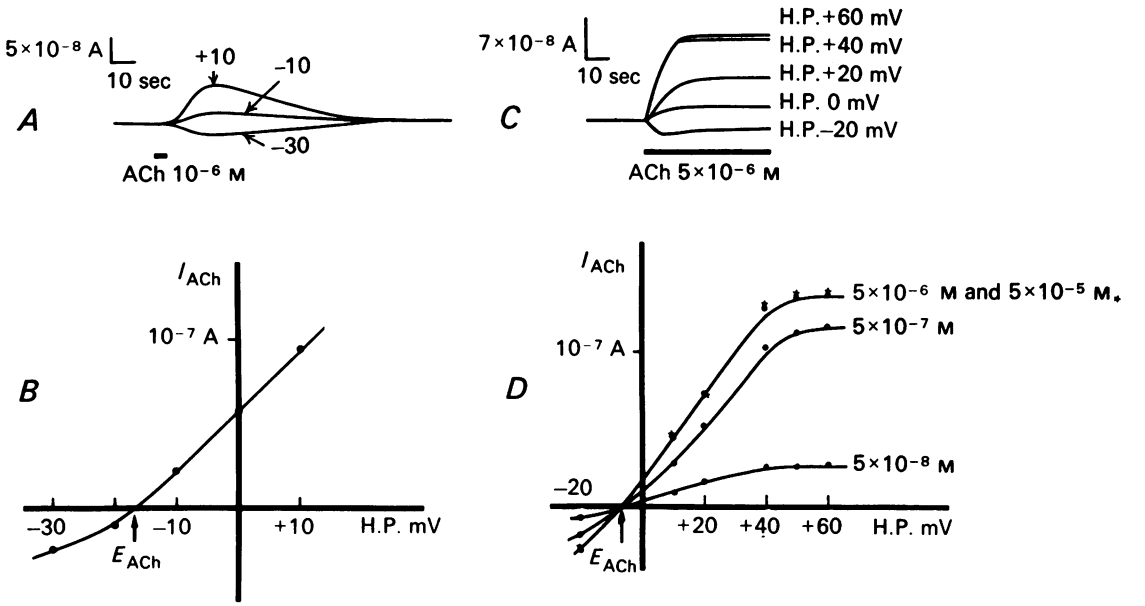

Fig. 3. *A*, measurement of the current induced by a short application (5 sec) of ACh (10^{-6} M) for three holding potentials.

Note that the current is outward for H.P. = − 10 mV and inward for H.P. = − 30 mV. *B*, determination of the reversal potential (E_{ACh}) of the ACh induced current. ACh is applied during 5 sec. E_{ACh} is located around − 15 mV in this preparation. *C*, measurement of the ACh induced current at various holding potentials for a longer ACh application (to obtain the current steady-state value). Only results for 5×10^{-6} M-ACh are presented. *D*, variation of the ACh-induced current with holding potential for various ACh concentrations (I_{ACh} measured at the steady-state value, see 3*C*). Note that (1) the ACh induced current is a function of ACh concentration and reaches a maximum value at positive holding potential (up to + 40 mV) and (2) E_{ACh} does not change with ACh concentration.

For different holding potentials, the I_{ACh} extra current was recorded for various ACh concentrations from $5 \cdot 10^{-8}$ to $5 \cdot 10^{-5}$ M. Fig. 2*C* shows that I_{ACh} extra current increases as a function of ACh concentration from $5 \cdot 10^{-8}$ to $5 \cdot 10^{-6}$ M and for concentrations beyond $5 \cdot 10^{-6}$ M reaches a maximal value (I_{ACh} extra current is measured at the steady-state value from the experiment reported Fig. 3*C*, *D*). The dose–response curves give an estimate of the threshold concentration around 10^{-8} M. This result is not in agreement with Giles & Noble (1976) who did not report an effect of ACh on background current at concentrations below 10^{-7} M.

3 *Ionic nature of the I_{ACh} extra current*

(*a*) *Reversal potential (E_{ACh}) of the I_{ACh} extra current.* One way to obtain indications about the ionic nature of a current is to estimate its reversal potential; this can easily be done with the experimental procedure described in the preceding paragraph.

Fig. 3*A* shows a set of records obtained by application of ACh during 5 sec at different holding potentials. It can be seen that the current induced by ACh reverses at a holding potential between −10 and −30 mV. A plot of the maximum value of the I_{ACh} extra current against potential (Fig. 3*B*) indicates by interpolation a reversal

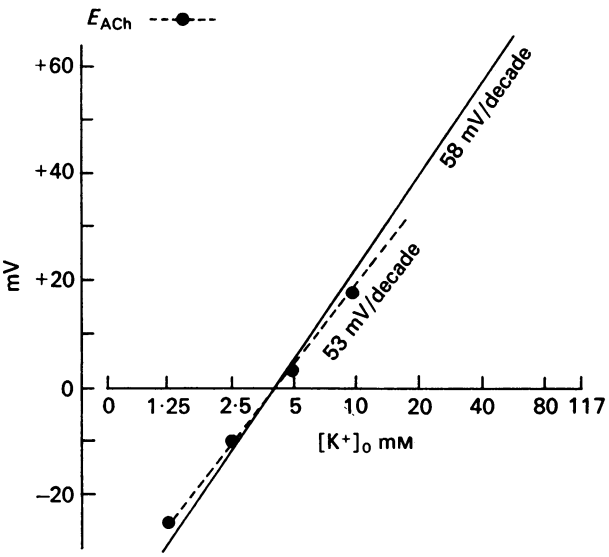

Fig. 4. Variation of the reversal potential E_{ACh} with the external K-concentration (logarithmic scale). Note that the experimental points can be fitted by a straight line with a slope of 53 mV/decade, very close to the slope of 58 mV/decade for a membrane behaving as a K electrode.

potential 15 mV more negative than the resting potential. Similar experiments, done on forty preparations have indicated that E_{ACh} is between 0 and 20 mV more negative than the resting potential. Furthermore the reversal potential measured by this method (5 sec of ACh application or a longer ACh application to have an I_{ACh} steady-state value: see Fig. 3*C*) does not depend on the concentration of ACh as shown in Fig. 3*D* (E_{ACh} around −8 mV). It is noticed that the relationships I_{ACh}/holding potential have a S shape. I_{ACh} extra current reaching a maximum value for positive holding potentials beyond +40 mV. These results are studied later (see paragraph 5 and Fig. 7).

(*b*) *Evolution of E_{ACh} against external potassium concentration.* The reversal potential of the I_{ACh} extra current was measured in different external potassium concentrations from 1·25 to 10 mM. The experimental data are of the kind of those presented Fig. 7*A*. The estimated reversal potentials are plotted against the logarithm of the external K concentration (Fig. 4). The experimental points can be fitted by a straight line with a slope of 53 mV/decade; this is very close to the 58 mV/decade obtained

for a K electrode. Moreover, it must be noticed that the potential measurements are not corrected by the short circuit factor, which can be estimated around 0·9 in most of the experiments; this indicates that our results are probably close to the theoretical prediction for a K electrode.

This result, together with the large decrease in membrane resistance described in Fig. 1*B*, can be considered as good experimental evidence that ACh increases specifically the K background conductance of frog auricular trabeculae.

It is interesting to compare this result with those of Rougier, Ildefonse & Gargouil (1966) which show a 45 mV/decade variation of the resting potential between 1·25 and 10 mM-K, and with those of Noble (1976) which indicate a shift of 40 mV of the reversal potential of the background current between 2 and 20 mM-K.

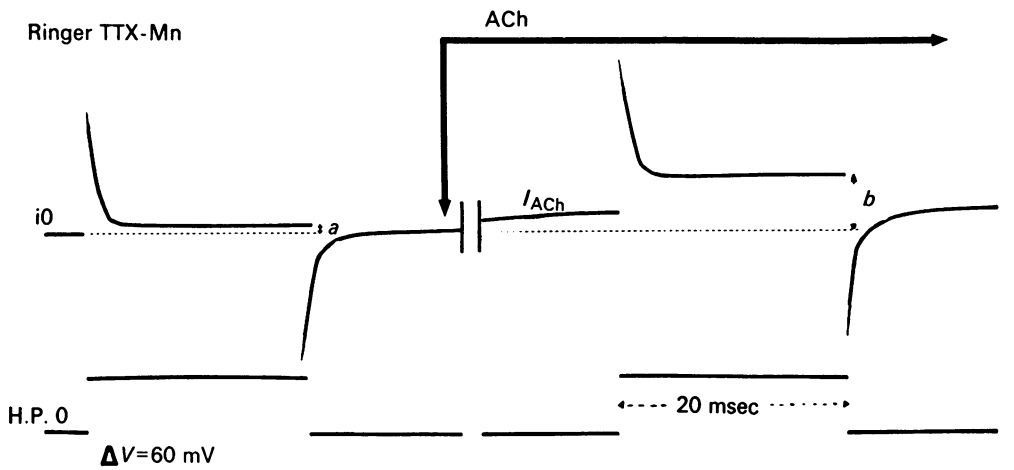

Fig. 5. Diagram showing the estimation of the current induced by ACh using the subtraction method (experiments performed in Ringer TTX-Mn).

Note that: (1) the application of ACh at the resting potential, induces an outward current; (2) the current at the end of a depolarizing pulse of +60 mV and 20 msec duration is considerably increased under the action of ACh (compare *a* and *b*); (3) the difference (*b* − *a*) gives ACh-induced current for this value of potential.

4 *Rectifying properties of the* I_{ACh} *extra current*

In order to understand the physiological role of the I_{ACh} current (during an action potential for example), we must get information about its rectifying properties. This has been done in two ways; first by plotting the difference between the quasi instantaneous current–voltage relation for the background current obtained with and without ACh and secondly by measuring the extra current induced at different holding potentials by a short ACh application.

(*a*) '*Quasi instantaneous*' *current voltage relationship of the* I_{ACh} *extra current.* The 'quasi instantaneous' current voltage relation for the background current can be obtained by plotting against potential the current measured after short duration pulses (Fig. 5), insufficient to activate any delayed outward current (20–40 msec), the inward currents being inhibited by TTX and Mn^{2+}. The current–voltage relation obtained (Fig. 6*Aa*) is S shaped showing a region of inward going rectification between

$V = 0$ and $V = +70$ mV and a region of outward going rectification for greater depolarizations.

When the preparation is perfused with ACh, the current is modified in the way presented in Fig. 5, the background current at the resting potential (H.P. = 0) becomes outward and the outward current given by a depolarization of +50 mV is dramatically increased (compare *a* and *b* in Fig. 5). In these conditions, plotting the current measured as shown in Fig. 5*B* gives the 'quasi instantaneous' current–voltage relationship under the action of ACh which is represented in Fig. 6*Ab*. Then by difference it is possible to obtain the 'quasi instantaneous' current–voltage

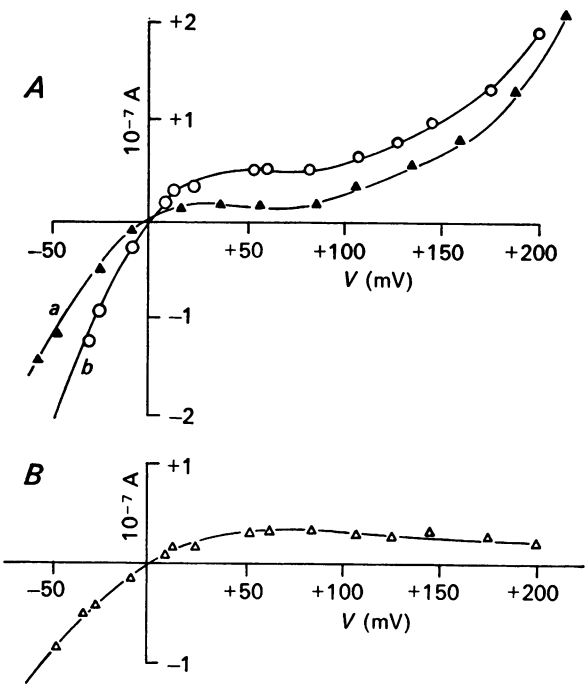

Fig. 6. *A*, 'quasi instantaneous' current–voltage relation in Ringer TTX-Mn (*a*) and under the action of ACh (10^{-7} M) (*b*). The current is measured as described in Fig. 5.

B, 'quasi instantaneous' current–voltage relation of the ACh line induced current obtained by the difference of the curves *b* and *a*. Note that for this preparation the value E_{ACh} is very close to the membrane potential.

relationship for the I_{ACh} extra current which is given in Fig. 6*B*. This curve has the characteristic shape of an inward rectifier and is very similar to the one given by Giles & Noble (1976). It must be noticed that the instantaneous or fully activated current–voltage relation of the $I_{x,1}$ current described by Noble & Tsien (1969) in Purkinje fibre is also very similar.

(*b*) *'Steady-state' current–voltage relation of the* I_{ACh} *extra current.* The current–voltage relation of the I_{ACh} extra current can also be obtained by plotting the peak value of the extra current induced at different holding potentials by short application of ACh (5 sec) as done in Fig. 3*A*, or by a longer ACh application to obtain the I_{ACh} steady-state value (Fig. 3*C*).

Such an experiment is illustrated in Fig. 7*A* left panel and in Fig. 7*Ba* which represent the I_{ACh} extra current and its current–voltage relation in normal Ringer (2·5 mM-K^+). It can be seen that the current–voltage relation rectifies in the inward direction (see also Fig. 3*D*).

The comparison of the 'quasi instantaneous' and of the 'steady-state' current–voltage relation shows that they differ in the range of potential more negative than the reversal potential, the 'quasi instantaneous' current being greater than the 'steady-state' one. A similar result is found in this range of potential for the 'quasi instantaneous' and 'steady-state' value of the background current as shown by Rougier *et al.* (1968, Fig. 5), Garnier & Rougier (1969, Fig. 2). Both these situations may be due to the existence, when the membrane is hyperpolarized, of an important inward current decaying with a time constant around 500 msec (Rougier *et al.* 1968). This current which is inhibited by TEA (Rougier *et al.* 1968; Garnier & Rougier, 1969) might correspond partly to a potassium depletion from the intercellular spaces as suggested by Maughan (1973).

These results indicate that the mechanism of specific K conductance increase produced by ACh seems to have properties of inward going rectification. These properties of inward going rectification are characteristic of most of the K currents in cardiac cells (Noble & Tsien, 1968–1969) and in muscle cells (Adrian, 1969). Furthermore the current–voltage relations of these inward rectifying systems obtained in different external potassium concentrations are characterized by a crossing over (see Noble, 1965).

In order to test the hypothesis that the ACh induced K conductance mechanism has properties of inward rectification, experiments were performed in different external K concentrations.

(*c*) *Rectifying properties of the I_{ACh} extra current in different external K concentrations.* Fig. 7*A* represents a set of records of I_{ACh} extra currents obtained in two different potassium concentrations (2·5 and 10 mM) at various holding potentials with the method described section 2. The plot of the maximum induced I_{ACh} extra current against holding potential gives current voltage relationships (Fig. 7*B*) which cross each other, the outward current being greater after the crossing over in 10 mM-K. The reversal potential E_{ACh} is about 30 mV more positive in 10 mM-K, in agreement with the results of Fig. 4. It must also be noticed that these observations are not due to a modification of the preparation under the action of a higher K concentration, because returning to Ringer (2·5 mM-K) as shown Fig. 7*A* right panel and Fig. 7*Bc*, indicates a good reversibility.

5 *Does ACh change the delayed $I_{x,1}$ current?*

Because it has been shown in the preceding results that ACh increases specifically the K conductance of atrial muscle fibre, it seemed interesting to look at whether ACh has also an action on the delayed K conductance $I_{x,1}$ described by Brown & Noble (1969), Ojeda & Rougier (1974), Brown, Clark & Noble (1976). It must be noticed that the instantaneous I/V relation of $I_{x,1}$ rectifies in the inward direction (Brown, Clark & Noble, 1976; Ojeda & Rougier, unpublished). Two kinds of experiments were performed for this purpose.

First, we have compared on the same preparation the reversal potential E_{ACh} of

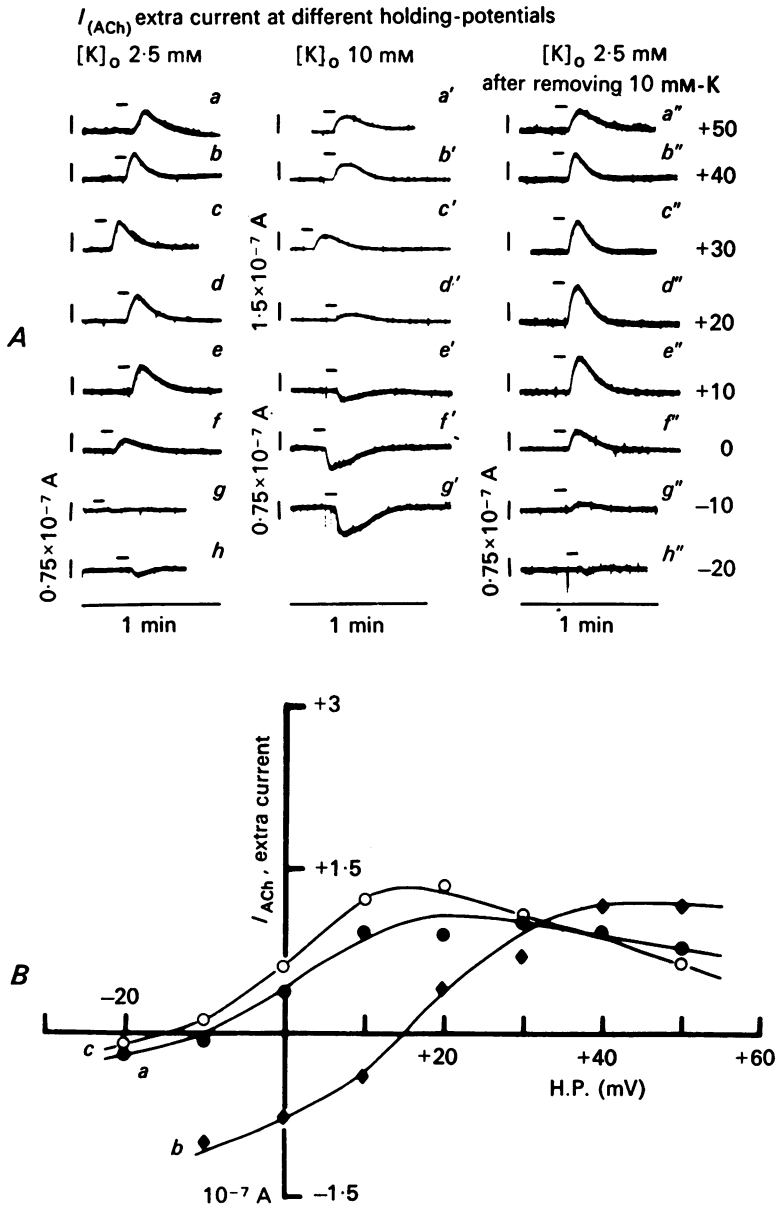

Fig. 7. Rectifying properties of the current induced by ACh in different external K concentrations (2·5 and 10 mM-K). *A*, records of the current induced by a short application (5 sec) of ACh (10^{-6} M) for two external K concentrations as a function of the holding potential. Left panel 2·5 mM-K: control experiment; central panel: 10 mM-K; right panel: 2·5 mM-K (recovery experiment). *B*, current–voltage relations for the maximum value of ACh-induced current as a function of extracellular K concentration. Note that (1) E_{ACh} is shifted about +30 mV from 2·5 to 10 mM-K and (2) the curves cross each other, the outward current in 10 mM-K (curve *b*) being greater than in 2·5 mM-K (curve *a* (control), curve *c* (recovery)).

the I_{ACh} extra current, with the reversal potential $E_{x,1}$ of the $I_{x,1}$ delayed outward current as described by Ojeda & Rougier (1974). The results on ten preparations show that E_{ACh} is always 10–20 mV more negative than $E_{x,1}$. Secondly, the membrane was clamped during 6 sec at different potentials in Ringer (Fig. 8*Aa*) and in Ringer with ACh (Fig. 8*Ab*), then the current values measured at the end of the pulse were

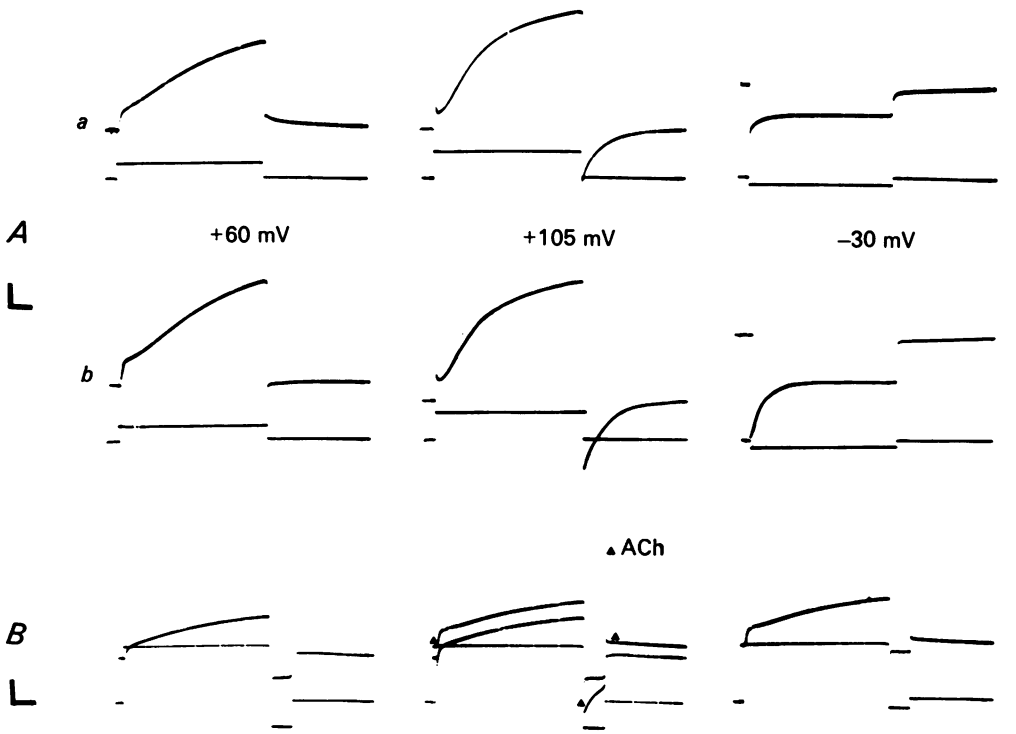

Fig. 8. Action of ACh on the delayed outward current. *A*, current records for three values of potential (+60, +105, −30 mV) from the resting potential, in Ringer (*a*) and under the action of ACh 10^{-6} M (*b*). Note that (1) the delayed outward current does not seem to be affected by ACh (see also *B*, central panel). (2) the tail current which is outward in Ringer becomes inward under the action of ACh; and (3) the 'quasi instantaneous' inward current during the hyperpolarizing pulse (−30 mV) is greatly increased under the action of ACh. Vertical scale: $I = 8{\cdot}10^{-8}$ A (+60 mV; −30 mV); $2{\cdot}10^{-7}$ A (+105 mV). Horizontal scale: 1 sec.

B, estimation of the reversal potential ($E_{x,1}$) of the delayed outward current in Ringer (left panel) and under the action of ACh (right panel) (from a H.P. = +10 mV). Note that (central panel) (1) the delayed outward current seems to bɔ only shifted by the presence of the ACh-induced current, and (2) the second step of potential in Ringer was chosen to give a zero tail current, the current tail becomes strongly inward at this potential under the action of ACh. Vertical scales: $V = 20$ mV; $I = 8{\cdot}10^{-8}$ A. Horizontal scale: 1 sec.

plotted against potential as shown in Fig. 9*A*. The two current–voltage relationships differ only slightly; the current is greater in Ringer ACh and the curves cross each other near −20 mV, which may represent the value of E_{ACh}. The difference between these two curves is represented in Fig. 9*B*; it gives a current–voltage relation which is very similar to the steady-state current–voltage relation for I_{ACh} current in Fig. 7.

This is good experimental evidence that the delayed rectifier is not modified by ACh, in agreement with the findings of Giles & Noble (1976).

But when looking at the tails of current following repolarization to the resting potential, it can be seen that the tail which is outward in Ringer is greatly diminished and even reversed under the action of ACh without any apparent modification in the amplitude of the delayed outward current (Fig. 8*B*). This result may indicate two things, (1) that there is probably a process of accumulation of K ions under the action of ACh and (2) that this tail is not directly related to the deactivation of the preceding delayed outward current.

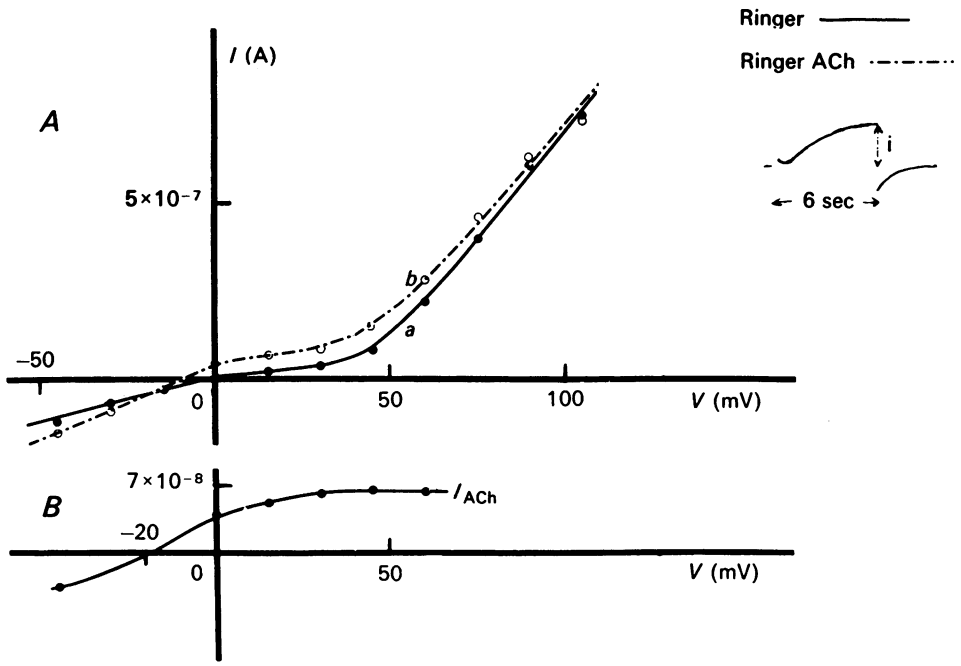

Fig. 9. *A*, current–voltage relation of the total outward current measured at the end of long (6 sec) clamp pulses in Ringer (*a*) and under the action of ACh (*b*). *B*, current–voltage relation of the current induced by ACh obtained by subtraction of the curves *b* and *a*. Note that this current–voltage relation is very similar to the one obtained in Fig. 7*B*.

In order to test the hypothesis of a K accumulation under the action of ACh, the $E_{x,1}$ potential was measured for different concentrations of ACh. The results show that the shift of the $E_{x,1}$ potential toward a positive value is proportional to the concentration of ACh (the $E_{x,1}$ potential is -10 mV in a Ringer solution, $+2$ mV with 10^{-7} M-ACh, $+12$ mV with 10^{-6} M-ACh and $+20$ mV with 10^{-5} M-ACh), and can be as great as 30 mV. This result leads to the following conclusion. The I_{ACh} extra current which is much weaker than the delayed outward current is able to induce a substantial accumulation of K ions in the intercellular spaces (a shift of about 20 mV in the reversal potential $E_{x,1}$, indicates that the external K concentration has reached twice its value). This can be understood if the I_{ACh} K current is flowing in a very restricted intercellular space.

It seems possible that the determination of E_{ACh}, at least in normal and low K concentration, might be complicated by some K accumulation or depletion. But the fact that E_{ACh} is independent of the concentration of ACh as shown in Fig. 3*D* can be considered as an indication that the complications are negligible with the method of estimation used in this case.

DISCUSSION

The results indicate that the application of ACh or carbachol to atrial muscle fibres induces a specific increase in K conductance which is demonstrated by the finding that the I_{ACh} reversal potential (E_{ACh}) behaves almost as a K electrode.

The ACh-induced K conductance mechanism seems to have properties of inward going rectification i.e. (1) its current–voltage relation rectifies in the inward direction, and (2) the current–voltage relations obtained in different external K concentrations cross each other.

One important question is, are the current–voltage relations of the I_{ACh} current the reflexion of the actual rectifying properties of the ACh channels or are they, entirely or partly the consequence of changes in the external K concentration described in the last paragraph of Results? It is difficult to answer this question entirely; if current–voltage relations like those in Fig. 7 can be obtained from a linear or from a constant field current–voltage relation as the consequence of accumulation and depletion, it seems difficult to explain the crossing over obtained by increasing the external K concentration (2·5 mM-K; 10 mM-K) in the same way.

It must be noticed that the ACh-induced K conductance mechanisms in other tissues also have current–voltage relations which seem to rectify in the inward direction. This is the case for the electroplaques of *Electrophorus* (Lester, Changeux & Sheridan, 1975) and for the junctional and extrajunctional receptors in frog muscle (Adams, 1976; Mallart, Dreyer & Peper, 1976). But it has been shown by Dionne & Stevens (1975) and Mallart *et al.* (1976) that this does not correspond to a property of the ACh-induced channel and that the deviation in non linearity observed in the current–voltage relations depends on the relative values of the mean channel life time and the duration of the application of ACh. It is impossible to know if the same explanation is valid for the I_{ACh} current–voltage relation in frog atrial because nothing is known about the life time of the ACh channel in this preparation.

Another important question arises now. Does ACh act by inducing the conductance of the inward rectifying time independent channel $I_{K,1}$, or by inducing a new specific K channel? This question is difficult to answer. Indeed both these channels can be considered as (time independent) K inward rectifiers and both seem to appear at the same time in the rat heart embryo as shown by Pager, Bernard & Gargouil (1965).

Nevertheless there is one major difference in the current–voltage relation of I_{ACh} and $I_{K,1}$; the latter rectifies also in the outward direction, not the former. But it must be remembered that there is almost always a confusion between the total background current and the $I_{K,1}$ current which only constitutes the major part of it. The total background current seems to be a mixture of currents ($I_B + I_{K,1}$) as theoretically postulated by McAllister *et al.* (1975); but, till now, nobody has tried to separate each component in any tissue. So it is not certain that the pure $I_{K,1}$ current–voltage

relation rectifies also in the outward direction. In fact the only specific K current which has been studied separately is the $I_{K,2}$ current in Purkinje fibre (Noble & Tsien, 1969) and it rectifies only in the inward direction. More information must be obtained about the properties of the $I_{K,1}$ system in frog atrial before the question might be definitively answered. For example it would be interesting to look at whether the frog ventricular fibres which also have inward K rectifying properties (Cleeman & Morad, 1976) are sensitive to ACh.

Finally, the study of the rectifying properties of the ACh-induced conductance mechanism indicates that the I_{ACh} current in the plateau range of potentials is too weak to explain the dramatic decrease in duration and amplitude of the action potential. In fact, it has been shown by Ikemoto & Goto (1975), Giles & Noble (1976), Garnier *et al.* (1976*a*) that ACh behaves also as if it was an inhibitor of the slow inward current.

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