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DESENSITIZATION OF THE ACETYLCHOLINE-INDUCED INCREASE OF POTASSIUM CONDUCTANCE IN RABBIT CARDIAC PURKINJE FIBRES

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SUMMARY

1. In rabbit cardiac Purkinje fibres, acetylcholine (ACh) changes membrane electrophysiological properties in a biphasic time course. On wash-out of ACh a rebound phenomenon is observed (Mubagwa & Carmeliet, 1983). The underlying mechanisms have been studied by the voltage-clamp technique.

2. The ACh-induced increase in K^+ current follows a biphasic time course during exposure to ACh. This time course is not due to intercellular accumulation or depletion of K^+, but results from a desensitization process. On wash-out a rebound is obtained, i.e. the membrane K^+ conductance transiently decreases below the control value. In contrast, the inhibition by ACh of the catecholamine-induced increase of slow inward current follows a monophasic time course.

3. The desensitization process or secondary decrease of ACh-induced change in K^+ current follows a mono- or a biexponential time course. The extent and rate of desensitization depend on ACh concentration. The rate of desensitization is not influenced by membrane potential but its extent seems to be increased by depolarization.

4. Recovery from desensitization is relatively rapid and has a half-time of about 2 min.

5. Different existing models for desensitization are discussed, no one of which accounts for all results in rabbit Purkinje fibres. Therefore, a three-state receptor model is proposed to explain the results. The model assumes that the K^+ channel is directly associated with the muscarinic receptor and that the channel–receptor complex may be in closed, open or desensitized state, in the presence as well as in the absence of agonist.

INTRODUCTION

The effects of acetylcholine (ACh) on transmembrane potentials of rabbit cardiac Purkinje fibres follow a biphasic time course. Within the first minute following the beginning of ACh application, a maximum is obtained for the hyperpolarization of the resting potential, the decrease of spontaneous activity and the shortening of the action potential duration. Afterwards, the magnitude of these changes decreases to a steady level. On wash-out of ACh, an overshoot (or rebound) is obtained and the

electrical properties of the preparations are transiently changed in the opposite direction (Mubagwa & Carmeliet, 1983).

The loss of response of a tissue to an agonist following prolonged exposure to this compound has been called fade or desensitization. This last term should be used for conditions in which only one receptor–effector system is involved in the loss of response to the agonist, i.e. it should not be used for situations in which a total response decreases due to production by one single agonist of two functionally antagonistic responses (following activation of different effector systems) with different time course.

With a few exceptions, the muscarinic response in cardiac preparations is believed to show little or no desensitization. For example, in frog sinus (Hartzell, Kuffler, Stickgold & Yoshikami, 1977) and in sheep Purkinje fibres (Carmeliet & Ramon, 1980), no decrease with time of the response to ACh has been found. In some preparations, very small decreases of the magnitude of the muscarinic cholinergic response have been observed, but these changes have usually been attributed to processes not involving the muscarinic receptor (Glitsch & Pott, 1978; Noma, Peper & Trautwein, 1979; Loeb, Dalton & Moran, 1981; Martin, Levy & Matsuda, 1982). However, in frog atrial (Tokimasa, Hasuo & Koketsu, 1981) and in mamalian atrial (Burke & Calaresu, 1972; Jalife, Hamilton & Moe, 1980; Nilius, 1983) or ventricular (Hollenberg, Carriere & Barger, 1965) preparations, pronounced decreases of the ACh-induced changes have been described which suggest that the ACh muscarinic response may undergo desensitization.

In this paper, it is shown that desensitization in rabbit cardiac Purkinje fibres affects the ACh-sensitive K^+ conductance. A tentative mechanism is discussed which may account for both the decrease in response during ACh application and the rebound during ACh wash-out.

METHODS

Short rabbit cardiac Purkinje fibres were voltage clamped by the two-micro-electrode technique (see preceding papers: Carmeliet & Mubagwa, 1986*a*, *b*).

In order to study the time course of ACh effects, membrane currents were continuously recorded before, during and after application of ACh. For analysing the effect of membrane potential on the time course of ACh effects, exposures to the same ACh concentration were repeated at different holding potentials. For studying the effect of ACh concentrations, a similar protocol was followed, but this time the holding potential was kept constant during exposure to various ACh concentrations. Each exposure to ACh lasted at least 5 min and was followed by a 10 min wash-out period.

The kinetics of desensitization were obtained by fitting the secondary decrease in current with one or two exponentials. For this purpose, membrane current chart recordings were digitized using an X–Y coordinate analyser (MDC Datalyser) and the fitting curves were obtained using a non-linear regression program (on a PDP-11 computer).

RESULTS

Time course of acetylcholine effects on membrane currents

Biphasic change of K^+ current. The time course of ACh action was studied by superfusing the preparations with an ACh-containing solution while holding the membrane potential at a constant level. Fig. 1*A* shows the membrane currents

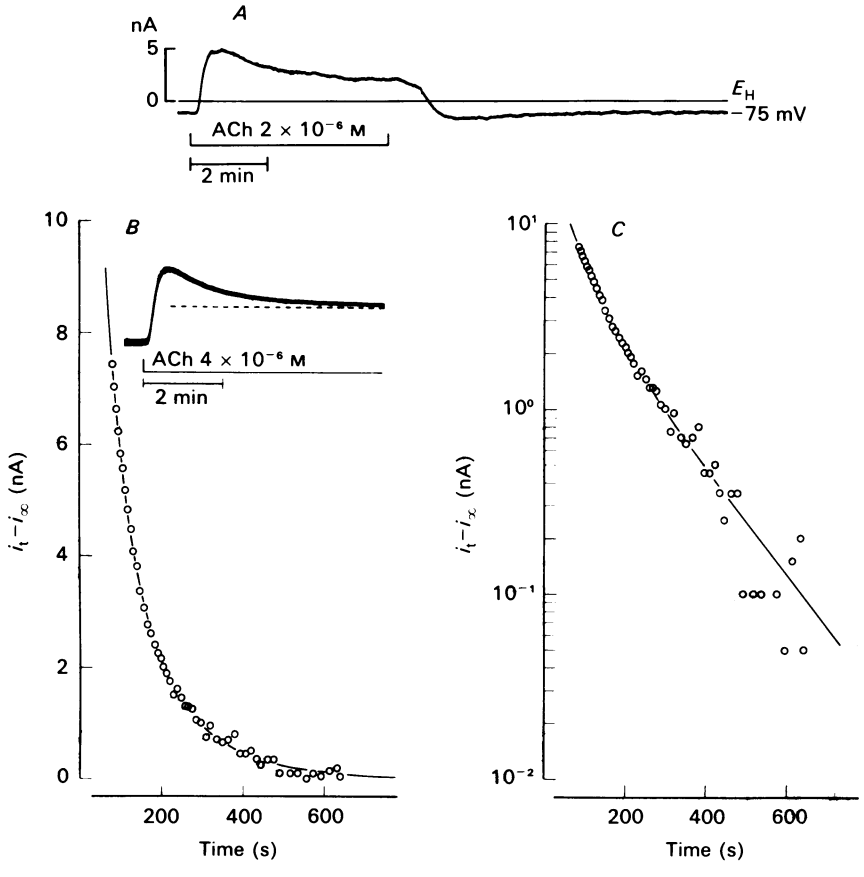

Fig. 1. Time course of the effects of ACh on membrane currents. *A*, biphasic time course of the ACh-induced current. *B*, linear plot of the difference between actual current (i_t) and steady state (i_∞) during the declining phase. The preparation is different from the one used in *A*. *C*, semilogarithmic plot of the same data as in *B*. The curves drawn through the points in *B* and *C* were obtained by double-exponential computer fitting. The inset in *B* shows part of the original current record from which measurements in *B* and *C* were taken. Holding potentials (E_H): −75 mV in *A*, −45 mV in *B* and *C*.

recorded in a fibre which was clamped at −75 mV during superfusion with 2×10^{-6} M-ACh.

After a certain delay following the change in solution, ACh produced an outward shift in membrane current. This change in current is due to an increase by ACh of an inward-rectifying K^+ current (see Carmeliet & Mubagwa, 1986*a*, *b*). The current rapidly reached a maximum, following which it decreased toward a new steady value, intermediate between the maximum and the pre-drug level. Upon wash-out of ACh, the current first decreased to a value which was lower than the control level. Thereafter, it increased and after 5 min wash-out, the control level was reached again.

Fig. 1*B* shows the deviation of the value of membrane current at different times from the steady-state value in another preparation. A semilogarithmic plot of the same data is given in Fig. 1*C*. The secondary decrease in current could be fitted by

a sum of two exponentials, with time constants of 55·9 s and 155·0 s, respectively. Similar experiments were carried out in five different fibres. In all these experiments the decay of the ACh-induced current could be fitted with one or two exponentials. One exponential was usually sufficient for decays during exposures to ACh concentrations up to 10^{-6} M, whereas two exponentials were needed for decays produced at higher concentrations (see below).

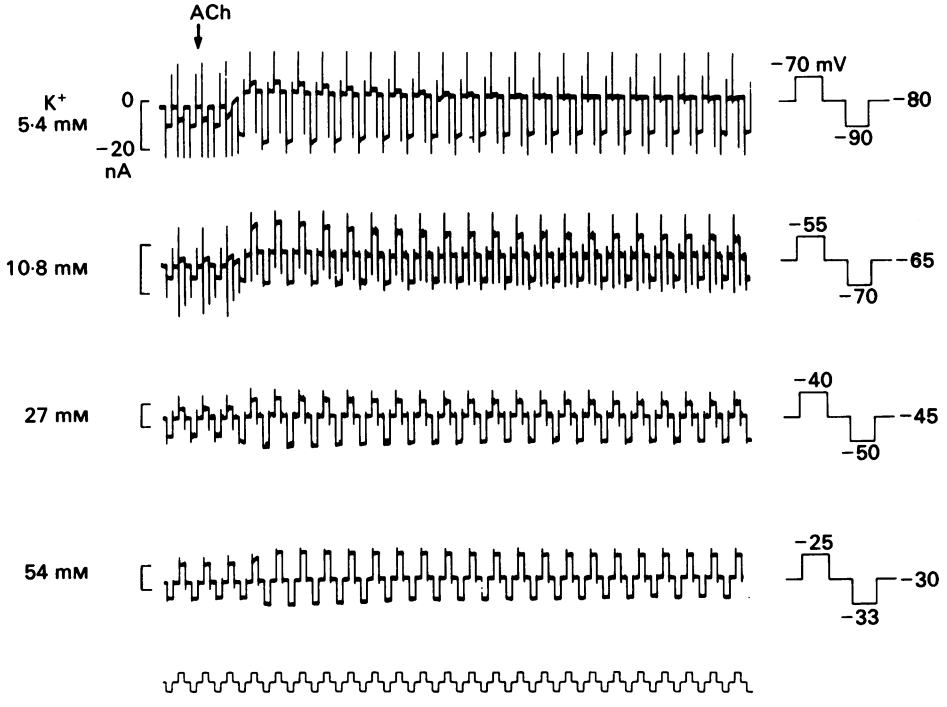

Fig. 2. Time course of the effects of ACh (2×10^{-6} M) in various $[K^+]_o$. 1 s depolarizing and hyperpolarizing pulses (sketched in the bottom trace) were given from the following holding potentials: −80 mV in 5·4 mM-K^+, −65 mV in 10·8 mM, −45 mV in 27 mM, −30 mV in 54 mM. The pulse potentials are given after each current trace. In front of each trace a 20 nA calibration is given, the upper level of which gives the zero current level.

The decrease with time of the ACh-induced current might be thought to result from a change in K^+ driving force following accumulation (for potentials positive to E_K) or depletion (for potentials negative to E_K) of K^+ in the intercellular spaces. This hypothesis is, however, not supported by experiments in which the effect of ACh on membrane currents was tested while clamping alternatively positive and negative to E_K. As shown in Mubagwa & Carmeliet (1983), when 1 s voltage-clamp pulses were repetitively (every 10 s) given from −75 to −95 mV, ACh shifted the currents in the outward direction at −75 mV and in the inward direction at −95 mV. The ACh-induced shifts in current decreased with time at both potentials. This indicates a secondary decrease of membrane K^+ conductance. If the biphasic time course were

due to K^+ accumulation, it should have been accompanied by an increase in membrane conductance. Depletion could neither have been present since the membrane potential was held positive to E_K for most of the time. A further argument against accumulation/depletion mechanism is provided by the presence of a biphasic time course when high $[K^+]_o$ were used. In the experiment illustrated in Fig. 2, $[K^+]_o$

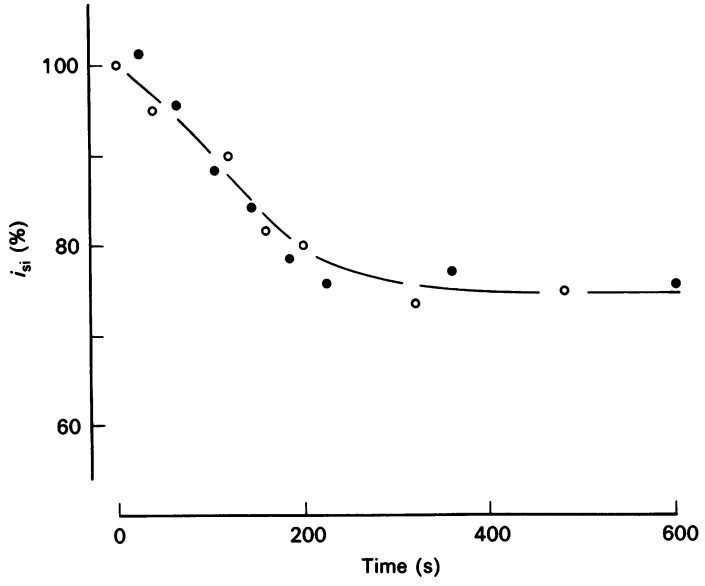

Fig. 3. Time course of the effect of ACh on catecholamine-stimulated ($1{\cdot}89 \times 10^{-6}$ M-isoprenaline) slow inward current (i_{si}). Voltage pulses from -40 to -20 mV were given every 40 s. i_{si} was estimated as the difference between peak inward current and current after 200 ms following voltage pulse. The current before addition of ACh was taken as 100%. (●): 4×10^{-6} M-ACh. (○): 10^{-4} M-ACh.

was increased to 10·8, 27 and 54 mM and, in each solution, depolarizing and hyperpolarizing voltage pulses near E_K were given. In such conditions, one expects that the small currents induced by ACh at potentials close to E_K are not sufficient to result in accumulation or depletion. In all solutions, however, the ACh-induced current changed in a biphasic way. The above experiments exclude a change in K^+ driving force as the main cause of the decline of ACh-induced current. Accordingly, the process underlying the secondary decrease of the ACh-induced current will from now on be considered to directly affect the conductance or kinetic properties of the ACh-sensitive channels. The primary process may occur either at the drug-binding sites (receptor), at the ionophore molecule (channel) or at eventual drug–receptor coupling structures.

Monophasic change of slow inward current. In preparations pre-treated with catecholamines, ACh becomes able to decrease the amplitude of the slow inward current, i_{si} (see Carmeliet & Mubagwa, 1986*a*), probably by antagonizing the stimulation produced by catecholamines on the adenylate cyclase system (Watanabe

& Besch, 1975; Biegon & Pappano, 1980). The time course of the inhibitory effect of ACh on i_{si} was investigated. In the experiment illustrated in Fig. 3, i_{si} was increased by superfusing the fibre with isoprenaline ($1{\cdot}89 \times 10^{-6}$ M)-containing Tyrode solution. Two ACh concentrations (2×10^{-6} and 10^{-4} M) were tested. ACh produced a decrease of i_{si}, the amplitude of which remained, however, higher than in control

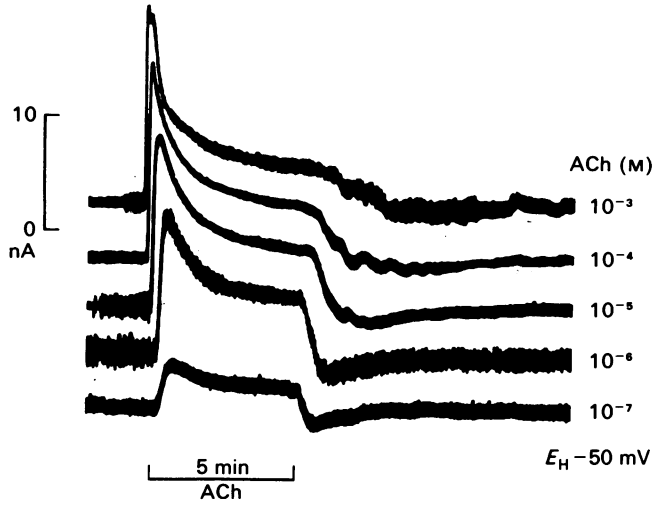

Fig. 4. Effect of various ACh concentrations on membrane currents. Holding potential (E_H): −50 mV. 5 min exposure of ACh in each case. Notice the pronounced decline in current at high concentrations and the biphasic time course during wash-out from low [ACh].

(i.e. in the absence of any drug). With either ACh concentration, i_{si} was decreased in a monophasic way, with a half-time of about 100 s, and the same steady decrease in i_{si} was obtained. This result suggests that, contrary to the activating effect of muscarinic receptors on K^+ channels, the inhibitory effect on adenylate cyclase does not desensitize. Therefore, for the analysis of the desensitization process, only the ACh-induced K^+ current will be studied further.

Dependence of desensitization on agonist concentration

Mubagwa & Carmeliet (1983) observed that the secondary effects of ACh were more pronounced and more rapid with increasing ACh concentrations. Since no one of the effects studied (hyperpolarization, decrease of spontaneous activity or shortening of action potential) is linearly related to the number of channels activated by ACh, it would have been inaccurate to use the extent and rate of their changes in order to characterize the steady-state and kinetic properties of desensitization. The change in membrane current produced by ACh may, on the contrary, be assumed to be proportional to the number of activated channels, which is probably closely related to the number of drug–receptor interactions.

In the following experiments the effect of varying ACh concentration on the extent and rate of desensitization was investigated. A preparation was exposed to different

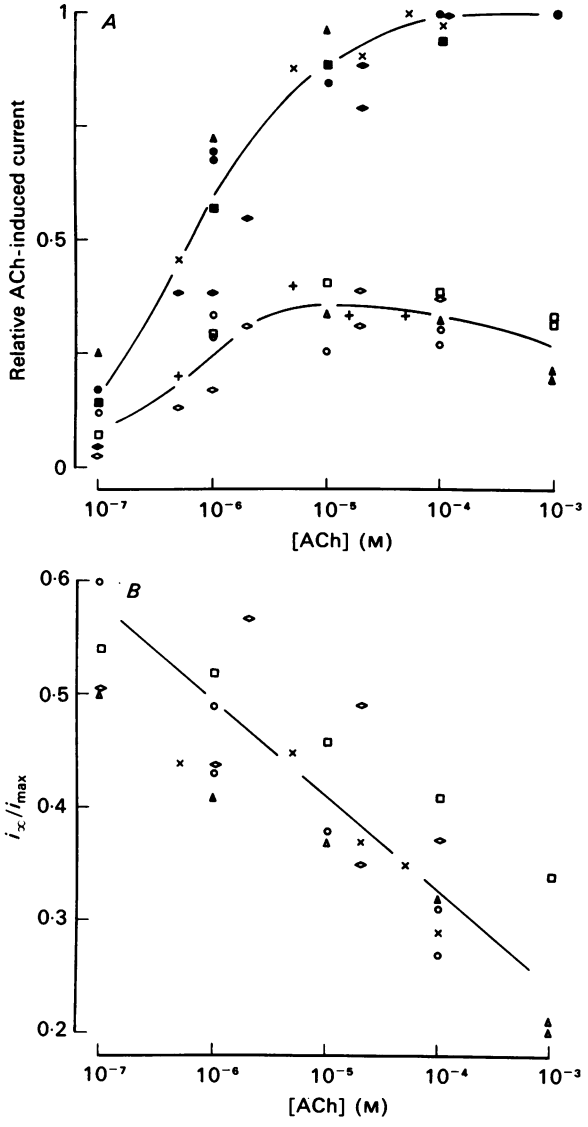

Fig. 5. Effect of ACh concentration on the extent of desensitization. *A*, concentration–effect curve of ACh on membrane currents. Results from four different fibres. The amplitudes of ACh-induced current were normalized taking the peak current induced by 10^{-3} M-ACh in each fibre as maximum. Closed symbols and x: peak effects. Open symbols and +: steady-state effects. *B*, ratio between steady effect and peak effect as a function of the ACh concentration. Same data as in *A*.

ACh concentrations while clamping the membrane potential at a constant value. In the experiment of Fig. 4, the potential was held at −50 mV during 5 min applications of 10^{-7}–10^{-3} M-ACh. ACh produced a biphasic change in membrane currents in all cases. Both the maximum and the steady-state values of the ACh-induced current depended on the ACh concentration used. Fig. 5*A* summarizes

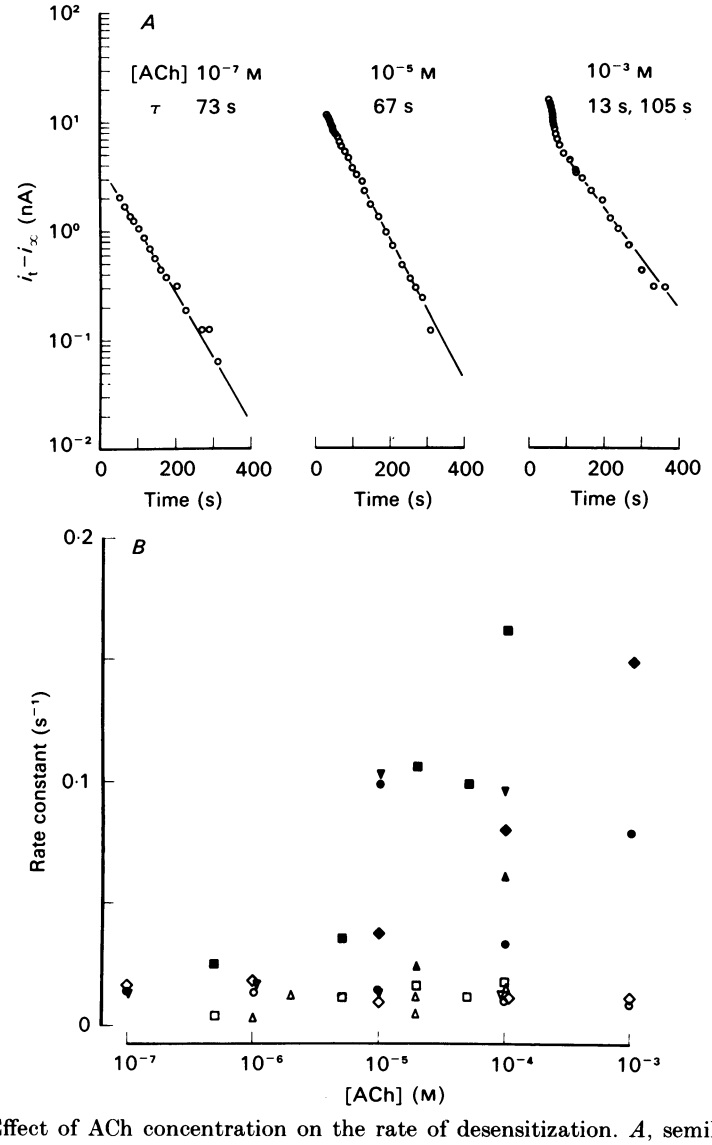

Fig. 6. Effect of ACh concentration on the rate of desensitization. *A*, semilogarithmic plots of current deviation from the steady levels. Holding potential = −50 mV. *B*, rate constants of ACh-induced current decline in five different fibres. The rate constants were obtained by double exponential fitting. Different types of symbols represent different experiments. Filled symbols: higher rate constants. Open symbols: lower or unique rate constants.

the results obtained in four different preparations. The maximum ACh-induced current was a sigmoid function of the logarithm of ACh concentration, increasing almost linearly between 10^{-7} and 10^{-5} M and saturating above 10^{-5} M. A least-squares fit of all the points with the Hill equation, $\Delta i = (1 + K_D/[\text{ACh}]^n)^{-1}$, gave an apparent affinity constant (K_D) of $0{\cdot}83 \times 10^{-6}$ M-ACh and a Hill coefficient (n) of 1·13. On the contrary, the steady-state effect, i.e. the ACh-induced current measured after

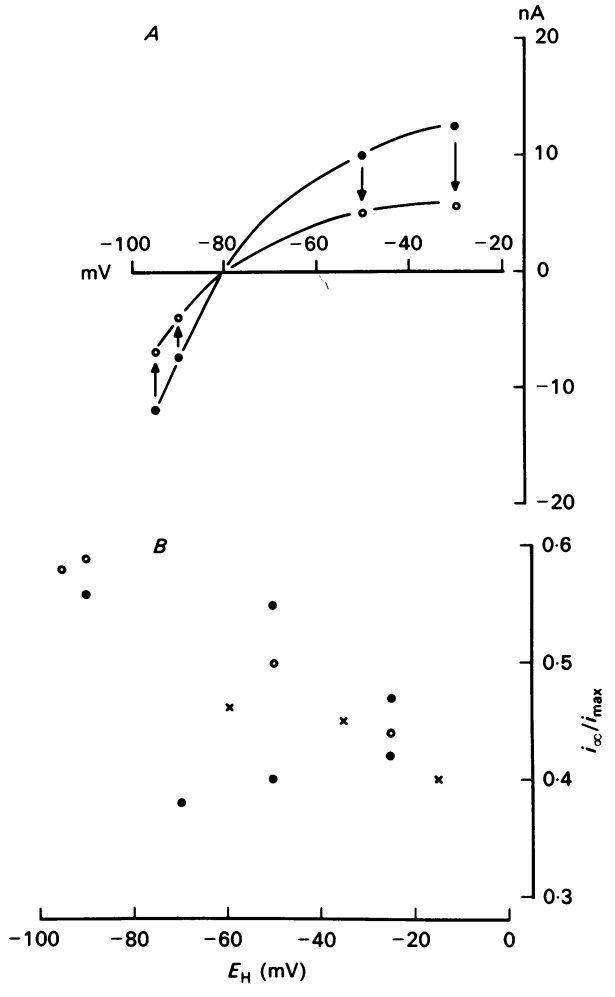

Fig. 7. Effect of membrane potential on the extent of desensitization. *A*, the ACh-induced current is plotted in function of the membrane potential at which it was measured. (●): peak effect. (○): steady-state effect. The arrows indicate the direction of current change during desensitization. *B*, ratio between steady-state and peak currents, in three different fibres (corresponding to the three different symbols).

desensitization had occurred, reached a maximum near 10^{-5} M-ACh and decreased at higher concentrations. Due to the limited number of experiments, a statistical test was not applied to measure the significance of the decrease in ACh-induced current at high concentrations. However, when the experiments were carried out, we took care to repeat the application of the highest ACh concentration in order to have more confidence in the measured value. This decrease of steady-state ACh-induced current at high concentrations is consistent with the decrease of steady-state shortening of action potential observed by Mubagwa & Carmeliet (1983) and suggests that the extent of desensitization becomes more pronounced when the ACh concentration is increased. Indeed, the ratio between the steady-state and the maximum ACh-induced

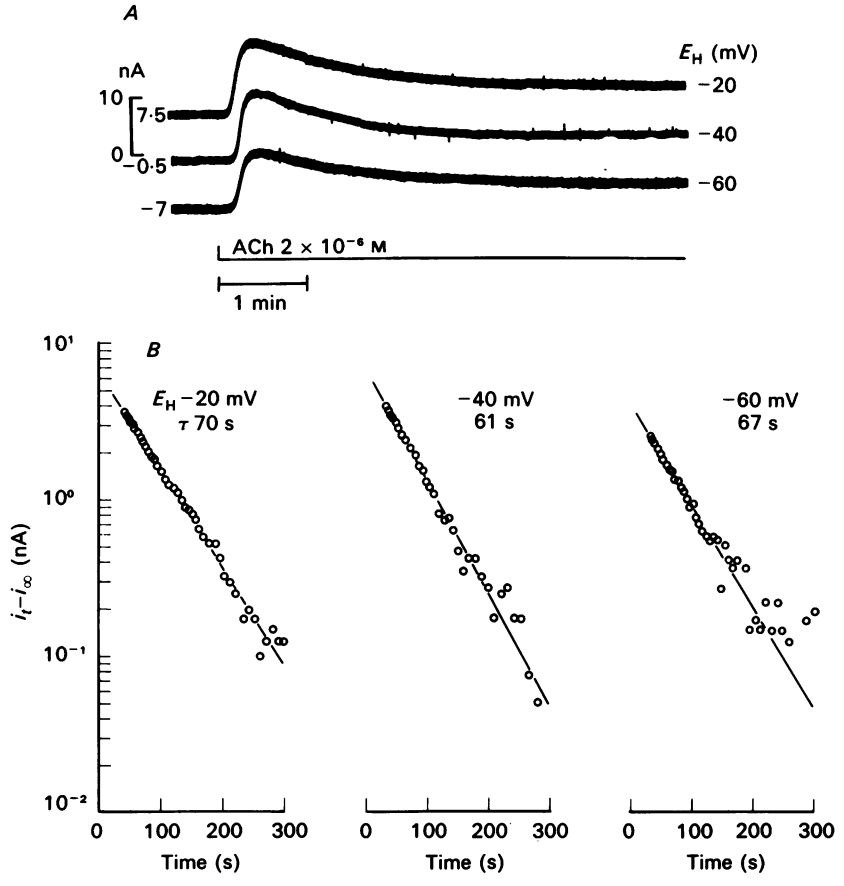

Fig. 8. Effect of membrane potential on the rate of desensitization. *A*, biphasic effect of ACh at three different holding potentials (E_H) *B*, semilogarithmic plot of current deviation from steady level during ACh exposure. Data obtained from *A*.

current, i_∞/i_{max}, which is presumably inversely related to the extent of desensitization, is shown to decrease with ACh concentration in Fig. 5*B*.

The effect of various ACh concentrations on the rate of desensitization was measured in the same experiments. In the example given in Fig. 6*A*, the decrease in ACh-induced current during desensitization followed similar time courses during exposure to 10^{-7} M or to 10^{-5} M-ACh. At 10^{-3} M, the current decrease was resolved by two exponentials, the slowest of which was comparable to the one observed with lower concentrations. The rate constants of current decline obtained from the five different preparations are given in Fig. 6*B*. As stated above, at ACh concentrations above 10^{-6} M, the ACh-induced current frequently declined with two rate constants (see Fig. 1). In this case, only the fast rate constant was increased by ACh. The smaller rate constant was not significantly modified with change in ACh concentration over many decades.

Dependence of desensitization on membrane potential

As mentioned above, the ACh-sensitive ionic channel itself is among the possible sites of the desensitization process. Since channel conformational changes are known to be sensitive to membrane potential, it was interesting to investigate the influence of membrane potential on the rate and extent of desensitization. Preparations were therefore repetitively superfused with the same ACh concentration whereas the membrane holding potential at which the current was measured was varied.

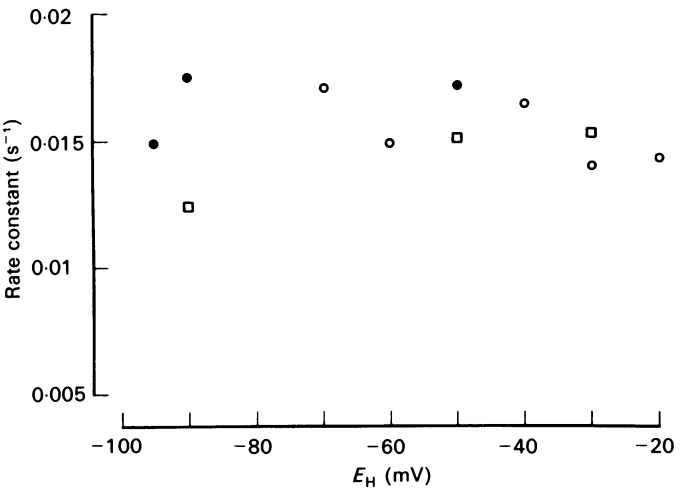

Fig. 9. Rate constant of ACh-induced current decline as a function of membrane potential. Data from three different fibres, represented by different symbols. Only one rate constant was present. [ACh]: $1–4 \times 10^{-6}$ M.

Fig. 7 *A* shows the current–voltage relations obtained by measuring the ACh-induced current at its peak and at its steady-state value. As expected, both the maximum and the steady-state ACh-induced currents varied with membrane potential, displaying the inward-rectifying properties of the ACh-sensitive channel. The rectification is more pronounced for the steady-state relation as a result of more marked relative decrease of ACh-induced current at less negative potentials. Fig. 7 *B* presents results from three different fibres, showing that the extent of desensitization tended to increase with depolarization.

The influence of holding membrane potential on the rate of desensitization in one fibre exposed to 2×10^{-6} M-ACh is illustrated in Fig. 8. During exposure to ACh, the ACh-induced current decreased with comparable time constants at −20, −40 and −60 mV. The rate constants obtained in three different fibres and presented in Fig. 9 show that no significant change by membrane potential of the desensitization kinetics was observed.

These results suggest that membrane potential does not influence the way desensitization is produced, but that it rather affects the number of channels undergoing the process.

Post-acetylcholine rebound

A rebound of the secondary decrease in ACh-induced current was observed when ACh was washed out. The membrane current first decreased to a value which was lower than the control, before rising again to the pre-drug level (see Figs. 1*A* and 4). This phenomenon was constantly observed but a quantitative study was difficult to carry out because of its small magnitude and of its slow time course. In Fig. 4,

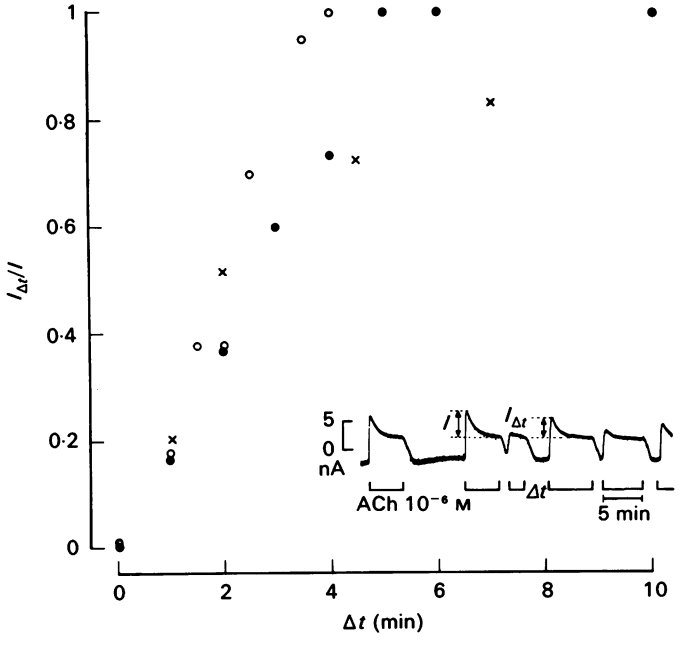

Fig. 10. Recovery from desensitization by ACh (2×10^{-6} M). The same ACh concentration was reapplied at different intervals during wash-out from a previous exposure. Different symbols represent different experiments. Inset: membrane current during repetitive exposure to the same ACh concentration, with varying wash-out intervals (Δt).

it is seen that the rebound was more marked at lower ACh concentrations (10^{-7}–10^{-5} M), probably because of the more rapid wash-out of ACh from the perfusing bath. The minimum current was usually reached in 1–3 min and the control level in 5–10 min. During wash-out of higher ACh concentrations, a long delay preceding the decrease of the ACh-induced current was usually observed and the return to control level was frequently preceded by oscillations in membrane current.

Recovery from desensitization

Desensitization probably results from a change of state of the receptors or channels which makes them refractory to further activation by the agonist. If the reverse process, i.e. the process by which the receptors or channels recover from refractoriness is slow, some time will elapse before a given ACh concentration can again produce its maximal effect. The time course of this recovery was investigated in three

experiments by applying a second ACh exposure at different wash-out intervals from a conditioning 5 min exposure to ACh and by comparing the amplitude of the decaying component during the second application to its amplitude in control (Fig. 10). The results show that recovery from desensitization is rapid. The desensitization produced by 10^{-6} M is removed with a half-time ($t_{1/2}$) of about 2 min.

DISCUSSION

Desensitization versus accumulation/depletion phenomena

The present results show that ACh changes the membrane K^+ conductance of rabbit cardiac Purkinje fibres in a biphasic way. Following exposure to ACh, the K^+ conductance is increased, but the magnitude of this change is reduced afterwards. The decrease with time of the current change produced by ACh follows an exponential course and is not due to accumulation/depletion phenomena. The biphasic time course was present when the fibres were superfused with high $[K^+]$ Tyrode solution, in which conditions accumulation/depletion phenomena following small changes in currents should be negligible. Accumulation/depletion phenomena should be very limited if not absent in rabbit Purkinje fibres because these preparations show usually wide intercellular clefts (Sommer & Johnson, 1968; Colatsky & Tsien, 1979). The fact that the biphasic time course of the ACh effect was always associated with a secondary lengthening of the action potential duration (Mubagwa & Carmeliet, 1983) is also against accumulation during ACh exposure since accumulation would normally result in a further shortening of the action potential. The results therefore strongly suggest a desensitization process.

Desensitization mechanisms

Various mechanisms have been proposed in the literature to explain desensitization. In order to see whether these models can be applied to the electrophysiological effects mediated by muscarinic receptors, the implications of different models will be confronted with the experimental findings in rabbit cardiac Purkinje fibres.

Receptor models. The most frequently used models are those which propose that desensitization occurs at the receptor level.

(1) A first type of receptor model assumes that desensitization is due to a slow transformation, following conformational change, of the active (conducting) agonist–receptor–channel complex into an inactive (non-conducting) form (Katz & Thesleff, 1957; Rang & Ritter, 1970).

(2) A second receptor model for desensitization proposes that the decrease in number of active receptors is due to agonist-induced removal of receptors from the cell membrane (receptor internalization). Muscarinic receptors may be 'down-regulated' following prolonged exposure to agonists (Nathason, Klein & Nirenberg, 1978; Siman & Klein, 1979). For cardiac muscarinic receptors, an agonist-induced decrease in receptor number has been observed in chick embryonic cultured heart cells (Galper, Dziekan, Miura & Smith, 1982).

(3) Another possible receptor mechanism for desensitization is uncoupling of the receptor (i.e. the agonist-binding structure) from the effector system (ionic channel, enzyme) without internalization of the receptors. For the systems where receptor

uncoupling has been observed (e.g. for β-adrenergic receptors; see Stiles, Caron & Lefkowitz, 1984), the process is rapid in its onset and in its recovery and may account for short-term desensitization.

The kinetic and steady-state properties of the above models are not fundamentally different (for the conformational model, see Rang & Ritter, 1970; Gero, 1983). (i) The rate of desensitization is a function of rate constants of the reactions in the models and of ACh concentration. Desensitization proceeds slowly because of the slow rate constants of the steps leading to the formation of the desensitized state. (ii) The extent of desensitization is a function of the ACh concentration. At equilibrium, the active receptor fraction, i.e. the ratio of the number of non-desensitized occupied receptors over the total number of receptors, is a monotonically increasing function of ACh concentration. (iii) The removal of agonist should not result in a rebound, as the unliganded receptor is usually assumed to be inactive.

Post-receptor models. In contrast to the previous models which propose that desensitization is the result of a decrease in number of active receptors, the following models suppose that desensitization is produced by mechanisms which interfere with activated effectors (ionic channel, enzyme) but which do not reduce agonist binding and do not inhibit the process of activation.

(1) According to one model, desensitization would be a consequence of changes in concentration of an intracellular or membrane-bound substance with secondary effects on ACh-sensitive channels. For example, Magazanik & Vyskočil (1970) have proposed that the fade of electrophysiological effects mediated by nicotinic ACh receptors in skeletal muscle may be due to an increase of membrane-bound Ca^{2+}, with secondary effect on membrane permeability. In heart, a change in concentration of intracellular (e.g. cyclic GMP) or membrane-bound substances (e.g. phosphatidyl-inositide metabolites) with possible secondary inhibitory effects on the ACh-activated channel is not excluded.

(2) In another model, blockade of the ACh-sensitive channel may be produced by excess agonist molecules themselves. Such a mechanism has been proposed for the frog end-plate (Adams, 1975). This last model predicts (i) that in function of the ACh concentration, the open-channel fraction will reach a maximum and will decrease to zero at very high agonist concentrations, and (ii) that the rate constants of desensitization will be increasing functions of agonist concentration.

Which model accounts for the results? The main results obtained from the experiments described in the present paper are the following, (1) desensitization appears grossly as a second-order process, since the time course of the current decline can be described by at most two exponentials (Figs. 1, 6 and 8). (2) The magnitude of desensitization is a varying function of agonist concentration and membrane potential; an increase in ACh concentration (Figs. 4–5) as well as membrane depolarization (Fig. 7) promotes desensitization. (3) The rate of desensitization is roughly constant with membrane potential (Figs. 8–9). The fast component of desensitization is modified by a change in ACh concentration (Fig. 6). (4) A rebound, i.e. a decrease of membrane conductance below the control level usually accompanies the ACh wash-out (Figs. 1 and 4).

For a reaction model to satisfactorily explain desensitization, it must account for all the above results. The models presented above usually assume that the agonist-

sensitive channel is closed in the absence of agonist. With this assumption, the receptor models cannot account for the maximum observed in the steady-state current–log [ACh] relation nor for the rebound phenomenon during ACh wash-out. The only model which predicts a maximum in dose–response curve is the agonist-induced, open-channel block (Adams, 1975). For this model, the ACh-induced current should decay to zero at very high ACh concentrations. However, while explaining such a convex effect–concentration relation, the agonist-induced block model does not account for the rebound nor for the dependence of only one rate constant of desensitization on agonist concentration. The decrease of extent of desensitization with hyperpolarization is also against ACh molecules entering the channels and producing a block, since hyperpolarization, which favours the inward movement of ACh, should enhance this process. It seems, therefore, that new assumptions have to be introduced in the previous models or that a different model has to be used in order to account for the observed effects.

Modified model. It is necessary, in order to account for the rebound, to assume that, during ACh wash-out, some ACh-sensitive channels which normally contribute to the normal K^+ conductance (i.e. which are already open in the absence of the agonist) remain temporarily closed due to desensitization. This implies that, contrary to the assumption in the above models, the presence of the agonist is not necessary for the channel to open (see Soejima & Noma, 1984). In the same way, it can be conceived that some desensitization may exist in the absence of agonist. Finally, it is possible that the agonist binds to the different states (resting, activated and desensitized) of the receptors.

With the above possibilities in mind, the probability of the ACh-sensitive channel to open, will depend on the rates of transition between the various states. The transition to the desensitized state must be very slow compared to the rate of transition to the other states. For explaining the increase in current produced by ACh, we have to assume that the channels stay predominantly in the closed state in the absence of agonist, and that they move into open state after binding of ACh. Soon after application of ACh, an equilibrium is established between ACh-bound closed and open states (this last state being the most likely). However, this equilibrium is only transient since the channels further move into a desensitized state (and the macroscopic current decreases) if the agonist is maintained present for enough time. During wash-out, ACh dissociates from the channels. Open channels move into the preferential closed state of unbound channels. A transient equilibrium is again obtained, during which the total number of open channels is lower than before ACh application, since a good number of other channels have not yet had enough time to leave the desensitized state. As wash-out is continued, the desensitized channels redistribute in the pool of unbound open and closed channels and the initial number of open channels is recovered.

For such a model, the open probability at equilibrium (after desensitization has occurred) may reach a maximum at a certain ACh concentration. The kinetics of the system are complex due to the presence of many steps. The rate of desensitization will still be monoexponential if the step leading to the desensitized state is much slower than the other steps, as assumed above.

The way membrane potential influences the extent of desensitization remains

unclear, but it might be related to the way it influences the number of channels already open in the absence of agonist.

Our results can be interpreted following the receptor models. Which one of the above-mentioned mechanisms (receptor conformational change, receptor–effector uncoupling or receptor internalization) is involved in the desensitization process remains to be elucidated. The quite rapid kinetics of desensitization found in the present results are against receptor internalization process, the onset and offset of which are usually slow.

In the present results, no biphasic time course was found for the effect of ACh on catecholamine-stimulated i_{si}. This might indicate an absence of desensitization for the process by which muscarinic receptors inhibit catecholamine-activated adenylate cyclase. Muscarinic receptors exert their inhibitory action on this enzyme probably via a GTP-regulated coupling protein (see Biegon & Pappano, 1980). It is possible that desensitized muscarinic receptors remain coupled to the intermediate protein. In view of the absence of a biphasic time course for the effect of ACh on catecholamine-stimulated i_{si}, a receptor internalization is unlikely. If internalization was the cause of desensitization, the inhibitory effect of ACh on the adenylate cyclase system, which is localized in the cell membrane, should decrease with time.

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