# Desensitization of the muscarinic receptor of bullfrog atrial muscle[1]

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*Summary.* The muscarinic ACh receptors, which hyperpolarize the resting membrane and also depress the action potential of bullfrog atrial muscle, show desensitization to the action of ACh. This suggests that the molecular mechanism of these muscarinic ACh receptor-ionic channel (voltage-dependent) complexes is comparable to that of the nicotinic ACh receptor-ionic channel (voltage-independent) complex of the end-plate.

In the neuromuscular junction of skeletal muscle, the sensitivity of the end-plate to acetylcholine (ACh) is depressed during continuous or repeated applications of ACh or its agonist, and such a decrease of the sensitivity of end-plate to transmitter substances is called desensitization[2,3]. The desensitization is causally related to the processes occurring at the receptor-ionic channel complex (RICC) which produces the changes in the membrane permeability and consequently those in the membrane potential. Although the desensitization of the nicotinic RICC at the end-plate has been studied in detail by many investigators, that of the muscarinic receptor has been little studied. In the case of amphibian or mammalian atrial muscle, ACh hyperpolarizes the resting membrane and decreases both amplitude and duration of the action potential by its muscarinic action. A question arises if the muscarinic receptors responsible for these ACh actions could be considered to compose a RICC, the nature of which is comparable to that of the nicotinic ACh receptor of the end-plate. In connection with this question, it was unexpectedly reported in 2 sets of recent experiments[4,5] that the hyperpolarization of amphibian and mammalian atrial muscles in ACh-containing solution did not show any sign of desensitization, unlike the depolarization of the end-plate. The present experiments were carried out using the voltage-clamp method for the purpose of examining whether the muscarinic receptor of amphibian atrial muscle indeed shows no desensitization. According to the present results, the membrane conductance changes inducing both the hyperpolarization and the depression of action potentials of atrium clearly show a desensitization.

*Material and methods.* Strips (size 0.3–0.5 × 5 mm) of quiescent muscle fibre bundles excized from the atria of bullfrog *(Rana catesbeiana)* heart were used. The experimental apparatus, including a chamber for mounting preparations, was the same as that described elsewhere[6], which was essentially similar to that reported by Beeler and Reuter[7]. The change of the resting membrane conductance in the ACh-containing Ringer's solution was measured by the voltage-clamp experiment, which was performed by recording membrane potential with an intracellular microelectrode and by applying the clamp current across the sucrose-gap. Action potentials were induced by applying an electrical pulse across the sucrose-gap, and they were simply recorded by an intracellular microelectrode. Ionic compositions of solutions used in the present experiment are as follows. Sucrose solution: 240 mM sucrose. Ringer's solution: 112 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 2.4 mM $NaHCO_3$ and 2.5 mM glucose. Drugs added to Ringer's solution was acetylcholine chloride (Wako Pure Chem. Ind.), carbachol (Tokyo Kasei), bethanechol chloride (Sigma) and d-tubocurarine chloride (Wako Pure Chem. Ind.).

*Results.* A preparation was continuously perfused with a solution. When superfusion solution was changed from the Ringer's solution to that containing ACh or carbachol, the membrane of quiescent atrial muscle (resting potential, $-90 \sim -95$ mV) was hyperpolarized and resting membrane conductance was increased; the maximum effect was observed with ACh or carbachol in concentrations more than $10^{-4}$ M. The membrane conductance change was measured at the potential level of $-95$ mV by applying test command pulses (less than 5 mV, 500 msec duration). The membrane conductance showed a marked increase within 30–60 sec after an application of a drug ($1 \times 10^{-4}$ M). Increased membrane conductance, however, showed a slow recovery during a prolonged application of drug, indicating a sign of desensitization. An example of these results obtained by use of carbachol is shown in figure 1. As seen in this figure, the rate of the onset of desensitization is slow. After the removal of drug from the external solution, the membrane conductance returned to normal within several min. The sensitivity to ACh or carbachol, however, was depressed for an extended period after a removal of drug. Thus, the full sensitivity to ACh or carbachol was restored within 10–15 min after a previous application. A similar result was also obtained under the condition where the resting potential was clamped at $K^+$-equlibrium potential ($E_K$) level; no steady ACh or carbachol current was elicited at this potential level.

Action potentials of quiescent atrial muscle were elicited by repeated electrical pulses (3 msec duration, 0.1–0.2 Hz).

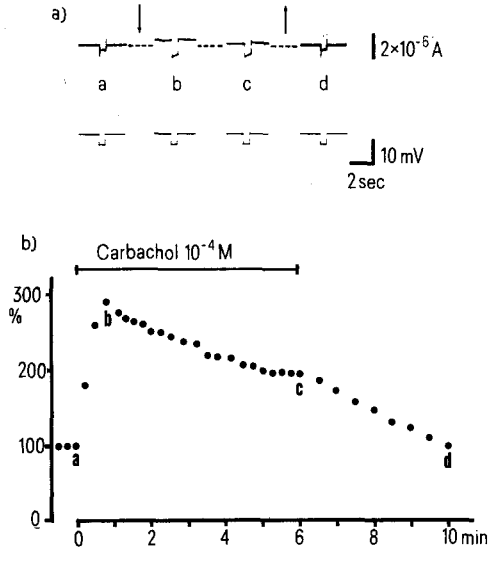


Fig. 1. Desensitization of membrane conductance changes caused by the action of carbachol ($1 \times 10^{-4}$ M). The membrane potential was clamped at $-95$ mV and anodal command pulses (4 mV, 500 msec) were applied at intervals of 5 sec. *a*) Upper and lower traces are current and voltage recordings, respectively. Record *a* was a control in Ringer solution and records *b* and *c* were 0.8 and 6 min after an application of carbachol. Record *d* was 4 min after withdrawal of carbachol. Application and withdrawal of drug are shown by arrows; note an increase of membrane conductance and a steady outward current during an application of carbachol. *b*) Membrane conductances measured by test command pulses are plotted against time; the membrane conductance in Ringer solution is taken as 100%. Alphabetical marks correspond to those in *a*). Note a large increase of membrane conductance and its restoration during an application of carbachol.

When the superfusion solution was changed from the Ringer's solution to that containing ACh ($5.5 \times 10^{-7}$ M) or carbachol ($5 \times 10^{-7}$ M), both amplitude and duration of action potentials were markedly depressed; changes in resting membrane potential or conductance were too small to detect their desensitization by such a small concentration. Interestingly, the decrease in amplitude and duration of action potentials were found to gradually recover during a sustained application of ACh or carbachol. This suggested that the changes in the action potential by ACh or carbachol shows desensitization to the action of these drugs. An example of these results obtained with carbachol is shown in figure 2. As seen in this figure, the rate of the onset of desensitization is slow. When the ACh was removed from the superfusion solution, the configuration of action potentials was rapidly restored to normal in a few min. A 2nd application of ACh, however, induced an effect which was less than that of a 1st application. Thus, it took 10–15 min for a full restoration of the sensitivity to ACh after a removal of ACh applied previously. Similar results were obtained with bethanechol ($1 \times 10^{-5}$ M) and also with ACh in the presence of d-tubocurarine chloride (d-TC, $1.2 \times 10^{-4}$ M).

Changes in both resting and action potentials of atrial muscle in ACh-containing solution were thought to be caused by an increase of the membrane permeability to K ions; the resulting increase in background $K^+$ current causes a hyperpolarization toward the $E_K$ and simultaneously decreases both amplitude and duration of the action potential[8]. According to the recent papers[9,10], however, the decreases in the amplitude and duration of the action potential have been explained as a result of the reduction of the slow inward current.

The present result demonstrated that the muscarinic receptor producing hyperpolarization of the resting membrane shows a desensitization to the action of ACh. Since the rate of the onset of the desensitization of this receptor is slow, caution must be used in its demonstration. Observations of a decay of the membrane hyperpolarization[5] or the steady membrane current during a continuous application of ACh or its agonist may not be pertinent for a demonstration of the desensitization, because such a decay may be simply reflecting a change of $E_K$ (diffusional polarization[11]). The experimental results, firstly that a slow recovery of the membrane conductance in carbachol-containing solution was observed even when the resting membrane potential was clamped at $E_K$ where no net steady membrane current was induced, and secondly that the sensitivity of the muscarinic receptors was depressed for an extended period whereas the membrane conductance was quickly restored to normal after a withdrawal of carbachol, clearly demonstrated the existence of the desensitization of the muscarinic receptor. It should be noted here that an application of ACh or its agonist for a short period may not allow a full development of the desensitization[4] because of a slow rate of onset of desensitization.

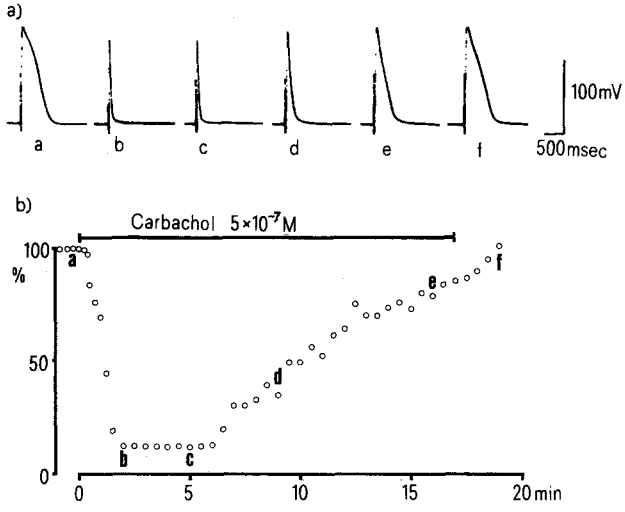


Fig. 2. Desensitization of action potential changes caused by the action of carbachol ($5 \times 10^{-7}$ M); resting potential was −95 mV. *a*) Record *a* was a control action potential in Ringer solution and records *b, c, d, e* were 2, 5, 9, 15 min after an application of carbachol, respectively. Record *f* was taken 2 min after withdrawal of carbachol. *b*) Action potential durations measured at the potential level of −75 mV are plotted against time; the duration of a control action potential is taken as 100%. Alphabetical marks correspond to those in *a*).

The changes in the action potential in a solution containing ACh or its agonist also showed signs of a desensitization. The fact that the sensitivity of the muscarinic receptors was depressed for an extended period whereas the configuration of the action potential was rapidly and fully restored to normal after a withdrawal of carbachol, suggested that a partial restoration of action potential in a solution containing carbachol was not simply due to a change of ionic distribution across the membrane. Another fact, that the desensitization could be observed under the action of bethanechol or ACh (with d-TC), suggested that the restoration of the action potential was not due to nicotinic action on the sympathetic nerve terminals, releasing a catecholamine.

The signs of the desensitization of the action potential was clearly observed under the effect of an agonist in a small concentration which was too small for the detection of the desensitization of resting membrane conductance. Action potential changes produced by the action of ACh in such low concentrations have been thought to be caused mainly by a depression of the slow inward current[10]. Thus, the desensitization of the muscarinic receptors responsible for the changes in the action potential of the atrium seems to represent that associated with the slow inward current.

In conclusion, the muscarinic receptors of atrial muscle, which increase the background $K^+$ current and also depress the slow inward current, exhibit desensitization to the action of ACh. If this is the case, one may be able to assume that these muscarinic receptors compose an RICC, of which the nature is comparable to that of the nicotinic RICC of the end-plate. In the case of the muscarinic RICC, its ionic channels would be for 2 kinds of voltage-dependent current, viz., the background $K^+$ current and the slow inward current. Whether these two types of muscarinic RICC of bullfrog atrium are separate, or form a single unit, is not known at present.

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