sites for activation and fast desensitization in *Torpedo* receptor is being further investigated.

The effects of different ligands, lipids, and anesthetics on the kinetic parameters are being explored in an effort to work out the relationships among the activation and inactivation pathways. The chemical modification techniques begun here will be extended to include subsequent alkylation with affinity reagents. In addition, the quantitative relationship of ligand binding and ligand affinity changes to the flux kinetics is being analyzed.

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## REFERENCES

1. McNamee, M. G., and E. L. M. Ochoa. 1982. Reconstitution of acetylcholine receptor function in model membranes. *Neuroscience*. 7:2305–2319.
2. Epstein, M., and E. Racker. 1978. Reconstitution of carbamylcholine-dependent sodium ion flux and desensitization of the acetylcholine receptor from *Torpedo californica*. *J. Biol. Chem.* 253:6660–6662.
3. Hess, G. P., D. J. Cash, and H. Aoshima. 1979. Acetylcholine receptor-controlled ion fluxes in membrane vesicles investigated by fast reaction techniques. *Nature (Lond.)*. 282:329–331.
4. Cash, D. J., and G. P. Hess. 1981. Quench flow technique with plasma membrane vesicles: acetylcholine receptor-mediated transmembrane ion flux. *Anal. Biochem.* 112:39–51.
5. Walker, J. W., K. Takeyasu, and M. G. McNamee. 1982. Activation and inactivation kinetics of *Torpedo californica* acetylcholine receptor in reconstituted membranes. *Biochemistry*. 21:5384–5389.
6. Walker, J. W., R. J. Lukas, and M. G. McNamee. 1981. Effects of thio-group modifications on the ion permeability control and ligand binding properties of *Torpedo californica* acetylcholine receptor. *Biochemistry*. 20:2191–2199.
7. Aoshima, H., D. J. Cash, and G. P. Hess. 1981. Mechanism of inactivation (desensitization) of acetylcholine receptor. Investigations by fast reaction techniques with membrane vesicles. *Biochemistry*. 20:3467–3474.

# ACETYLCHOLINE-INDUCED $K^+$ CURRENT IN AMPHIBIAN ATRIAL CELLS

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Although the inotropic and chronotropic actions of acetylcholine on the heart are of fundamental importance, many of their biophysical properties remain incompletely understood. In bullfrog (*Rana catesbeiana*) atrial trabeculae, voltage-clamp data have shown that acetylcholine (ACh) may decrease an inward $Ca^{++}$ current and/or increase an outward $K^+$ current (1, 2), and that the *I-V* relation of this ACh-induced $K^+$ current exhibits strong inward rectification. We have combined an enzymatic dispersion procedure that yields single cells from bullfrog atria (3) with whole-cell voltage-clamp (4–5) and patch-clamp techniques to study the ion transfer characteristics of the steady (noninactivating, nondesensitizing) outward ACh-induced $K^+$ current in single atrial myocytes.

## RESULTS AND DISCUSSION

Fig. 1 *A* illustrates our whole-cell/patch-clamp technique, which is an adaptation of that described by Hamill et al. (1982). Fig. 1 *B* shows three superimposed current records elicited by noncumulative bath application of acetylcholine ($10^{-7}$, $10^{-6}$, $10^{-4}$ M). It is apparent that these maintained outward currents, induced by ACh, are dose-dependent and do not desensitize. Additional measurements (not shown) indicate that these currents can be inhibited completely by atropine ($3 \times 10^{-8}$ M).

Fig. 2 summarizes results which define the reversal potential, and hence the ionic nature, of the ACh-induced current. Short (100 ms) "puffs" of ACh were delivered repeatedly from a pressure-phoresis device and the membrane potential (holding potential) was clamped in the range $-80$ mV to $-120$ mV. The data in the left-hand panel show a reversal potential near $-105$ mV in 2.5 mM $K^+$ Ringers; the results in the right-hand panel show that this reversal potential shifts 58 mV/10-fold change in $(K^+)_0$ when $(K^+)_0$ is increased in the range 2.5–50 mM. Thus, the classical muscarinic receptor-mediated increase in $K^+$ "permeability" (6) can be demonstrated in individual, enzymatically-dispersed atrial cells.

Subsequent whole-cell voltage-clamp and patch-clamp experiments have attempted to define the ion transfer (current-voltage, *I-V*) characteristics of this ACh-induced $K^+$ current. The data in Fig. 3 show that the *I-V* relation for the ACh-induced $K^+$ current exhibits strong inward rectification, in agreement with earlier data obtained from atrial trabeculae (1, 2). In isolated atrial cells we consistently find that ACh-induced $K^+$ current strongly resembles the previously identified background inwardly-rectifying $K^+$ current, $i_{K_1}$, in that both currents (*a*) have a high selectivity for $K^+$; (*b*) exhibit prominent inward rectification positive to the reversal potential; and (*c*) are inhibited



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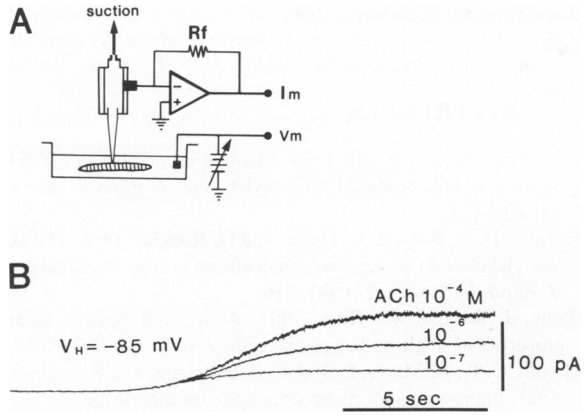


FIGURE 1 *A*, diagram of macroscopic current and patch-clamp recording apparatus. The pipette may be either placed against the cell surface (patch recording) or "popped" inside the cell (whole-cell voltage clamp) by varying the applied suction. $R_f$ is usually $10^{10}$ MΩ; transmembrane potential is displaced with a simple battery-powered DC source. Resting potential is assumed to be −85 mV in normal Ringers. *B*, whole cell outward currents elicited by successive bath application of acetylcholine (ACh). Variations in bath exchange times prevent meaningful determination of the time-course of the ACh effect.

by bath application of $Ba^{++}$ ($10^{-5}$–$10^{-4}$ M). These similarities suggest that the muscarinic action of acetylcholine is brought about by an increased activity of $i_{K_1}$ channels, either by way of increasing the number of open channels or by increasing the conductance and mean open time of existing channels. A different, but still plausible, supposition would be that a distinct population of $i_{K_1}$-like channels are "induced" by activation of the muscarinic receptor.

Single-channel measurements, using the "patch clamp"

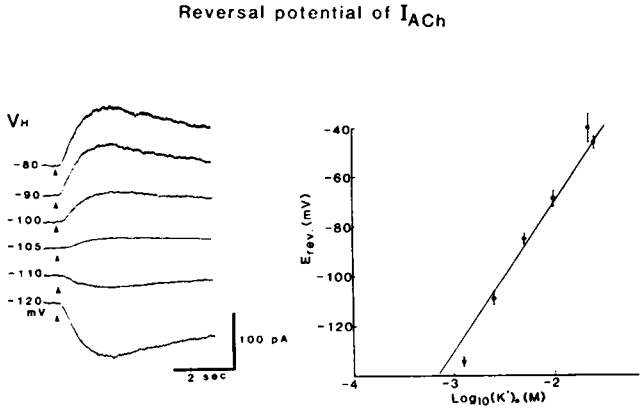


FIGURE 2 Determination of the reversal potential of $I_{ACh}$ and illustration of the dependence of $I_{ACh}$ on $[K^+]_0$. The left panel shows raw data used in determining the reversal potential in 2.5 mM $(K^+)_0$. 100 ms pressure-phoretic pulses (picospritzer) were "puffed" from an electrode containing $10^{-6}$ M ACh, following graded changes (10 mV) of the holding potential ($V_H$) in the range −80 mV to −120 mV. The right panel summarizes the results of repeating this procedure in five different $[K^+]_0$. The straight line has a slope of 58 mV/10-fold change in $[K^+]_0$, indicating that $I_{ACh}$ is a potassium current. Means ± SEM were calculated from repeating the test ($n$ = 4–7) in a number of different cells in each $(K^+)_0$.

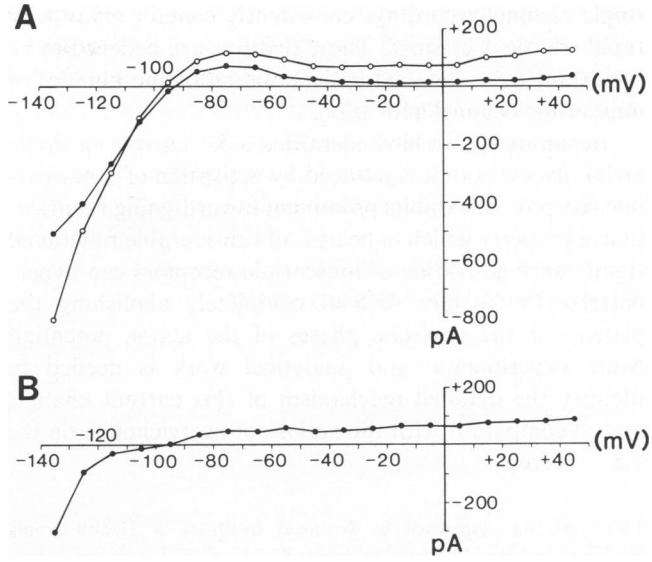


FIGURE 3 *A*, current-voltage (*I-V*) relationships for the conventional inwardly rectifying background $K^+$ current, $i_{K_1}$ (•), and the changes produced by ACh $10^{-6}$ M (○) in 2.5 $(K^+)_0$ Ringers. *B* is a plot of the difference in the control $i_{K_1}$ and ACh-induced current. Note that the apparent reversal potential is very similar to that determined in Fig. 2, and that $I_{ACh}$ also exhibits marked inward rectification.

technique described by Hamill et al. (1981), were made to study the action of ACh at the single channel level. When ACh or muscarine is included in the patch pipette, transitions such as those illustrated in Fig. 4 can be recorded routinely. Sudden increases in the inward current, corresponding to downward deflections, indicate channel opening. Similar, less well-defined transitions are seen when $(K^+_0)$ is reduced from 113 mM. Construction of single channel *I-V* relations for various $(K^+_0)$ yields evidence that the single channel current is carried by $K^+$ and that it also exhibits inward rectification. Furthermore, the number of channels activated in a cell by ACh may be estimated by comparing whole cell $I_{ACh}$–$V$ relationships with that of a single channel, yielding ~3,500 ACh activated channels per cell. Finally, it is important to note that ACh-induced

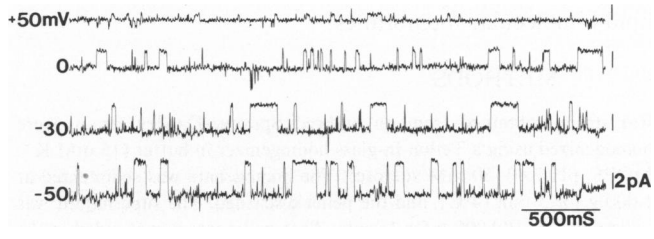


FIGURE 4 Patch-clamp records of ACh-induced current in isolated bullfrog atrial myocytes. The "patch" pipette contained 113 mM $(K^+)$, $10^{-5}$ M TTX, $10^{-4}$ M $CdCl_2$ $10^{-5}$ M ACh and 3 mM HEPES buffer, pH 7.4. Denoted potentials are the $\Delta V$ applied to the bath; the potential across the patch will be this value plus the resting potential, assumed to be −85 mV. Open channels produce a downward current deflection or inward current.

single channel recordings consistently contain many very rapid transient closings. These features are believed to be important for a detailed understanding of the kinetics of muscarinic channel activation.

In summary, we have identified a $K^+$ current in single atrial myocytes that is induced by activation of a muscarinic receptor. It exhibits prominent inward-going rectification, a property which in heart is of considerable functional significance activation of muscarinic receptors can hyperpolarize the atrium without completely abolishing the plateau or the upstroke phases of the action potential. More experimental and analytical work is needed to identify the detailed mechanism of this current change, and to compare it with the actions of acetylcholine on the $Ca^{++}$ current.

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## REFERENCES

1. Giles, W., and S. J. Noble. 1976. Changes in membrane currents in bullfrog atrium produced by acetylcholine. *J. Physiol.* (*Lond.*). 261:103–123.
2. Garnier, D., J. Narjeot, C. Ojeda, and O. Rougier. 1978. The action acetylcholine on background conductance in frog atrial trabeculae. *J. Physiol.* (*Lond.*). 274:381–396.
3. Hume, J. R., and W. Giles. 1981. Active and passive electrical properties of single bullfrog atrial cells. *J. Gen. Physiol.* 78:18–43.
4. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers. Arch. Eur. J. Physiol.* 391:85–100.
5. Hume, J. R., and W. Giles. 1983. Ionic currents in single isolated bullfrog atrial cells. *J. Gen. Physiol.* 81:153–194.
6. Hutter, O. F. 1961. Ion movements during vagus inhibition of the heart. *In* Nervous Inhibition. E. Florey, editor. Pergamon Press, Oxford. 114–124.

# FUNCTIONAL RECONSTITUTION OF RAT STRIATAL DOPAMINE AGONIST RECEPTORS INTO ARTIFICIAL LIPID BIMOLECULAR MEMBRANES

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Functional reconstitution of ionic-channel–linked receptor systems into lipid bimolecular membranes (BLM) allows, in principle, the examination of single channels not only from highly purified preparations but also from essentially unenriched membrane fragments. Appropriate pharmacological criteria must be used to define the receptor operationally. The present study reports the reconstitution of an ionic-channel–linked dopamine agonist binding site from rat corpus striatum into artificial, essentially solvent-free lipid bimolecular membranes.

## METHODS

Rat striata, obtained from an outbred Sprague-Dawley strain, were homogenized using a Teflon-in-glass homogenizer in buffer (15 mM $K^+$-MOPS, pH 7.40, 50 mM sucrose). The homogenate was sedimented at 1,000 g for 5 min (4°C), and the pellet discarded. The supernatant was sedimented at 100,000 g for 15 min. That pellet was resuspended in the above buffer, processed in a sonicator for 2 min, and sedimented (2 min, 12,000 g); the resulting supernatant was "the homogenate" in all subsequent procedures.

The homogenate can be shown to bind dopaminergic ligands such as [$^3$H]-spiroperidol (in the presence of 200 nM ketanserin) with a picomolar $K_d$.[1] We prepared essentially solvent-free lipid bimolecular membranes, 2 mm$^2$, added homogenate, and measured changes in current under voltage-clamp conditions using previously described methods (2–4).

## RESULTS

A striking increase in DC conductance occurs when the dopaminergic agonist apomorphine (7.5 nM final concentration) is added to the *cis* side of homogenate-treated striatal membrane. This change is noticeable ~30 s after agonist addition, and reaches a maximum within 2–4 min. Addition of a higher (25 nM) concentration of apomorphine resulted in a still greater increase in conductivity. Pretreating the membrane with the potent dopaminergic antagonist (+)−butaclamol (5) 5 min prior to adding apomorphine (final butaclamol concentration, *cis* side, 500 nM), we observed no statistically significant activation of the DC conductance by subsequent application of apomorphine up to 300 nM. However, the addition of (−) − butaclamol (final concentration 500 nM), which is ~10,000-fold less potent in its binding to dopamine receptors than its enantiomer (6), had no significant effect upon the dose-dependence of the apomorphine-induced conductance increase. These data are illustrated in Fig. 1.

[1]Murphy, R. B. Unpublished observations.



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