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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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).

[1]Murphy, R. B. Unpublished observations.

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.

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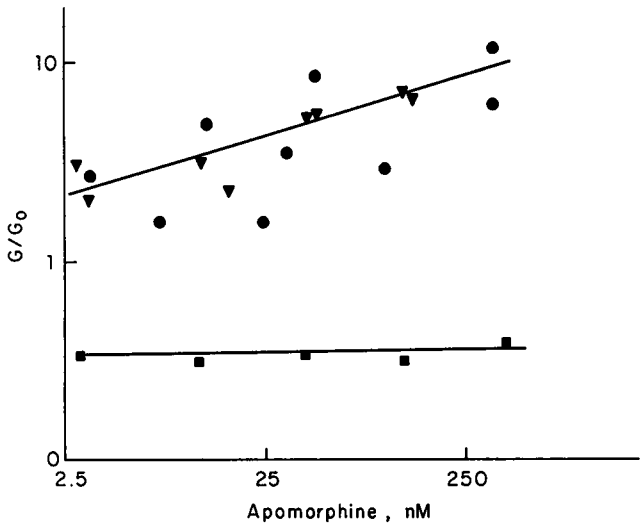

FIGURE 1 Effect of (+)− and (−)−butaclamol on apomorphine-induced conductance increase in lipid bimolecular membranes containing membrane material from rat striatum. Data are illustrated from 11 different membranes all treated with homogenate at a Lowry protein concentration of 50 μg/ml. •, no added butaclamol; ▲, 500 nM (−)-butaclamol added 5 min before apomorphine treatment; ■, 500 nM (+)-butaclamol added 5 min before apomorphine treatment. A straight line within the limits of experimental precision at $G/G_0 = 0.85$ was observed if pretreatment was carried out with (+)-butaclamol at a concentration of 100 nM. Membrane: egg PC, 20 mM KCl, 20 mM NaCl, 2 mM $CaCl_2$, pH 7.4. Clamp voltage: +20 mV.

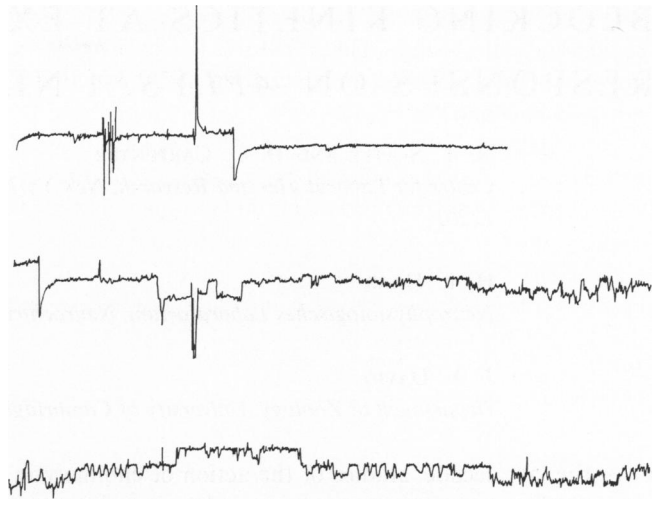

FIGURE 2 Single-channel fluctuations from apomorphine treatment of planar lipid bimolecular membranes containing material from membrane fraction of rat striatal homogenates. Bathing electrolyte as in Fig. 1: Membrane: essentially solvent-free bacterial phosphatidylethanolamine (Avanti Biochemicals, Inc., Birmingham, AL); initial capacitance 0.75 $\mu F/cm^2$. One vertical mm represents 1.43×10^{-13} *A*, one horizontal cm represents 10.0 s. *A*, no homogenate incorporation (bare membrane). *B*, spontaneous fluctuations induced by the addition of striatal homogenate (final protein concentration 50 μg/mL). *C*, the steady current fluctuations after the addition of 5 nM apomorphine. All records were obtained under a clamp voltage of +60 mV, which was reduced to 0 mV at the end of each record. Only a portion of each record is illustrated.

We also examined the effect of the selective dopaminergic agonist ADTN (7), and observed a qualitatively similar activation to that produced by apomorphine. Adding apomorphine and ADTN up to 1,000 nM concentration to lipid BLM that was untreated with striatal homogenates produced no statistically significant changes in DC conductivity or membrane capacitance.

It is possible to express the concentration dependence of the observed current changes as a linearized Scatchard isotherm. For the high-affinity region the apomorphine K_d is ~1.5 nM; the ADTN is ~1.2 nM.

Fig. 2 is a record of single-channel activity elicited by apomorphine addition to a membrane treated with striatal homogenate. Analysis of these records yields a conductance of 12.1 ± 0.3 pS (SEM) ($N = 52$) for the apomorphine-elicited channels. The mean open time of these channels is 12 ± 2.6 s (SEM).

Although activation remains unchanged in a variety of bathing media containing chloride, no activation is observed when 40 mM potassium sodium tartarate is used. With choline chloride (40 mM) we observed single channel fluctuations excited by apomorphine (10 nM).

These data are consistent with the hypothesis that a dopamine receptor site can be functionally reconstituted into an artificial, planar, and essentially solvent-free lipid bimolecular membrane. The question remains as to which dopamine receptor subtype we observe. The agonist dissociation constant values that we see are inconsistent with the association of the agonists with a putative D1 site, and suggest association with either D2 or D3 sites.

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