brief communication

Single delayed rectifier channels in frog atrial cells

Effects of β-adrenergic stimulation

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ABSTRACT The patch-clamp technique with two pipettes was used to record single delayed K^+ channels (cell-attached electrode) and to control the potential and the composition of the intracellular compartment (whole-cell electrode). With 30 μM cAMP in the cell and physiological potassium concentrations inside and outside the patch, a channel carrying an outward current was characterized. Its open probability was very low and the channel was recorded in only 5% of patches under control conditions. Increasing intracellular cAMP increased the probability of finding a channel in a patch 10-fold. The channel had the characteristics expected of a delayed rectifier channel. The time-course of its ensemble average resembled the whole-cell current in the same cell. The current-voltage relationship exhibited inward rectification, with a slope conductance of 20 pS in the linear portion and a reversal potential close to E_K. Both the open- and the closed-time distributions were described by the sum of two exponentials, suggesting a complicated gating scheme involving two closed states and two open states. The β-adrenergic stimulation did not change the conductance of the channel, but increased its probability of opening.

INTRODUCTION

The present experiments were designed to characterize the single channels underlying the delayed rectifier potassium current in frog atrial myocytes and to examine the effects of β-adrenergic agonists on these channels. Much is known about the macroscopic I_K current in various species (Giles and Shibata, 1985; Bennett et al., 1985; Hume et al., 1986; Simmons et al., 1986; Matsuura et al., 1987). In frog heart, I_K has been described as a single Hodgkin-Huxley conductance having high selectivity for K^+. The threshold for activation is near -40 mV and the instantaneous current-voltage relationship exhibits inward-going rectification (Duchatelle-Gourdon et al., 1989). Despite the fact that the macroscopic I_K current appears simple, the nature of the underlying single channels remains unclear.

Several different conductance states for delayed rectifier channels have been reported, suggesting that delayed rectifier channels may be composed of a family of different channel types. Single delayed rectifier K channels were first identified in nerve cells by Conti and Neher (1980) as a 17.5-pS channel in high $[K^+]_e$. Under similar conditions, Shibasaki (1987) has identified a single channel with a conductance of 11.5 pS in rabbit sinoatrial node cells that has the properties of a delayed rectifier channel. In normal $[K^+]_e$, a 15-pS conductance has been reported in skeletal muscle (Standen et al., 1985) and cardiac cells (Clapham and Logothetis, 1988), but several other conductance levels have also been found. In nerve cells, 10-, 20-, and 40-pS conductance states were found by Llano et al. (1988) and in cardiac muscle cells, 60 pS (Clapham and DeFelice, 1984) as well as 15- and 30-pS (Mazzanti and DeFelice, 1988) states have been described. This variability may reflect different types of channels or the lipid environment surrounding the same channel type because Coronado et al. (1984) have shown that the conductance of the neuronal delayed rectifier channel reconstituted in planar lipid bilayer depends on the phospholipid composition of the bilayer. In cardiac cells, this channel appears to have a relatively high selectivity for potassium ions (Shibasaki, 1987; Clapham and Logothetis, 1988; Mazzanti and DeFelice, 1988) and to be present at very low density. Mazzanti and DeFelice (1988) note that they can record the 15- and 60-pS conductance channels in only one patch out of 40. This observation is consistent with the expected channel density calculated by Clapham and DeFelice (1984) from whole cell data.

The β-adrenergic regulation of the delayed rectifier current has been described in frog heart (Brown and Noble, 1974; Duchatelle-Gourdon et al., 1989; Giles et al., 1989) as well as in other cardiac tissues (Tsien et al., 1972; Noma et al., 1980; Bennett et al., 1986; Walsh et al., 1989). The macroscopic I_K is enhanced ~100% by cAMP-dependent phosphorylation (Duchatelle-Gourdon et al., 1989). The slope conductance is increased, suggesting an increase in the number of channels, the probability

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of channel opening, or single channel conductance. Bennett et al. (1989), performing a noise analysis on the delayed K current in Purkinje fibers, suggest that β-adrenergic agonists increase the number of channels that open during a depolarization. The effects of the β-adrenergic stimulation at the single channel level, however, have not been reported.

Thus, it remains unclear whether there are several kinds of channels underlying the delayed I_K current and how they are regulated by the cAMP cascade at the single channel level. We report here that, in frog atrial cells, a 20-pS delayed rectifier channel activity can be identified using the cell-attached patch-clamp technique (Hamill et al., 1981), but at the very low frequency of two observations out of 40 patches under standard conditions. If we activate the cAMP cascade at room temperature, the frequency of channel detection jumps to 21 out of 36 patches. We have been able to see channel openings in previously silent patches after activation of the cAMP system.

METHODS

Cell isolation

The experiments have been performed on isolated atrial cells from frog (*Rana catesbeiana*). The isolation procedure is essentially the same as described in Duchatelle-Gourdon et al. (1989). Briefly, the heart was first perfused with 1 mg/ml collagenase and 0.5 mg/ml trypsin in a Langendhorf set-up for 1.5 h. Then the atria were removed and a subsequent mechanical dissociation in the presence of 1.4 mg/ml collagenase and 0.6 mg/ml trypsin was run for 1 h. The atrial cells were kept at room temperature before use.

Electrophysiology

In all cases, two patch clamp electrodes were used, one in whole-cell mode and one in cell-attached mode. Both whole cell currents and single channel events were studied simultaneously in the same cell. This was done to control the composition of the intracellular medium as well as the transmembrane potential while recording the single channel activities. All experiments were carried out at room temperature (22–26°C). The bath solution was a Ringer solution containing in mM: 115 NaCl; 2.5 KCl; 1.8 $CaCl_2$; 1.8 $MgCl_2$; 10 Hepes; 5 glucose; 5 sodium pyruvate; 0.003 TTX (tetrodotoxin); pH 7.4 (with NaOH). The whole-cell recording component of the set-up was very much like the one described in Duchatelle-Gourdon et al. (1989). The same pipettes were used (Soft glass; Drummond Scientific Co., Broomall, PA, resistance ranging between 0.8 and 2.5 MΩ), and in some experiments the whole-cell pipette was perfused to apply cAMP intracellularly (Fischmeister and Hartzell, 1987). The standard intracellular solution in the whole-cell electrode was the same as previously used (Duchatelle-Gourdon et al., 1989), and contained in mM: 118 KCl; 4.04 $MgCl_2$; 5 K_2EGTA; 2.8 Na_2K_2ATP; 5 sodium creatine phosphate; 10 Pipes (piperazine-*N*-*N'*-bis[2-ethanesulfonic acid]); pH 7.15 (with KOH). The free Mg^{2+} concentration calculated from the Godt and Lindley (1982) program was 1 mM. The cell-attached patch electrode contained the same solution as the bath solution except that no $CaCl_2$ was added, the $[Mg^{2+}]$ was raised to 2 mM, and the TTX concentration was 600 nM. The cell-attached electrodes (Hard glass; Garner Glass Co., Claremont, CA) had resistances of 3–5 MΩ and were coated with Sylgard 184 (Dow Corning Corp., Midland, MI) and fire polished before use. Usually, the resistance of the seals were on the order of 20–35 GΩ. A dual channel programmable digital stimulator allowed us to control simultaneously and independently the potentials in the whole cell and in the cell-attached patch electrodes (design and concept by Goolsby, W. N., Department of Anatomy and Cell Biology, Emory University, Atlanta, GA). Long 12-s pulses are usually needed to reach the steady-state of I_K in our conditions. The same pulses were applied in the cell-attached electrode, usually to 0, +20, or +40 mV (all the potentials are expressed as true transmembrane potential), at the rate of one pulse every 20 s (same protocol conditions as in the previous study of whole-cell currents; Duchatelle-Gourdon et al., 1989). The current traces were stored on a model 1220 VCR (Panasonic Co., Secaucus, NJ) at 3 KHz, and analyzed later on model AT 80386 microcomputer (IBM Instruments, Inc., Danbury, CT). The programs used to analyze the digitized waveforms were created by Mr. Bill Goolsby here in our department.

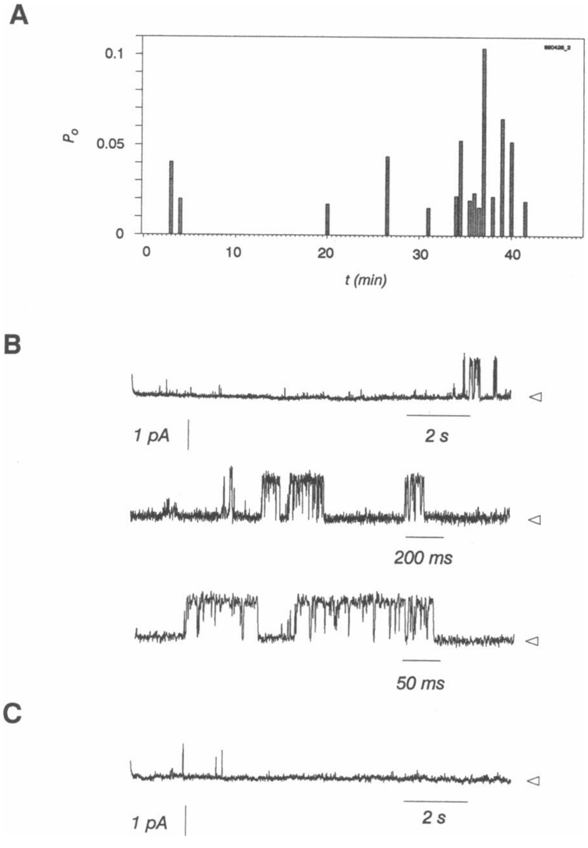

FIGURE 1 The delayed rectifier channel. (*A*) Channel open probability (p_o) is plotted as a function of time during an experiment. Note that the channel seldom opens. The last 10 min of the experiment are not represented because no channel openings were recorded. (*B*) (*Top trace*) Typical recording of a burst during a 12-s step from −80 to 0 mV transmembrane potential. (*Lower traces*) Same channels on faster time scales. Filtering: 2 kHz, sampling: 8 kHz. Arrow heads indicate the closed state (*C*). Example of a trace where the channel opens without bursting. 30 μM cAMP present throughout.

RESULTS

Properties of I_K single channels

The single delayed rectifier channels were recorded with physiological potassium concentrations inside and outside the patch membrane, (166 and 2.5 mM, respectively). Under these conditions, we identified the delayed rectifier channel on the basis of four criteria: (*a*) The channel should carry outward current at positive potentials; (*b*) The channel should activate at potentials positive to −40 mV; (*c*) The ensemble average of the single channel current should resemble the macroscopic I_K in time-course; (*d*) The reversal potential of the channel should be near E_K.

In our initial search for single I_K channels, we found that I_K channels were rarely observed in the absence of conditions stimulating cAMP-dependent phosphorylation (see below). Because the whole-cell current is increased by cAMP-dependent phosphorylation (Duchatelle-Gourdon et al., 1989), 30 μM cAMP was added to the whole-cell pipette to increase the likelihood of seeing a channel. Channels having the characteristics expected for the delayed rectifier under these conditions were observed in 15 patches out of 36 (Fig. 1). The channel had a chord conductance of 13.5 pS at 0 mV transmembrane potential. The probability of channel opening was very low. Fig. 1 *A* shows the probability of channel opening as a function of time. Each vertical bar represents the open probability of the channel during one 12-s depolarization. Most of the pulses do not elicit channel opening, and even when the channel opens, its open probability is quite low. In seven patches, the channel exhibited a prominent bursting behavior (Fig. 1 *B*), whereas in eight patches, the bursting behavior was minimal (Fig. 1 *C*). In the example of Fig. 1 *B*, the open probability estimated from the relative areas of closed and open states of the all-points histogram was 0.05 (see Fig. 2 *A*). The open probability was often even lower in patches such as that shown in Fig. 1 *C*.

The ensemble average of these channels is shown in Fig. 2 *B* and is compared with the whole-cell current in the same cell. The onset of the ensemble average and the whole-cell currents exhibit a lag period after the onset of the pulse. The time-to-first opening in the 12 traces used to construct the ensemble average was 603 ± 498 ms (mean ± SD). The ensemble average current increases with a time course consistent with that of the whole-cell current.

The open- and closed-time histograms for these channels are illustrated in Fig. 2, *C* and *D*. Two exponentials were required to fit both the open- and the closed-time distributions. The open time distribution was fit with two

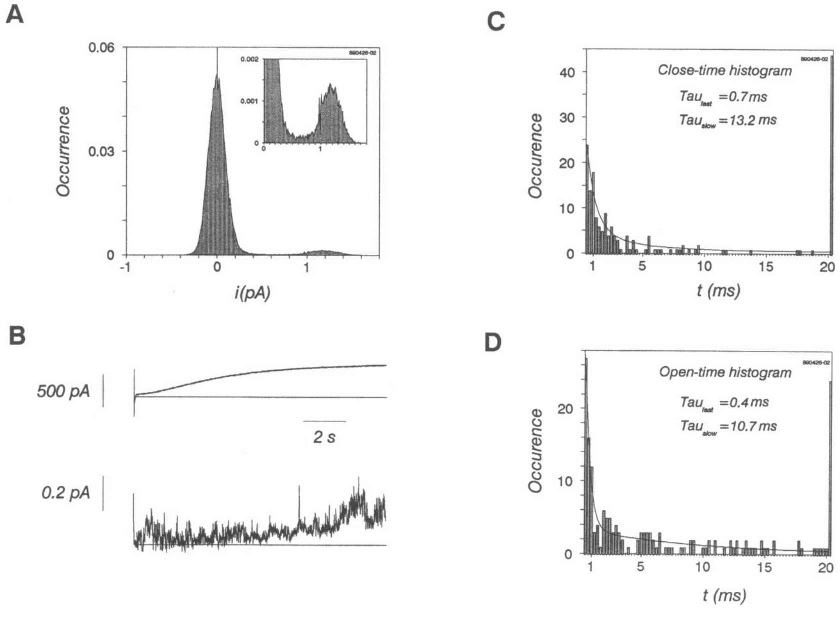

FIGURE 2 Kinetic properties of the delayed rectifier channel shown in Fig. 1. (*A*) All-points amplitude histogram constructed from six traces exhibiting channel activity at 0 mV transmembrane potential. The open probability is very low, but the open state is clearly separated from the closed state as shown in the inset. (*B*) (*Top trace*) Whole-cell current in the same cell. (*Bottom trace*) Ensemble average constructed from 12 traces. (*C*) Closed-time distribution. Bars are experimental data, solid line is a least-squares fit of the sum of two exponentials with time constants of 0.7 and 13.2 ms to the data. (*D*) Open-time distribution. The solid line shows the fit to the data of the sum of two exponentials with time constants of 0.4 and 10.7 ms. 30 μM cAMP present throughout.

exponentials with time constants of 0.4 ms and 10.7 ms. The closed-time distribution was fit with two exponentials with time constants of 0.7 ms and 13.2 ms. Double openings were never observed. These data are consistent with a gating model involving two open states and two closed states. The bursting activity that we observe is consistent with two closed states, but the presence of another channel of similar conductance providing the second exponential component in the open-time distribution cannot be ruled out. The time distributions could not be analyzed on traces like in Fig. 1 *C* because of a too small number of events.

The current-voltage (IV) relationship for this channel was determined in another cell (Fig. 3). Fig. 3 *A* shows samples of the traces used to generate the IV curve in Fig. 3 *B*. Each point on the curve represents the mean amplitude of the open state, determined from the amplitude histogram. The single-channel current inwardly rectified. The extrapolated reversal potential was −82 mV, ~20 mV positive to the K^+ reversal potential (−106 mV in our conditions). These features compare quite closely to the properties of the macroscopic I_K current, as we have recently described (Duchatelle-Gourdon et al., 1989). Fig. 3 *C* shows traces used to construct the macroscopic I_K IV relationship, and Fig. 3 *D* compares the IV relationship obtained from the single channel and from the whole-cell currents. The reversal potential for the single channel current (−82 mV) is close to the one recorded in the whole-cell experiments (−88 ± 3 mV in the presence of cAMP, Duchatelle-Gourdon et al., 1989) under the same conditions. This indicates that the channel is mainly selective to K^+ ions. Between −80 and −20 mV, the slope conductance was estimated to be 20 pS.

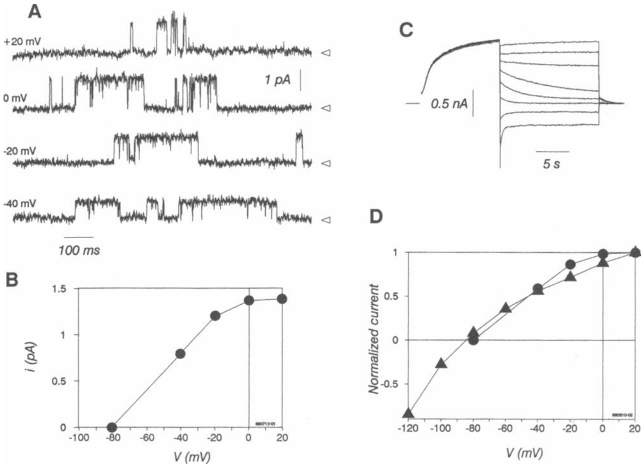

FIGURE 3 Current-voltage (IV) relation of the delayed rectifier. (*A*) Single channels recorded at various transmembrane potentials (indicated on each trace). Filtering 1 kHz, sampling: 4 kHz. (*B*) IV relation for the currents in (*A*) Each point is the mean value of the peak of the open state on the amplitude histogram. (*C*) Whole cell currents recorded with a double-pulse protocol. The cell was first depolarized from −80 to +40 mV for 12 s to fully activate I_K, and then repolarized to various test potentials (from +20 to −120 mV in 20 mV steps) for 15 s. (*D*) The filled triangles represent the instantaneous IV relation for the cell in (*C*) (see Duchatelle-Gourdon et al. [1989] for details). The curve has been normalized to the potential at +20 mV for comparison with the single channel IV relation (*solid circles*, same data as in *B*). Note that the shapes of the curves and the reversal potentials are very similar. 30 μM cAMP present in both cells.

Effects of cAMP

When we omit cAMP from the whole-cell electrode, the probability of finding a channel in the patch is greatly reduced (2 out of 40 patches). Fig. 4 *A* shows the probability of channel opening as a function of time in a patch in which cAMP levels were initially at a basal level before 30 μM cAMP was perfused internally. During the first 20 min before adding cAMP, no channel openings were observed. After adding cAMP, channel activity significantly increased. Fig. 4 *B* shows a similar experiment except that isoproterenol was added extracellularly instead of cAMP internally. In this case, the channel opened only once before addition of isoproterenol, but opened much more frequently during isoproterenol application. Application of cAMP inside the cell or isoproterenol outside the cell during a two-electrode experiment was difficult. In ~75% of the cases, the patch and/or the cell was lost when we moved the intra- or extracellular capillaries, because of mechanical problems. Furthermore, we never could change the solutions more than once and therefore could not address the question of reversibility.

In experiments where we could maintain both the whole cell and the patch in fairly good conditions while adding 30 μM cAMP to the intracellular solution or 1 μM isoproterenol to the extracellular solution, the channel opened in 10 out of 27 silent patches (Fig. 4 *A*). In all 27 cells, internal perfusion with cAMP increased the whole cell I_K. In two other experiments, a single channel could be detected in control conditions (Fig. 4 *B*). After adding 1 μM isoproterenol to the extracellular solution, the conductance of the channel did not change, but its open probability did. In these experiments, a total of 305 12-s duration sweeps were recorded under control conditions and 365 sweeps were recorded after simulation with cAMP, isoproterenol, or forskolin. Channels were observed in 2/305 sweeps in the absence of stimulation and in 33/365 sweeps in the presence of stimulation. Thus, stimulation of cAMP-dependent phosphorylation increases the percentage of sweeps exhibiting a channel ~10-fold, from 0.7 to 9%. These percentages compare favorably with the percentages of sweeps having a chan-

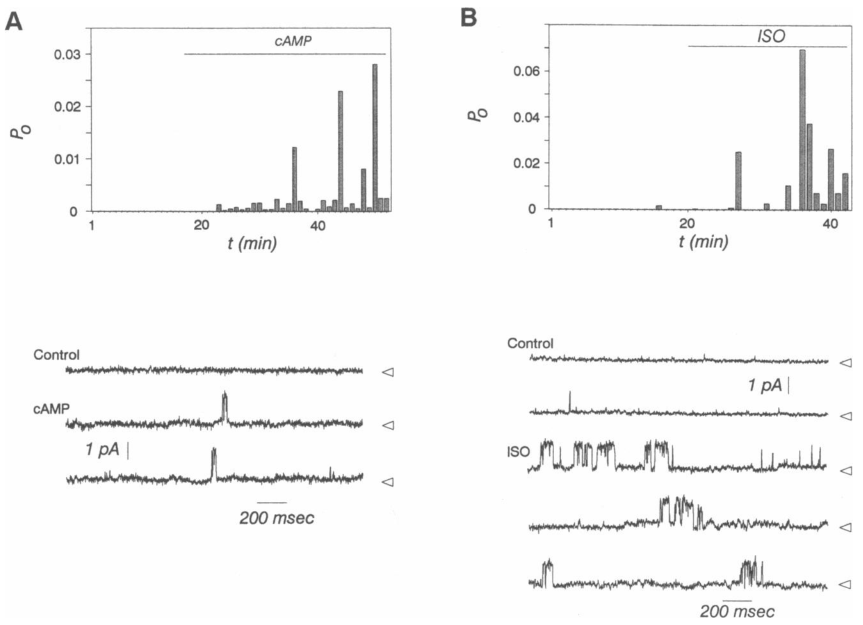

FIGURE 4 Effects of cAMP and isoproterenol on I_K single channels. (*A*) Effect of internal cAMP on single I_K channels. The perfusable whole-cell electrode was used to introduce 30 μM cAMP into the cell. (*Upper panel*) Channel open probability (p_o) during the experiment. 30 μM was added to the whole-cell pipette during the period indicated by the bar. (*Lower panel*) typical traces. The top trace shows a control recording during a pulse, with no detectable channel present. About 14 min after perfusion with cAMP into the well was begun, channels shown in the two lower traces were recorded. The channels opened 1.5 s (*middle trace*) and 4.7 s (*lower trace*) after the beginning of two different pulses. (*B*) Effects of application of 1 μM isoproterenol to the external solution on single I_K channels. (*Upper panel*) Time course of p_o during the superfusion with ISO. (*Lower panel*) Examples of recordings. The two top traces were recorded in control conditions. One channel was recorded 4.4 s after the beginning of a pulse. The same trace contained two other similar openings. 15 min after adding 1 μM isoproterenol outside the cell, a channel started to burst (three *lower traces*). The three traces belong to the same pulse and are consecutive. The first opening is 1.15 s after the beginning of the pulse. Arrow heads indicate the closed state. Potential: +20 mV. Filtering: 1 kHz, sampling: 4 kHz.

nel in separate patches that were either continually exposed to cAMP or were maintained under control conditions for the duration of the experiment. In cells where cAMP was present throughout the experiment, channels were recorded in 115 sweeps out of 1,238 (9%), whereas in the absence of cAMP stimulation, channels opened in only two sweeps out of 541 (<0.4%).

DISCUSSION

The present experiments were carried out on frog atrial myocytes to explain at the single channel level certain interesting features of the macroscopic delayed rectifier channel. We report here that we found a 20-pS channel that has the basic characteristics of the delayed rectifier. The reversal potential, virtually identical to the macroscopic I_K reversal potential, shows that the channel carries mainly K^+ ions. The IV curve is not linear but exhibits the same inward going rectification as the instantaneous whole cell IV curve (Duchatelle-Gourdon et al., 1989). This channel was recorded when the cell had been exposed to cAMP inside or isoproterenol outside the cell. In the absence of cAMP or isoproterenol, the channel was very rarely observed.

If we compare our results with what has been previously found on some other systems, this very low frequency of recording of the delayed rectifier K channel seems to be a common property of cardiac cells. For example, Mazzanti and DeFelice (1988) observed this channel in one patch out of 40 in embryonic chick ventricle and Clapham and Logothetis (1988) in one patch out of 10 in the same cell. The channel described here has a conductance similar to the smallest I_K conductance state reported in other systems. We have not observed higher conductance states in our preparation.

In the absence of β-adrenergic stimulation, the channel can be recorded in 5% of the patches tested. This is ~two-fold higher than that reported by Clapham and DeFelice (1984) and Clapham and Logothetis (1988) in chick ventricle, but the density of the macroscopic I_K in frog atrium is ~two times greater than that in chick ventricle. In the presence of β-adrenergic stimulation, the probability of finding a channel in the patch increases

~10-fold. This was surprising because the whole-cell current only doubles in response to cAMP (Duchatelle-Gourdon et al., 1989). However, it should be recognized that our estimates of the probability of channel opening are inaccurate, considering the very low probability of opening of these channels, even in the presence of cAMP. Another possibility is that the channel did open in control conditions but too briefly to be detected by our recording system (traces were filtered at 2 kHz). A final possibility that should be considered is that the macroscopic I_K is produced by several different conductances and that we have found only one of these in our single channel recording experiments. In any case, the effects of isoproterenol and cAMP on the channel described here are very similar to the effects we have previously found on the macroscopic current. These data demonstrate that a phosphorylation induced by the β-adrenergic system most likely increases the probability of opening of an I_K channel with no change in channel conductance in frog atrial cells.

The analysis of the open and closed time histograms suggests that the I_K channel can be described as having two closed states and two open states. Two closed states have already been reported for this channel (Bennett et al., 1985; Llano et al., 1988). Llano et al. (1988) also found two components in the open-time distribution and, as they suggested, the presence of another channel of similar amplitude cannot be excluded. However, this seems unlikely given the very low open probability of the channel that we observe.

The open- and closed-time histograms are consistent with the I_K channel having two open states and two closed states. A variety of different reaction schemes can be proposed with four states, but our data does not permit unambiguous differentiation between these schemes. A C1 — C2 — O1 — O2 model of channel gating, however, would be consistent with these data. If we assume this model is correct, the total probability of opening of the channel represents the product of the number of available channels (C2 state) and the rate constant of channel opening. Because cAMP drastically decreased the number of null sweeps, we conclude that the main effect of cAMP is either to increase the number of I_K channels in the C2 state, available to be opened by depolarization, or to increase the rate of opening. This supports the conclusion reached by Bennett et al. (1989) from their analysis of the effects of β-adrenergic agonists on whole-cell I_K current.

In summary, we have described a channel that can account for the macroscopic delayed rectifier current with the β-adrenergic system activated. The same channel was rarely detectable in control conditions, probably because of an extremely low probability of opening. The channel can open in a silent patch if we activate the β-adrenergic system during the experiment. The action of either isoproterenol or cAMP is to increase the probability of opening of the channel, leading to an increase in the number of open channels at a given potential.

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REFERENCES

Bennett, P. B., L. McKinney, R. S. Kass, and T. Begenisich. 1985. Delayed rectification in calf cardiac Purkinje fibers: evidence for multiple state kinetics. *Biophys. J.* 48:839–848.

Bennett, P. B., L. C. McKinney, T. B. Begenisich, and R. S. Kass. 1986. Adrenergic modulation of the delayed rectifier potassium channel in calf cardiac Purkinje fibers. *Biophys. J.* 49:839–848.

Bennett, P. B., R. Kass, and T. Begenisich. 1989. Nonstationary fluctuation analysis of the delayed rectifier K channel in cardiac Purkinje fibers. Actions of norepinephrine on single-channel current. *Biophys. J.* 55:731–738.

Brown, H. F., and S. J. Noble. 1974. Effects of adrenaline on membrane currents underlying pacemaker activity in frog atrial muscle. *J. Physiol.* (*Lond.*). 238:51–52P.

Clapham, D. E., and L. J. DeFelice. 1984. Voltage activated K channels in embryonic chick heart. *Biophys. J.* 45:40–42.

Clapham, D. E., and D. E. Logothetis. 1988. Delayed rectifier K^+ current in embryonic chick heart ventricule. *Am. J. Physiol. Heart.* 254:H192–H197.

Conti, F., and E. Neher. 1980. Single channel recordings of K^+ currents in squid axons. *Nature.* (*Lond.*). 285:140–143.

Coronado, R., R. Latorre, and H. G. Mautner. 1984. Single potassium channels with delayed rectifier behavior from lobster axon membranes. *Biophys. J.* 45:289–299.

Duchatelle-Gourdon, I., H. C. Hartzell, and A. L. Lagrutta. 1989. Modulation of the delayed rectifier potassium current in frog cardiomyocytes by β-adrenergic agonists and magnesium. *J. Physiol.* (*Lond.*). 415:251–274.

Fischmeister, R., and H. C. Hartzell. 1987. Cyclic guanosine 3′,5′-monophosphate regulates the calcium current in single cells from frog ventricle. *J. Physiol.* (*Lond.*). 387:453–472.

Giles, W., and E. F. Shibata. 1985. Voltage clamp of bull-frog cardiac pace-maker cells: a quantitative analysis of potassium currents. *J. Physiol.* (*Lond.*). 368:265–292.

Giles, W., T. Nakajima, K. Ono, and E. F. Shibata. 1989. Modulation of the delayed rectifier K^+ current by isoprenaline in bull-frong atrial myocytes. *J. Physiol.* (*Lond.*). 415:233–249.

Godt, R. E., and B. D. Lindley. 1982. Influence of temperature upon contractile activation and isometric force production in mechanically skinned muscle fibers of the frog. *J. Gen. Physiol.* 89:279–297.

Hamill, O., 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. *Pfluegers Arch. Eur. J. Physiol.* 391:85–100.

Hume, J. R., W. Giles, K. Robinson, E. F. Shibata, R. D. Nathan, K. Kanai, and R. Rasmusson. 1986. A time- and voltage-dependent K^+ current in single cardiac cells from bullfrog atrium. *J. Gen. Physiol.* 88:777–798.

Llano, I., C. K. Webb, and F. Bezanilla. 1988. Potassium conductance of the squid giant axon. Single channel studies. *J. Gen. Physiol.* 92:179–196.

Matsuura, H., T. Ehara, and Y. Imoto. 1987. An analysis of the delayed outward current in single ventricular cells of the guinea-pig. *Pfluegers Arch. Eur. J. Physiol.* 410:596–603.

Mazzanti, M., and L. J. DeFelice. 1988. K channel kinetics during the spontaneous heart beat in embryonic chick ventricle cells. *Biophys. J.* 54:1139–1148.

Noma, A., H. Irisawa, S. Kokobun, H. Kotake, M. Nishimura, and Y. Watanabe. 1980. Slow current systems in the A-V node of the rabbit heart. *Nature* (*Lond.*). 285:228–229.

Shibasaki, T. 1987. Conductance and kinetics of delayed rectifier potassium channels in nodal cells of the rabbit heart. *J. Physiol.* (*Lond.*). 387:227–250.

Simmons, M. A., T. Creazzo, and H. C. Hartzell. 1986. A time-dependent and voltage-sensitive K^+ current in single cells from frog atrium. *J. Gen. Physiol.* 88:739–755.

Standen, N. B., P. R. Stanfield, and T. A. Ward. 1985. Properties of single potassium channels in vesicles formed from the sarcolemma of frog skeletal muscle. *J. Physiol.* (*Lond.*). 364:339–358.

Tsien, R. W., W. R. Giles, and P. Greengard. 1972. Cyclic AMP mediates the action of norepinephrine on the action potential plateau of cardiac Purkinje fibres. *Nature* (*Lond.*). 240:181–183.

Walsh, K. B., T. B. Begenisich, and R. S. Kass. 1989. β-adrenergic modulation of cardiac ion channels. Differential temperature sensitivity of potassium and calcium currents. *J. Gen. Physiol.* 93:841–854.