The Journal of Neuroscience, December 1991, 11(12): 3727–3737

D_2 Dopamine Receptor Activation of Potassium Channels in Identified Rat Lactotrophs: Whole-Cell and Single-Channel Recording

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Dopamine (DA) is the major physiological regulator of prolactin secretion from the anterior pituitary, exerting a tonic inhibitory control that is mediated by D_2 DA receptors. D_2 receptors in both the anterior pituitary and CNS are thought to produce some of their inhibitory effects via a coupling to potassium (K^+) channels to increase K^+ conductance. Utilizing the reverse hemolytic plaque assay and patch-clamp techniques, we characterize the actions of DA on membrane potential and associated DA-activated whole-cell current, as well as the single K^+ channels that underlie the response in primary rat lactotrophs. We demonstrate that DA (5 nM to 1 μM) or D_2-selective agonists (RU24213 and quinpirole) evoke a hyperpolarization of membrane potential that was blocked by D_2 antagonists and associated with an increased K^+ conductance. Whole-cell current responses to ramp voltage commands revealed a DA-activated current whose reversal potential was near the calculated Nernst potential for K^+, varied as a function of K^+ concentration, exhibited some inward rectification, and was Ca^{2+} independent. The current was insensitive to tetraethylammonium (TEA; 10 mM), partially blocked by 4-aminopyridine (4-AP; 5 mM), and almost completely inhibited by quinine (100 μM). Cell-attached recordings in the presence of DA or a D_2 agonist revealed the opening of a K^+ channel that was not present in the absence of DA or when a D_2 receptor antagonist was included with DA. Analysis of the single-channel current showed the current–voltage relationship to be linear at negative patch potentials and yielded a unitary conductance of 40.2 pS in the presence of 150 mM KCl. The channels were not blocked by TEA (10 mM), were slightly suppressed by 4-AP (5 mM), and were almost completely inhibited by quinine (100 μM). These experiments establish that in primary rat lactotrophs, DA acts at D_2 receptors to activate the opening of single K^+ channels, which results in an increase in K^+ conductance and associated membrane hyperpolarization. This is the first characterization of single DA-activated K^+ channels in an endocrine cell.

Received Jan. 14, 1991; revised June 12, 1991; accepted June 27, 1991.

This work was supported by NIH Grant NS18788 to G.S.O. We thank Michele Smith and Scott MacMurdo for excellent technical assistance with tissue culture and reverse hemolytic plaque assay.

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In the CNS, dopamine (DA) has been hypothesized to play an intimate role in the organization of thought, locomotion, and reward. Accordingly, it has been implicated in the etiology of such disorders as schizophrenia, Parkinsonism, and most recently in the biological basis of drug addiction (Seeman et al., 1978; Bunney, 1984; Barnes, 1988). In the anterior pituitary, DA is well established to be the primary regulator of prolactin (PRL) secretion by exerting a tonic inhibitory control (Ben-Jonathan, 1985). DA inhibits PRL secretion by activating D_2 receptors located on the PRL-secreting cells of the pituitary, that is, lactotrophs (Caron et al., 1978; Enjalbert and Bockaert, 1983). The anterior pituitary also serves as an excellent model system for studying events coupled to D_2 receptor stimulation in the CNS, as it is considered to express the prototypical D_2 receptor while lacking D_1 binding sites (Kebabian and Calne, 1979; Creese et al., 1984).

The mechanism(s) by which DA inhibits PRL secretion at a cellular level is still a matter of controversy (Tam and Dannies, 1981; Canonico et al., 1982; Enjalbert and Bockaert, 1983; Vallar et al., 1988; Canonico, 1989). It is well established that pituitary cells display spontaneous electrical activity, including spontaneous membrane potential fluctuations and calcium-dependent action potentials (Ozawa and Sand, 1986). Furthermore, the ionic conductances underlying this electrical activity have been characterized in lactotrophs identified with the reverse hemolytic plaque assay (RHPA) (Lingle et al., 1986; DeRiemer and Sakmann, 1987; Cota et al., 1990; Lledo et al., 1990a). As is the case in neurons, the stimulus–secretion coupling processes involved in PRL secretion appear to involve these electrical events (Tam and Dannies, 1980; Ray and Wallis, 1982). Elucidation of the mechanisms underlying regulation of PRL secretion by DA will therefore involve characterization of the ionic mechanisms by which DA regulates lactotroph excitability. Previous investigations of this issue have suggested that stimulation of D_2 receptors on lactotrophs results in an inhibition of Ca^{2+}-dependent action potentials because of a hyperpolarization of membrane potential mediated by an increase in potassium (K^+) conductance (Israel et al., 1985, 1987; Ingram et al., 1986). Similar findings have been reported for intermediate lobe cells (Douglas and Taraskevich, 1978; Taraskevich and Douglas, 1990). These studies were conducted in primary cultures enriched in lactotrophs and utilized standard intracellular microelectrode recordings. Therefore, only the actions of DA on membrane potential were examined. More recent studies have directly explored the possibility that DA might activate voltage-dependent outward K^+ currents (Lledo et al., 1990a) that are known to exist in these cells (Lingle et al., 1986).

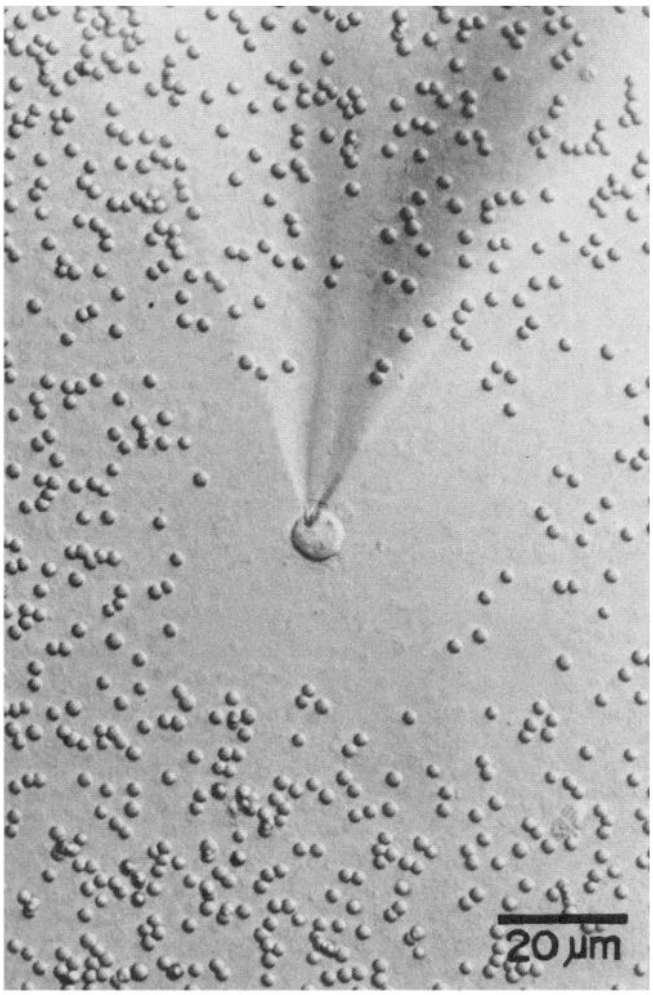

Figure 1. Identification of lactotrophs for patch-clamp studies by the reverse hemolytic plaque assay. A viable PRL-secreting cell dissociated from rat anterior pituitary tissue was identified in a plaque assay by the surrounding area of lysed ovine red blood cells. The tip of a patch recording electrode was sealed onto the membrane surface. Photomicrograph of unstained, unfixed cell was taken through Hoffmann modulation contrast optics. Scale bar, 20 μm.

Increases in K^+ conductance may be a common mechanism by which D_2 receptor stimulation inhibits electrical activity in many cell types. In this regard, regulation of K^+ channels by D_2 receptors has been reported for neurons of the substantia nigra and striatum from rat (Lacey et al., 1987; Freedman and Weight, 1988) as well as abdominal ganglion neurons of *Aplysia* (Sasaki and Sato, 1987). Using patch-clamp techniques, Freedman and Weight (1988) reported the opening of single 85 pS, nonrectifying K^+ channels upon D_2 receptor activation in primary neuronal cultures of rat striatum. To date, there have been no other reports on the regulation of ion channels by DA at the single-channel level in neurons. Furthermore, the DA response in endocrine cells has not been previously examined at the single-channel level. Given the apparent similarities between K conductance responses to D_2 agonists in neurons and lactotrophs, a detailed characterization of K^+ channel properties in pituitary cells might reveal common elements of D_2 receptor–effector coupling. Therefore, we have combined the RHPA with patch-clamp electrophysiological measurements to examine the actions of DA on K^+ channel activity in identified rat lactotrophs at both the whole-cell and single-channel level.

A preliminary report of this work has been published (Einhorn and Oxford, 1989).

Materials and Methods

Anterior pituitary dissociation. Anterior pituitary glands were dissected from adult, female Sprague–Dawley rats in the proestrus phase of the estrous cycle. Cells were dispersed enzymatically using a nontrypsin dissociation protocol modified from that used by Weiner et al. (1983). In brief, the glands were removed and minced in sterile Hank's balanced salt solution, which was free of calcium and magnesium (Hanks CMF). After washing, the fragments were incubated in a shaking water bath for 1 hr at 37°C in Hanks CMF containing trypsin inhibitor (0.1 mg%), collagenase (Worthington Biochemicals, 0.3%), and DNase I (1 mg%). Fragments were then mechanically dispersed by trituration with a siliconized Pasteur pipette, washed with Dulbecco's Modified Eagle's Medium containing 0.1% bovine serum albumin (DMEM-BSA), filtered through a nylon mesh (20 μM), and harvested via centrifugation.

Reverse hemolytic plaque assay. The reverse hemolytic plaque assay was performed as a means to unambiguously identify functionally secreting lactotrophs in a mixed cell culture. A detailed description of the procedure has appeared previously (Smith et al., 1986). Immediately following the dissociation, pituitary cells suspended in DMEM-BSA (4×10^5 cells/ml) were mixed with an equal volume of a 9% suspension of ovine erythrocytes (Colorado Serum) previously coupled to *Staphylococcus* protein A with aged chromium chloride hexahydrate (0.9 mg/ml). Aliquots of this mixture were infused into modified Cunningham chambers that were constructed by affixing a polylysine-coated glass coverslip to a microscope slide by means of two parallel pieces of double-stick tape. The chambers were inverted and incubated for 45 min at 37°C. The chambers were then rinsed with DMEM-BSA, and the assay was initiated by infusion of DMEM-BSA containing rabbit antiserum to rat PRL (PRL-1-5 generated in our laboratory; 1:120 dilution) and placed in the incubator for 1 hr. Following antiserum incubation, the plaques were developed by infusion of guinea pig complement (dilution, 1:50–1:100; 30 min). The complement reaction was terminated by repeated rinses with DMEM-BSA, and the chambers were dismantled. The coverslips with cells attached were maintained in culture in DMEM containing 10% equine serum and gentamycin (40 μg/ml) for electrophysiological experiments 1–3 d following the assay (Fig. 1). The optimum dilution of antiserum was determined independently in "titer assays" in which known quantities of rat PRL were used to promote the hemolysis of erythrocytes in parallel lanes in Cunningham chambers simulating an actual experiment. Preabsorption of the antiserum with prolactin (PRL I-3 standard from NIDDK; 100 μg/ml) abolished plaque formation, while preabsorption with purified rat growth hormone (GH I-1 standard from NIDDK; 100 μg/ml) did not diminish plaque formation.

Electrophysiological experiments. Whole-cell and cell-attached, single-channel recordings were made utilizing the gigaohm seal patch-clamp technique described by Hamill et al. (1981). Patch electrodes with 2–6 MΩ tip resistances were constructed from N51A capillary glass (Drummond Scientific, Broomall, PA) and coated with Sylgard (Dow Corning). Recordings were made using an Axopatch 1A patch clamp (Axon Instruments) or a Dagan 3900 integrating patch clamp (Dagan Corp.) and low-pass filtered at 1–2 kHz with a four-pole Bessel filter (Ithaco model 4302). Single-channel currents under voltage clamp or whole-cell voltage responses under current clamp were recorded wideband (~50 kHz) on videotape using a digital audio processor interface sampling at 44 kHz (Sony PCM701). Stored videotape recordings were subsequently filtered at 2 kHz and redigitized by a computer for subsequent analysis of single-channel current data using Axotape and pCLAMP software (Axon Instruments). Opening events were discriminated using a 50% threshold criterion excluding all events less than the sampling interval (200 μsec). Closing events excluded all transitions less than 400 μsec. Events were screened manually in most cases to eliminate spurious current changes and to adjust for baseline drift. Whole-cell macroscopic currents were sampled at 12-bit resolution by an A/D converter (LabMaster, Scientific Solutions); the data were stored on a 80286-based microcomputer and analyzed with custom programs written in C (C-Lab, INDEC).

The basic extracellular solution for whole-cell and single-channel recording consisted of (in mM): 145 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10

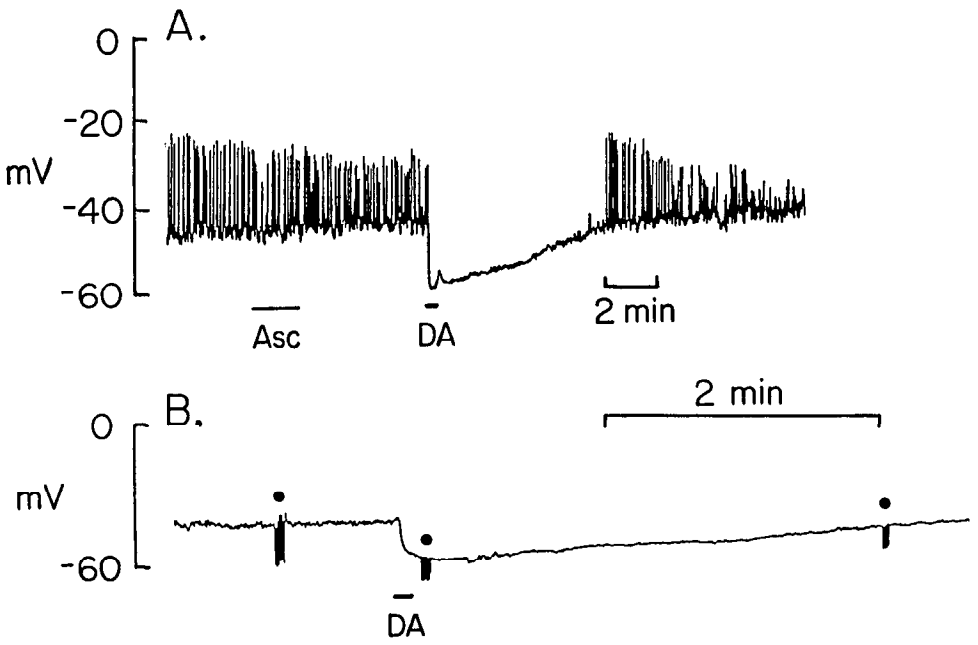

Figure 2. Membrane potential responses of a primary rat lactotroph to DA under whole-cell current clamp. Brief application (*bars*) of DA (4 μM) elicited a rapidly developing, long-lasting hyperpolarization in identified lactotrophs. *A*, In a spontaneously active cell, the hyperpolarization was accompanied by cessation in spiking activity that resumed with recovery of the membrane potential. Application of the ascorbate vesicle (*Asc*; 0.5 mM) alone had no effect. *B*, In a quiescent cell, DA (4 μM) also elicited a hyperpolarization. Attenuated voltage responses to the injection of a series of −5 pA hyperpolarizing current pulses (*dots*) indicate a decreased membrane resistance during the DA-induced hyperpolarization. Note the change in time scale between *A* and *B*.

glucose, and 10 HEPES buffer adjusted to pH 7.4 and osmolarity 295–300 mOsm. In some experiments, K^+ currents were isolated with solutions containing TTX (1 μM) to block voltage-dependent sodium channels and EGTA (1 mM) while omitting $CaCl_2$ to reduce calcium channel currents. The basic pipette solution for whole-cell recordings contained (in mM) 130 K-aspartate, 20 KCl, 10 glucose, and 10 HEPES adjusted to pH 7.4 and osmolarity 300–305 mOsm. For single-channel experiments with cell-attached patches, the pipette solution contained 150 mM KCl, 3.1 mM $MgCl_2$, and 15.5 mM HEPES. Drug solutions were made fresh each day just prior to experiments. DA was prepared as a stock solution diluted with ascorbic acid in 100-fold excess to prevent oxidation of the catecholamine. All experiments were performed at room temperature (20–23°C), and the control external bath solution was continuously perfused into the recording chamber by gravity flow. Application of test drugs was accomplished through a U-tube device that could be positioned next to a cell to apply and withdraw a solution rapidly while minimizing mechanical disturbance (Oxford and Wagoner, 1989). Agonist applications of less than 5 sec in duration were used with associated time constants for application and withdrawal in the range of 10–30 μsec.

Results

Membrane potential response to DA and D_2-selective agonists. Recordings were made from lactotrophs that had a range of different cell diameters and that formed plaques of different sizes. No qualitative differences in responses were observed among the cells examined. Following establishment of the whole-cell configuration, the majority (~70%) of lactotrophs exhibited a relatively steady resting membrane potential while others (~30%) were spontaneously active, exhibiting repetitive voltage-dependent action potentials (Fig. 2). In 85% of identified lactotrophs, a brief application of DA (0.05–5 μM) elicited an immediate hyperpolarization of the membrane potential that recovered slowly. In a spontaneously active cell, the hyperpolarization resulted in an immediate cessation of spiking activity that reinitiated with recovery of membrane potential. The hyperpolarization was accompanied by a 50% reduction in cell input resistance as assessed by attenuated voltage responses to hyperpolarizing current injections (e.g., Fig. 2*B*). No systematic differences in the responses were seen in firing versus quiescent cells. This response could also be induced by lower DA concentrations that are in the range of those measured in hypothalamic-pituitary portal blood (Gibbs and Neill, 1978; Plotsky et al., 1978). The effects of DA were observable at 5 nM, and the magnitude of hyperpolarization appeared to saturate at concentrations greater than 50 nM (Fig. 3*A*). However, further increases in DA concentration retarded the rate of recovery of the potential following DA application. As the U-tube system permits withdrawal of unbound agonist within 10–30 μsec and the applications were of only 1 sec in duration, the retardation of recovery likely reflects both dissociation of the bound agonist and decay of the activated effector system. The saturation of the response magnitude probably reflects a thermodynamic limitation imposed by an ion equilibrium potential, most likely E_K (see below). Under voltage-clamp conditions, changes in whole-cell holding currents in either inward or outward directions could be induced by DA at different membrane potentials, while prolonged applications at voltages near E_K elicited only negligible responses (Fig. 3*C*).

Several pharmacological experiments confirm that D_2 receptors mediate the DA-induced responses. The specific D_2 agonists RU24213 (1 μM; $n = 9$) and quinpirole (10 μM; $n = 10$) mimicked the membrane potential response. An example of a membrane hyperpolarization induced by 10 μM quinpirole is shown in Figure 3*B*. The specific D_2 receptor antagonists sulpiride (5 μM; $n = 9$) and (+)-butaclamol (100 nM; $n = 7$) blocked or attenuated (>90%) the response to DA. The inactive (−)-isomer of butaclamol (100 nM; $n = 5$) was unable to suppress the hyperpolarization induced by DA.

Ionic basis of the DA-induced hyperpolarization. Under whole-cell current clamp, membrane potentials were balanced at hyperpolarizing or depolarizing levels by current injection and the amplitude of the responses elicited by 2 sec applications of DA (1 or 5 μM) or RU24213 (1 μM) was assessed (Fig. 4*A*). Maximum changes in membrane potential elicited by DA were plotted as a function of the membrane potential just prior to drug application. These data demonstrate that the magnitude and direction of the DA response varied linearly as a function of membrane potential. A linear regression fit of the data yielded a

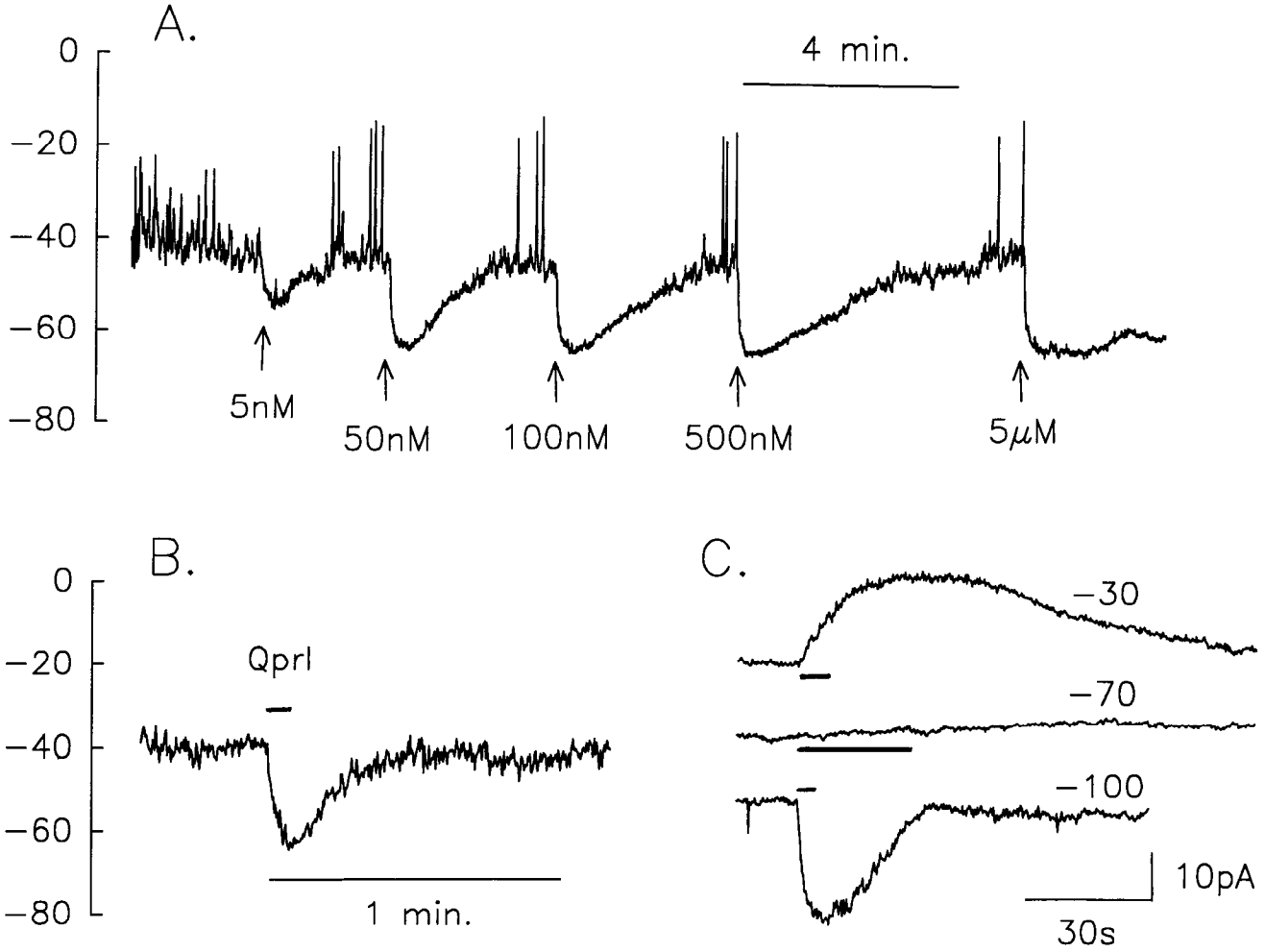

Figure 3. Membrane potential and current responses to varying concentrations of DA or quinpirole. *A,* Whole-cell current-clamp recordings of membrane potential demonstrating transient hyperpolarizations with recovery of baseline spiking activity following consecutive 1 sec applications of DA (*arrows*) from 5 nM to 5 μM in a single primary rat lactotroph. While the hyperpolarization appears to saturate, the recovery of membrane potential is clearly retarded for increasing doses of DA. *B,* Lactotroph membrane potential hyperpolarizes to the application of 10 μM quinpirole (*Qprl*), a selective D_2 receptor agonist. Duration of *Qprl* application is indicated by the *bar. C,* Whole-cell membrane current responses to DA under voltage clamp. DA (100 nM) was applied three times (*bars*) via a U-tube to a single RHPA-identified lactotroph held at the indicated membrane potentials (−30, −70, −100 mV). Outward currents are upward deflections. Baseline currents prior to DA application are vertically displaced to distinguish each response more clearly.

reversal potential for the DA-induced hyperpolarization of −77.6 mV, which closely approximated the calculated equilibrium potential for potassium (E_K = −78 mV).

DA-activated K^+ current measured in voltage clamp. The DA-activated conductance was further investigated by directly determining the DA-sensitive current under whole-cell voltage-clamp conditions. Current responses were elicited to ramp voltage commands from −120 to +20 mV before and during D_2 receptor activation (e.g., Fig. 3*C*). The resulting control responses were subtracted from the agonist-induced responses to yield a current–voltage relationship for the DA-induced conductance. The DA-induced current exhibited a reversal potential coincident with E_K (−76.7 mV) and dependent on external K^+ concentration (Fig. 4*B*). The current-voltage curve shifted to more depolarized potentials and reversed at the new predicted value for E_K (−15 mV) when the external K^+ concentration was increased from 5 to 50 mM (n = 6). The current–voltage relationship for the DA response was relatively linear (from −120 to +20 mV) when experiments were conducted in 5 mM external K^+ but began to exhibit a slight inward rectification when external [K^+] was increased to 50 mM. Furthermore, the DA-activated K^+ current was not calcium dependent, as normal responses could be elicited when both extracellular and intracellular [Ca^{2+}] were buffered with EGTA to less than 100 nM (n = 8; e.g., Fig. 4*B*).

Pharmacology of the DA-induced K^+ conductance. The sensitivity of the DA-induced K^+ current to the known K^+ channel blockers tetraethylammonium (TEA), 4-aminopyridine (4-AP), and quinine was investigated in order to characterize further the K^+ current underlying the DA response (Fig. 5). The DA-activated current was assessed in the absence or the presence of each blocker that was administered by inclusion in the external bathing solution several minutes prior to challenge by DA (100 nM). The DA-induced current was not blocked by TEA (10 mM; n = 7; Fig. 5*A*). 4-AP had no effect at 1 mM (n = 4), but at a high dose of 5 mM (n = 6) partially suppressed both the inward (44% decrease) and outward (26% decrease) DA-induced current (Fig. 5*B*). Quinine (100 μM; n = 6) completely blocked the

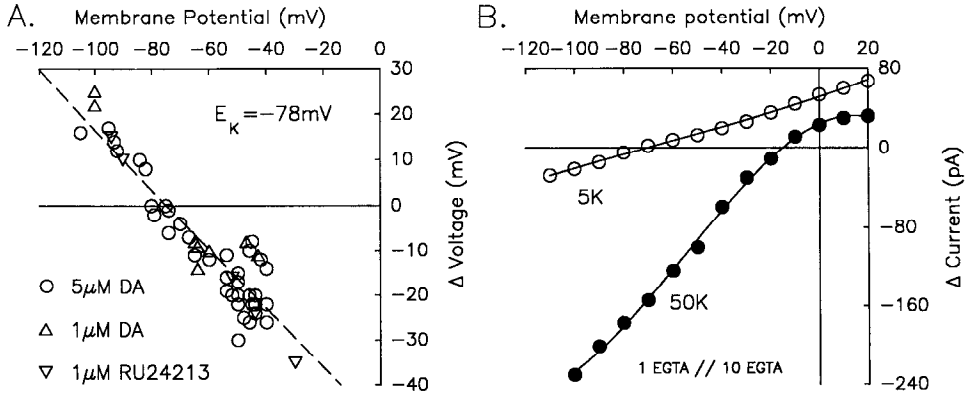

Figure 4. The DA-induced membrane potential response reverses at the potassium equilibrium potential. *A,* Changes in membrane potential induced by DA or RU24213 at the indicated concentrations were plotted as a function of membrane potential in the absence of agonist. The steady membrane potential prior to agonist application was maintained by current injection. The *broken line* is a linear regression fit of the data yielding a voltage intercept of −77.6 mV, in good agreement with a predicted equilibrium potential for potassium of −78 mV. *B,* Current–voltage relations of the DA-induced current in one lactotroph under whole-cell voltage clamp. Membrane voltage was commanded by ramps from −120 to +20 mV, and the current responses to three ramps were averaged. Average responses prior to agonist application were subtracted from those obtained at the peak DA (100 nM) response to yield the DA-sensitive current. Data were obtained in the same cell in the presence of either 5 mM (*open circles*) or 50 mM K^+ (*solid circles*) substituted for external sodium. *Smooth curves* through each data set represent third-order polynomial fits to the data points. Both external and internal solutions contained EGTA (1 and 10 mM, respectively) to reduce free Ca^{2+} to negligible levels.

inward current and partially blocked the outward current (55% decrease; Fig. 5*C*). The effects of quinine on the DA-induced current were partially reversible. Following removal of quinine and a washout period of several minutes, the DA-induced inward and outward currents recovered to 49% and 93% of their original magnitudes, respectively. Furthermore, the effects of quinine are apparently dose dependent since at a lower concentration (10 μM) only 15% of the inward current was inhibited and the outward current remained unchanged.

D_2 receptor-activated K^+ channels. The cell-attached patch-clamp technique was used to identify single DA-activated K^+ channels. KCl (150 mM) was used in the electrode to increase single-channel current amplitude at positive pipette potentials (negative patch membrane potentials). These potentials were employed, as typical voltage-gated channels have a lower probability of opening and thus would be less likely to interfere with the measurements. At positive pipette potentials, and in the absence of DA, there was relatively little channel activity. On only rare occasions were brief spontaneous channel openings observed that appeared of a similar conductance as channels observed in the presence of DA. In contrast, when DA (100 nM) was applied to the extracellular face of the patch, by inclusion in the pipette solution, frequent bursts of channel openings were observed in 80% of the cells recorded (n = 19; Fig. 6*A,B*). In general, a patch contained more than one channel and simultaneous openings were often observed. Like the macroscopic DA-induced K^+ current, DA-induced K^+ channels were coupled to D_2 receptors. In 85% of the cell-attached membrane patches examined, the D_2-selective agonist quinpirole (10 μM) activated a K^+ channel with a conductance essentially identical to those induced by DA (Fig. 6*B*). In contrast, when sulpiride (25 μM), a selective D_2 receptor antagonist, was included in the pipette solution together with quinpirole (10 μM), diminished channel activity was observed in only 20% of membrane patches (n = 9).

Initial characterization of the DA-activated K^+ channels. DA-activated channel openings were discriminated from continuous data records using a 50% amplitude threshold criterion. Amplitude histograms of channel opening events in a given patch were adequately fit by a Gaussian distribution, suggesting only a single-channel type. (An example is shown in Fig. 8*A* for one cell where the mean current amplitude at −40 mV pipette potential was 2.51 ± 0.321 pA as determined by the fit.) Similar fits for at least 3000 opening events at each of several membrane potentials were performed to yield current–voltage relationships of the D_2 agonist-activated K^+ channel. These *I/V* relationships were linear at negative patch potentials, yielding a predicted reversal potential of −10 to 0 mV (average resting potential − pipette potential) and a single-channel conductance of 40.6 ± 2.2 pS (Fig. 7*B*). When patches were held at positive potentials, larger-conductance channels (likely Ca^{2+}-activated K^+) were frequently observed that obscured the analysis of the DA-activated K^+ channel. For this reason, it was impossible to determine the voltage dependence of the channel at positive patch potentials. In those few patches in which large conductance channels were absent, D_2 receptor-coupled channels were not readily distinguished, suggesting that the channels may be strictly inwardly rectifying. This would be unexpected as outward whole-cell currents can be easily evoked by DA application. Our efforts to resolve this point have been unsuccessful thus far, but we continue attempts to clarify the rectification properties of this channel.

Open-dwell time histograms for DA-activated channel events were best fit by single exponential functions, indicating the presence of a single, relatively brief, open state with an average time constant of about 0.84 ± 0.08 msec. An example of such a fit is shown in Figure 7*C* for a patch held at a pipette potential of +40 mV. The mean channel open time was independent of voltage, as the time constant for a given patch did not vary significantly with a change in pipette potential (Fig. 8*A*). Since a patch usually contained more than one DA-activated K^+ channel, it was impossible to calculate accurately the open probability for a given channel. We instead estimated the percent open time (fractional time that channels were open/number of channels in patch) and found that a channel was open on average about 2.49 ± 0.37% of the time. This estimate should be close

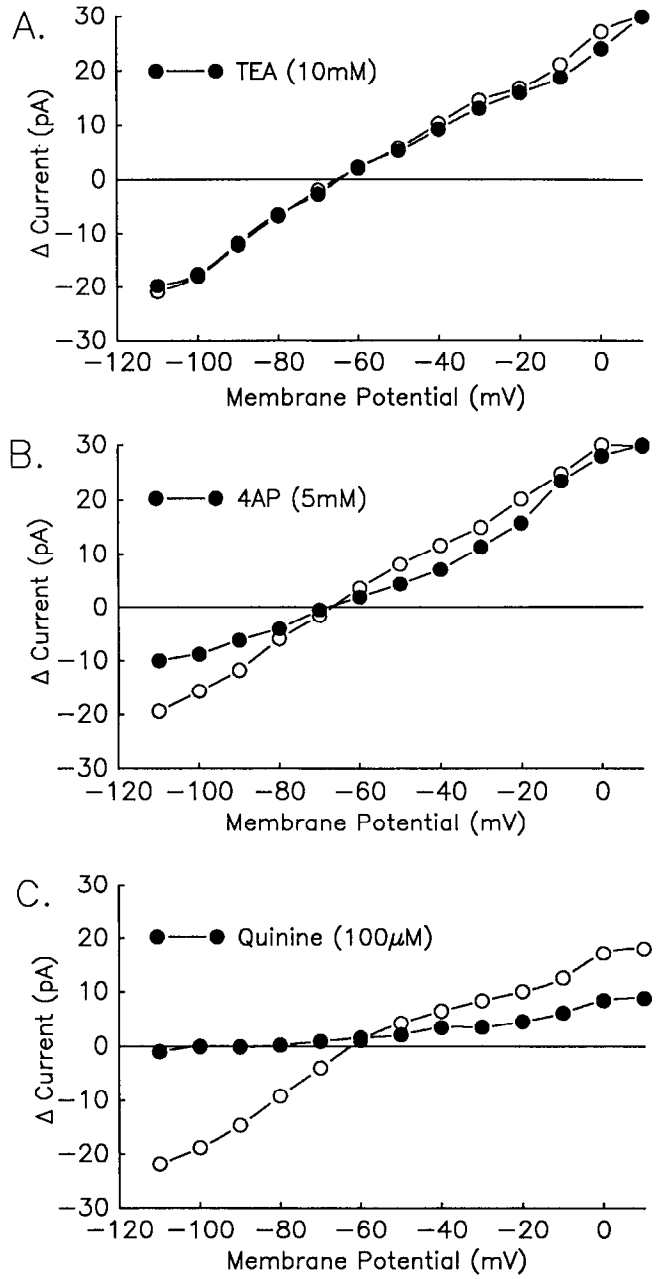

Figure 5. Sensitivity of the DA-activated K^+ current to several K^+ channel blockers. Current–voltage curves were obtained in three separate lactotrophs as described in Figure 4. Control DA (100 nM) responses (*open circles*) were compared to responses following the 3–5 min external application of 10 mM TEA-Cl (*A*), 5 mM 4-AP (*B*) or 100 μM quinine-HCl (*C*). Note the insensitivity of the DA-activated K^+ current to TEA, partial blockade by 4-AP, and subtantial inhibition (complete blockade of inward current) by quinine. The apparent shift in the reversal potential by quinine in *C* was not a consistent finding and reflects the errors inherent in the small currents (<2 pA) involved in these measurements.

to the open probability, as the occurrences of multiple simultaneous openings was low. Furthermore, the percent open time was independent of voltage between + 30 and +80 mV (pipette potential) (Fig. 8*B*). Closed-dwell time histograms were best fit by multiexponential functions indicating the presence of two or three closed states with time constants, for example in one patch, of 3.67 and 20.43 msec (Fig. 7*D*). Due to the variability and complexity of the closed state distributions, it was not prudent to attempt to define the voltage dependence of the mean closed times from our data. It was, nonetheless, clear from qualitative examination of the data that the time constant values were relatively independent of pipette potential. Furthermore, one can generally infer the absence of significant voltage dependence for closed state distributions if both the open times and open probabilities are likewise voltage independent.

Pharmacology of the DA-activated K^+ channels. The sensitivities of single DA-activated channels to the K^+ channel blockers TEA, 4-AP, and quinine were investigated to help establish a causal relationship between the channel activity and the whole-cell DA response. In cell-attached patches in which TEA (10 mM; $n = 5$) was included in the pipette solution, mean channel open time and percent open time were not significantly different from control patches ($n = 6$; Fig. 8). When the pipette contained 4-AP (5 mM; $n = 5$), both the mean open time and percent open time were only slightly decreased to 0.65 ± 0.08 msec and 1.92 ± 0.76%, respectively, as compared to control values of 0.84 msec and 2.5%. In contrast, quinine (100 μM; $n = 5$) almost completely blocked channel activity reducing the percent open time to approximately 0.01%. In fact, due to the low level of channel activity it was impossible to accumulate an open time histogram with enough events to determine if mean open time was also decreased.

Discussion

The present studies were conducted in order to characterize the actions of DA on K^+ channels in RHPA-identified rat lactotrophs at both the whole-cell and single-channel level. At concentrations that inhibit PRL secretion *in vivo,* DA altered the electrical activity of lactotrophs by eliciting a rapid, sustained hyperpolarization of the membrane potential that was accompanied by a cessation of calcium-dependent spiking activity in spontaneously active cells. The receptor mediating the response to DA was shown to be pharmacologically classified as a D_2 receptor as assessed by the mimicry and blockade of DA effects by selective D_2 receptor agonists and antagonists, respectively. An increased potassium conductance underlies the hyperpolarization as evidenced by (1) a decrease in membrane resistance that accompanied the hyperpolarization, (2) a reversal potential for the DA effects on both current and voltage that was coincident with the calculated E_K, and (3) a dependence of the DA-induced current on extracellular $[K^+]$.

DA-induced membrane hyperpolarizations likely mediated via K^+ have been previously reported using electrophysiological techniques in human PRL-secreting adenoma cells, as well as bovine and rat pituitary cell populations enriched for PRL-secreting cells (Israel et al., 1985, 1987; Ingram et al., 1986; Lledo et al., 1990a). Other groups have reported DA-induced increases in K^+ conductance based on ion flux studies using ^{86}Rb as a probe of K^+ permeability (Memo et al., 1987; Castelletti et al., 1989). By employing voltage-clamp techniques to assess and characterize directly the DA-activated K^+ current, our findings confirm and extend several of these previous observations. Castelletti et al. (1989) described two types of DA-activated ^{86}Rb fluxes: one was voltage sensitive but calcium independent, whereas the other was calcium dependent. Lledo et al. (1990a) have recently reported that in RHPA-identified lactotrophs DA increased two voltage-dependent outwardly rectifying K^+ currents, I_K (delayed) and I_A (transient), which are present in the

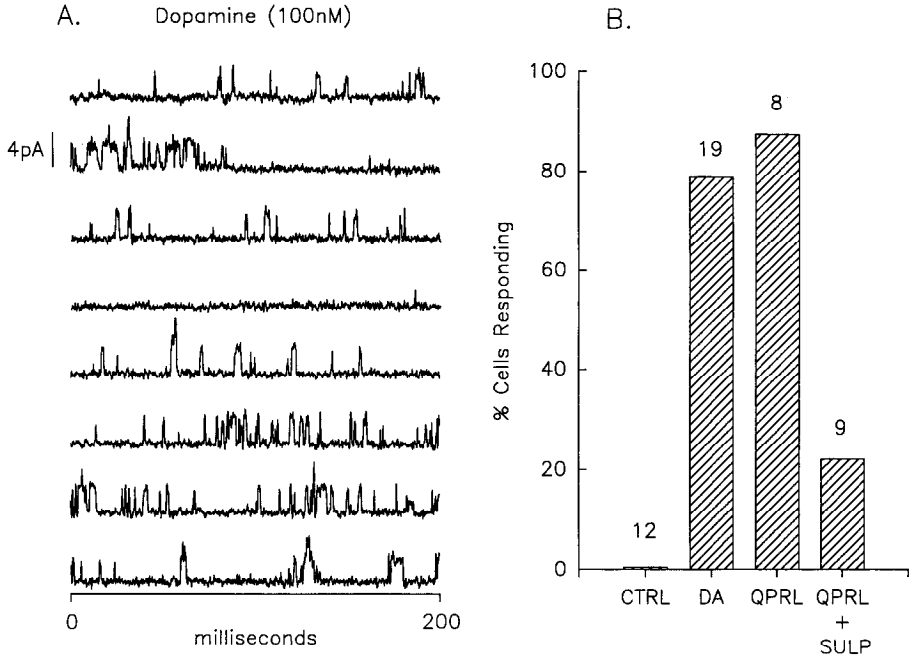

Figure 6. D_2 receptor-activated single K^+ channels can be recorded in cell-attached membrane patches. *A,* Representative records of channel activity in a patch exposed to 100 nM DA plus 10 μM ascorbate (pipette potential, +50 mV). The traces shown are consecutive records from a much longer experimental epoch. Upward deflections represent inward current. Currents were filtered at 1 kHz and sampled at 5 kHz. Pipette solutions contained (in mM) 150 KCl, 3.1 $MgCl_2$, and 15.5 HEPES. *B,* Summary of D_2 receptor pharmacology for cell-attached single-channel recordings. Data represent percentage of patches in which any channel openings were observed for 2 min (minimum) recordings. The number of cells examined for each condition in the patch electrode is indicated *above* the *bars.* Control (*CTRL*), either no drug, 10 μM ascorbate, or 1% dimethyl sulfoxide; *DA,* 100 nM DA + 10 μM ascorbate; *QPRL,* 10 μM quinpirole; *QPRL + SULP,* 10 μM quinpirole + 25 μM sulpiride.

absence of DA. In contrast, the current–voltage relationship for the DA-activated K^+ current we observed in whole cells was only slightly dependent on voltage (slight inward rectification) and persisted in the absence of Ca^{2+}. More importantly, perhaps, is the fact that the conductance we describe does not require membrane depolarization and thus can be activated at or near the normal resting potential of the cell to impact on its physiology. A hypothesis thus supported by our results is that a major action of DA in the lactotroph is to activate a K^+ conductance that is not normally manifest in the absence of DA. While the data reported here do not, however, exclude the possibility that DA has additional effects on other types of K^+ channels, our preliminary attempts to detect changes in voltage-gated K^+ currents have thus far yielded only negative results.

The DA-activated current can be characterized by its sensitivity to several known K^+ channel blockers. We have found it to be insensitive to TEA (10 mM) and only mildly inhibited by 4-AP (5 mM). These findings are consistent with those reported

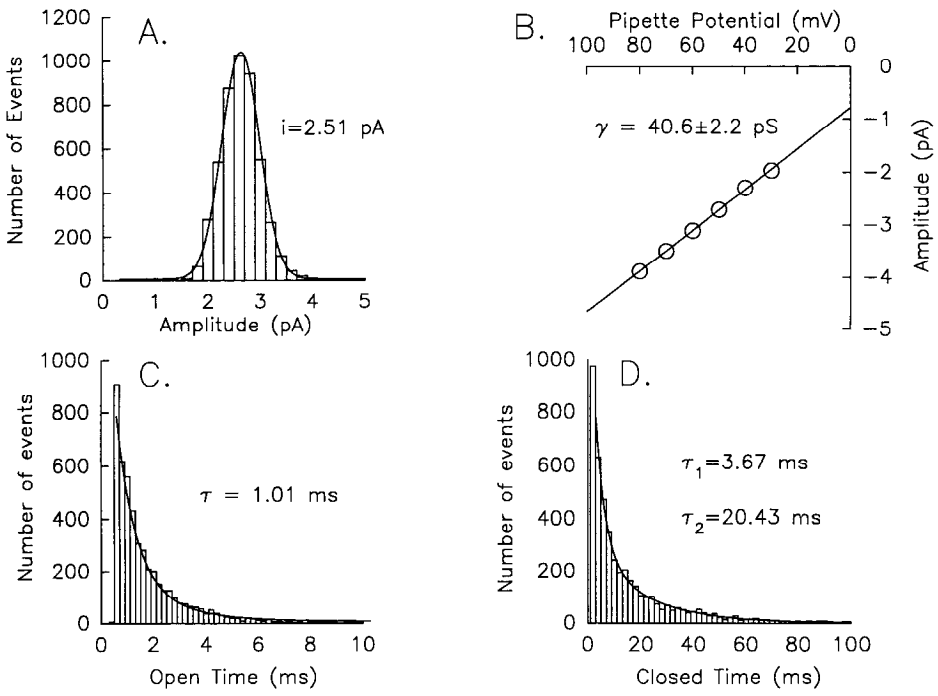

Figure 7. Characteristics of single DA-activated channels in cell-attached patches. Experiments were performed as described in Figure 6. *A,* Amplitude histogram of channel opening events (50% amplitude criterion) in one patch recorded in the presence of 100 nM DA at a pipette potential of 40 mV. A Gaussian function was fit to the event distribution (0.2 pA bin width) as indicated by the *smooth curve* through the data, yielding a unitary current of 2.51 pA. *B,* Current–voltage relationship for DA-stimulated channels. Each data point represents the mean current amplitude determined by histogram fits performed in *A* from six patches exposed to 100 nM DA. SE are within the symbol dimensions. A linear fit of the data yields a single-channel conductance of 40.6 pS. *C,* Open dwell time histogram of idealized channel openings in one patch at a pipette potential of 40 mV. The data were adequately fit by a single exponential function (*solid line*) yielding a time constant of 1.01 msec. Bins less than 400 μsec were excluded from the fit. *D,* Closed dwell time histogram of idealized channel closings in one patch at a pipette potential of 40 mV. The data were best fit to a multiexponential decay curve (*solid line*) with two time constants as indicated.

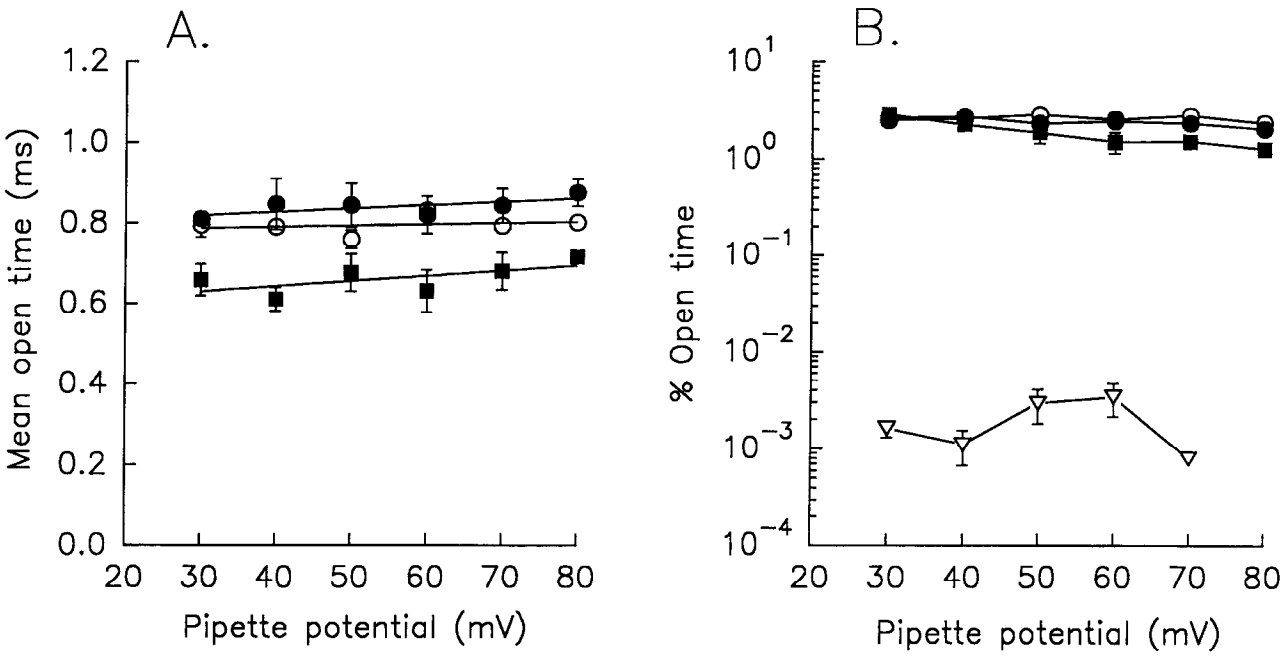

Figure 8. Voltage-dependence of gating kinetics and channel pharmacology. Sensitivity of mean channel open time and percent open time for the DA-activated K^+ channels to K^+ channel blockers was determined. Control values (*solid circles*) were compared to values obtained when the pipette solution also contained 10 mM TEA (*open circles*), 5 mM 4-AP (*solid squares*), or 100 μM quinine (*B, open triangles*). Each data point is the mean value (±SEM) from three to five cells. *A,* Mean channel open time was determined from open time histograms as described in Figure 7*C* and plotted as a function of pipette potential. Note the relative lack of voltage dependence, insensitivity to TEA, and slight reduction by 4-AP. *Straight lines* are linear regression fits to each set of data points. *B,* Percent open time was determined from the fractional amount of time channels spent in the open state during a 1–2 min recording divided by the number of channels in a patch. Percent open time was plotted on a logarithmic scale as a function of pipette potential in order to visualize the inhibition by quinine clearly. Note the weak voltage dependence, insensitivity to TEA, slight sensitivity to 4-AP, and dramatic reduction of channel activity by quinine.

by Israel et al. (1987), in which changes in membrane potential induced by DA were also unaffected by cobalt (a Ca^{2+} channel blocker) or TEA, while 4-AP induced a weak modification of the response. We observed that while the inward DA-induced current was almost completely blocked by extracellular quinine (100 μM), the outward DA-induced K^+ current was only partially inhibited. The increased sensitivity of inward currents to quinine block may be accounted for by the zwitterionic properties of the molecule (pK values of the amino groups of 5.1 and 9.7; Grygorczyk and Schwarz, 1985) assuming that positively charged quinine is the effective form. At pH 7.4, quinine is predominantly in the charged form and would presumably be driven into the channel by hyperpolarization to block inward K^+ current. The smaller fraction of neutral quinine molecules would permeate the cell membrane and become protonated and trapped in the cell interior to block outward K^+ current at a lower effective dose. Further experiments beyond the present scope are required, however, at various pH values and patch configurations to evaluate this hypothesis.

Previous attempts to characterize the population of K^+ channels underlying the whole-cell DA response have been based solely on the sensitivity of membrane potential changes to known K^+ channel blockers. In the present study, we have advanced this point using cell-attached patch recordings to identify and describe single K^+ channels associated with D_2 receptor stimulation. To our knowledge, this is the first characterization of single DA-activated K^+ channels in an endocrine cell. When DA or a selective D_2 receptor agonist was included in the pipette solution (150 mM KCl), we observed inward single-channel currents with a unitary conductance of about 40 pS. The single channels appear to be K^+ selective, as the projected reversal potential for the response approximated the expected value for E_K (depending on resting membrane potential), and channel activity was blocked by quinine, a known K^+ channel blocker. The present results, however, do not directly examine ion selectivity, and thus the exact degree to which these channels are very slightly permeable to other cations is not precisely known. Analysis of single-channel gating kinetics suggests that the channel exhibits a single, very brief, open state and two or three closed states of several milliseconds duration. The dramatic decrease in channel open probability in the presence of quinine appears to reflect a great increase in closed times, but any change in the open channel lifetime was not possible to document owing to insufficient opening events.

While it is not yet possible to provide *direct* experimental evidence linking the DA-induced whole-cell responses and the observed DA-activated single K^+ channel events, several of our observations support this role for the channel. Both whole-cell current (and voltage) responses and single channels (1) reflect an increased K^+ conductance; (2) are coupled to D_2 receptor stimulation; (3) exhibit identical channel pharmacology by being insensitive to TEA, partially inhibited by 4-AP, and more potently blocked by quinine; and (4) occur with comparably high frequency and are robust. DA elicited a hyperpolarization of membrane potential in about 85% of identified lactotrophs, while DA-activated K^+ channels were observed in 80–85% of cell-attached membrane patches (with usually at least two channels per patch). We hypothesize that while a single DA-activated K^+

channel is open for only a small percentage of the time, its activity summates with that of many similarly coupled channels to yield a robust whole-cell response. The number of simultaneously active channels required to produce a 10–20 mV hyperpolarization would not, however, be great considering the extremely high input impedance of the cells (≥5 GΩ). Assuming a mean open time of 1 msec, a unitary current of 2 pA near the resting potential (−40 mV), and a membrane time constant of 100 msec, one can roughly calculate that only 20 active channels could hyperpolarize a cell by 15–20 mV.

There is one feature of our results, however, that indicates the channel we observed may not solely underlie the DA-induced hyperpolarization. We found that while DA-activated K^+ channel activity was evident at negative patch potentials, openings were not observed at positive patch potentials. One interpretation of these results would be that the channel inwardly rectifies. In contrast, the DA-activated whole-cell current showed no significant rectification from −120 to +20 mV when experiments were conducted in 5 mM external K^+. Several possibilities exist that may help to resolve the apparent discrepancy. First, the inward rectification of the DA-activated K^+ current may have been precluded by the recording conditions. While it was possible to record at positive pipette potentials (+30 mV or greater) where the driving force was large enough to identify clearly single channel events for a 40 pS conductance channel, recording at equivalently negative pipette potentials (−110 mV or greater) was often difficult due to frequent breakdown of the gigaohm seal. Alternatively, the apparent linearity of the current–voltage relationship for the DA-activated whole-cell current may also be a function of the recording conditions. We began to observe some inward rectification of the DA-activated K^+ current when the external K^+ concentration was increased from 5 to 50 mM. It is possible that with larger increases in the concentration of K^+ in the external bathing solution (analogous to the pipette solution), inward rectification would become more pronounced. Ultimately, this disparity may be resolved by recording outward single DA-activated K^+ channels in inside-out patches where both intracellular and extracellular solutions can be manipulated.

While previous studies have established that anterior pituitary and CNS D_2 receptors are pharmacologically similar, it might also be speculated that the physiological actions of DA at D_2 receptors are based on their coupling to similar effector mechanisms in cell types expressing the receptors. In support of this hypothesis, several groups have reported that stimulation of D_2 receptors in either striatum or anterior pituitary inhibits adenylyl cyclase activity and phosphoinositide metabolism (Giannattasio et al., 1981; Cooper et al., 1985; Vallar and Meldolesi, 1989). Electrophysiologically, D_2 receptors in the CNS and anterior pituitary have now been demonstrated to trigger inhibitory responses mediated through coupling to K^+ channels. Increases in K^+ conductance have been reported to underlie the inhibitory response to stimulation of somatodendritic autoreceptors on cell bodies of substantia nigra neurons (Lacey et al., 1987, 1988), autoreceptors on nerve terminals in the striatum (Cass and Zahniser, 1990), and postsynaptic receptors in the striatum (Freedman and Weight, 1988, 1989). In the latter case, direct support for a D_2-mediated increase in K^+ conductance was also based on an examination of single K^+ channel events. The regulation of K^+ channels by D_2 receptors in the anterior pituitary described in this article confirms the previous reports in CNS neurons and raises the possibility that an identical K^+ channel mediates both CNS and pituitary inhibitory responses. The D_2 receptor-activated K^+ channel we describe in lactotrophs, however, differs from that reported by Freedman and Weight (1988) in the striatum in a number of ways. The striatal K^+ channel was reported to exhibit a unitary conductance of 85 pS and a linear current–voltage relationship and spent a large percentage of the time in the open state in the presence of agonist. In contrast, under similar recording conditions and comparable agonist concentrations the channel in lactotrophs exhibits a lower unitary conductance (40 pS), a current–voltage relationship that appears to inwardly rectify, and a much lower open state probability. Both the striatal and pituitary DA-activated K^+ channels were completely blocked by quinine. The striatal K^+ channel, however, is reportedly more sensitive (~1000-fold) to quinine than the lactotroph K^+ channel (Freedman and Weight, 1989). Interestingly, in a recent report, quinine was also demonstrated to block the inhibition of DA release mediated by D_2 autoreceptors located on terminals of nigrostriatal neurons (Cass and Zahniser, 1990), suggesting that K^+ channels coupled to D_2 receptors are integral to the functional consequences of DA autoreceptor activation.

Agonist-activated K^+-selective channels have also been observed in clonal rat pituitary cells (Yatani et al., 1987). In these cells, somatostatin and muscarinic receptors activate a 55 pS channel that appears very similar to the channel we described as evidenced by brief channel open times and possible inward rectification. Unlike the lactotroph K^+ channel, however, TEA^+ completely blocks these channels. Taken together these observations seem to indicate that D_2 receptor-activated K^+ channels may share a similar pharmacological profile but can exhibit tissue-specific properties, such as conductance and gating kinetics, which may be a reflection of the plasma membrane in which they are located rather than channel primary structure. Several voltage-dependent K^+ channels have now been cloned via homologies to the *Drosophila Shaker* locus gene product, and it is now apparent that some of the diversity in properties of these channels may arise from heterologous subunit combination and multiple gene products (e.g., Stühmer et al., 1989). Although no voltage-independent K^+ channel has yet been cloned, similar molecular mechanisms might also be responsible for variations in conductance and gating properties of such channels in different cell types.

After many years of investigation, the mechanisms underlying the supression of prolactin secretion by DA remain unknown. While D_2 receptor stimulation is linked to inhibition of cAMP, the involvement of this event in the regulation of secretion is still a matter of dispute (Swennen and Denef, 1982; Ray et al., 1986). One hypothesis suggested from our electrophysiological observations is that the DA-induced K^+ current leads to a membrane hyperpolarization that dramatically decreases voltage-gated Ca^{2+} channel activity. A subsequent decline in intracellular Ca^{2+} concentration would in turn inhibit PRL secretion. Consistent with this hypothesis, measurements of intracellular calcium in single lactotrophs using fluorescent indicators have indicated significant decreases in response to DA (Malgaroli et al., 1987). The present observations that the DA-induced hyperpolarization halts calcium-dependent action potential activity are consistent with this hypothesis and suggest a critical role for K^+ channel activation in the modulation of PRL secretion from rat lactotrophs by DA. Our observations, however, do not exclude the possibility that other ion channels in lactotrophs may also be regulated by DA. In fact, there is some indirect

evidence from nonelectrophysiological investigations that lactotroph D_2 receptors may also couple to Ca^{2+} channels (Malgaroli et al., 1987; Enjalbert et al., 1988). In this regard, D_2 receptors have recently been reported to modulate Ca^{2+} conductances in bovine adrenal chromaffin cells (Bigornia et al., 1990), rat pars intermedia (Williams et al., 1990), and rat pituitary melanocytes (Cota and Hiriart, 1989). A recent investigation in rat lactotrophs also suggests a direct modulation of Ca^{2+} channels by DA (Lledo et al., 1990b). Further experiments will be needed to explore whether other ion channels are coupled to D_2 receptors in lactotrophs as well as the signal transduction mechanism that may link receptors to effector channels.

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