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Depletion of intracellular polyamines relieves inward rectification of potassium channels

(K^+ current/spermine/spermidine/putrescine/ornithine decarboxylase)

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ABSTRACT **Two different approaches were used to examine the *in vivo* role of polyamines in causing inward rectification of potassium channels. In two-microelectrode voltage-clamp experiments, 24-hr incubation of *Xenopus* oocytes injected with 50 nl of difluoromethylornithine (5 mM) and methylglyoxal bis(guanylhydrazone) (1 mM) caused an approximate doubling of expressed Kir2.1 currents and relieved rectification by causing an approximately +10-mV shift of the voltage at which currents are half-maximally inhibited. Second, a putrescine auxotrophic, ornithine decarboxylase-deficient Chinese hamster ovary (O-CHO) cell line was stably transfected with the cDNA encoding Kir2.3. Withdrawal of putrescine from the medium led to rapid (1-day) loss of the instantaneous phase of Kir2.3 channel activation, consistent with a decline of intracellular putrescine levels. Four days after putrescine withdrawal, macroscopic conductance, assessed using an $^{86}Rb^+$ flux assay, was approximately doubled, and this corresponded to a +30-mV shift of $V_{1/2}$ of rectification. With increasing time after putrescine withdrawal, there was an increase in the slowest phase of current activation, corresponding to an increase in the spermine-to-spermidine ratio over time. These results provide direct evidence for a role of each polyamine in induction of rectification, and they further demonstrate that *in vivo* modulation of rectification is possible by manipulation of polyamine levels using genetic and pharmacological approaches.**

Inward rectifying potassium channels are present in a wide variety of cell types. As their name implies, they conduct cations in the inward direction more easily than in the outward direction, and this property is critical to their role in shaping the electrical properties of a cell (1). In addition to a voltage-dependent block by intracellular Mg^{2+} (2, 3), a steep voltage-dependent block by intracellular polyamines (PAs) has recently been shown to cause the inward rectification of several strong inward rectifying potassium channels, including the cloned Kir2.1 (IRK1) and Kir2.3 (HRK1) channels (4–7). Since these initial reports, PAs have also been shown to cause rectification in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor channels (8–11). The naturally occurring PAs (putrescine, spermine, spermidine) are small positively charged molecules synthesized from decarboxylation of amino acids (see Fig. 1*A*). Intracellular PA levels are regulated through biosynthesis, degradation, uptake, and release. Intracellular PAs are essential for cell growth and have important roles in stabilizing DNA and RNA (12–14). Given the role of PAs in causing inward rectification, it is possible that physiological regulation of inward rectification, and hence cell excitability, might occur through alterations in PA levels.

Although ion channel block by PAs has been studied extensively using excised cell membrane patches, direct evidence demonstrating the *in vivo* role of PAs in channel rectification is still lacking. Here, we used two different approaches to obtain such evidence. First, pharmacological agents that are known to inhibit ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (SAMDC), enzymes involved in the synthesis of PAs, are shown to relieve inward rectification of Kir2.3 current expressed in *Xenopus* oocytes. Second, a mutant Chinese hamster ovary (O-CHO) cell line deficient in ODC (15, 16) was transfected with the cDNA encoding Kir2.3. Expressed Kir2.3 currents show altered inward rectification kinetics when putrescine (Put) is removed from the cultured medium. The results provide direct evidence for PA involvement in rectification *in vivo*, and furthermore demonstrate that rectification can be modulated *in vivo* by changes in cellular PA concentrations.

MATERIALS AND METHODS

Oocyte Expression of Kir2.3. Kir2.1 cDNA was propagated in the transcription-competent vector pBluescript SK(−) in *Escherichia coli* XL-1 blue. cRNA was transcribed *in vitro* using T7 RNA polymerase and capping from linearized cDNA. Stage V–VI *Xenopus* oocytes were isolated by partial ovariectomy while the animals were under tricaine anesthesia, and then defolliculated by treatment with 1 mg/ml collagenase (type 1A; Sigma) in 0 mM Ca^{2+} ND96 (below) for 1 hr. From 2 to 24 hr after defolliculation, oocytes were pressure injected with ≈50 nl of cRNA (1–100 ng/ml). Oocytes were maintained at room temperature in ND96 containing 2 mM Ca^{2+} (added as $CaCl_2$) and supplemented with penicillin (100 units/ml) and streptomycin (100 mg/ml) for 1–7 days prior to recording. Kir2.1 currents were measured at room temperature using a two-electrode voltage clamp (OC-725 Oocyte Clamp; Warner Instruments, Hamden, CT). Intracellular electrodes were filled with 3 M KCl and had initial tip resistances from 0.5 to 5 MΩ. Experiments were controlled by using pClamp software to control a Digidata 1200 D/A interface (Axon Instruments) running on a 80486-based microcomputer (486/33C, Gateway 2000). Data were digitized on-line and stored on hard disk, or digitized at 22 kHz and stored on videotape (Neuro-corder DR-890, Neuro-Data Instruments, New York) for later playback onto a chart recorder (Gould Instruments) or computer capture with Axotape software (Axon Instruments, Foster City, CA). Solutions used in these experiments were either ND96 (96 mM NaCl/2 mM KCl/1 mM $MgCl_2$/5 mM Na-Hepes, pH 7.5) or KD98 (98 mM KCl/1 mM $MgCl_2$/5 mM KHepes, pH 7.5), with additions as indicated.

Expression of Kir2.3 in O-CHO Cells. O-CHO cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, nonessential amino acids, penicillin (100 units/ml), streptomycin (100 mg/ml), and 0.1 mM Put in a 37°C incubator with an atomosphere of 5%

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Abbreviations: PA, polyamine; ODC, ornithine decarboxylase; SAMDC, *S*-adenosylmethionine decarboxylase; Put, putrescine; Spd, spermidine; Spm, spermine; DFMO, difluoromethylornithine; MGBG, methylglyoxal bis(guanylhydrazone).

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CO_2/95% air. Kir2.3 was subcloned in the mammalian expression vector pECE, and transfected into O-CHO cells with pRSVneo (17) at a ratio of 10:1 using Lipofectin (BRL) according to the supplier's instructions. Antibiotic-resistant clones were selected in 600 μg/ml G418 and analyzed for Kir2.3 channel as described below.

Rubidium-86 Flux. Cells were plated in medium supplemented with 0.1 mM Put on six-well culture plates 5 days before the experiment. To deplete intracellular PAs, Put-supplemented medium was replaced with medium without Put at various times. Cells were incubated with $^{86}Rb^+$ (chloride) at 0.1 mCi/ml (1 mCi = 37 MBq) for 24 hr before the efflux measurements. To measure the rate of $^{86}Rb^+$ efflux, cells were rinsed briefly twice with room-temperature Ringer's solution (103 mM NaCl/20 mM KCl/2.5 mM $CaCl_2$/1.2 mM KH_2PO_4/25 mM $NaHCO_3$/1.2 mM $MgSO_4$/10 mM Hepes, pH 7.4). $^{86}Rb^+$ released into the Ringer's solution was collected at 1, 2.5, 5, 15, 25, and 40 min. Cells were then lysed in 2% SDS and cell lysates assayed for $^{86}Rb^+$ remaining in the cells. The data are presented as percentage efflux, defined as the percentage of total cellular $^{86}Rb^+$ released.

Electrophysiology. O-CHO cells were voltage-clamped in the whole-cell patch-clamp configuration by using an Axopatch 1D patch clamp amplifier (Axon Instruments). Micropipettes were pulled from thin-walled glass (WPI, New Haven, CT) on a horizontal puller (Sutter Instruments, Novato, CA) and fire-polished, and the tips were coated with a 1:1 mixture of light mineral oil and Parafilm (American National Can, Greenwich, CT), to reduce capacitative currents. Electrode resistance was typically 0.5–1 MΩ when filled with K-INT solution (140 mM KCl/1 mM KEGTA/10 mM KHepes, pH 7.35). Experiments were performed at room temperature in a chamber mounted on the stage of an inverted microscope (Nikon Diaphot). CHO cells were continuously perfused with K-INT solution, and we did not find any effect of inclusion of Put in this solution on currents. Unless specifically stated, current recordings were made within 5–10 sec after attaining the whole-cell configuration, to avoid washing out of cytosolic components by intracellular perfusion with K-INT solution. Only cells expressing inward currents > 1 nA (at −80 mV) were selected for analysis, and hence endogenous currents (<50 pA at −80 mV) were negligible. pClamp software and a Labmaster TL125 D/A converter were used to generate voltage pulses and to collect data. Data were normally filtered at 5 kHz. Off-line analysis was performed using ClampFit and Microsoft Excel programs. Leak current and capacity transients were corrected off-line with a P/1 procedure (+50 mV or higher conditional prepulse). Currents were corrected for rundown wherever possible and necessary.

Data Analysis. *Estimation of contribution of "instantaneous" and "time-dependent" components of activation.* The time-dependent component of "activation" was fitted with two exponentials and extrapolated back to the beginning of a −50-mV test pulse to estimate the amplitude of the time-dependent part of "activation." The instantaneously activating current at −50 mV was then calculated by subtraction of the current at +50 mV (leak and pre-activated currents) from the extrapolated zero-time current. The relative contribution (R_{Inst}) of the instantaneous component to the total activated current was calculated as

$$R_{Inst} = \text{instantaneous}/(\text{instantaneous} + \text{time-dependent}). \quad [1]$$

Relative chord conductance (G_C). The degree of rectification of the channel was estimated as the relative chord conductance—i.e., the conductance relative to that expected for an unrectified current. Two assumptions were made to estimate the relative chord conductance (G_C): (*i*) Kir2.3 channels are fully unblocked at 80 mV negative to E_K, and (*ii*) the current–voltage (*I-V*) relationship of fully unblocked channels is linear in symmetrical potassium solutions. Both assumptions may be reasonable for the experimental conditions under consideration, although as discussed below, there may be a very shallow or voltage-independent component of PA block that is ignored by this analysis. G_C was calculated as the ratio between current and the current predicted assuming a linear unblocked current. G_C relationships were then fitted by the sum of two Boltzmann equations (Eq. **2**) with the sum of amplitudes A_1 and A_2 normalized to 1 ($A_1 + A_2 = 1$).

$$R(V_M) = A_1/[1 + \exp\{-\lambda_1\cdot(V_M - V^1_{1/2})\}] + A_2/[1 + \exp\{-\lambda_2\cdot(V_M - V^2_{1/2})\}], \quad [2]$$

where $\lambda_{1,2} = ZF/RT$, V_M is membrane potential, and $V^{1,2}_{1/2}$ is a parameter. Z stands for effective valency (steepness of rectification) of a blocking particle (PA or Mg^{2+}), and F, R, and T have their usual meanings. After the fitting procedure, the membrane potential at which currents were half blocked, $V_{1/2}$ (no upper index), was calculated.

RESULTS

Blocking PA Synthesis Relieves Kir2.1 Rectification in Intact *Xenopus* Oocytes. PAs are synthesized by ODC and SAMDC (Fig. 1*A*). ODC is specifically and irreversibly inhibited by DFMO. SAMDC is inhibited by MGBG, and both drugs have been widely used to study the functional roles of PAs (18). We used DFMO and MGBG to inhibit the synthesis of Put, spermidine (Spd), and spermine (Spm) in *Xenopus* oocytes, and we examined the electrophysiological effects of these drugs on outward current through Kir2.1 channels expressed at high density in these cells. Bath application of these agents did not alter Kir2.1 channel activity (not shown), and so DFMO and MGBG were applied by direct injection (50 nl of 5 mM DFMO and 1 mM MGBG per oocyte). Twenty-four hours later, Kir2.1 currents expressed in these cells were examined under voltage clamp. Fig. 1 shows original current records and the corresponding current–voltage relationship of control (water-injected) and DFMO + MGBG-treated cells. A significant increase in outward currents was observed in PA-depleted cells, corresponding to an increase of the voltage at which the conductance was half-maximally inhibited ($V_{1/2}$) of ≈+10 mV (Fig. 1*D*). Interestingly, treatment by DFMO and MGBG caused a significant increase in the amplitude of inward potassium currents (Fig. 1), suggesting that there may be a very shallowly voltage-independent component of PA block, such that at very negative membrane potentials, some block exists, as was observed in patch-clamp experiments (4). We cannot discount, however, that MGBG and DFMO treatment somehow led to increased expression of Kir2.1 channels, possibly as a result of effects on translation of the cRNA.

Increased Kir2.3 Channel Activity After Inhibition of PA Synthesis in O-CHO Cells. An ODC-deficient mutant CHO cell line (O-CHO) has been described previously by Steglich and Scheffler (15). These cells have only 3% of the ODC activity of normal CHO cells, and they require at least 10 μM Put in the medium to maintain a normal growth rate. To investigate the effects of PA depletion on channel rectification, without pharmacological intervention, we established stable clones of O-CHO cells expressing Kir2.3 channels. Three different clones were used for our experiments to avoid potential artifacts caused by any specific clone. Removal of Put from the medium has been shown to cause intracellular Put and Spd to be depleted within 24 and 48 hr (15), respectively, although Spm concentration remains relatively constant for up to 4 days. In Fig. 2*A* we have replotted the data from Steglich and Scheffler (15) to show the time course of total cellular PA levels after Put withdrawal. To assess the effects of PA depletion on inward rectification we have analyzed Kir2.3 currents by Rb^+ efflux, or whole-cell patch clamp, on succes-

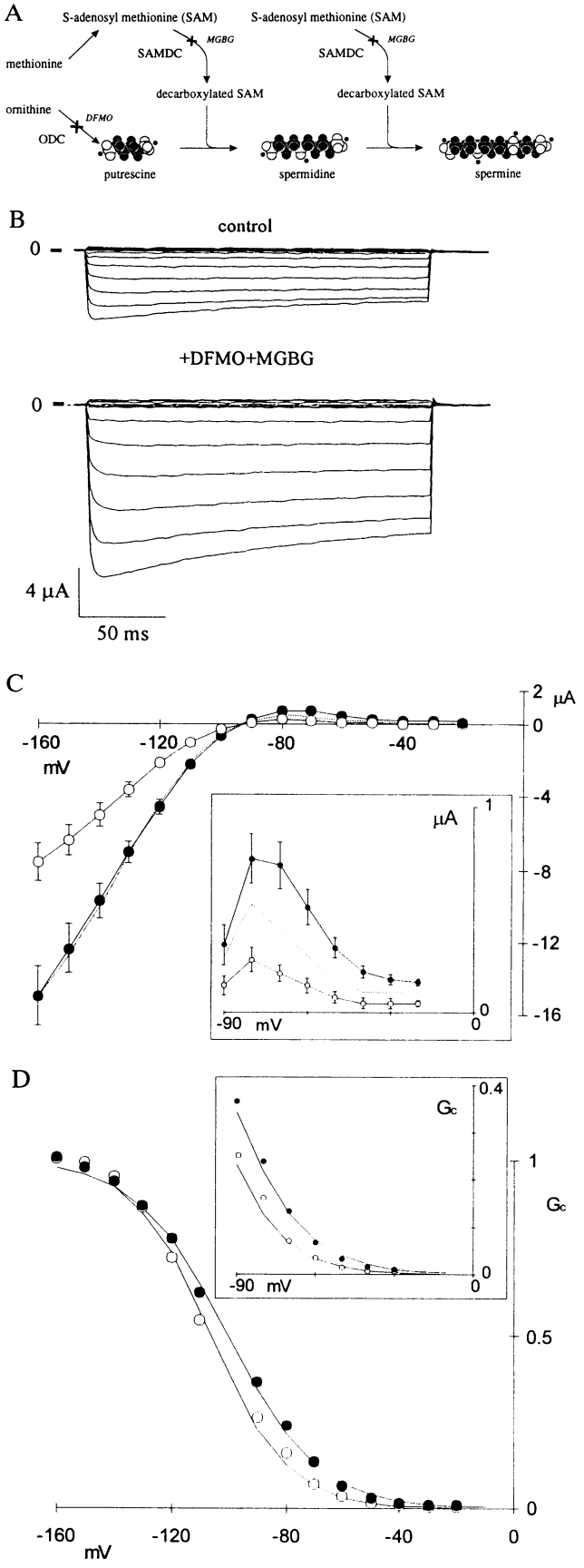

FIG. 1. PA metabolism and Kir2.1 channel activity. (*A*) Biosynthetic pathway of PAs in mammalian cells. The synthetic enzymes ODC and SAMDC are inhibited by difluoromethylornithine (DFMO) and methylglyoxal bis(guanylhydrazone) (MGBG), respectively. (*B*) Current records from *Xenopus* oocytes expressing Kir2.1 channels in response to voltage steps from −60 mV holding potential to voltages between −160 and 0 mV in 10-mV increments in ND96 (2 mM K^+) solution. (*C*) Current–voltage relationship for control (○) and DFMO + MGBG-treated (●) oocytes. Each data point is averaged from six oocytes. Standard errors are shown where larger than the symbol. The broken line corresponds to control data multiplied by 2.2. (*D*) G_C–voltage relationship for the averaged currents in *C*. The solid lines are fits of a single Boltzmann equation with $V_{1/2}$ = 107 mV and 100 mV, and Z = 1.86 and 1.60 for control and drug-treated oocytes, respectively. The *Insets* in *C* and *D* show data only for the voltage range −90 to −20 mV with expanded ordinates.

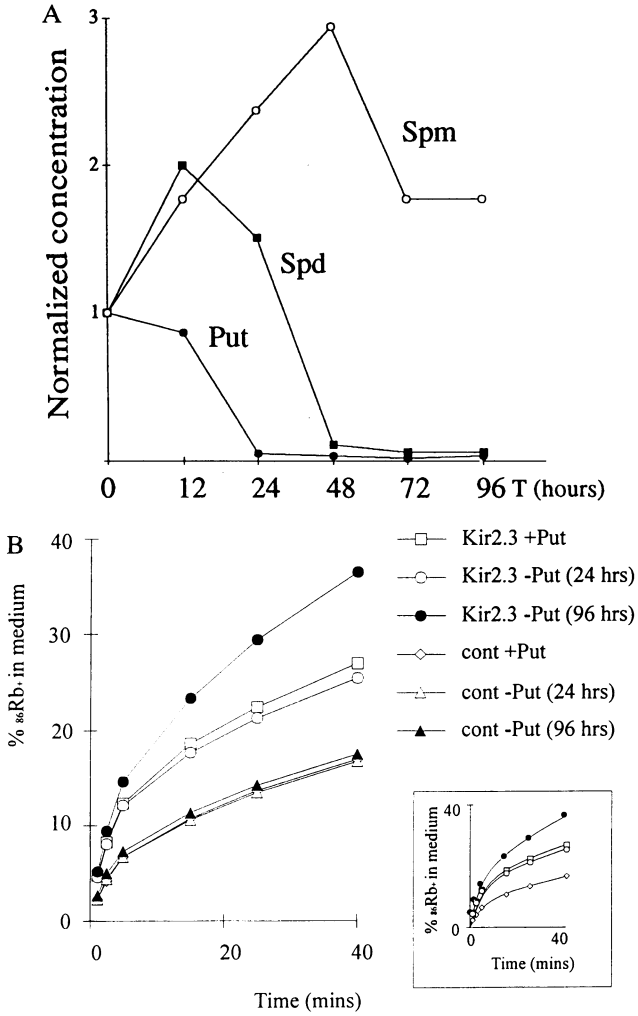

FIG. 2. Put withdrawal causes an increase in the activity of Kir2.3 channels expressed in O-CHO cells. (*A*) Total cellular PA levels in O-CHO cells at various times after the removal of Put from the culture medium, normalized to the level in cells grown in the presence of Put. The data are plotted from those provided in table 2 of Steglich and Scheffler (15). (*B*) Percentage $^{86}Rb^+$ released into the medium as a function of time in control cells (cont) and in cells expressing Kir2.3 channels (Kir2.3). Put was removed from the culture medium at 0 days (+Put), 1 day [−Put (24 hr)], or 4 days [−Put (96 hr)] before the assay. Each data point represents the average of three measurements. Standard deviations are all smaller than the symbol size. (*Inset*) The Rb^+ efflux was modeled on the assumption that it occurs as simple diffusion through an endogenous background conductance pathway (G_{bg}) and through Kir2.3 channels (G_K). An additional decaying exponential term was added to account for the rapid initial efflux that is always present, and which is assumed to represent incomplete washout of Rb^+ from the medium at the beginning of the assay.

sive days after Put withdrawal. Rb^+ is a weakly permeant analog of K^+ in inward rectifier channels (19, 20) and hence

should permeate Kir2.3 channels. Fig. 2*B* compares the rates of Rb^+ efflux from untransfected cells and cells expressing Kir2.3 channels. Put was removed from the culture medium at various times during a 5-day growth period. In untransfected cells, ≈15% of accumulated Rb^+ left the cells in 40 min, representing the flux through endogenous channels. This efflux was unaffected by Put withdrawal (Fig. 2*B*). In cells expressing Kir2.3 channels, the control efflux was increased, representing an estimated increase in Rb^+ conductance of 2.2-fold over untransfected cells (as estimated using the model described in Fig. 2B). While Put withdrawal had no effect on the Rb^+ efflux from untransfected cells, 4 days after withdrawal, the efflux from Kir2.3-expressing cells was considerably increased, representing an increase of the Kir2.3 conductance (G_K) of 2.5-fold. Interestingly, there was a small but reproducible decrease in efflux (representing a 15% decrease in G_K) from Kir2.3-expressing cells after 1 day of Put withdrawal (see *Discussion*).

Kir2.3 Channel Rectification Kinetics Can Be Directly Modulated by Intracellular PAs. We next examined the kinetic and voltage-dependent properties of Kir2.3 channels in O-CHO cells under whole-cell voltage clamp. During internal perfusion with Mg^{2+}-free solutions (+1 mM EGTA) the amplitude of inward Kir2.3 currents consistently increased over the first 2 or 3 minutes, before declining (Fig. 3*A Upper*, $n = 5$). Inclusion of 10 mM $MgCl_2$ in the pipette solution caused extremely fast channel rundown (Fig. 3*A Lower*, $n = 2$), precluding a detailed analysis of the effects of Mg^{2+} perfusion on rectification.

Fig. 3*B* shows currents in response to hyperpolarizing voltage steps from a cell perfused with 10 mM EDTA, immediately after attaining the whole-cell configuration. Inward Kir2.3 currents activate with instantaneous and time-dependent components. In cell-attached patches from *Xenopus* oocytes expressing Kir2.3 channels, the time-dependent activation of inward current can also be resolved into instantaneous and time-dependent phases (7). The instantaneous phase matches instantaneous unblocking of the channel resulting from exit of Mg^{2+} or Put, and the slower phases quantitatively match the time-dependent unblocking resulting from exit of Spd and Spm in inside-out patches (7). Fig. 3 *B* and *C* provides direct evidence that this correlation is indeed causal, and it further suggests that the instantaneous phase of unblocking may actually be predominantly due to exit of Put. The relative amplitude of the instantaneous component of rectification seemed to be insensitive to the presence of Mg^{2+}-chelating EDTA in the pipette. In control cells, even after more than 5 min of intracellular perfusion with 10 mM EDTA, there is clearly still an instantaneous component of channel activation and a time-dependent component (Fig. 3*C*), although the time-dependent component is slowed (see below). Further evidence that Put is likely to be primarily responsible for the instantaneous part of "activation" is provided in Fig. 3 *D* and *E*. Even short (≈2-min) intracellular perfusion with 10 mM Put (and 10 mM EDTA) leads to considerable increase of the amplitude of the "instantaneous" component (Fig. 3*D*), and the steady-state G_C–voltage relationship (Fig. 3*E*) becomes shallower.

Rectification and Kinetics of Kir2.3 Channels Can Be Modulated by Altering PA Biosynthesis. The time-dependent depletion of intracellular PAs after Put withdrawal (Fig. 2*B*) strongly affects inward rectification of Kir2.3 channels expressed in O-CHO cells. Fig. 4*A* illustrates the characteristic changes in "activation" kinetics that result from Put withdrawal. Within 1 day of Put withdrawal, the instantaneous component of activation is greatly reduced (Fig. 5*A*), and by the second day, the "fast" time-dependent component is also absent and only a "slow" component is observed (Fig. 4 *A*

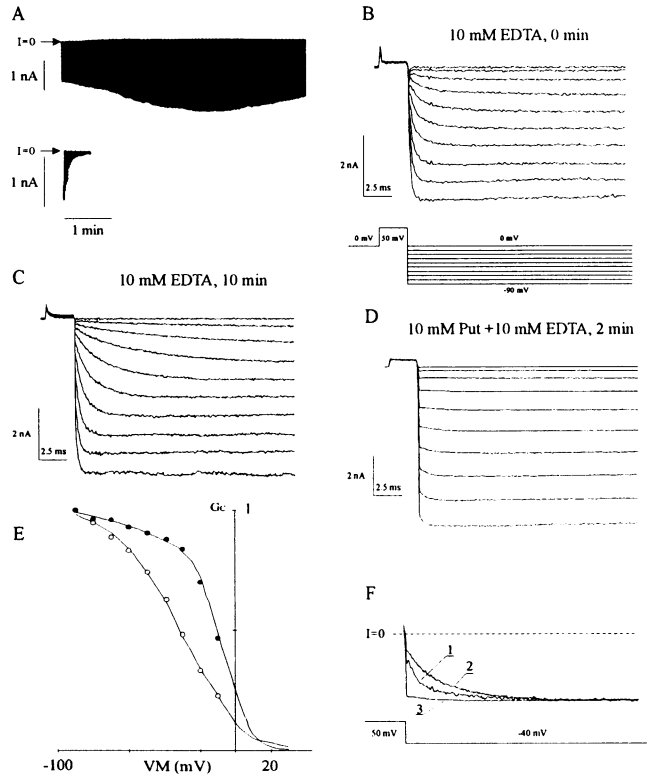

FIG. 3. Direct manipulation of PA levels causes changes of inward rectification. (*A*) Slow time-base recording of membrane currents in response to voltage steps from a holding potential of 0 mV to 50 mV and then to −90 mV in O-CHO cells (day 0) with pipette solutions containing 10 mM EDTA (*Upper*) or 10 mM Mg^{2+} (*Lower*). (*B*) Family of current traces recorded immediately (<20 sec) after establishing whole-cell configuration in response to the voltage protocol shown beneath. The pipette solution contained KINT, 1 mM EGTA, and 10 mM EDTA. (*C* and *D*) Current traces like those shown in *B* were recorded after a long period (about 10 min in *B* and 2 min in *D*) of intracellular perfusion with 10 mM EDTA (*C*) and 10 mM EDTA + 10 mM Put (*D*). (*E*) Steady-state G_C–voltage relationships from *B* (●) and *D* (○). The solid curves are fitted as the sum of two Boltzmann functions (see text). (*F*) Currents in response to −40-mV voltage step were selected from *B* (trace 1), *C* (trace 2), and *D* (trace 3), normalized to the same amplitude and superimposed.

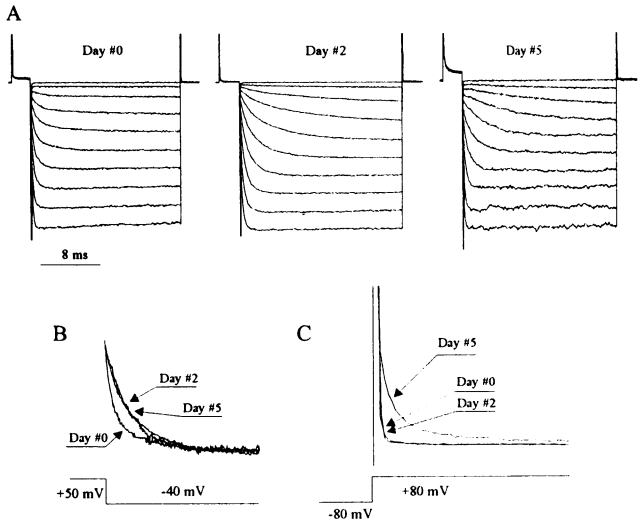

FIG. 4. Put withdrawal causes changes in Kir2.3 current kinetics. (*A*) Currents in response to voltage steps from a holding potential of 0 mV to +50 mV and then to voltages bewteen 0 and −90 mV at day 0 (control), day 2, and day 5 after Put withdrawal. (*B*) Time-dependent components of currents at −40 mV are scaled to the same amplitude and superimposed. (*C*) Currents at +80 mV, following an "activating" voltage step to −80 mV.

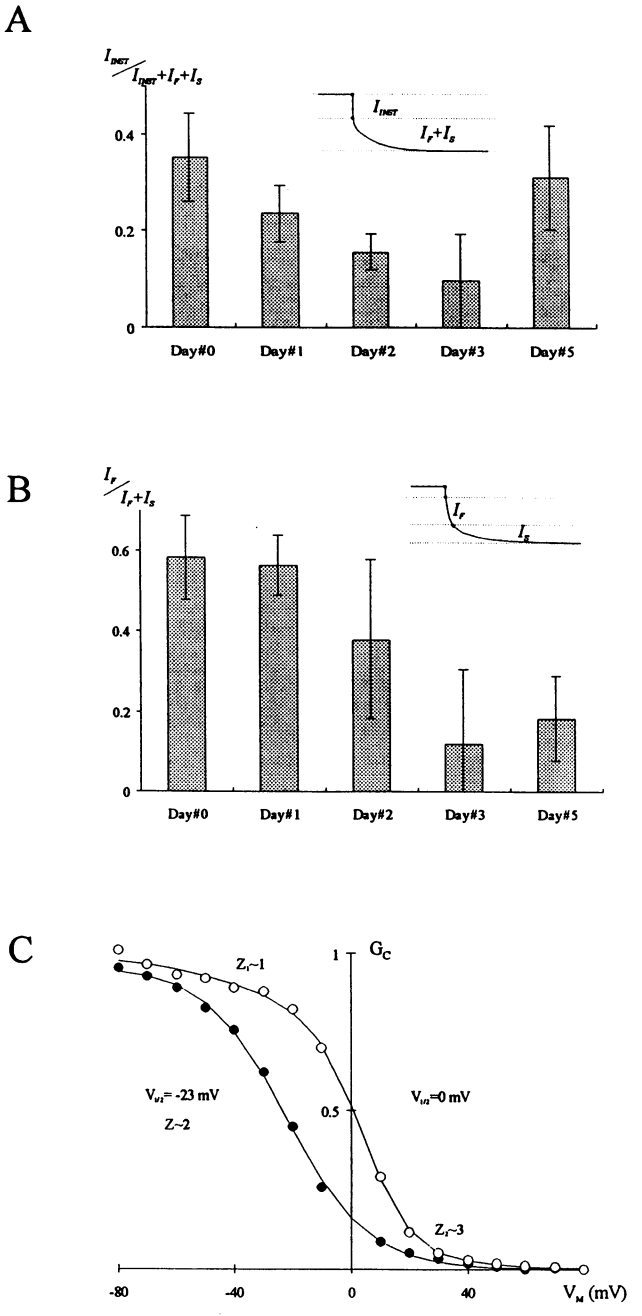

FIG. 5. Depletion of PAs relieves and slows down rectification. (*A*) The relative amplitude of the instantaneous component [$I_{INST}/(I_{INST} + I_F + I_S)$] is plotted as a function of time after Put withdrawal. (*B*) The contribution of the fast component of activation [$I_F/(I_F + I_S)$] is plotted as a function of time after Put withdrawal. (*C*) Representative G_C–voltage relationships at day 0 (●) and day 5 (○) after Put withdrawal. Each bar in *A* and *B* corresponds to three to five experiments. G_C–voltage relationships in *C* were fitted with single (●) and double (○) Boltzmann functions on day 0 and day 5, respectively.

Center and *B*). On the basis of results obtained in excised patch experiments (7), the changes in *kinetics* over time (i.e., a shift to only slow activation) can be explained as a reflection of the changes in Put:Spd:Spm *ratios* (i.e., Put and Spd disappear, Spm levels are maintained), since Spm causes slow activation, whereas activation of channels blocked by Put is instantaneous, and that of channels blocked by Spd is fast (7).

Five days after Put withdrawal, significant changes in the steady-state currents are observed (Fig. 4). In control (in the presence of Put in the extracellular medium) rectification is so strong that virtually no outward currents can be measured at positive (>+50 mV) membrane potentials, but 5 days after Put withdrawal, fast inactivating outward currents can be detected even at +80 mV (Fig. 4*C*). These results are consistent with reduction of *absolute* PA concentrations. We have previously shown that the steepness of rectification (effective valency Z) depends on the valency of the blocking particle ($Z_{Mg^{2+}} = Z_{Put} = 2$, $Z_{Spd} = 3$, $Z_{Spm} = 4$). As shown in Fig. 5*C*, G_C–V relationships become steeper during PA depletion as the highly charged Spm becomes predominant, while the midpoint of rectification ($V_{1/2}$) shifts to more positive potentials, reflecting the relief of steady-state rectification due to lowering of absolute PA levels.

DISCUSSION

Physiological Regulation of Inward Rectification. There is now considerable evidence that PAs can cause inward rectification by voltage-dependent block of potassium channels when applied exogenously to the intracellular side of the membrane (4–7, 21, 22). Analysis of the kinetics of PA unblocking and the multiphase "activation" of currents in intact cells is consistent with each PA contributing to rectification in intact cells (7). These results raise the possibility that inward rectification might be modulated physiologically by changes in intracellular PA concentrations and that pharmacological manipulation of inward rectification and hence excitability might be possible by manipulation of PA levels (23). The present results provide *in vivo* evidence that modulation of PA levels results in changes of inward rectification that can be predicted from the changes in PAs that occur. First, pharmacological manipulations that block PA synthesis relieved inward rectification of Kir2.1 channels expressed in *Xenopus* oocytes (Fig. 1). Second, although control potassium conductance (as assessed macroscopically using Rb^+ flux measurements) was unaffected in control O-CHO cells after Put withdrawal, the rate of Rb^+ efflux was significantly increased in O-CHO cells expressing Kir2.3 currents (Fig. 2). Rb^+ ions can act as blockers of inward rectifier channels and have only weak permeance in the absence of external K^+ ions (19, 20). Therefore the 2.2-fold increase of Rb^+ conductance resulting from expression of Kir2.3 channels and the subsequent 2.5-fold increase resulting from Put withdrawal are probably underestimations of the increases in K^+ conductance. Finally, the kinetics of Kir2.3 currents were altered after Put withdrawal, and after intracellular Put perfusion (Figs. 4 and 5) in ways that were directly predictable from inside-out patch-clamp recordings.

In *Xenopus* oocytes expressing Kir2.1 channels, injection of DFMO and MGBG caused significant increase of channel current in 24 hr, and this resulted from a shift in the midpoint of rectification by about +10 mV (Fig. 1*D*). Computer simulations of the cardiac action potential demonstrate that shifts in the midpoint of rectification of as little as 10 mV could cause significant effects on the repolarization phase of the action potential (23), and such shifts as we see here should cause a profound shortening of the action potential and decrease in excitability.

Expression of Kir2.3 channels in O-CHO cells resulted in expression of Kir channels with essentially the same properties as those seen in *Xenopus* oocytes (7). In untransfected O-CHO cells, the Rb^+ efflux rate was essentially unaltered between day 0 and day 4 after Put withdrawal from the medium. With Put in the incubation medium, expression of Kir2.3 channels caused an increase in the rate of Rb^+ efflux (Fig. 2*B*). Four days after withdrawal of Put from the medium, there was a significant increase in the Rb^+ efflux rate from Kir2.3-expressing cells, consistent with relief of rectification resulting from the decline in Put and Spd levels. Interestingly, in these Kir2.3-expressing O-CHO cells, there was a small but very reproducible *reduction* in the Rb^+ efflux rate 1 day after Put withdrawal (Fig. 2), At this time, Put levels have declined, and Spd and Spm should be largely unaltered (Fig. 2*A*). There are suggestions that Mg^{2+} can cause a paradoxical increase in

inward rectifier current by relieving intrinsic rectification (24, 25). Put block appears to be similar in nature to Mg^{2+} block (7), and it might also relieve intrinsic (i.e. Spm- or Spd-induced) rectification. In this case, lowering Put levels might permit enhanced channel blocking by Spm and Spd and hence a reduction in macroscopic Rb^+ efflux rates.

Voltage-clamp experiments on O-CHO cells revealed important additional information regarding the nature of the inward rectification (Figs. 4 and 5). Before Put withdrawal, the kinetics of currents immediately after attaining the whole-cell configuration were virtually indistinguishable from the kinetics of Kir2.3 currents in on-cell macropatch recordings from *Xenopus* oocytes (7). Withdrawal of Put led to a rapid loss of the instantaneous component of current activation (Fig. 5) that correlates with the expected time course of intracellular Put depletion (Fig. 2*A*; ref 15). The surprisingly complete disappearance of instantaneous activation with time after Put withdrawal, together with the lack of effect of EDTA on instantaneous activation, suggests that essentially all of the instantaneous phase of activation in control conditions is due to unblocking resulting from Put exit. We cannot formally rule out, however, the possibility that the fall of Put is accompanied by a decline of free Mg^{2+} concentration as a result of Mg^{2+} replacing Put on divalent cation binding sites. It is also clear that, with progressive time after Put withdrawal, the current activation becomes increasingly monoexponential, with time constants predicted from the time course of Spm exit, based on inside-out patch-clamp data (Fig. 5; ref. 7).

Physiological and Pharmacological Implications. The present results have important physiological implications. Dynamic effects of altered PA levels on inward rectification leads to the speculation that regulation of PA synthesis may be an active cellular mechanism for controlling ion channel activities, and hence cell excitability (23). It has been shown that PA synthesis is regulated in tissues under different physiological conditions. For instance, the activity of ODC is dramatically increased in regenerating liver after partial hepatectomy (26). The intracellular level of Put and Spm has been reported to decrease during *Xenopus laevis* oogenesis due to a decrease in ODC activity (27). PAs are elevated in many tumors (28, 29), and both epilepsy and cardiac hypertrophy are each associated with increased PA levels and with enhanced excitability (30–34). In addition, cells can take up and release PAs through specific mechanisms (see ref. 35 for review). There is a wide variety of inwardly rectifying potassium channel properties when examined in different tissues, particularly in the nervous system. Although some channels show almost instantaneous and steep rectification, others have very slow kinetics or shallow rectification (36–42). Although no correlative studies have yet been performed, intrinsic differences in the levels of PAs in different tissues (e.g., see ref. 43) might underlie some of this diversity. The present results further emphasize the hypothesis that regulation of intracellular PA levels will regulate cellular excitability by regulating the degree of rectification. The demonstration that pharmacological modulators of PA synthesis can alter rectification (Fig. 1) provides direct evidence for the possibility of therapeutic modulation of inward rectifier currents via tissue-directed modulation of PA synthesis.

Note Added in Proof. In confirmation of the present results, Bianchi *et al.* (44) have also recently demonstrated that rectification of endogenous Kir channels in rat basophil leukemia cells is relieved when the cells are cultured in the presence of inhibitors of ODC and SAMDC.

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