Acute Modulation of Adrenal Chromaffin Cell BK Channel Gating and Cell Excitability by Glucocorticoids

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Lovell, Peter V., Jonathan T. King, and David P. McCobb. Acute modulation of adrenal chromaffin cell BK channel gating and cell excitability by glucocorticoids. J Neurophysiol 91: 561–570, 2004. First published August 6, 2003; 10.1152/jn.01101.2002. Although adrenal glucocorticoids cortisol and corticosterone (CORT) have numerous “genomic” effects on adrenomedullary chromaffin cells, acute modulatory actions remain largely unknown, despite rapid stress-related changes in secretion. We report that 1 μM glucocorticoids rapidly modulate gating of chromaffin cell BK channels and action potential firing. In general, CORT, or the analog dexamethasone (DEX), increased channel activity in inside-out bovine patches, an effect not blocked by the glucocorticoid receptor (GR) antagonist RU38486. By contrast, these steroids could profoundly inhibit BK activation in many rat patches, while facilitating activation in others. We show that BK inhibition arises from a negative shift in the voltage dependence of BK inactivation paralleling that for activation. We report that rat cells characteristically exhibit greater repetitive firing ability than bovine cells in the absence of glucocorticoids. In both species, steroid application typically increased firing responses to smaller current injections, attributable to BK-enhanced repolarization and NaK channel deinactivation. However, in rat cells, where BK inactivation is generally faster and more complete, glucocorticoids tended to dampen responses to stronger stimuli. Thus, in the context of natural variation in BK gating, glucocorticoids can either promote or limit firing responses. We suggest that steroids exploit BK gating variety to tailor catecholamine output in a species- and context-specific fashion.

INTRODUCTION

Regulation of protein expression by steroid hormones has long been recognized as an important mechanism underlying cellular plasticity (for review see McEwen 1991). However, increasing evidence for much more rapid steroid-dependent modulation of voltage- and ligand-gated ion channels has begun to reshape our view of the physiological roles of steroids in the short-term (Falkenstein et al. 2000; Makara and Haller 2001; Moore and Evans 1999; Wehling 1997). Among these are the glucocorticoids produced by the adrenal cortex, especially cortisol (in bovine and human) and corticosterone (in rat), together abbreviated CORT. CORT synthesis is under the regulatory control of pituitary ACTH and represents the most important link between stress-related hypothalamic function and a myriad of body and brain responses. CORT secretion exhibits wide diurnal and stress-related fluctuations and has an enormous variety of regulatory effects on, for example, metabolism, storage, and mobilization of nutrients; cardiovascular function; growth and reproductive function; and hippocampal structure and function (Sapolsky et al. 2000).

Historically, glucocorticoids have been credited with “genomic” effects that initiate and maintain the phenotypic differences distinguishing chromaffin cells from sympathetic preganglionic neurons, for example, spherical shape (lack of axon outgrowth) and epinephrine versus norepinephrine synthesis (Hodel 2001). Stress experience-related “tuning” of chromaffin cell protein expression by CORT, initially described for enzymes (Betito et al. 1992, 1994; Stachowiak et al. 1998; Tank et al. 1986) has begun to be extended to ion channels, including Ca2+ channels (Fuller et al. 1997a,b) and BK channel splice variants (Lai and McCobb 2002). However, rapid surges in CORT release from adrenal cortex, to which underlying chromaffin cells are immediately exposed, may produce peak concentrations as high as 100 μM within the rat adrenal medulla (Betito et al. 1992, 1994). Potential acute effects of these surges have received very little attention. Dexamethasone (DEX) has been shown to decrease the amplitude of nicotinic acetylcholine-induced currents in porcine adrenal chromaffin cells (Inoue and Kuriyama 1989), dramatically reducing nicotine-induced catecholamine secretion (Wagner et al. 1999).

In chromaffin cells, large conductance calcium- and voltage-dependent potassium channels (BK for Big K+/H11001) are uniquely positioned to influence features of electrical excitability (Lingle et al. 1996; Lovell and McCobb 2001; Solaro et al. 1995). Glucocorticoids have been shown to regulate BK channel sensitivity to phosphatase activity in pituitary-related cells, however, this regulatory effect requires changes in protein synthesis (Tian et al. 2001a,b). In contrast, BK channels expressed in smooth muscle can be modulated immediately by 17β-estradiol (Dick et al. 2001; Valverde et al. 1999) and dehydroepiandrosterone (DHEA) (Farrukh et al. 1998; Peng et al. 1999). To our knowledge similar modulation of BK channels by corticosteroids has not been shown in chromaffin cells or any other cells. We demonstrate here that these steroids provide a proximate link through which the principle endocrine stress-response system, the hypothalamic—pituitary—adrenocortical (HPA) axis, can exert a rapid, fine-control of the adrenomedullary branch of the sympathetic response system. Heterogeneity in BK activation and inactivation gating shape the repertoire of CORT responses at cell and species levels.

METHODS

Chromaffin cell isolation and culture

Bovine and rat chromaffin cells were isolated and cultured using procedures described by Lovell et al. (2000) and Lai and McCobb

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(2002). Briefly, bovine adrenal glands (Cudlin’s Meat Market, Newfield, NY) were perfused for 30 min at 20°C with a Ca²⁺- and Mg²⁺-free Locke’s buffer (in mM: 154 NaCl, 5.6 KCl, 3.6 NaHCO₃, 5.6 glucose, and 5 mM HEPES at pH 7.4) followed by perfusion with a modified Ca²⁺- and Mg²⁺-free Locke’s solution containing an additional 1 X MEM vitamins (GIBCO), 1 X MEM amino acids (GIBCO), Na-Pyruvate (11g/L), l-glutamine (0.2M), penicillin (80 U/ml), streptomycin (80 μg/ml), nystatin (0.8%), collagenase B (1 mg/ml; Boehringer Mannheim), and 0.0875% BSA for 1 h at 37°C. For perfusion with steps, an O₂ and CO₂ (Carbogen 41%/4/92.5%) mix was continuously bubbled into solution. Following digestion, medullary tissue was manually removed from the adrenal, minced, and filtered through a plastic mesh strainer (13 mm pore size). Tissue was placed in a second saline containing collagenase B (see above) for 1 h at 37°C, filtered through cheesecloth, and washed several times using pellet centrifugation (120g for 10 min) and a modified Locke’s solution with 0.5% added BSA. Isolated chromaffin cells were filtered through a 70-μM nylon mesh filter (Falcon 2350, Fisher Scientific, Pittsburgh, PA), resuspended in prewarmed cell culture medium (GIBCO, RPMI 1640 with 10% horse serum, 5% fetal calf serum, 2% H9262/H9024), and filtered through cheesecloth, and washed several times using a modiﬁcation of clamp data was performed using custom software written for a Macintosh G3. Data were acquired and digitized at 20 kHz for Pulse software (Heka Elektronik, Lambrecht, Germany) for the analysis of clamp data. Data and standard clamp protocols designed with fi al. 2000; Lovell and McCobb 2001) using a List EPC-7 or EPC-9 electrophysiology data were collected as described previously (Lovell et al. 2000; Lovell and McCobb 2001) coated with collagen (Vitrogen, Collagen Corporation, Carlsbad, CA: 0.6 mg/ml in ddH₂O) or onto the glass bottom of a 35-mm plastic dish (Plastek Cultureware) coated with 0.1% poly-l-lysine (Sigma) in water. Cell cultures were maintained at 37°C in a 5% CO₂ atmosphere and used for several days.

Electrophysiological methods

Single and macroscopic currents were recorded using standard patch clamp recording techniques (Hamill et al. 1981; Sakmann and Neher 1985). Borosilicate glass patch electrodes (World Precision Instruments, Sarasota, FL; inside/outside 1.5/1.12 mm; 3–6 MΩ) were pulled and coated with Sylgard 184 (Dow Corning, Midland, MI) to decrease capacitance. Voltage-clamp and current-clamp electrophysiology data were collected as described previously (Lovell et al. 2000; Lovell and McCobb 2001) using a List EPC-7 or EPC-9 patch-clamp amplifier and standard clamp protocols designed with Pulse software (Heka Elektronik, Lambrcht, Germany) for the Macintosh G3. Data were acquired and digitized at 20 kHz for voltage-clamp data and at 10 kHz for current-clamp traces. Off-line analysis of clamp data was performed using custom software written for Igor Pro (Wavemetrics, Lake Oswego, OR).

All experiments were conducted at room temperature (20°C). As described previously (Lovell et al. 2000), seals with resistances of 3–6 GΩ were obtained, following which zero-Ca²⁺ saline was perfused onto the cell for excision of inside-out patches. Solution exchange was accomplished with a seven-barrel gravity-fed perfusion pipette.

Current-clamp recordings were made using the perforated patch technique as described by Lovell and McCobb (2001). Briefly, high resistance seals were achieved as described above and recordings were made when the apparent input resistance had dropped to between 50 and 150 MΩ. For some cells, a small holding current was used to maintain cell resting potentials between −65 and −75 mV. To verify that the perforated patch was not ruptured during recording, input resistance was monitored during deliberate rupture at the end of the recording. For excitability measurements, a series of 2-s current pulses of increasing strength was used to elicit repetitive firing. The magnitude of current and serial increment of steps was adjusted by trial and error to fit the varying input resistances of the cells.

Solutions

For inside-out patches, symmetrical K⁺ solutions were used to eliminate a potassium driving force and to allow any DC offset to be cancelled at 0 mV. The usual pipette and bath saline solutions contained (in mM): 160 KCl, 10 HEPEs, 1 HEDTA, and 0.0375 CaOH, pH adjusted with KOH to 7.2 to make approximately 500 nM free [Ca²⁺]. The free [Ca²⁺] was calculated using MaxChelator software (WebMaxC v2.10) (Bers et al. 1994). Zero-Ca⁺² solution contained an additional 5 mM EGTA (Sigma). When pharmacology experiments were performed, iberiotoxin (IBTX, 10 nM, Alomone Labs, Jerusalem, Israel) was added to the bath perfused salines and pH adjusted to 7.2. DEX and corticosterone (Sigma) were dissolved in 100% DMSO, stored in small aliquots at −20°C, and added fresh to recording salines as needed. In previous experiments, inclusion of DMSO in the recording saline did not appreciably alter BK channel activity. The osmolarity of salines was measured by dew point osmometer and adjusted to 300 osM.

For current-clamp recordings, the bath salines contained the following (in mM): 145 NaCl, 5 KCl, 10 HEPEs, 2 CaCl₂, and 1 MgCl₂, pH adjusted to 7.4 with 2 M NaOH. The technique used for obtaining perforated patches has been described elsewhere (Lovell and McCobb 2001). Briefly, the tips of patch electrodes (2–4 MΩ) were first filled with whole cell saline containing (in mM): 140 KCl, 5 MgCl₂, 10 EGTA, and 10 HEPEs, pH 7.4. The electrode barrel was then back-filled with a solution containing 20 μl of fresh stock amphotericin B (6 mg/100 μl DMSO; GIBCO) and 40 μl of stock Pluronic acid F-127 (2.5 mg/100 μl DMSO; Molecular Probes) added to 1 ml of whole cell recording saline. To maintain perforating efficacy, fresh aliquots were used during each hour of recording.

Data collection and statistical analysis

Single and multichannel currents were linear leak subtracted as previously described (Lovell and McCobb 2001). The fraction of inactivated current (BK/BKtotal) was estimated by calculating a ratio of the BK current at 350 ms as a function of the estimated peak BK current. The calcium-/voltage-dependence of activation (G–V) and inactivation (H–V) were evaluated by measuring peak amplitudes at increasing voltage steps of 20 mV, converting these to conductances by dividing out the driving force, and fitting G–V and H–V curves to a single term Boltzmann of the form

\[ G_{max} = G_{max}(1 + \exp(V_n - V_{1/2})/s) \]

with parameters for maximum conductance (Gmax), voltage of half-activation (V_{1/2}), and slope (s; the steepness of the voltage-dependence of activation, in mV/e-fold change in voltage). Effects of steroids on currents were normalized by dividing currents after exposure by currents before exposure. Natural logs of fractional changes were taken for averaging and statistical comparisons, to avoid the confounding effects of averaging unbounded values for increases with values for decreases bounded between 0 and 1. Means were compared using a standard Student’s t-test that assumed either equal or unequal variances as appropriate (α = 0.05). Statistical outcomes on nontransformed data did not alter conclusions drawn.

RESULTS

Rapid modulation of chromaffin BK channel function by corticosteroids

In inside-out patch recordings from bovine or rat adrenal chromaffin cells, BK channels overshadow any other voltage-gated potassium channels when a low micromolar Ca²⁺ concentration is present on both sides of the patch and K⁺ is the only permeant cation (Lovell et al. 2000; Solario et al. 1995). This has been confirmed by pharmacological block with 1 mM TEA⁺ and with iberiotoxin and charybdotoxin. In the bovine patch in Fig. 1, a small number of channels were activated by 350-ms voltage-steps to +80 mV from a holding potential of
−100 mV ($V_{hold}$), including both inactivating and noninactivating BK channels (BK$_{i}$ and BK$_{s}$) (Lovell et al. 2000; Solaro et al. 1995). Such patches typically exhibit very little other channel activity in the absence or presence of Ca$^{2+}$, with the exception of infrequent, very brief openings in the 50–75 pS range or channels too small to resolve at this gain. The BK channels seen here open with a low probability when exposed to zero-Ca$^{2+}$ solution buffered with EGTA. In the center and right panels, the perfusion solution contains 500 nM Ca$^{2+}$ and 0.1% DMSO, the vehicle used to dissolve steroids. Addition of 10 μM cortisol resulted in an immediate increase in the number of channel opening events, with no observable change in the types of channel events observed. No significant change in the amplitude of single channel currents was noted, making it unlikely that effects on more macroscopic or ensemble-averaged currents described below resulted from a change in single channel conductance, as has been suggested for the effect of estradiol-like tamoxifen (Dick and Sanders 2001; Dick et al. 2001).

The modulation of BK current by CORT is very rapid; a sharp increase in channel activity is detectable within seconds of exposure (Fig. 1A). Similar results were obtained with cortisol in six of eight patches tested. In six of seven patches tested, current was increased by simultaneous exposure to 10 μM CORT and 10 μM RU38486 (Fig. 1B). The latter is a selective antagonist of the cytosolic glucocorticoid receptor (GR, also known as GR-II, to distinguish it from the mineralocorticoid receptor, MR or GR-I). These observations and the fact that many isolated, continuously perfused patches responded to CORT or DEX (to which MR is not sensitive) argue strongly that these steroid effects are mediated via a novel, membrane-associated receptor or on the channel directly.

At the top of Fig. 1C, a multichannel patch containing predominantly noninactivating BK channels showed nearly a 70% increase in amplitude in response to application of the synthetic glucocorticoid DEX at a concentration of 1 μM. Increasing DEX to 10 and then to 100 μM led to further increases to 130 and 200% above control. This progressive effect of increasing doses was typical of patches exposed to multiple concentrations. DEX effects could be partially reversed with a brief washout, though reversal of the sustained component was typically less efficacious. Figure 1D illustrates the negative shift in the voltage-dependence of activation of BK channels by DEX. Further evidence for a negative shift, as opposed to an increase in the maximum achievable $P_{o}$, or the number of channels participating in the current, is provided by currents in rat chromaffin patches, where the plot of conductance as a function of test potential (G–V curve) typically
plateaus in a more negative range, allowing more accurate determination of \( G_{\text{max}} \) and the half-activation point (\( V_{0.5} \); see Fig. 3).

**Glucocorticoid effects on BK channels in rat chromaffin cells**

Starting at the high end of the concentration range, we made the seemingly paradoxical observation that 100 \( \mu M \) DEX or CORT tended to have an effect on rat patches that was the opposite of that seen on bovine patches, profoundly inhibiting BK channel activity (Fig. 1, E and F). This effect was usually largely reversed by washing and was repeatable. Of 18 rat patches tested at 100 \( \mu M \), a net decrease in current was observed in 14 (Fig. 2B). By contrast, only 3 of 21 bovine patches showed a decrease in peak current at this DEX concentration, whereas 16 showed an increase. As explained under METHODS, drug responses were averaged after natural log-transformation of fractional changes in current. Thus, in rat, 100 \( \mu M \) DEX reduced peak current amplitude by an average of 57.1\%, whereas it increased peak current in bovine by 47.7\%.

One and 10 \( \mu M \) concentrations of DEX had more varied and complex effects. In the majority of patches representing both species, DEX increased peak BK current (38 of 54 patches, see Fig. 1B for a breakdown). Average peak currents in 1 and 10 \( \mu M \) DEX were increased by 35.7 and 29.6\% in bovine and 8.9 and 39.8\% in rat. However, the quantitative variation was sufficient that net responses did not differ statistically between doses. Changes in peak current amplitudes in the negative direction, as well as in the positive direction, were typically at least partially reversed by washout and qualitatively reproduced on reintroduction of DEX.

BK currents of both species are very heterogeneous with respect to the rate and voltage range of activation, and the rate, extent, and the voltage range of inactivation. Although both species exhibit cells covering the full range from BK\(_s\) to BK\(_i\) channels exclusively, bovine currents are much more likely to be of the former type or to have a mix of channels with a resultant macroscopic or ensemble current that is slowly and/or incompletely inactivating (Lovell et al. 2000; Lovell and McCobb 2001). Moreover, a relatively negative voltage-dependence of activation correlates with a greater proportion of BK\(_i\) channels, and faster inactivation, across patches of both species (Lovell et al. 2000).

Differential responses of inactivating and sustained subcomponents of BK current can explain much of the variation within and between species; subdividing revealed a more consistent pattern of DEX responses. In the present study, we subdivided currents from both species before and after DEX: inactivation was virtually complete well before 350 ms, thus the current remaining at that point was taken as the BK\(_i\) current, with the difference between it and the total representing BK\(_s\) current. In absolute terms, the BK\(_s\) component of current in bovine patches was increased by DEX in 42 of 48 cases, while it was reduced in 2 and unaffected in 4. Rat BK\(_s\) current was increased in 22 of 34 patches having a sustained component, reduced in 8, and unaffected in 4 (Fig. 2). Five of 8 reductions were in 100 \( \mu M \) DEX. Compared with currents from the same patches before DEX, BK\(_s\) currents after 1, 10, and 100 \( \mu M \) DEX, respectively, exhibited an average increase in amplitude of 187, 269, and 5.4\% for rat and 84, 102, and 101\% for bovine. In contrast to the BK\(_s\) component, the absolute amplitude of the BK\(_i\) component was reduced in 28 of 45 and 23 of 41 bovine and rat patches, respectively. As illustrated in Fig. 3C, the proportion of BK\(_i\) current was much more often increased than decreased by DEX.

Experiments with very negative "prepulse" potentials preceding BK activation steps further demonstrate the confounding effects introduced by BK channel inactivation. Inactivation, a process conferred by one or more accessory "\( \beta \)" subunits interacting with the Slo gene-encoded \( \alpha \) subunit, is voltage-dependent. Inactivation more rapidly follows activation (elicited by a depolarizing voltage step) at more depolarized step potentials, deinactivation is faster at more negative

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**FIG. 2.** Glucocorticoids at lower concentrations typically increased peak BK current in rat chromaffin cells, although substantial variability was seen at all concentrations in both species. Importantly, sustained (BK\(_s\)) and inactivating (BK\(_i\)) components were differentially affected. A: representative traces from rat (left) and bovine (right) are shown. Pie diagrams (right of traces) illustrate the relative proportion of the BK subcomponents. Note the tendency of BK\(_s\) and BK\(_i\) components to increase and decrease, respectively, regardless of species, net effect on peak current, or relative sizes of the two components prior to DEX. B: summary of direction of change in absolute size of peak current and two subcomponents following exposure to 3 DEX concentrations. Black bars represent that proportion of patches showing increases, and gray bars that showing decreases, with total numbers of patches tallied for each category given on the right. Note that some patches lacked one or the other of the 2 subcomponents. C: the relative proportion of the peak current that was noninactivating was much more likely to increase with DEX exposure for both rat (filled circles) and bovine patches (unfilled circles). The diagonal line represents no change.
return potentials, and the number of channels inactivated at a “steady state” holding potential is a sigmoidal function of that potential.

At the top of Fig. 3A, the membrane potential was stepped from −100 mV to +80 mV before and after application of 100 µM DEX. DEX reduced peak current amplitude dramatically. However, when the membrane potential was stepped briefly to −140 before stepping to +80, we observed that 1) the peak current was slightly greater (610 pA vs. 550 pA, or about 10%), demonstrating that some channels remained inactivated even at a steady state of −100 mV, and 2) the negative effect of DEX was virtually eliminated. With a series of test potentials stepping from −140 mV, it was clear that DEX, even at 100 µM on rat cells, shifted the half-activation voltage strongly in the negative direction (by approximately 25 mV) and without affecting the maximum activatable current (\(G_{\text{max}}\), Fig. 3B). Furthermore, as seen at the bottom of Fig. 3C, a full series of steps to +80 mV from prepulse potentials as low as −140 mV revealed that DEX shifted the voltage-dependence of inactivation dramatically; in this case by about 40 mV. This clearly indicates that the apparent negative effect of DEX is explained by a reduction in the number of channels available for activation from starting potentials positive to −140 mV. There is no compensatory increase in channels activated during the test step to +80 mV, despite the negative shift in the \(G-V\) curve, because the plateau had been reached even without the DEX.

While in bovine cells we could not conclusively rule out that DEX acted to increase \(G_{\text{max}}\) independently of the shift in the \(G-V\) curve because the curve was shifted too far right to reach saturation; the consistent observation in rat cells that \(G_{\text{max}}\) was not increased provides strong support for a common mechanism of action involving a negative shift in channel open probability.

In the experiment shown in 3D, DEX has a small negative effect on peak amplitude when the current is elicited from −60 mV, although when it is elicited from −100 mV it has a strong positive effect. Because the range of voltages spanned in this case encompasses that of probable physiological “resting membrane potential,” the opposite effects of DEX on one patch argues strongly that the impact of DEX on channel function in vivo will depend critically on the starting membrane potential, as it interacts with channel gating parameters.

In summary, the combination of inherent complexity in BK gating and the intra- and interspecies heterogeneity in various aspects of this gating, as observed in chromaffin cells from two species, ensures a complex landscape on which glucocorticoids can have quite varied effects. The more frequent occurrence of inhibitory effects of DEX on rat cells than bovine cells and the detailed patterns of current changes in cells of different BK channel compositions (particularly with respect to inactivation) are consistent with a relatively simple effect of glucocorticoids in facilitating the activation (and inactivation) of BK channels at more negative potentials.

**Bovine chromaffin cells tend to fire at lower frequencies than rat cells**

Before studying the effects of steroids on action potential (AP) generation, we characterized responses to current injection using perforated-patch current clamp recording in the absence of glucocorticoids. Prior rat–bovine comparisons of BK gating in voltage clamp revealed striking differences (Lovell et al. 2000), thus we characterized chromaffin cell excitability in the two species in parallel.

Trains of APs could be elicited reliably by 2-s suprathreshold depolarizing current injections in bovine and rat cells (Fig. 4). For uniformity in our quantitative comparisons, a small holding current was applied, where necessary, to maintain membrane potentials between −65 and −70 mV, thereby minimizing variation in preexisting Na⁺ or Ca²⁺ channel inactivation. Cell-to-cell variation in input resistance was controlled for by altering the initial amplitude and serial increment of the injected current. Solaro et al. (1995) and Lovell and McCobb (2001) have shown previously that incremental increases in the amount of depolarizing current injected increases the number of APs fired (or average frequency) up to a peak, after which further increases in current produced fewer and fewer spikes. We were therefore careful to increment currents finely enough, and over a wide enough range, to determine the point of peak response accurately. Because studies have suggested that Ca²⁺ APs, relatively broad APs that do not necessarily cross the 0 mV axis, produce calcium transients that elicit catecholamine exocytosis (as do Na⁺ APs (Artalejo et al. 1994; López et al. 1994), they were counted (after Lovell and McCobb 2001).

AP response patterns of bovine and rat chromaffin cells varied over a wide range. While it was not uncommon for bovine cells to respond with a single AP over a wide range of...
inputs, most were able to vary their response with current amplitude. Examples of maximal responses to graded series of inputs are shown in Fig. 4. Relationships between input current and response frequency were roughly parabolic in virtually all cases. Examples are shown in Fig. 5. Under the parameters defined above, rat cells were consistently found to fire many more spikes at the peak of the curve than bovine cells (Fig. 4) in the absence of steroids. On average, the number of spikes that could be elicited during a 2-s pulse was 4.49 ± 0.39 (mean ± SE; n = 55) for bovine cells compared with 9.96 ± 0.67 for rat cells (Fig. 5B; n = 58; P < 0.0001). The frequency distributions in Fig. 4C illustrate characteristic differences between the rat and bovine chromaffin cell populations.

Corticosteroids enhance and depress features of excitability in bovine and rat chromaffin cells

Using the techniques described above to assess excitability, multiple series of AP trains were elicited from bovine and rat chromaffin cells before and after exposure to corticosteroid or DEX for approximately 2 min. The bovine traces presented in Fig. 5A illustrate the effects of various concentrations of DEX on repetitive firing elicited by an intermediate intensity current step of 9 pA. At this stimulus intensity, the cell fired four distinct spikes in control saline (Fig. 5A, top trace). Relatively low concentrations of DEX (1 and 10 μM) favored improved firing of five and seven spikes, respectively. In contrast, 100 μM DEX dramatically reduced the ability of this cell to sustain firing. Figure 5B shows the frequency of spikes elicited from the same cell in response to representative series of test current pulses (frequency values represent the number of spikes elic-
exhibited a pattern of responses similar to that seen in Fig. 5. In these cells, lower DEX concentrations also increased in absolute terms by DEX at low concentrations, whereas 100 nM DEX was out of range (too positive) in control saline. Nevertheless, in those in which \( G_{\text{max}} \) was determined, the full curve was shifted left substantially, with no significant change in \( G_{\text{max}} \) or in the steepness of the voltage-dependence.

Accompanying the negative shift in the \( G-V \) curve in the case depicted in Fig. 3C was a robust negative shift (approximately 40 mV) in the curve defining the voltage-dependence of inactivation at steady state (the \( h \_c \) curve). Such a shift can dramatically reduce the number of channels available for activation, an effect that directly counteracts the effect of shifting the \( G-V \) curve left, and leads to an underestimation of activation-promoting effects of glucocorticoids. Even in control saline, a substantial number of BK channels were inactivated at \( V_{\text{hold}} = -100 \) mV in some cells, and a few at \(-120 \) and even \(-140 \) mV, ([Ca\(^{2+}\)]\_c = 500 nM). It is not surprising therefore that a profound inhibition of current was observed at this holding potential in patches with predominantly inactivating BK channels nor, by extension, that a reduction was seen in the inactivating fraction of the current in many additional patches from both species.

BK channels differ from 0 to 4 in the number of inactivation-conferring subunits associated with them (Wang et al. 2002). They also vary widely, within and between cells, as well as between species, in the voltage-dependence of activation and inactivation gating, reflecting diverse factors such as subunit and splice variant composition, phosphorylation state, redox state, and other unknowns. By differentially altering the subset of a heterogeneous population of channels that respond to a stimulus, different concentrations of steroids are likely to have markedly nonlinear dose-dependent effects. Moreover, patch-to-patch heterogeneity can be expected to obscure any dose dependency in averaged voltage-clamp data. In our experiments, dose-sensitivity was evident in individual patches and in current-clamp recordings, despite the increasingly negative effects of higher doses seen particularly in averaged rat patches.

Based on comparisons between patches from bovine, rat, and hypophysectomized rat chromaffin cells, we suggested that BK channels tend to have a more negative voltage-dependence of activation than BK channels (Lovell et al. 2000; Lovell and McCobb 2001). This must derive in part from the negative shift in the voltage-dependence and kinetics of activation conferred by inactivation-conferring \( \beta \) subunits (Wallner et al. 1999; Wang et al. 2002; Xia et al. 1999). One consequence is that steroid exposure will increase the relative representation of others in which they are expressed are influenced by glucocorticoids will be of interest to determine.

**Steroid effects on BK currents in voltage clamp mode**

The opposite effects of high glucocorticoid concentrations on rat and bovine BK channels presented an initial paradox. However, further analysis suggested a common mechanism, in which activation gating of channels in both species is enhanced by exposure to the steroids in a roughly dose-dependent manner, while the inhibition is an important consequence of facilitated channel inactivation. In the many records in which individual channel events were easily measured, no change in single channel conductance was observed. The possibility that new channels could be recruited from an otherwise inactive pool of channels cannot be excluded in some cases, because \( G_{\text{max}} \) was out of range (too positive) in control saline. Nevertheless, in those in which \( G_{\text{max}} \) was determined, the full curve was shifted left substantially, with no significant change in \( G_{\text{max}} \) or in the steepness of the voltage-dependence.

**Discussion**

Immediate effects of glucocorticoids on chromaffin cells have been reported only for nicotinic acetylcholine receptor activation and catecholamine secretion (Inoue and Kuriyama 1989; Wagner et al. 1999). We report that physiological concentrations of glucocorticoids have immediate effects on BK-type K\(^+\) channels in chromaffin cells and that these impact directly on AP firing. With this discovery, we add to the short list of steroids, including 17\(\beta\)-estradiol and DHEA, known to acutely modulate BK channels (Farrukh et al. 1998; Valverde et al. 1999). Whether BK channels in any of the many other

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slowly activating and slowly inactivating and noninactivating channels. This view explains kinetic changes seen in many patches (see Figs. 2 and 3).

There may be a closer link between steroid sensitivity and β subunits. Rapid effects on isolated patches, and failure of classical GR antagonists to block them, suggest a membrane-associated receptor. Steroids can interact with members of several superfamilies of membrane proteins, including G protein–coupled receptors, ligand-gated ion channels, and voltage-gated channels (Falkenstein et al. 2000; Makara and Haller 2001; Moore and Evans 1999; Wehling 1997). The discovery that estradiol modulates smooth muscle BK channel gating by interacting with the Slo-β1 subunit makes related members of the Slo-β family particularly attractive candidate glucocorticoid receptors.

Steroid effects on cellular excitability

Chromaffin BK channels are very prominent and contribute heavily to shaping intrinsic excitability. Rapid BK activation minimizes AP duration and augments the brief afterhyperpolarization. These effects minimize Na+ channel entry into an inactivated state and optimize deinactivation, thus preparing the cell to fire again (Fig. 4, top) (Lingle et al. 1996; Lovell and McCobb 2001). Our data suggest that glucocorticoid augmentation of BK channel activation will increase the number of spikes elicited by a wide range of stimulus intensities in vivo and thus augment catecholamine secretion.

The effect of CORT on BK channel inactivation raises the provocative hypothesis that CORT may, under some circumstances, suppress firing. We report negative effects of even lower concentrations of CORT or DEX, including 1) reduction in the number of APs that could be elicited at peak and 2) narrowing of the range of stimulus strengths that effectively elicit spikes.

The strongest arguments that the aforementioned effects will have consequences in vivo come from species comparisons. Both species’ channels are heterogeneous, however, J) rat cells have more inactivating BK channels than their bovine equivalents, and 2) rat channels tend to activate at more negative voltages and more rapidly at equivalent voltages (Lovell et al. 2000). In the present study, we report that rat cells are substantially more effective at repetitive firing. While channels other than BK channels probably enter into the species difference, the general picture is consistent with the idea that greater ease of activation of rat BK channels outweighs the potentially negative effect of easier BK inactivation, in the absence of steroids. However, suppressive effects of steroids on firing were clearly more pronounced in rat cells. The maximum number of APs that could be elicited was reduced more and reduced by lower concentrations, whereas even 100 μM DEX could raise the maximal spiking frequency in bovine cells. The range of stimulus strengths that could effectively elicit spikes was, though shifted to a lower intensity range, also more consistently narrowed in rat cells at all concentrations.

Are in vivo firing and secretion suppressed at the peak of large episodic swings in rat (or bovine) CORT levels, which may exceed 100 μM within the rat adrenal medulla (Betito et al. 1992, 1994)? Five- to 10-fold higher levels make suppression more likely in rats than cows (el-Nouty et al. 1978; Koehl et al. 1999; Manzanares et al. 1999; Veissier et al. 1999; Viau et al. 1999). Bovine chromaffin cells generally reside lower on the scale of responsiveness but have more room for increase. Rat cells, comparatively speaking, may be on chronic high alert. While speculative, a damping effect on potential “runaway firing” may be offered by BK channel inactivation. This could serve to pace, or restrain, catecholamine output to protect against too rapid exhaustion of stores, especially under intense stress.

Moderating effects of corticosteroids at high concentrations or stimulus intensities are not incompatible with a facilitatory role at lower concentrations or stimulus intensities; glucocorticoids could exert important modulatory effects at both ends of the firing range. Relative emphasis probably differs between species. From another vantage point, the relative lack of inactivation in bovine BK channels allows more channels to be open at rest. The low input resistance will then make the cells harder to bring to threshold (with the converse true in rat cells). In this context, BK channels would be playing an “antiexcitatory” role, and glucocorticoids, by shifting inactivation of rat channels in the negative direction, would help ensure that the negative effects of BK channels can still be overridden. Roles in threshold regulation of BK or CORT are not necessarily incompatible with roles in regulation of repetitive firing but would apply in a different range of voltage (or [Ca2+]i).

It seems likely that HPA-driven diurnal and stress-related increases in CORT synthesis will rapidly prime epinephrine-secreting chromaffin cells to respond more efficaciously to sympathetic stimulation than they would otherwise. Given the gating complexity of BK channels and their relationship to excitability, we suggest that a capping or suppressive effect is also likely to accrue in the high end of the response range. How moment-to-moment changes in CORT affect cellular excitability and catecholamine output in vivo remains an open and challenging question. Species comparisons will continue to help elucidate the electrophysiological, molecular, and, ultimately, the adaptive significance of acute BK channel interactions with stress steroids.

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