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Reciprocal Modulation of Voltage-Gated and Background K^+ Channels Mediated by Nucleotides and Corticotropin

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ABSTRACT

Bovine adrenal zona fasciculata (AZF) cells express two types of K^+-selective ion channels including a rapidly inactivating bKv1.4 current (I_A) and an ATP-dependent noninactivating background current (I_{AC}) that sets the resting membrane potential. Whole-cell, patch-clamp recording from cultured AZF cells was used to demonstrate a novel reciprocal modulation of these two K^+ channels by intracellular nucleotides and corticotropin. Specifically, increases in I_{AC} activity induced by intracellular ATP, as well as GTP and 5′-adenylyl-imidodiphosphate (AMP-PNP), were accompanied by a corresponding decrease in the amplitude of the voltage-gated I_A current. The reduction in I_A current was observed only when patch pipettes contained ATP or other nucleotides at concentrations sufficient to support activation of I_{AC}. Conversely, the nearly complete inhibition of I_{AC} by corticotropin was accompanied by the coincident reappearance of functional I_A channels. In the absence of I_{AC} current, corticotropin failed to alter I_A. The reciprocal modulation of AZF cell K^+ channels by nucleotides and corticotropin was independent of membrane voltage. These results demonstrate a new form of channel modulation in which the activity of two different K^+ channels is reciprocally modulated in tandem through hormonal and metabolic signaling pathways. They further suggest that I_A and I_{AC} K^+ channels may be functionally coupled in a dynamic equilibrium driven by intracellular ATP and G-protein-coupled receptors. This may represent a unique mechanism for transducing biochemical signals to ionic events involved in cortisol secretion.

Several large families of K^+-selective ion channels have been identified that are expressed throughout the plant and animal kingdoms. These include voltage-gated and metabolically regulated "background" K^+ channels that regulate the frequency and duration of action potentials and set the resting membrane potential in various cells (Chandy and Gutman, 1995; Goldstein et al., 1998). Consequently, K^+ channels function critically in regulating cellular functions, including hormone secretion, muscle contraction, and neural conduction and transmission. Although many cells express multiple K^+-channel subtypes that are modulated through a variety of signaling pathways, functional coupling between these K^+ channels under physiological conditions has not been described.

Bovine adrenocortical cells express two distinct types of K^+ selective channels. These include a voltage-gated, rapidly inactivating A-type channel (I_A) and a noninactivating background K^+ channel (I_{AC}) that set the resting membrane potential (Mlinar et al., 1993; Mlinar and Enyeart, 1993; Enyeart et al., 1996, 1997). I_{AC} is unique among K^+ channels described thus far and seems to act pivotally in the physiology of cortisol secretion. In whole-cell, patch-clamp recordings from AZF cells, I_{AC} grows continuously over many minutes when ATP is present in the patch electrode at concentrations greater than 1 mM (Mlinar et al., 1993; Enyeart et al., 1996, 1997; Gomora and Enyeart, 1998).

I_{AC} channels are potently inhibited by corticotropin (IC_{50} = 5.4 pM) at concentrations identical with those that depolarize AZF cells and stimulate cortisol secretion (Mlinar et al., 1993; Enyeart et al., 1996). The inhibition of I_{AC} by corticotropin is independent of A-kinase but requires the presence of hydrolyzable ATP, suggesting that the gating of I_{AC} channels could be coupled to an ATP hydrolysis cycle (Enyeart et al., 1996). Regardless, I_{AC} channels function as sensors, coupling hormonal and metabolic signals to membrane potential, Ca^{2+} entry, and cortisol secretion (Enyeart et al., 1993).

Molecular cloning of the rapidly inactivating I_A K^+-channel cDNA shows that it belongs to the bKv1.4 K^+-channel family (Enyeart et al., 2000). Although this voltage-gated channel is prominently expressed in virtually every AZF cell,

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ABBREVIATIONS: I_A, rapidly inactivating bKv1.4 current in bovine adrenal fasciculata cells; I_{AC}, ATP-activated, noninactivating potassium current in bovine adrenal fasciculata cells; AZF, bovine adrenal zona fasciculata; AMP-PNP, 5′-adenylyl-imidodiphosphate; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid; DPBP, diphenylbutylpiperidine; î, the unitary current; N, the number of active channels in any given patch; P_o, channel open probability; T_o, initial time of recording; T_{MAX}, time after I_{AC} K^+ current reaches a stable maximum amplitude; I-V, current-voltage.

its function has not been determined (Mlinar and Enyeart, 1993). I_A channels have not been shown to be modulated by corticotropin.

Previous studies examining the activation of I_{AC} by nucleotides and its inhibition by corticotropin have not uncovered a link between the activity of I_A and I_{AC} K^+ channels in AZF cells (Enyeart et al., 1996, 1997). However, in recent experiments, we have discovered compelling evidence for a unique form of channel regulation in which the gating of I_A and I_{AC} K^+ channels are reciprocally controlled in tandem by nucleotides and corticotropin receptors.

Materials and Methods

Tissue culture media, antibiotics, fibronectin, and fetal bovine sera were obtained from Life Technologies (Grand Island, NY). Coverslips were purchased from Bellco Glass, Inc. (Vineland, NJ). Enzymes, corticotropin(1–24), MgATP, NaATP, NaUTP, 5-adenylylimido-diphosphate (AMP-PNP, lithium salt), NaGTP, guanosine-5′-*O*-(2-thio)diphosphate, BAPTA, and pimozide were obtained from Sigma Chemical Company (St. Louis, MO). Penfluridol and fluspirilene were obtained from Janssen Pharmaceuticals (Beerse, Belgium).

Isolation and Culture of AZF Cells. Bovine adrenal glands were obtained from steers (age range, 1 to 3 years) within 30 min of slaughter at a local slaughterhouse. Fatty tissue was removed immediately and the glands were transported to the laboratory in ice-cold phosphate-buffered saline containing 0.2% dextrose. Isolated AZF cells were prepared as described previously (Enyeart et al., 1996). After isolation, cells were either resuspended in Dulbecco's modified Eagle's medium/Ham's F12 medium (1:1) with 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and antioxidants 1 μM tocopherol, 20 nM selenite, and 100 μM ascorbic acid and plated for immediate use, or resuspended in fetal bovine serum/5% dimethyl sulfoxide, divided into 1-ml aliquots, each containing about 2×10^6 cells, and stored in liquid nitrogen for future use. Cells were plated in 35-mm dishes containing 9-mm^2 glass coverslips that had been treated with 10 μg/ml fibronectin at 37°C for 30 min then rinsed with warm, sterile phosphate-buffered saline immediately before adding cells. Dishes were maintained at 37°C in a humidified atmosphere of 95% air/5% CO_2.

Patch-Clamp Experiments. Patch-clamp recordings of K^+-channel currents were made in the whole-cell configuration. The standard pipette solution was 120 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 20 mM HEPES, 11 mM BAPTA, 200 μM GTP, and 5 mM MgATP, pH buffered to 7.2 using KOH. Deviations from the standard solution are described in the text. The external solution consisted of 140 mM NaCl, 5 mM KCl, 2 mM CaCl, 2 mM $MgCl_2$, 10 mM HEPES, and 5 mM glucose, pH buffered to 7.4 using NaOH. All solutions were filtered through 0.22-μm cellulose acetate filters. Drugs were applied externally by bath perfusion controlled manually by a six-way rotary valve.

AZF cells were used for patch-clamp experiments 2 to 12 h after plating. Typically, cells with diameters of <15 μm and capacitances of 8 to 15 pF were selected. Coverslips were transferred from 35-mm culture dishes to the recording chamber (volume, 1.5 ml), which was continuously gravity-perfused at a rate of 3 to 5 ml/min. To minimize series resistance errors, patch electrodes with resistances of <1.5 MΩ were fabricated from Corning 0010 glass (Garner Glass Co., Claremont, CA). These routinely yielded access resistances of <3 MΩ. K^+ currents were recorded at room temperature (22–25°C) following the procedure of Hamill et al. (1981) using an Axopatch 1D patch-clamp amplifier (Axon Instruments, Inc., Burlingame, CA).

Pulse generation and data acquisition were done using a personal computer and PCLAMP software with a TL-1 interface (Axon Instruments). Currents were digitized at 5 to 20 kHz after filtering with an eight-pole Bessel filter (Frequency Devices, Haverhill, MA). Linear leak and capacity currents were subtracted from current records using scaled hyperpolarizing steps of one-third to one-fourth amplitude. Data were analyzed and plotted using pCLAMP 5.5 and 6.04 (Clampan and Clampfit) and SigmaPlot (ver 4.0; SPSS, Chicago, IL).

Results

Reciprocal Effects of ATP on I_A and I_{AC}. Differences in the nucleotide dependence and the voltage-dependent gating and kinetics of I_A and I_{AC} K^+ channels allow them to be isolated and measured in whole-cell recordings. In this study, K^+ currents from AZF cells were elicited using either of two voltage clamp protocols. Voltage steps to +20 mV, applied from a holding potential of −80 mV, elicited combined I_A and I_{AC} currents (Fig. 1, left voltage protocol). With this protocol, I_{AC} could be measured near the end of the voltage step at a time when I_A current had completely inactivated. Identical voltage steps preceded by a 10 s prepulse to −20 mV inactivate I_A channels, allowing I_{AC} current to be recorded and measured in isolation (Fig. 1, right voltage protocol).

When pipette solutions contained ATP at concentrations ≤1 mM, I_{AC} was poorly expressed, as reported previously (Enyeart et al., 1997), and voltage steps to +20 mV primarily activated the rapidly inactivating bKv1.4 current (I_A), the amplitude of which remained nearly constant over many minutes of recording (Fig. 1, left traces). In these experiments, isolation of I_{AC} with a depolarizing prepulse confirmed that this K^+ current was very small (<50 pA) and did not increase during the course of the experiment (Fig. 1A, right traces and graph). Overall, with pipettes containing 1 mM MgATP, I_A showed no measurable decrease in whole-cell recordings lasting from 15 to 25 min ($n = 6$).

In recordings made with pipettes containing ATP at concentrations >1 mM, I_{AC} typically increases dramatically over a period of minutes, as reported (Enyeart et al., 1997; Xu and Enyeart, 2001). In the experiment illustrated in Fig. 1B, I_{AC} increased more than 20-fold to a maximum of >2630 pA during 17 min of recording with a patch pipette containing 5 mM MgATP (middle trace). In whole-cell recordings, I_{AC} appears as a noninactivating current composed of an instantaneous and a smaller, time-dependent component (Enyeart et al., 1997).

Furthermore, in recordings such as those shown in Fig. 1B, left traces, the combined I_A plus I_{AC} K^+ current recorded when I_{AC} had reached a maximum value was less than predicted by the simple addition of the initial I_A current to the maximum I_{AC} current. Specifically, it appeared as if I_A amplitude had been reduced.

Digital subtraction of I_{AC} currents from combined I_A plus I_{AC} currents demonstrated that the development of I_{AC} current is accompanied by a decrease in the amplitude of I_A current. In the experiment illustrated in Fig. 2A, combined currents ($I_A + I_{AC}$) and isolated I_{AC} current are shown immediately after initiating whole-cell recording (T_o) and then approximately 20 min later, when I_{AC} had reached a stable maximum value (T_{MAX}). During this interval, I_{AC} grew from its initial value of 120 pA to a maximum amplitude of 2451 pA, while peak I_A current decreased from 2159 pA to 1081 pA during the same interval. I_A currents at T_o and T_{MAX} are superimposed for comparison in Fig. 2, top left traces. The difference between I_A and I_{AC} is shown as ΔI_A (1078 pA) and represents the quantity of I_A current that was lost between

T_o and T_{MAX}. In 27 experiments, I_{AC} amplitude increased by an average of 1208 ± 117 pA during whole-cell recordings, whereas peak I_A currents were reduced by 507 ± 58 pA (Fig. 2B). The magnitude of I_{AC} increase was positively correlated with I_A decrease with a correlation coefficient of 0.548 and a slope factor of 2.05 ± 0.20 (n = 27) (Fig. 2C).

Higher ATP concentration alone was insufficient to induce a time-dependent rundown of I_A. In some cells, I_{AC} K^+ current fails to grow dramatically even when pipettes contain ATP at concentrations greater than 1 mM. I_A current also showed little time-dependent decrease in these recordings. In a total of seven cells in which I_{AC} reached a maximum of <50 pA with 2 or 5 mM MgATP in the pipette, peak I_A current decreased only 2.5 ± 1.7% during recordings lasting at least 15 min.

Time Dependence of Reciprocal Changes in K^+-Current Amplitudes. If the development of I_{AC} in whole-cell recordings is coupled to a decrease in the number of functional I_A channels, then the temporal pattern for these reciprocal changes should be similar. Figure 3 shows that the time-dependent development of I_{AC} was paralleled by a corresponding decrease in I_A. Over a 15-min period, I_{AC} grew gradually from an initial value of 150 pA to a maximum amplitude of 1110 pA. Over this same time interval, I_A decreased monotonically from its initial value of 2375 pA to a final value of 1848 pA. Once I_{AC} reached a stable maximum amplitude, no further decrease in I_A was observed. Similar results were obtained in each of nine experiments.

Reciprocal Modulation of I_{AC} by Other Nucleotides. Other nucleotides, including the poorly hydrolyzable ATP analog AMP-PNP, UTP, and GTP, each activate I_{AC} when present in the recording pipette at millimolar concentrations (Enyeart et al., 1997; Xu and Enyeart, 2001). The time-dependent increases in I_{AC} amplitude observed with these nucleotides in the pipette were also accompanied by a corresponding decrease in I_A current. In the experiment illustrated in Fig. 4, increases in I_{AC} current observed with AMP-PNP (1 mM) and GTP (5 mM) resulted in reductions of I_A of 599 pA and 362 pA, respectively, at T_{MAX}. Similar results were obtained in each of eight experiments with pipettes containing AMP-PNP, GTP, or UTP.

Selective Block of I_{AC} Reveals Reduction of I_A. Digital subtraction of I_{AC} from combined (I_A+ I_{AC}) currents indicated that the growth of I_{AC} in whole-cell recordings was accompanied by a simultaneous decrease in I_A current. This point was demonstrated by a second method using diphenylbutylpiperidine (DPBP) antipsychotics that potently and selectively block I_{AC} channels in AZF cells. The DPBPs pimozide, penfluridol, and fluspirilene inhibit I_{AC} channels with IC_{50} values of 0.35, 0.19, and 0.23 μM, respectively, while ≅ 200-fold higher concentrations are required to inhibit I_A channels (Gomora and Enyeart, 1999).

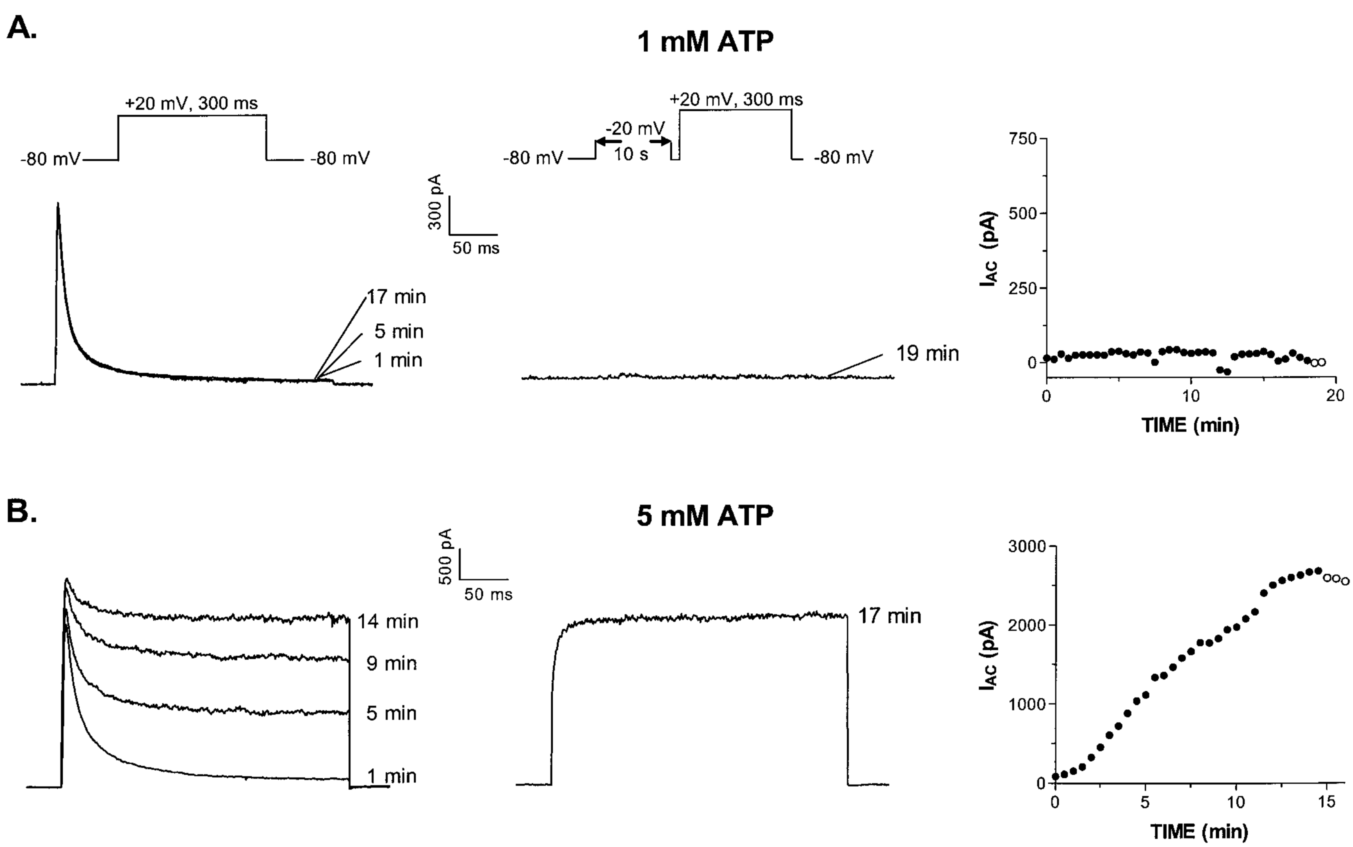

Fig. 1. Effect of ATP on the time-dependent expression of I_A and I_{AC} K^+ currents. Whole-cell K^+ currents were recorded from bovine AZF cells at 30-s intervals with pipettes containing 1 or 5 mM MgATP in response to voltage steps to +20 mV, applied from a holding potential of −80 mV, with (right traces) or without (left traces) 10-s prepulses to −20 mV. A, 1 mM ATP: K^+ currents were activated with either of the illustrated voltage protocols over 19 min. Left and middle traces show currents at indicated times. I_{AC} amplitudes recorded with (○) and without (●) depolarizing prepulses are plotted at right. B, 5 mM ATP: K^+ currents were activated with either of the voltage protocols over 17 min. Left and middle traces show currents at indicated times. Shown at right are I_{AC} amplitudes recorded with (○) and without (●) depolarizing prepulses.

In the experiment shown in Fig. 5, I_{AC} was allowed to grow to a maximum value before superfusing the cell with pimozide (2.5 μM) to selectively inhibit I_{AC}. Digital subtraction of I_{AC} from combined K^+ currents indicated that I_A decreased by 509 pA, or 26%, between T_o and T_{MAX}. Measurement of I_A after this cell was superfused with pimozide (2.5 μM) at a concentration that inhibits I_{AC} almost completely and reduces I_A by approximately 5% showed a reduction in I_A at T_{MAX} by an amount similar to that calculated by digital subtraction of I_{AC} from the combined current. The additional small reduction of I_A current from 1433 to 1322 pA would probably occur through a direct action of pimozide on I_A channels. In a total of nine cells, determined from digital subtraction, I_A at T_{MAX} was reduced to 63.3 ± 5.4% of its original amplitude. By comparison, direct measurement of I_A in these same cells after preferential block of I_{AC} with DBPBs at 2.5 μM showed that I_A was reduced to 52.4 ± 4.3% (n = 9) of its original value. Most of this difference can be attributed to a direct inhibition of a small fraction of I_A channels by the DPBPs.

Inhibition of I_{AC} by Corticotropin is Coincident with Recovery of I_A. Although I_{AC} is activated in the presence of intracellular nucleotides, this current is potently inhibited (IC_{50} = 5.4 pM) by corticotropin (Mlinar et al., 1993). Interestingly, the inhibition of I_{AC} by corticotropin appeared to differ significantly from that observed with the DPBPs. Specifically, the corticotropin-mediated inhibition of I_{AC} was accompanied by recovery of I_A current that was lost during the development of I_{AC}.

In the experiment illustrated in Fig. 6A, I_{AC} grew to a maximum value of nearly 1600 pA after 16 min of whole-cell recording (T_{MAX}). During this same interval, I_A decreased by 766 pA to 70% of its initial value. Superfusion of this cell with corticotropin (200 pM) inhibited I_{AC} almost completely, and I_A amplitude increased by 346 pA to 84% of its initial value. Similar results were obtained in each of 10 experiments, where inhibition of I_{AC} by corticotropin was associated with an increase in I_A from 71 ± 4% to 89 ± 4% of its original value (Table 1).

The corticotropin-stimulated increase in I_A current seemed

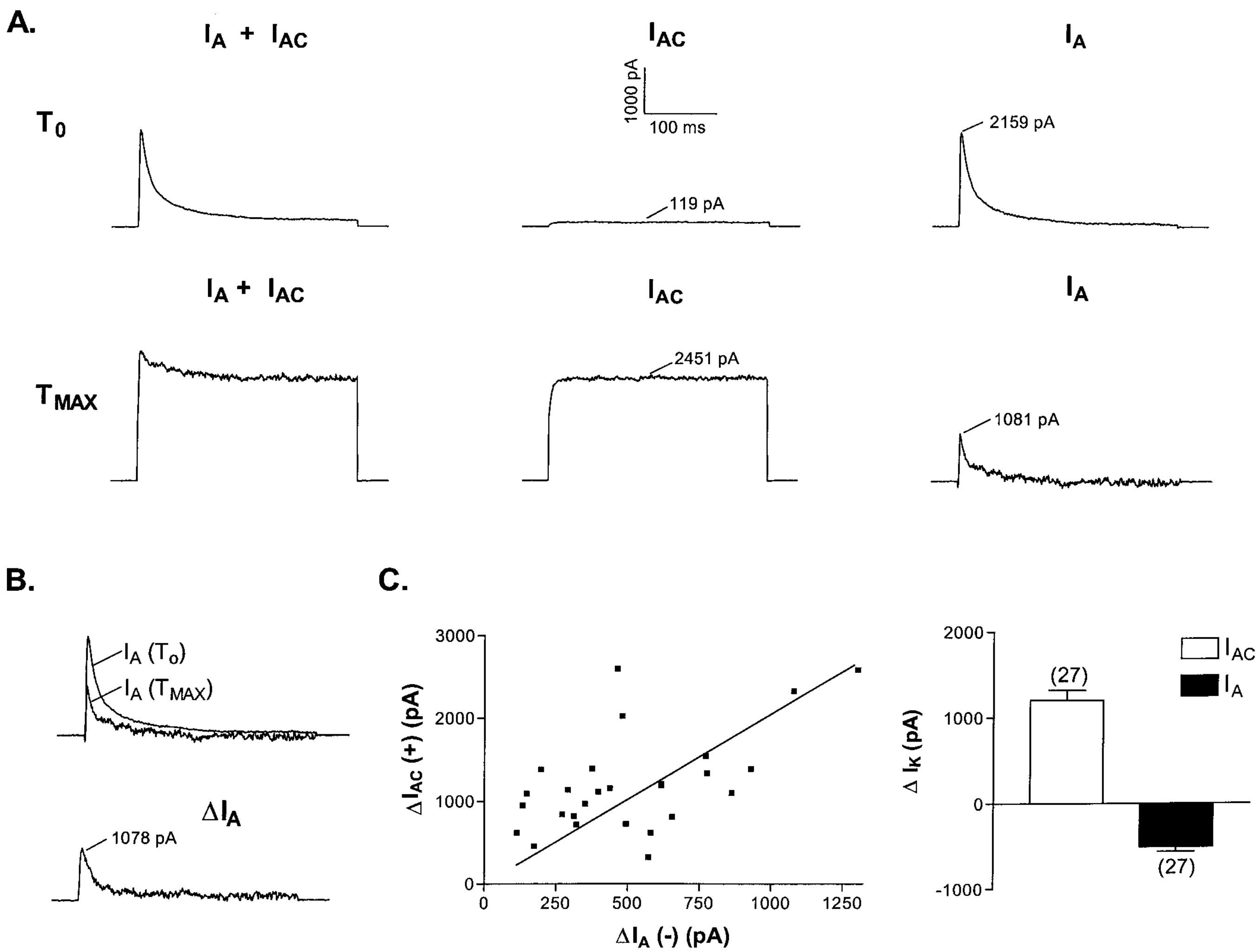

Fig. 2. Reciprocal relationship between I_A and I_{AC} K^+ currents. Combined I_A and I_{AC} or isolated I_{AC} K^+ currents were recorded using the two voltage protocols shown in the legend of Fig. 1. I_A current traces were determined initially (T_o) and after I_{AC} had reached a stable maximum amplitude (T_{MAX}) by point-for-point digital subtraction of I_{AC} current traces from combined I_A + I_{AC} current traces. A, combined K^+ currents (I_A + I_{AC}) and isolated I_{AC} currents were recorded at T_o and T_{MAX}. I_A traces were obtained as described above by digital subtraction. B, ΔI_A was determined from digital subtraction of I_A (T_{MAX}) from I_A (T_o). C, comparison of time-dependent increases in I_{AC} versus decreases in I_A K^+ current in AZF cells obtained from current measurements at T_o and T_{MAX}. Maximum decreases in I_A (ΔI_A) are plotted versus corresponding increases in I_{AC} (41 I_{AC}) for each of 27 cells. Linear regression analysis yielded a slope factor of 2.05 ± 0.20 and a correlation coefficient of 0.548. Changes in K^+ currents ΔI_K) are plotted at right as mean ± S.E.M.

to be tightly linked to the inhibition of I_{AC} current. When whole-cell recordings were made with pipette solutions containing low ATP (≤ 1 mM) to retard the development of I_{AC} current, subsequent superfusion of corticotropin did not significantly increase the amplitude of I_A K^+ current in any of the eight cells tested (Fig. 6B).

Reciprocal Changes in I_A K^+ Current are Independent of Voltage. The decrease in I_A current amplitude associated with development of I_{AC} and the corticotropin-stimulated increase in I_A current associated with I_{AC} inhibition were found to be present over a wide range of test potentials. In the experiments illustrated in Fig. 7A, current-voltage relationships were first obtained in control saline immediately after initiating whole-cell recording (left traces). After I_{AC} had grown to a stable maximum value of at least 500 pA, cells were superfused with corticotropin (200 pM) or penfluridol (2.5 μM), producing nearly complete inhibition of I_{AC} (middle traces), and current-voltage relationships were again recorded (right traces).

Penfluridol inhibits I_{AC} and I_A with respective IC_{50} values of 187 nM and 42 μM (Gomora and Enyeart, 1999). At a concentration of 2.5 μM, penfluridol (2.5 μM) produces nearly complete inhibition of I_{AC} and reduces I_A by approximately 5%. Current-voltage relationships obtained after block of I_{AC} by penfluridol showed that I_A had decreased by 39 to 45% at potentials between −10 and +50 mV, compared with respective control values (n = 4) (Fig. 7). Besides demonstrating that the reduction of I_A current coincident with I_{AC} development is not voltage dependent, these results also indicate that it is not an artifact attributable to a spontaneous rightward shift in the voltage dependence of I_A activation. Accordingly, when the I_A current values shown in Fig.

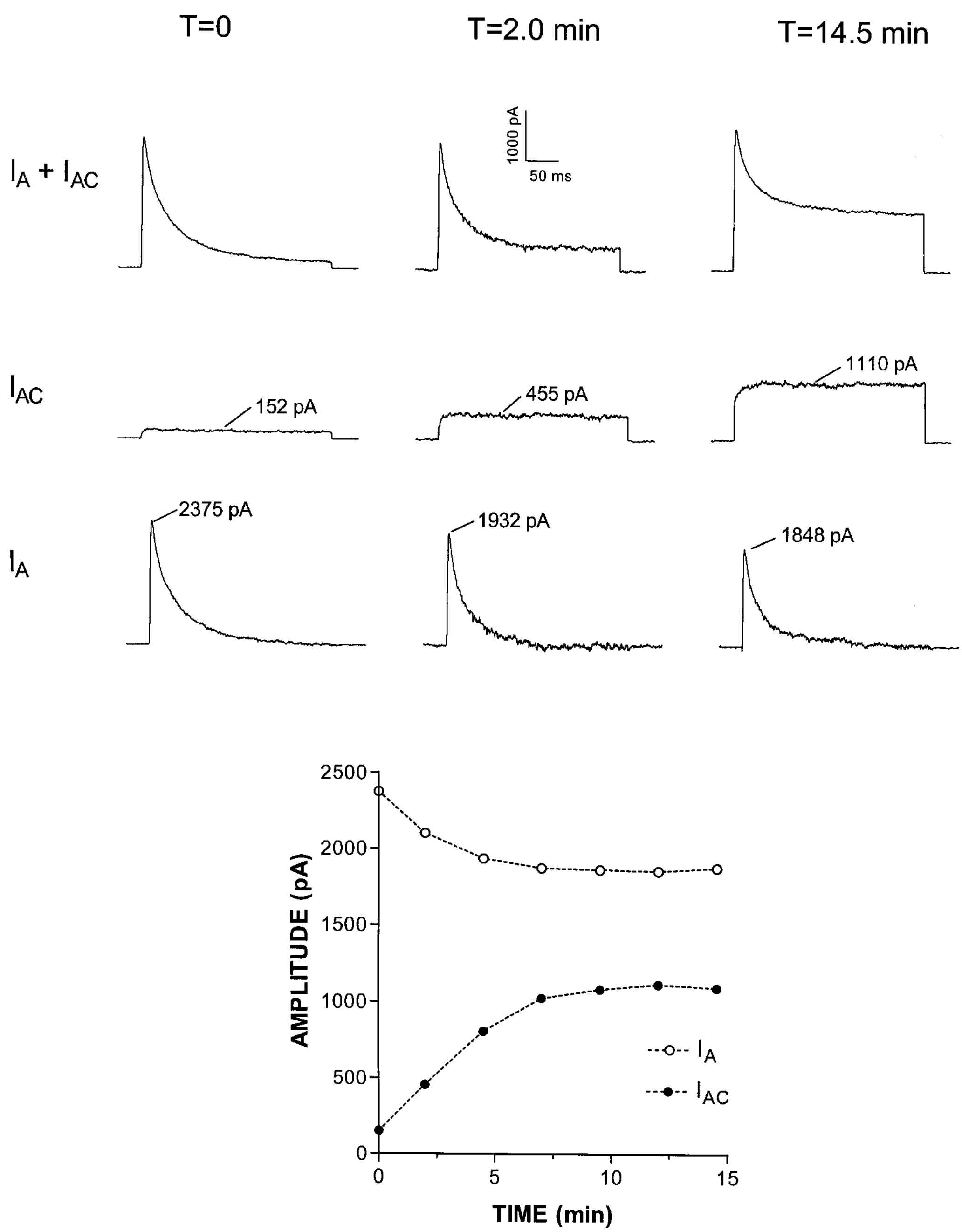

Fig. 3. Temporal pattern for reciprocal changes in I_A and I_{AC} K^+ currents. The time-dependent changes in I_A and I_{AC} currents were monitored at seven time points during a 15-min recording. At each time point, K^+ currents were recorded in the absence (I_A + I_{AC}) and presence (I_{AC}) of 10-s depolarizing steps to −20 mV as described in the legend of Fig. 1. I_A current at each time was determined by digital subtraction of I_{AC} from combined I_A + I_{AC} currents. Traces show measured and calculated currents immediately after initiating recording and at t = 2 and 14.5 min. Measured I_{AC} and calculated I_A currents are plotted against time at bottom.

7B were normalized against the maximum, both before and after penfluridol, and then plotted on the same graph, these values were nearly identical, indicating no shift in voltage dependence (data not shown).

In contrast to the voltage-independent reduction of I_A current associated with I_{AC} development that was unmasked by selective inhibition of I_{AC} current with penfluridol, the nearly complete inhibition of I_{AC} current with corticotropin (200 pM) was not accompanied by a decrease in I_A current over the entire range of test voltages. In four experiments, inhibition of I_{AC} current with 200 pM corticotropin produced I_A currents that did not differ significantly from control currents over the entire range of test potentials. This result demonstrates that I_A current lost during the development of I_{AC} is

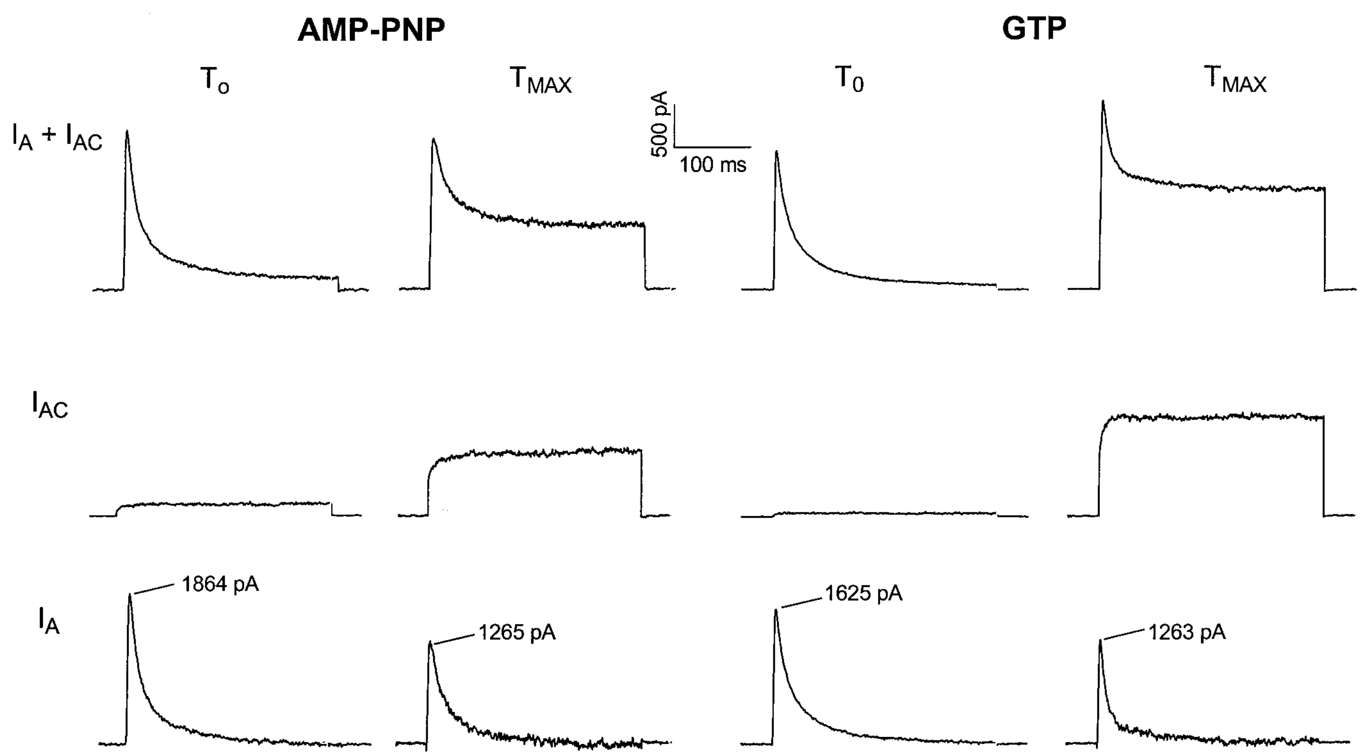

Fig. 4. Reciprocal effects of AMP-PNP and GTP on I_A and I_{AC} currents. Patch pipettes containing AMP-PNP (2 mM) or GTP (5 mM) were used to record combined ($I_A + I_{AC}$) and isolated I_{AC} K^+ currents at T_o and T_{MAX} with the two voltage protocols described in the legend of Fig. 1. I_A traces were obtained by digital subtraction of I_{AC} current traces from combined current traces.

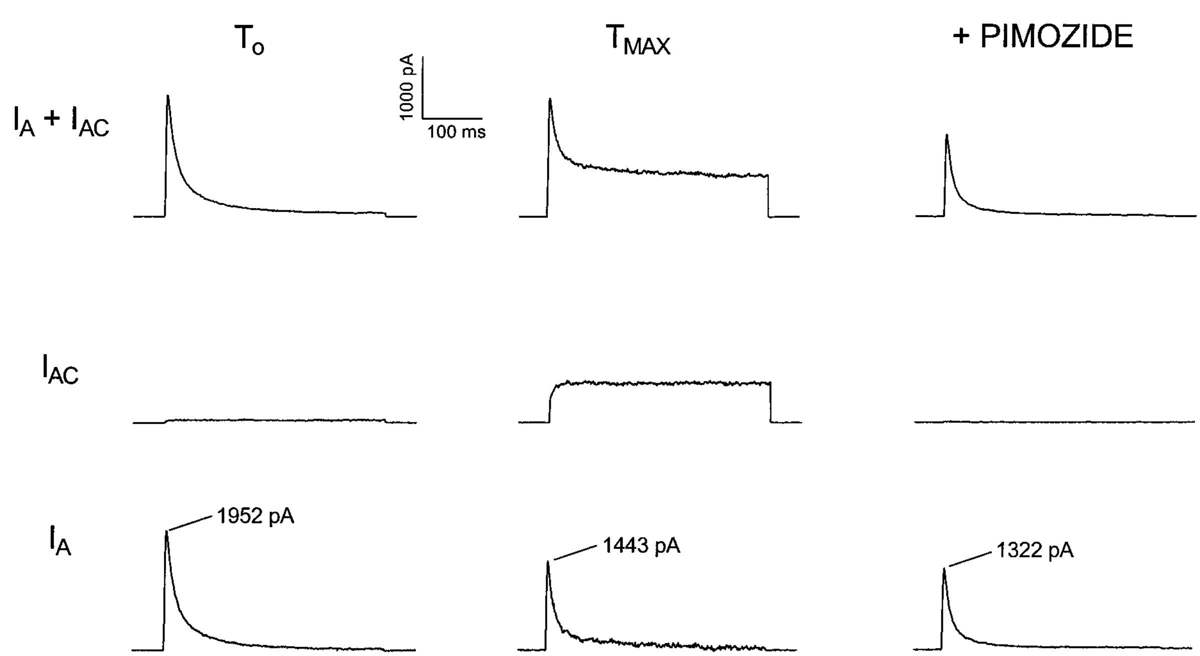

Fig. 5. Reciprocal relationship between I_A and I_{AC} K^+ channels revealed by pimozide. Combined ($I_A + I_{AC}$) and isolated I_{AC} K^+ currents were recorded using the two voltage protocols described in the legend of Fig. 1 initially (T_o), when I_{AC} reached a maximum value (T_{MAX}) and after steady-state block of I_{AC} K^+ current by 2.5 μM pimozide. I_A current traces were obtained by digital subtraction of isolated I_{AC} currents from combined K^+ currents.

restored over a range of test potentials when corticotropin inhibits I_{AC}.

Discussion

In this study, we discovered that the activity of voltage-gated rapidly inactivating bKv1.4 A-type K^+ channels and noninactivating "background" K^+ channels in AZF cells were reciprocally modulated through intracellular nucleotides and G-protein-coupled corticotropin receptors. Specifically, in whole-cell recordings, the nucleotide-dependent increase of I_{AC} K^+ channel activity was accompanied by a coincident decrease in the number of functional I_A channels. Conversely, the nearly complete inhibition of I_{AC} current by corticotropin was associated with the reappearance of functional I_A channels. Regardless of the mechanism that may couple these two K^+ channels, this is the first report demonstrating the modulation of I_A activity by nucleotides and corticotropin.

Model for I_A-I_{AC} Coupling. Overall, the results of the current study in combination with our previous work on I_{AC} suggest a novel form of channel modulation in which the activity of I_A and I_{AC} K^+ channels is reciprocally coupled in a dynamic equilibrium. In this model, shown in Fig. 8, the binding of ATP or other nucleotides to the I_{AC} channel, or associated protein, increases the number of active I_{AC} channels and reduces the number of functional I_A channels. In contrast, the activation of corticotropin receptors shifts the equilibrium in the reverse direction,

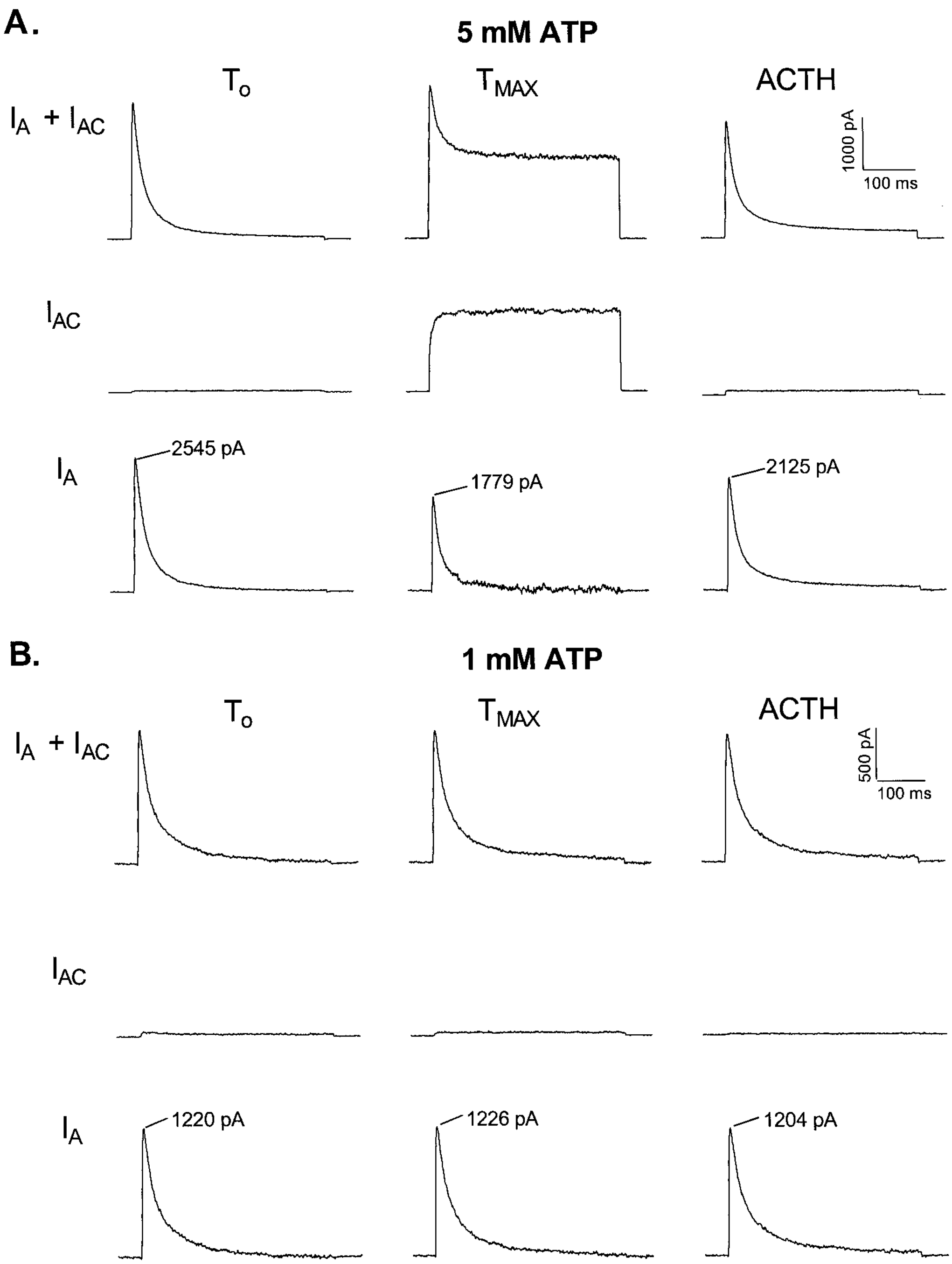

Fig. 6. Corticotropin-stimulated inhibition of I_{AC} and recovery of I_A K^+ current. Combined (I_A + I_{AC}) and isolated I_{AC} K^+ currents were recorded using the two voltage protocols described in the legend of Fig. 1 with pipettes containing 5 mM (A) or 1 mM ATP (B). Traces show combined and isolated currents recorded immediately after initiating recording (T_o), after I_{AC} reached a maximum value (T_{MAX}), and after steady-state inhibition of I_{AC} by corticotropin. I_A current traces were obtained by digital subtraction of isolated I_{AC} currents from combined K^+ currents.

reducing the number of active I_{AC} channels and increasing the pool of I_A channels.

Considerable evidence supporting the model for tight reciprocal coupling between the two different K^+ channels was presented. The nucleotide-induced increase in I_{AC} channel activity was consistently paralleled in time by a decrease in I_A current. Conversely, in the absence of I_{AC} growth, I_A amplitude remained nearly constant over many minutes.

TABLE 1
Effect of DPBPs and corticotropin on I_A K^+ current

I_A K^+ current amplitudes are expressed as a percentage of its initial value (T_o), after I_{AC} had reached a maximum value (T_{MAX}) and after superfusion of a DPBP or corticotropin (Treatment). Values are expressed as mean ± S.E.M.

	I_A (T_0)	I_A (T_{MAX})	I_A (Treatment)
		%	
DPBP	100	63 ± 5	52 ± 4 (*n*= 9)
Corticotropin	100	71 ± 4	89 ± 4 (*n*=10)

Furthermore, the inhibition of I_{AC} current by corticotropin was always accompanied by the recovery of I_A current in whole-cell recordings. In the absence of I_{AC} current, corticotropin has no effect on I_A K^+ current. Despite these findings, questions remain regarding the nature of the relationship that links these two K^+ channels.

Molecular Basis of K^+-Channel Coupling. Our findings, as depicted in Fig. 8, suggest that I_A and I_{AC} K^+ channels are physically linked by signaling pathways involving nucleotide binding and the G-protein-coupled corticotropin receptor. In previous studies, we showed that I_{AC} channels' activity was enhanced by hydrolyzable and nonhydrolyzable nucleotides, as well as polytriphosphates (Enyeart et al., 1997; Xu and Enyeart, 2001). Furthermore, inhibition of I_{AC} channels by corticotropin was independent of A-kinase but required hydrolyzable forms of ATP (Enyeart et al., 1996). Taken together, these results sug-

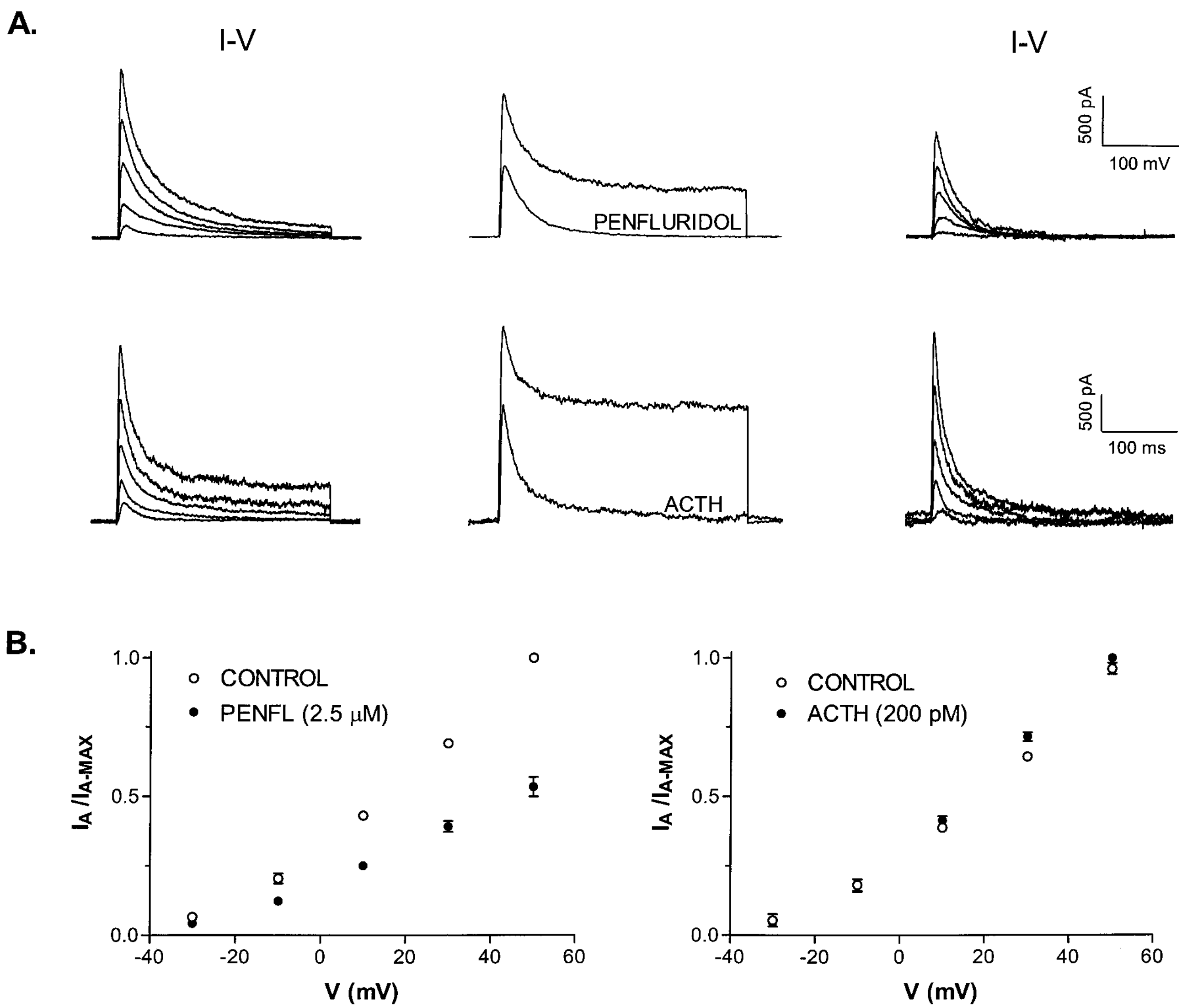

Fig. 7. Reciprocal relationship between I_A and I_{AC} K^+ currents is voltage-independent. The current-voltage (I-V) relationship was obtained immediately after initiating whole-cell recording by applying voltage steps to test potentials between −30 and +50 mV. I_{AC} was then allowed to reach a maximum amplitude before superfusing the cell with penfluridol (2.5 μM) or corticotropin (200 pM). I-V relationships were again obtained after steady-state block was reached. A, traces show initial I-versus (left), combined I_A + I_{AC} currents before (1) and after (2) superfusion of corticotropin or penfluridol as indicated (middle traces), and I-V after steady-state block by corticotropin or penfluridol (right traces). B, I-V plots: maximum peak currents are plotted against test potential before and after superfusion of penfluridol (left) or corticotropin (right). Results are mean ± S.E.M. for four separate determinations.

gest a model for I_{AC} gating that involves an ATP hydrolysis cycle: channel activity is enhanced by the binding of ATP and inhibited through corticotropin-stimulated ATP hydrolysis.

In the present study, we found that in addition to enhancing I_{AC} channel activity, hydrolyzable and nonhydrolyzable nucleotides also promoted the "rundown" of I_A K^+ currents. Furthermore, corticotropin-mediated inhibition of I_{AC} was accompanied by an increase in I_A. Taken together, these results suggest that the reciprocal, coupled gating of both of these K^+ channels could be mediated through a cycle of ATP binding and hydrolysis involving G-protein-coupled receptors.

The molecular basis for functional coupling between I_A and I_{AC} K^+ channels is unknown. Perhaps these two K^+ channels exist within a protein complex in the plasma membrane in close association with corticotropin receptors. The binding and hydrolysis of ATP might be linked to the shuttling of a common, shared subunit, leading to the activation of one K^+ channel and the coincident inactivation of the coupled channel.

In this regard, auxiliary subunits that could modulate the function of I_A and I_{AC} K^+ channels are yet to be identified. The molecular identity of the primary ± subunit of I_{AC} channels is also unknown. Unlike other ATP-gated K^+ channels, I_{AC} channels are insensitive to sulfonylureas (Gomora and Enyeart, 1999). Thus, it is unlikely that these channels include a sulfonylurea receptor as the β subunit. No β subunit common to voltage-gated and background K^+ channels has yet been identified. In this regard, most of the background K^+ channels that set the resting membrane potential in mammalian cells belong to a large family of two-pore, four-membrane-spanning channels for which no auxiliary subunits have been described (Goldstein et al., 1998).

Stoichiometry of K^+-Channel Coupling. If I_A and I_{AC} K^+ channels are in close proximity within functional complexes of the AZF cell membrane, the channel number and stoichiometric ratio will be an important consideration. In this regard, the average increase in macroscopic I_{AC} current was 2.38 times larger than the corresponding mean decrease in I_A K^+ current in the same experiments. This data might

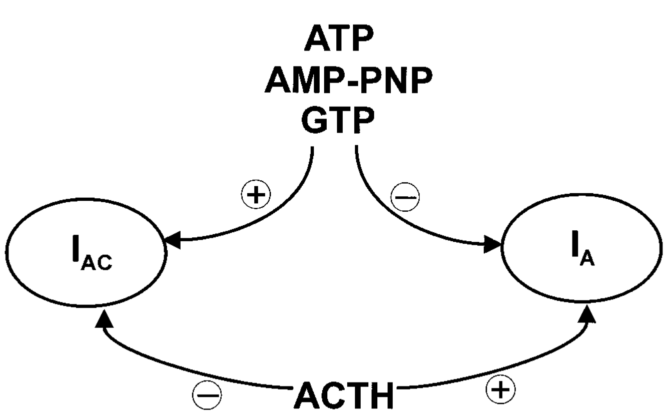

Fig. 8. Model for reciprocal modulation of I_{AC} by nucleotides and corticotropin. Schematic depicts the reciprocal coupling of I_A and I_{AC} K^+ channels controlled by nucleotides and corticotropin. Nucleotides, including ATP, AMP-PNP, and GTP increase the number of active I_{AC} channels and simultaneously decrease the number of active I_A channels. Conversely, corticotropin reduces the number of functional I_{AC} channels and increases the pool of available I_A channels.

suggest that the stoichiometry involved in I_A-I_{AC} channel coupling could be calculated by comparing the observed changes in the two macroscopic currents to the relative unitary conductances measured under similar conditions. For example, if the two K^+ channels are functionally coupled in a one-to-one stoichiometry, the ratio of the measured changes in the macroscopic K^+ currents might be equal to the ratio of unitary current amplitudes for I_A and I_{AC} channels.

However, macroscopic currents are a product of NP_oî, where N is the number of functioning channels, P_o is the channel open probability, and î is the unitary current. Therefore, even if the activity of I_A and I_{AC} channels were tightly coupled in a 1:1 reciprocal relationship, this would not be evident from macroscopic recordings unless P_o was identical for the two channels. Because P_o is generally quite variable, it is unlikely that averaged P_o values for I_A and I_{AC} channels would be equal in a single cell. Accordingly, although they were positively correlated, considerable variability was present in the ratios of measured I_{AC} increases to I_A decreases measured from cell to cell. Nevertheless, the average ratio of 2.38 for I_{AC} increase compared with I_A decrease is consistent with the fact that the unitary I_{AC} current amplitude is severalfold larger than unitary I_A currents measured under similar conditions (Latorre and Miller, 1983; Xu and Enyeart, 2001).

Although corticotropin (200 pM) inhibited I_{AC} almost completely, the corresponding recovery of I_A was often less efficient. Again, this could be caused by time-dependent decreases in P_o for I_A channels, as a result of rightward shifts in the voltage dependence of activation that can occur with cell dialysis. Alternatively, it is possible that the inhibition of an I_{AC} channel by corticotropin is not absolutely tied to the activation of a coupled I_A channel.

Functional Significance. In addition to corticotropin receptors, bovine AZF cells express several other receptors, the activation of which is coupled to I_{AC} inhibition and membrane depolarization (Mlinar et al., 1993; Mlinar et al., 1995; Xu and Enyeart, 1999a,b). It will be interesting to determine whether the inhibition of I_{AC} by angiotensin II, external ATP, and adenosine is also linked to an increase in the number of available I_A channels.

Regardless, the results of this study describe a novel form of channel modulation where the activity of voltage-gated and metabolically regulated background K^+ channels are reciprocally controlled in tandem by intracellular ATP and a G-protein-coupled peptide hormone receptor. How this unique form of ion channel modulation functions in the physiology of cortisol secretion has not been determined.

In a previous study, we showed that corticotropin-stimulated increases in cortisol secretion from bovine AZF cells require Ca^{2+} entry through low-voltage activated T-type Ca^{2+} channels (Enyeart et al., 1993). Perhaps, the opposing action of corticotropin on I_A and I_{AC} K^+ channels promotes electrical activity such as an oscillating membrane potential that maximizes Ca^{2+} entry through the rapidly inactivating T-type channels.

It is not known whether K^+ channels in other types of cells might be functionally linked as they are in AZF cells. In cell-attached patch recordings from dendrites of rat hippocampal neurons, arachidonic acid was found to reduce the amplitude of a transient K^+ current and increase that of a sustained K^+ current (Colbert and Pan, 1999). Perhaps the activity of these two K^+ channels could also be linked in a reciprocal relationship. Coupled modulation of voltage- and non–voltage-gated K^+ channels could represent a new form of modulation operating in a wide range of cells.

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