A-type potassium current modulated by A_1 adenosine receptor in frog melanotrophs

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1. Transient outward current was recorded in cultured frog melanotrophs with the whole-cell configuration of the patch-clamp technique. The ionic dependence, kinetics and pharmacological properties of the current were studied. The effects of the A_1 adenosine receptor agonist *R*-N^6-phenylisopropyl-adenosine (*R*-PIA) on this current were also investigated.

2. In tetrodotoxin- and cobalt-containing solution, depolarization from −120 mV elicited both transient and delayed outward currents. Pulses from −60 mV activated only a sustained late current.

3. 4-Aminopyridine (4 mM) reduced the transient outward current much more than the delayed outward current. In contrast, tetraethylammonium (10–20 mM) selectively reduced the delayed current.

4. Tail current measurements showed a positive shift in the reversal potential when external K^+ concentration was increased, indicating that K^+ was the predominant charge carrier.

5. Steady-state inactivation was complete at potentials positive to −10 mV and removed by hyperpolarization.

6. Inactivation of the transient current was slowed and accelerated in oxidizing and reducing conditions, respectively, confirming the involvement of an inactivating 'ball and chain' peptide.

7. *R*-PIA increased the transient current. The steady-state inactivation curve was shifted towards more positive potentials without changing the activation kinetics. Pretreatment with pertussis toxin (1 μg ml^{-1}) blocked the response to *R*-PIA.

8. It is concluded that frog melanotrophs possess an A-type current that is likely to play an important role in excitability. This current, which is directly modulated by A_1 adenosine receptors through a G_i/G_o protein, appears to be responsible for the inhibitory effects of adenosine on electrical activity.

Adenosine is recognized as an important neuromodulator or neurotransmitter in the central and peripheral nervous systems (Phillis & Wu, 1981; Simpson, O'Regan, Perkins & Phillis, 1992). Adenosine also regulates pituitary hormone secretion (Dorflinger & Schonbrunn, 1985; Anand-Srivastava, Cantin & Gutkowska, 1989). In particular, adenosine is a potent inhibitor of α-melanocyte-stimulating hormone (α-MSH) release from frog pituitary melanotrophs (Chartrel, Tonon, Lamacz & Vaudry, 1992).

The effects of adenosine on A_1 adenosine receptors are mediated via different transduction pathways, including inhibition of calcium currents (Zhu & Ikeda, 1993) and activation of potassium conductances (Trussell & Jackson, 1987; Alzheimer & ten Bruggencate, 1991). Neurones and secretory cells express multiple K^+ channels which collectively regulate membrane potential, determine action potential frequency and duration, and thereby control transmitter and hormone release (Shimahara, 1983; Augustine, 1990). Among the different K^+ currents, the Q- and M-currents regulate the resting membrane potential (Halliwell & Adams, 1982; Spain, Schwindt & Crill, 1987) while the delayed rectifier and A-type currents influence

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repolarization and discharge behaviour of action potentials (Connor & Stevens, 1971; Madison & Nicoll, 1984).

A number of studies have demonstrated that adenosine can modulate voltage-independent and voltage-dependent potassium conductances (Trussel *et al.* 1987; Bennett & Ho, 1992). Until recently, however, little was known about the effects of adenosine on the transient outward A-current (Pan, Osmanovic & Shefner, 1994). In a previous electrophysiological study, we have shown that adenosine and the A_1 receptor agonist *R*-PIA inhibit spontaneous action potentials in cultured frog melanotrophs (Mei, Vaudry & Cazin, 1994), suggesting that modulation of potassium conductances could be involved in the mechanism of action of adenosine. In the present report, we explored the possibility that an A-type current exists in frog melanotrophs and that adenosine could activate this current.

METHODS

Cell cultures and reagents

Primary cultures of frog pituitary melanotrophs were performed as described previously (Louiset, Cazin, Lamacz, Tonon & Vaudry, 1988; Valentijn, Louiset, Vaudry & Cazin, 1991). Adult male frogs, *Rana ridibunda* (Couétard, St Hilaire de Riez, France) were killed by cervical dislocation and decapitated. The neurointermediate lobes of the pituitary were dissected and washed in Leibowitz L-15 culture medium adjusted to *Rana ridibunda* osmolality and supplemented with $CaCl_2$ (0·1 g l^{-1}), glucose (0·2 g l^{-1}), and a kanamycin and antimycotic–antibiotic solution (1% v/v). The tissues were then enzymatically dissociated in the same medium containing 0·15% protease Type IX and 0·15% collagenase Type IA, for 15 min at room temperature. After mechanical dispersion, the cells were centrifuged (50 *g*) for 5 min, rinsed three times and suspended in Leibowitz medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics. Cells were then plated at a density of 10 000 cells per 35 mm tissue culture dishes. Cultured cells were incubated at 26 °C and used 5–10 days after plating.

Leibowitz medium, protease and collagenase were purchased from Sigma. Kanamycin, the antibiotic–antimycotic solution and fetal calf serum were obtained from Boehringer Mannheim. Tissue culture dishes were supplied by Costar (Cambridge, MA, USA). *R*-N^6-Phenylisopropyl-adenosine (*R*-PIA), tetrodotoxin (TTX), tetraethylammonium (TEA), 4-aminopyridine (4-AP), pertussis toxin (PTX), guanosine triphosphate (GTP) and ethylene glycol tetraacetic acid (EGTA) were purchased from Sigma.

Electrophysiological recordings and analysis

Electrophysiological recordings were performed at room temperature on 5- to 10-day-old cultured frog melanotrophs using the patch-clamp technique in the whole-cell configuration. For recording, the culture medium was replaced with a bathing solution containing (mM): NaCl, 112; KCl, 2; $CoCl_2$, 1; Hepes, 15; TTX, 0·001 (pH adjusted to 7·4 using NaOH). In some experiments, TEA (10 and 20 mM) was added to the external solution and the osmolality was adjusted by reducing the NaCl concentration. Soft glass patch electrodes were made on a vertical pipette puller (List Electronic) and the tip of the electrodes was fire polished. The solution used to fill patch pipettes had the following composition (mM): KCl, 100; $MgCl_2$, 2; GTP, 0·1; Hepes, 10; EGTA, 10 (pH adjusted to 7·4 using KOH). The resistance of the electrodes filled with this solution was 2–4 MΩ. *R*-PIA solutions (50 μM) were prepared extemporaneously and pressure-ejected for 10 s from a glass pipette in the vicinity of the cells under study. The bathing solution was continuously renewed using a peristaltic pump. In some experiments, the cells were pre-incubated with PTX (1 μg ml^{-1}) for 12 h before electrophysiological recording.

All current signals were recorded with an EPC-7 patch-clamp amplifier (List Electronic) operated in the voltage-clamp mode. Step voltage commands, data acquisition and analysis were performed with the aid of a computer running pCLAMP 5.51 software (Axon Instruments). The currents were corrected on-line for leak and residual capacitance transients by a P/4 protocol.

Quantitative data are given as means ± S.E.M. Statistical analysis was carried out using Student's *t* test.

RESULTS

Separation of the transient and delayed outward K^+ currents

Outward K^+ currents were evoked in a total of 121 melanotrophs by step depolarizations to +60 mV from a holding potential of either −60 or −120 mV. When the membrane potential was held at −60 mV, depolarizing voltage pulses evoked a slowly rising outward current. At depolarizing potentials ranging between +20 and +60 mV, the current reached a maximal amplitude within 30–50 ms and thereafter very slightly inactivated with time (Fig. 1*A*). This current resembled the late, slowly decaying outward K^+ current (I_K) which had previously been characterized (Louiset *et al.* 1988). When a 1 s hyperpolarizing prepulse from −60 to −120 mV was applied, the depolarizing voltage steps evoked an outward current which activated rapidly (5–10 ms) and then clearly decayed with time, particularly at depolarizing pulses above +10 mV (Fig. 1*B*). The decay phase was fitted with a single exponential function. Typically, the inactivation time constant became shorter at more depolarized potentials. The mean time constant values were 403·5 ± 21·5, 197·4 ± 28·3 and 107·7 ± 17·8 ms (means ± S.E.M.; $n = 9$) at 0, +30 and +60 mV, respectively. Addition of 4-AP (2 mM) to the extracellular solution produced a reversible decrease of the overall outward current with a predominant effect on the early inactivating current. At a lower concentration of 4-AP (1 mM), the current was unaffected (not shown). In contrast, at a higher concentration of 4-AP (4 mM), the inactivating component was virtually abolished to leave a residual sustained current (Fig. 1*C* and *D*). 4-AP affected the sustained component as well (Fig. 1*E*). The early current was reduced by 50–60% while the late current was inhibited by 20–30%. The percentage of inhibition of each

component was constant over the entire range of voltages tested (Fig. 1*F*).

In order to characterize the early component of the outward current evoked from a hyperpolarizing prepulse of −120 mV to various levels of depolarization, the cells were incubated with TEA which has been previously described as a relatively specific blocker of the delayed current (Louiset, Cazin, Lamacz, Tonon & Vaudry, 1988). Figure 2*A* illustrates a family of outward currents recorded in the absence and presence of 10 and 20 mM TEA. Addition of TEA to the external solution rapidly resulted in a marked acceleration of the inactivation and a reduction of the amplitude of the current. The speed-up of inactivation increased with the concentration of TEA (Fig. 2*B*). For this representative example, the decay phase of the current, evoked by step depolarization to +30 mV, was best fitted by a single exponential, whose time constants were 205·3, 87·2 and 51·4 ms at 0, 10 and 20 mM TEA, respectively. TEA strongly reduced the amplitude of the late current but had only a small effect on the early transient peak current. In all cells tested ($n = 20$), the effect of TEA was much more pronounced at a 20 mM than at a 10 mM concentration (Fig. 2*C* and *D*). Exposure of the cells to 20 mM TEA reduced the amplitude of the late and early currents by 75 ± 3 and $30 \pm 2\%$, respectively, at a potential of +20 mV. The percentage of inhibition of both currents was constant over the range of potentials studied (Fig. 2*E*).

Reversal potential of the transient outward current

All the results described hereafter were obtained using a bath solution containing 20 mM TEA. To determine the ionic component of the current, the reversal potential of the transient current was investigated with low and high external K^+ concentrations. The transient outward current

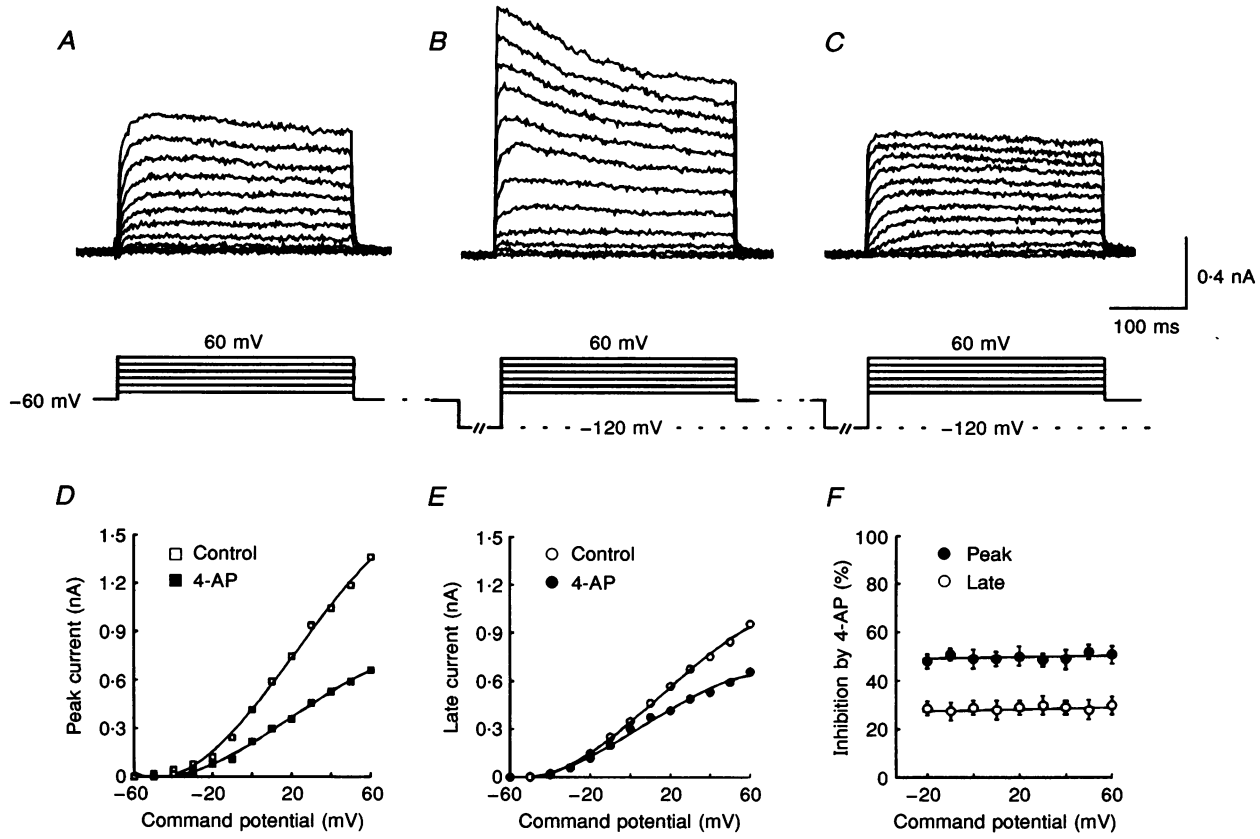

Figure 1. Effects of 4-AP on outward K^+ currents in a cultured frog melanotrophs

A, outward currents evoked by depolarizations from a holding potential of −60 mV by steps of 10 mV. *B*, currents evoked by a series of 300 ms voltage steps from −50 to +60 mV in 10 mV increments. A 1 s hyperpolarizing prepulse to −120 mV was applied to remove resting inactivation. Note that the transient outward current is now present. *C*, currents evoked by using an identical voltage protocol to that in *B* in the presence of 4 mM 4-AP in the bath solution. The peak outward current was markedly reduced and a slight reduction in the late current was also observed. *D–E*, current–voltage relationships for the peak transient current (*D*) and for the late current (*E*) in the absence or presence of 4-AP. *F*, relative inhibition of the peak and late currents induced by 4-AP *versus* membrane potential.

was evoked from a holding potential of −120 mV by a brief (3 ms) depolarizing step to +50 mV. The depolarization was followed by potential levels from +30 to −110 mV and the amplitude of the tail current was then examined (Fig. 3). In a normal extracellular K^+ concentration (2 mM), instantaneous outward current was recorded during repolarizing potential steps more positive than −100 mV. At more negative potentials, a small inward current was evoked (Fig. 3*A*). When the external solution contained 20 mM K^+, the inward current became more evident (Fig. 3*B*). The reversal potential was shifted rightward from −99·6 ± 3·2 mV ($n = 7$) for the low K^+ concentration to −40·8 ± 2·1 mV ($n = 6$) for the high K^+ concentration (Fig. 3*C*), two values which correspond to the respective K^+ equilibrium potential values (101·4 and 43·4 mV) predicted by the Nernst equation.

Activation and inactivation properties of the transient outward current

Activation and inactivation of the transient outward current were examined in a total of eleven melanotrophs. Activation was studied using the same protocol as shown in Fig. 2, while inactivation was investigated by applying a 1 s conditioning pulse to various potentials between −120 and +20 mV, followed by a test pulse to +40 mV (Fig. 4*A* and *B*). The activation threshold occurred between −60 and −50 mV and half-maximal activation was observed at −6·3 ± 1·5 mV ($n = 6$). The current was half-inactivated at −50·9 ± 2·4 mV ($n = 5$) and inactivation was completely removed at potentials negative to −90 mV. The activation and inactivation curves displayed an overlap with a window conductance between −60 and +10 mV and a maximum at −32 mV (Fig. 4*C*).

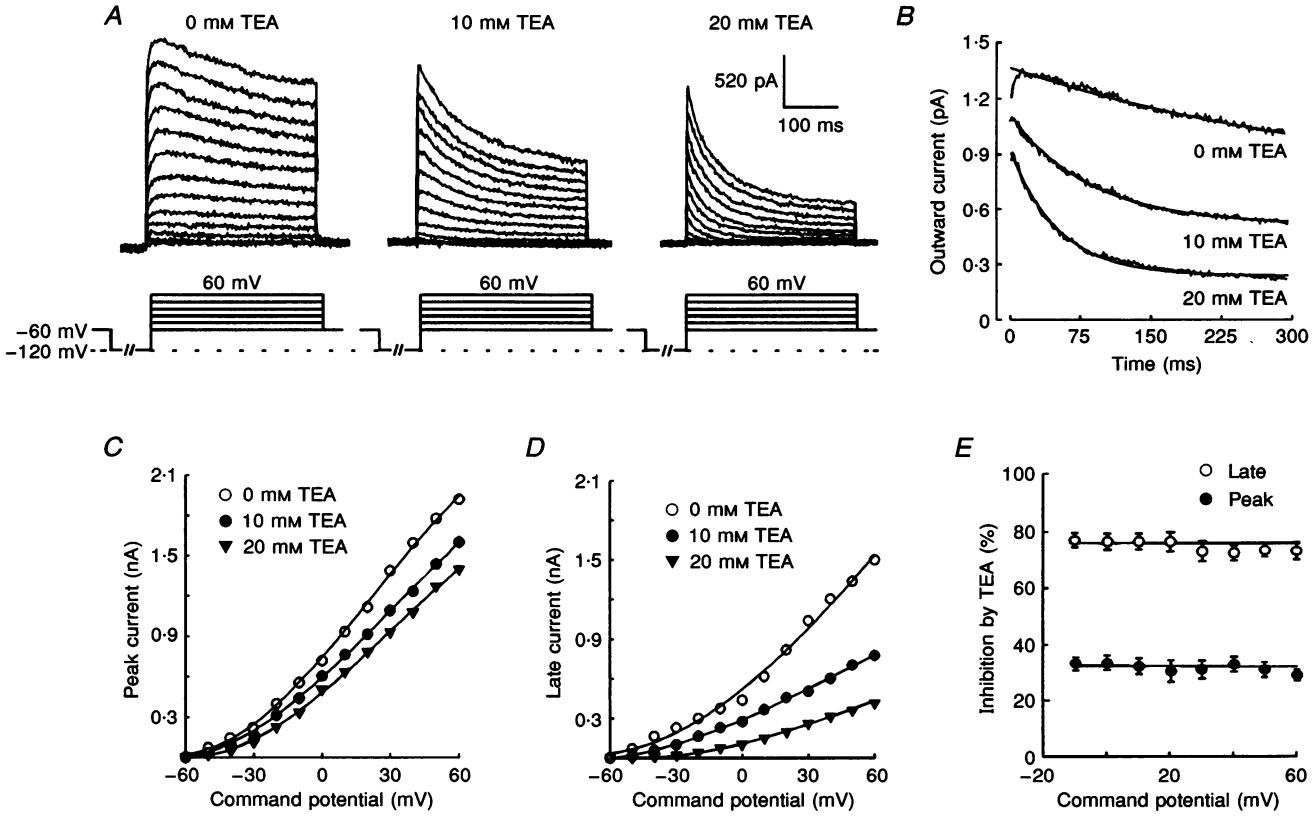

Figure 2. Effects of TEA on outward K^+currents

A, outward currents evoked in the absence or presence of TEA (10 or 20 mM) in the bath solution. A 1 s hyperpolarizing prepulse to −120 mV was applied before each depolarizing test pulse to potentials between −60 and +60 mV. *B*, time course of the inactivation of the transient current in the absence or presence of TEA. The current traces obtained with a constant depolarizing pulse from −100 to +30 mV were well-fitted by a single exponential function of the form $y = A_0 + A_1\exp(-t/\tau)$. The time constants for the three traces were 205·3, 89·1 and 51·5 ms for 0, 10 and 20 mM TEA, respectively. *C–D*, current–voltage relationships for the peak (*C*) and the late outward currents (*D*) in the absence (○) or presence of 10 mM (●) or 20 mM (▼) TEA. Note that TEA predominantly reduced the late component and only slightly diminished the early component. *E*, relative inhibition of the early and late K^+ currents by TEA (20 mM) *versus* membrane potential.

In another set of experiments, removal of inactivation was determined by using the double-pulse protocol illustrated in Fig. 5. Membrane potential was held at −50 mV, which inactivated 70–80% of the transient outward current. For current activation, the membrane potential was stepped to −120 mV for periods between 0 and 500 ms, in 25 ms increments before a constant test pulse to +40 mV. As shown in Fig. 5*A*, peak transient current increased with the length of the prepulse. The maximal magnitude of the current was reached when the duration of the hyperpolarizing prepulse exceeded 350 ms (Fig. 5*B*). This observation justified the use of a longer conditioning prepulse to −120 mV in all experiments performed on maximal transient outward current.

Effects of H_2O_2 and mercaptoethanol on inactivation of the transient outward current

Oxidation and reduction of the intracellular domain of the channel protein are known to influence the inactivation behaviour of mammalian A-type channels (Ruppersberg, Stocker, Pongs, Heinemann, Frank & Koenen, 1991). In order to determine whether, in frog melanotrophs, transient outward K^+ current channels could be regulated by compounds that oxidize or reduce cysteine residues, experiments were performed with oxidizing (1% H_2O_2) and reducing (5 mM 2-mercaptoethanol) intracellular solutions. Cells were held at −60 mV and submitted to 600 ms depolarizing test pulses to +30 mV from a 1 s hyperpolarizing potential of −120 mV, at 10 s intervals. In cells dialysed with H_2O_2, inactivation was irreversibly slowed over a period of 1–3 min. The mean time constants of inactivation in the dialysed and control cells were 144·5 ± 8·4 ms ($n = 5$) and 86·8 ± 6·6 ms ($n = 6$), respectively (Fig. 6*A*). The change in the inactivation time course induced by H_2O_2 occurred after a delay of 1 min following the establishment of the whole-cell configuration (Fig. 6*B*). Conversely, in cells dialysed with 2-mercaptoethanol, inactivation was accelerated: the time constant of the decay phase of the current was reduced to 55·3 ± 4·3 ms ($n = 5$). The amplitude of the current measured at the end of the test pulse gradually declined 1 min after rupture of the cell membrane and stabilized within 1 min (Fig. 6*C*).

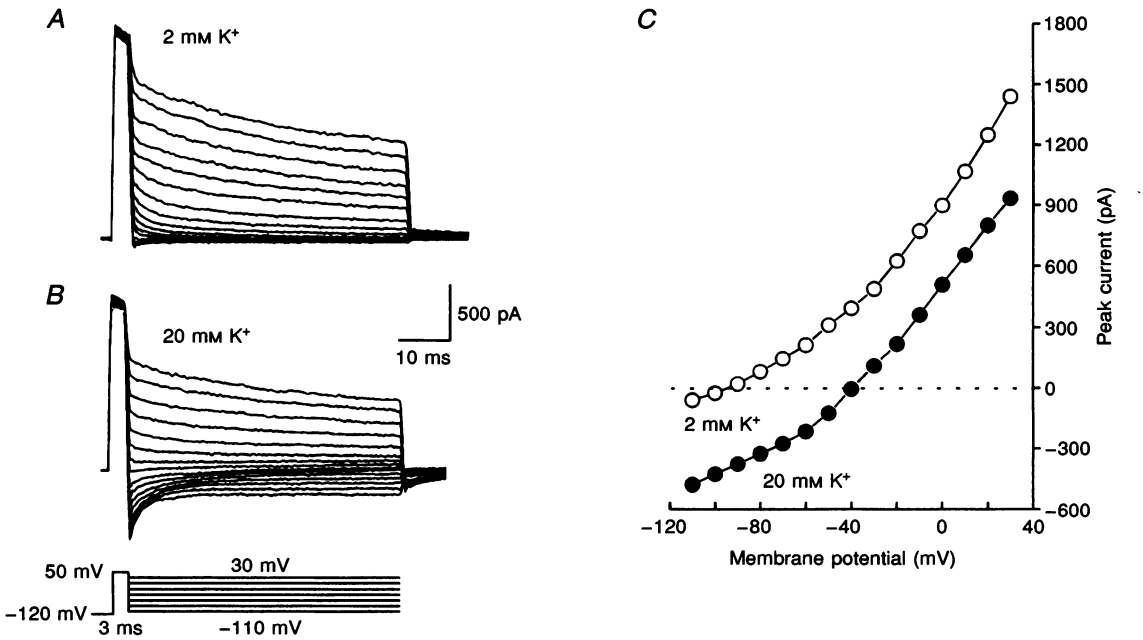

Figure 3. Determination of the reversal potential of the transient current at two different concentrations of extracellular K^+

The cell was held at −120 mV, and the transient current was evoked every 10 s by a 3 ms test pulse to +50 mV. At the end of the test pulse, the membrane potential was repolarized by 10 mV steps to the new potential levels (between +30 and −110 mV) and the tail currents were then recorded. The voltage traces below the current records in *A* and *B* illustrate the stimulation protocol. *A*, transient currents evoked with a normal extracellular solution containing 2 mM K^+. *B*, A-current recorded with 20 mM K^+ in the extracellular solution (Na^+ concentration was reduced to 94 mM to maintain constant osmolality). The peak outward current was diminished at a repolarizing potential of +30 mV and increased at −100 mV. *C*, instantaneous current–voltage relationships for the transient outward current plotted from current traces shown in *A* and *B*. The reversal potentials were estimated to be −99·6 ± 3·2 mV in normal external K^+ (○) and −40·8 ± 2·1 mV in 20 mM K^+ solution (●).

Effects of *R*-PIA

Modulation of the fast transient current by the adenosine A_1 receptor agonist *R*-PIA was investigated using the activation and inactivation protocols described above. In cells ($n = 6$) tested with the activation protocol, *R*-PIA (50 μM) enhanced the amplitude of the peak current evoked by depolarizing pulses positive to −10 mV (Fig. 7*A* and *B*). After washing, current amplitudes returned to control levels (not shown). Surprisingly, the activation curves were unchanged by the addition of *R*-PIA. The current was half-activated at −6·3 ± 1·5 and −3·5 ± 1·7 mV ($P > 0·05$) in the absence and presence of *R*-PIA, respectively (Fig. 7*C*). In contrast, in cells ($n = 5$) tested with the inactivation protocol, *R*-PIA both induced an enhancement of the evoked transient current (Fig. 8*A*) and altered the voltage dependence of the current (Fig. 8*B*). *R*-PIA significantly ($P < 0·005$) shifted the inactivation curve towards the depolarizing potentials by 14 mV. The current was half-inactivated at −37·2 ± 2·7 mV in the presence of *R*-PIA.

Effect of PTX-pretreatment on the adenosine-induced increase of A-current

To determine whether a G protein was involved in the enhancement of the fast transient current induced by the A_1 receptor agonist *R*-PIA, cells were pre-incubated with PTX (1 μg ml^{-1} for 12 h). In this set of experiments, the current activation protocol described above was applied. In all cells tested ($n = 11$), PTX pretreatment abolished the effect of *R*-PIA on the transient current (Fig. 9).

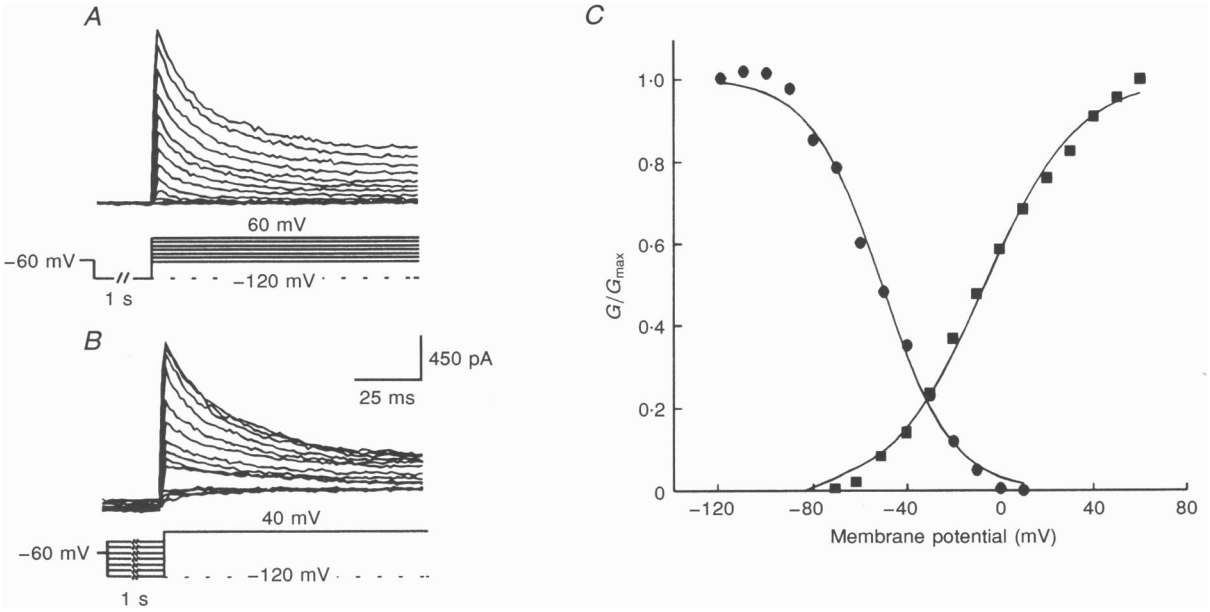

Figure 4. Comparison of steady-state activation and inactivation properties of the transient outward current

A, voltage-dependent activation evoked by depolarizing step commands between −50 and +60 mV after a 1 s hyperpolarizing prepulse to −120 mV from a holding potential of −60 mV. The current activated between −50 and −40 mV, and increased in amplitude with incremental depolarizing commands. *B*, voltage-dependent inactivation of the transient current. The current records were obtained from the same cell as in *A*. The membrane potential was held at −60 mV, and 1 s conditioning prepulses to various potentials (between −120 and +10 mV) were applied before the test pulse to +40 mV. The current amplitude gradually decreased with decremental hyperpolarizing prepulses. The voltage traces below the current records in *A* and *B* illustrate the stimulation protocols. *C*, activation (■) and inactivation curves (●) of the transient current. The abscissa indicates test pulse voltages for activation and conditioning prepulse voltages for inactivation. Conductances were computed and normalized on the assumption that currents were carried by potassium. Each curve was fitted with the Boltzmann equation:

$$G/G_{max} = (1 + \exp(V - V_{½})/k)^{-1},$$

where G_{max} is the maximal conductance; V, the command potential for activation or the conditioning potential for inactivation; $V_{½}$, the half-activation potential or inactivation voltage; and k, the Boltzmann slope factor. For activation the best-fit line had a half-activation potential of −6·3 ± 1·5 mV and a slope factor of +19·8 ± 0·4 mV. For inactivation, the best-fit line had a half-inactivation potential of −50·9 ± 2·4 mV and a slope factor of −15·1 ± 0·6 mV.

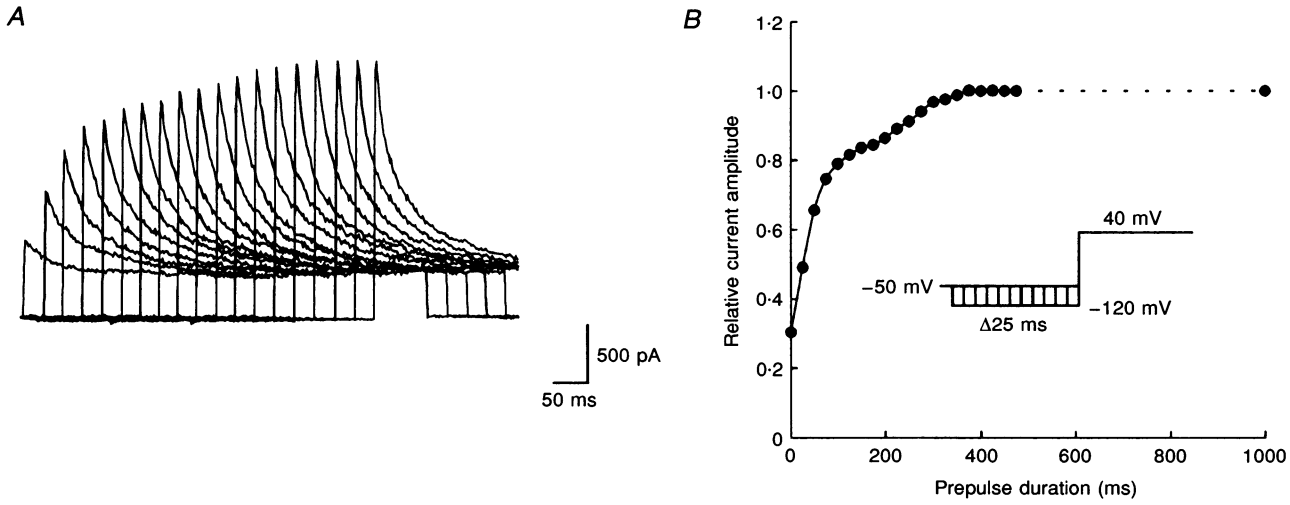

Figure 5. Removal of inactivation of the transient outward current

A, superimposed current traces obtained from a single melanotroph. The cell was clamped at −50 mV, and a series of hyperpolarizing prepulses to −120 mV was applied for various durations (between 0 and 500 ms). Then, a depolarizing test pulse to +40 mV was applied. Note that the amplitude of the peak current gradually increased with the duration of the prepulse and reached its maximum for prepulses lasting from 450 to 500 ms. *B*, normalized peak current plotted against prepulse duration. The inset illustrates the stimulation protocol.

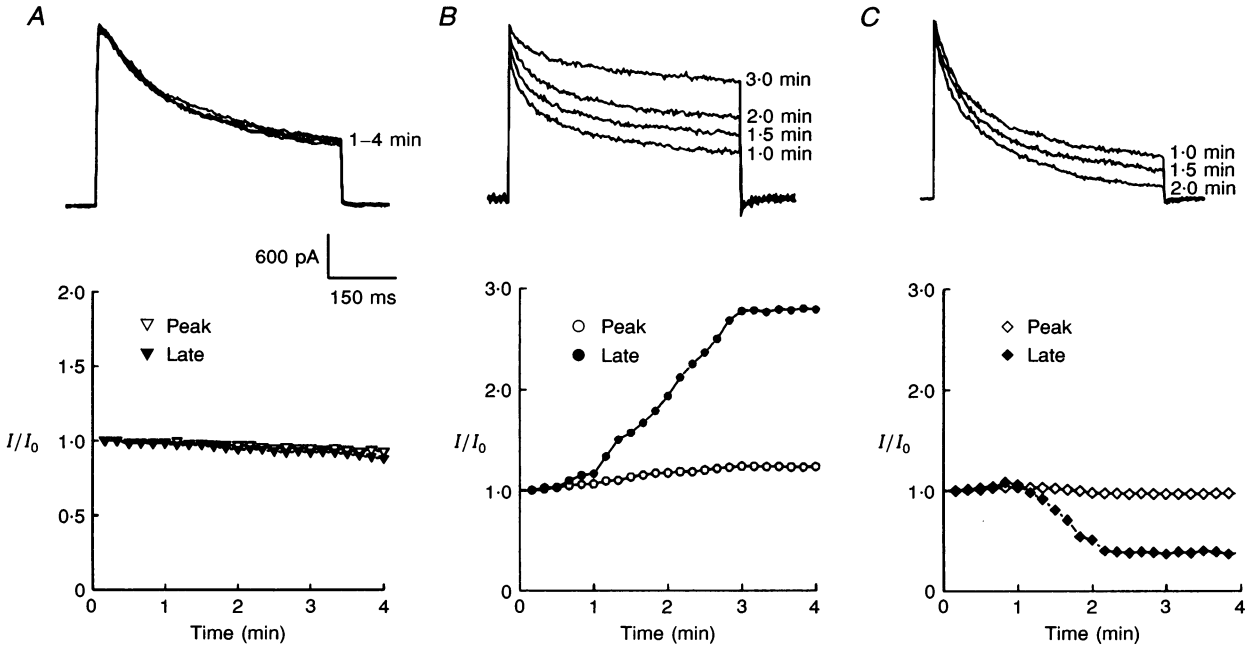

Figure 6. Effects of cell dialysis with H_2O_2 and 2-mercaptoethanol on the fast inactivation of the transient outward current

The cells were held at −60 mV, and the transient current was evoked by 600 ms depolarizing pulses to +30 mV after 1 s hyperpolarizing prepulses to −120 mV at 10 s intervals. In the graphs shown in the lower panels in *A*, *B* and *C*, the current amplitude at each time point is expressed as the ratio to the initial current measured just after the establishment of the whole-cell configuration. In *A*, *B* and *C*, the superimposed current recordings obtained at different times, indicated near each trace, correspond to time points noted on the graphs. *A*, the current traces recorded with a normal internal solution did not show any changes in inactivation during the recording period. *B*, the recordings were obtained with an oxidazing intracellular solution (1 % H_2O_2). Note that the loss of fast inactivation gradually augmented with the duration of cell dialysis. *C*, the recordings were performed in another cell with a reducing intracellular solution (5 mM 2-mercaptoethanol). The antioxidant substance accelerated inactivation after cell dialysis.

DISCUSSION

Previous studies have shown that melanotrophs of the frog pituitary exhibit spontaneous action potentials generated by voltage-activated currents reminiscent of those observed in neurones (Louiset *et al.* 1988; Valentijn *et al.* 1991). The bioelectrical activity of melanotrophs is held responsible for the secretion of α-MSH (Tomiko, Taraskevich & Douglas, 1984) since the neuroendocrine factors which increase or decrease the frequency of action potentials respectively stimulate or inhibit α-MSH release (Louiset *et al.* 1989; Louiset, Cazin, Duval, Lamacz, Tonon & Vaudry, 1990; Chartrel, Conlon, Danger, Fournier, Tonon & Vaudry, 1991; Desrues, Lamacz, Jenks, Vaudry & Tonon, 1993; Valentijn, Vaudry, Kloas & Cazin, 1994). We have recently shown that adenosine is a potent inhibitor of both bioelectrical activity and α-MSH secretion in frog melanotrophs (Chartrel *et al.* 1992; Mei *et al.* 1994). The present report provides the first evidence for a stimulatory effect of adenosine on a transient A-type outward K^+ current, a K^+ conductance which so far has never been characterized in melanotrophs.

Application of depolarizing pulses from −60 to +60 mV elicited only a slowly inactivating outward current which has already been described (Louiset *et al.* 1988). In contrast, application of depolarizing voltage steps from hyperpolarizing potentials evoked a large, inactivating A-current. The involvement of any Ca^{2+}-dependent K^+ current could be ruled out, since calcium was omitted in the pipette solution and cobalt was added to the external medium. The transient current reversed at the K^+ equilibrium potential in both normal and elevated potassium concentrations, indicating that K^+ ions were the charge carriers. This current, which was relatively insensitive to TEA but could be blocked by 4-AP, exhibited

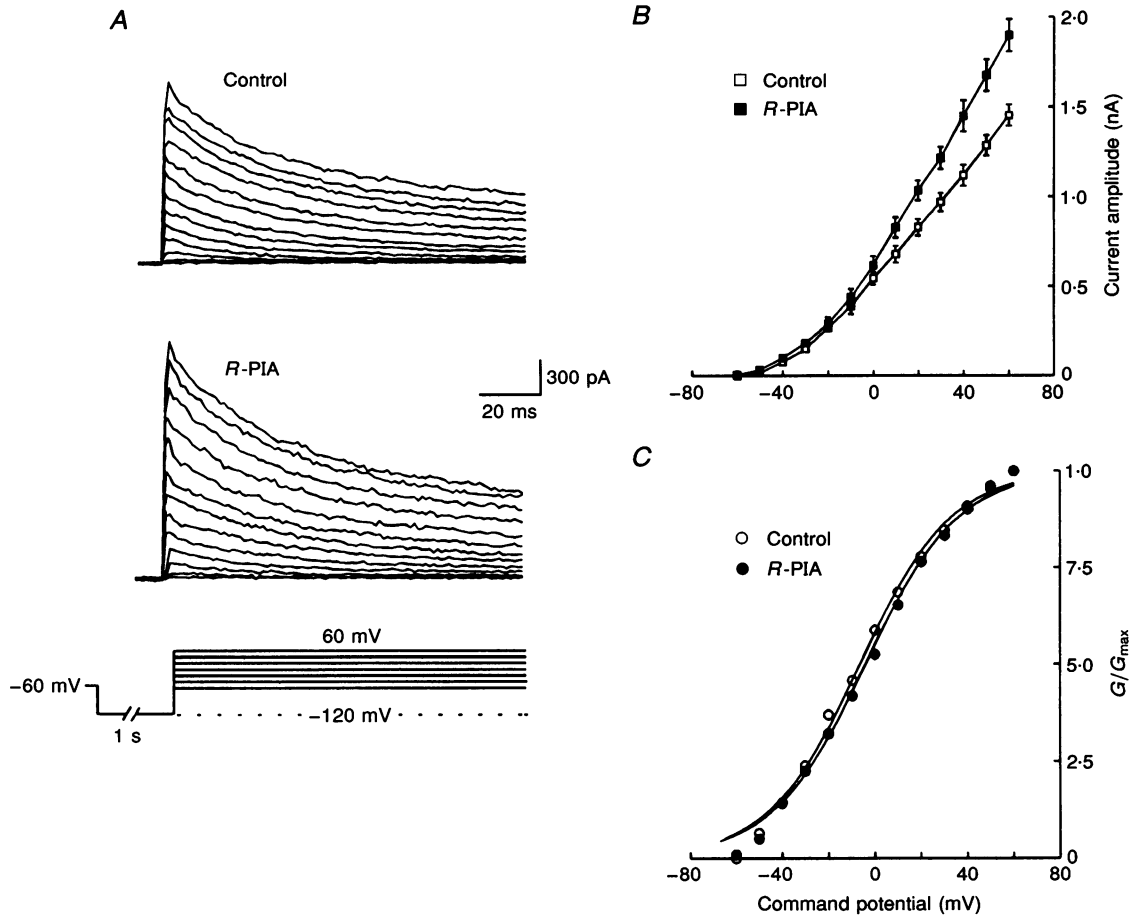

Figure 7. Effect of *R*-PIA on the voltage-dependent steady-state activation of the transient outward K^+ current

A, top traces are control currents evoked by depolarizing step commands shown below the current records. Bottom traces are currents evoked from the same cell after application of *R*-PIA (50 μM). *B*, voltage-dependent activation curve constructed from the data of 6 independent experiments in the absence (□) or presence of *R*-PIA (■). Values are means ± S.E.M. Note that *R*-PIA augmented outward current amplitude at each depolarizing pulse above −10 mV. *C*, plot of the normalized conductance as a function of the command potential in the absence or presence of *R*-PIA. The data points were fitted with a Boltzmann function (continuous line). *R*-PIA failed to shift the activation curve.

the same pharmacological profile as the A-current previously characterized in neurones (Hermann & Gorman, 1981; Belluzzi, Sacchi & Wanke, 1985; Wang, Strahlendorf & Strahlendorf, 1991; Fickler & Heinemann, 1992), adrenal cells (Mlinar & Enyeart, 1993), lactotrophs (Lledo, Legendre, Zhang, Israel & Vincent, 1990) and lymphocytes (Choquet & Korn, 1992). Complete abolition of the peak current required a high concentration of 4-AP (4 mM) while application of low concentrations of 4-AP (1 mM) affected neither the early peak nor the late decay phase of the current. It thus appears that the transient outward current presently characterized in frog melanotrophs does not correspond to the slowly inactivating K^+ current termed I_D, previously described in hippocampal neurones (Storm, 1988), which exhibits a much higher sensitivity to 4-AP.

The kinetic properties of the transient K^+ current in frog melanotrophs were similar to those described for the A-current in several other cell types (Oxford & Wagoner, 1989; Lledo *et al.* 1990; Bardoni & Belluzzi, 1993) since: (1) the steady-state activation and inactivation curves showed a range of overlap close to resting potential; (2) the current activated upon subsequent depolarizing step following hyperpolarization and exponentially decayed; (3) inactivation was removed by hyperpolarization; and (4) the inactivation behaviour, observed with reducing and oxidizing intracellular solutions, corresponded to that previously reported for inactivating K^+ channel proteins, including A-type K^+ channels (Ruppersberg *et al.* 1991; Rettig *et al.* 1994). Reducing conditions, promoting the formation of free sulfhydryl groups in the cysteine residues at the *N*-terminal sequence of the channel protein, induced a maximal state of inactivation. Conversely, oxidizing conditions decreased the inactivation rate. These observations strongly suggest that the inactivating K^+ channels in frog melanotrophs possess a 'ball and chain' domain underlying the fast inactivation of the transient outward K^+ current. Taken together, the present biophysical and pharmacological data support the existence, in our cell model, of a current exhibiting the major characteristics of the A-type current.

The overall properties of the transient outward K^+ current make it a very sensitive target for any modulatory mechanism influencing cell excitability and action potential firing. The present study has shown that, when the steady-state inactivation was eliminated by using a conditioning potential of −120 mV, the A_1 adenosine receptor agonist *R*-PIA enhanced the amplitude of the peak current without any significant shift of the steady-state activation curve. In this respect, these data differ from those obtained in rat locus coeruleus neurones, in which adenosine had no effect

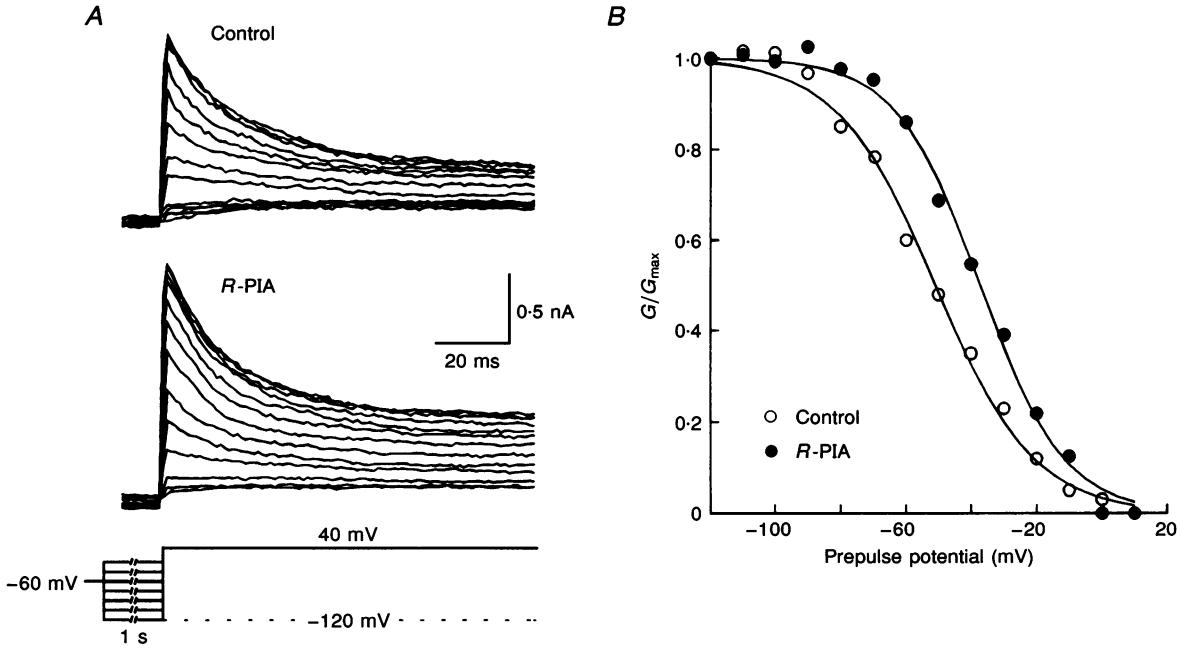

Figure 8. Effect of *R*-PIA on the voltage-dependent steady-state inactivation of the transient outward K^+ current

A, top traces are control currents and bottom traces are currents after application of *R*-PIA (50 μM). The membrane potential was held at −60 mV, and 1 s conditioning prepulses to various potentials (between −120 and +10 mV) were applied before the test to +40 mV. The voltage protocol is shown below the current records. Note that *R*-PIA enhanced the amplitude of the evoked current. *B*, steady-state inactivation curve plot in the absence (○) or presence of *R*-PIA (●). Values are means ± S.E.M. ($n = 5$). Normalized points were fitted with a Boltzmann function (continuous line). *R*-PIA shifted the steady-state inactivation rightward by 14 mV.

on the maximum amplitude of the A-current (Pan *et al.* 1994). Furthermore, modulation of the A-current by *R*-PIA was characterized, in agreement with Pan *et al.* (1994) by a shift of the steady-state inactivation curve towards the depolarizing potentials, suggesting that the effects of *R*-PIA can be accounted for by partial deinactivation of the current.

We have recently reported that, in frog melanotrophs, adenosine causes a marked reduction of spontaneous action potential discharge associated with a very modest hyperpolarization (Mei *et al.* 1994), suggesting that adenosine can suppress spike firing independently of a lowering of the membrane potential. In addition, we also observed that adenosine altered the action potential by reducing both its amplitude and duration and by increasing the after-hyperpolarizing potential (Y. A. Mei, E. Louiset, H. Vaudry & L. Cazin, unpublished data). These effects could result from the enhancement of the transient outward K^+ current described herein: the adenosine-induced increase of the A-current would provoke a faster repolarization of the action potentials and a stronger after-hyperpolarizing potential leading to a reduction of the action potential frequency.

Previous studies have demonstrated that adenosine-induced inhibition of the electrical activity in frog melanotrophs was mediated through the A_1 receptor subtype (Mei *et al.* 1994). The facilitatory action of *R*-PIA observed on the transient outward K^+ current provides additional evidence for the involvement of purinergic A_1 receptors in the control of membrane potential and action potential discharge in this cell type.

The present results have shown for the first time that pretreatment with PTX suppressed the stimulatory action of *R*-PIA on the A-current, indicating that the coupling of A_1 adenosine receptors to the fast inactivating K^+ channel

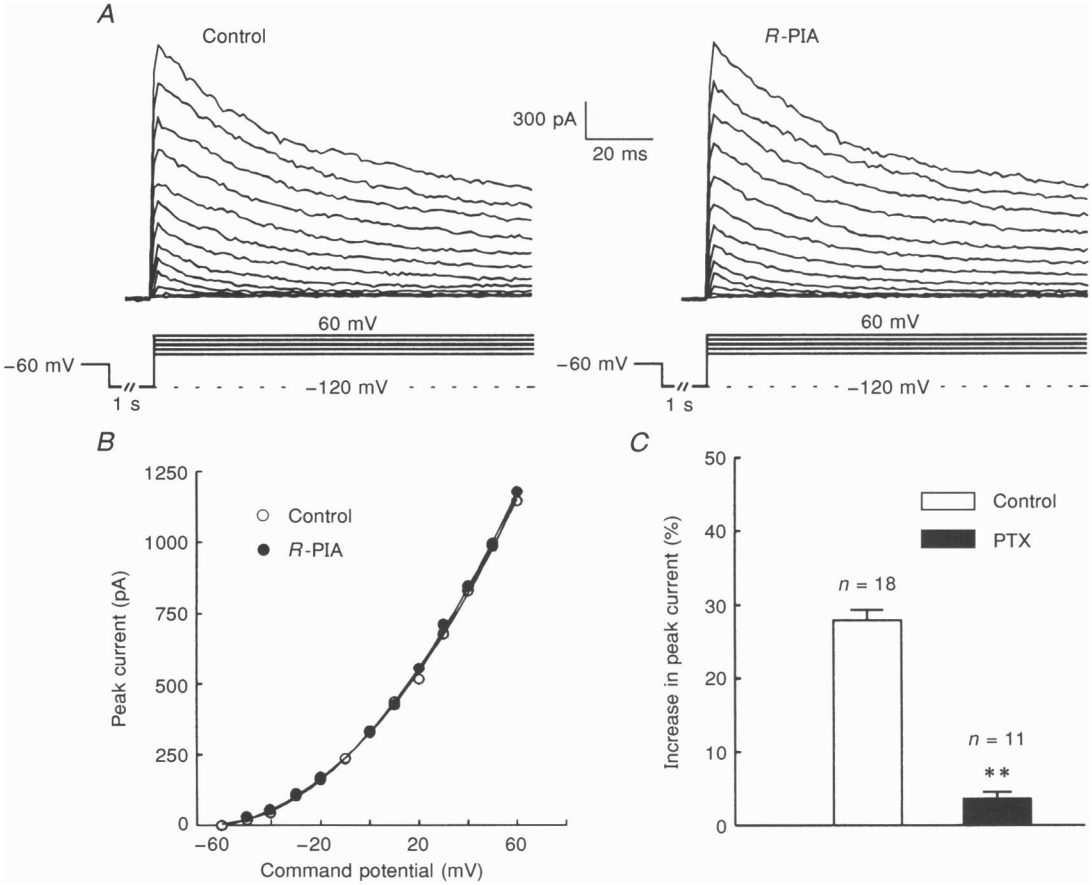

Figure 9. Effect of pertussis toxin pretreatment on the response to *R*-PIA

A, transient outward K^+ current evoked by depolarizing step commands in a melanotroph pre-incubated with PTX (1 μg ml^{-1}) for 12 h, in the absence (left) or presence (right) of *R*-PIA (50 μM). *B*, plot of the peak amplitude of the current as a function of the command potential in the absence (○) or presence of *R*-PIA (●) in PTX-pretreated cells. *C*, effect of *R*-PIA on the increase of the peak current in cells which were pre-incubated with normal medium ($n = 18$) or with 1 μg ml^{-1} PTX ($n = 11$). The values represent the percentage (mean ± S.E.M.) of the increase in the peak current. $^{**}P < 0{\cdot}001$.

was mediated through a G_i or G_o protein. Previous studies suggested that a PTX-sensitive G protein could be involved in the electrophysiological effects produced by adenosine (Trussel & Jackson, 1987; Cooper, Caldwell, Boyajian, Petcoff & Schlegel, 1989; Thompson, Haas & Gähwiler, 1992; Mei *et al.* 1994), but the mechanism of action of adenosine on the A-type current has never been described.

In conclusion, the present study has shown that pituitary melanotrophs exhibit an A-type current which is probably involved in the regulation of the threshold and discharge rate of action potentials. This transient K^+ current could play a crucial role in purinergic A_1 receptor-mediated inhibition of electrical and secretory activities in endocrine cells.

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