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SPECIAL REPORT

Augmentation by intracellular ATP of the delayed rectifier current independently of the glibenclamide-sensitive K-current in rabbit arterial myocytes

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Elevation of intracellular ATP levels by flash photolysis of caged ATP augmented the delayed rectifier K-current (I_{KDR}) in rabbit pulmonary artery myocytes. The percentage augmentation was unaffected when I_{KDR} was inactivated by 50% (holding potential −40 mV), although the magnitude of the ATP-induced current was substantially reduced. Inactivation of 90% I_{KDR} (holding potential −20 mV) virtually abolished the ATP-dependent augmentation. We conclude that modulation of I_{KDR} by ATP does not require conversion of the glibenclamide-sensitive K-current ($I_{K(ATP)}$).

Keywords: ATP; delayed rectifier; potassium channel; pulmonary artery; rabbit arterial myocytes

Introduction In smooth muscle cells, increases in a glibenclamide-sensitive K-current ($I_{K(ATP)}$) are often associated with concomitant decreases in the delayed rectifier current (I_{KDR}). For this reason it was suggested that $I_{K(ATP)}$ may be a voltage-insensitive form of I_{KDR} (Beech & Bolton, 1989). Recently, Edwards *et al.* (1993) proposed that channel phosphorylation converts $I_{K(ATP)}$ to I_{KDR} with dephosphorylation reversing the process. We present data that contradict this hypothesis and suggest that I_{KDR} is augmented by ATP, independently of any action on $I_{K(ATP)}$.

Methods Rabbit pulmonary artery myocytes were isolated and whole-cell voltage-clamp recordings made as previously described (Clapp & Gurney, 1991). Solutions contained (mM): Bath–Hank's balanced salt solution with $NaHCO_3$ 10, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, pH 7.4; Pipette–KCl 130, $MgCl_2$ 1, EGTA 1, HEPES 20, Na_3GTP 0.5, pH 7.2. 1-(2-Nitrophenyl)ethyl (caged) ATP (10 mM), Na_2ATP (1 mM) and glutathione (5 mM) were included in the pipette solution and dialysed into cells as required. A single light flash was estimated to generate millimolar intracellular ATP from caged ATP. Experiments were performed at room temperature (22–26°C).

Results Figure 1a shows the current-voltage relationship for sustained outward current elicited from a holding potential of −80 mV, in the presence and absence of 1 mM internal ATP. At positive potentials, ATP significantly enhanced the outward current. We previously suggested that this was due to augmentation of I_{KDR} (Evans *et al.*, 1993). I_{KDR} was studied at +40 mV, when the Ca^{2+}-activated K current and $I_{K(ATP)}$ were blocked with extracellular tetraethylammonium chloride (10 mM) and glibenclamide (10 μM), and the transient A-like current was inactivated by using 100 ms test pulses (Clapp & Gurney, 1991). Under these conditions, photolysis of caged ATP augmented I_{KDR} by 89 + 14% ($n = 5$; Figure 1b), in the absence of decreases associated with inhibition of $I_{K(ATP)}$ (Clapp & Gurney, 1992). This augmentation was unaffected in the presence of 5 mM intracellular glutathione (Figure 1c; 66 ± 17%, $n = 4$), but was not produced by a light flash alone or by photolysis of caged ATP in the presence of 20 mM intracellular ATP. Thus ATP appears to mediate the photolysis-induced augmentation of I_{KDR}.

Figure 2 a and b show the voltage- and time-dependence of I_{KDR} inactivation. I_{KDR} was inactivated by >50% at −40 mV, and almost completely at −20 mV (Figure 2a),

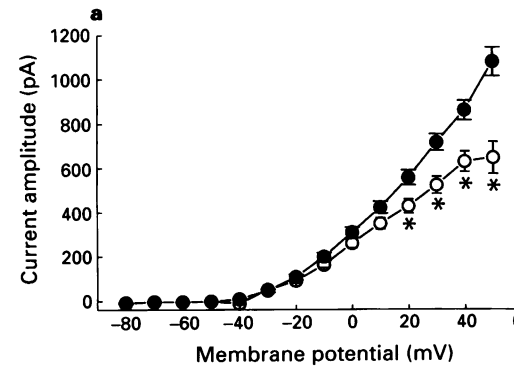

Figure 1 (a) Relationship between sustained outward current evoked from −80 mV and the test potential, with (●) and without (○) 1 mM intracellular ATP. Mean ± s.e.mean, >25 cells. *$P < 0.01$, two-tailed t test. Some experiments omitted pipette GTP. (b) Superimposed outward currents evoked by steps from −80 mV to +40 mV before (1), at photolysis (2; arrow), 5 s (3) and 25 s (4) after photolysis of intracellular caged ATP (10 mM). (c) Superimposed currents as in (b), with 5 mM intracellular glutathione, before (1) and 25 s after (4) photolysis. Extracellular tetraethylammonium (10 mM) and glibenclamide (10 μM) present in (b) and (c). Dotted lines indicate zero current.

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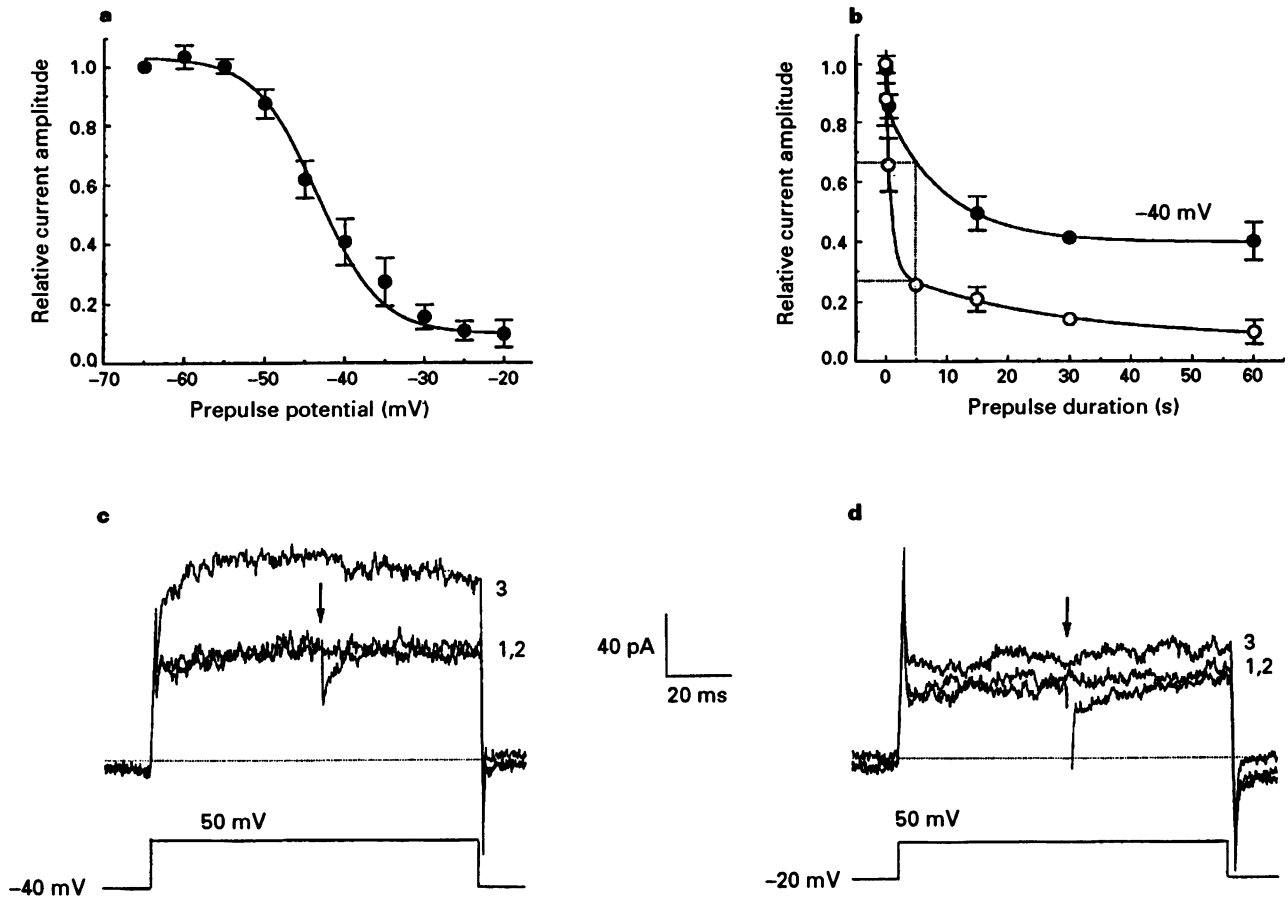

Figure 2 (a) Voltage-dependence of inactivation of I_{KDR} evoked by steps to + 40 mV or + 50 mV following 1 min pre-pulses to the potentials shown, measured relative to the maximum current activated with the same test pulse. Curve is best fit to $I = \{1 + \exp[(V-V_{0.5})/k]\}^{-1}$, with $V_{0.5} = -43.5$ mV, $k = 3.9$ mV, I the relative current and V the pre-pulse potential. Mean ± s.e.mean, 3 cells. (b) Development of inactivation at − 40 mV (●) and − 20 mV (○). Curves are bi-exponential fits, with time constants of 0.25 s and 9.6 s at − 40 mV and 0.84 s and 26.1 s at − 20 mV. Symbols represent means of 2–3 cells. Vertical bars show s.e.mean. (c) Current evoked by a step to + 50 mV after inducing steady-state inactivation by holding the cell at − 40 mV for > 60 s, before (1), at (2) and 5 s after (3) photolysis (arrow) of caged ATP (10 mM). (d) As (c) but holding potential − 20 mV. External tetraethylammonium (10 mM) and glibenclamide (10 μM) present. Dotted lines indicate zero current for (c) and (d).

with 60 s required to reach steady-state (Figure 2b). Measured 5 s after photo-release of ATP, the outward current at + 50 mV was increased by 38 ± 1 pA ($n = 3$) or 53 ± 5%, when cells were held at − 40 mV (Figure 2c). This compares with an increase of 159 ± 22 pA ($n = 5$) at 5 s, equivalent to 48 ± 6%, when cells were held at − 80 mV. When I_{KDR} was almost completely inactivated by holding cells at − 20 mV (Figure 2d), there was little time-dependent outward current evoked at + 50 mV and 5 s after photorelease of ATP the current was increased by only 8 ± 6 pA ($n = 3$).

Discussion We present the first demonstration of ATP-dependent augmentation of a delayed rectifier current in mammalian cells, and provide evidence that enhancement of I_{KDR} by ATP does not result from the modification of non-inactivating, glibenclamide-sensitive $I_{K(ATP)}$ channels.

Based on their findings in rat portal vein, Edwards and coworkers (1993) proposed that the channels underlying $I_{K(ATP)}$ are a dephosphorylated form of I_{KDR} channels. They suggested that when phosphorylated, $I_{K(ATP)}$ channels develop voltage-dependent gating and lose sensitivity to glibenclamide. Since the gating of $I_{K(ATP)}$ is voltage-independent, this hypothesis predicts that the number of channels converted to I_{KDR}, and hence the magnitude of the current induced by ATP, would not vary with the holding potential. On the contrary, the ATP-induced current was greatly reduced at a holding potential of − 40 mV and virtually abolished at − 20 mV. Rapid inactivation of newly converted channels cannot account for the reduced current at positive potentials. During the 5 s delay between photolysis and measurement of I_{KDR}, only 33% of the converted channels would have inactivated at − 40 mV and 73% at − 20 mV (Figure 2b), corresponding to an ATP-inducible current of 106 pA and 43 pA, respectively. The observed currents were substantially smaller than that. It is more likely that ATP modulated pre-existing I_{KDR} channels, and that inactivation reduced the number of channels available to respond at positive holding potentials. The similar percentage augmentation observed at − 80 mV and − 40 mV supports this view.

We conclude that in pulmonary artery myocytes, intracellular ATP modulates I_{KDR} independently of $I_{K(ATP)}$. Both phosphorylation-dependent (Edwards *et al.*, 1993) and independent (Evans *et al.*, 1993) mechanisms have been proposed to regulate these channels. We suggest that the effects of K-channel openers and dephosphorylating conditions on I_{KDR} and $I_{K(ATP)}$ (Edwards *et al.*, 1993) result from independent effects on each channel through inhibition of phosphorylation and/or ATP binding.

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