Journal of Physiology (1993), **471**, *pp.* 767–786
With 9 figures
Printed in Great Britain

ADENOSINE-ACTIVATED POTASSIUM CURRENT IN SMOOTH MUSCLE CELLS ISOLATED FROM THE PIG CORONARY ARTERY

BY C. DART AND N. B. STANDEN

From the Ion Channel Group, Department of Physiology, University of Leicester, PO Box 138, Leicester LE1 9HN

(*Received 4 February 1993*)

SUMMARY

1. The perforated patch technique with nystatin or amphotericin was used to record whole cell currents activated by adenosine in smooth muscle cells isolated enzymatically from pig coronary arteries.

2. Adenosine (5–40 μM) activated an outward current at a holding potential of 0 mV in 5 mM $[K^+]_o$ and an inward current at −60 mV in 143 mM $[K^+]_o$. The dependence of the reversal potential for the adenosine-activated current on $[K^+]_o$ suggests that it flows through K^+ channels, while its current–voltage relation is consistent with the channels showing little voltage dependence.

3. The adenosine-activated current was inhibited by the sulphonylurea glibenclamide (5 μM) and by phencyclidine (5 μM). It was unaffected by charybdotoxin (50 nM) or apamin (100 nM), blockers of large and small conductance Ca^{2+}-activated K^+ channels respectively.

4. At −60 mV in 143 mM K^+ solution, openings of single channels passing a current of just over −2 pA could sometimes be detected in the absence of adenosine. Openings became more frequent after the application of adenosine, with several levels then being detected. Openings of channels with a larger conductance were sometimes also seen in the presence of adenosine. Fluctuation analysis gave somewhat lower estimates of unitary current than did direct measurements.

5. The effect of adenosine could be mimicked by the A_1 receptor agonist CCPA (2-chloro-N^6-cyclopentyladenosine), while the A_2 agonist CGS 21680 (2-*p*-(2-carboxethyl)phenethylamino-5′-*N*-ethylcarboxamido adenosine hydrochloride) was without effect. The response to adenosine was inhibited by the A_1 antagonist DPCPX (8-cyclopentyl-1,3-dipropylxanthine), but was unaffected by the A_2 antagonist CGS 15943A (5-amino-9-chloro-2-(2-furanyl)-1,2,4-triazolo[1,5-C]quinazoline monomethanesulphonate).

6. Our results suggest that adenosine acts at an A_1 receptor to activate K^+ channels. We consider it most likely that these are ATP-dependent K^+ channels. We discuss the mechanism by which K^+ channel activation may lead to hyperpolarization and so vasorelaxation.

MS 2097

INTRODUCTION

The suggestion that adenosine released from active cardiac muscle acts as a vasodilator in the coronary circulation, and so helps match coronary blood flow to metabolic demand, was first made by Berne (1962). Since then, adenosine has been shown to be a vasodilator in most vascular beds, and to be especially potent in the heart and cerebral circulation (see reviews by Berne, 1980; Olsson & Pearson, 1990). The vasodilator action of adenosine has generally been ascribed to stimulation of the A_2 adenosine receptor subtype, probably acting by activation of adenylyl cyclase leading to elevation of cyclic AMP levels (Olsson & Pearson, 1990). However, both A_1 and A_2 receptors have been shown to occur in smooth muscle, including cells cultured from coronary artery (Mills & Gewirtz, 1990).

Recently Daut and colleagues have shown in the isolated guinea-pig heart that part of the vasodilator action of adenosine is blocked by the sulphonylurea glibenclamide, and have suggested that this component of the vasodilatation occurs by way of activation of ATP-dependent K^+ channels (K^+_{ATP} channels) in coronary smooth muscle cells (Daut, Maier-Rudolph, von Beckerath, Mehrke, Günther & Goedel-Meinen, 1990). A similar effect of glibenclamide has been reported in the dog heart (Clayton, Hess, Smith & Grover, 1992), while in the guinea-pig the vasodilatory response to dipyridamole, an inhibitor of adenosine reuptake, is also strongly inhibited by glibenclamide (von Beckerath, Cyrys, Dischner & Daut, 1991). Measurements of the relaxation of porcine coronary arterial rings suggest that the glibenclamide-sensitive component of vasorelaxation occurs by way of stimulation of A_1 receptors, since the response to a selective A_2 agonist was unaffected by glibenclamide (Merkel, Lappe, Rivera, Cox & Perrone, 1992).

In the present study, we have used the perforated patch technique to investigate the action of adenosine at the level of single coronary smooth muscle cells. We find that adenosine activates a K^+ current, and that this effect is inhibited by glibenclamide, but unaffected by blockers of Ca^{2+}-activated K^+ channels. Our results suggest that activation occurs via stimulation of an A_1 receptor, and are consistent with adenosine causing activation of K^+_{ATP} channels.

METHODS

Preparation and cell isolation

Fresh pig hearts were collected from a local abattoir and transported back to the laboratory in ice-cold buffer of composition (mM): NaCl, 140; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1; Hepes, 10; glucose, 10; pH adjusted to 7·4 at 4 °C using NaOH. The left descending coronary arteries were dissected out and cleaned of blood and connective tissue. Once dissected, the artery was placed in a cold physiological salt solution of composition (mM): NaCl, 110; KCl, 5; $CaCl_2$, 1·8; $MgCl_2$, 2; NaH_2PO_4, 0·5; KH_2PO_4, 0·5; $NaHCO_3$, 15; Hepes, 10; glucose, 10; Phenol Red, 0·04; pH adjusted to 7·4 at 4 °C using NaOH, and was stored at 4 °C.

Cells were isolated using a method adapted from that described by Clapp & Gurney (1991). Briefly, this involved selecting two sections of artery, typically 5–10 mm in length, from one of the smaller branches. One section was used on the same day and the other maintained in an 'overnight' enzyme solution and dispersed for experimentation on the following day. On the first day, the section of artery was cut open along its length and dissected into strips, about 1 mm wide. These were placed in 5 ml of cold isolation buffer of composition (mM): NaCl, 110; KCl, 5; $CaCl_2$, 0·16; $MgCl_2$, 2; NaH_2PO_4, 0·5; KH_2PO_4, 0·5; $NaHCO_3$, 10; Hepes, 10; glucose, 10; Phenol Red, 0·04; EDTA, 0·49; taurine, 10; containing 0·02 % bovine serum albumin (essentially fatty acid free, Sigma), 0·8 mg/ml collagenase (Type IA, Sigma, UK) and 0·2 mg/ml elastase (Type

IIA, Sigma). The strips were incubated in this enzyme cocktail for 20–25 min at 4 °C, which depresses enzyme activity and allows the enzymes to diffuse into the extracellular matrix of the tissue. The enzyme cocktail was then warmed to 37 °C in a shaking water bath for 60–70 min, after which time the muscle strips were moved into fresh, enzyme-free isolation buffer and gently triturated with a wide-bore Pasteur pipette. The muscle strips and dispersed cells were stored in isolation buffer at 4 °C and used over the next 8–10 h. For the 'overnight' dissociation, strips of artery were placed in isolation buffer containing 0·02% BSA; 0·2 mg/ml collagenase and 0·1 mg/ml elastase, and stored overnight at 4 °C. The following morning, this enzyme cocktail was warmed to 37 °C for 10–12 min, and the tissue dispersed as described above. Both isolation procedures produced high yields of relaxed coronary smooth muscle cells of dimensions ~ 100 μm long by ~ 10 μm maximum diameter.

Solutions

Single isolated myocytes were allowed to settle in isolation buffer on the bottom of a small plastic Petri dish and were then continuously superfused with a bath solution containing (mM): NaCl, 140; KCl, 5; $CaCl_2$, 1·8; $MgCl_2$, 1; Hepes, 10; glucose, 10; pH adjusted to 7·4 at 22 °C using NaOH. During the course of an experiment, a cell could be superfused with any one of up to seven different solutions using a flow system designed by Dr P. D. Langton in which seven lines fed a common outflow placed near the cell. The flow of each line was controlled by an electrically switched valve (Lee, Westbrook, CT, USA). The diameter of the flow system aperture was about 200 μm and the dead volume < 5 μl, thus facilitating the rapid change of solutions. The flow solutions used were either of the same composition as the bath solution above, a 143 mM K^+ solution containing (mM): KCl, 143; $CaCl_2$, 1·8; $MgCl_2$, 1; Hepes, 10; pH adjusted to 7·4 at 22 °C using KOH, or occasionally a 40 mM K^+ solution in which 103 mM KCl was replaced by NaCl and NaOH was used to adjust the pH.

Adenosine (Sigma, UK) was made up as a 10 mM stock solution daily and diluted into the flow solution to give a final concentration of between 5 and 40 μM. The following pharmacological agents were also used: CCPA (2-chloro-N^6-cyclopentyladenosine; Research Biochemicals Inc. (RBI), St Albans, Herts); CGS 21680 (2-*p*-(2-carboxethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine hydrochloride; RBI); DPCPX (8-cyclopentyl-1,3-dipropylxanthine; RBI); CGS 15943A (5-amino-9-chloro-2-(2-furanyl)-1,2,4-triazolo[1,5-C]quinazoline monomethanesulphonate; a gift from Ciba-Geigy, Summit, NJ, USA); glibenclamide (Sigma); phencyclidine (Sigma); charybdotoxin (a gift from ICI Pharmaceuticals, Macclesfield, Cheshire), apamin (Sigma), and adenosine deaminase (Boehringer, Lewes, East Sussex). CGS 21680, DPCPX and CGS 15943A were dissolved in dimethyl sulphoxide (DMSO; Sigma) to give stock solutions of 10 mM. CCPA, charybdotoxin and phencyclidine were dissolved in distilled water to give stock solutions of 10, 0·1 and 20 mM respectively. Glibenclamide was dissolved in 50% DMSO:50% polyethyleneglycol to give a stock solution of 10 mM. Apamin was dissolved in dilute acetic acid to give a stock of 1 mM. All stock solutions were stored in aliquots at −20 °C. All compounds were diluted into the flow solution immediately prior to use; the final concentrations are given throughout.

The pipette-filling solution had the following composition (mM): KCl, 143; $MgCl_2$, 1; EGTA, 0·5; Hepes, 10; pH adjusted to 7·2 at 22 °C using KOH. For experiments in which we used outside-out patches (Fig. 5*A*), we used the same pipette solution, and excised patches in the 5 mM K^+ bath solution described above. Patches were then superfused with 143 mM K^+ solution of the composition given above.

Recording methods

Recordings were made using the perforated patch method, either with nystatin (Horn & Marty, 1988) or amphotericin B (Rae, Cooper, Gates & Watsky, 1991). Nystatin (Sigma) was made up in methanol as described by Amédée, Large & Wang (1990) and diluted into the pipette solution to give a final concentration in the range 50–100 μg/ml. Amphotericin B (Sigma) was made up as a stock (6 mg/100 μl) in DMSO (Tissue Culture Grade, Sigma) and stored at −20 °C. Twenty microlitres of the stock was diluted into 5 ml of the pipette-filling solution immediately prior to use.

Patch pipettes were pulled from borosilicate tubing (o.d. 1·5 mm; Clarke Electromedical, Pangbourne, Berks), coated with dental sticky wax (Kemdent, Swindon, Wilts) and fire polished. Their resistance when filled with pipette solution was in the range 5–10 MΩ. Seals, formed by the application of negative pressure, were in the order of 10 GΩ. Whole cell access was achieved by

the antibiotics within 5–15 min of seal formation. Series resistance (R_s), input resistance, and whole cell capacitance were measured using a 10 mV depolarization. R_s was 20·1 ± 1·5 MΩ ($n = 30$). Since the currents measured here were normally small (< 50 pA) the voltage error caused by R_s will be less than 1 mV and so we did not routinely compensate for R_s. In experiments where voltage ramps were used, however, up to 80 % compensation for R_s was used.

Whole cell currents were recorded using a List EPC-7 amplifier and stored on a modified Sony digital audio tape (DAT) recorder. Current records obtained from ramp protocols were digitized at 10 kHz, using a TL-125 labmaster A–D interface (Axon Instruments, Foster City, CA, USA) and a Dell 325 microcomputer, or a CED 502 A–D converter and PDP 11/73 computer. A suite of programs developed using the AxoBASIC library (Davies, 1993) was used both to apply voltage-clamp command potentials and for analysis of whole cell currents. Histograms of current amplitude were fitted with Gaussian curves using a Marquardt algorithm as has been described previously (Standen, Stanfield & Ward, 1985). For the measurement of current noise we used programs provided by Professor D. Colquhoun to measure the mean current and variance of 4 s segments of recording filtered at 250 Hz (8-pole Butterworth) and sampled at 512 Hz. Regression lines or parabolic functions were fitted to the variance–mean plots using Sigmaplot software (Jandel, Corte Madera, CA, USA). For display purposes whole cell currents have been low-pass filtered at 100 Hz (8-pole Bessel) unless otherwise stated. Membrane potentials are expressed as inside (of the membrane) relative to outside, and outward currents are defined positive and plotted upwards. Experiments were done at room temperature, 18–22 °C, and results are given as means ± S.E.M. Student's t test was used to test the significance of results where indicated.

RESULTS

Myocytes isolated from the pig coronary artery had membrane capacitances of 24·2 ± 1·1 pF and input resistances of 8·0 ± 1·0 GΩ ($n = 30$ in each case).

Extracellular adenosine activates a K^+ conductance

Figure 1*A* shows the effect of adenosine on the whole cell current recorded from an isolated porcine coronary arterial cell. Throughout the experiment the cell was continuously superfused with flow solution with a physiological $[K^+]_o$ of 5 mM, and the cell was held at 0 mV. The addition of 20 μM adenosine to the superfusate activated an outward current and increased current noise. In this experiment the induced current reached a maximum value of 17 pA about 2 min after the introduction of extracellular adenosine. Upon removal of adenosine, the increased levels of both current and current noise persisted for almost 1 min before declining gradually. Similar results were seen in experiments on four other cells in this solution.

Figure 1*B* shows the effect of adenosine on a cell superfused with 143 mM K^+ solution (so that the K^+ equilibrium potential, E_K, should be 0 mV), and held at −60 mV. Under these conditions, the application of 20 μM adenosine induced an inward current which was again associated with an increase in current noise. The adenosine-activated current reached its maximal value of −26 pA within 1–2 min of the introduction of adenosine, and both the inward current and the noise declined upon adenosine removal. The results described in this paper were all obtained using the perforated patch method with either nystatin or amphotericin B. Adenosine sometimes activated a current when conventional whole cell recording was used, but its effect was much more consistent with the perforated patch method.

In order to investigate the ionic basis of the current induced by adenosine, we used voltage ramps to measure its reversal potential. Figure 2*A* shows the currents obtained in response to such ramps in a cell bathed in 5 mM K^+ solution both at the

peak of the response to adenosine (20 μM), and in its absence. The voltage was ramped from −100 to 0 mV over 50 ms, and the currents shown are averages from five identical ramps, and are plotted against the membrane potential. Figure 2*B* shows the current–voltage relation for the adenosine-activated current, obtained

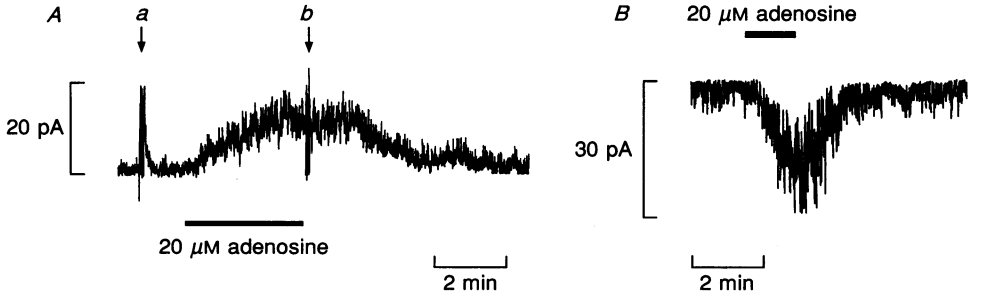

Fig. 1. Whole cell currents induced by adenosine. *A*, the effect of 20 μM adenosine on a cell bathed in extracellular solution containing 5 mM K^+, and held at 0 mV. Adenosine activates an outward current under these conditions. The arrows indicate periods when voltage ramps were applied, and correspond to the current–voltage relations of Fig. 2*A*. *B*, the inward current activated by 20 μM adenosine in a cell bathed in 143 mM K^+ solution and held at −60 mV.

by subtracting the ramped current in the absence of adenosine from that in its presence. The reversal potential, measured by increasing the gain of the current axis, was −86 mV in this cell, close to the calculated value for the equilibrium potential for K^+ of −84 mV. Figure 2*C* and *D* shows a similar experiment, but on a cell bathed in 143 mM K^+. In this case the voltage was ramped from −60 to +30 mV over 50 ms, and the current–voltage relation for the adenosine-activated current shown in Fig. 2*D* reverses close to 0 mV.

Figure 3 shows the results of a number of experiments like those of Fig. 2 on cells bathed in solutions with a $[K^+]_o$ of either 5, 40 or 143 mM. The reversal potential of the adenosine-activated current is plotted against the extracellular K^+ concentration. The data are well fitted by a line drawn to the Nernst equation for K^+ (making the assumption that the intracellular $[K^+]$ is equal to that in the pipette, 143 mM), strongly suggesting that the current activated by adenosine is carried by the movement of K^+ through K^+ channels.

The current–voltage relations of Fig. 2 also suggest that open-state probability of the channels activated by adenosine shows little voltage dependence. The relation in 5 mM K^+ (Fig. 2*B*) can be well fitted assuming that K^+ flow through open channels obeys constant field theory (Goldman, 1943) to give the expression:

$$I_K = P_K \frac{VF^2}{RT} \frac{[K^+]_i \exp(VF/RT) - [K^+]_o}{\exp(VF/RT) - 1}, \quad (1)$$

where P_K is the potassium permeability and R, T and F have their usual thermodynamic meanings. In symmetrical K^+ the current–voltage relation is linear over most of the voltage range (Fig. 2*D*), again as expected if the channels show little voltage dependence.

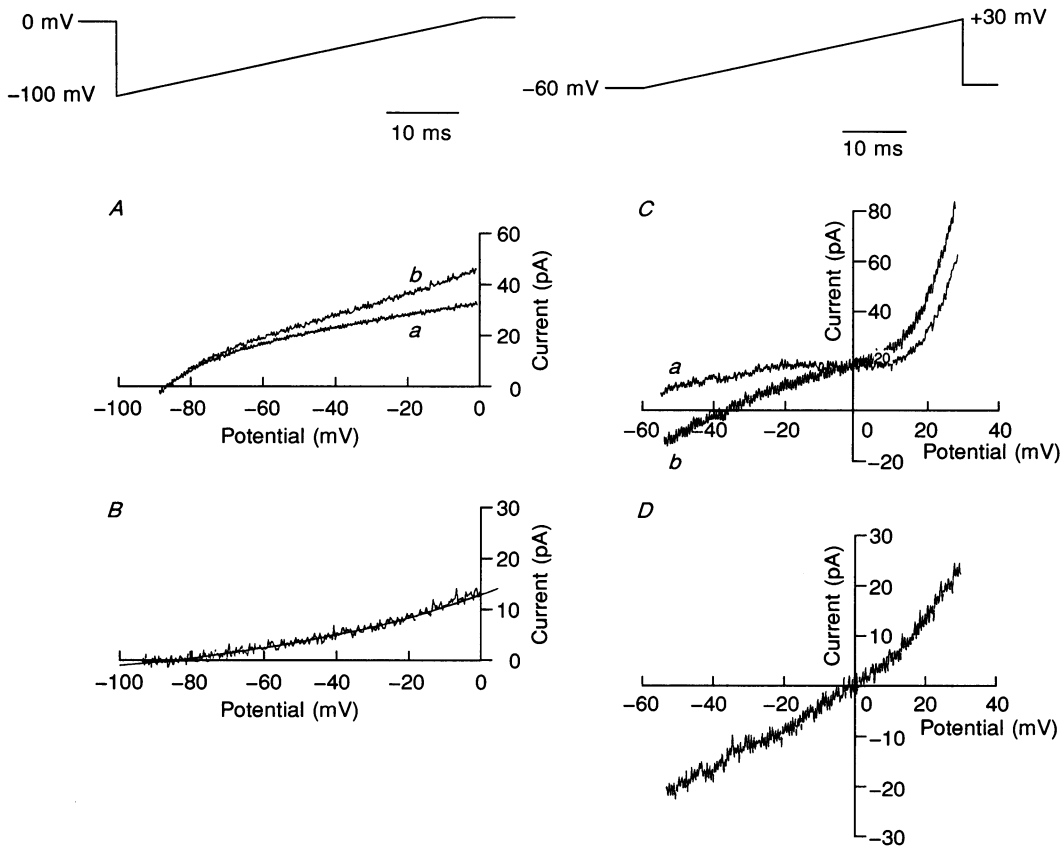

Fig. 2. Current–voltage relations for the adenosine-activated current. *A*, currents recorded in response to a linear voltage ramp (shown above) in the absence (*a*) and presence (*b*) of 20 μM adenosine (*a* and *b* correspond to the periods marked in Fig. 1*A*). The cell was superfused with 5 mM K^+ solution, and the currents are averages from 5 records. *B*, current–voltage relation for the adenosine-activated current obtained by subtracting record *a* from record *b* in Fig. 2*A*. The curve through the record is drawn to the constant field equation (eqn (1) of the text) with $P_K = 9{\cdot}76 \times 10^{-13}$ cm/s. *C*, currents in response to a voltage ramp (shown above) in a cell superfused with 143 mM K^+ solution in the absence (*a*) and presence (*b*) of 20 μM adenosine. Currents are averages from 7 records, and are offset on the current axis because of the time-invariant capacity current in response to the relatively rapid ramp. *D*, current–voltage relation for the adenosine-activated current obtained by subtraction of *a* from *b* in Fig. 2*C*.

We did not make a detailed study of the dependence of the adenosine-activated current on adenosine concentration because it was difficult to get consistent responses to repeated applications of adenosine in the same cell. However, in three cells a current was elicited by 5 μM adenosine, the lowest concentration that we used, while there appeared to be little difference between the effects of 20 and 40 μM adenosine. The peak current elicited by 20 μM adenosine in 5 mM K^+ at 0 mV

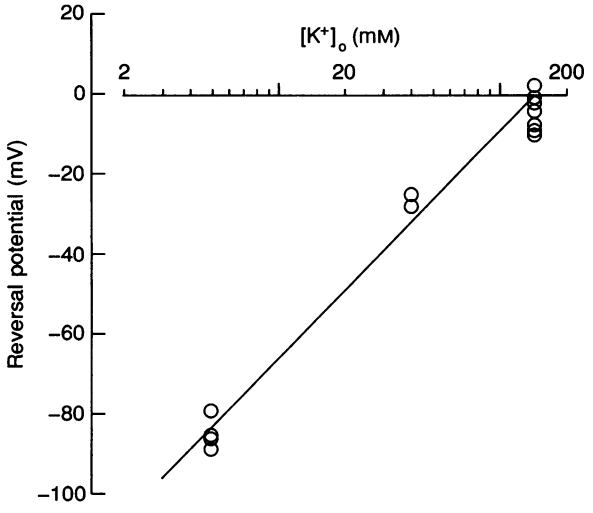

Fig. 3. Relation between the reversal potential for the adenosine-activated current and extracellular K^+ concentration. Reversal potentials were determined from ramps as illustrated in Fig. 2. Each point is from a different cell. The line is drawn to the Nernst equation for K^+, $E_K = RT/F\ln([K^+]_o/143\text{ mM})$, where R, T and F have their usual thermodynamic meanings.

was $15{\cdot}2 \pm 1{\cdot}7$ pA ($n = 5$), while in 143 mM K^+ at -60 mV it was $-21{\cdot}6 \pm 1{\cdot}7$ pA ($n = 9$). Because the current was larger under the latter conditions, and because the negative holding potential made the activation of voltage-dependent K^+ channels less likely and resulted in a quieter baseline, we made the recordings of adenosine-activated currents described in the rest of this paper at -60 mV with 143 mM extracellular K^+.

Effects of potassium channel blockers

To characterize further the potassium channel activated by adenosine, we looked at the effects of a number of blockers of K^+ channels on the adenosine-activated current.

Glibenclamide

A number of vasodilating agents that give rise to some or all of their effects by causing membrane hyperpolarization act by way of a mechanism that is sensitive to the antidiabetic sulphonylurea glibenclamide (Standen, Quayle, Davies, Brayden, Huang & Nelson, 1989; Standen, 1992). Glibenclamide has been shown to reduce the open probability of ATP-sensitive K^+ channels (K^+_{ATP} channels) in a variety of tissues (e.g. De Weille, Fosset, Mourre, Schmid-Antomarchi, Bernardi & Lazdunski, 1989; Cook & Quast, 1990). In the present study we found that the adenosine-activated K^+ current was also inhibited by glibenclamide.

Figure 4 shows an experiment illustrating the effect of glibenclamide. The cell was bathed in 143 mM K^+ and held at −60 mV throughout. Adenosine (20 μM) evoked a small inward current and an increase in current noise (*a*), both of which gradually declined when adenosine was removed. The application of 5 μM

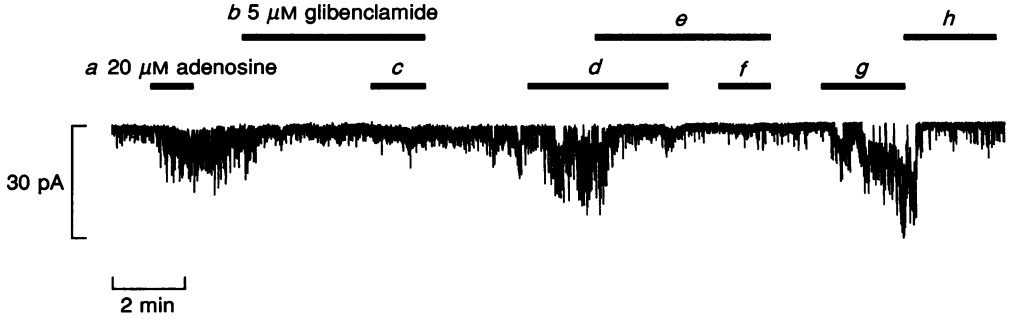

Fig. 4. Inhibition of adenosine-activated current by glibenclamide. Current recorded from a cell bathed in 143 mM K^+ and held at −60 mV. Where indicated, the solution superfusing the cell was switched to one containing either adenosine (20 μM; *a*, *c*, *d*, *f* and *g*), glibenclamide (5 μM; *b*, *e* and *h*), or both. *a*, *b*, etc. refer to the description in the text.

glibenclamide (*b*) almost immediately abolished residual noise and returned the current to the resting level. When adenosine (20 μM) was reapplied in the presence of glibenclamide (*c*) it elicited no inward current. Both the adenosine and the glibenclamide were then washed from the cell for a period of 3 min, after which time the reapplication of adenosine (*d*) activated an inward current. Glibenclamide (5 μM) was then added in the presence of the adenosine (*e*). Despite the continued presence of the extracellular adenosine, both the noise and the inward current rapidly declined to resting levels. A subsequent addition of adenosine in the presence of glibenclamide again failed to elicit any response (*f*). The effect of glibenclamide was clearly reversible, for on washing the cell for a period of 1·5 min, a third adenosine response was evoked (*g*). The application of glibenclamide simultaneously with the removal of adenosine (*h*) caused an instantaneous block of the inward current and current noise, which may be compared with the usual slow decline of current and noise observed upon adenosine removal (e.g. response *a* in Fig. 4; Fig. 1). The failure of adenosine to activate current in the presence of 5 μM glibenclamide was confirmed in a further six cells, all of which responded to adenosine in the absence of glibenclamide.

The effect of glibenclamide in reducing background current noise in the absence of adenosine, and the rapid decline in current seen when glibenclamide was applied immediately on the removal of adenosine, led us to consider whether such background noise and the normal slow decline of current after removal of adenosine resulted from the effects of residual adenosine in the immediate vicinity of the cell. Experiments were therefore repeated in the presence of the enzyme adenosine deaminase (1–2 units/ml), which will rapidly break down any such residual

adenosine. The presence of the enzyme had no effect on either background noise or on the decline of the response after adenosine removal ($n = 4$, data not shown). We conclude that the noise represents a low level of channel activity in the absence of adenosine, and that the decline of the adenosine-induced current on removal of exogenous adenosine represents a relaxation of the cellular mechanisms linking the adenosine receptor to channel activation, rather than a slow decline in the adenosine concentration in the immediate vicinity of the cell membrane.

Since phencyclidine (PCP) is known to antagonize the effects of the K^+ channel opener cromakalim in myocytes from the rabbit portal vein (Beech & Bolton, 1989), we tested its effect on the adenosine-activated K^+ current. PCP (5 μM) blocked the current activated by adenosine in five cells (not shown).

Charybdotoxin and apamin

Large conductance Ca^{2+}-activated K^+ channels (BK channels) appear to be present in most if not all smooth muscles, and are the easiest channels to detect in excised patches. This type of K^+ channel is activated by an increase in cytoplasmic free Ca^{2+} and is also voltage dependent, its open-state probability (P_o) increasing with depolarization (Latorre, Oberhauser, Labarca & Alvarez, 1989). Current–voltage relations for the adenosine-induced current (Fig. 2*B* and *D*) suggest that the underlying channels show little voltage dependence, arguing against the involvement of BK channels. Since BK channels are very effectively blocked by charybdotoxin (CTX), a peptide from scorpion venom (Miller, Moczydlowski, Latorre & Phillips, 1985), which acts with a K_i (concentration for half-inhibition) in the range of a few nanomolar, we also used CTX to check whether BK channels were involved in the action of adenosine.

The effectiveness of CTX on BK channels of coronary arterial myocytes was confirmed by applying it to outside-out patches excised from the cells, as illustrated in Fig. 5*A*. Under these conditions 50 nM CTX rapidly reduced the P_o of BK channels to zero. CTX was equally effective on BK channels in patches excised from rabbit pulmonary arterial myocytes (P. D. Langton, personal communication). Figure 5*B* shows that 50 nM CTX did not, however, affect the K^+ current induced by the application of 40 μM adenosine. Similar results were obtained in six other cells, and we conclude that BK channels do not contribute to the current activated by adenosine.

We also investigated the effect of apamin, a potent and specific inhibitor of small conductance Ca^{2+}-activated K^+ (or SK) channels (Hugues, Romey, Duval, Vincent & Lazdunski, 1982). Such channels exhibit little or no voltage dependence (Latorre *et al.* 1989). In experiments on five cells, 100 nM apamin had no discernible effect upon adenosine-activated currents.

Single channels activated by adenosine

In many cells held at −60 mV in 143 mM K^+ solution the current noise in whole cell recording was low. This meant that with appropriate filtering (usually 250 Hz), single channel openings could be detected easily. An example is shown in Fig. 6. In this cell, occasional openings of a channel passing an inward current of −2·06 pA

occurred before the addition of adenosine (Fig. 6*A a* and *A b*). Addition of adenosine led to an increase of channel activity, as can be seen in the records of Fig. 6*A* taken during the onset of the adenosine response (records *A c* and *A d*). Fig. 6*B* shows a histogram of current amplitude made during the onset of the response to adenosine

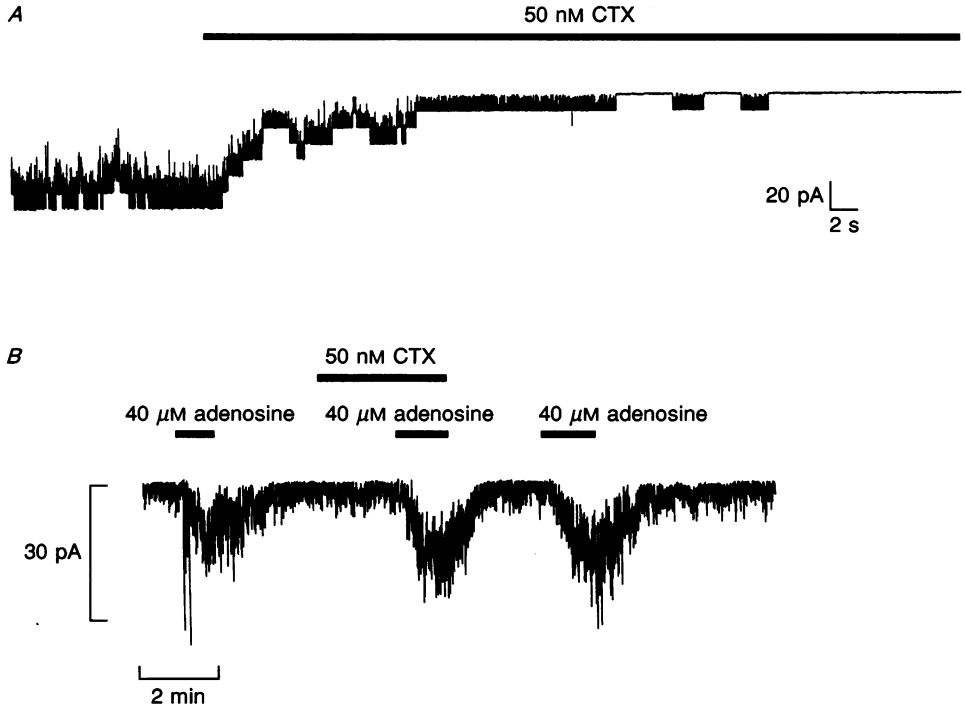

Fig. 5. Lack of effect of charybdotoxin on adenosine-activated current. *A*, CTX block of coronary BK channels. Recording from an outside-out patch from a coronary myocyte. The external solution was 143 mM K^+ solution, and the patch was held at −60 mV throughout. Under these conditions openings of BK channels lead to inward current steps of −13·0 pA in amplitude, corresponding to a conductance of 217 pS. CTX (50 nM) was applied where indicated. Record filtered at 900 Hz. *B*, test of CTX on the adenosine-activated current recorded from a cell bathed in 143 mM K^+ and held at −60 mV. Where indicated, the solution superfusing the cell was switched to one containing either adenosine (40 μM), CTX (100 nM), or both.

in another cell. The histogram shows clear peaks corresponding to current levels with one or two channels open, with a broader peak corresponding to a third level. Later in the adenosine response, when the current through more channels is superimposed, the underlying channel openings cannot be resolved as clearly (Fig. 6*A e* and *A f*). Larger steps in current were also sometimes seen (e.g. arrow in Fig. 6*A e*), which may correspond to openings of channels with larger unitary conductance. The channels with unitary current around −2 pA were seen consistently during adenosine activation when the recording noise level was sufficiently low. In eight cells exposed to adenosine the mean unitary current,

measured by fitting Gaussian curves to amplitude histograms like those of Fig. 6*B*, and under the same recording conditions was $-2{\cdot}08 \pm 0{\cdot}02$ pA. Assuming a reversal potential of 0 mV (see Fig. 3), this corresponds to a unitary conductance of 35 pS.

An alternative method that has often been used to estimate the size of unitary

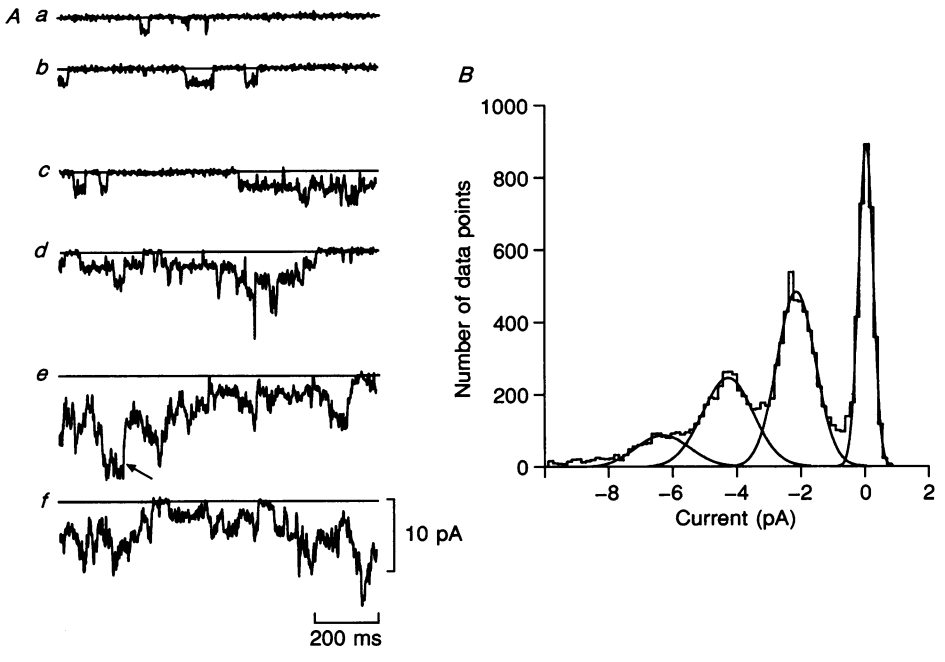

Fig. 6. K^+ channels activated by adenosine. *A*, records of whole cell current from a cell held at −60 mV and bathed in 143 mM K^+ solution. Records *a* and *b* were made before the addition of adenosine, records *c* and *d* during the early part of the response (about 1 min after the addition of 40 μM adenosine), and records *e* and *f* later in the response. The zero current level is indicated by a continuous line in each case, and the records have been filtered at 250 Hz for display. *B*, histogram of current amplitude taken during the onset of the response to adenosine in another cell under the same recording conditions as in *A*. The continuous line shows the best fit Gaussian curves, drawn with means and S.D.s (pA) of 0, 0·23; −2·17, 0·60; −4·29, 0·79; −6·32, 0·83 respectively.

events underlying macroscopic currents is the analysis of fluctuations, or noise, in the current (Anderson & Stevens, 1973). The variance of the macroscopic current, σ_I^2, will be related to its mean value $\bar{I}$ by the expression:

$$\sigma_I^2 = i\bar{I} - \frac{\bar{I}^2}{N}, \tag{2}$$

where i is the unitary current and N the number of channels. The expression gives a parabolic relation between variance and mean current as the channel open-state probability (P_o) varies from 0 to 1. When P_o is $\ll 1$ the variance–mean relation will approximate a straight line with a slope equal to the unitary current. The method

will give a lower estimate of unitary current if the variance is reduced significantly by filtering. This effect may be particularly important in whole cell patch clamp recording where the series resistance of the pipette and the capacitance of the cell membrane combine to form a low-pass filter, which may have a low cut-off

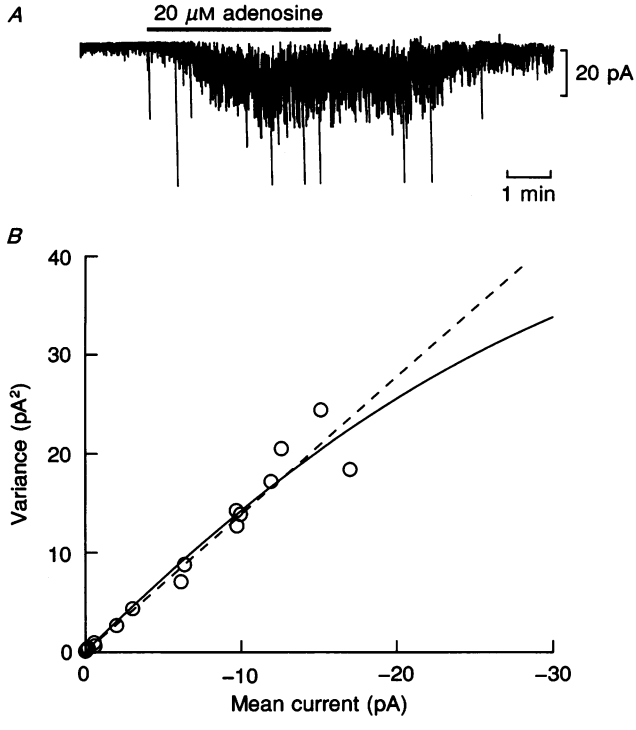

Fig. 7. Current noise induced by adenosine. *A*, current recorded from a cell bathed in 143 mM K^+ and held at −60 mV. The record has been filtered at 900 Hz to show the noise activated by adenosine. *B*, plot of current variance against mean current for the experiment illustrated in *A*. The dashed line shows the best fit to a straight line, while the continuous curve gives the best fit to eqn (2) of the text, with $i = -1{\cdot}58$ pA, $N = 66$.

frequency. We were interested to compare the values of unitary current derived from noise analysis with the currents we observed directly. Figure 7 shows an example of such analysis. In this experiment the unitary current i measured from the slope of the straight line fitted to the variance–mean plot (dashed line of Fig. 7*B*) was −1·38 pA, while the best fit to eqn (2), shown by the continuous curve, gave a value of −1·58 pA. Direct measurement of single channel currents seen at the onset of the adenosine response in the same cell gave a single channel current of −2·14 pA. We found in general that noise analysis gave lower estimates for unitary currents than those we obtained directly. In six cells analysed in this way the mean i obtained from the best fit straight line to the variance–mean plot was $-1{\cdot}25 \pm 0{\cdot}13$ pA, while fitting eqn (2) gave $-1{\cdot}73 \pm 0{\cdot}2$ pA.

The action of adenosine is mediated by an A_1 receptor

Adenosine initiates its relaxation of vascular smooth muscle by binding to specific cell surface receptors. These receptors, termed P_1 purinergic receptors (Burnstock,

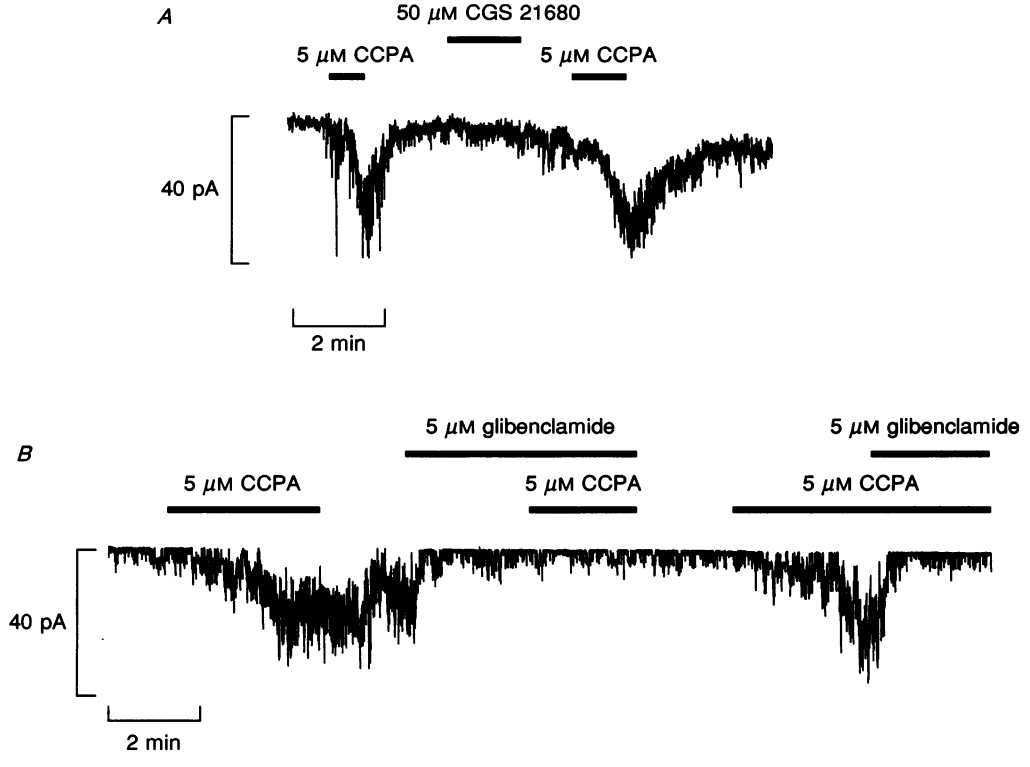

Fig. 8. Effects of A_1 and A_2 agonists. *A*, current recorded from a cell bathed in 143 mM K^+ and held at −60 mV. Where indicated, the solution superfusing the cell was switched to one containing either the A_1 agonist CCPA (5 μM) or the A_2 agonist CGS 21680 (50 μM). *B*, inhibition of the effect of CCPA by glibenclamide in a cell under the same recording conditions as in *A* above. Where indicated, the superfusing solution was switched to one containing 5 μM CCPA, 5 μM glibenclamide, or both.

1978), can be divided into two pharmacologically and biochemically defined subclasses, A_1 and A_2 receptors, both of which have been demonstrated in arterial smooth muscle (see Olsson & Pearson, 1990). To investigate the type of receptor involved in the activation of K^+ channels by adenosine, we tested the ability of selective A_1 and A_2 agonists to activate K^+ current, and the effects of A_1 and A_2 antagonists on the action of adenosine.

Effects of A_1 and A_2 receptor agonists

As an A_1 receptor agonist we chose the potent and highly selective compound CCPA. In striatal membranes from the rat brain CCPA has almost 10000-fold A_1

selectivity (Lohse, Klotz, Schwabe, Cristalli, Vittori & Grifantini, 1988). We also used the selective A_2 receptor agonist CGS 21680, which is reported to be 100- to 140-fold selective for the A_2 receptor (Hutchison, Webb, Oei, Ghai, Zimmerman & Williams, 1989).

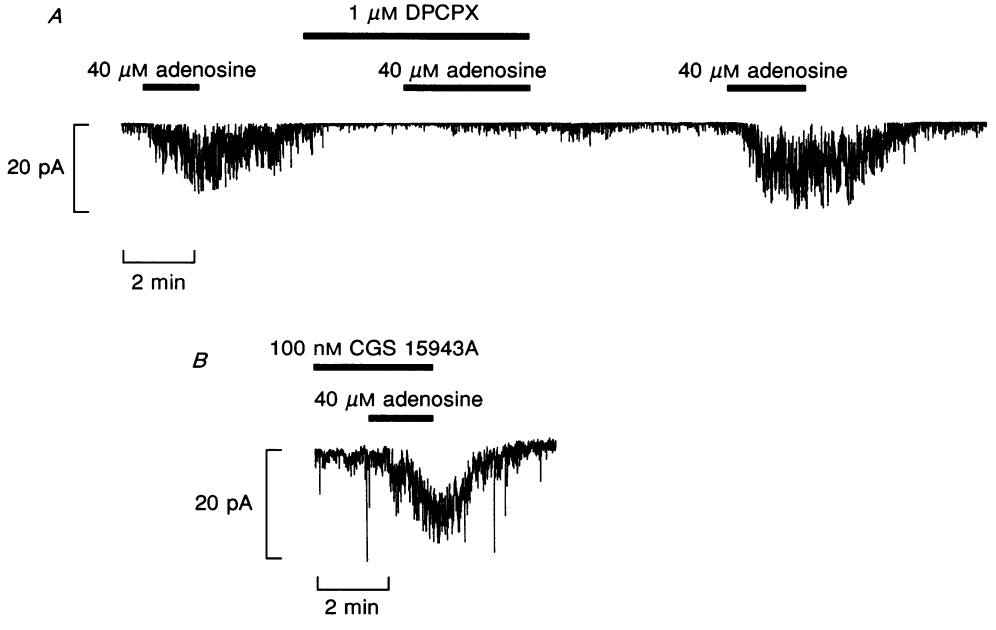

Fig. 9. Effects of A_1 and A_2 antagonists. *A*, block of the response to adenosine by DPCPX in a cell bathed in 143 mM K^+ and held at −60 mV. Where indicated, the solution superfusing the outside of the cell was switched to one containing either adenosine (40 μM), the A_1 antagonist DPCPX (1 μM), or both. *B*, response to adenosine in the presence of CGS 15943A in a cell under the same recording conditions as in *A* above. Where indicated, the superfusing solution was switched to one containing CGS 15943A (100 nM) or adenosine (40 μM) + CGS 15943A.

Figure 8*A* shows an experiment in which both the A_1 and the A_2 agonist were applied to the same cell. Five micromolar of the A_1 agonist CCPA activated an inward current of −25 pA after about 1 min and also increased current noise; a response very similar to that elicited by adenosine in other cells under the same recording conditions (−60 mV, 143 mM K^+). On removal of CCPA both current and noise declined to its baseline level. Addition of the A_2 agonist CGS 21680 (50 μM) was without effect despite the continued ability of the cell to respond, as shown by the response to a subsequent application of CCPA. The ability of 5 μM CCPA to activate a current was confirmed in experiments on six other cells (mean $-22{\cdot}3 \pm 2{\cdot}3$ pA, $n = 7$), while 10 μM had similar effects ($n = 3$). In contrast, neither 50 ($n = 5$) nor 100 μM ($n = 3$) of the A_2 agonist CGS 21680 activated currents in any of the cells tested.

To confirm the similarity of the whole cell current activated by the A_1 agonist CCPA to that induced by adenosine itself, we tested the effect of glibenclamide on

the CCPA-activated current. Figure 8*B* illustrates such an experiment. In the absence of glibenclamide, 5 μM CCPA activated an inward current of peak amplitude -23 pA together with an increase in noise, both of which declined gradually after the removal of CCPA. The addition of 5 μM glibenclamide led to a rapid return of both current and noise to their baseline levels, and subsequent application of 5 μM CCPA in the continued presence of glibenclamide elicited no response. Both the CCPA and the glibenclamide were then removed, and the cell perfused with 143 mM K^+ solution free of these agents for 2 min. The readdition of 5 μM CCPA after this time evoked an inward current of -27 pA and an associated increase in noise. Glibenclamide (5 μM) was then added to the cell in the presence of the CCPA. Despite the continued presence of the agonist, both the noise and the current rapidly returned to the resting level. The ability of glibenclamide (5 μM) to block the response to CCPA was confirmed in experiments on four other cells. Unitary currents measured with CCPA also suggest that it activates the same channels as adenosine. In six experiments where we could resolve the openings of single channels early in the response to CCPA (as illustrated for the response to adenosine in Fig. 6), unitary currents did not differ from those measured when adenosine itself was used as an agonist ($2{\cdot}04 \pm 0{\cdot}25$ pA, $P = 0{\cdot}76$). These results strongly suggest that in the pig coronary artery A_1 receptors are linked to glibenclamide-sensitive K^+ channels.

Effects of adenosine receptor antagonists

To investigate further the type of receptor involved in the response to adenosine, we tested the effects of A_1 and A_2 antagonists upon the ability of adenosine to activate a K^+ current. We used the potent A_1 receptor antagonist DPCPX, which is more than 500-fold selective for the A_1 receptor (Haleen, Steffen & Hamilton, 1987). In five cells we found that 40 μM adenosine failed to activate a K^+ current when it was applied in the presence of 1 μM DPCPX, though they responded to adenosine in its absence. An example of such an experiment is shown in Fig. 9*A*.

Highly selective A_2 receptor antagonists are not available at present. However, we examined the effect of the triazoloquinazole CGS 15943A, which is a potent A_2 receptor antagonist, though it may only show 15- to 30-fold selectivity for A_2 over A_1 receptors (Ghai *et al.* 1987; Olsson & Pearson, 1990). In four cells in which it was tested, adenosine was able to activate a current in the presence of 100 nM CGS 15943A (Fig. 9*B*). The mean current in the presence of the antagonist was $-18{\cdot}3 \pm 2{\cdot}7$ pA ($n = 4$), not significantly different from the mean current activated by adenosine in its absence ($P = 0{\cdot}31$).

DISCUSSION

Adenosine and K^+ channel activation

Our results show that adenosine activates a K^+ conductance in coronary artery smooth muscle cells, and that this activation does not occur in the presence of the sulphonylurea K^+ channel inhibitor glibenclamide. The pharmacological profile of the effect is consistent with an action of adenosine at receptors of the A_1 subtype. These findings provide evidence at the single cell level for the suggestion that adenosine may exert part of its vasodilator action in the coronary circulation by

causing activation of K^+_{ATP} channels (Daut *et al.* 1990; von Beckerath *et al.* 1991). They are also in agreement with the conclusion of Merkel *et al.* (1992), on the basis of studies of the relaxation of coronary arterial rings, that the component of relaxation that involves K^+ channel activation is mediated by A_1 receptors.

Adenosine is known to activate K^+ channels in both atrial muscle and in sino-atrial node, leading to a shortening of the atrial action potential and a decrease in the heart rate respectively (see e.g. Belardinelli, Linden & Berne, 1989). The effect does not involve cyclic nucleotides (Böhm *et al.* 1984), and is mediated by the G_i subtype of GTP-binding protein (Kurachi, Nakajima & Sugimoto, 1986). Adenosine appears to act at A_1 receptors to exert these effects, and the K^+ channel activated appears to be the same as that activated by stimulation of muscarinic receptors (see Olsson & Pearson, 1990).

Adenosine has also been shown to activate K^+_{ATP} channels in ventricular myocytes (Kirsch, Codina, Birnbaumer & Brown, 1990). Again the effect is mediated by A_1 receptors and G_i appears to be involved, since the channel is also activated by α_i G-protein subunits applied directly to the cytoplasmic side of the membrane in excised patches. Since activation can occur in such cell-free patches, the authors suggest that the pathway linking the A_1 receptor to the K^+_{ATP} channel is membrane-delimited. In the present study we have not investigated the mechanism linking A_1 receptors to K^+ channels in coronary arterial cells. By analogy with the effects discussed above, a G-protein link that does not involve cyclic nucleotides seems likely. The greater reliability of the response to adenosine when permeabilized patch recording rather than conventional whole cell recording was used suggests, however, that some metabolic support or cytoplasmic constituent may be necessary either for the activation pathway or to maintain the channels in a functional state.

The type of K^+ channel activated by adenosine

The sensitivity of the K^+ current activated by adenosine to the sulphonylurea glibenclamide suggests that the type of channel involved is an ATP-sensitive K^+ channel. Glibenclamide can no longer be regarded as completely selective for K^+_{ATP} channels, since it has been found to block D-current in hippocampal neurones (Crépel, Krnjević & Ben-Ari, 1992). The D-current is not very sensitive to the sulphonylurea, however, 10 μM glibenclamide causing only 35% inhibition. We found that 5 μM glibenclamide completely abolished the response to adenosine, while Cyrys & Daut (1991) have shown that nanomolar concentrations of glibenclamide are sufficient to inhibit hypoxic vasodilatation in isolated guinea-pig heart. Involvement of Ca^{2+}-activated K^+ channels of either large or small conductance in the adenosine response seen in our experiments is very unlikely in view of the lack of effect of either charybdotoxin or apamin.

K^+_{ATP} channels of both large and small conductance have also been reported in patches excised from smooth muscle, or after incorporation into bilayers, but they are recorded less readily than in other muscle tissues and the details of their regulation are not fully understood (Standen *et al.* 1989; Kovacs & Nelson, 1990; Kajioka, Kitamura & Kuriyama, 1991; Ottolia & Toro, 1993). A K^+_{ATP} channel of 30 pS conductance has been described in membrane patches of cultured porcine

coronary artery cells, and is inhibited by angiotensin II and endothelin (Miyoshi & Nakaya, 1991; Miyoshi *et al.* 1992). In the present study, we could resolve unitary currents at the onset of the adenosine response in many cells that correspond to a channel with a conductance of 35 pS in symmetrical 143 mM K^+ (Fig. 6). These channels appear to be consistently activated by adenosine, though we cannot exclude the possibility that channels of larger conductance are also activated, since we sometimes saw openings of larger channels during the response.

The mechanisms of adenosine-induced vasodilatation

The contractile tone of arterial smooth muscle is controlled, at least in part, by the myoplasmic Ca^{2+} concentration. Ca^{2+} forms a Ca^{2+}–calmodulin complex that activates a myosin light chain kinase (MLCK) which phosphorylates the light chain of myosin. The rate of actomyosin cross-bridge formation, and so the force developed, depends on the extent of myosin light chain phosphorylation (Hai & Murphy, 1989). The activity of MLCK itself is also controlled by phosphorylation by a cAMP-dependent protein kinase, phosphorylation in this case reducing the ability of MLCK to phosphorylate myosin by reducing its affinity for Ca^{2+}–calmodulin. Thus contractile tone may be modulated either by changes in $[Ca^{2+}]_i$, or by a change in the Ca^{2+} sensitivity of the contractile mechanism mediated through MLCK phosphorylation. Activation of A_2 receptors leads to activation of adenylate cyclase, and it has been suggested that the A_2 vasorelaxing action of adenosine occurs by way of MLCK phosphorylation (see Olsson & Pearson, 1990). However, such an effect may not be important under physiological conditions, and decreases in $[Ca^{2+}]_i$ may account for the relaxation responses to cAMP formation (Stull, Hsu, Tansey & Kamm, 1990). In tracheal myocytes, cAMP has been shown to activate large-conductance Ca^{2+}-activated K^+ channels, and β-adrenergic agonists and forskolin appear to act by this route to cause hyperpolarization and relaxation (Kume, Takai, Tokuno & Tomita, 1989). We did not, however, see evidence for activation of these channels in coronary myocytes by adenosine in the present study, and adenosine-activated currents were not blocked by CTX.

The results of studies in whole hearts and coronary arterial rings (Daut *et al.* 1990; Clayton *et al.* 1992; Merkel *et al.* 1992), together with our findings, show that adenosine also activates K^+ channels to cause vasodilatation in the coronary circulation. The pharmacology of this action of adenosine is consistent with its being mediated by an A_1 receptor. Because of the high affinity of these receptors for adenosine, the effect may form a substantial component of vasodilatation. Thus Merkel *et al.* (1992) showed that 0·3 μM glibenclamide shifted the dose–response curve for the vasorelaxing effect of adenosine on pig coronary arteries to higher concentrations by > 75-fold, while von Beckerath *et al.* (1991) found that 2 μM glibenclamide reduced the vasodilator response to adenosine in guinea-pig heart to 29 % of its control value.

The vasodilator response to K^+ channel activation is likely to occur by way of hyperpolarization of the smooth muscle membrane. Although the adenosine-activated currents we report here are small, the high input resistance of the smooth muscle cells (8 GΩ) means that the underlying relative change in membrane

conductance is substantial. The 15 pA current we measure in 5 mM K^+ solution represents a more than doubling of the membrane conductance, which, since the extra conductance represents opening of K^+ channels, would be expected to cause substantial hyperpolarization (Nelson, Patlak, Worley & Standen, 1990). It is also possible that the cell isolation method itself may reduce the size of the responses to adenosine. In a microelectrode study on bovine coronary artery 10 μM adenosine was found to produce a hyperpolarization of about 17 mV (Sabouni, Hargittai, Lieberman & Mustafa, 1989). Hyperpolarization will lead to decreased Ca^{2+} entry through voltage-dependent Ca^{2+} channels. In arterial smooth muscle these channels are very sensitive to changes in membrane potential at voltages around the resting potential, and so quite small hyperpolarizations will reduce Ca^{2+} channel open-state probability and so Ca^{2+} entry, $[Ca^{2+}]_i$ and contractile force (Nelson *et al.* 1990). Hyperpolarization may also affect $[Ca^{2+}]_i$ independently of effects on Ca^{2+} entry, since Itoh, Seki, Suzuki, Ito, Kajikuri & Kuriyama (1992) have recently shown that hyperpolarization can inhibit the noradrenaline-induced synthesis of inositol trisphosphate in rabbit mesenteric artery, and so inhibit Ca^{2+} release from storage sites within the smooth muscle cells.

We thank Drs J. P. Boyle, N. W. Davies, R. A. J. Challis, P. D. Langton and M. T. Nelson for helpful discussions during the course of this work, and the staff of Parker's Ltd for supplying pig hearts. The work was supported by the MRC, Wellcome Trust and British Heart Foundation.

REFERENCES

AMÉDÉE, T., LARGE, W. A. & WANG, Q. (1990). Characteristics of chloride currents activated by noradrenaline in rabbit ear artery cells. *Journal of Physiology* **428**, 501–516.

ANDERSON, C. R. & STEVENS, C. F. (1973). Voltage clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction. *Journal of Physiology* **235**, 655–691.

BEECH, D. J. & BOLTON, T. B. (1989). Properties of the cromakalim-induced potassium conductance in smooth muscle cells isolated from the rabbit portal vein. *British Journal of Pharmacology* **98**, 851–864.

BELARDINELLI, L., LINDEN, J. & BERNE, R. M. (1989). The cardiac effects of adenosine. *Progress in Cardiovascular Disease* **32**, 73–97.

BERNE, R. M. (1962). Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. *American Journal of Physiology* **204**, 317–322.

BERNE, R. M. (1980). The role of adenosine in the regulation of coronary bloodflow. *Circulation Research* **47**, 807–813.

BÖHM, M., BRÜCKNER, R., HACKBARTH, I., HAUBITZ, B., LINHART, R., MEYER, W., SCHMIDT, B., SCHMITZ, W. & SCHOLZ, H. (1984). Adenosine inhibition of catecholamine-induced increase in force of contraction in guinea-pig atrial and ventricular heart preparations. Evidence against a cyclic AMP- and cyclic GMP-dependent effect. *Journal of Pharmacology and Experimental Therapeutics* **230**, 483–492.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptor. In *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach*, ed. BOLIS, L. & STRAUB, R. W., pp. 107–118. Raven, New York.

CLAPP, L. H. & GURNEY, A. M. (1991). Outward currents in rabbit pulmonary artery cells dissociated with a new technique. *Experimental Physiology* **76**, 677–693.

CLAYTON, F. C., HESS, T. A., SMITH, M. A. & GROVER, G. J. (1992). Coronary reactive hyperemia and adenosine-induced vasodilatation are mediated partially by a glyburide-sensitive mechanism. *Pharmacology* **44**, 92–100.

COOK, N. S. & QUAST, U. (1990). Potassium channel pharmacology. In *Potassium Channels. Structure, Classification, Function and Therapeutic Potential*, ed. COOK, N. S., pp. 181–255. Ellis Horwood, Chichester.

CRÉPEL, V., KRNJEVIĆ, K. & BEN-ARI, Y. (1992). Glibenclamide depresses the slowly inactivating outward current (I_D) in hippocampal neurones. *Canadian Journal of Physiology and Pharmacology* **70**, 306–307.

CYRYS, S. & DAUT, J. (1991). Hypoxic vasodilation in isolated guinea-pig heart is inhibited by nanomolar concentrations of the antidiabetic sulphonylurea glibenclamide. *Pflügers Archiv* **419**, suppl. 1, R107.

DAUT, J., MAIER-RUDOLPH, W., VON BECKERATH, N., MEHRKE, G., GÜNTHER, K. & GOEDEL-MEINEN, L. (1990). Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. *Science* **247**, 1341–1344.

DAVIES, N. W. (1993). A suite of programs for acquisition and analysis of voltage- and patch-clamp data developed using the Axobasic library. *Journal of Physiology* **459**, 111*P*.

DE WEILLE, J. R., FOSSET, M., MOURRE, C., SCHMID-ANTOMARCHI, H., BERNARDI, H. & LAZDUNSKI, M. (1989). Regulation and pharmacology of ATP-sensitive K^+ channels. *Pflügers Archiv* **414**, suppl. 1, S80–87.

GHAI, G., FRANCIS, J. E., WILLIAMS, M., DOTSON, R. A., HOPKINS, M. F., COTE, D. T., GOODMAN, F. R. & ZIMMERMAN, M. B. (1987). Pharmacological characterization of CGS 15943A: a novel nonxanthine adenosine antagonist. *Journal of Pharmacology and Experimental Therapeutics* **242**, 784–790.

GOLDMAN, D. E. (1943). Potential, impedance, and rectification in membranes. *Journal of General Physiology* **27**, 37–60.

HAI, C.-M. & MURPHY, R. A. (1989). Ca^{2+}, crossbridge phosphorylation, and contraction. *Annual Review of Physiology* **51**, 285–298.

HALEEN, S. J., STEFFEN, R. P. & HAMILTON, H. W. (1987). DPCPX, a highly-selective A_1 adenosine receptor antagonist. *Life Sciences* **40**, 555–563.

HORN, R. & MARTY, A. (1988). Muscarinic activation of ionic currents measured by a new whole-cell recording method. *Journal of General Physiology* **92**, 145–159.

HUGUES, M., ROMEY, G., DUVAL, D., VINCENT, J. P. & LAZDUNSKI, M. (1982). Apamin as a selective blocker of the calcium-dependent potassium channel in neuroblastoma cells. *Proceedings of the National Academy of Sciences of the USA* **79**, 1308–1312.

HUTCHISON, A. J., WEBB, R. L., OEI, H. H., GHAI, G. R., ZIMMERMAN, M. B. & WILLIAMS, M. (1989). CGS 21,680C, an A_2 selective adenosine receptor agonist with preferential hypotensive activity. *Journal of Pharmacology and Experimental Therapeutics* **251**, 47–55.

ITOH, T., SEKI, N., SUZUKI, S., ITO, S., KAJIKURI, J. & KURIYAMA, H. (1992). Membrane hyperpolarization inhibits agonist-induced synthesis of inositol 1,4,5-trisphosphate in rabbit mesenteric artery. *Journal of Physiology* **451**, 307–328.

KAJIOKA, S., KITAMURA, K. & KURIYAMA, H. (1991). Guanosine diphosphate activates an adenosine-5′-triphosphate-sensitive K^+ channel in the rabbit portal vein. *Journal of Physiology* **444**, 397–418.

KIRSCH, G. E., CODINA, J., BIRNBAUMER, L. & BROWN, A. M. (1990). Coupling of ATP-sensitive K^+ channels to A_1 receptors by G proteins in rat ventricular myocytes. *American Journal of Physiology* **259**, H820–826.

KOVACS, R. & NELSON, M. T. (1990). ATP-sensitive K^+ channels from aortic smooth muscle incorporated into planar lipid bilayers. *American Journal of Physiology* **261**, H604–609.

KUME, H., TAKAI, A., TOKUNO, H. & TOMITA, T. (1989). Regulation of Ca^{2+}-dependent K^+ channel activity in tracheal myocytes by phosphorylation. *Nature* **341**, 152–154.

KURACHI, Y., NAKAJIMA, T. & SUGIMOTO, T. (1986). On the mechanism of activation of muscarinic K^+ channels by adenosine in isolated atrial cells: involvement of GTP-binding proteins. *Pflügers Archiv* **407**, 264–274.

LATORRE, R., OBERHAUSER, A., LABARCA, P. & ALVAREZ, O. (1989). Varieties of calcium-activated potassium channels. *Annual Review of Physiology* **51**, 385–399.

LOHSE, M. J., KLOTZ, K.-N., SCHWABE, U., CRISTALLI, G., VITTORI, S. & GRIFANTINI, M. (1988). 2-Chloro-N^6-cyclopentyladenosine: a highly selective agonist at A_1 adenosine receptors. *Naunyn-Schmiedeberg's Archives of Pharmacology* **328**, 687–689.

MERKEL, L. A., LAPPE, R. W., RIVERA, L. M., COX, B. F. & PERRONE, M. H. (1992). Demonstration of vasorelaxant activity with an A_1-selective adenosine agonist in porcine coronary artery: involvement of potassium channels. *Journal of Pharmacology and Experimental Therapeutics* **260**, 437–443.

MILLER, C., MOCZYDLOWSKI, E., LATORRE, R. & PHILLIPS, M. (1985). Charybdotoxin, a protein inhibitor of single Ca^{2+}-activated K^+ channels from mammalian skeletal muscle. *Nature* **313**, 316–318.

MILLS, I. & GEWIRTZ, H. (1990). Cultured vascular smooth muscle cells from porcine coronary artery possess A_1 and A_2 adenosine receptor activity. *Biochemical and Biophysical Research Communications* **168**, 1297–1302.

MIYOSHI, Y. & NAKAYA, Y. (1991). Angiotensin II blocks ATP-sensitive K^+ channels in porcine coronary artery smooth muscle cells. *Biochemical and Biophysical Research Communications* **181**, 700–706.

MIYOSHI, Y., NAKAYA, Y., WAKATSUKI, T., NAKAYA, S., FUJINO, K., SAITO, K. & INOUE, I. (1992). Endothelin II blocks ATP-sensitive K^+ channels and depolarizes smooth muscle cells of porcine coronary artery. *Circulation Research* **70**, 612–616.

NELSON, M. T., PATLAK, J. B., WORLEY, J. F. & STANDEN, N. B. (1990). Calcium channels, potassium channels and the voltage dependence of arterial smooth muscle tone. *American Journal of Physiology* **259**, C3–18.

OLSSON, R. A. & PEARSON, J. D. (1990). Cardiovascular purinoceptors. *Physiological Reviews* **70**, 761–845.

OTTOLIA, M. & TORO, L. (1993). ATP-sensitive potassium channels from coronary smooth muscle cells reconstituted in lipid bilayers. *Biophysical Journal* **64**, A311.

RAE, J., COOPER, K., GATES, P. & WATSKY, M. (1991). Low access resistance perforated patch recordings using amphotericin B. *Journal of Neuroscience Methods* **57**, 15–26.

SABOUNI, M. H., HARGITTAI, P. T., LIEBERMAN, E. M. & MUSTAFA, S. J. (1989). Evidence for adenosine receptor-mediated hyperpolarization in coronary smooth muscle. *American Journal of Physiology* **257**, H1750–1752.

STANDEN, N. B. (1992). Potassium channels, metabolism and muscle. *Experimental Physiology* **77**, 1–25.

STANDEN, N. B., QUAYLE, J. M., DAVIES, N. W., BRAYDEN, J. E., HUANG, Y. & NELSON, M. T. (1989). Hyperpolarising vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science* **245**, 177–180.

STANDEN, N. B., STANFIELD, P. R. & WARD, T. A. (1985). Properties of single potassium channels in vesicles formed from the sarcolemma of frog skeletal muscle. *Journal of Physiology* **364**, 339–358.

STULL, J. T., HSU, L.-C., TANSEY, M. G. & KAMM, K. E. (1990). Myosin light chain kinase phosphorylation in tracheal smooth muscle. *Journal of Biological Chemistry* **265**, 16683–16690.

VON BECKERATH, N., CYRYS, S., DISCHNER, A. & DAUT, T. (1991). Hypoxic vasodilatation in isolated perfused guinea-pig heart: an analysis of the underlying mechanisms. *Journal of Physiology* **442**, 297–319.