Adenosine-induced hyperpolarization in guinea pig coronary artery involves A2b receptors and KATP channels

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Mutafova-Yambolieva, Violeta N., and Kathleen D. Kee. Adenosine-induced hyperpolarization in guinea pig coronary artery involves A2b receptors and KATP channels. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2687–H2695, 1997.—The role of P1 purinoceptor subtypes, the adenylyl cyclase (AC) pathway, and ATP-sensitive K⁺ (KATP) channels in adenosine (Ado)-induced membrane hyperpolarization was investigated in isolated segments of the guinea pig coronary artery using conventional microelectrode techniques. Ado (1–100 µM) elicited concentration-dependent hyperpolarization (half-maximal effective concentration 7.5 ± 0.5 µM) that averaged 28.6 ± 2.9 mV at 100 µM Ado. The A1 selective agonist N6-cyclopentyladenosine (CPA), the A2A/A2B agonist 2-chloroadenosine, and the A2B/A2A agonist 5-(N-ethylcarboxamido)adenosine (NECA) each induced glibenclamide (3 µM)-sensitive hyperpolarization at 10 µM. However, the selective A2b receptor agonists CGS-21680 and N6-[2-(3,5-dimethoxyphenyl)-2-(2-methoxyphenyl)]ethyladenosine (10 µM each) were without effect. Responses to CPA and NECA were significantly reduced by the AC inhibitor SQ-22,536 (100 µM). Activation of the AC-adenosine 3′,5′-cyclic monophosphate (cAMP)-protein kinase A (PKA) pathway by four additional methods, i.e., 1) forskolin (0.3–1 µM), 2) isoproterenol (0.1–1 µM), 3) combined milrinone (0.4 µM) and rolipram (30 µM), and 4) combined N6-phenyladenosine 3′,5′-monophosphate, 8-(6-aminohexyl)aminoadenosine 3′,5′-cyclic monophosphate, and the Sp-isomer of 5,6-dichloro-1-β-butyranosylbenzimidazole-3′,5′-cyclic monophosphothioate (100 µM each), also gave rise to glibenclamide-sensitive hyperpolarization. These results suggest that stimulation of A2b receptors coupled to AC represents the predominant mechanism by which Ado elicits hyperpolarization in this vessel. The ensuing increase in cAMP activates PKA, which then increases the activity of KATP channels. Our results further suggest that KATP channels are an important target for numerous pathways that raise cAMP levels in the coronary artery.

A1 receptors; A2a receptors; glibenclamide; adenylyl cyclase; protein kinase A; adenosine-5′-triphosphate-dependent potassium channel

IN 1962 IN A SEMINAL PAPER Berne (3) postulated that adenosine (Ado) released from active cardiac muscle acts as a vasodilator in the coronary circulation and so matches coronary blood flow to metabolic demand. Since then, Ado has been shown to be a vasodilator in most vascular beds and is particularly potent in the heart and cerebral circulations (3, 33). Ado is believed to mediate its effects via four Ado receptor subtypes, termed A1, A2a, A2b, and A3 (14). All of the Ado receptors are members of the G protein-coupled receptor family and possess seven transmembrane helical regions. A1 and A3 receptors mediate inhibition of adenylyl cyclase (AC), whereas A2 receptors enhance AC activity (14). Both A1 and A2 receptors have been identified in smooth muscle, including cells cultured from coronary artery (27).

In some vascular preparations, the vasodilator action of Ado is reduced by the sulfonylurea glibenclamide, suggesting that Ado leads to activation of ATP-sensitive potassium channels (KATP channels; see Refs. 7, 10). However, there is still controversy regarding the subtype of Ado receptor that mediates this effect. In cardiac muscle, A1 receptors lead to activation of KATP channels (20), and some studies have suggested that a similar pathway is present in the coronary artery as well. For example, Merkel et al. (26) concluded that, in porcine coronary artery, the glibenclamide-sensitive component of relaxation to Ado occurs via stimulation of A1 receptors, since the response to a selective A2 receptor agonist was unaffected by glibenclamide, whereas the response to an A1 receptor agonist was reduced. A similar conclusion was reached from studies of isolated, saline-perfused rabbit hearts (30). Additional support for this hypothesis came from patch-clamp studies of porcine coronary artery cells, which reported that the A1 receptor agonist N6-cyclopentyladenosine (CPA) induced a glibenclamide-sensitive current, whereas the A2 receptor agonist 2-(p-carboxyethyl)phenethylamino-5′-N-ethylcarboxamidoadenosine (CGS-21680) was without effect (9). Because A1 receptors lead to a reduction in AC activity, an important implication of these studies is that activation of KATP channels is independent of the AC-adenosine 3′,5′-cyclic monophosphate (cAMP) pathway. A similar conclusion was reached from microcirculatory studies that reported that the Ado-induced relaxation in the hamster cheek pouch was blocked by glibenclamide, whereas relaxation with forskolin (FSK) was not (16).

In contrast to these studies, substantial literature exists linking the vasodilator action of Ado to A2 receptors that stimulate AC and raise cAMP levels (33). Furthermore, there are studies that suggest that Ado-induced activation of KATP channels is mediated in large part via A2 receptors and the AC-cAMP-CAMP-dependent protein kinase (PKA) pathway. For example, in the dog coronary artery, the A2-selective agonist NECA was reported to produce glibenclamide-sensitive dilation (1). In addition, the A2 receptor agonist CGS-21680 elicited glibenclamide-sensitive K⁺ currents, although the A1-receptor agonist CPA was without effect in isolated rabbit mesenteric artery cells. The CGS-21680-induced currents were blocked by inhibitors of PKA (21). Glibenclamide-sensitive outward currents can also be elicited in these cells with application of calcitonin gene-related peptide (34), which is also coupled to stimulation of AC. Finally, in cultured cells from the porcine coronary artery, KATP channel activity
was enhanced by the catalytic subunit of PKA (28). A similar link between AC and activation of KATP channels has also been proposed in canine saphenous vein in which both direct activation of AC with FSK as well as the G protein-coupled stimulation of AC by isopropenol (Iso) gave rise to glibenclamide-sensitive hyperpolarization (29).

In summary, there is substantial evidence linking the AC-cAMP-PKA pathway to activation of KATP channels in smooth muscle. However, controversy still remains regarding the contribution of this pathway to the actions of Ado in the coronary artery. The goal of the present study was therefore to explore the relationship of this pathway to Ado-induced changes in membrane potential in the guinea pig coronary artery and to obtain functional evidence for the receptor subtype involved. To accomplish this goal, we determined the glibenclamide sensitivity of changes in membrane potential elicited by various Ado analogs as well as activators and inhibitors of the AC-cAMP-PKA pathway.

METHODS

General procedures. Male guinea pigs were killed by CO2 overdose followed by exsanguination. The heart was immediately removed from the animal and placed in cold (10°C) oxygenated Krebs solution for further dissection of the coronary artery. Segments of the left circumflex and descending coronary arteries were dissected out and cleaned of all adhering connective and myocardial tissue. Ring segments (3–5 mm long; 200–300 µm external diameter) were pinned in the sylgard bottom of a 2-ml recording chamber perfused with Krebs solution (3 ml/min; 37°C; aerated with 95% O2-5% CO2) of the following composition (in mM): 120.2 NaCl, 3.0 KCl, 1.2 MgCl2, 23.8 NaHCO3, 1.2 KH2PO4, 11.0 dextrose, and 2.5 CaCl2.

The endothelium of the guinea pig coronary artery appears to be tightly attached to the elastic lamina. Thus the success rate for either mechanical or chemical disruption of the endothelium is quite low (<10%). Therefore, in the majority of experiments in this study, the endothelium was left intact, and indomethacin (10 µM) and Nω-nitro-L-arginine (L-NNA; 100 µM) were included in the superfusate to eliminate the possible effects of endothelium-derived nitric oxide or prostacyclin. All examples in Figs. 1–7 are for endothelium-intact tissues, unless otherwise stated. In a few experiments, the endothelium was successfully removed by gentle rubbing of the lumen with a stainless steel pin (0.1 mm diameter). Removal of the endothelium was confirmed by a lack of hyperpolarization in response to acetylcholine (ACh; 0.5–1 µM). The smooth muscle was regarded as “undamaged” by the following criteria: 1) absence of visible contracture, 2) membrane potential more negative than -40 mV, and 3) membrane hyperpolarization in response to activators of AC.

Intracellular measurements were made through the adventitia of the vessel with fiber-containing borosilicate electrodes filled with 3 M KCl and having resistance between 70 and 100 MΩ. The electrode was attached to a dual high-input impedance differential electrometer (Duo 773; World Precision Instruments), and the signal was then amplified before viewing on a digital oscilloscope (Hitachi VC-6025) and stored on a tape with a Vetter PCM recording adapter attached to a Panasonic videocassette recorder.
Intracellular measurements of membrane potential were made in the absence and the presence of the drug tested. Impalements were judged on the basis of a rapid drop in potential upon entering the cell, a low noise level and minimum change in the electrode resistance, and zero potential before and after impalement. Membrane potential changes were analyzed by AcqKnowledge 3.2 software (Biopac System).

**Experimental design.** The effects of Ado, Ado analogs, Iso, FSK, phosphodiesterase (PDE) inhibitors, and PKA activators were examined by recording membrane potential before and during a 2- to 6-min superfusion of the tissue with Krebs solution containing the test drug. When the involvement of \( K_{ATP} \) channels or the AC pathway was examined, the tissue was superfused with either glibenclamide or the AC inhibitor 9-(tetrahydro-2-furanyl)-9H-purin-6-amine (SQ-22,536; see Refs. 15, 35) for at least 15 min before the test drug was added. In some experiments, the tissue was superfused for 10–15 min with BaCl\(_2\) and ibotenic acid (IbTx) before Ado was added.

**Drugs used.** ACh hydrochloride, Ado, glibenclamide, IbTx, Iso, indomethacin, L-NNA, FSK, and 1,9-dideoxyforskolin were from Sigma (St. Louis, MO). 2-Chloroadenosine (2-CAdo), CPA, CGS-21680, N\(^6\)-(2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl))ethyl adenosine (DPMA), 8-cyclopentyl-1,3-
isolated segments of guinea pig coronary artery was \(-47.2 \pm 0.5 \text{ mV} (n = 143, N = 56)\). Ado at concentrations between 0.1 and 0.3 \text{ mM} was without effect \((n = 4)\), whereas higher concentrations gave rise to significant hyperpolarization \((\text{Fig. 1, A and B})\). At 100 \text{ mM}, Ado hyperpolarized cells by 28.6 \pm 2.9 \text{ mV}. The EC\(_{50}\) for Ado was 7.5 \pm 0.5 \text{ mM} \((n = 4)\).

The \(A_1\) selective Ado agonist CPA \([\text{inhibitory constant (K}_{i}) = 0.3-3 \text{ nM} \text{ and } 200-500 \text{ nM} \text{ for } A_1 \text{ and } A_2 \text{ receptors, respectively; see Ref. 14}]\) had no effect on membrane potential at concentrations <3 \text{ mM} \((n = 4)\). At 10 \text{ mM}, CPA elicited a 15.8 \pm 1.3 \text{ mV} \((n = 16)\) hyperpolarization \((\text{Fig. 2})\). The hyperpolarization elicited with 10 \text{ mM} CPA was not reduced by the 10- to 30-min preexposure to the \(A_1\) antagonist DPCPX \((50 \text{ nM}; 15.0 \pm 3.0 \text{ mV}, n = 3)\). Additional experiments were performed with the Ado analog 2-CAdo \((A_2_1 > A_2_2 \text{ agonist}; K_i = 3-30 \text{ nM}, 20-200 \text{ nM}, \text{ and } 5-20 \text{ mM} \text{ for } A_3, A_2_{2a}, \text{ and } A_2_{2b} \text{ receptors, respectively; see Ref. 14})\). 2-CAdo \((10 \text{ mM})\) hyperpolarized cells by 15.8 \pm 1.8 \text{ mV} \((n = 9)\). There was no significant difference in the amplitude of hyperpolarization observed with 10 \text{ mM} Ado \((17.6 \pm 1.5 \text{ mV}, n = 12)\), CPA, or 2-CAdo. The combined application of BaCl\(_2\) \((30 \text{ mM})\) and IbTx \((0.1 \text{ mM})\) substantially reduced or abolished the hyperpolarization elicited with each of the Ado analogs \((\text{Fig. 3})\). Glibenclamide by itself depolarized the cells by 6.3 \pm 0.7 \text{ mV} \((n = 7)\).

The nonselective \(A_1/A_2_{2a}/A_2_{2b} \text{ receptor agonist NECA (10 \text{ mM}, n = 8) hyperpolarized cells by 15.7 \pm 3.0 \text{ mV} \text{ (e.g., Figs. 3 and 4)}\). This hyperpolarization was entirely blocked by glibenclamide \((3 \text{ mM}; n = 3; \text{ Fig. 3D})\). In contrast to the other Ado analogs tested, CGS-21680 and DPMA \((\text{each at } 10 \text{ mM})\), which are selective \(A_2_{2a}\)-receptor agonists, were without effect on membrane potential \((n = 4; \text{ Fig. 4, A and B})\). The \(K_i\) values for NECA at \(A_1, A_2_{2a}\), and \(A_2_{2b}\) receptors is 3–30 \text{ nM} and at \(A_2_{2a}\) receptors is 0.5–5 \text{ mM} \((14)\). The \(K_i\) values for CGS-21680 at \(A_1, A_2_{2a}, \text{ and } A_2_{2b}\) receptors are 2,600 \text{ nM}, 15 \text{ nM}, and >100 \text{ mM}, respectively \((8, 14)\), and the \(K_i\) values for DPMA are 140 \text{ nM}, 4.4 \text{ nM}, and >100 \text{ mM}, respectively \((2, 5)\).

Ado-induced hyperpolarization is independent of the endothelium. Although L-NNA \((100 \text{ mM})\) and indomethacin \((1 \text{ mM})\) were present throughout these experiments, it is still possible that the Ado-induced hyperpolarization could be due to release of an additional factor such as endothelium-derived hyperpolarizing factor \((\text{EDHF}; 13)\). To distinguish the actions of Ado from those of EDHF, we also tested AcH \((0.5 \text{ mM})\) before and after exposure to glibenclamide. The hyperpolarization elicited with AcH was not reduced by glibenclamide \((i.e., 18.7 \pm 5.2 \text{ mV control vs. } 22.0 \pm 4.0 \text{ mV with glibenclamide, } n = 3)\) in the same tissues in which the Ado-
induced hyperpolarization was reduced by ~95%. In some tissues, the endothelium was successfully removed (as assessed by a lack of response to ACh). In these, Ado analogs produced comparable membrane hyperpolarization (Fig. 5A) to that observed in endothelium-intact tissues (e.g. Fig. 3C).

Hyperpolarization with Ado analogs is dependent on AC activity. The experiments with Ado analogs suggest that a large glibenclamide-sensitive hyperpolarization can be elicited with the A<sub>2</sub> selective agonist CPA and the A<sub>2</sub> selective agonist NECA. To determine whether one or both of these responses is coupled to stimulation of AC, we tested the AC inhibitor SQ-22,536. SQ-22,536 did not significantly affect membrane potential. However, SQ-22,536 reduced the hyperpolarization elicited with CPA from 15.8 ± 1.4 mV (n = 16) to 4.6 ± 0.8 mV (n = 4; Fig. 5, A and C) and reduced the hyperpolarization with NECA from 15.7 ± 3.0 to 5.3 ± 3.2 mV (Fig. 5, B and C). The actions of SQ-22,536 appear to be specific, since it had no significant effect on the hyperpolarization elicited with addition of the K<sub>ATP</sub> channel activator lemakalim (1 µM; 29.3 ± 2.9 mV control vs. 31.0 ± 3.9 mV in the presence of SQ-22,536, n = 3).

Raising cAMP levels leads to glibenclamide-sensitive hyperpolarization. The results with glibenclamide, SQ-22,536, and Ado analogs suggest that stimulation of AC and production of cAMP lead to activation of K<sub>ATP</sub> channels. If this is the case, then other maneuvers that also increase cAMP levels in the tissue should hyperpolarize the membrane in a glibenclamide-sensitive manner. To determine whether this is the case, three additional methods were tested, including 1) direct activation of AC with FSK (0.3–1 µM), 2) β-adrenoceptor activation of AC with Iso (0.1–1 µM), and 3) inhibition of PDE III with milrinone (0.4 µM) and PDE IV with rolipram (30 µM). FSK (1 µM, n = 7), Iso (0.3 µM, n = 6), and combined milrinone (0.4 µM) and rolipram (30 µM, n = 3) hyperpolarized the membrane by 16.4 ± 2.5, 16.8 ± 3.3, and 12.3 ± 5.0 mV, respectively. Glibenclamide (3 µM) reduced the amplitude of these responses to 0.6 ± 0.5 mV (n = 5), 0.3 ± 0.3 mV (n = 3), and 1.0 ± 1.0 mV (n = 3), respectively (Fig. 6). Application of the inactive analog of FSK, i.e., 1,9-dideoxyforskolin (10 µM), was without effect in this tissue (data not shown, n = 3). SQ-22,536 (100 µM) reduced the hyperpolarization elicited with Iso from 16.7 ± 3.3 to 0.7 ± 0.3 mV (n = 3), indicating that the actions of SQ-22,536 are not limited to Ado analogs. These results suggest that a number of different pathways that enhance cAMP levels also give rise to activation of K<sub>ATP</sub> channels.

Glibenclamide-sensitive hyperpolarization can be elicited with direct activation of PKA. Combined adminis-

Fig. 5. Effects of 9-(tetrahydro-2-furanyl)-9H-purin-6-amine (SQ-22,536) on the hyperpolarization induced by CPA and NECA in guinea pig coronary arteries. A and B show recordings obtained from 2 different vessels. Time of drug exposure is indicated by horizontal bars. A: example of hyperpolarization obtained with CPA in an endothelium-denuded segment of coronary artery. This hyperpolarization (16 mV) was not different from that observed in endothelium-intact tissues (e.g., Fig. 3C). After washing out of CPA, the successful removal of the endothelium was confirmed by application of ACh, which failed to initiate a hyperpolarization. In middle trace, the response to CPA is shown after 15 min preexposure to SQ-22,536. In the presence of SQ-22,536, the response to CPA was greatly attenuated. After 45 min of washout, CPA was reapplied, and the response to CPA had recovered. B: in another tissue containing endothelium, the response to NECA was tested. Initial application of NECA gave rise to a 9-mV hyperpolarization. After 15 min pretreatment with SQ-22,536, the response to NECA was abolished as seen in middle trace. After 8 min of washout, the response to NECA was still blocked. However, after 45 min of washout, the response to NECA had recovered. C: graph showing the mean hyperpolarization to CPA and NECA in the absence (open bars) and presence (filled bars) of SQ-22,536 (100 µM). *Significant differences from hyperpolarization observed in the absence of SQ-22,536 (P < 0.05). Values are expressed as means ± SE from 3–7 experiments.
tation of the site-selective PKA activators 6-Phe-cAMP, 8-AHA-cAMP, and Sp-5,6-DCl-cBIMPS (each at 100 µM) hyperpolarized the membrane by 16.2 ± 3.1 mV (n = 6). However, in the presence of glibenclamide, these activators did not induce a significant change in membrane potential (i.e., 0.7 ± 3.7 mV, n = 3; Fig. 7). These results suggest that activation of K\textsubscript{ATP} channels is mediated by PKA.

**DISCUSSION**

Ado has long been implicated as an important mediator of blood flow in the heart (3, 4). However, there is still controversy concerning its mechanism of action. Ado has been reported to hyperpolarize the rabbit carotid artery (6) as well as the pig and rabbit coronary artery (11, 32). Furthermore, studies in isolated smooth muscle cells (9, 21), as well as contractile studies of isolated blood vessels and flow studies from isolated hearts, all suggest that Ado acts at least in part through activation of K\textsubscript{ATP} channels (7, 10, 20, 30). In the present study, we have expanded upon this previous work by directly measuring membrane potential in the intact coronary artery. Our results suggest that Ado leads to hyperpolarization of the coronary artery via stimulation of A\textsubscript{2b} receptors, which are coupled to stimulation of AC. This hyperpolarization is blocked by...
glibenclamide but is unaffected by either BaCl₂ or IbTx. Experiments using a variety of different activators and inhibitors of the AC-cAMP-PKA pathway all support the hypothesis that KATP channels are an important target of this pathway.

As discussed in the introduction, there is still considerable controversy regarding the subtype of purine receptor that is responsible for the effects of Ado on the ionic currents that underlie membrane potential (i.e., see Refs. 9 and 21). In the present study, we found that glibenclamide-sensitive hyperpolarization could be elicited with two different Ado analogs that are more potent at A₂ than A₁ receptors (i.e., CPA and 2-CAdo). One of these (i.e., CPA) has previously been used to argue that Ado directly activates KATP channels via an A₂/G protein-mediated pathway (9). However, the following observations suggest that the actions of CPA and 2-CAdo are due to stimulation of A₂ rather than A₁ receptors. First, we found that the effects of CPA were greatly reduced by the AC inhibitor SQ-22,536 and unaffected by the selective A₁-receptor antagonist DPCPX (50 nM), suggesting that CPA stimulates A₂ receptors (which activate AC) rather than A₁ receptors (which inhibit AC). Second, we found that relatively high concentrations of these analogs were required to elicit hyperpolarization (i.e., 1–10 µM), although the dissociation constant for stimulation of A₁ receptors with these analogs is in the nanomolar range (14, 17).

The Ado analogs CGS-21680 and DPMA have been used extensively as selective A₂-receptor agonists (14, 33). Neither of these analogs produced membrane hyperpolarization in the guinea pig coronary artery. In a similar manner, CGS-21680 did not activate KATP channels in the porcine coronary artery, leading to the conclusion that A₁ receptors were not involved (9, 26). However, these analogs are now known to be quite selective for the A₂b receptors and to have little potency at A₂a receptors (2, 14, 17). The lack of effect of CGS-21680 and DPMA therefore suggests that A₂a receptors are not involved in the Ado-induced hyperpolarization in the guinea pig coronary artery but leave open the possibility that A₂b receptors are involved.

To explore the possible role of A₂b receptors, we tested the nonselective A₂a/A₂b receptor agonist NECA. This analog was chosen since selective A₂b receptor agonists and antagonists are not available (17). NECA gave rise to glibenclamide-sensitive hyperpolarization, which was reduced ~70% by the AC inhibitor SQ-22,536. This observation provides functional evidence that Ado-induced hyperpolarization is due in large part to activation of A₂b receptors, which increase AC activity. In the previous study of KATP currents in isolated porcine coronary artery cells, activators of A₂b receptors were not tested (9). Furthermore, the concentration of the A₁-receptor antagonist DPCPX tested in this study (i.e., 1,000 nM) was sufficient to block both A₁ and A₂ receptors, since the Kᵣ for DPCPX at the A₁ receptor is in the 0.5–to 6-nM range (2, 14), whereas the Kᵣ for A₂b receptors in guinea pig aorta is 171 nM (2). Thus A₂b receptors may contribute to the responses observed in the porcine coronary artery as well.

The concentration of SQ-22,536 used in our study (i.e., 100 µM) did not entirely block the response to either NECA or CPA. SQ-22,536 produces half-maximal inhibition of agonist-stimulated AC activity in the micromolar range (i.e., 5–15 µM; see Refs. 15, 35). However, millimolar concentrations have sometimes been used to achieve complete blockade (23). Thus the hyperpolarization to NECA and CPA that persists in the presence of SQ-22,536 may be due to incomplete blockade of AC. Alternatively, the remaining hyperpolarization may be due to some other AC-independent mechanism.

Our conclusion that Ado activates A₂b receptors in the guinea pig coronary artery is in agreement with a recent study of relaxation responses to Ado agonists and antagonists in the guinea pig aorta (2). However, our results differ from studies of the rabbit mesenteric artery in which A₂b receptors appear to be involved (21). In isolated mesenteric artery cells, the A₂a receptor agonist CGS-21680 activated glibenclamide-sensitive currents (21), whereas, in the present study, CGS-21680 was without effect on membrane potential. The
significance of this difference in distribution of Ado receptor subtypes is unclear, since both A2a and A2b receptors are coupled to activation of AC (14). It is possible that other second messenger pathways unrelated to either KATP channels or membrane potential may be differentially activated by these receptor subtypes. Additional studies are required to address this issue.

Additional experiments were directed toward characterizing the various steps in the AC-cAMP-PKA pathway. Because the coupling between Ado and AC involves the guanine nucleotide-binding protein Gs, we investigated whether a similar membrane hyperpolarization could be elicited with β-adrenoceptor stimulation, which also activates Gs. Iso led to glibenclamide-sensitive hyperpolarization that was blocked by SQ-22,536, suggesting that a similar pathway is involved in the actions of both Ado and Iso. Likewise, we found that direct activation of AC with FSK hyperpolarized cells in a glibenclamide-sensitive manner. Similar effects of FSK and Ido have previously been reported in the canine saphenous vein (29). FSK has also been shown to activate KATP currents in isolated mesenteric artery cells (34).

The levels of cAMP in the tissue are modulated by the activity of various PDEs. Of particular importance for cAMP are PDE III and PDE IV (24). Blockade of the PDE III and PDE IV activity in the coronary artery also led to glibenclamide-sensitive hyperpolarization. This observation is particularly intriguing since it suggests that the basal production of cAMP, unchecked by metabolism, is sufficient to lead to activation of KATP channels. This result also raises the interesting possibility that an additional manner in which KATP channel activity may be regulated is through modulation of PDE activity.

A major target activated by cAMP is PKA. To provide direct evidence for the involvement of this kinase in activation of KATP channels, we applied three different site-selective activators of PKA (i.e., 6-Phe-cAMP, 8-AHA-cAMP, and Sp-5,6-DCl-cBIMPS). This combination of drugs together activate both the “A” and “B” sites of both type I and type II PKA (12, 31). We found that this combination of drugs led to glibenclamide-sensitive hyperpolarization, providing further evidence that activation of KATP channels is mediated by PKA. This approach differs somewhat from the previous study of mesenteric arterial cells in that we used direct activation of endogenous PKA to enhance KATP channel activity, whereas the previous study used PKA inhibitors to suppress KATP channel activity (21).

At present, there is controversy concerning the role of the endothelium in Ado-induced responses. In some blood vessels, it has been reported that the endothelium contributes to the relaxation and/or the membrane hyperpolarization produced by Ado (22, 25, 32). However, this does not appear to be the case in the guinea pig coronary artery. In this vessel, ACh releases at least three different factors from the endothelium that hyperpolarize the smooth muscle, i.e., nitric oxide, prostacyclin, and EDHF. The present experiments were done in the presence of L-NNA and indomethacin, eliminating the possible role of nitric oxide and prostacyclin in the Ado-induced hyperpolarization. Furthermore, we found that the Ado-induced hyperpolarization is blocked by glibenclamide, whereas the ACh-induced hyperpolarization is glibenclamide insensitive (13). Finally, we found that Ado analogs still hyperpolarized endothelium-denuded tissues (e.g., Fig. 5A). This conclusion is compatible with the results of a previous study in which we showed that a brief (50-ms) picospritz application of 2-CAdo to the endothelial surface of the guinea pig coronary artery did not hyperpolarize the smooth muscle, whereas application of either ATP or ACh by the same method resulted in a 10- to 20-mV endothelium-dependent hyperpolarization (18, 19). The Ado-induced hyperpolarization recorded in the rabbit carotid artery also appears to be endothelium independent (6).

In conclusion, we have provided functional evidence that the predominant mechanism by which Ado hyperpolarizes the guinea pig coronary artery is via stimulation of A2b receptors. The binding of Ado to these receptors activates the AC-cAMP-PKA pathway, which leads to an increase in the activity of KATP channels. Our results further suggest that KATP channels are an important target for numerous pathways that raise cAMP levels in the coronary artery.

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