Involvement of p38-mitogen-activated protein kinase in adenosine receptor-mediated relaxation of coronary artery

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Teng, Bunyen, Weixi Qin, Habib R. Ansari, and S. Jamal Mustafa. Involvement of p38-mitogen-activated protein kinase in adenosine receptor-mediated relaxation of coronary artery. Am J Physiol Heart Circ Physiol 288: H2574–H2580, 2005. First published January 14, 2005; doi:10.1152/ajpheart.00912.2004.—The purpose of this study was to explore the involvement of adenosine receptor(s) in porcine coronary artery (PCA) relaxation and to define the role of MAPK signaling pathways. Isometric tensions were recorded in denuded PCA rings. 5′-(N-ethylcarboxamido)adenosine (NECA), a nonselective adenosine receptor agonist, induced a concentration-dependent relaxation (EC50 = 16.8 nM) of PGF2α (10 μM)-precontracted arterial rings. NECA-induced relaxation was completely blocked by 0.1 μM SCH-58261 (A2A antagonist) at lower doses (1–40 nM) but not at higher doses (80–1,000 nM). MRS-1706 (1 μM, A2B antagonist) was able to shift the NECA concentration-response curve to the right. CGS-21680 (selective A2A agonist) induced responses similarly to NECA, whereas 6-cyclopentyladenosine (A1 agonist) and CI-IB-MECA (A3 agonist) did not. Furthermore, the effect of NECA was attenuated by the addition of SB-203580 (10 μM, p8 MAPK inhibitor) but not by PD-98059 (10 μM, ERK, or p42/44, MEK inhibitor). Interestingly, SB-203580 had no effect on CGS-21680-induced relaxation. Western blot analysis demonstrated that PGF2α, and adenosine agonists stimulated p38 MAPK at a concentration of 40 nM in PCA smooth muscle cells. MRS-1706 (1 μM) significantly reduced NECA-induced p38 MAPK phosphorylation. Addition of NECA and SB-203580 alone or in combination inhibited PGF2α-induced p38 MAPK. Western blot data were further confirmed by p38 MAPK activity measurement using activating transcription factor-2 assay. Our results suggest that the adenosine receptor subtype involved in causing relaxation of porcine coronary smooth muscle is mainly A2A subtype, although A2B also may play a role, possibly through p38 MAPK pathway.

adenosine receptors; vascular smooth muscle; p42/44; c-Jun NH2-terminal kinase; prostaglandin F2α

ADENOSINE IS KNOWN TO CAUSE dilation of coronary artery. Four adenosine receptor subtypes, A1, A2A, A2B, and A3, have been identified and cloned (14). It has been suggested that both A2A and A2B adenosine receptors mediate hyperpolarization and nitric oxide (NO) release from coronary artery endothelium (2, 30, 47). The adenosine receptor subtypes responsible for endothelium-independent vasodilation in coronary artery smooth muscle are still not fully understood; however, both A2A and A2B adenosine receptors have been implicated (28, 40, 41). Upon activation, both A2A and A2B receptors, which are coupled to G protein and subsequently activate adenyl cyclase, cause an activation of various second messenger systems, including mitogen-activated protein kinases (MAPKs). There are three well-characterized MAPKs: extracellular signal-regulated kinases (ERKs, or p42/44), p38, and c-Jun NH2-terminal kinase (JNK). These MAPKs have been linked to ischemic preconditioning, smooth muscle cell growth, vascular smooth muscle migration, and vascular contraction (16, 19, 45). Adenosine is reported to stimulate all MAPKs in perfused rat hearts (16). However, their role in the regulation of vascular tone remains unclear. Most of the attention for these MAPKs is focused on their role in causing vasoconstriction. For instance, p42/44 has been found to be involved in angiotensin II- and endothelin-1-induced contraction in rat aorta (17, 43) but not in phenylephrine-induced contraction in canine pulmonary artery smooth muscle (49). Recent studies have suggested that p38, not ERK, is involved in the modulation of smooth muscle force and the regulation of angiotensin II-induced contraction (27, 49). There is no clear evidence for their role in vasodilatory mechanisms related to the vascular smooth muscle, especially adenosine-mediated signaling. Therefore, the purpose of our study was to explore the adenosine receptor subtype(s) involved in porcine coronary artery relaxation by using A2A and A2B receptor-selective antagonists and to define the involvement of MAPKs.

MATERIALS AND METHODS

Tissue bath experiments. Porcine hearts were obtained from a local slaughterhouse (n = 15). Coronary artery vascular rings (CAVR) from the left descending coronary artery branch of each porcine heart were dissected and placed in Krebs-Henseleit solution with the following composition (in mM): 118 NaCl, 1.2 MgSO4, 1.2 KH2PO4, 2.5 NaHCO3, 2.5 CaCl2, and 11 glucose. Eight rings (2–4 mm outer diameter) from left descending coronary arteries were obtained from each heart. Individual vascular rings were then dissected free of surrounding tissues. Two stainless steel-tungsten triangles (hooks) were inserted through the center of each vascular ring. The endothelial cell lining of each ring was removed by gentle rubbing of the intimal surface with tungsten wire. The vascular rings were immediately transversely mounted on the tissue bath. The removal of endothelium-dependent vasodilation in coronary artery smooth muscle is mainly A2A subtype, although A2B also may play a role, possibly through p38 MAPK pathway.

adenosine receptors; vascular smooth muscle; p42/44; c-Jun NH2-terminal kinase; prostaglandin F2α

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dependent vasodilator bradykinin (10⁻⁷ M) during the plateau phase of KCl-induced contraction. Vascular rings that did not contract after the addition of KCl or that relaxed after the addition of bradykinin were eliminated from further study.

CAVR were then divided into four groups. In the first experiment, 10 μM PGF₂α was added to all the tissue baths to precontract the rings. After a plateau was reached, cumulative dosages of S⁻⁵(N-ethylcarboxamidoadenosine (NECA), N⁻⁵-cyclopentyladenosine (CPA), 2⁻¹(phenylethylamino)-S⁻⁵-N-ethylcarboxamidoadenosine (CGS-21680), and 2-chloro-N²-(3-iodobenzyl)adenosine-5²-N-methyluronamide (CI-IB-MECA) were added to different baths to construct dose-response curves. In the second experiment, A₂₅ adenosine receptor antagonist (SCH-58261, 0.1 μM), p38 MAPK inhibitor (SB-203580, 10 μM), JNK inhibitor (SP-600125, 10 μM), and p42/44 MAPK inhibitor (PD-98059, 10 μM) were used to inhibit the NECA-induced relaxation. In the third experiment, CGS-21680 was used instead of NECA. Finally, in the fourth experiment, the newly available A₂₅ antagonist MRS-1706 (1 μM) was used to block NECA-induced relaxation. All the antagonists were incubated for 30 min before the application of PGF₂α, NECA, and CGS-21680.

Cell cultures. Smooth muscle cells from porcine coronary artery were isolated and cultured as described previously (26, 35) in this laboratory with minor modifications. Briefly, the left descending and left circumflex coronary arteries were isolated from five porcine hearts. Surrounding fat and connective tissue were cleaned from each CAVR. The endothelial cells were scraped off by opening up the vascular tree and gently rubbing the endothelial surface with metal wire. The isolated vascular tissue was soaked in Hanks' balanced salt solution containing 2% (vol/vol) antibiotic-antimycotic (200 units of penicillin, 200 μg of streptomycin, and 0.5 μg of amphotericin B per ml in final solution) for 15 min. The tissues were then cut into small pieces and digested with enzyme solution containing 1 mg/ml collagenase type I, 0.5 mg/ml soya trypsin inhibitor, 3% bovine serum albumin, and 2% antibiotic. The digested tissues were filtered and collected at 1-, 1.5-, and 2- and 3-hour intervals and centrifuged at 1,000 rpm for 10 min. The supernatant was discarded. The cell pellets were reconstituted in DMEM with 10% fetal bovine serum (FBS) and 2% bovine serum albumin, and 2% antibiotic-antimycotic and plated in 100-mm culture plates. The media were changed at least once a week. When the cells became confluent, they were split at a 1:5 ratio by using trypsin (0.25%).

Western blot analysis. The cells were starved off FBS-containing medium for 24 h before the experiment proceeded. Time courses for activating the MAPKs for adenosine agonists and PGF₂α were determined at 0, 1, 5, 10, 30, and 60 min. For the antagonist experiments, all the drugs were incubated for 30 min, followed by the addition of agonists for 10 min. PGF₂α was added after the addition of agonists, followed by incubation for 10 min (based on the preliminary results from the time course experiments). Cells were lysed in 20 mM Tris (pH 7.5) containing 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na₃VO₄, and 1 μg/ml leupeptin, and 0.1 mM PMSF was added to the lysis buffer (before it was added to the dish). Cells were scraped, transferred to a microcentrifuge tube, and boiled for an additional 5 min. The samples were then sonicated briefly and centrifuged at 12,000 rpm for 5 min at 4°C. Protein was measured using a Bio-Rad protein assay based on the Bradford dye procedure by using bovine serum albumin as a standard. The sample proteins were aliquoted and stored at −80°C. At the time of analysis, experimental samples as well as positive controls (anisomycin-treated cell lysate) were thawed, and 60 μg protein/lanes were separated on 10% SDS-PAGE gel. Prestained SDS-PAGE (low or high range, 20, 50 or 112 kDa) were run in parallel as protein molecular mass markers. The proteins were then transferred to polyvinylidene difluoride membrane (Schleicher and Schuell, Keene, NH) and probed with rabbit polyclonal anti-p38 (1:1,000 dilution) or anti-phospho-p38 antibodies (1:1,000 dilution). The secondary antibody was a horseradish peroxidase-conjugated anti-rabbit IgG. The membranes were developed using enhanced chemiluminescence (Amersham Life Sciences) and exposed to X-ray film for appropriate time.

p38 MAPK activity. p38 MAPK activity was measured as the phosphorylation of activating transcription factor-2 (ATF-2) by using a commercial p38 MAPK activity kit according to the manufacturer’s instructions. Briefly, control and treated cells were lysed with lysis solution (similar to Western blot experiments except that 1 mM PMSF was added before use). Cells were scraped, transferred to a microcentrifuge tube, and sonicated on ice four times for 5 s each time. The lysate was centrifuged (10,000 rpm) for 10 min at 4°C, and the supernatant was transferred to a new tube. The supernatant lysate (200 μl, 2.3 ± 0.6 μg) and 20 μl of the immobilized phospho-p38 MAPK monoclonal antibody were mixed and incubated with gentle shaking overnight at 4°C. The mixture was centrifuged for 30 s at 4°C. The pellets were washed twice with lysis solution and then twice again with kinase buffer containing 25 mM Tris (pH 7.5), 5 mM β-glycerophosphate, 2 mM DTT, 10 mM Na₃VO₄, and 10 mM MgCl₂. After being washed, the pellets were suspended in 50 μl of kinase buffer supplemented with 200 μM ATP and 2 μg of ATF-2 fusion protein and were incubated for 30 min at 30°C. The reaction was terminated with 25 μl of 3X SDS sample buffer containing 187.5 mM Tris·HCl (pH 6.8 at 25°C), 6% (wt/vol) SDS, 30% glycerol, 150 mM DTT, and 0.03% (wt/vol) bromphenol blue. The samples were boiled for 5 min, vortexed, and centrifuged for 2 min before the samples were loaded to 10% SDS-PAGE gel for Western blotting of phospho-ATF-2. Equal volumes of the samples were used to load the gel. The final densitometric readings were normalized to the total protein of the original samples before being normalized to the control values in this part of the experiments (more explanation in Data and statistical analysis).

Materials. The adenosine agonists NECA, CPA, CGS-21680, and CI-IB-MECA were purchased from Sigma (St. Louis, MO). The A₂₅ antagonist SCH-58261 was a generous gift from Dr. A. Monopoli (Shearing Plough, Milan, Italy). All three MAPK inhibitors, SB-203580, SP-600125, and PD-98059, were obtained from Calbiochem (La Jolla, CA). The A₂₅ antagonist MRS-1706 was purchased from Tocris (Ellisville, MO). All of the chemicals were dissolved in 100% DMSO as 10 mM stock solution unless indicated otherwise. The final concentration of DMSO in the tissue bath was <1%.

For the cell culture, Hanks’ balanced salt solution, DMEM medium, FBS, antibiotic-antimycotic, collagenase type I, and trypsin inhibitor were purchased from GIBCO (Carlsbad, CA). Anti-rabbit polyclonal anti-p38 antibody, the horseradish peroxidase-conjugated secondary anti-rabbit IgG antibodies, and the p38 MAPK activity kit were obtained from Cell Signaling Technology (Beverly, MA). Anti-phospho-p38 antibody was purchased from Biosource (Camarillo, CA).

Data and statistical analysis. For tissue bath experiments, the data are expressed as percentages of relaxation from the PGF₂α-induced maximal contraction and are presented as means ± SE. One-way ANOVA was used to compare data between groups at the same concentration. For Western blot experiments (including the p38 MAPK activity assay), relative band intensities were determined using a densitometer (Alpha Imager TM 2200 documentation and analysis system; Alpha Inotech, San Leandro, CA) (1). Densitometric analysis of the bands on the film was normalized to the protein loaded and the control (nontreated) value, and data are presented as percentages of the control. The data presented are the means of three separate experiments. One-way ANOVA and Student’s t-test were used to compare data between groups.

RESULTS

Tissue bath experiments. The CAVR were incubated with various adenosine receptor agonists to obtain concentration-response curves. Our results show that both NECA (97.19 ± 2.32%) and CGS-21680 (96.18 ± 1.42%) produced dose-
dependent relaxation of the porcine coronary artery rings with an ED50 of 16.81 and 9.69 nM, respectively. CPA and Cl-IB-MECA, on the other hand, induced only minimal relaxation of 27.18 ± 9.29% and 30.54 ± 14.87%, respectively, at high concentrations (Fig. 1). These results demonstrate that NECA and CGS-21680 are quite potent in relaxing the porcine coronary artery rings, whereas CPA and Cl-IB-MECA are less potent.

The addition of a selective A2A antagonist, SCH-58261, significantly blocked the NECA- and CGS-21680-induced relaxations (Figs. 2 and 3). The addition of a selective A2B antagonist, MRS-1706, significantly blocked NECA-induced relaxation (Fig. 4); however, it was less effective than SCH-58261. These data demonstrate a major role for A2A and a minor role for A2B in NECA-induced relaxation. Furthermore, the addition of p38 MAPK inhibitor SB-203580 significantly reduced the NECA-induced relaxation at 40, 80, and 120 nM but did not reduce the CGS-21680-induced relaxation. The addition of p42/44 MAPK inhibitor PD-98059 had no effect on NECA- and CGS-21680-induced relaxations (Figs. 2 and 3). These data demonstrate that p38 MAPK may be involved in NECA-induced relaxation.

**Western blot analysis.** All adenosine agonists used in our experiments activated p38 MAPK. The p38 MAPK exists in at least four subtypes: p38α, p38β, p38γ, and p38δ. The polyclonal antibodies we used in Western blot experiments were capable of detecting p38α, p38β, and p38γ. The cell extract as a positive control (C6 cell stimulated by anisomycin) consists of only the p38α subtype and was confirmed by our experiments, which showed only one band. Experiments performed using PGF2α, NECA, CPA, CGS-21680, and Cl-IB-MECA showed three bands, corresponding to p38α, p38β, and p38γ, respectively. For densitometric analysis of the bands, all bands were taken into account for comparative analysis.

Our Western blot results with porcine coronary arterial smooth muscle cells show that PGF2α (10 μM) and all adenosine analogs used (NECA, CPA, CGS-21680, and Cl-IB-MECA at a concentration of 40 nM) showed maximum phospho-p38 MAPK phosphorylation at 10 min (Figs. 5 and 6). These results demonstrate that NECA-, CPA-, CGS-21680-, and Cl-IB-MECA-induced p38 MAPK phosphorylation occurred rapidly (within minutes) and reached a peak at 10 min. The increased p38 MAPK phosphorylation by these agonists lasted for at least 60 min (except Cl-IB-MECA). In the antagonist experiments, MRS-1706 significantly blocked the NECA-induced p38 MAPK phosphorylation from 126.05 ± 7.93% to 106.51 ± 13.65% (Fig. 7). The addition of NECA significantly blocked the PGE2α-induced p38 MAPK phosphorylation from 118.5 ± 1.29% to 103.5 ± 0.9%. Furthermore, the addition of NECA with SB-203580 also significantly reduced the PGE2α-induced phosphor-p38 MAPK increases from 118.5 ± 1.3% to 99.3 ± 3.5% (Fig. 8).

In p38 MAPK activity experiments, PGE2α significantly enhanced the p38 MAPK activity by 152.3 ± 4.9% from the controls. NECA alone did not significantly increase p38 MAPK activity (109.0 ± 21.3% of control). However, when in combination with PGE2α, NECA alone or in combination with SB-203680 inhibited PGE2α-induced activity of the phosphor-p38 MAPK increase to 100.9 ± 23.8% and 82.87 ± 16.3% of the control, respectively (Fig. 9).

**DISCUSSION**

We investigated the effects of all four subtypes of selective adenosine receptor agonists to explore the possible role of...
MAPK signaling pathways in porcine coronary artery. As expected, we demonstrated that A2A may be the predominant adenosine receptor subtype that mediates the coronary smooth muscle relaxation. Furthermore, our results showed for the first time that these agonists induce a concentration- and time-dependent relaxation that was inhibited by p38 MAPK inhibitor (SB-203580) in a concentration-dependent manner (Figs. 1 and 2). This finding is further supported by the relaxation response to CGS-21680 (selective A2A agonist) and near complete blockade of NECA- and CGS-21680-induced relaxation by a selective A2A antagonist, SCH-58261. This is consistent with our previous findings and those of others (2, 6, 28, 40). Furthermore, the rightward shift of the concentration-response curve for NECA in response to the A2B specific antagonist MRS-1706 provides the first direct evidence for the existence of A2B adenosine receptor in coronary arteries, which was only implied in previous studies in human small resistance-like coronary arteries and in isolated mouse coronary flow (21, 28).

NECA-induced concentration (40, 80 and 120 nM)-dependent relaxation of coronary artery was significantly inhibited by the p38 MAPK inhibitor SB-203580 (10 μM) but was unaffected by the p42/44 inhibitor PD-98059 (10 μM; Fig. 2) and the JNK inhibitor SP-600125 (10 μM) (data not shown).

Because SB-203580 significantly reduced NECA-induced re-

![Fig. 4. Effect of A2B antagonist MRS-1706 (MRS) on NECA-induced relaxation of porcine coronary artery vascular rings preconstricted with PGE2. Values are means ± SE; n = no. of pigs used. *P < 0.05 compared with NECA alone at the same concentration.](image)

![Fig. 5. Time-dependent effect of PGE2- and NECA-induced p38 MAPK phosphorylation in cultured porcine coronary arterial smooth muscle cells. The cell extract of C6 cell stimulated by anisomycin was used as positive control (+Control). A: representative blot of total p38 MAPK activated by 10 μM PGE2. B: representative blot of phospho-p38 MAPK activated by 10 μM PGE2. C: representative blot of phospho-p38 MAPK with 40 nM NECA treatment.](image)

![Fig. 6. Phospho-p38 MAPK time-dependent effects of CPA (an A1 agonist, A), CGS-21680 (an A2A agonist, B), and CI-IB-MECA (an A3 agonist, C) in cultured porcine coronary arterial smooth muscle cells. The optical density of phospho-p38 activity at 0 min was taken as 100% (control). Data are presented as percentages of control. Values are means ± SE from 3 experiments performed in cells (10-cm plates) from 5 different pigs. *P < 0.05 compared with control.](image)
laxation and not CGS-21680-induced relaxation (Figs. 2 and 3), our results suggest that SB-203580 is most likely blocking the effect of A2B receptors. Furthermore, the \( K_i \) values for NECA range from 3 to 20 nM at A1 and A2A receptors and from 50 to 500 nM at A2B receptors (12, 14). These data further support the suggestion that SB-203580 is blocking the effect of A2B receptors. A2B receptors are known to activate p38 MAPK in various cells, such as Chinese hamster ovary cells (36) and human mast cells (13). There is evidence that p38 MAPK plays a role in receptor-mediated vasoconstriction (20, 27, 49). For instance, p38 MAPK has been found to mediate norepinephrine (NE)- and angiotensin II-induced vasoconstriction (20, 27, 49). Recently, it was also suggested that the cytosolic PLA2-generated arachidonic acid and its lipoygenase metabolites mediate NE-induced p38 MAPK stimulation (19). p38 MAPK activation also partly contributes to impairment of N-methyl-D-aspartate-induced cerebrovasoconstriction after brain injury (3). Our study is the first report demonstrating that p38 MAPK activation modulates A2B receptor-mediated smooth muscle relaxation in coronary artery.

All of the four adenosine receptor subtypes, A1, A2A, A2B, and A3, have been demonstrated to activate p38 MAPK (37). In our experiments, all the adenosine agonists activate p38 MAPK at a concentration of 40 nM. Addition of the A2B antagonist MRS-1706 significantly blocked the NECA-induced p38 MAPK phosphorylation. These data suggest that p38 MAPK is involved in A2B signaling in porcine coronary smooth muscle cells. An interesting finding in our study is that whereas NECA activated p38 MAPK, NECA also blocked PGF2\(_a\)-induced phosphorylation of p38 MAPK in our Western blot experiments. As mentioned previously, there are four known p38 MAPK subtypes, and their function in smooth muscle remains unclear. The antibody we used in our experiments was able to detect three bands of p38 MAPK (p38\(_{11021}\), p38\(_{11021}\), and p38\(_{11021}\)); therefore, one possible explanation is that NECA activated one (or multiple) adenosine receptor subtype(s) and subsequently activated different p38 MAPK subtypes that are responsible for different physiological function(s), and that one of them is responsible for modulating the NECA-induced vasodilation while others are responsible for other unknown function(s). Different isoforms of p38 MAPK that generate opposite effects also have been reported (29, 32, 42). It is possible that A2B receptor activates one of the p38 MAPK subtypes to induce subsequent vasodilation, whereas another p38 MAPK subtype(s) may be responsible for cell proliferation and differentiation, as reported in a recent review (10). Because SB-203580, the p38 MAPK inhibitor we used, inhibits only p38\(_{11021}\) and p38\(_{11021}\) (9, 25), one or both of these two p38 MAPK subtypes may be responsible for the NECA-induced vasodilation. Furthermore, there are reports that suggest cross talk between p38 and other second messenger pathways, such as PKC (11), phosphatidylinositol 3-kinase (PI3-kinase) (15), and ERK (38, 48) also may exist.

Fig. 7. Effect of MRS-1706, an A2B antagonist, on NECA-induced phosphorylation of p38 MAPK in cultured porcine coronary arterial smooth muscle cells. Data are presented as percentages of control (no drug) in optical density. All 3 bands were included in the optical density measurements. *P < 0.05 compared with control. $P < 0.05 compared with NECA.

Fig. 8. Effects of NECA and SB-203580, a p38 MAPK inhibitor, on PGF2\(_a\) (PG)-induced activation of phospho-p38 in cultured porcine coronary arterial smooth muscle cells. Data are represented as percentages of control (no drug) in optical density. *P < 0.05 compared with control, $P < 0.05 compared with PG.

Fig. 9. Effects of NECA (NE) and SB-203580, a p38 MAPK inhibitor, on PGF2\(_a\) (PG)-induced activity of phospho-p38 MAPK, measured as the change in phospho-activating transcription factor-2, in cultured porcine coronary arterial smooth muscle cells. Data are normalized to total protein and are represented as percentages of control (no drug) in optical density. *P < 0.05 compared with control. $P < 0.05 compared with PG.
The involvement of p38 MAPK in NECA-induced relaxation remains unclear. There are few reports that link adenosine receptors to p38 MAPK and fewer clues as to which mediator is involved between receptors and p38 MAPK activation. A recent report (33) demonstrated that cAMP, a major second messenger for A2A and A2B receptors, inhibits p38 MAPK activation in human umbilical vein endothelial cells. In contrast, PKA was found to activate p38 MAPK in macrophages (8). Furthermore, the signaling pathways up- and downstream of MAPK/p38 pathway are diverse, which may explain why p38 pathway can be activated by various stimuli (10, 31) and create cross talk among various stimuli. For instance, recent data suggest that the activation of the PI3-kinase/Akt pathway in unstimulated smooth muscle may modulate vascular smooth muscle tone through inhibition of the cAMP/heat shock-related protein 20 (HSP20) pathways (23, 24). Although another heat shock-related protein, HSP27, has been shown to be involved in vascular smooth muscle contraction, whether HSP20, a substrate for protein kinases A and G, mediates vascular relaxation, possibly via a direct interaction of large HSP20, a substrate for protein kinases A and G, mediates another heat shock-related protein, HSP27, has been shown to mediate vascular smooth muscle contraction, possibly through PKC and receptor-operated Ca\textsuperscript{2+} channels (39). Recently, it was reported that adenosine A2B receptor activates p38 MAPK (36). Taken together, these results indicate that the adenosine receptors (presumably A2B) may possibly activate cAMP, (36). Taken together, these results indicate that the adenosine receptors (presumably A2B) may possibly activate cAMP, (36). Taken together, these results indicate that the adenosine receptors (presumably A2B) may possibly activate cAMP, which interferes with PGF\textsubscript{2α}-activated p38 kinase pathway through p38 MAPK. An alternate explanation may be that cAMP or its downstream effectors phosphorylate one of the p38 MAPK subtypes, which subsequently phosphorylates HSP20, as in the well-known p38/HSP27 pathway. Further investigations are needed to establish a relationship between p38 and HSP20.

Our Western blot experiments show increases in phospho-p38 MAPK after the application of agonists. We wanted to make sure that the phospho-p38 MAPK is also able to activate downstream effectors. The p38 MAPK activity experiments further confirm the Western blot data. An interesting discrepancy between the phospho-p38 MAPK Western blot experiments and p38 MAPK activity measurements is that NECA did not significantly activate the p38 MAPK activity but did increase the phospho-p38 MAPK. By looking at these data more closely, it is noted that the increase in phospho-p38 MAPK in the Western blot experiments is very small (106.5 ± 2.23%), whereas the standard error in the p38 MAPK activity experiments is large (109.0 ± 37.04%). This is an indication that NECA may activate conflicting signaling pathways.

In summary, our results demonstrate that the major adenosine subtype mediating vasodilation in porcine coronary artery is the A2A subtype, and we have shown the first direct evidence for the involvement of A2B receptor in coronary artery by using A2B receptor antagonist. We also have demonstrated involvement of the p38 MAPK signaling pathway in NECA-induced relaxation in a concentration- and time-dependent manner in porcine coronary artery, possibly through A2B receptors. This evidence is based on the fact that NECA-induced relaxation can be partially blocked by p38 MAPK inhibitor (SB-203580) but not by ERK inhibitor (PD-98059) or JNK inhibitor; however, the relaxation response to CGS-21680 was not blocked by SB-203580. In addition, a selective A2B antagonist, MRS-1706, blocked NECA-induced p38 MAPK phosphorylation. This finding further suggests a possible involvement of A2B subtype through p38 MAPK pathway. PGF\textsubscript{2α}, NECA, CPA, CGS-21680, and CI-IB-MECA all were found to activate p38 MAPK. Combined with the results from our tissue bath experiments, these results suggest that activation of p38 MAPK by different receptor subtypes may have different functions. The mechanism(s) of NECA-induced vasodilation in porcine coronary artery needs further investigation.

GRANTS

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