5-(N-ethylcarboxamido)adenosine desensitizes the A$_{2b}$-adenosine receptor in lung circulation

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Haynes, J R. , Boniface Obiako, Pavel Babal, and Troy Stevens. 5-(N-ethylcarboxamido)adenosine desensitizes the A$_{2b}$-adenosine receptor in lung circulation. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1877–H1883, 1999.—The adenosine agonist 5-(N-ethylcarboxamido)adenosine (NECA) induces vasodilation in the pulmonary circulation via A$_{2b}$-adenosine-receptor activation. We addressed whether prolonged treatment with NECA desensitizes in A$_{2b}$-adenosine-receptor function in isolated lung and pulmonary artery smooth muscle cells (PASMC). In lung microcirculation pre-constricted with a hypoxic gas, initial administration of NECA caused a 57% vasodilatory response after 3–4 min. Readministration of NECA after 45 min resulted in minimal vasodilatation. The highest accumulation of PASMC cAMP occurred 3–5 min after NECA, coincident with NECA-induced vasodilation. In PASMCs treated with NECA for 45 min, cAMP did not increase. Isoproterenol- and indolidan-induced vasodilation remained intact in NECA-desensitized lungs. In NECA-desensitized PASMCs, isoproterenol-induced cAMP accumulation was decreased, suggesting a common mechanism of desensitization. cAMP accumulation was decreased in cholera toxin-treated NECA-desensitized PASMCs compared with cholera toxin-treated control PASMCs, demonstrating that G$_{o}$-adenyl cyclase signaling contributes to desensitization. The A$_{2b}$-adenosine-receptor agonist CGS-21680C neither increased cAMP accumulation in PASMCs nor attenuated NECA-induced vasodilation. These data support that the A$_{2b}$-adenosine receptor is responsible for pulmonary vasodilation and desensitization through mechanisms involving G$_{o}$-adenyl cyclase signaling. G$_{o}$-adenyl cyclase signaling; adenosine 3′,5′-cyclic monophosphate; pulmonary artery vasodilation

A problem with the clinical utility of Ado is its potential to produce A$_{2}$-adenosine-receptor desensitization after sustained infusion. Although desensitization of the A$_{2}$-adenosine receptor has not been demonstrated in the pulmonary circulation, the downregulation of G$_{o}$ protein, inhibition of adenylyl cyclase, and activation of phosphodiesterase have been observed in other cell lines as mechanisms of A$_{2}$-adenosine-receptor desensitization (2, 11, 12). We therefore sought to determine whether prolonged administration of Ado agonists results in sustained vasodilation or receptor desensitization in the pulmonary circulation.

On the basis of previous observations (3, 8, 16, 19, 25) in the precontracted pulmonary vascular bed, Ado-mediated vasodilation occurs after activation of the A$_{2}$-adenosine receptor, probably of the A$_{2b}$ subtype (8), which is coupled through a G$_{o}$ protein to adenylyl cyclase activation (19, 27, 28). Adenylyl cyclase activation increases intracellular cAMP and mediates vasodilation (25). In this study we investigate effect(s) of prolonged A$_{2}$-adenosine-receptor exposure to the nonmetabolizable nonselective Ado agonist 5-(N-ethylcarboxamido)adenosine (NECA) and the selective A$_{2}$-adenosine-receptor agonist CGS-21680C on Ado-mediated vasodilation in the lung and on cAMP accumulation in pulmonary artery smooth muscle cell (PASMC) cultures from the Sprague-Dawley rat.

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Materials. Ado, NECA, ANG II, and isoproterenol (Iso) were purchased from Sigma Chemical (St. Louis, MO). CGS-21680C was a kind gift from Ciba-Geigy (Summit, NJ), and indolidan (Ind) was a kind gift from Lilly Research Laboratories (Indianapolis, IN). All drugs were solubilized in normal saline except for NECA and CGS-21680C, which were dissolved in distilled H$_{2}$O, and Ind, which was dissolved in DMSO.

Isolated perfused lung. Male Sprague-Dawley rats (270–340 g) were anesthetized with Nembutal sodium (25 mg ip), and lungs were removed for extracorporeal perfusion as previously described (6, 17). A tracheostomy was performed that permitted ventilation with a Harvard rodent ventilator (model 683) at 55 breaths/min with a tidal volume of 2.5 ml and 2.0 cmH$_{2}$O positive end-expiratory pressure. The inspired gas mixture was 95% air-5% CO$_{2}$ (room air gas). During hypoxic challenges, lungs were ventilated with a 3% O$_{2}$-5% CO$_{2}$-balance N$_{2}$ (hypoxic gas) gas mixture. A median sternotomy was performed, heparin sodium (100 IU) was injected in the right ventricle, and cannulas were placed in the pulmonary artery and left ventricle. Heart, lungs, and mediastinal structures were removed en bloc and placed into a humidified chamber. Lungs were perfused by a Gilson Minipuls 2 peristaltic pump at a constant flow of 0.03 ml·g body wt$^{-1}$·min$^{-1}$. Lungs were perfused with homologous blood (30 ml) previously collected in a heparinized syringe by
cardiac puncture from three or four adult male Sprague-Dawley rats anesthetized with Nembutal sodium. The temperature of the recirculating blood was maintained at 37°C. Pulmonary arterial (Ppa) and venous pressures were continuously monitored with Cope pressure transducers (041-500–503) and were recorded on a Grass polygraph recorder (model 7E).

After a 30-min equilibration period, all lungs were challenged with 0.1 g ANG II injected as a bolus in the pulmonary artery. Ten minutes after the ANG II challenge, lungs were ventilated with the hypoxic gas mixture for 10 min, and the first hypoxic pressor response (HPR1) was assessed. Room air gas was reinstituted, and lungs were allowed to equilibrate for 10 min (Table 1). This was followed by two 25-min sequential hypoxic challenges (HPR2, HPR3, n = 16 each). At the peak (~10 min) of HPR2, NECA (10 µM, final perfusate concentration) was administered to the reservoir, and the change in Ppa was monitored for an additional 15 min. At the peak of HPR3, NECA (10 µM) was administered (total of 20 µM NECA in perfusate) and Ppa was again monitored for 15 min (group A). With the use of the identical protocol, five lungs previously exposed to 20 µM NECA were washed with 100 ml of a 4 g/100 g albumin-physiological salt solution and reperfused with fresh homologous blood (30 ml) in a recirculating fashion as described above (group B). After a 10-min equilibration period, lungs were subjected to a fourth hypoxic challenge (HPR4). At the peak of HPR4, NECA (10 µM, final perfusate concentration) was administered to the reservoir, and the decrease in Ppa was compared with the decrease in Ppa observed after NECA administration during the peak of HPR2.

In group A, 11 lungs that demonstrated an attenuation of NECA-mediated vasodilation were subjected to HPR4, and either Ado (7.5 mM, n = 4), the nonselective agonist, Iso (1 µM, n = 3), or the cGMP-inhibitable cAMP phosphodiesterase inhibitor (7) Ind (1 µM, n = 4) was administered, and any change in Ppa was observed over a 15 min period (Table 1). In control lungs that were subjected to four sequential hypoxic challenges and not administered NECA (group C), studies were performed with Ado (7.5 mM, n = 3), Iso (1 µM, n = 3), and Ind (1 µM, n = 3) administration during the plateau of HPR4 for comparison with NECA-pretreated lungs from group A that were either administered either Ado, Iso, or Ind. Whereas Iso and Ind were administered in the perfusate reservoir, Ado was infused in the pulmonary artery at a rate 0.125 ml/min.

In the final group of lungs, the selective A2a-adenosine-receptor agonist CGS-21680C was added to the perfusate in a concentration of either 10 µM (n = 4, group F) or 1,000 µM (n = 4, group G) during HPR2 (45 min before 10 µM NECA administration). NECA was administered at the peak of the HPR3 as described above, and any change in Ppa was observed over 15 min. This change in Ppa seen with NECA was compared with that seen with NECA administration during HPR2 and HPR3 in group A.

RESULTS

Isolated lung. Initial studies were designed to assess whether prolonged Ado receptor activation results in desensitization of the A1-adenosine receptor. In group A, HPR2 and HPR3 were 22.3 ± 1 and 20.8 ± 1 cmH2O, respectively. The administration of NECA (10 µM) during HPR2 resulted in a 12.7 ± 0.7 cmH2O decrease in Ppa below the peak Ppa at 2–4 min and an 8.7 ± 1 cmH2O decrease in Ppa at 15 min. In contrast to the initial 57% vasodilatory response with NECA, repeat administration of NECA resulted in a 0.6 ± 0.3 cmH2O decrease in Ppa at 2–4 min and a 2.4 ± 0.4 cmH2O increase in Ppa above the initial peak Ppa at 15 min (Fig. 1).

In lungs from group B, repeat administration of NECA (10 µM) during HPR4 decreased the peak Ppa by only 3.9 ± 1.3 cmH2O at 2–4 min and by 1.7 ± 2.2 cmH2O at 15 min. This NECA response was significantly diminished (P < 0.05) compared with the de-

| Table 1. Experimental design of studies in the isolated-perfused rat lung |
|-----------------|-----------------|-----------------|
| Group | HPR2 | HPR3 | HPR4 |
| A | NECA | NECA | Ado, Iso, Ind |
| B | NECA | NECA | NECA* |
| C | NECA | NECA | Ado, Iso, Ind |
| E | CGS 10 | CGS 1000 | NECA |

HPR, hypoxic pressor response; NECA, 5’-(N-ethylcarboxamido)adenosine (10 µM); Ado, 9-β-D-ribofuranosyladenine adenosine (7.5 mM) infused over 15 and 45 min at 0.125 ml/min; Iso, isoproterenol (1 µM); Ind, indomethacin (1 µM); CGS, 2,3-p-(carboxyethyl)-phenylaminol-5’-N-ethylcarboxamido adenosine (10 and 1,000 µM). *Lungs washed with 4 g/100 g physiological salt solution and reperfused with fresh homologous blood (30 ml).
crease in P\textsubscript{pa} observed during HPR2, which was 9.9 ± 1.3 cmH\textsubscript{2}O at 2–4 min and 8.2 ± 1.2 cmH\textsubscript{2}O at 15 min. Repeat administration of NECA in lungs from groups A and B resulted in an attenuation of NECA-induced vasodilation and is consistent with desensitization of the A\textsubscript{2}-adenosine receptor.

In group A where NECA-induced vasodilation could no longer be demonstrated, Ado, Iso, and Ind were administered (Fig. 2). Similar to NECA, Ado decreased P\textsubscript{pa} 1.3 ± 1.4 cmH\textsubscript{2}O compared with the 15.4 ± 1.5 cmH\textsubscript{2}O decrease in P\textsubscript{pa} observed in group C (no NECA pretreatment). Administration of Iso and Ind to lungs that were pretreated with NECA and refractory to NECA-induced vasodilation resulted in an attenuation of HPR4 comparable to that observed in lungs not previously treated with NECA (Table 2).

Table 2. Effect of NECA pretreatment on Ado-, Iso-, and Ind-induced vasodilation

<table>
<thead>
<tr>
<th>Groups</th>
<th>(n)</th>
<th>Peak response</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ado</td>
<td>8</td>
<td>20.1 ± 2.8</td>
<td>4.7 ± 1.5</td>
</tr>
<tr>
<td>NECA + Ado</td>
<td>4</td>
<td>20.8 ± 1.0</td>
<td>19.5 ± 0.4</td>
</tr>
<tr>
<td>Iso</td>
<td>3</td>
<td>25.7 ± 2.8</td>
<td>3.2 ± 1.2</td>
</tr>
<tr>
<td>NECA + Iso</td>
<td>3</td>
<td>26.7 ± 1.5</td>
<td>4.8 ± 1.8</td>
</tr>
<tr>
<td>Ind</td>
<td>3</td>
<td>19.7 ± 2.2</td>
<td>9.3 ± 0.8</td>
</tr>
<tr>
<td>NECA + Ind</td>
<td>4</td>
<td>25.8 ± 2.2</td>
<td>10.5 ± 1.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n\), no. of rats. Peak response, the maximum increase in pulmonary arterial pressure (P\textsubscript{pa}) seen when ventilating lungs with a hypoxic (fraction of inspired O\textsubscript{2} = 0.03) gas mixture. NECA (10 \(\mu\)M) was administered to the perfusate reservoir at the peak of the second (HPR2) and third (HPR3) HPRs. HPR + NECA represents the change in HPR from the peak response after NECA administration. *Significantly different at \(P<0.001\). NS, not significant.

To assess whether the A\textsubscript{2a}-adenosine receptor was involved in Ado receptor desensitization, lungs were pretreated with the selective A\textsubscript{2a}-adenosine-receptor agonist CGS-21680C (10 and 1,000 \(\mu\)M) during HPR2 and allowed to recirculate 45 min before NECA administration. CGS-21680C (10 \(\mu\)M) did not mediate a vasodilatory response when administered at the peak of HPR2. In contrast, CGS-21680C (1,000 \(\mu\)M) caused a 32 ± 6% decrease in HPR2. Unlike the relatively complete desensitization observed in group A with sequential NECA administration, NECA resulted in a 13.6 ± 2.2 cmH\textsubscript{2}O decrease in P\textsubscript{pa} in lungs pretreated with CGS-21680C (10 \(\mu\)M), which was not significantly different from the 12.7 ± 0.7 cmH\textsubscript{2}O decrease observed with HPR2 in group A. However, in lungs pretreated with CGS-21680C (1,000 \(\mu\)M) the decrease in P\textsubscript{pa} seen with NECA was 2.6 ± 0.5 cmH\textsubscript{2}O, which was significantly less than seen with HPR2 in group A and similar to the attenuation of NECA-mediated vasodilation observed with HPR3 in group A.

PASMC culture. To confirm whether NECA and CGS-21680C activation of the A\textsubscript{2}-adenosine receptor increased cAMP, PASMC cultures were exposed to either

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**Fig. 1.** Effects of sequential administration of 5-(N-ethylcarboxamido)adenosine (NECA, 10 \(\mu\)M) on the hypoxic pressor response (HPR). HPR is the increase in pulmonary arterial pressure (P\textsubscript{pa}) above the baseline P\textsubscript{pa} when lungs were ventilated with a hypoxic (fraction of inspired O\textsubscript{2} = 0.003) gas mixture. NECA (10 \(\mu\)M) was administered to the perfusate reservoir at the peak of the second (HPR2) and third (HPR3) HPRs. HPR + NECA represents the change in HPR from the peak response after NECA administration. *Significantly different at \(P<0.001\). NS, not significant.

**Fig. 2.** Representative tracings of sequential hypoxic pressor responses (HPR2–HPR4). HPR1 is not shown. HPR is the observed increase in P\textsubscript{pa} above baseline when lungs are ventilated with a 3% O\textsubscript{2}-balance N\textsubscript{2} gas mixture. In all studies, the nonmetabolizable, nonselective adenosine (Ado) agonist NECA (10 \(\mu\)M) was added to the perfusate reservoir at the peak of HPR2, resulting in a decrease in P\textsubscript{pa} that waned with time. On repeat administration of NECA during HPR3, lungs were noted to be refractory to NECA-induced vasodilation. Ado (7.5 mM) infused in the pulmonary artery at 0.125 ml/min resulted in a minimal decrease in P\textsubscript{pa} that was similar to NECA. In contrast, vasodilation with the \(\beta\)-adrenergic agonist isoproterenol (Iso, 1 \(\mu\)M) and the cGMP-inhibitable cAMP phosphodiesterase inhibitor indolidan (Ind, 1 \(\mu\)M) was maintained compared with controls (see Table 2).
NECA (10 µM), CGS-21680C (10 µM), or vehicle over a 45-min time course (Fig. 3). Activation of the A2b-adenosine receptor by NECA increased cAMP 876% above control at 5 min. After 45 min of NECA, PASMC cAMP accumulation decreased to 169% of control. In contrast, activation of the A2a-adenosine receptor with CGS-21680C produced no detectable increase in cAMP, indicating that activation of the A2b-adenosine receptor most likely accounted for NECA-induced increases in cAMP.

In the isolated lung, an ~45-min exposure to NECA desensitized the pulmonary circulation to subsequent NECA-induced vasodilatory challenges. We next examined whether PASMC were similarly desensitized to NECA-induced cAMP production. To test this idea, PASMC were exposed to either vehicle or NECA (10 µM) for 45 min (as in Fig. 2) and subsequently rechallenged with either vehicle, NECA (10 µM), Is0 (1 µM), or Ind (1 µM) for 3 min. Whereas vehicle pretreatment followed by application of NECA increased cAMP 239% above control, NECA pretreatment followed by application of NECA did not significantly increase cAMP content (Fig. 3). These data are consistent with desensitization of the NECA-induced cAMP signaling pathway observed in the pulmonary circulation.

Interestingly, in PASMC culture, 45-min exposure to NECA significantly attenuated responsiveness to β-adrenergic stimulation with Iso. Whereas vehicle pretreatment followed by application of Iso elicited a 340% increase in cAMP above control, NECA pretreatment followed by Iso produced a 234% increase in cAMP (Fig. 5). In contrast, NECA pretreatment did not influence cAMP responsiveness to Ind (Fig. 5).

We examined whether NECA desensitization of cAMP stimulation involved G protein activation by pretreating PASMC with either vehicle or NECA (10 µM) for 45 min followed by stimulation of Gsα proteins using cholera toxin (10−10 to 10−8 M). NECA pretreatment attenuated responsiveness to cholera toxin 312%, consistent with desensitization of Gsα proteins for activation of adenylyl cyclase (Fig. 6).

**DISCUSSION**

The purpose of this study was to investigate whether desensitization of the A2b-adenosine receptor occurs in the rat pulmonary circulation on prolonged exposure to the synthetic nonmetabolizable Ado agonist NECA. We found that sequential administration of NECA (10 µM each dose) in the isolated rat lung precontracted with hypoxic gas caused an acute vasodilatory response with a maximum decrease in Ppa 3–4 min after administration. However, 45 min after the initial administration, repeat administration of NECA resulted in only a minimal vasodilatory response. This effect of NECA in the lung closely paralleled cAMP accumulation in PASMC culture. In PASMCs, the highest accumulation of cAMP occurred 3–5 min after NECA administration, coincident with the vasodilatory response in isolated perfused lungs. Furthermore, in PASMCs pre-
Adenosine receptor desensitization in the lung

Figure 6. NECA desensitizes cholera toxin (CT)-induced cAMP accumulation in PASMC. Direct G\(_{\alpha}\)-activator, cholera toxin, was applied to PASMCs at the indicated doses. Whereas cholera toxin (3 min) produced a dose-dependent increase in cAMP, 45 min pretreatment with NECA abolished the G\(_{\alpha}\)-dependent elevation in cAMP. *Significantly different at P < 0.05; n = 6/group.

Desensitization of the A\(_2\)-adenosine receptor has been characterized in smooth muscle of porcine coronary artery ring (15), dithiothreitol, MF-2 cells (22), and rat pheochromocytoma PC-12 cells (2, 14). To date, studies characterizing A\(_2\)-adenosine-receptor desensitization have evaluated the A\(_{2a}\)-adenosine receptor (2, 14, 22). There is a paucity of studies characterizing A\(_{2b}\)-adenosine-receptor desensitization. Reported mechanisms of A\(_{2a}\)-adenosine-receptor desensitization include inhibition of adenylyl cyclase, downregulation of G\(_{\alpha}\) protein, and activation of phosphodiesterase. Interestingly, no studies demonstrate desensitization due to a change in receptor number or affinity. Common to all the reported studies, A\(_{2a}\)-adenosine-receptor desensitization occurs rapidly. Makujina and Mustafa (15) demonstrated that NECA and CGS-21680 caused rapid desensitization of A\(_{2a}\)-adenosine-receptor-mediated vasodilation in preconstricted porcine coronary artery rings. Tissues pretreated with NECA for 30 min exhibited a blunted relaxation response to Ado and NECA but not to other vasodilators such as Iso, forskolin, and sodium nitroprusside (15). This led to the conclusion that, in porcine coronary artery smooth muscle, A\(_{2a}\)-adenosine-receptor desensitization is homologous. Similar observations were made in the isolated lung in this study, suggesting a homologous pattern of rapid desensitization to NECA manifested by blunted NECA-induced vasodilation while maintaining Iso and Ind vasodilation in NECA-desensitized lungs. Despite evidence in the pulmonary circulation that prolonged exposure to NECA functionally induces homologous desensitization, data from in vitro studies indicated that NECA desensitization of PASMCs was heterologous. Prolonged NECA exposure eliminated NECA-induced increases in cAMP and also diminished Iso-stimulated rises in cAMP. Why the patterns of desensitization differed between PASMC culture and isolated lung cannot be answered from this study but suggest Iso-induced vasodilation is mediated only in part via G\(_{\alpha}\)-adenyl cyclase-cAMP signaling. We could speculate that the preservation of Iso-induced vasodilation in the lung reflects the capacity of the many different cells to make cAMP. Although this is true, vasodilation is a reflection of cAMP accumulation in smooth muscle cells and not the amount of cAMP in the circulation. The most plausible explanation for the

Treated with NECA for 45 min, washed, and rechallenged with NECA, no increase in cAMP was observed. These findings demonstrate that Ado receptor desensitization occurs, likely due to reduced activation of the Ado receptor-coupled G\(_{\alpha}\)-adenyl cyclase stimulation of cAMP.

NECA-induced desensitization was not limited to the Ado receptor. In PASMC culture treated with NECA for 45 min followed by Iso, cAMP accumulation was significantly reduced compared with PASMCs treated with Iso alone. A plausible explanation for the observed decrease in cAMP accumulation in NECA-desensitized PASMCs treated with Iso is β\(_2\)-adrenergic receptor phosphorylation by protein kinase A (PKA; see Ref. 5). NECA-stimulated increase in cAMP production acutely activates PKA, which phosphorylates the β\(_2\)-adrenergic receptor and uncouples the receptor from G\(_{\alpha}\) protein. PKA activation by NECA-generated increases in intracellular cAMP may be the mechanism responsible for β\(_2\)-adrenergic receptor phosphorylation and desensitization in this study. Penn et al. (21) have recently reported that agents which stimulate cAMP production such as forskolin and PGE\(_2\) activate PKA, which results in β\(_2\)-adrenergic receptor desensitization in human airway smooth muscle cell culture. This cannot be excluded as a possible mechanism for the observed decrease in cAMP accumulation with Iso in PASMC culture. Data derived from NECA-desensitized PASMCs treated with cholera toxin (irreversibly couples the A\(_2\)-adenosine receptor to G\(_{\alpha}\)) revealed a decrease in stimulation of cAMP accumulation when compared with cAMP accumulation in PASMCs treated with cholera toxin alone. This observation coupled with the decreased cAMP accumulation after Iso in NECA-desensitized PASMCs suggests that a common mechanism of desensitization is likely that involves G\(_{\alpha}\)-adenyl cyclase signaling. Potential mechanisms of G\(_{\alpha}\)-involvement may include dissociation of ligand-receptor complex from G\(_{\alpha}\), impaired G\(_{\alpha}\)-binding to and activation of adenyl cyclase, and downregulation of G\(_{\alpha}\) protein. Downregulation of G\(_{\alpha}\) protein as a mechanism of A\(_{2a}\)-adenosine-receptor desensitization has been described in rat pheochromocytoma PC-12 cells when exposed to the selective A\(_{2a}\)-adenosine agonist CGS-21680 for 12-20 h (2).
reported differences observed in this study is perhaps related to the modulation of vascular smooth muscle K⁺ channels by Ado and β-adrenoreceptor agonists. Several investigators (24, 26) have reported that Ado, calcitonin gene-related peptide, and β-adrenoreceptor agonists activate the ATP-sensitive K⁺ channel through a CAMP-dependent protein kinase, which results in vasodilation. In addition, Iso has been shown to also activate the Ca²⁺-activated K⁺ channels (BKCa) through Gs, independent of phosphorylation by PKA (23). It is possible that vasodilation in the isolated lung was preserved through activation of the BKCa and explains why Iso-induced CAMP accumulation in NECA-desensitized PASMC culture was blunted while Iso-induced vasodilation was preserved. Further studies will be necessary to address this question.

Because the A₂a-adenosine receptor has two subtypes, A₂a₁ and A₂a₂, their relative individual roles in desensitization were assessed. NECA binds the A₂a₁ receptor with high affinity and the A₂a₂ receptor with low affinity (1, 9, 11). The concentration of NECA utilized in this study exceeds the previously reported dissociation constant for NECA binding to the low-affinity A₂a₂-adenosine receptor in rat striatum (286 nM; see Ref. 9) and human peripheral lung (200 nM; see Ref. 11). Thus it could not be used to distinguish which A₂-adenosine-receptor subtype(s) is involved in the process of Ado agonist desensitization. CGS-21680 is now the current ligand of choice for the characterization of the high-affinity A₂a₁ receptor (10). In this study, pretreatment with CGS-21680 (10 μM) neither caused desensitization to NECA-induced vasodilation in the isolated lung nor caused a significant accumulation of CAMP in PASMC culture. We previously reported (8) that pretreatment with CGS-21680 (1 mM) was required to significantly promote desensitization to NECA-induced vasodilation in the isolated lung. The requirement of a millimolar concentration of CGS-21680 to desensitize the lung to NECA and its lack of effect at a micromolar concentration on CAMP accumulation in PASMC culture strongly supports that the low-affinity A₂b receptor is the predominant receptor involved in Ado-mediated vasodilation and subsequent desensitization in the rat pulmonary circulation. This differs from the observation in the porcine coronary artery ring where a desensitization to CGS-21680 parallels that observed with NECA (15). This difference in response to CGS-21680 suggests that the presence of the A₂b-adenosine receptor may either be species specific and/or vary according to the vascular bed studied, i.e., pulmonary vs. coronary. Using immunohistochemical staining, we have been able to demonstrate the presence of both the A₂a₁-adenosine receptor and A₂a₂-adenosine receptor in rat pulmonary vascular beds (data not shown).

In summary, this study demonstrates that the A₂b-adenosine receptor is involved in Ado-mediated vasodilation through increasing intracellular CAMP and that relatively prolonged agonist exposure results in desensitization via Gs, adenyl cyclase coupling. The findings in this study provide a functional model for expanding our understanding of the various effects of Ado on pulmonary vascular hemodynamics and desensitization.

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