Postreceptor defects in alveolar epithelial β-adrenergic signaling after prolonged isoproterenol infusion

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Postreceptor defects in alveolar epithelial β-adrenergic signaling after prolonged isoproterenol infusion. Am J Physiol Lung Cell Mol Physiol 285: L578–L583, 2003. First published May 16, 2003; 10.1152/ajplung.00339.2002.—We previously found that prolonged isoproterenol (Iso) infusion in rats impaired the ability of β-adrenoceptor (β-AR) agonists to increase alveolar liquid clearance (ALC). Here, we determined if postreceptor defects in β-AR signaling contribute to this impairment. Iso was infused using subcutaneous miniosmotic pumps (4, 40, or 400 μg·kg⁻¹·h⁻¹) in rats for 48 h. At this time, forskolin-stimulated ALC was measured by mass balance. Forskolin-stimulated ALC (33.4 ± 2.1% in vehicle-infused rats was reduced by 25 and 38%, respectively, after the 40 and 400 μg·kg⁻¹·h⁻¹ Iso infusions. The ability of forskolin to increase cAMP was reduced by 70% in alveolar type II (ATII) cells isolated from rats infused with 400 μg·kg⁻¹·h⁻¹ Iso. Additionally, the ability of the stable cAMP analog 8-bromoadenosine-3',5'-cyclic monophosphorothioate, Sp-isomer, to increase ALC (48.7 ± 3.0% in vehicle-infused rats) was reduced by 25 and 51%, respectively, after the 40 and 400 μg·kg⁻¹·h⁻¹ infusions. Finally, the ability of cAMP to increase protein kinase A activity was eliminated in ATII cells isolated from rats infused with Iso at 400 μg·kg⁻¹·h⁻¹. These data demonstrate that prolonged β-AR agonist exposure can impair alveolar epithelial β-AR signaling downstream of the β-AR.

PULMONARY EDEMA; β-adrenergic receptor signaling pathway; receptor desensitization; alveolar epithelial type II cells; adenylyl cyclase; adenosine 3',5'-cyclic monophosphate; protein kinase A

IT IS NOW WELL ACCEPTED THAT administration of β-adrenoceptor (β-AR) agonists significantly increases the rate of alveolar transepithelial Na⁺ and, consequently, water transport in most species, including humans (12). These observations have suggested that β-AR agonists might be valuable if used clinically to accelerate the recovery from pulmonary edema (1, 6). A possible limitation of β-AR agonist therapy for the treatment of pulmonary edema, however, is the potential for β-AR desensitization [a regulated process in which continued agonist exposure attenuates the receptor’s biological effect] and downregulation [a form of desensitization in which receptor number decreases; see Refs. 2 and 15]. Thus, if the alveolar epithelial cell β-ARs undergo desensitization, the efficacy of β-AR agonist therapy could diminish over time. We recently evaluated this possibility and observed a dose-dependent impairment in the ability of β-AR agonists to increase the rate of alveolar liquid clearance (ALC) after continuous isoproterenol (Iso) infusion (14).

This functional impairment was accompanied by a reduction in the number of total β-ARs on alveolar epithelial type II (ATII) cells freshly isolated from rats infused with Iso (14), but a lack of correlation between the ability of terbutaline to stimulate ALC and the β-AR density suggested that additional desensitization mechanisms must have played a role in producing the dose-dependent ALC responses. Although significant attention has been focused on examining desensitization events at the β-AR and G protein levels, there is emerging evidence that desensitization may be a phenomenon that affects additional downstream points in the β-AR signaling pathway. For example, McMartin and Summers (13) recently reported that left atrial tissue and left and right ventricular papillary muscles obtained from rats infused with Iso for 14 days exhibited an impaired ability to contract in response to both forskolin (a direct activator of adenylyl cyclase) and dibutyryl-cAMP (a stable cAMP analog). Accordingly, the objective of this study was to determine if additional impairments in the lung β-AR signaling pathway developed downstream of the β-AR in rats infused with Iso for 48 h. To do so, we examined the ability of forskolin to increase ALC and to stimulate cAMP production in ATII cells isolated from rats infused with Iso. We also evaluated the abilities of a stable cAMP analog to increase ALC and of cAMP to increase protein kinase A (PKA) activity in ATII cells isolated from rats infused with Iso.

METHODS

Experimental Model

Male Sprague-Dawley rats (n = 96) weighing 250–300 g (Harlan, Chicago, IL) were used in this study. The rats were

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housed in the Comparative Medicine Unit at the Northeastern Ohio Universities College of Medicine for at least 1 wk under temperature-controlled conditions (20 ± 2°C (mean ± SD) and at a relative humidity of 50 ± 10% before experimental use. The rats were fed a standard rat chow and had water ad libitum. All experiments were approved by the Northeastern Ohio Universities College of Medicine Institutional Animal Care and Use Committee.

Miniosmotic pumps (Alzet model 1001; Durect, Cupertino, CA) were filled under sterile conditions with either the β-AR agonist (-)-isoproterenol (+)-bitartrate (Iso; Sigma Chemical, St. Louis, MO) or its vehicle (0.001 N HCl) and primed in sterile saline at 37°C overnight. The next morning, the filled pumps were aseptically implanted subcutaneously under halothane anesthesia as previously described (14). Iso concentrations were selected to allow the pumps to deliver the drug at infusion rates of 4, 40, or 400 μg Iso base·kg⁻¹·h⁻¹. These Iso administration rates were selected because they represent the range of infusion rates that have been used in previous studies to evaluate the ability of Iso to produce β-AR downregulation (8, 14, 24, 25).

Determination of ALC

ALC was measured as previously described (3, 14). The rats were anesthetized with 80 mg/kg ip pentobarbital sodium (Abbott Laboratories, N. Chicago, IL), with the anesthetic being supplemented as needed. Body temperature was monitored using a rectal temperature probe and was maintained using a water-perfused heating pad. A polyethylene tracheal cannula (PE-240; Clay Adams, Becton-Dickinson, Sparks, MD) was placed in the rat’s airway via a tracheotomy and connected to a mechanical ventilator (Harvard Apparatus, Nantucket, MA). The lungs were ventilated with a FIO₂ = 1.0 at a respiratory rate of 40 breaths/min with an average tidal volume of 2.6 ± 0.2 (SD) ml. Peak inspiratory pressure was 9.2 ± 1.2 Torr under baseline conditions, and end-expiratory pressure was atmospheric. The rat was placed at a 45° angle (head elevated), and a polyethylene catheter (PE-50; Clay Adams) was inserted through a port in the tracheal cannula and in the lungs for liquid instillation. The rats were allowed to stabilize for 10 min after surgery before starting the experiment. At this time, 3 ml/kg of a 5% BSA (Sigma) solution in Ringer lactate (Baxter Healthcare, Deerfield, IL) was instilled in the lung for measurement of ALC. After the 48-h Iso infusion period, the rats were anesthetized with pentobarbital sodium and an external jugular vein was cannulated. An intravenous forskolin (Sigma) infusion was started (16.6 μg·kg⁻¹·min⁻¹ in a volume flow of 0.026 ml/min) and maintained for the duration of the experiment. Forskolin was dissolved in 5% DMSO (Sigma) in saline. The 5% BSA solution, containing 10⁻¹⁴ M dl-propranolol (Sigma), was instilled in the lung for measurement of ALC 15 min after starting the forskolin infusion. These experiments were done in the presence of propranolol to ensure that the forskolin-stimulated increase in ALC was not mediated by β-AR activation. Low forskolin concentrations have been shown to potentiate accumulation of cAMP produced by a variety of agents, including adrenergic agonists (22). ALC was also measured in vehicle-infused rats administered forskolin and propranolol (n = 12) and those treated with 5% DMSO (forskolin vehicle) and propranolol (n = 6).

Effect of prolonged Iso infusion on the ability of forskolin to increase ALC. In this set of experiments, we determined if 48 h of Iso infusion (at rates of either 4, 40, or 400 μg·kg⁻¹·h⁻¹, n = 6 for each infusion rate) affected the ability of the adenylyl cyclase activator, forskolin, to increase ALC. After the 48-h Iso infusion period, the rats were anesthetized with pentobarbital sodium, and an external jugular vein was cannulated. An intravenous forskolin (Sigma) infusion was started (16.6 μg·kg⁻¹·min⁻¹ in a volume flow of 0.026 ml/min) and maintained for the duration of the experiment. Forskolin was dissolved in 5% DMSO (Sigma) in saline. The 5% BSA solution, containing 10⁻¹⁴ M dl-propranolol (Sigma), was instilled in the lung for measurement of ALC 15 min after starting the forskolin infusion. These experiments were done in the presence of propranolol to ensure that the forskolin-stimulated increase in ALC was not mediated by β-AR activation. Low forskolin concentrations have been shown to potentiate accumulation of cAMP produced by a variety of agents, including adrenergic agonists (22). ALC was also measured in vehicle-infused rats administered forskolin and propranolol (n = 12) and those treated with 5% DMSO (forskolin vehicle) and propranolol (n = 6).

Effect of prolonged Iso infusion on the ability of forskolin to increase cAMP concentration in isolated ATII cells. For these experiments, rats were infused with Iso (at rates of 4, 40, or 400 μg·kg⁻¹·h⁻¹) or vehicle (0.001 N HCl) for 48 h. After 48 h, the animals were heparinized and anesthetized as described in ATII Cell Isolation, and the lungs were removed for isolation of ATII cells. A sufficient number of ATII cells could be isolated from the lungs of each rat to allow a complete cAMP analysis for each animal. Four rats were used for each infusion rate.

Immediately after isolation, the cells were assayed for cAMP concentrations, as previously described (16) using a commercially available enzyme immunoassay (EIA) kit (Direct cAMP Correlate-EIA; Assay Designs, Ann Arbor, MI). We used the same number of cells (1.5 × 10⁶) for each assay, thus ensuring that the cell number would not be a variable. cAMP was always measured in the presence of the phosphodiesterase inhibitor IBMX (10⁻³ M; Sigma) at

ATII Cell Isolation

The ATII cells were isolated using the technique of Dobbs et al. (4). Briefly, the rats were anesthetized with pentobarbital sodium (80 mg/kg ip) and heparinized (1,000 units). A tracheotomy was done, and an 18-gauge angiocatheter (Becton-Dickinson Infusion Therapy Systems, Sandy, UT) was inserted. The chest was opened, and the lungs were removed. The lungs were perfused through the pulmonary artery to remove blood and lavaged to remove alveolar macrophages. The lungs were then digested with 20 ml elastase (3 U/ml; Worthington Biochemical, Lakewood, NJ) for 20 min at 37°C. The digested tissue was minced in the presence of FBS (Hyclone, Logan, UT) and DNase (Sigma) and filtered through sterile gauze and then through 70-μm nylon mesh filters (Becton-Dickinson Labware, Franklin Lakes, NJ). The filtrate was centrifuged at 400 g, and the cell pellet was resuspended in DMEM (Irvin Scientific, Santa Ana, CA) containing an antibiotic-antimycotic cocktail of penicillin G, streptomycin sulfate, and amphotericin B (GIBCO-BRL, Grand Island, NY) and glutamine (Irvin Scientific). The type II cells were purified by differential adherence to IgG (Sigma)-coated plates. Cell yield and purity (85%) were determined by using a Beckman Coulter Z1 Coulter particle counter and by tannic acid staining (11).

Experimental Design

Effect of prolonged Iso infusion on the ability of forskolin to increase ALC. In this set of experiments, we determined if 48 h of Iso infusion (at rates of either 4, 40, or 400 μg·kg⁻¹·h⁻¹, n = 6 for each infusion rate) affected the ability of the adenylyl cyclase activator, forskolin, to increase ALC. After the 48-h Iso infusion period, the rats were anesthetized with pentobarbital sodium, and an external jugular vein was cannulated. An intravenous forskolin (Sigma) infusion was started (16.6 μg·kg⁻¹·min⁻¹ in a volume flow of 0.026 ml/min) and maintained for the duration of the experiment. Forskolin was dissolved in 5% DMSO (Sigma) in saline. The 5% BSA solution, containing 10⁻¹⁴ M dl-propranolol (Sigma), was instilled in the lung for measurement of ALC 15 min after starting the forskolin infusion. These experiments were done in the presence of propranolol to ensure that the forskolin-stimulated increase in ALC was not mediated by β-AR activation. Low forskolin concentrations have been shown to potentiate accumulation of cAMP produced by a variety of agents, including adrenergic agonists (22). ALC was also measured in vehicle-infused rats administered forskolin and propranolol (n = 12) and those treated with 5% DMSO (forskolin vehicle) and propranolol (n = 6).

Effect of prolonged Iso infusion on the ability of forskolin to increase cAMP concentration in isolated ATII cells. For these experiments, rats were infused with Iso (at rates of 4, 40, or 400 μg·kg⁻¹·h⁻¹) or vehicle (0.001 N HCl) for 48 h. After 48 h, the animals were heparinized and anesthetized as described in ATII Cell Isolation, and the lungs were removed for isolation of ATII cells. A sufficient number of ATII cells could be isolated from the lungs of each rat to allow a complete cAMP analysis for each animal. Four rats were used for each infusion rate.

Immediately after isolation, the cells were assayed for cAMP concentrations, as previously described (16) using a commercially available enzyme immunoassay (EIA) kit (Direct cAMP Correlate-EIA; Assay Designs, Ann Arbor, MI). We used the same number of cells (1.5 × 10⁶) for each assay, thus ensuring that the cell number would not be a variable. cAMP was always measured in the presence of the phosphodiesterase inhibitor IBMX (10⁻³ M; Sigma) at
37°C under baseline conditions and after a 10-min forskolin stimulation (10⁻⁴ M). Additional cAMP determinations were also made at 4°C and in the presence of 1% DMSO (forskolin vehicle). DMSO was found to have no effect on cAMP production. The EIA had a sensitivity of 0.39 pmol/ml and intra- and interassay variabilities of 8.9 and 13.1%, respectively.

Effect of prolonged Iso infusion on the ability of 8-bromoadenosine-3',5'-cyclic monophosphorothioate, Sp-isomer to increase ALC.

In this set of experiments, we determined if a prolonged Iso infusion (at rates of either 4, 40, or 400 µg·kg⁻¹·h⁻¹, n = 6–7) to rats affected the ability of the stable cAMP-dependent protein kinase activator 8-bromoadenosine-3',5'-cyclic monophosphorothioate, Sp-isomer (Sp-8-bromo-cAMPS; 10⁻⁴ M dissolved in the instillate; Biolog Life Science Institute, Bremen, Germany), to increase ALC. ALC was also measured under baseline conditions (n = 7) and after Sp-8-bromo-cAMPS stimulation (n = 6) in groups of rats receiving a 48-h vehicle infusion.

Effect of prolonged Iso infusion on the ability of cAMP to stimulate PKA activity in isolated ATII cells. Rats were infused with Iso (at rates of 4, 40, or 400 µg·kg⁻¹·h⁻¹) or vehicle (0.001 N HCl) for 48 h (n = 3 for each infusion rate). After 48 h, ATII cells were isolated as described in ATII Cell Isolation. A sufficient number of ATII cells could be isolated from the lungs of each rat to allow an analysis of baseline and cAMP-stimulated PKA activity in each animal. PKA activity was determined in the freshly isolated ATII cells using a commercially available ELISA (Calbiochem, San Diego, CA) that uses a synthetic PKA pseudosubstrate peptide and a monoclonal antibody that recognizes only the phosphorylated form of the pseudosubstrate peptide. Briefly, ~4.0 × 10⁷ cells were used for the assay. The cells were washed in ice-cold DMEM and pelleted at 11,600 g, and the supernatant was removed. The cell pellet was then snap-frozen in liquid nitrogen and stored at −80°C until the assay was run. On the day of assay, the cell pellet was resuspended in 1 ml ice-cold sample preparation buffer according to the protocol of the assay kit. The cell membranes were disrupted by sonication five times for 10 s each. The cell suspension was then centrifuged at 100,000 g for 1 h at +4°C, and the supernatant was collected. The assay was then immediately run according to the kit protocol.

Statistical Analysis

Multigroup comparisons of data were done by ANOVA followed by the Student Newman-Keul’s post hoc test. Comparisons between two groups were made by unpaired Student’s t-test.

RESULTS

Systemic effects of Iso infusion

The pattern of weight changes observed in the rats of this study after 48 h of vehicle or Iso infusion was similar to that observed in our previous study (14). Rats infused with vehicle or Iso at 4 µg·kg⁻¹·h⁻¹ exhibited, respectively, 3.9 ± 0.3% and 6.6 ± 0.4% (means ± SE) weight gains during the 48-h period after pump implantation. In contrast, rats infused with Iso at rates of 40 and 400 µg·kg⁻¹·h⁻¹ exhibited, respectively, a weight increase of 1.4 ± 0.6% or a weight loss of 6.1 ± 0.8% during the 48-h period.

Effects of Prolonged Iso Infusion on the Ability of Forskolin to Increase ALC and cAMP Concentration in Isolated ATII Cells

The average baseline ALC (in the presence of propranolol) observed in vehicle-infused (0 µg·kg⁻¹·h⁻¹ Iso) rats was 16.6 ± 1.7% (mean ± SE) of the instilled volume absorbed per hour. ALC in vehicle-infused rats administered forskolin and propranolol was 101% greater (33.4 ± 2.1%, P < 0.05, Fig. 1A) than that observed under baseline conditions. In rats infused with Iso at the 40 and 400 µg·kg⁻¹·h⁻¹ rates, forskolin-stimulated ALC was reduced by 25 and 38%, respectively (both P < 0.05, Fig. 1A). ALC measured after forskolin administration (20.7 ± 2.0%) in the 400 µg·kg⁻¹·h⁻¹ Iso infusion group was not different from that observed in the vehicle-infused rats under baseline conditions.

Although the ability of forskolin to stimulate ALC was reduced in rats infused with Iso at the 40 µg·kg⁻¹·h⁻¹ infusion rate, an impairment in ATII cell forskolin-stimulated cAMP production (70%, P < 0.05) was only observed at the highest Iso infusion rate (Fig. 1B).

![Fig. 1. Effects of prolonged isoproterenol (Iso) infusion on the ability of forskolin to increase alveolar liquid clearance (ALC) in rats (A) and cAMP in isolated alveolar type II (ATII) cells (B). *P < 0.05 compared with Iso infusion rate of 0 µg·kg⁻¹·h⁻¹ (vehicle). †P < 0.05 compared with Iso infusion rates of 0, 4, and 40 µg·kg⁻¹·h⁻¹.](http://ajplung.physiology.org/)

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Effects of Prolonged Iso Infusion on the Ability of Sp-8-bromo-cAMPS to Increase ALC and of cAMP to Stimulate PKA Activity in Isolated ATII Cells

The average baseline ALC observed in vehicle-infused rats was 22.0 ± 1.4% (mean ± SE) of the instilled volume absorbed per hour. ALC in vehicle-infused (0 μg·kg⁻¹·h⁻¹ Iso in Fig. 2) rats administered Sp-8-bromo-cAMPS was 121% higher (48.7 ± 3.0%, P < 0.05) than that observed under baseline conditions. In rats infused with Iso at 40 and 400 μg·kg⁻¹·h⁻¹, Sp-8-bromo-cAMPS-stimulated ALC was reduced by 25 and 51%, respectively (both P < 0.05, Fig. 2) compared with Sp-8-bromo-cAMPS-stimulated ALC in vehicle-infused rats. ALC measured after Sp-8-bromo-cAMPS administration in the 400 μg·kg⁻¹·h⁻¹ Iso infusion group (23.8 ± 1.2%) was not different from that observed in the vehicle-infused rats under baseline conditions. ALCs observed in the 4, 40, and 400 μg·kg⁻¹·h⁻¹ groups were all significantly different (P < 0.05) from one another.

Basal PKA activity of freshly isolated ATII cells from rats infused with any of the three Iso infusion rates did not differ from that observed in ATII cells obtained from vehicle-infused rats (Fig. 3). In ATII cells harvested from vehicle-infused rats, cAMP stimulated the PKA activity by 387% (Fig. 3). Iso infusion inhibited the ability of cAMP to stimulate PKA activity in a dose-dependent manner, with no stimulation being observed from vehicle-infused rats, cAMP stimulated the PKA activity by 387% (Fig. 3). Accordingly, in this study, we determined if prolonged Iso infusion caused desensitization at additional downstream points in the alveolar epithelial β-AR signaling pathway and identified two sites downstream of the β-AR that were susceptible to desensitization.

In initial experiments, we administered the adenylyl cyclase activator forskolin to rats infused with Iso and observed that the ability of forskolin to increase ALC was reduced in a dose-dependent pattern (Fig. 1A) that was reminiscent of that previously observed with β-AR agonists (14). Because forskolin can potentiate the action of β-AR agonists (22), we coadministered propranolol with forskolin in these experiments to ensure that the action of forskolin was not the result of β-AR activation by endogenous catecholamines or Iso. Because we were able to eliminate this as a possibility, the results of these experiments demonstrate the existence of at least one additional site of desensitization in the β-AR signaling pathway downstream of the β-AR.

Although forskolin is a direct adenylyl cyclase activator, these observations cannot in themselves be interpreted to mean that the site of desensitization is at the level of adenylyl cyclase. A similar pattern of ALC responses to forskolin could have also been obtained if the desensitization site was located at some point downstream of the enzyme. To answer this question, we conducted additional experiments in which we evaluated the ability of forskolin to increase cAMP in ATII cells freshly isolated from rats infused with Iso and found that forskolin-stimulated cAMP production was impaired in ATII cells obtained from rats that had 

Fig. 2. Effects of prolonged Iso infusion on the ability of 8-bromo-cAMP to increase ALC and of cAMP to stimulate PKA activity in isolated ATII cells (open bars) from the same animal.

Fig. 3. Effects of prolonged Iso infusion on the ability of cAMP to increase protein kinase A activity in isolated ATII cells (filled bars). OD, optical density. *P < 0.05 compared with non-cAMP-treated cells (open bars) from the same animal.
received the highest Iso (400 $\mu$g·kg$^{-1}$·h$^{-1}$) infusion rate (Fig. 1B), but not at the lower Iso infusion rates. Although there is evidence that prolonged $\beta$-AR stimulation may lead to increased phosphodiesterase activity in whole lung tissue (5), which in turn might decrease cAMP accumulation, this seemed unlikely since our experiments were conducted in the presence of relatively high concentrations of the phosphodiesterase inhibitor IBMX. These data thus indicate that the impaired forskolin-stimulated cAMP accumulation was the result of a decreased cAMP production rather than an enhanced degradation. The mechanism responsible for this impairment is unknown but is presumably the result of a reduction in adenylyl cyclase expression or activity. With respect to activity, adenylyl cyclase is capable of receiving and integrating both stimulatory and inhibitory inputs from a variety of sources, with the nine known mammalian isoforms exhibiting variable responsiveness to different stimuli (23). It is thus possible that prolonged Iso infusion may have altered the balance of afferent stimulatory and inhibitory signaling to adenylyl cyclase within the ATII cell.

These observations suggest that a limitation in the ATII cell’s ability to produce cAMP could have contributed to the impaired ability of forskolin (as well as $\beta$-AR agonists) to increase ALC in rats infused with the highest Iso infusion rate (400 $\mu$g·kg$^{-1}$·h$^{-1}$). In contrast, the ability of forskolin to stimulate ALC in vivo was reduced at the 40 $\mu$g·kg$^{-1}$·h$^{-1}$ Iso infusion rate (Fig. 1A), but there was no accompanying impairment in the ability of forskolin to increase cAMP in the isolated ATII cells (Fig. 1B). This disparity suggests that the reduced ability of forskolin to increase ALC in rats infused with Iso at 40 $\mu$g·kg$^{-1}$·h$^{-1}$ (and perhaps at higher Iso infusion rates as well) resulted from additional desensitization events occurring downstream of the point of cAMP production.

To investigate this latter possibility, we administered the stable cAMP analog and PKA activator Sp-8-bromo-cAMPS to rats infused with Iso and observed that the ability of Sp-8-bromo-cAMPS to increase ALC was decreased in rats infused with 40 and 400 $\mu$g·kg$^{-1}$·h$^{-1}$ Iso (Fig. 2). These data indicate that prolonged Iso infusion also interferes with alveolar epithelial $\beta$-AR signaling at some site in the signaling pathway at the level of PKA or beyond. This site could represent PKA itself, the membrane Na$^+$ transport proteins (i.e., apical Na$^+$ channels or Na$^+$-K$^+$-ATPase), and/or some intermediate step.

To determine if PKA became desensitized, we evaluated the ability of cAMP to stimulate PKA activity in ATII cells isolated from rats infused with vehicle or Iso. We observed that Iso infusion resulted in a dose-dependent impairment in the ability of cAMP to stimulate PKA activity, with a total loss of PKA stimulation at the 400 $\mu$g·kg$^{-1}$·h$^{-1}$ Iso infusion rate (Fig. 3). These observations suggest that the Iso-induced impaired ability of Sp-8-bromo-cAMPS to increase ALC resulted from desensitization of PKA. It is important to point out, however, that this conclusion is predicated on the assumption of an intact transalveolar epithelial Na$^+$ transport ability. In this regard, the level of PKA stimulation (or any other component of the $\beta$-AR signaling pathway) would be of little consequence if Iso infusion impaired the function of the alveolar epithelial Na$^+$ transport pathways.

The combined results of our previous (14) and current studies show that by 48 h of 400 $\mu$g·kg$^{-1}$·h$^{-1}$ Iso infusion, alterations in the ATII cell $\beta$-AR signaling pathway develop at the receptor, adenylyl cyclase, and PKA levels. The inability of Sp-8-bromo-cAMPS to increase ALC and of cAMP to stimulate PKA activity that developed at this infusion rate suggests that an important rate-limiting step in the ability of $\beta$-AR agonists to increase ALC develops at the PKA level. Our data do not provide insight, however, about the physiological importance of the receptor downregulation and impaired adenylyl cyclase activity, because it is not known if these changes by themselves are of sufficient magnitude to impair signaling. It is thus not clear if the overall impairment in $\beta$-AR agonist-stimulated ALC reflects the contribution of the effects of multiple desensitization events or of a single rate-limiting step residing at the level of PKA.

The development of downstream $\beta$-AR signaling pathway defects after prolonged $\beta$-AR agonist exposure has been found to occur in other tissues and cell types and is consistent with our observations. For example, a number of studies have reported the development of impaired cardiac $\beta$-AR signaling downstream of the $\beta$-AR in animals and cultured heart cells administered $\beta$-AR agonists for prolonged time periods (9, 13, 18, 19) as well as in myocytes obtained from patients with congestive heart disease (7). These signaling defects include impaired forskolin- and stable cAMP-stimulated contractile responses and reduced forskolin-stimulated cAMP production. Additionally, exposure of a variety of cultured cell types to cAMP for prolonged periods produces desensitization of PKA mediated by a variety of mechanisms (10, 17, 20, 26). The results of this study extend this concept to the alveolar epithelium and indicate that the development of such impairments in postreceptor signaling can have functional significance.

The ability of $\beta$-AR agonists to increase ALC suggests that $\beta$-AR agonist therapy might be used clinically for the treatment of alveolar edema (1, 6). The combined results of the current and our previous study (14) indicate that prolonged Iso infusion results in impaired signaling at multiple sites in the alveolar epithelial $\beta$-AR signaling pathway, which exhibit differing desensitization thresholds. For example, $\beta$-AR downregulation and impaired PKA stimulation were apparent at the 40 $\mu$g·kg$^{-1}$·h$^{-1}$ infusion rate, whereas an impaired adenylyl cyclase function did not develop until the Iso infusion rate was increased to 400 $\mu$g·kg$^{-1}$·h$^{-1}$. These observations suggest that attention might need to be given to tailoring $\beta$-AR agonist therapy to maximize the increase in ALC while minimizing the potential for desensitization. Sartori et al. (21) have recently reported that mice infused with the
specific β2-AR partial agonist albuterol for up to 6 days exhibited a reduction in β-AR density and an impaired β-AR agonist-stimulated cAMP accumulation in peripheral lung tissue but still retained a maximum ability to increase ALC in response to terbutaline administration. These data suggest that the mice did not undergo PKA desensitization. The reason for these differences is unclear but could relate to species differences, agonist characteristics, or drug doses. An answer to this question may provide additional important insight into the potential efficacy of β-AR agonist therapy for treatment of severe pulmonary edema.

In summary, we previously identified β-AR down-regulation as a potential mechanism that may have played a role in producing the impaired ability of β-AR agonists to increase ALC observed in rats after 48 h of iso infusion. The results of the current study extend this observation by showing that additional sites of the β-AR signaling pathway undergo desensitization as well as clinically relevant agonist doses. These sites include the enzymes adenylyl cyclase and PKA, with the latter being a potential rate-limiting step.

DISCLOSURES

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