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Effects of SQ 22536, an adenylyl cyclase inhibitor, on isoproterenol-induced cyclic AMP elevation and relaxation in newborn ovine pulmonary veins

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Abstract

The effects of inhibition of adenylyl cyclase on isoproterenol-induced relaxation were determined in isolated pulmonary veins of newborn lambs (7–12 days old). In veins constricted with endothelin-1, isoproterenol at concentrations $\leq 3 \times 10^{-9}$ M had no effect on the cyclic AMP (cAMP) content but caused up to 56% relaxation. At higher concentrations ($\geq 10^{-8}$ M), isoproterenol elevated cAMP content and caused further relaxation. In veins constricted with endothelin-1 or U46619 (9,11-dideoxy-11, 9-epoxymethanoprostaglandin prostaglandin F2α), the cAMP elevation but not relaxation caused by isoproterenol was abolished by SQ 22536 [9-(tetrahydro-2-furanyl)-9*H*-purin-6-amine; an adenylyl cyclase inhibitor]. The effects of isoproterenol on vessel tension and cAMP content were inhibited by propranolol. Rp-8-CPT-cAMPS [8-(4-Chlorophenylthio)-adenosine-3′,5′-cyclic monophosphorothioate, Rp-isomer] and Rp-8-Br-PET-cGMPS [β-phenyl-1, N^2-etheno-8-bromoguanosine-3′,5′-cyclic monophosphorothioate, Rp-isomer], inhibitors of cAMP- and guanosine-3′,5′-cyclic monophosphate (cGMP)-dependent protein kinases, respectively, attenuated relaxation caused by a cAMP analog but not that by isoproterenol. In the crude membrane preparations of pulmonary veins, an increase in the activity of adenylyl cyclase caused by isoproterenol was abolished by propranolol and SQ 22536. These results suggest that cAMP may not play a critical role in isoproterenol-induced relaxation of pulmonary veins of newborn lambs.

Keywords: β-Adrenoceptor agonist; cAMP; Adenylyl cyclases; SQ 22536; Smooth muscle, vascular; Pulmonary vein

1. Introduction

β-Adrenoceptor agonists are potent dilators of various smooth muscle types (Bülbring and Tomita, 1987). It is thought that relaxation caused by these agonists is mainly mediated by cyclic AMP (cAMP). This belief is supported by the findings that β-adrenoceptor-mediated relaxation is accompanied by an increase in the intracellular cAMP content and that cAMP analogs cause smooth muscle cell to relax (Bülbring and Tomita, 1987; Murray, 1990; Torphy, 1994).

β-Adrenoceptor agonists elevate the intracellular content of cAMP by activating adenylyl cyclases (Murray, 1990). If cAMP plays an obligatory role in β-adrenoceptor-mediated relaxation, an inhibition of adenylyl cyclase activity should attenuate the relaxation. Such a possibility has not been examined. In the present study, SQ 22536 [9-(tetrahydro-2-furanyl)-9*H*-purin-6-amine], an adenylyl cyclase inhibitor (Haslam et al., 1978), was employed to inhibit the activity of adenylyl cyclases and the response of isolated pulmonary veins of newborn lambs to isoproterenol [a β-adrenoceptor agonist (Bylund et al., 1994)] was determined. Our previous study shows that isoproterenol is a potent dilator of ovine pulmonary veins (Gao et al., 1998).

2. Materials and methods

2.1. Pulmonary vein preparations

Twenty-three newborn lambs (7–12 days old, either sex) from Nebeker Ranch (Lancaster, CA, USA) were used.

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They were anesthetized with ketamine hydrochloride (30 mg/kg, i.m.) and sacrificed with an overdose of pentobarbital (100 mg/kg, i.v.). The lungs were immediately removed and fourth generation pulmonary veins (outside diameter: 1.5–2.5 mm) were dissected free of parenchyma and cut into rings (length: 5 mm). To eliminate the involvement of endogenous nitric oxide, the endothelium was removed by gently rubbing the luminal surface with the tips of a watchmaker's forceps. Removal of endothelium was confirmed by lack of relaxation to 3×10^{-5} M acetylcholine (Gao et al., 1995).

2.2. Vessel tension study

Vessel rings were suspended in organ chambers filled with 10 ml of modified Krebs–Ringer bicarbonate solution [composition (in mM): NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.1] maintained at 37 ± 0.5 °C and aerated with 95% O_2–5% CO_2 (pH = 7.4). Each ring was suspended by two stirrups passed through the lumen. One stirrup was anchored to the bottom of the organ chamber; the other one connected to a strain gauge (model FT03C, Grass Instrument, Braintree, MA, USA) for the measurement of isometric force (Gao et al., 1995).

At the beginning of the experiment, each vessel ring was stretched to its optimal resting tension. This was achieved by step-by-step stretching in 0.1-g increments until the active contraction of the vessel ring to 100 mM KCl reached a plateau. The optimal resting tension of pulmonary veins was 0.25 ± 0.07 g ($n = 13$).

After the vessels were brought to their optimal resting tension, 1 h of equilibration was allowed. Indomethacin (10^{-5} M) and phentolamine (5×10^{-6} M) were then added to exclude the possible involvement of endogenous prostanoids and α-adrenoceptor receptors (Vane, 1978; Bylund et al., 1995). Indomethacin caused an increase in resting tension by 0.31 ± 0.07g ($n = 13$) of these veins. Phentolamine had no significant effect on the basal tension of the veins.

The effects of isoproterenol were determined in vessels constricted with endothelin-1 (3×10^{-9} M) or U46619 [9,11-dideoxy-11, 9-epoxymethanoprostaglandin prostaglandin F2α, 3×10^{-8} M; a stable analog of thromboxane A_2 (Coleman et al., 1981)]. The addition of different concentrations of isoproterenol was non-cumulative. Relaxations of similarly sized vessel rings from the same animal were determined with varying concentrations of isoproterenol simultaneously. In some experiments, the effects of various inhibitors on isoproterenol-induced relaxation were determined, in a parallel manner. The following inhibitors were used: SQ 22536 [3×10^{-4} M, an adenylyl cyclase inhibitor (Haslam et al., 1978)]; Rp-8-CPT-cAMPS [8-(4-chlorophenylthio)-adenosine-3′,5′-cyclic monophosphorothioate, Rp-isomer (3×10^{-5} M), an inhibitor of cAMP-dependent protein kinase (Gjertsen et al., 1995)]; Rp-8-Br-PET-cGMPS [β-phenyl-1, N^2-etheno-8-bromoguanosine-3′,5′-cyclic monophosphorothioate; Rp-isomer ($3x10^{-5}$ M); an inhibitor of guanosine-3′,5′-cyclic monophosphate (cGMP)-dependent protein kinase (Butt et al., 1995)]; and propranolol [(10^{-5} M); a β-adrenoceptor antagonist (Bylund et al., 1994)]. These inhibitors were administrated 30 min before the vessels were constricted with endothelin-1 or U46619.

2.3. Cyclic AMP assay

Vessel rings of pulmonary veins without endothelium were incubated in modified Krebs–Ringer bicarbonate solution (37 °C, 95% O_2 and 5% CO_2, pH 7.4) in the presence of indomethacin (10^{-5} M) and phentolamine (5×10^{-6} M) to prevent the interference of endogenous prostanoids and α-adrenoceptor receptors, respectively (Vane, 1978; Bylund et al., 1995). After 1 h of equilibration, SQ 22536 (3×10^{-4} M), propranolol (10^{-5} M), or control solvent was added. Thirty minutes later, endothelin-1 (3×10^{-9} M) or U46619 (3×10^{-8} M) was added. The reason for the addition of endothelin-1 or U46619 was to make the conditions of cAMP assay similar to those of vessel tension studies where the constrictors were used. Thirty minutes later, isoproterenol, in various concentrations (3×10^{-10} M to 10^{-7} M), was added. The vessel rings were rapidly frozen in liquid nitrogen immediately before the addition of endothelin-1 or U46619, before the administration of isoproterenol, or 2 min after the addition of isoproterenol. Preliminary studies showed that maximal accumulation of cAMP in the vessels in response to isoproterenol occurred at 2 min. The frozen vessels were thawed in trichloroacetic acid (6%), homogenized in glass with a motor-driven teflon pestle, sonicated for 5 s, and centrifuged at $13{,}000 \times g$ for 15 min. The supernatant was extracted with four volumes of water-saturated diethyl ether and lyophilized. The lyophilized samples were resuspended in 0.5 ml of sodium acetate buffer (0.05 M, pH 6.2) and the content of cAMP was determined using a cAMP radioimmunoassay kit (Biomedical Technologies, Stoughton, MA, USA). The content of the cyclic nucleotide is expressed as pmol/mg protein of the tissues. The protein content was determined by Bradford method using bovine serum albumin as the standard (Bradford, 1973).

2.4. Adenylyl cyclase assay

Isolated pulmonary veins were homogenized in 5 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.6) containing dithiothreitol (2 mM), EDTA (5 mM), phenylmethylsulfonyl fluoride (1 mM), antipain (10 μg/ml), aprotinin (10 μg/ml), leupeptin (10 μg/ml), and pepstatin (10 μg/ml). The homogenate was centrifuged at $500 \times g$ for 10 min to remove the unbroken cells and cell nuclei. Further purification of the membrane was not performed because isoproterenol-sensitive adenylyl cyclase activity decreases with subsequent steps of smooth muscle membrane preparation (Popovich et al., 1984; Shaul et al., 1990). The protein concentrations

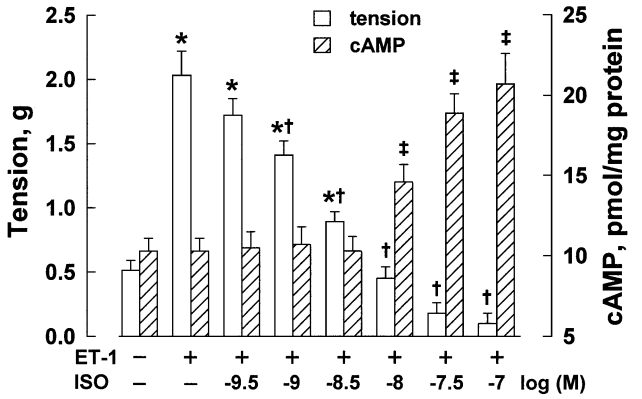

Fig. 1. Effect of isoproterenol (ISO) on tension and the intracellular content of cAMP of isolated newborn ovine pulmonary veins. ET-1, endothelin-1 (3×10^{-9} M). +, treated with endothelin-1. –, not treated with either endothelin-1 or isoproterenol. Data are shown as means ± S.E.M.; $n = 6$ for each group. *, significantly greater in tension from vessels not treated with endothelin-1; †, significantly smaller in tension from vessels not treated with isoproterenol; ‡, significantly increase in cAMP content from vessels not treated with isoproterenol ($P < 0.05$).

of the homogenate were determined by Bradford method using bovine serum albumin as the standard (Bradford, 1973).

Pulmonary vessel homogenate (30 μg protein) was incubated at 30 °C for 10 min in 50 mM Tris–HCl (pH 7.4), dithiothreitol (0.1 mM), ATP (1 mM), $MgCl_2$ (10 mM), creatine phosphate (20 mM), creatine phosphokinase (150 units/ml), ascorbic acid (0.5 mM), and isobutylmethylxanthine (1 mM) in the presence or absence of isoproterenol (10^{-7} M). In some vials, SQ 22536 (3×10^{-4} M) or propranolol (10^{-5} M) was included. The total incubation volume was 150 μl. The reaction was terminated by placing the assay tubes in boiling water for 5 min. The samples were then centrifuged at $13{,}000 \times g$ for 15 min. The supernatant was taken for cAMP measurement using a radioimmunoassay kit (Biomedical Technologies, Stoughton, MA, USA). Adenylyl cyclase activity is expressed as pmol cAMP/min/mg protein. Preliminary experiments confirmed the linearity of adenylyl cyclase activity at the protein concentrations and incubation times mentioned above.

2.5. Drugs

The following drugs were used (unless otherwise specified, all were obtained from Sigma, St. Louis, MO, USA): L-ascorbic acid, 8-Br-cAMP (8-bromoadenosine-3′,5′-cyclic monophosphate), Rp-8-Br-PET-cGMPS (Biolog Life Science Institute, La Jolla, CA, USA), Rp-8-CPT-cAMPS (Biolog Life Science Institute), endothelin-1 (American Peptide Company, Sunnyvale, CA, USA), indomethacin, isoproterenol bitartrate salt, SQ 22536 (Biomol Research Laboratories, Plymouth Meeting, PA, USA), phentolamine mesylate, propranolol hydrochloride, and U46619.

SQ22536 was dissolved in dimethyl sulfoxide (final concentration: 0.18%). Preliminary experiments indicated that the solvent at this concentration did not significantly affect contraction of the tissue to endothelin-1 and U46619 nor the relaxation to isoproterenol (data not shown). Indomethacin (10^{-5} M) was prepared in equal molar Na_2CO_3. This concentration of Na_2CO_3 did not significantly affect the pH of the solution in the organ chamber. Isoproterenol was prepared in 0.1% ascorbic acid stock solution to prevent the oxidation of the agent. The other drugs were prepared using distilled water. Vessels were exposed to inhibitors and antagonists for at least 30 min prior to testing their effects.

2.6. Data analyses

Data are shown as means ± S.E.M.. When the mean values of two groups were compared, Student's t test for unpaired observations was used. When the mean values of the same group before and after stimulation were compared, Student's t test for paired observations was used. Comparisons of mean values of more than two groups were performed with one-way analysis of variance (ANOVA) test with Student–Newman–Keuls test for post hoc testing of multiple comparison. All analyses were performed using a commercially available statistics package (SigmaStat, Jandel Scientific, San Rafael, CA, USA). Statistical significance was accepted when the P value (two tailed) was less

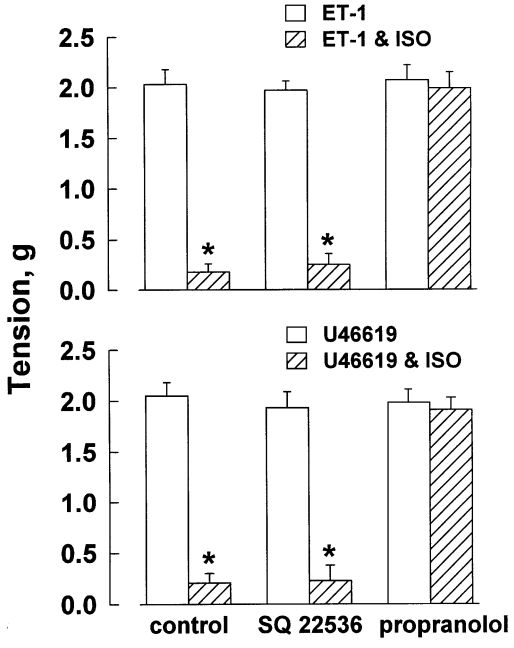

Fig. 2. Effects of SQ 22536 (3×10^{-4} M) and propranolol (10^{-5} M) on relaxation induced by isoproterenol (3×10^{-8} M) in newborn ovine pulmonary veins constricted with endothelin-1 (3×10^{-9} M; upper panel) or with U46619 (3×10^{-8} M; lower panel). Data are shown as means ± S.E.M.; $n = 5$ for each group. *, significantly different from vessels not treated with isoproterenol ($P < 0.05$).

than 0.05. In all experiments, n represents the number of animals.

3. Results

3.1. Effect of isoproterenol on vessel tension and cAMP

In pulmonary veins constricted with endothelin-1 [3×10^{-9} M; tension: 2.03 ± 0.19 g ($n=6$)], isoproterenol induced a concentration-dependent relaxation with a threshold concentration at 10^{-9} M. At basal conditions, the intracellular content of cAMP of pulmonary veins was 10.3 ± 0.8 pmol/mg protein, which was not affected by endothelin-1 (3×10^{-9} M). In the presence of endothelin-1, isoproterenol at concentrations $\leq 3 \times 10^{-9}$ M reduced vessel tension by up to 56% without significant effect on cAMP content. At higher concentrations ($\geq 10^{-8}$ M), the β-adrenoceptor agonist elevated cAMP content and caused further relaxation (Fig. 1).

3.2. Effects of SQ 22536 on isoproterenol-induced relaxation

Isoproterenol (3×10^{-8} M) caused a comparable relaxation of pulmonary veins when the vessel tension was raised to a similar level with either endothelin-1 (3×10^{-9} M) or U46619 (3×10^{-8} M). Relaxation induced by isoproterenol was not significantly affected by SQ 22536 (3×10^{-4} M) but was abolished by propranolol (10^{-5} M) (Fig. 2). SQ 22536 (3×10^{-4}) and propranolol (10^{-5} M) had no significant effect on the basal tension (data not shown; $n=5$ for each group, $P>0.05$) nor the contraction of the veins to endothelin-1 and U46619 (Fig. 2).

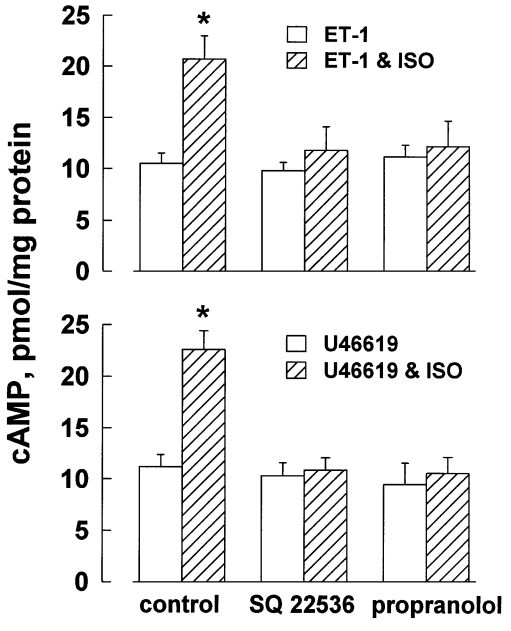

Fig. 3. Effects of SQ 22536 (3×10^{-4} M) and propranolol (10^{-5} M) on cAMP elevation caused by isoproterenol (3×10^{-8} M) in newborn ovine pulmonary veins in the presence of endothelin-1 (3×10^{-9} M; upper panel) or U46619 (3×10^{-8} M; lower panel). Data are shown as means ± S.E.M.; $n=6$ for each group. *, significantly different from vessels not treated with isoproterenol ($P<0.05$).

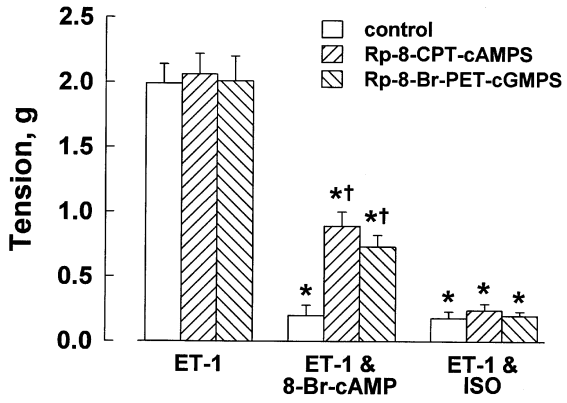

Fig. 4. Effect of Rp-8-CPT-cAMP (3×10^{-5} M) and Rp-8-Br-PET-cGMPS (3×10^{-5} M) on relaxation induced by 8-Br-cAMP (3×10^{-4} M) or isoproterenol (3×10^{-8} M) in newborn ovine pulmonary veins constricted with endothelin-1 (3×10^{-9} M). Data are shown as means ± S.E.M.; $n=6$ for each group. *, significantly different from vessels not treated with 8-Br-cAMP or isoproterenol; †, significantly different from control ($P<0.05$).

3.3. Effect of SQ 22536 on isoproterenol-induced cAMP elevation

In the presence of endothelin-1 (3×10^{-9} M) or U46619 (3×10^{-8} M), the intracellular contents of cAMP of pulmonary veins were not significantly different from their basal values (Figs. 1 and 3). Under these conditions, cAMP elevation induced by isoproterenol (3×10^{-8} M) was eliminated by SQ 22536 (3×10^{-4} M) and propranolol (10^{-5} M) (Fig. 3). SQ 22536 and propranolol had no significant effect on the basal cAMP (data not shown; $n=6$ for each group).

3.4. Effects of Rp-8-CPT-cAMPS and Rp-8-Br-PET-cGMPS on isoproterenol-induced relaxation

8-Br-cAMP [3×10^{-4} M; a cell membrane permeable analog of cAMP (Meyer and Miller. 1974)] caused a significant relaxation of pulmonary veins preconstricted with endothelin-1 (3×10^{-9} M). The effect of 8-Br-cAMP was attenuated by Rp-8-CPT-cAMPS (3×10^{-5} M) and Rp-8-Br-PET-cGMPS (3×10^{-5} M), selective inhibitors of cAMP- and cGMP-dependent protein kinase, respectively (Gjertsen et al., 1995; Butt et al., 1995). These inhibitors had no significant effect on relaxation caused by isoproterenol (3×10^{-8} M) (Fig. 4). Rp-8-CPT-cAMPS (3×10^{-5} M) and Rp-8-Br-PET-cGMPS (3×10^{-5} M) had no significant effects on either the basal tension vessels (data not shown, $n=6$ for each group, $P>0.05$) or constriction induced by endothelin-1 (Fig. 4).

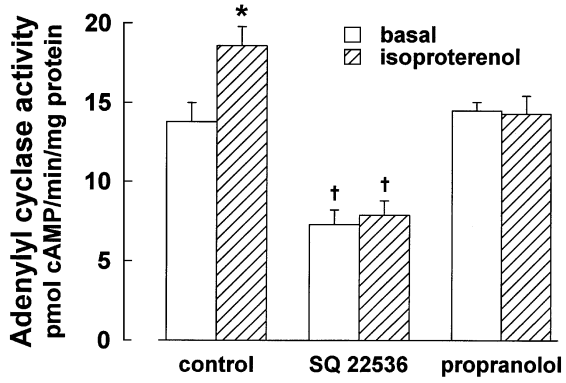

Fig. 5. Effect of isoproterenol (10^{-7} M) on adenylyl cyclase activity of crude membrane preparations of newborn ovine pulmonary veins under control conditions and in the presence of SQ 22536 (3×10^{-4} M) or propranolol (10^{-5} M). Data are shown as means ± S.E.M.; $n = 6$ for each group. *, significantly different from basal; †, significantly different from control ($P < 0.05$).

3.5. Effect of SQ 22536 on isoproterenol-induced increase in adenylyl cyclase activity

In the crude membrane preparations of pulmonary veins, the basal activity of adenylyl cyclase was 13.8 ± 1.2 pmol/min/mg protein ($n = 6$). The basal activity of adenylyl cyclase was significantly reduced by SQ 22536 (3×10^{-4} M) but was not affected by propranolol (10^{-5} M). Isoproterenol (10^{-7} M) caused a significant increase in adenylyl cyclase activity. The increase was inhibited by SQ 22536 (3×10^{-4} M) and propranolol (10^{-5} M) (Fig. 5).

4. Discussion

In some smooth muscle types, β-adrenoceptor agonists cause marked relaxation without affecting cAMP level (Marshall and Fain, 1984; Nesheim et al., 1975; Verma and McNeill, 1976; Lau and Lum, 1983). For instance, in rabbit uterine muscle, isoproterenol (5×10^{-9} M to 5×10^{-7} M) attenuates acetylcholine-induced contraction by up to 80% without affecting cAMP levels (Marshall and Fain, 1984). In bovine tracheal muscle, β_2-adrenoceptor agonists (salbutamol at 2.83×10^{-7} M and carbuterol at 2.0×10^{-7} M) cause a maximal or near maximal inhibition of methacholine-evoked contraction but have no effect on the cAMP level (Lau and Lum, 1983). In the present study, isoproterenol at concentrations $\leq 3 \times 10^{-9}$ M had no effect on cAMP levels of newborn ovine pulmonary veins but attenuated endothelin-induced contraction of the vessels by up to 56%. These findings would suggest that, in certain smooth muscle types, cAMP elevation is not a necessity for the relaxation effects induced by β-adrenoceptor agonists.

In our study, isoproterenol at higher concentrations ($\geq 10^{-8}$ M) attenuated vessel tension and elevated the intracellular content of cAMP. The effects were eliminated by propranolol, indicating that the actions of isoproterenol were via β-adrenoreceptors (Bylund et al., 1994). In the presence of SQ 22536, a specific inhibitor of adenylyl cyclases (Haslam et al., 1978), the effect of isoproterenol on cAMP content was abolished. However, its effect on vessel tension was not affected. This phenomenon was observed in vessels constricted with either endothelin-1 or U46619, suggesting that it is not an artifact due to the type of constrictor used. Rather, it indicates that, in ovine pulmonary veins, an increase in cAMP level may not be critical to β-adrenoceptor-mediated relaxation. The inhibition of isoproterenol-induced cAMP elevation by SQ 22536 seems to be due to a direct inhibition of the activity of adenylyl cyclases, as evident from the results of adenylyl cyclase activity assay.

In ovine newborn pulmonary veins, inhibition of adenylyl cyclase abolishes the increase in cAMP content but not the relaxation induced by forskolin (Gao and Raj, 2001). In guinea-pig aortas, SQ 22536 eliminates the elevation of cAMP caused by iloprost but not relaxation induced by this PGI_2 analog (Turcato and Clapp, 1999). These results are in line with our present data with isoproterenol. Since cAMP analogs cause smooth muscle to relax (Murray, 1990), it is interesting that the cAMP elevation in these studies does not significantly affect vessel tension. Different smooth muscle types vary in their sensitivity to cAMP. Thus, a certain level of cAMP that is sufficient to relax one type of smooth muscle may have no effect on another smooth muscle type (Kikkawa et al., 1986). Secondly, the components of cAMP pathway exist in distinct subcellular compartments such that cAMP generated by certain stimuli may not have access to enzymes which are involved in tension modulation (Zhou et al., 1992; An et al., 1999). This could also contribute to the dissociation of cAMP elevation from relaxation observed in certain smooth muscle types.

Smooth muscle relaxation caused by cAMP may result from stimulation of cAMP- and cGMP-dependent protein kinases (Jiang et al., 1992; Dhanakoti et al., 2000). In the present study, relaxation of the veins to 8-Br-cAMP but not to isoproterenol was attenuated by selective inhibitors of cAMP- and cGMP-dependent protein kinase [Rp-8-CPT-cAMPS and Rp-8-Br-PET-cGMPS, respectively; (Butt et al., 1995; Gjertsen et al., 1995)]. These data provide further evidence against a pivotal role of cAMP in β-adrenoceptor-mediated relaxation of pulmonary veins of newborn lambs.

The proposal that cAMP acts as a mediator of catecholamine-induced responses was introduced in 1960 (Sutherland and Robison, 1966). The observation that smooth muscle relaxation caused by β-adrenoceptor agonists was accompanied by an elevation of cAMP was first made in rat uterus (Triner et al., 1970). This phenomenon has since been reported in many smooth muscle types (Bülbring and Tomita, 1987; Murray, 1990; Torphy, 1994). The notion that cAMP mediates β-adrenoceptor-mediated relaxation is also supported by the evidence that cAMP analogs cause

smooth muscle relaxation and that β-adrenoceptor-mediated relaxation is potentiated by inhibition of phosphodiesterases which degrade cAMP (Bülbring and Tomita, 1987; Murray, 1990; Torphy, 1994). Despite substantial favorable evidence, an obligatory role for cAMP in β-adrenoceptor-mediated relaxation has not been established. Many studies suggest that β-adrenoceptor agonists can cause smooth muscle relaxation via cAMP-independent mechanisms. In canine tracheal smooth muscle cells (Kume et al., 1992, 1994), rat mesentery arteries (Huang and Kwok, 1997), and rabbit coronary arteries (Ahn et al., 1995), β-adrenoceptor agonists stimulate calcium-activated K^+ channels without the involvement of cAMP. In rat pulmonary arteries (Sheridan et al., 1997), mesentery arteries (Randall and McCulloch, 1995), and aortas (Husken et al., 1997) β-adrenoceptor agonists active ATP_{ase}-sensitive K^+ channels in a cAMP-independent manner. The activation of K^+ channels may result in cell membrane hyperpolarization, decrease in Ca^{2+} influx into the cells, and thus attenuation of smooth muscle tone (Nelson and Quayle, 1995). It should be pointed out that β-adrenoceptor agonists may cause smooth muscle relaxation via both cAMP-dependent and cAMP-independent pathways (Kume et al., 1994; Overweg and Schiff, 1978; Scornik et al., 1993; Torphy, 1994). It is likely that the relative contribution of these pathways may vary depending on tissue type, species, and other factors.

β-Adrenoceptor agonists are potent dilators of the pulmonary vasculature (Barnes and Liu, 1995). Our present study demonstrates that cAMP is not critical in the relaxation of newborn ovine pulmonary veins induced by isoproterenol. It is likely that heterogeneity exists for the role of cAMP in β-adrenoceptor-mediated relaxation among different smooth muscle cell types and that the relative importance of this cyclic nucleotide can be evaluated by using inhibitors of adenylyl cyclases.

Acknowledgements

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