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Effects of phosphodiesterase inhibitors on hypoxic pulmonary vasoconstriction. Influence of K^+ channels and nitric oxide

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Abstract

We studied the relaxant effects of the cyclic nucleotide phosphodiesterase inhibitors theophylline (non-selective), rolipram (type IV, 3′,5′-cyclic monophosphate (cAMP)-specific) and zaprinast (type V, 3′,5′-cyclic monophosphate (cGMP)-specific) on the hypoxic vasoconstriction in the isolated perfused rat lung and the involvement of K^+ channels and nitric oxide (NO) in these effects. K^+ channels were inhibited by glibenclamide, charybdotoxin, apamin and 4-aminopyridine and nitric oxide synthase by L-N^G-nitroarginine methyl ester (L-NAME). Hypoxic ventilation produced a significant pressure response. L-NAME and 4-aminopyridine increased this response. Rolipram, zaprinast and theophylline shared the ability to oppose the hypoxic pulmonary vasoconstriction. The order of potency was zaprinast > rolipram > theophylline. Glibenclamide partially inhibited the relaxant effects of rolipram and theophylline. Charybdotoxin inhibited the dilator response to rolipram. Apamin inhibited partially the vasodilation induced by rolipram and zaprinast. 4-Aminopyridine inhibited partially the relaxant effects of theophylline. L-NAME failed to block the effects of the three compounds. These data illustrate different pharmacological profiles according to the phosphodiesterase inhibitors and support the potential interest of selective inhibitors as relaxant agents in pulmonary vessels.

Keywords: Hypoxic pulmonary vasoconstriction; Phosphodiesterase inhibitor; K^+ channel; Nitric oxide

1. Introduction

Pulmonary hypertension may be secondary to hypoxic vasoconstriction, resulting in complex changes in the pulmonary vascular bed. Despite extensive research, the mechanism underlying hypoxic pulmonary vasoconstriction remains unclear. Suppression of endogenous vasodilator substances such as endothelium-derived relaxing factor (nitric oxide, NO) may be one mechanism mediating the hypoxic vasoconstriction (Liu et al., 1991). In recent years, a body of evidences has emerged indicating that oxygen level per se can regulate ion channel activity (Weir and Archer, 1995). Results from patch-clamp studies on isolated pulmonary vascular myocytes have shown that acute hypoxia inhibits outward K^+ currents, thereby causing depolarization (Post et al., 1995). Most subsequent studies have indicated that the K^+ current inhibited by hypoxia is voltage-gated and sensitive to 4-aminopyridine (Archer et al., 1996; Osipenko et al., 1997).

Currently, medical treatment of hypoxic pulmonary vasoconstriction remains unsatisfactory. The recent development of inhaled therapy [nitric oxide, prostacyclin and analogs] represents a significant advance in its management (Olschewski et al., 1996; Manktelow et al., 1997). However, the therapeutic effects of this approach are limited by the short half-life of the drug effects. A new pharmacological approach consists in prolonging the effects of inhaled prostacyclin or nitric oxide by combination with dilator agents mediating their effects through inhibition of the hydrolysis of adenosine 3′,5′-cyclic monophosphate (cAMP) and/or guanosine 3′,5′-cyclic monophosphate (cGMP) (Ghofrani et al., 1998; Ichinose et al., 1998).

If numerous agents increase cAMP and cGMP levels through stimulation of adenylate cyclase such as β-adrenoceptor agonists, prostacyclin receptor agonists, adenosine A_2 receptor activators or guanylate cyclase such as nitric oxide, the hydrolysis of cyclic nucleotides is catalyzed

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solely by phosphodiesterases. There are at least seven isoforms of mammalian phosphodiesterases with varying specificity for hydrolysis of cyclic nucleotides, for regulation and for tissue localization (Beavo, 1995; Torphy, 1998). Four families (I, III, IV and V) have been identified in the human pulmonary circulation (Rabe et al., 1994). Phosphodiesterase type I and type V catalyze the breakdown of cGMP whereas phosphodiesterase type III and type IV that of cAMP. Isozyme-selective inhibitors are available for most phosphodiesterase families (Torphy, 1998). The inhibition of cGMP and cAMP hydrolysis by the phosphodiesterase inhibitors has been shown to promote vasodilation (De Witt et al., 2000) and to oppose pulmonary vasoconstriction (Haynes et al., 1991; Cohen et al., 1996; Ichinose et al., 1998). In this context, the development of selective inhibitors of phosphodiesterase isozymes may be of interest. For example, inhibition of cGMP phosphodiesterase selectively vasodilates the pulmonary circulation (Cohen et al., 1996). Recent evidence suggests the involvement of K^+ channels in the relaxant effects of phosphodiesterase inhibitors in coronary artery (Chen et al., 1997) and small mesenteric artery (Taylor and Benoit, 1999). To our knowledge, very few studies have investigated these various mechanisms of action in the pulmonary circulation (Barman, 1997; Crilley et al., 1998).

The purpose of this study was to investigate (1) the relaxant effects of cyclic nucleotide phosphodiesterase inhibitors theophylline (non-selective), rolipram (type IV, cAMP specific, cGMP insensitive), zaprinast (type V, cGMP-specific) on hypoxic vasoconstriction in rat lungs perfused with salt solution and (2) the role of endogenous nitric oxide, ATP-sensitive K^+ channels (K_{ATP}), small conductance Ca^+-activated K^+ channels (SK_{Ca}), large conductance Ca^+-activated K^+ channels (BK_{Ca}) and 4-aminopyridine-sensitive voltage-gated K^+ channels (K_V) in these effects.

2. Materials and methods

2.1. Rat perfused isolated lung preparation

Twenty eight groups ($n = 5–10$ per group) of male Wistar rats (Dépré, St. Doulchard, France), weighing 270–370 g, were anaesthetized with sodium pentobarbitone (100 mg kg^{-1}) and tracheotomized. After thoracotomy, a polyethylene cannula was inserted into the main pulmonary artery and the lungs were removed quickly and allowed to equilibrate in the perfusion circuit maintained at 38°C by a surrounding water bath and consisting of a perfusion reservoir, a roller pump (Harvard 77, Ealing, Les Ullis, France), connecting tubing and bubble trap. Mean perfusion pressure which was measured from a side-arm of the arterial line (Harvard transducer, −50 to 300 mm Hg), was recorded continuously (Ankersmit WR 3701 recorder, Graphtec, Japan) and reflected pulmonary vascular resistances because the flow rate was constant (0.025 ml g^{-1} min^{-1}). The lungs were perfused with a salt solution containing (mM): NaCl 116, KCl 5.4, NaH_2PO_4 1.04, $MgSO_4$ 0.83, $CaCl_2$ 1.8, $NaHCO_3$ 19 and D-glucose 5.5. Ficoll (1 g 100 ml^{-1}, type 70; Sigma, La Verpillère, France) was included as a colloid (Hasunuma et al., 1991). The lungs were ventilated with a Harvard rodent ventilator at a tidal volume of 10 ml kg^{-1} body weight and a frequency of 55 breaths min^{-1}. The end expiratory pressure was set at 2.5 cm H_2O. The pressure of airways was measured with a Validyne DP45 (0 to 88 cm H_2O) differential pressure transducer. A 20-min equilibration period was allowed to establish a stable baseline for pulmonary airway and vascular pressures before experiments were started. During this period the lungs were ventilated with a humid mixture of 21% O_2, 5% CO_2, 74% N_2 (normoxia). Lungs of which the weight had increased in excess of 10% (indicative of oedema) at the end of the experiments were discarded.

2.2. Vasoconstrictor responses to hypoxia

After the equilibration period, the pulmonary vasculature was precontracted twice, using a bolus of 0.25–0.5 μg angiotensin II to prime the otherwise low vascular reactivity seen in salt solution-perfused lungs. The lungs were then challenged with a hypoxic gas mixture (5% CO_2, 95% N_2) as described previously (Dumas et al., 1997). Each hypoxic challenge (4 min) was followed by the addition of 0.25 μg angiotensin II in normoxic ventilation (4 min) and the pressure was allowed to return to baseline before the initiation of hypoxic ventilation. The perfusate gas tensions were measured during hypoxic challenges, by collecting perfusate anaerobically from the arterial cannula and analyzing it immediately (Corning 170 pH/blood gas analyzer). During hypoxic periods, P_{O_2} was maintained below 35 mm Hg. After four hypoxic pulmonary vasoconstrictions, the responses became reproducible (Dumas et al., 1997). Drugs were tested after a stable response to hypoxia was reached.

2.3. Effects of theophylline, rolipram and zaprinast on pulmonary vasoreactivity: influence of K^+ channels and NO synthase inhibitors

Concentration–response curves to the three phosphodiesterase inhibitors were obtained by perfusing lungs during 8–10 hypoxic periods with a salt solution containing theophylline (0.1–1000 μM), rolipram (0.01–30 μM) or zaprinast (0.001–30 μM). In a second series of experiments, concentration–response curves for the three phosphodiesterase inhibitors were obtained in the presence of glibenclamide (1 μM), a K_{ATP} channel blocker. Charybdotoxin, a BK_{Ca} channel blocker and apamin, a SK_{Ca} channel

blocker, could not be administered for more than three hypoxic periods because of subsequent lung injury development (oedema). The hypoxic pressure response obtained with 4-aminopyridine, the K_V channel blocker was significantly increased only during three periods of infusion of this compound. The hypoxic pressure response obtained with L-N^G-nitroarginine methyl ester (L-NAME), the nitric oxide-synthase inhibitor was reproducible and significantly increased only during the second and third periods of infusion of this compound (Dumas et al., 1997). Consequently, in others series of experiments, charybdotoxin (0.1 μM), apamin (0.05 μM), 4-aminopyridine (1 mM), and L-NAME (100 μM), were administered during the fifth, sixth and seventh hypoxic periods (Dumas et al., 1997) whereas phosphodiesterase inhibitors were used during the sixth and seventh periods, at concentrations inducing about 70% inhibition of the pressure response to hypoxia (theophylline 100 μM, rolipram 10 μM and zaprinast 1 μM). Because of the close correspondence between theophylline concentration and phosphodiesterase inhibition (Rabe et al., 1995), additional experiments were made with theophylline 1 mM, to obtain a more potent non-specific phosphodiesterase inhibition (Wu et al., 1982; Landells et al., 2000).

2.4. Chemicals / drugs

The drugs used were as follows: theophylline sodium anisate (Theophylline Bruneau, Laboratoires Delalande, Dijon, France), glibenclamide (Laboratoire Hoechst, Paris la Défense, France), 4-aminopyridine, angiotensin II, L-N^G-nitroarginine methyl ester, rolipram (4-[3-(cyclopentyloxy)-4-methoxy-phenyl]-2-pyrrolidinone) (Sigma), zaprinast (2-*o*-propoxyphenyl-8-azapurin-6-one) (Rhône Poulenc-Rohrer, France), charybdotoxin and apamin (Latoxan, Rosans, France). Apamin, angiotensin II, 4-aminopyridine, L-N^G-nitroarginine methyl ester and theophylline were dissolved in distilled water, charybdotoxin in saline, glibenclamide in a mixture of dimethylsulfoxide/distilled water (1:1), rolipram and zaprinast in a mixture of dimethylsulfoxide/ethanol/distilled water (0.15:0.5:0.35); the stock solutions were then diluted in distilled water. All the stock solutions were kept frozen until use and all the diluted solutions were prepared just before administration. The maximal concentrations of dimethylsulfoxide (0.45%) or ethanol (1.5%) added to the bath did not by themselves exert any effect and did not modify the reactivity of the preparation.

2.5. Data analysis

Hypoxic pressure response was measured at the time of the peak increase and expressed as absolute changes from baseline values. The maximal response was determined as the relaxation reversing the hypoxic pressure response. The relaxant effect obtained at the highest concentration of drugs being lower than 50% of the maximal effect in certain experiments, this potency was expressed in the negative logarithm to base 10 form of the concentration inducing 30% of the maximal effect ($-\log EC_{30}$) determined from individual concentration–response curves. Because K^+ channels and NO synthase inhibitors modulate by themselves the pressure response to hypoxia, their influence on the relaxation induced by phosphodiesterase inhibitors is expressed as percentage of inhibition of the relaxation and calculated as:

$$\left[1 - \frac{\frac{(R_I - R_{D+I})}{R_I}}{\frac{(R - R_D)}{R}}\right] \times 100$$

were (a) $(R_I - R_{D+I})/R_I$ is the relaxant effect elicited by the phosphodiesterase inhibitor in presence of the K^+ channel or NO synthase inhibitor. R_{D+I} and R_I are the hypoxic pressure responses observed in presence of the inhibitor with and without the phosphodiesterase inhibitor, respectively, and (b) $(R - R_D)/R$ is the relaxant effect elicited by the phosphodiesterase inhibitor in the control experiments. R_D and R are the corresponding hypoxic

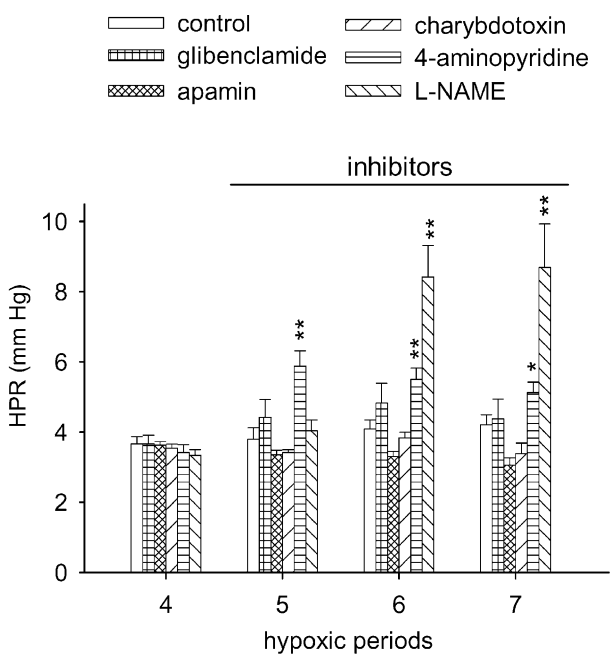

Fig. 1. Influence of normal saline and inhibitors: glibenclamide (1 μM), charybdotoxin (0.1 μM), apamin (0.05 μM), 4-aminopyridine (1000 μM) and L-NAME (100 μM) on hypoxic pressure response ($n = 6, 6, 6, 10, 6$ and 9, respectively) in the isolated perfused lung. Values shown are increases of perfusion pressure above basal values from the fourth to the seventh hypoxic periods with infusion of the inhibitors from the fifth to the seventh hypoxic periods (hypoxic pressure response: HPR). Data represent mean ± S.E.M. Asterisks indicate responses that were different from corresponding responses in control experiments ($^{**}P < 0.01$, $^{*}P < 0.05$).

pressure responses observed in control experiments with and without the phosphodiesterase inhibitors, respectively.

Data are shown as mean ± S.E.M. Statistical significance was assessed with the Mann–Withney *U*-test for simple comparisons and the Analysis of Variance (ANOVA) Bonferroni multiple *t*-test for multiple comparisons; P values of < 0.05 were considered significant.

3. Results

In lung preparations, the mean baseline inflation pressure was 11.38 ± 0.07 cm H_2O ($n = 195$) and was not significantly modified by hypoxic ventilation or addition of the various drugs. After equilibration of the preparation, the baseline perfusion pressure in normoxic ventilation was similar in all series of rats (6.42 ± 0.07 mm Hg, $n = 195$). Ventilation with a hypoxic mixture of gas produced a significant increase of the perfusion pressure ($+3.63 \pm 0.05$ mm Hg, $n = 181$, $+56.54\%$ from baseline values, $P < 0.001$), which, starting from the fourth period of hypoxia, was reproducible for at least nine subsequent periods.

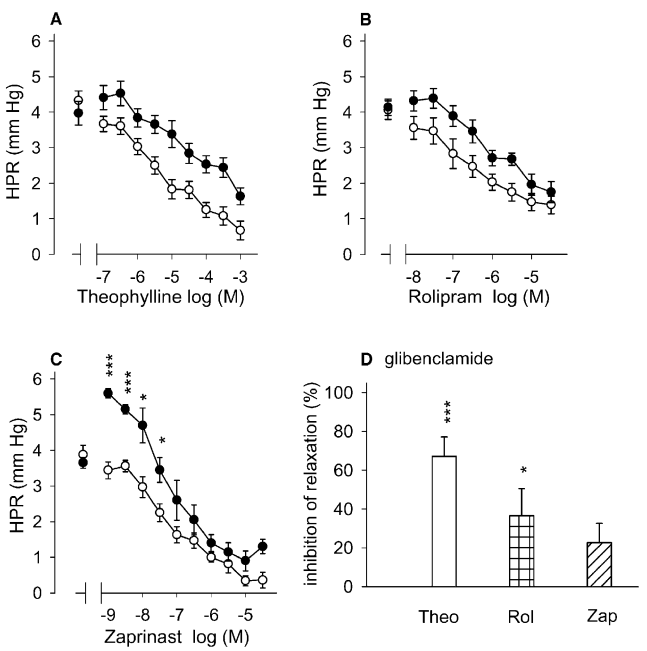

Fig. 2. Effects of infusions of (A) theophylline (0.1–1000 μM), (B) rolipram (0.01–30 μM) and (C) zaprinast (0.001–30 μM) on hypoxic pressure response (HPR) in the absence (○) ($n = 9$, 9 and 8, respectively) and in the presence of glibenclamide (1 μM) (●) ($n = 8$, 7 and 5, respectively), in the isolated perfused lung. Values shown are increases of perfusion pressure above basal values. Influence of (D) glibenclamide (1 μM) on the relaxant effects of theophylline (Theo, 100 μM), rolipram (Rol, 10 μM) and zaprinast (Zap, 1μM) ($n = 9$, 9 and 8, respectively) in the isolated perfused lung; values shown are percentages of inhibition of the relaxation. Data represent the mean ± S.E.M. Asterisks indicate responses with glibenclamide that were significantly different from corresponding responses obtained without glibenclamide (A, B, C) or percentages of inhibition that were statistically significant (D) ($^{***}P < 0.001$, $^{*}P < 0.05$).

Table 1
Potencies expressed as ($-\log EC_{30}$) values of theophylline, rolipram and zaprinast in pulmonary vessels contracted by hypoxia. Influence of glibenclamide
Values are mean ± S.E.M. from five to nine separate experiments per group.

$-\log EC_{30}$		
Drugs	Control	Glibenclamide (1 μM)
Theophylline	5.97 ± 0.27	4.56 ± 0.27^{a}
Rolipram	6.74 ± 0.21^{b}	6.15 ± 0.21^{c}
Zaprinast	$7.76 \pm 0.13^{d,e}$	$6.89 \pm 0.3^{c,f}$

[a] $P < 0.01$ vs. theophylline alone.
[b] $P < 0.05$ vs. theophylline.
[c] $P < 0.01$ vs. theophylline.
[d] $P < 0.001$ vs. theophylline.
[e] $P < 0.01$ vs. rolipram.
[f] $P < 0.05$ vs. zaprinast alone.

3.1. Effects of K^+ channel and NO synthase inhibitors on pulmonary reactivity

As shown in Fig. 1, glibenclamide at 1 μM, charybdotoxin at 0.1 μM, and apamin at 0.05 μM did not by themselves affect the hypoxic pressure response. In contrast, the hypoxic pressure response was significantly increased by L-NAME at 100 μM ($+8.42 \pm 0.9$ vs. 4.08 ± 0.27 mm Hg, $P < 0.01$ at the sixth hypoxic period and $+8.69 \pm 1.24$ vs. 4.21 ± 0.28 mm Hg, $P < 0.01$ at the seventh hypoxic period) and by 4-aminopyridine at 1 mM ($+5.88 \pm 0.44$ vs. 3.79 ± 0.33 mm Hg, $P < 0.01$ at the fifth hypoxic period, $+5.50 \pm 0.32$ vs. 4.08 ± 0.27 mm Hg, $P < 0.01$ at the sixth hypoxic period and $+5.13 \pm 0.29$ vs. 4.21 ± 0.28 mm Hg, $P < 0.05$ at the seventh hypoxic period).

3.2. Effects of theophylline, rolipram and zaprinast on pulmonary reactivity; influence of K^+ channel and NO synthase inhibitors

As shown in Fig. 2, theophylline, rolipram and zaprinast concentration-dependently decreased the hypoxic pressure response ($P < 0.001$). Percent inhibition of this response was $74 \pm 3\%$ with zaprinast 1 μM, $63 \pm 5\%$ with rolipram 10 μM and $70 \pm 5\%$ with theophylline 100 μM. Table 1 shows that zaprinast was significantly more potent than theophylline ($P < 0.001$) and rolipram ($P < 0.05$) at decreasing the hypoxic pressure response, the decreasing order of potency being: zaprinast > rolipram > theophylline. During the hypoxic pressure response, the K_{ATP} channel inhibitor, glibenclamide produced a rightward shift of the concentration–response curves to theophylline, rolipram and zaprinast ($P < 0.001$, Fig. 2A, B, C), resulting in a significantly lower EC_{30} value of theophylline and zaprinast ($P < 0.01$ and $P < 0.05$, respectively) than in the control conditions (Table 1). Glibenclamide inhibited the response to 100 μM theophylline, 10 μM rolipram and 1

μM zaprinast by 67% ($P < 0.001$), 37% ($P < 0.05$) and 23% (NS) (Fig. 2D), respectively.

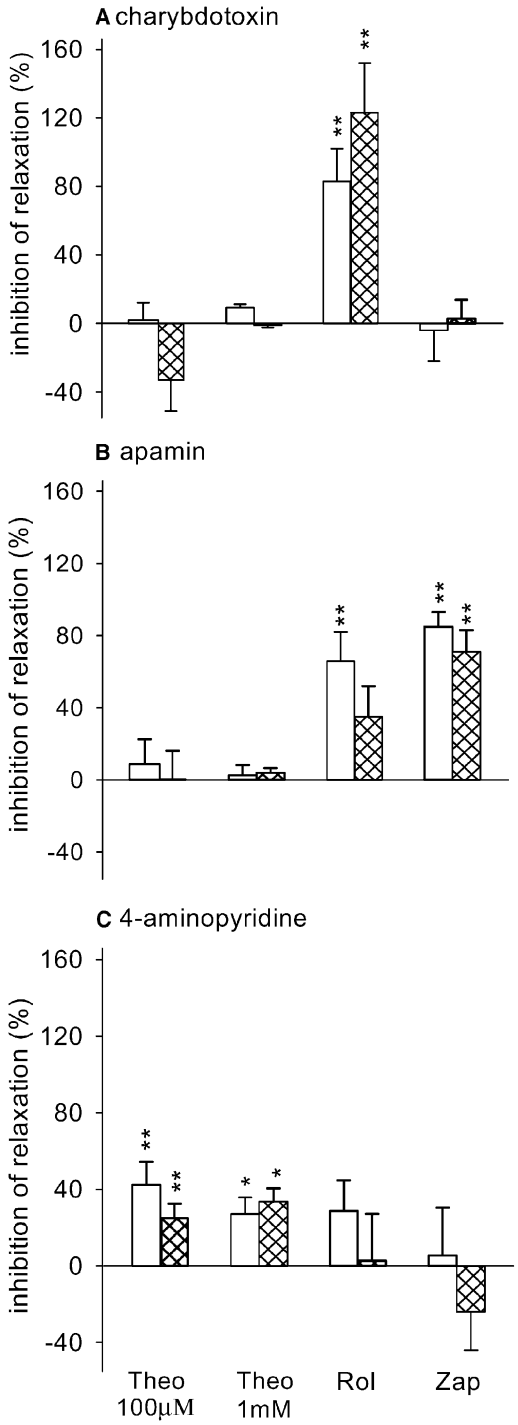

Fig. 3. Influence of (A) charybdotoxin (0.1 μM), (B) apamin (0.05 μM) and (C) 4-aminopyridine (1 mM) on the relaxant effects of theophylline (Theo, 100 μM) ($n = 6$, 7, and 6, respectively), theophylline (Theo, 1000 μM) ($n = 4$, 4, 5, respectively), rolipram (Rol, 10 μM) ($n = 7$, 8 and 7, respectively) and zaprinast (Zap, 1 μM) ($n = 6$, 6 and 8, respectively) in the rat isolated perfused lung at the sixth (white columns) and the seventh (cross-hatched columns) hypoxic periods. Values shown are percentages of inhibition of the relaxation. Data represent the mean ± S.E.M. Asterisks indicate percentages of inhibition that were statistically significant ($^{*}P < 0.05$, $^{**}P < 0.01$).

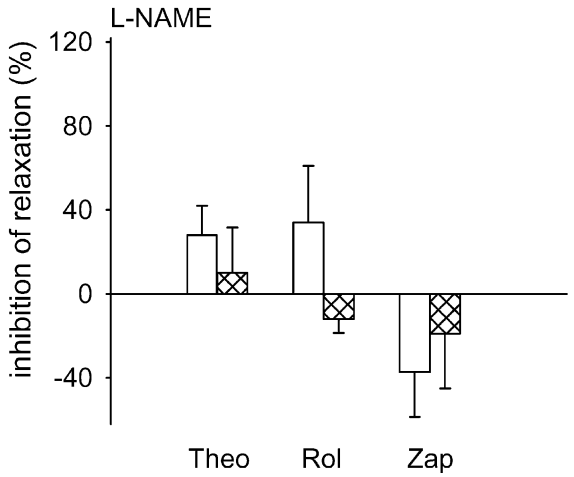

Fig. 4. Influence of L-NAME (100 μM) on the relaxant effects of theophylline (Theo, 100 μM) ($n = 9$), rolipram (Rol, 10 μM) ($n = 5$) and zaprinast (Zap, 1 μM) ($n = 9$) in the rat isolated perfused lung at the sixth (white columns) and the seventh (cross-hatched columns) hypoxic periods. Values shown are percentages of inhibition of the relaxation. Data represent the mean ± S.E.M.

The BK_{Ca} channel inhibitor, charybdotoxin, was devoid of any effect on the response to theophylline and zaprinast but reduced that to rolipram by approximately 103% ($P < 0.01$, Fig. 3A). The SK_{Ca} channel inhibitor, apamin significantly decreased the relaxant effects of zaprinast by 78% ($P < 0.01$, Fig. 3B) and that of rolipram at the 6th hypoxia by 66% ($P < 0.01$, Fig. 3B) but showed no effect on the response to theophylline (Fig. 3B). Relaxation induced by theophylline 100 μM and 1 mM was partially inhibited by 4-aminopyridine by 38% and 26% ($P < 0.01$ and $P < 0.05$, respectively, Fig. 3C), whereas that of rolipram and zaprinast remained unaffected. L-NAME did not significantly affect the relaxant effects of theophylline, rolipram and zaprinast (Fig. 4).

4. Discussion

In this study, we investigated the effects of theophylline, rolipram and zaprinast on the pulmonary vascular response to hypoxia in a rat model of isolated perfused lung according to a method based on that described by McMurtry (1984). This model allows the investigation of the vasomotor tone in all pulmonary vessels and particularly in the small arteries and veins which are known to account for the greatest part of pulmonary vascular resistances (Madden et al., 1985).

We observed that glibenclamide, charybdotoxin and apamin did not affect significantly the hypoxic vasoconstriction thus suggesting the absence of any modulating effects of K_{ATP}, BK_{Ca} or SK_{Ca} channels, as previously described (Hasunuma et al., 1991; Dumas et al., 1999). In

contrast, 4-aminopyridine potentiated the pressure response to hypoxia as in isolated lung of dog (Barman, 1998) suggesting that, at least a part of 4-aminopyridine-sensitive voltage-gated K^+ channels are activated. As described previously in isolated perfused lungs, L-NAME enhanced hypoxic pulmonary vasoconstriction thus confirming that endogenous nitric oxide decreases the hypoxic pulmonary vasoconstriction (Liu et al., 1991; Dumas et al., 1997). Furthermore, the relaxant effects of nitric oxide have been shown to be mediated in part by activation of voltage-gated K^+ channels (Zhao et al., 1997). In consequence, the potentiation of hypoxic pulmonary vasoconstriction by 4-aminopyridine may also be explained by an inhibition of the relaxant effects of nitric oxide.

In our study, the three phosphodiesterase inhibitors shared the ability to oppose the pulmonary vasoconstriction during hypoxia and in doing so zaprinast was more potent than rolipram and theophylline. These results are consistent with previous biochemical investigations that showed high phosphodiesterase III and phosphodiesterase V and low phosphodiesterase IV isozyme activities in the human pulmonary artery (Rabe et al., 1994). Our results support the potential interest of type V phosphodiesterase inhibitors as pulmonary vasodilators as previously described (Ichinose et al., 1998; Hanasato et al., 1999). Regarding the effects of rolipram, many studies have shown a weak potency of this agent to induce pulmonary vasodilation in human pulmonary artery (Rabe et al., 1995) or in the isolated lamb lung (Braner et al., 1993). Nevertheless, despite the lower potency of rolipram compared to zaprinast, recent studies have exhibited the interest of using type IV phosphodiesterase inhibitors in combination with other relaxant drugs for pulmonary vasodilation (Schermuly et al., 2000).

Our experiments with glibenclamide clearly show that K_{ATP} channels contribute to the vasodilation induced by theophylline and rolipram. To our knowledge, the role of K_{ATP} channels in the effects of phosphodiesterase inhibitors has not yet been investigated in the pulmonary circulation. These results are consistent with previous studies that showed the involvement of K_{ATP} channels in the relaxant effects of agents acting through adenylate cyclase pathway such as β-adrenoceptor agonists (Dumas et al., 1999). In contrast, in other studies, glibenclamide failed to inhibit the cAMP-induced relaxation with forskolin (Zhao et al., 1998) or prostaglandin E_1 (Dumas et al., 1997). These discrepancies confirm the possible involvement of cAMP compartments that are coupled to different functional responses (Murray et al., 1989). Regarding zaprinast, it may be argued that the significant increase of tone observed with non-efficient concentrations in presence of glibenclamide is responsible of the decreased vasodilation. Consequently, we cannot conclude that K_{ATP} channels are involved in the relaxant effects of zaprinast.

As regards BK_{Ca} channels, charybdotoxin failed to block the relaxant effects of zaprinast but inhibited those of rolipram, thus demonstrating that cGMP-insensitive cAMP phosphodiesterase inhibition acts through BK_{Ca} channels as observed previously in the guinea-pig trachea (Thirstrup et al., 1998). In contrast, vasorelaxant effects of 6-bromo-8(methylamino)imidazo[1,2 *a*]pyrazine-2-carbonitrile (SCA40), a cGMP-sensitive cAMP phosphodiesterase inhibitor were not inhibited by charybdotoxin (Crilley et al., 1998). It is to note that these results have been obtained in isolated rings from main pulmonary artery of rat whereas our study was performed in isolated whole lung preparation. As regards the nitric oxide/cGMP pathway, many previous studies concluded that nitric oxide-induced relaxation is mediated in part by BK_{Ca} channels through a cGMP-dependent mechanism (Carrier et al., 1997). This conclusion, however has been challenged by other studies that demonstrated that nitric oxide directly actives BK_{Ca} channels in vascular smooth muscle without requiring cGMP production (Zhao et al., 1997). Our report is in accordance with the latter hypothesis.

Our experiments with apamin showed that SK_{Ca} channels contribute to the vasodilation induced by rolipram and zaprinast. These results suggest that the cAMP- or cGMP-mediated relaxation in the pulmonary circulation may be modulated by SK_{Ca} channels. Similar results were obtained in other tissues with zaprinast and nitric oxide (Mule et al., 1999).

As regards the lack of selectivity of theophylline for phosphodiesterase isozymes, it might be expected to observe with this compound the whole effects exhibited by rolipram and zaprinast. This was not confirmed insofar as the relaxant effects of theophylline were not inhibited by charybdotoxin or apamin. The discrepancies observed between theophylline and the selective phosphodiesterase inhibitors suggest preferential mechanisms of relaxation in the pulmonary circulation different from type IV and type V phosphodiesterase inhibition for theophylline. Moreover, in our study, 4-aminopyridine partially inhibited the relaxant effects of theophylline, but not those of rolipram and zaprinast. These results confirm the lack of clear pharmacological profile of theophylline (Vassallo and Lipsky, 1998) and support reports that recommend to use selective phosphodiesterase inhibitors to obtain pulmonary vasodilation (Cohen et al., 1996; Ichinose et al., 1998; Dukarm et al., 1999; Hanasato et al., 1999). Regarding the influence of 4-aminopyridine-sensitive voltage-gated K^+ channels on agents acting through the cGMP or cAMP pathways, contrasting results have been observed between zaprinast in our study and nitric oxide in isolated pulmonary arterial rings of rat (Zhao et al., 1997). These discrepancies may be explained by the possible involvement of cGMP-independent pathway for nitric oxide and/or differences according to the arterial size. Similar differences have been observed between rolipram in our study and forskolin (Zhao et al., 1998). Once more it is to note that the role of cyclic nucleotides in these effects is not clear. Cross-activation should be also possibly related to compartmen-

talization between cGMP and protein kinase A and also between cAMP and protein kinase G (Haynes et al., 1992).

L-NAME failed to inhibit the effects of the three PDE inhibitors in our study. Activation of guanylate cyclase by nitric oxide is a well-established mechanism for the effects of NO in vascular smooth muscle (Moncada et al., 1991). It was therefore somewhat unexpected that L-NAME did not block the relaxant effects of zaprinast. Ineffectiveness of L-NAME and endothelium dysfunction can be ruled out as far as it potentiated the hypoxic pulmonary vasoconstriction (Dumas et al., 1997; Sato et al., 2000). Moreover, used in previous studies according to the same experimental protocol, L-NAME inhibited the relaxant effects of various drugs (Dumas et al., 1997, 1999). Guanylate cyclase has been shown to be stimulated not only by NO but also by calcium, oxygen radicals (Gerzer et al., 1981) and natriuretic peptides (Eddahibi et al., 1998; Muramatsu et al., 1997). It is to note that alternative hypoxic and normoxic challenges may have favoured the production of oxygen radicals in our model. Concerning natriuretic peptides-stimulated cGMP production, our report is to compare to studies that exhibited in hypoxia-induced hypertensive rat lungs an increased cyclic GMP generation by atrial natriuretic peptide independently of NO production (Muramatsu et al., 1997) and a lack of inhibition by L-NAME of the pulmonary vasorelaxation induced by 1.3 dimethyl-6-(2-propoxy-5-methane sulfonylamidophenyl)-pyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one (DMPPO), a type 5 PDE inhibitor (Eddahibi et al., 1998). Furthermore, the relaxant effects of DMPPO were potentiated by an inhibitor of neutral endopeptidase involved in the degradation of natriuretic peptides (Eddahibi et al., 1998). Thus, these studies suggested the great role of natriuretic peptides in the modulation of vascular tone through cGMP formation. Nevertheless, in our model this attractive hypothesis has to be confirmed by further investigations.

In conclusion, it appears from our data that (a) nitric oxide and 4-aminopyridine-sensitive voltage-gated K^+ channels exert a permanent limitation against hypoxic pulmonary vasoconstriction, (b) theophylline, rolipram and zaprinast share the ability to oppose the hypoxic pulmonary vasoconstriction, (c) zaprinast exhibits the higher potency suggesting the interest of type V phosphodiesterase inhibitors in the modulation of hypoxic pulmonary vasoconstriction, (d) the relaxant effects of the three investigated phosphodiesterase inhibitors on the hypoxic pulmonary vasoconstriction in the isolated perfused rat lung involve differential activation of K^+ channels.

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