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Rolipram, but not siguazodan or zaprinast, inhibits the excitatory noncholinergic neurotransmission in guinea-pig bronchi

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Rolipram, but not siguazodan or zaprinast, inhibits the excitatory noncholinergic neurotransmission in guinea-pig bronchi. Y. Qian, V. Girard C.A.E. Martin, M. Molimard, C. Advenier. ©ERS Journals Ltd 1994.

ABSTRACT: Theophylline has been reported to inhibit excitatory noncholinergic but not cholinergic-neurotransmission in guinea-pig bronchi. As theophylline might exert this effect through an inhibition of phosphodiesterases (PDE), and since many types of PDE have now been described, the aim of this study was to investigate the effects of three specific inhibitors of PDE on the electrical field stimulation (EFS) of the guinea-pig isolated main bronchus *in vitro*. The drugs used were siguazodan, rolipram and zaprinast, which specifically inhibit PDE types, III, IV and V, respectively.

Guinea-pig bronchi were stimulated transmurally with biphasic pulses (16 Hz, 1 ms, 320 mA for 10 s) in the presence of indomethacin 10^{-6} M and propranolol 10^{-6} M. Two successive contractile responses were observed: a rapid cholinergic contraction, followed by a long-lasting contraction due to a local release of neuropeptides from C-fibre endings.

Rolipram (10^{-9} to 10^{-6} M) but not siguazodan or zaprinast, inhibited the peptidergic contraction in a concentration-dependent manner. Conversely, the cholinergic response was unaffected. Contractile responses induced by exogenous acetylcholine (10^{-8} to 10^{-3} M) or [Nle^{10}]NKA(4-10) (10^{-10} to 10^{-6} M) were also unaffected by rolipram, siguazodan and zaprinast (10^{-7} M).

These results demonstrate that concentrations of rolipram, similar to those which inhibit PDE, reduce the release of sensory neuropeptides from C-fibre endings, and suggest that the cyclic adenosine monophosphate (AMP) PDE type IV is specifically involved in this effect, as in other anti-inflammatory effects.
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Stimulation of bronchial C-fibres induces bronchoconstriction and inflammation, by means of central reflex pathways and local release of the sensory neuropeptides, substance P, neurokinin A and calcitonin gene-related peptide [1]. These peptides cause multiple effects, including contraction of airway smooth muscle, mucus hypersecretion, increase in microvascular permeability, release of inflammatory mediators, and inflammatory cell chemotaxis [2–4]. These proinflammatory effects may play a role in the pathogenesis of asthma, thereby suggesting that control of the local release of neuropeptides might be effective in the management of this disease.

One experimental approach to C-fibre stimulation and control is the study of guinea-pig bronchial reactivity to electrical field stimulation (EFS) *in vitro*, since EFS causes both a rapid cholinergic and a long-lasting noncholinergic contraction of bronchial smooth muscle, due to release of sensory neuropeptides from C-fibre endings [1, 5–8].

It has been shown that several neural or inflammatory mediators exert marked effects on neurotransmitter release [9]. MANZINI *et al.* [10] and, more recently, BARLINSKI *et al.* [11] have observed that theophylline (10–100 μM) also inhibited the peptidergic contraction in a concentration-dependent manner, but did not affect the cholinergic response, so that this effect might be involved in the previously reported anti-inflammatory action of this substance [12, 13].

As theophylline might exert its effect through inhibition of phosphodiesterases (PDE) and accumulation of cyclic adenosine monophosphate (cAMP) in the cells, and since many types of phosphodiesterase have now been described [14, 15], the aim of this study was to investigate the effects of three specific inhibitors of PDE on the EFS of the guinea-pig main bronchi *in vitro*. The drugs used were siguazodan, rolipram and zaprinast, which selectively inhibit the phosphodiesterase types III, IV and V, respectively.

Methods

Tissue preparation

Guinea-pig main bronchial rings were obtained from tricoloured Hartley guinea-pigs of either sex (250–350 g) anaesthetized with urethane (1.25 g·kg^{-1}, *i.p.*), and were suspended under an initial load of 2.0 g, in Krebs solution, at 37°C gassed with 95% O_2 - 5% CO_2. After 1 h of equilibration, resting force was between 1.5 and 2.0 g. Under these conditions, responses to agonists were reproducible over several hours. Changes in force of contraction were measured isometrically with Pioden strain gauges (UF-1), and amplifiers (Dei Lierre Electronique, France), and displayed on a recorder (Linseis L65514, France). The composition of the Krebs solution was (mM): NaCl 118.0; KCl 5.4; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 25.0 and glucose 11.7.

In all experiments, after 1 h of rest, guinea-pig bronchial rings were contracted to maximal tension with acetylcholine (ACh) 1 mM, and relaxed to maximal relaxation with theophylline 3 mM, and then allowed to equilibrate for 60 min whilst they were washed with Krebs solution every 15 min.

Electrical field stimulation

Experiments were performed in organ baths, fitted with two platinum plate electrodes (1 cm^2) placed alongside the tissue (10 mm apart) for transmural EFS (biphasic pulse width 1 ms, constant current of 320 mA for 10 s) [6, 16]. In all experiments, propranolol 10^{-6} M was added to the buffer solution at the start of the experiment to avoid the influence of adrenergic nerve stimulation, and indomethacin 10^{-6} M was added to the bath to avoid indirect effects of prostaglandins on the neuronal responses. Following the return of the tissue to baseline tone, the preparation was stimulated every 30–45 min, using a stimulator (Dei Lierre Electronique, France) in which the voltage output was adjusted to give a constant current of 320 mA and which produced biphasic rectangular pulses of alternating polarity. Control experiments (n=10) showed no significant fading of the response to field stimulation during the experimental period. These stimulus parameters caused an optimal reproducible biphasic contraction, which consisted of a fast contraction, followed by a sustained contractile response [7, 8]. These procedures were repeated in the absence or presence of rolipram (10^{-9} to 10^{-6} M), siguazodan (10^{-9} to 10^{-6} M) or zaprinast (10^{-9} to 10^{-6} M), administered 30 min before transmural stimulation was applied.

Cumulative concentration-response curves

The inhibitory effects of rolipram (10^{-7} M), siguazodan (10^{-7} M) and zaprinast (10^{-7} M), used as preventive treatment, were studied. After 30 min of contact, cumulative concentration-response curves to ACh (10^{-8} to 10^{-3} M) or [Nle^{10}]NKA(4-10) (10^{-10} to 10^{-6} M) were obtained by addition of these compounds every 5–10 min until a plateau was reached. Spasmogen induced contractions were expressed as percentage of maximal contraction induced by control ACh (1 mM). The experiments with [Nle^{10}]NKA(4-10) were performed in the presence of phosphoramidon (10^{-5} M) to avoid an inhibition of its effect by metabolism [17]. The effects of zaprinast, siguazodan and rolipram on [Nle^{10}]NKA(4-10) were studied, firstly, because this substance is a selective agonist of neurokinins NK_2 receptors and, secondly, because it has been shown that what is principally involved in the guinea-pig bronchial contraction in response to electrical stimulation is stimulation of NK_2 receptors [5, 7, 8, 18, 19].

Statistical analysis of results

Data are expressed as mean±SEM. EC_{50} values represent the concentration producing 50% of the maximal effect. Statistical analysis of the results was performed with variance analysis and Student's t-test for paired or unpaired data, as appropriate. Probability values of $p<0.05$ were considered significant.

Drugs

The substances used were: acetylcholine HCl (PCH, Paris, France); indomethacin (Merck); [Nle^{10}]NKA(4-10) (Novabiochem, Paris, France); propranolol (Sigma, St Louis, USA); rolipram, siguazodan, zaprinast (gift from Rhône Poulenc Rorer, Dagenham, UK); theophylline sodium anisate (Bruneau, Paris, France). All drugs were dissolved in distilled water and then diluted in Krebs solution, except for indomethacin and rolipram, which were dissolved in ethanol, and for siguazodan, which was dissolved in dimethyl sulphoxide (DMSO), and then diluted in Krebs solution. The maximal amount of ethanol or DMSO added to the bath (0.4%) did not alter the reactivity of the preparation to acetylcholine.

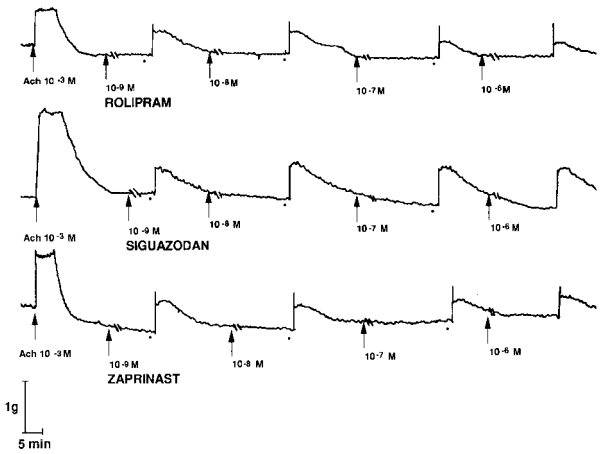

Fig. 1. – Representative traces showing the biphasic response of the guinea-pig isolated main bronchus (propranolol 10^{-6} M and indomethacin 10^{-6} M in the bath) to electrical field stimulation (•) (EFS)(16 Hz, 1 ms, 320 mA for 10 s) and the effects of rolipram (10^{-9} to 10^{-6} M) (upper trace), siguazodan (10^{-9} to 10^{-6} M) (middle trace) and zaprinast (10^{-9} to 10^{-6} M) (lower trace). Ach: acetylcholine.

Results

Figure 1 shows representative traces of the comparative effects of rolipram (10^{-9} to 10^{-6} M), siguazodan (10^{-9} to 10^{-6} M) and zaprinast (10^{-9} to 10^{-6} M) on guinea-pig bronchial contraction induced by EFS.

Figure 2 shows the results obtained globally in our experiments. The PDE inhibitors tested did not significantly modify the cholinergic response to EFS. Furthermore, it clearly appears that only rolipram, in concentrations of 10^{-8} to 10^{-7} M, exerts a significant and concentration-dependent inhibitory effect on the late and prolonged guinea-pig bronchial contraction induced by EFS, involving the noncholinergic excitatory system, with 74% (10^{-7} M, n=8) of inhibition. In contrast, siguazodan and zaprinast did not significantly reduce the noncholinergic response to EFS, reaching only 38% (10^{-7} M, n=9) and 42% (10^{-7} M, n=7) of inhibition, respectively.

Figure 3 shows that rolipram (10^{-7} M), siguazodan (10^{-7} M) and zaprinast (10^{-7} M) have no inhibitory effects preventing the contractions induced by [Nle^{10}]NKA(4-10) and by acetylcholine. In the presence of PDE inhibitors, EC_{50} (-log M) of acetylcholine and [Nle^{10}]NKA(4-10) were not significantly different from control (table 1).

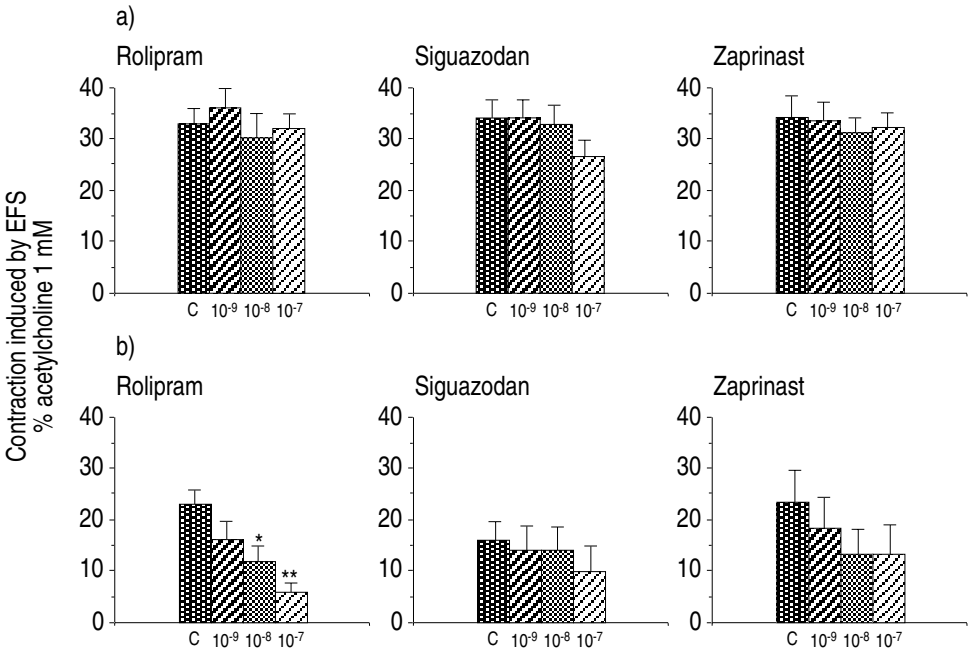

Fig. 2. – Histograms illustrating the effects of rolipram, siguazodan and zaprinast: a) on the cholinergic responses to electrical field stimulation (EFS) (16 Hz, 1 ms, 320 mA for 10 s) of guinea-pig isolated main bronchi; and b) on the NANC responses to EFS. Columns represent contractions expressed as percentages or the contraction induced by acetylcholine (1 mM). Control (C) ( ) or in the presence of rolipram, siguazodan or zaprinast 10^{-9} M ( ), 10^{-8} M ( ) or 10^{-7} M ( ). Experiments were performed in the presence of propranolol (10^{-6} M) plus indomethacin (10^{-6} M). Mean±SEM of 7 to 9 animals are shown. Significant differences from control: *: $p<0.05$; **: $p<0.001$. NANC: noncholinergic nonadrenergic.

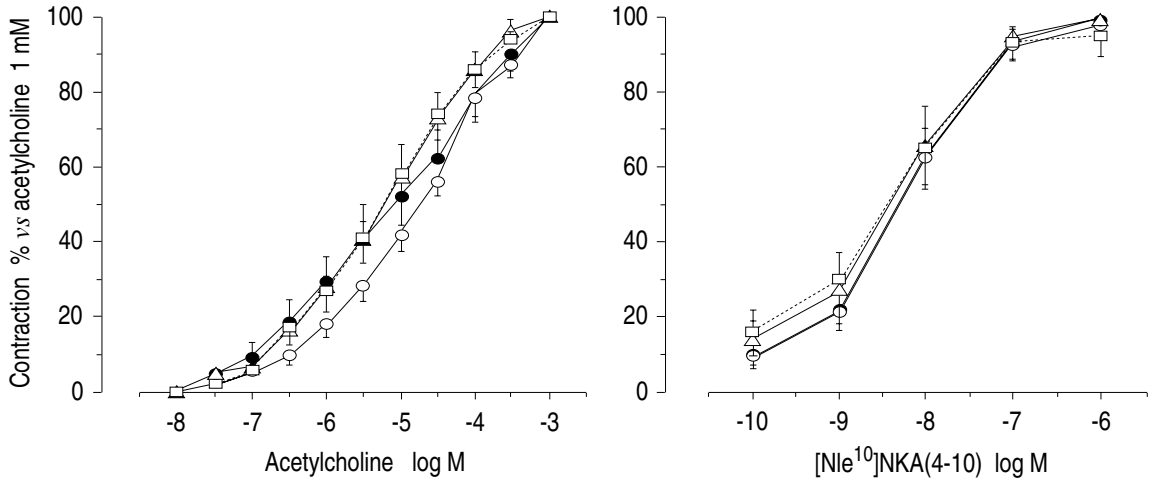

Fig. 3. – Cumulative concentration-response curves for acetylcholine and [Nle^{10}]NKA(4-10). ●: control; ○: in presence of rolipram 10^{-7} M; Δ: in presence of siguazodan 10^{-7} M; : in presence of zaprinast 10^{-7} M. Mean±SEM of 4 to 5 animals are shown. No significant difference from control can be shown.

Table 1. – -log EC_{50} values of acetylcholine and [Nle^{10}]NKA(4-10) in the absence (control) or in the presence of rolipram (10^{-7} M), siguazodan (10^{-7} M) and zaprinast (10^{-7} M)

	Control	Rolipram 10^{-7} M	Siguazodan 10^{-7} M	Zaprinast 10^{-7} M
Acetylcholine	5.14±0.33 (n=5)	4.92±0.08 (n=4)	5.20±0.15 (n=4)	5.19±0.21 (n=4)
[Nle^{10}]NKA(4-10)	8.24±0.17 (n=5)	8.24±0.17 (n=4)	8.38±0.25 (n=4)	8.36±0.15 (n=4)

Data are presented as mean±SEM. EC_{50}: concentration producing 50% of the maximum effect.

Discussion

At least five distinct phosphodiesterase isoenzymes are present in mammalian airway smooth muscle cells, each having different selectivities and Km values of cAMP and cyclic guanosine monophosphate (cGMP). Among these, type III (low Km, cGMP inhibited) and type IV (high Km, cAMP selective) isoenzymes appear to be important for the regulation of cAMP breakdown in airway from guinea-pig, and in canine, bovine or human airways, whereas, type V is involved in the regulation of cGMP breakdown [14, 15, 20–22].

It has recently been demonstrated that theophylline can inhibit the excitatory noncholinergic neurotransmission in guinea-pig bronchi [11]. Since one of the mechanisms of the theophylline action might be inhibition of PDEs, although theophylline has no specific action on any of the PDE isoenzymes, we endeavoured, in this study, to determine precisely whether inhibition of one of these PDE enzymes was more specifically involved in the inhibition of the excitatory nonadrenergic noncholinergic (NANC) response observed in electrical stimulation of guinea-pig isolated bronchi. For this purpose, we used three specific inhibitors of PDE types III, IV and V, namely siguazodan [23], rolipram [24] and zaprinast [25].

Our results show that only rolipram, in concentrations that inhibit PDEs - thereby potentiating the effects of isoprenaline on the bronchial smooth muscle in guinea-pigs and man [26, 27] - significantly reduces the NANC contraction of guinea-pig bronchi, without having any effect on cholinergic EFS-mediated contraction. One hypothesis to explain the absence of inhibitory effect of rolipram on cholinergic response might be that cholinergic contraction is higher in intensity than the NANC contraction. Additional experiments at low frequencies of stimulation might provide a response. The inhibitory effect of rolipram is not due to a reduced contractile response of bronchial smooth muscle, since exogenous ACh contractions were unaffected. Moreover, the contractile response to [Nle^{10}]NKA(4-10) was also unaffected, suggesting that neither affinity nor responsiveness of receptors was modified by rolipram. Altogether, these results suggest that rolipram reduces the release of neuropeptides from NANC nerve endings.

The absence of inhibitory effect of siguazodan and zaprinast, in comparison to inhibition induced by rolipram, could be explained by a lesser activity of these PDE inhibitors at the concentrations tested. However, it has previously been shown that each of these three PDE inhibitors are approximatively equipotent (EC_{50} = 1 µM) for their corresponding isoenzymes [14, 15].

Under similar conditions, siguazodan and zaprinast, which inhibit PDE III and V, respectively, had no inhibitory effects on these two responses.

Thus, in the airways at least, PDE type IV inhibition may result in an inhibition of local release of neuropeptides. This is of interest for substances to be used in the treatment of asthma and goes side-by-side with other specific and potentially interesting effects of PDE type IV inhibitors, such as inhibition of the microvascular leakage induced by platelet-activating factor (PAF) in the guinea-pig [28, 29], of the N-formylmethionyl-leucyl-phenylalanine (fMLP)-stimulated superoxide release, and fMLP/thiomerosal elicited leukotriene biosynthesis by human polymorphonuclear leucocytes [30], as well as inhibition of mediator release from human basophils, mast cells, monocytes or neutrophils [31, 32], or of superoxide formation in guinea-pig eosinophils [33].

In conclusion, our results suggest that of the specific inhibitors of PDE tested, only rolipram, an inhibitor of PDE IV subtype, is capable of reducing the release of sensory neuropeptides from C-fibre endings. This property could be an additional component of the anti-inflammatory effects described with this type of substance, and of their potential value in the treatment of asthma.

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