Anti-Inflammatory Activities of a New Series of Selective Phosphodiesterase 4 Inhibitors Derived from 9-Benzyladenine

ELISABETH BOICHOT, JOHN L. WALLACE, NOËLLA GERMAIN, MARIANNE CORBEL, CLAIRE LUGNIER, VINCENT LAGENTE, and JEAN-JACQUES BOURGUIGNON

Institut National de la Santé et de la Recherche Médicale U456, Laboratoire de Pharmacodynamie et de Pharmacologie Moléculaire, Faculté des Sciences Pharmaceutiques et Biologiques, Université de Rennes 1, Rennes, France (E.B., N.G., M.C., V.L.); Mucosal Inflammation Research Group, University of Calgary, Calgary, Alberta, Canada (J.L.W.); Laboratoire de Pharmacologie et Physico-Chimie des Interactions Cellulaires et Moléculaires, Faculté de Pharmacie, Illkirch, France (C.L.); and Laboratoire de Pharmacochimie de la Communication Cellulaire, Faculté de Pharmacie, Illkirch, France (J.J.B.)

Accepted for publication October 18, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

Adenine derivatives substituted in position 9 have been demonstrated to have potent phosphodiesterase (PDE) inhibition properties with high selectivity toward PDE4. We compared the effects of various compounds derived from 9-benzyladenine with those of the selective PDE4 inhibitor RP 73401 on the inhibition of PDE4 isolated from bovine aorta, arachidonic acid, and tumor necrosis factor-α release by mononuclear cells from healthy subjects. The rank order of potency of the various compounds for in vitro activities on arachidonic acid release is RP 73401 > NCS 613 > NCS 630 > NCS 632 > BWA 78U = NCS 631. The most effective compounds in vitro (RP 73401 and NCS 613) were further investigated in vivo. Both PDE inhibitors dose dependently (1, 10, and 30 mg/kg per os) inhibited the recruitment of neutrophils in the bronchoalveolar lavage fluid of mice exposed to endotoxin via aerosol. Significant differences were observed with 10 and 30 mg/kg RP 73401 and 30 mg/kg NCS 613. In rats, RP 73401, but not NCS 613, significantly increased basal acid secretion at 30 mg/kg i.v. and pentagastrin-stimulated acid secretion at 0.3, 1, and 10 mg/kg. These results demonstrate that the compounds derived from 9-benzyladenine, namely NCS 613, elicit anti-inflammatory activities. It is also suggested that their activities have been mediated through the inhibition of PDE4 isoenzyme. The fact that NCS 613 did not stimulate the gastric acid secretion suggests that this compound may produce fewer gastrointestinal side effects than second-generation PDE4 inhibitors, such as RP 73401.

Cyclic nucleotide phosphodiesterase (PDE) includes at least seven families of isoenzymes that hydrolyze the 3',5'-cyclic nucleotides to 5'-nucleotide monophosphates (Beavo, 1995). Among these isoenzymes, type 4 PDE (PDE4) appears to be a molecular target for new anti-inflammatory drugs (Torphy, 1998). Indeed, PDE4 enzyme has a major cAMP-hydrolyzing activity in immune and anti-inflammatory cells (Tenor and Schudt, 1996), and the elevation of intracellular cAMP in these cell types reduces their activity and the release of inflammatory mediators (Alvarez et al., 1996). Moreover, the selective inhibition of PDE type 4 isoenzyme by PDE4 inhibitors leads to marked anti-inflammatory activities in vitro and in vivo in several animal models (for a review, see Teixeira et al., 1997).

The use of prototypical PDE4 inhibitors, such as rolipram and analogs, was limited by various side effects, such as nausea, emesis, gastric acid secretion, or central nervous system activation, that are not yet completely identified as linked to the mechanism of action (Torphy, 1998). Side effects of PDE4 inhibitors may also be correlated with the possibility that rolipram or other structurally related compounds may bind to the high-affinity rolipram binding sites of the enzyme (Torphy et al., 1993; Jacobitz et al., 1996). This was strongly suggested to account for emesis (Duplantier et al., 1996), gastric acid secretion (Barnette et al., 1995), and psychotropic activity (Saccomano et al., 1991). However, the majority of the in vitro anti-inflammatory activities are correlated with the capacity of the compounds to inhibit PDE4 formers that bind rolipram with low affinity (Barnette et al., 1998; Torphy, 1998). Therefore, one way to obtain potent selective PDE4 inhibitors with an improvement in the therapeutic index is to develop new PDE inhibitors with original chemical structures compared with known PDE4 inhibitors (Cavalla and Frith, 1995).

Adenine derivatives substituted in position 9 constitute

ABBREVIATIONS: PDE, cyclic nucleotide phosphodiesterase; BAL, bronchoalveolar lavage; TNF-α, tumor necrosis factor-α; HPDE4, high-affinity rolipram-binding site; LPS, lipopolysaccharide; LPDE4, low-affinity rolipram-binding site.
putative ligands that may compete with specific targets of adenosine and other nucleotides, including AMP and its corresponding cyclic nucleotides, diaphosphate and triphosphate (cAMP, AMP, ADP, and ATP). More recently, these compounds have been shown to present potent PDE inhibitory properties, with high selectivity toward PDE4 (Bourguignon et al., 1997). The aim of the present study was to compare the inhibitory activity of PDE4 isoenzyme and the anti-inflammatory activity of compounds belonging to the 9-benzyladename (Fig. 1) with that of the potent selective PDE4 inhibitor, RP 73401 (Karlsson et al., 1995). We also studied the capacity of these compounds to induce in vivo gastrointestinal side effects, namely gastric acid secretion in rats.

Experimental Procedures

PDE Inhibition. PDE4 was isolated from the media layer of bovine aorta according to a modification of the method of Lugnier et al. (1986). PDE activities were measured by the two-step assay described by Keravis et al. (1980) at a [3H]cAMP concentration of 1 μM as substrate in a buffer solution of 50 mM Tris-HCl, pH 7.5, 2 mM magnesium acetate, 1 mg/ml BSA, and 1 mM EDTA.

To prevent the influence of PDE4 contamination by PDE3, the experiments were performed in the presence of 100 μM cGMP. PDE inhibitors were dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 1%. At this concentration, DMSO had no significant effects on PDE activity. The concentration of drugs that produced 50% inhibition of substrate hydrolysis (IC50) was calculated by linear regression analysis from concentration-response curves and included different concentrations of inhibitors. The results represent the mean of three determinations obtained for three different enzymatic preparations. The experimental error is about 15%.

Preparation of Peripheral Human Blood Mononuclear Cells. Mononuclear cells were isolated from peripheral blood obtained from several healthy donors through density gradient centrifugation (20 min at 1100g) on Ficoll-Hypaque. Cells recovered at the interface were then washed twice with PBS without Ca2+ and Mg2+.

Mononuclear cells were resuspended in RPMI 1640 supplemented with 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1% heat-inactivated fetal calf serum (RPMI-FCS). The cells were counted and assessed for viability by trypan blue exclusion. Under these conditions, the viability of cells exceeded 95%.

Arachidonate Release. For arachidonic acid incorporation, mononuclear cells were labeled with [3H]arachidonic acid (2 μCi/2 x 10⁶ cells) for 2 h as previously described (Hichami et al., 1995). Aliquots of cell suspension (350 μl, 7 x 10⁶ cells) were distributed in 5-ml polypropylene tubes and incubated at 37°C in an atmosphere of 5% CO2 at 100% humidity for 30 min. Cells were then treated with 50 μl of one of the compounds at the appropriate concentrations (10⁻⁸ to 10⁻⁶ M). All drugs were first dissolved in 0.1% DMSO and then diluted in RPMI supplemented with 0.2% fatty acid-free BSA. Vehicle controls were included in the experimental design. Cells were stimulated with N-formyl-Met-Leu-Phe (fMLP; 1 μM) for 10 min in the presence or absence of drugs. Samples were then centrifuged for 3 min at 1250g, and 0.4 ml of supernatant was added to 2 ml of scintillation cocktail (Packard Instruments, Meriden, CT) in Pico vials (Packard), and samples were counted in a liquid scintillation analyzer (Packard).

Tumor Necrosis Factor-α (TNF-α) Production. Mononuclear cells were incubated for 30 min with each tested PDE4 inhibitor at the concentration of 10⁻⁸ to 10⁻⁶ M. The cells were then stimulated with lipopolysaccharide (LPS of Escherichia coli, 10 μg/ml) overnight at 37°C in an atmosphere of 5% CO2 at 100% humidity as previously described (Hichami et al., 1996). Cell-free supernatants were collected, centrifuged (2000g), and stored frozen at −20°C before TNF-α determination. TNF-α concentrations in cell culture supernatants were determined by specific ELISA using a commercial kit (Genzyme Corp., Cambridge, MA). Sensitivity of the assay was 1 pg/ml. The absorbance at 450 nm was assessed with an ELISA reader (DynaTech, Alexandria, VA).

LPS Exposure and BAL. Ten-week-old male BALB/c mice (CERJ, Le Genest Saint Isle, France) were exposed for 60 min via aerosol to LPS (100 μg/ml) in saline solution (0.9% NaCl) or to the saline solution alone (control group). For exposure, nonanesthetized mice were placed into a Plexiglas chamber (30 x 50 x 30 cm) directly connected to a DeVilbiss ultrasonic nebulizer (Ultraneb 99; Sommerset, PA) that generated particles with an average aerodynamic diameter of 0.5 to 3 μm. Twenty-four hours after the LPS aerosol, mice were anesthetized with pentobarbital sodium (60 mg/kg i.p.). After semicirossion of the trachea, a plastic cannula was inserted, and airspaces were washed with 0.5 ml of 0.9% NaCl containing 2.6 mM EDTA using a 1-ml syringe. This operation was repeated nine times. Bronchoalveolar lavage (BAL) was centrifuged (600g for 10 min at 4°C). After the lysis of erythrocytes with distilled water, cell pellets were resuspended in 500 μl of RPMI 1640 medium, and the total cell count was evaluated using an hemacytometer chamber. After cytocentrifugation (Cytospot 7620 WESCOR) at 700 rpm for 10 min, the cells were stained with May-Grünewald and Giemsa. Differential counts on 200 cells were made using standard morphological criteria. RP 73401 or NCS 613 (1, 10, or 30 mg/kg) was administered per os 2 h before LPS or saline aerosol exposure.

Basal and Pentagastrin-Stimulated Gastric Secretion. Basal and pentagastrin-stimulated gastric secretions were assessed as previously described (Wallace et al., 1991). Briefly, male Wistar rats (180–240 g) were obtained from Charles-River Breeding Farms (Montreal, Quebec, Canada). Before an experiment, the rats were

![Chemical structures of PDE4 inhibitors derived from 9-benzyladename](image-url)
deprived of food for 18 to 22 h but were allowed water ad libitum. This procedure involving animals was performed in accordance with the guidelines of the Canadian Council on Animal Care. The rats were anesthetized with urethane [6 ml/kg of a 20% (w/v) solution in normal saline i.p.], and a tracheotomy was performed. The stomach was continuously perfused with 37°C isotonic saline (3 ml/h) through an orogastric catheter, and the perfusate was collected from a second polyethylene catheter inserted through the duodenum into the stomach. The esophagus and pylorus were ligated to prevent contamination by saliva or duodenogastric reflux. At the beginning of the experiment, the stomach was flushed with saline to remove any residual matter. Once the surgical preparation had been completed, a period of 15 min was allowed for stabilization. Thereafter, the gastric perfusates were collected every 30 min and titrated to pH 7 with 0.01 M NaOH using an automatic titration system (Metrohm; Brinkmann Instruments, Rexdale, Ontario, Canada). Results are expressed as microequivalent of acid per unit time.

At the end of the stabilization period, the rats were treated via the i.v. route with RP 73401 or NCS 613 (0.3–10 mg/kg) or with an equal volume of vehicle. The gastric perfusate was collected for the ensuing 30 min, which represented “basal” acid secretion. At the end of that period, pentagastrin was injected i.v. as a bolus (20 μg/kg), and a continuous infusion of pentagastrin (20 μg/kg/h) was started that ran for 60 min. The gastric perfusate was collected at the end of 30 and 60 min (i.e., two periods of pentagastrin-stimulated acid secretion).

Materials. [3H]cAMP (1.1–1.85 TBq/nmol, TRK 498) and [3H]arachidonic acid were obtained from Amersham (Les Ulis, France). Tris-HCl was obtained from Merck (Darmstadt, Germany). Ficoll-Hypaque was obtained from Pharmacia (Uppsala, Sweden). PBS, RPMI 1640, glutamine, penicillin, and streptomycin were obtained from Life Technologies (Cergy-Pontoise, France). FCS was purchased from Flow Laboratories (Irving, UK). DMSO, BSA, EDTA, fMLP, pentagastrin, urethane, and LPS from E. coli (0.55 B5) were purchased from Sigma Chemical Co. (St. Louis, MO). May-Grünwald and Giemsa stains were obtained from RAL (Paris, France). Sodium pentobarbital was purchased from Sanofi santé Animale (Libourne, France). Rolipram was a generous gift from Schering (Berlin, Germany). Compounds derived from 9-benzyladenine, NCS 613, and the rank order of potency of the various compounds for the inhibition of arachidonate is RP 73401 > NCS 613 > NCS 631.

Effects of Various Compounds on TNF-α Release. Overnight incubation of mononuclear cells with LPS induced a marked production of TNF-α. RP 73401, NCS 613, and NCS 631 (10^-8 to 10^-5 M) elicited a concentration-dependent inhibition of the fMLP-induced arachidonate release (Fig. 2). In contrast, BWA 78U and NCS 631 failed to significantly inhibit arachidonate release. IC_{50} values are presented in Table 1, and the rank order of potency of the various compounds for the inhibition of arachidonate is RP 73401 > NCS 613 > NCS 630 > NCS 632 > BWA 78U = NCS 631.

Total Number of Cells and Cellular Composition in BAL. Only the most potent compound derived from 9-benzyladenine, NCS 613, was tested in vivo and compared with RP 73401. Exposure to LPS aerosol led to a significant increase in the total number of BAL cells compared with saline-exposed mice (control group). Treatment of mice with RP 73401 (10 and 30 mg/kg) or NCS 613 (30 mg/kg) produced a significant inhibition of the increase in the total number of BAL cells after LPS exposure (data not shown). The most significant increase in the number of cells after LPS exposure in mice was noted for neutrophils (Fig. 4). In mice treated with either RP 73401 or NCS 613, a dose-dependent reduction of the neutrophil recruitment due to LPS was observed. This inhibition was significant with 10 and 30 mg/kg RP 73401 and 30 mg/kg NCS 613.

TABLE 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>PDE4</th>
<th>AA</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP 73401</td>
<td>0.001</td>
<td>0.47</td>
<td>0.0007</td>
</tr>
<tr>
<td>BWA 78U</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>NCS 613</td>
<td>0.042</td>
<td>0.91</td>
<td>0.018</td>
</tr>
<tr>
<td>NCS 630</td>
<td>0.1</td>
<td>1.99</td>
<td>ND</td>
</tr>
<tr>
<td>NCS 631</td>
<td>11</td>
<td>NA</td>
<td>0.036</td>
</tr>
<tr>
<td>NCS 632</td>
<td>0.75</td>
<td>5.68</td>
<td>ND</td>
</tr>
</tbody>
</table>

AA, arachidonate; ND, not determined; NA, not active at 10 μM.

Results

Effects of Various Compounds on PDE4 Isoenzyme Inhibition. The results of the inhibitory activity of the compounds on PDE4 isoenzyme in vitro are presented in Table 1. All compounds elicited a marked inhibition of PDE4 isoenzyme and the rank order of potency is RP 73401 > NCS 613 > NCS 630 > NCS 632 > BWA 78U > NCS 631.

Effects of Various Compounds on Arachidonate Release. As previously described by Hichami et al. (1995), a 10-min stimulation of mononuclear cells with 1 μM fMLP resulted in a sustained release of [3H]arachidonate compared with that obtained with cells incubated with the vehicle alone.

RP 73401, NCS 630, NCS 613, and NCS 632 (10^-8 to 10^-5 M) elicited a concentration-dependent inhibition of the fMLP-induced arachidonate release (Fig. 2). In contrast, BWA 78U and NCS 631 failed to significantly inhibit arachidonate release. IC_{50} values are presented in Table 1, and the rank order of potency of the various compounds for the inhibition of arachidonate is RP 73401 > NCS 613 > NCS 630 > NCS 632 > BWA 78U = NCS 631.
In contrast, the i.v. administration of NCS 613 (0.3, 1, 10, and 30 mg/kg) failed to significantly modify the basal or the pentagastrin-stimulated acid secretion (Fig. 5).

**Discussion**

The present results demonstrate that PDE4 inhibitors derived from 9-benzyladenine elicit in vitro and in vivo anti-inflammatory activities, which have been related to their capacities to inhibit the PDE4 isoenzyme. Furthermore, in comparison with the well known PDE4 inhibitor RP 73401, NCS 613, the most potent of the compounds tested, failed to increase the basal and the pentagastrin-stimulated gastric acid secretion in rats.

The interest in selective PDE4 inhibitors as potent anti-inflammatory drugs and as a molecular target for new anti-asthmatic compounds has increased greatly in the past few years (for a review, see Teixeira et al., 1997; Torphy, 1998). However, the use of such compounds was limited by side effects such as emesis, gastric acid secretion, and psychotropic activity (Torry, 1998). It has also been proposed that these side effects may be an extension of their pharmacological mechanism of action (i.e., inhibition of PDE4 in inappropriate tissues; Torphy and Undem, 1991). Therefore, it appears necessary to develop new PDE4 inhibitors that may possess potent anti-inflammatory activities with reduced side effects.

In the present study, we studied the anti-inflammatory activities of various compounds derived from 9-benzyladenine: BWA 78U, NCS 613, NCS 630, NCS 631, and NCS 632. These compounds have been preliminarily described as selective PDE4 inhibitors (Bourguignon et al., 1997).

We previously demonstrated that PDE4 inhibitors, but not PDE3 and PDE5 inhibitors, elicit a marked inhibition of arachidonate release from human mononuclear cells stimulated with fMLP (Hichami et al., 1995); consequently, this model is suitable to investigate the in vitro anti-inflammatory activity of PDE4 inhibitors. The present data showed that NCS compounds derived from 9-benzyladenine were also able to reduce,
in a dose-dependent manner, arachidonate release. However, only NCS 613, NCS 630, and NCS 632 reach a statistically significant inhibitory activity. The results obtained for each compound allowed us to establish a rank order of potency of NCS 613 $>$ NCS 630 $>$ NCS 632 $>$ BWA 78U = NCS 631. It appears that NCS 613 is the most potent drug in reducing arachidonate release, with an $IC_{50}$ value of 0.91 $\mu$M, and is only twice less potent as the well known PDE4 inhibitor RP 73401 ($IC_{50} = 0.47$ $\mu$M). The inhibitory activities of compounds derived from 9-benzyladenine on arachidonate release are significantly correlated ($r = 0.9, P = .0065$) with the $IC_{50}$ value obtained on the PDE4 isoenzyme inhibition. This would strongly suggest that the PDE4 isoenzyme inhibition represents the

Fig. 3. Effects of PDE4 inhibitors on TNF-$\alpha$ release from mononuclear cells stimulated with LPS (10 $\mu$g/ml). Cells were incubated in vitro with one of the PDE4 inhibitors for 30 min before stimulation. Results are expressed as means $\pm$ S.E. of percent release of TNF-$\alpha$ compared with the release by nonstimulated cells. Each experiment was performed with three to five different donors and was performed in triplicate. Statistical differences were assessed using the paired Student’s $t$ test. *$P < .05$, **$P < .01$.

Fig. 4. Influence of RP 73401 and NCS 613 (1, 10, and 30 mg/kg per os) on the number of neutrophils ($\times 10^5$) in the BAL fluid of mice exposed to an aerosol of saline solution containing 100 $\mu$g/ml LPS. Control: mice exposed to saline aerosol alone. None: mice receiving no pretreatment before LPS aerosol. BAL was performed 24 h after the LPS aerosol. Statistical differences were assessed using the nonparametric Mann-Whitney $U$ test. #$P < .01$, compared with control mice. *$P < .05$, **$P < .01$, compared with untreated mice (none) ($n = 4–9$).
TNF-α benzyladenine derivatives were more effective in reducing lease. Indeed, the less potent PDE4 inhibitor within this by the Newman-Keuls test. *P significant differences were determined using one-way ANOVA followed
Results are expressed as microequivalent of acid per 30 min. Statistically
One of the most striking actions of PDE inhibitors on
mononuclear cells.

The onset of acute lung injury is often an early symptom of multiple organ failure associated with sepsis, and sepsis is associated with elevated blood levels of endotoxin or LPS. LPS has therefore been implicated as an important inducer of lung injury (Parsons et al., 1989). Acute lung injury is accompanied by sequestration of neutrophils in the pulmonary microcirculation and their migration in the airways. Thus, their activation appears to be a key event in the development of lung injury (Worthen et al., 1987). Inhibition of LPS-induced inflammatory cell recruitment in airways is an available model to provide an initial assessment of the in vivo activity of PDE4 inhibitors (Turner et al., 1993, Escofier et al., 1999). In the present study, RP 73401 and NCS 613 significantly reduced the increase in the number of total cells as well as the number of neutrophils in the BAL fluid of mice exposed to LPS. However, RP 73401 appears to be more effective than NCS 613, because in contrast to this latter compound, RP 73401 significantly reduced the increase in neutrophil number at 10 mg/kg. These data strongly support that both PDE 4 inhibitors elicit anti-inflammatory activities in vivo after oral administration and suggest that they are candidate as drugs that might reduce lung injury.

Finally, we compared the ability of NCS 613 to alter the basal and pentagastrin-stimulated gastric acid secretion in rats. Our results clearly showed that NCS 613 (0.3–30 mg/kg) failed to modify the gastric acid secretion. This should be compared with the significant, although moderate, increase in both basal and pentagastrin-stimulated acid secretion obtained after the administration of RP 73401.

Schneider et al. (1986) were the first to report the presence of a high-affinity, stereoselective, and saturable [3H]rolipram-binding site in rat brain homogenates. The role of this binding site is not clear, because rolipram inhibits PDE4 catalytic activity only at greater concentrations than those needed to interact with its high-affinity binding site. Moreover, the rank order of potency of various compounds for inhibition of PDE4 catalytic activity is distinct from that for competition at the high-affinity rolipram-binding site (Koe et al., 1990; Torphy et al., 1992; Torphy, 1998). Recent studies support the proposal that two distinct and catalytically active conformers of PDE4 coexist (Jacobitz et al., 1996; Rocque et al., 1997; Souness and Rao, 1997). One of them binds rolipram at the catalytic site with high affinity, and a second binds rolipram with lower affinity. These conformers are termed high-affinity rolipram-binding site (HPDE4) and low-affinity rolipram-binding site (LPDE4; Torphy, 1998).

Some therapeutic effects of PDE4 inhibitors appear to be related to inhibition of LPDE4, whereas the side effects of these compounds appear to be related to HPDE4 inhibition. For example, inhibition of LPDE4 is associated with suppression of TNF-α generation from monocytes (Barnette et al., 1996; Souness et al., 1996), superoxide production from eosinophils (Barnette et al., 1995), and interleukin-2 synthesis from splenocytes (Souness et al., 1997). In contrast, the side effects of these compounds appear to be linked exclusively to inhibition of HPDE4; these side effects include emesis (Duplantier et al., 1996), psychotopic activity (Saccomano et al., 1991), and gastric acid secretion (Barnette et al., 1995). Therefore, these data led to the proposal that the therapeutic index of PDE4 inhibitors could be improved by either decreasing the affinity of newly synthesized compounds for HPDE4 or increasing their affinity for LPDE4 (Hughes et al.,
Phosphodiesterase 4 Inhibitors and Inflammation

Inhibitors of the previous generation. PDE4 inhibitor RP 73401, the most potent compound, NCS 613, failed to elicit gastric acid secretion in rats, suggesting that NCS 613 could not elicit gastrointestinal side effects.

In summary, these results demonstrate that compounds derived from 9-benzyladenine have anti-inflammatory properties, which can be related to their capacities to inhibit PDE4 isoenzyme. This anti-inflammatory activity of NCS 613 has been also demonstrated in vivo in LPS-induced neutrophil recruitment in BAL fluid of mice. Furthermore, compared with the well known PDE4 inhibitor RP 73401, the most potent compound, NCS 613, fails to induce acid gastric secretion in rats, suggesting that NCS 613 may have fewer side effects than the suggested PDE4 inhibitors of the previous generation.

Acknowledgments

We thank Webb McKnight, Michael Dicap, Nathalie Donval, Marion Hab, and Dr. A. Le Bec for their technical assistance and Dr. Tardivel and Mrs. Massot (CRTS Rennes) for the supply of buffy coats.

References

Alvarez R, Daniels DV, Shelton ER, Baecker PA, Fong AT, Devens B, Wilhem R, and Mrs. Massot (CRTS: Rennes) for the supply of buffy coats.

Am J Respir Crit Care Med 157:1411–1420.


