

## Analgesic and anti-inflammatory effects of phosphodiesterase inhibitors

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The aim of the present study was to investigate the role of phosphodiesterase (PDE) enzyme inhibitors namely rolipram and theophylline in pain and inflammation in experimental animals. Rolipram, a selective PDE IV inhibitor and theophylline a nonspecific PDE inhibitor exerted dose dependent analgesic and anti-inflammatory effect against acetic acid-induced writhing in mice and carrageenan-induced paw edema in rats, respectively. Nimesulide (1, 2 mg/kg) produced significant anti-inflammatory effect. Further, nimesulide (0.5 mg/kg) potentiated analgesic effect of rolipram but it failed to modulate the anti-inflammatory effect of PDE inhibitors. Present study suggests that PDE enzymes might be playing a role in nociceptive and inflammatory responses in animals.

Phosphodiesterase (PDE) enzymes occur widely in the biological system and present in the mammalian tissues except in red blood cells<sup>1</sup>. The cyclic nucleotide phosphodiesterases are responsible for degrading the second messenger cyclic nucleotides cAMP and cGMP which are involved in a wide range of physiological processes<sup>2,3</sup>. At least 8 distinct cyclic nucleotide phosphodiesterase isoenzymes (PDE 1-8) have been identified on the basis of their functional characteristics, such as substrate specificity, cellular distribution and susceptibility to selective inhibitors<sup>4</sup>. PDE type 4 (PDE 4) is characterised by cAMP specificity, and sensitivity to inhibition by rolipram<sup>5,6</sup>. PDE 4 inhibitors have shown anti-inflammatory effects due to induction of adenylate cyclase which leads to increase in the intracellular level of cAMP. Since cAMP suppresses inflammatory cells activities thus inducing relaxation of airway smooth muscle. Furthermore, PDE inhibitors are also able to block production of cytokines i.e. IL-5, IL-6, IL-8 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), a proinflammatory mediators which appears to be closely related to inflammatory response<sup>7</sup>.

Present study mainly investigate the analgesic, anti-inflammatory effects of selective PDE4 inhibitor rolipram, and nonspecific PDE inhibitor theophylline in acetic acid induced writhing reflex and carrageenan induced paw edema, respectively.

### Materials and Methods

**Animals**—Albino mice (Laka strain, 20-30 g) and rats (Portan strain, 200-250 g) of either sex, bred in Central Animal House, Panjab University, Chandigarh, were housed under standard laboratory

conditions with free access to food and water. All experiments were carried out between 0900 and 1700 hr.

### Procedure

**Analgesic study**—*Writhing test (acetic acid writhing assay)*

1% acetic acid solution (1 ml/100 g) was used to produce writhing in mice. The severity of pain response (writhing) was assessed by counting number of wriths (constriction of abdomen, turning of trunk (twist) and extension of hind legs) in mice<sup>8</sup>. Number of writh per animal was counted during a 20 min session beginning 3 min after the injection of acetic acid.

Analgesic activity was calculated as % maximum possible effect (% MPE) using the following relation<sup>9</sup>.

$$\% \text{ MPE} = \frac{100 \times (\text{Mean of wriths in control group} - \text{Mean of wriths in treated group})}{\text{Mean of wriths in control group}}$$

**Anti-inflammatory study**—*Carrageenan-induced paw edema*

Acute edema was induced in the right hind paw of rats by injecting 0.1 ml of freshly prepared 1% solution of carrageenan (type IV). The left paw served as control (non-inflamed paw) for comparison (0.9% 0.1 ml saline injected).

The carrageenan was injected under the planter region of right hind paw and paw volumes were measured using plethysmometer (water displacement, UGO BASILE, Italy) at 15, 30, 60, 90, 120, 150, 180, 210 and 240 min. after carrageenan challenge. Percent increase in paw volumes were calculated and expressed as amount of inflammation<sup>10,11</sup>.

$$\% \text{ increase in paw volume} = \frac{100 \times (\text{Paw volume of right paw} - \text{Paw volume of left paw})}{\text{Paw volume of left paw}}$$

### Drugs

Rolipram (Schering, Aktiengesellschaft, Berlin), Theophylline (Sigma), Nimesulide (Panacea Biotech, New Delhi), Carrageenan type IV (Sigma), Acetic acid (Sarabhai chemicals, Baroda). Rolipram was suspended in 0.5% carboxymethyl cellulose. Rolipram and theophylline were given intraperitoneally (ip) 30 min before acetic acid or carrageenan challenge. Carrageenan (1% w/v) was injected in single dose 0.1 ml per paw constantly.

### Statistical analysis

Results were expressed as mean  $\pm$  SEM. and subjected to one way analysis of variance (ANOVA) followed by Student's t-test.  $P < 0.05$  were considered significant.

### Results

#### Analgesic activity: Writhing assay

Intraperitoneally administered theophylline (2, 4, 8

mg/kg) produced significantly increase ( $P < 0.05$ ) in the pain threshold against acetic acid induced writhing in a dose-dependent manner. Theophylline (8 mg/kg) produced 51.05% MPE.

Rolipram (1, 2 mg/kg ip) also produced significant analgesic effect ( $P < 0.05$ ) against acetic acid-induced writhing response. Nimesulide (0.5, 1 mg/kg) exerted analgesic effect. Co-administration of nimesulide (1 mg/kg) with varying doses of theophylline (4, 8 mg/kg) did not potentiate the analgesic effect of theophylline, however it (nimesulide 0.5 mg/kg) significantly potentiated the analgesic effect of rolipram (1, 2 mg/kg) (Table 1, 2).

#### Anti-inflammatory activity: Carrageenan induced paw edema

Carrageenan (1%) produced significant increase in paw volume in control group. Theophylline (4, 8, 10 mg/kg ip) produced dose dependent (after 90 min) protective effect against carrageenan-induced inflammation as compared to control group ( $P < 0.05$ , Fig. 1). Rolipram (2, 5 mg/kg, ip) produced significant anti-inflammatory effect (Fig. 2). Nimesulide (0.5, 1 mg/kg) also produced

Table 1—Effect of theophylline, nimesulide and their combinations on acetic acid induced writhing in mice

Treatment	Dose (mg/kg, ip)	n	No. of wriths in 20 min (Mean $\pm$ S.E)	Antinociceptive action (% MPE)
Control	(1% acetic acid 10 ml/kg)	24	53.83 $\pm$ 1.14	—
Theophylline	2	6	40.50 $\pm$ 1.28*	24.76
Theophylline	4	6	31.16 $\pm$ 4.88*	42.11
Theophylline	8	6	26.50 $\pm$ 0.84*	50.77
Nimesulide	1	6	34.83 $\pm$ 4.26*	35.29
Nimesulide + Theophylline	1, 4	6	36.5 $\pm$ 1.99 <sup>NS</sup>	32.19
Nimesulide + Theophylline	1, 8	6	29.80 $\pm$ 3.68 <sup>NS</sup>	44.64

\*  $P < 0.05$  as compared with control  
<sup>NS</sup> : theophylline *per se*.

Table 2—Effect of rolipram, nimesulide and their combinations on acetic-acid induced writhing in mice

Treatment	Dose (mg/kg, ip)	n	Number of wriths in 20 min (Mean $\pm$ S.E)	Antinociceptive action (% MPE)
Control	(1% acetic acid 10 ml/kg)	24	53.83 $\pm$ 1.14	
Rolipram	1	6	38.83 $\pm$ 6.04*	27.86
Rolipram	2	6	29.00 $\pm$ 4.09*	46.12
Nimesulide	0.5	6	50.33 $\pm$ 4.95*	6.50
Nimesulide + Rolipram	0.5, 1	6	24.83 $\pm$ 3.44* <sup>0</sup>	53.87
Nimesulide + Rolipram	0.5, 2	6	15.83 $\pm$ 1.04* <sup>0</sup>	70.59

\*  $P < 0.05$  as compared with control  
<sup>0</sup>  $P < 0.05$  compared with rolipram *per se*.

significant anti-inflammatory effect as compared to control ( $P < 0.05$ ). When nimesulide (0.5, 1 mg/kg) was given in combination with rolipram (2 mg/kg) or theophylline (8 mg/kg), it failed to modify the anti-inflammatory effect of rolipram and theophylline (Figs 3, 4).

**Discussion**

Cyclic nucleotide phosphodiesterases play a key role in the metabolism of cAMP and cGMP. Many agents modulate tissue function via stimulation of adenylyl or guanylyl cyclase activity and hence via the elevation of cellular levels of cAMP and cGMP<sup>12</sup>.

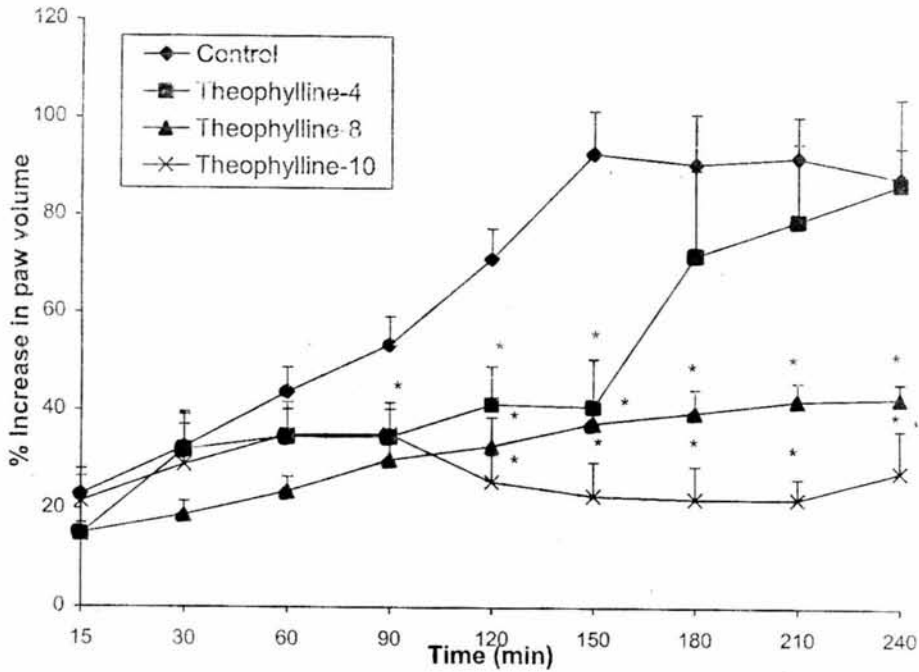


Fig. 1—Effect of various doses (4, 8, 10 mg/kg, i.p) of theophylline on carrageenan-induced paw edema in rats. Vertical lines show mean  $\pm$  SE \*  $P < 0.05$  as compared with control. (n = 6)

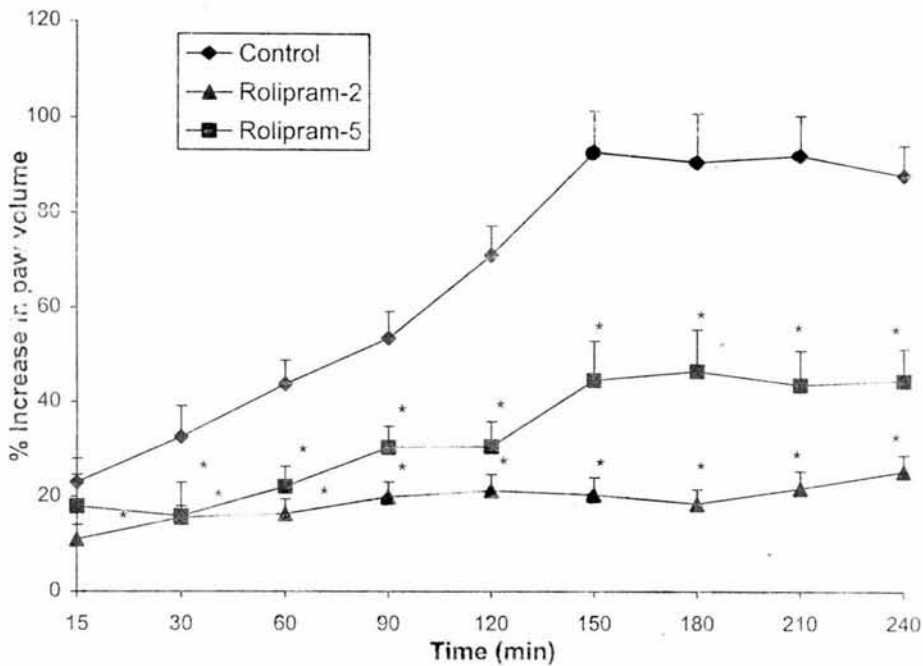


Fig. 2—Effect of rolipram (2, 5 mg/kg, i.p) on carrageenan-induced paw edema in rats. Vertical lines show mean  $\pm$  SE \*  $P < 0.05$  as compared with control. (n = 6)

Rolipram is a selective PDE IV inhibitor which is known as antidepressant and effective in asthma<sup>13</sup> and theophylline a non-specific PDE inhibitor<sup>14</sup>, is a one of the most effective drug for the treatment of asthma due to combination of bronchodilatory, pulmonary

antiallergic and pulmonary anti-inflammatory activity<sup>15</sup>. The present investigation demonstrated that theophylline (2, 4, 8 mg/kg) and rolipram 1, 2 mg/kg a selective PDE IV inhibitor produced significant analgesic, anti-inflammatory effect against acetic acid

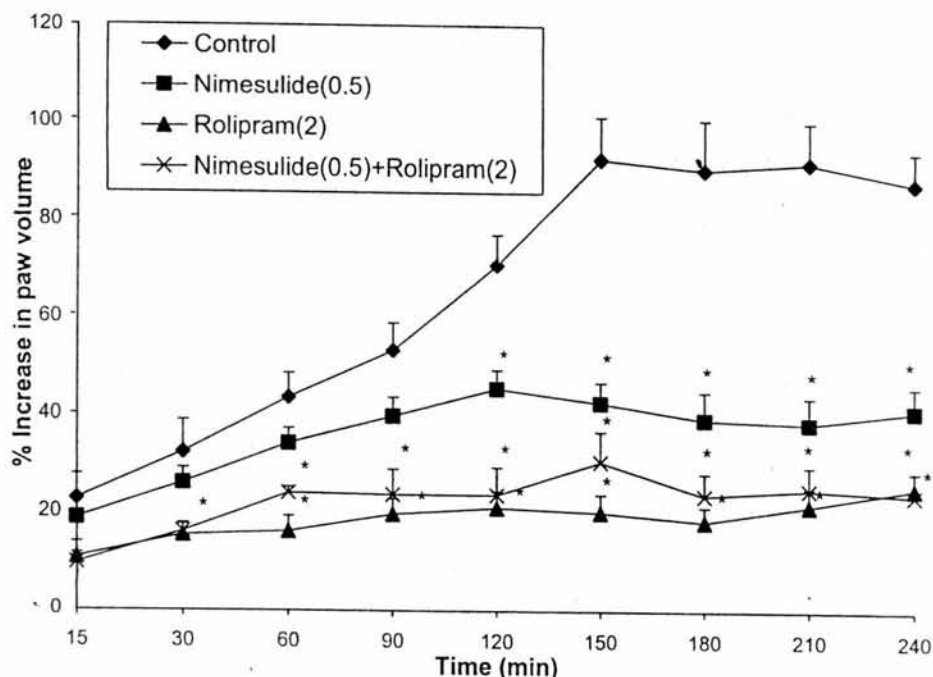


Fig. 3—Influence of annealing on initial modulus of MJS yarns [Grey: A—11.5 tex, 48:52 P/V; B—11.5 tex, 80:20 P/V; C—29.5 tex, 48:52 P/V; D—29.5 tex, 80:20 P/V; and Annealed: E—11.5 tex, 48:52 P/V; F—11.5 tex, 80:20 P/V; G—29.5 tex, 48:52 P/V; H—29.5 tex, 80:20 P/V]

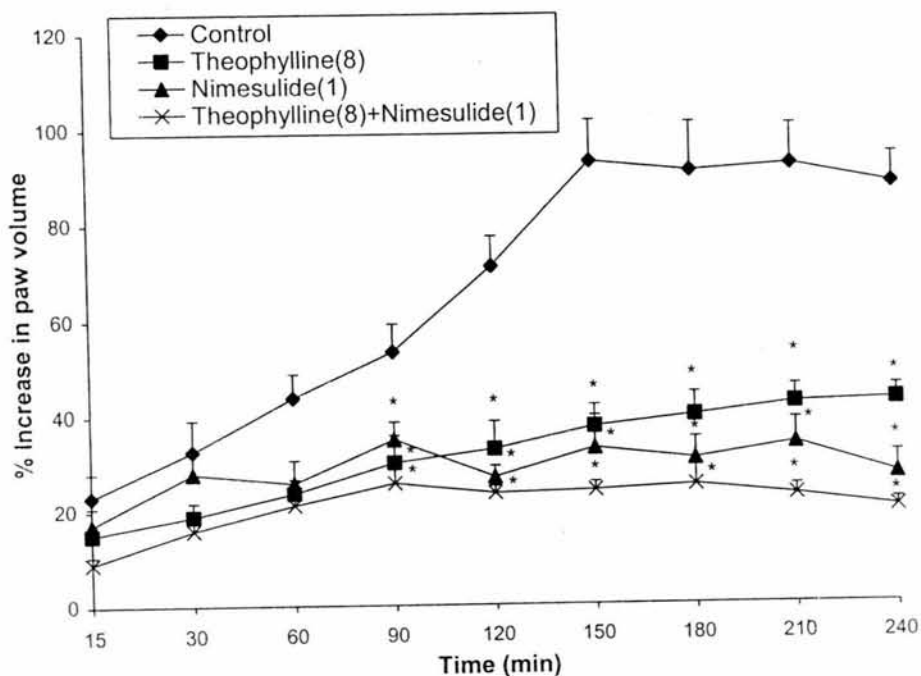


Fig. 4—Thermal shrinkage of MJS yarns [A—11.5 tex, 48:52 P/V; B—11.5 tex, 80:20 P/V; C—29.5 tex, 48:52 P/V; and D—29.5 tex, 80:20 P/V]

induced writhing reflex and carrageenan induced paw edema, respectively. It is reported that PDE IV inhibitors elevate cAMP level in cells that participate in the inflammatory process. The elevation of intracellular cAMP has been associated with inhibition of the function of various types of cells including lymphocytes, monocytes, macrophages, neutrophils, eosinophils, mast cells, basophils, endothelial cells and lung epithelial cells<sup>16</sup>. Above mentioned mechanism may be participating in the analgesic and anti-inflammatory effects of rolipram and theophylline, because mediators of these cells could be involved in various painful and inflammatory conditions<sup>17</sup>.

Anti-inflammatory effect of theophylline in airway inflammation has been well reported<sup>17</sup> so it may be speculated that theophylline exerted anti-inflammatory effect in carrageenan induced paw edema through inhibition of phosphodiesterase activity. Nimesulide, a selective cyclo-oxygenase-2 inhibitor, is also known to have PDE IV inhibiting property<sup>19</sup>. The rationale of combining the nimesulide with theophylline or rolipram was to assess whether nimesulide showed synergistic or potentiating effects in analgesic and anti-inflammatory activity. Co-administration of nimesulide with theophylline did not modify pain threshold and anti-inflammatory activity of theophylline but when nimesulide given with rolipram, it potentiated analgesic activity. It's suggested that nimesulide by PDE IV inhibition potentiates analgesic activity of rolipram.

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