

## IC<sub>50</sub> of trifluoperazine and RMI 12330A for the Theophylline and Phloretin - Induced Increase in Intestinal D-Galactose Accumulation

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The effect of trifluoperazine and RMI 12330A on D-Galactose accumulation was studied in isolated rat intestinal mucosa. Both drugs inhibited the theophylline and phloretin-induced increase in tissue sugar accumulation in a concentration-dependent fashion, with IC<sub>50</sub> values close to 10<sup>-6</sup> M. These findings suggest that calmodulin might mediate the theophylline and phloretin actions on galactose transport in intact rat ileum.

**Key words:** Trifluoperazine, RMI 12330A, Intestine, Sugar transport.

In isolated chicken enterocytes, it has been shown that sugar efflux across the basolateral cell boundary takes place via a Na<sup>+</sup>-independent, facilitated diffusion system (10, 14) which is inhibited by theophylline and phloretin (10, 12, 14). Theophylline also reduced serosal sugar permeability in intact rabbit ileum (6). Both theophylline and phloretin (1, 7) were previously found to reduce mucosal to serosal galactose fluxes and increase tissue sugar accumulation in intact rat ileum. These findings agree with the view that theophylline and phloretin might act

by reducing sugar permeability across the serosal border. Furthermore, the anticalmodulin agents trifluoperazine (11) and RMI 12330A (8) prevented the theophylline and phloretin effects on intestinal sugar transport (1, 7).

In the current study we have evaluated the IC<sub>50</sub> of RMI 12330A and trifluoperazine for the theophylline and phloretin-induced increase in D-galactose accumulation in intact rat ileum.

### Materials and Methods

*Animals, incubation solutions.* — Male albino Wistar rats (150-200 g) were anaesthetized with ether and killed by

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ether overdose. Following laparotomy, the distal small intestine was removed and rinsed free of intestinal contents with ice-cold Ringer's solution. The tissue was then stripped of its serosal and external muscle layers using the method of POWELL *et al.* (13). The Ringer's solution contained (in mM): 140 NaCl, 10 KHCO<sub>3</sub>, 0.4 KH<sub>2</sub>PO<sub>4</sub>, 2.4 K<sub>2</sub>HPO<sub>4</sub>, 1.2 CaCl<sub>2</sub> and 1.2 MgCl<sub>2</sub> and was continuously bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub>.

**Experimental protocol.** — Pieces of the stripped mucosa, weighing about 50 mg, were incubated at 37°C in 0.5 mM galactose Ringer's solution containing tracers of <sup>14</sup>C-galactose. After a single 20 min incubation period, the tissues were washed with gentle shaking in ice-cold Ringer's solution, lightly blotted and weighed. The radioactivity was extracted from the tissue by continuous shaking for 15 h in 1 ml 0.1 N HNO<sub>3</sub>. Samples were taken from the bathing solutions and from the extracts of the tissues for radioactivity counting. The modifiers were added to the incubation solution at the beginning of the incubation period. The total amount of sugar sequestered within 1 g of wet tissue was corrected for the phosphorylated galactose and extracellular space as previously reported (7).

The IC<sub>50</sub> value is defined as the concentration of the drug required to produce a 50% inhibition of either theophylline- or phloretin-induced increase in tissue sugar accumulation.

**Statistics.** — Data are expressed as mean ± S.E. Statistical significance was evaluated by the two-tailed Student's t-test.

**Materials.** — Galactose, theophylline and phloretin were obtained from Sigma. Trifluoperazine was from Smith, Kline and French Laboratories and R.M.I. 12330A from Merrell Dow Pharmaceuticals INC (Cincinnati).

## Results and Discussion

When sugar efflux across the basolateral cell boundary of isolated chicken enterocytes is inhibited by either theophylline or phloretin, the cells establish much greater sugar concentration gradients (10, 14). It has been previously shown (1, 7) that both theophylline and phloretin increased cell water sugar accumulation and reduced mucosal to serosal sugar flux in intact rat small intestine, and that these effects were prevented by 0.1 mM trifluoperazine and by 0.1 mM RMI 12330A.

In the current study, as previously (1, 7) the C/M ratio (cell water free sugar concentration / sugar concentration in the bathing solution) measured in control conditions was 2.83 ± 0.07 (n = 22). Theophylline (3 mM) and phloretin (0.1 mM) caused a two-fold increase in the C/M ratio.

Trifluoperazine inhibited both the theophylline (3 mM) and phloretin (0.1 mM)-stimulated cell water sugar accumulation in a concentration-dependent manner. The potency of trifluoperazine in reducing cell water sugar concentration in rat small intestine was estimated from the dose response curve (figure 1). The drug concentrations required to achieve a half-maximal response (IC<sub>50</sub>) were close to 1 μM. Similar results were obtained with RMI 12330A (fig. 2).

Trifluoperazine is a phenothiazine which binds with high affinity and specificity to the calcium-calmodulin complex (11) preventing the calmodulin from activating a wide variety of cellular processes.

RMI 12330 A has been shown to inhibit adenylyl cyclase activity (16) and to compete with <sup>3</sup>H-trifluoperazine for the calcium-dependent binding site of calmodulin (8).

The results presented in the current study agree with previous work on the affinity of trifluoperazine for purified calmodulin (11, 8) and suggest that both

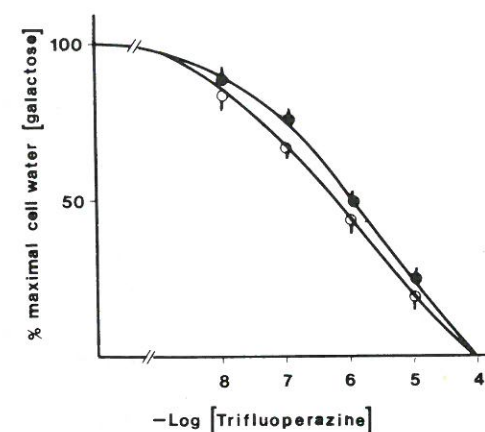


Fig. 1. Effect of increased concentrations of trifluoperazine on either theophylline or phloretin-dependent increase in cell water galactose concentration.

3 mM theophylline (O), 0.1 mM phloretin (●). The concentration of sugar in the incubation solutions was 0.5 mM. The data are plotted as mean ± S.E. of 20 independent determinations. The IC<sub>50</sub> of trifluoperazine for the theophylline and phloretin-induced increase in D-galactose accumulation were 0.7 and 1 μM respectively.

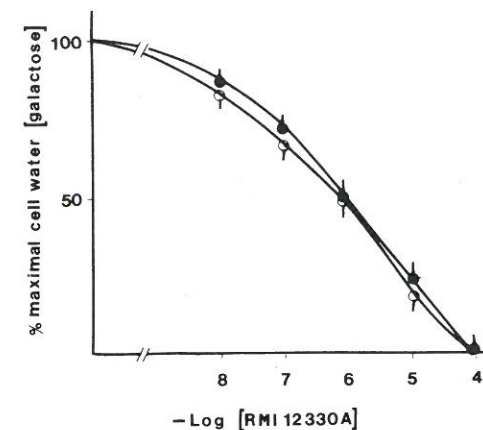


Fig. 2. Effects of increasing concentration of RMI 12330A on either theophylline or phloretin-stimulated cell water sugar concentration.

Foot notes of figure 1 also applied to this figure. The IC<sub>50</sub> of RMI 12330A was 1 μM in both cases.

potential. It could be postulated that Ca<sup>2+</sup> permeability might also be modified by this mechanism. On the other hand, RANGLES and KIMMICH (14) have reported that phloretin produced a 23% decrease in total cellular ATP, and it is well known that low cytosolic free calcium concentration is maintained by the action of ATPases, which actively extrude calcium at expenses of ATP hydrolysis. At least in lymphocytes a reduction in ATP levels caused a rise in intracellular Ca<sup>2+</sup> (18).

The results presented in the current work appear to suggest that both theophylline and phloretin may reduce serosal sugar efflux in rat ileum via an increase in Ca-calmodulin complex. However, in interpreting these findings it is worth pointing out that the tissue preparation studied contains both intestinal epithelial cells and muscularis mucosa. Therefore, the muscle could play a role in the sugar transport changes herein reported. It has been shown that theophylline increases smooth muscle tone (19) whereas trifluoperazine reduces it (17).



Studies with isolated intestinal epithelial cells are presently carried out to find out whether the effects of theophylline and phloretin on sugar movements across the basolateral cell boundary are mediated by changes in intracellular  $Ca^{2+}$ .

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#### Resumen

Se estudia el efecto de trifluoperazina y RMI 12330 A sobre la acumulación de galactosa por la mucosa de íleon de rata. Ambos agentes inhiben el aumento en la acumulación del azúcar inducido por la teofilina y la floretina, con valores de  $IC_{50}$  próximos a  $10^{-6}$  M. Los resultados sugieren que la calmodulina podría mediar la acción de la teofilina y floretina sobre el transporte de galactosa en íleon de rata.

**Palabras clave:** Trifluoperazina, RMI 12330 A, Intestino, Transporte de azúcares.

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## Liver and M (Vicia faba)

S. SANTIDRIAN, M.  
*Activity in Field Bean* (1987)  
375-380, 1987.

Liver and muscle protein synthesis in chickens fed *ad libitum* heated soybean (HSB) protein. Vitamin E (250 IU/kg) that in comparison to control, increased in the rate of growth, and D in liver and muscle protein synthesis on either weight gain or effects is discussed.

**Key words:** Antinutritional factors

Legumes are widely used as protein sources both in human nutrition. In the Mediterranean region, in many other countries, they are extensively harvested and used as relatively inexpensive protein sources for domestic animals and humans. However, the utilization of legumes in animal nutrition produces

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