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Influence of VIP on D-Galactose Transport Across Rabbit Jejunum *in vivo*

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D-galactose absorption during 1 min perfusion periods was not affected by the presence of 10^{-7}-10^{-8} M VIP in the sugar solution, but exposure of mucosa to VIP for 5 min inhibited sugar absorption in the subsequent periods of perfusion. This inhibition is reversed after washing with saline solution. The effect of VIP disappeared when 10^{-6} M RMI 12330A was added to the incubation solution together with 10^{-7} M VIP and 1 mM D-galactose solution. These results suggest the existence of VIP receptors on the brush border membrane. The action of VIP could be mediated through the cAMP system.

Key words: Galactose absorption, VIP, Rabbit jejunum.

Vasoactive intestinal peptide (VIP) is widely distributed throughout the body but is most highly concentrated in the nervous system and the gut.

The biological actions of VIP in the gastrointestinal tract include a potent relaxing effect on the gut muscle, specially the sphincter (6), a potent vasodilatation stimulating the flow of blood to splanchnic and other organs, a suppression of gastric acid secretion and stimulation of water and electrolyte secretion, most probably mediated by the increase of 3'5'-cyclic monophosphate (cAMP) in intestinal mucosa (3, 12, 14, 18, 19).

There is evidence of the existence of VIP receptors on the basolateral membrane of rabbit and rat intestinal cells (4). On the other hand, many hormones, including VIP, have been detected in the intestinal lumen; the action of these intraluminal hormones is currently being investigated (9, 17). The presence of intraluminal hormones raises the question of whether there are hormone receptors on the brush border membrane or not.

This study investigates the effect of VIP on D-galactose absorption across the rabbit jejunum *in vivo*.

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Materials and Methods

The experiments were carried out on male crossed New Zealand and Australian rabbit species, with a body weight of 1 kg and fasted for 24 h. The animals were anesthetized with thiopental. *In vivo* intestinal absorption was measured by the PONZ *et al.* method (11) with *in situ* cannulated jejunum segments of 10-20 cm in length, perfused at 7 ml/min rate.

For each animal, the sugar absorbed for single pass perfusion and 1 min absorption periods were adopted, and sugar absorption was calculated as the difference in the sugar content of the solution at entry and exit of the jejunum and expressed in nanomoles per cm length per minute (11).

The Ringer solution used contained in mM: 140 NaCl; 10 $KHCO_3$; 0.4 KH_2PO_4; 1.2 $CaCl_2$ and 1.2 $MgCl_2$, and was always adjusted to pH 7.4.

The amount of sugar was chemically estimated by the NELSON and SOMOGYI method (10, 16) with a spectrophotometer Kontron Uvikon 810.

Materials: D-galactose (Merck), VIP (Sigma) and RMI 12330 A (Merrell Dow).

Statistics: Results are expressed as mean ± SE. Statistical significance was evaluated by the two-tail Student's «t» test for paired variates.

Results

Several successive absorption periods were used for each animal in order to find the control values for D-galactose absorption. Another series was then carried out with VIP added to the sugar solution, followed by another series with hormone-free solution. The absorption of 1 mM D-galactose did not diminish significantly when the sugar solution contained 10^{-7} or 10^{-8} M VIP (fig. 1).

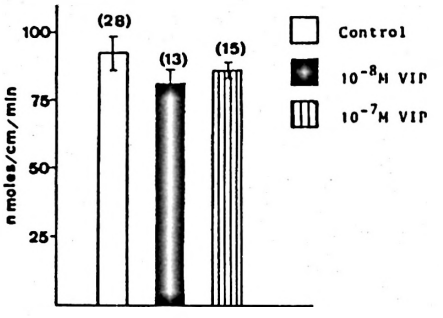

Fig. 1. *Absorption of 1 mM D-galactose (nmoles/cm/min) by rabbit jejunum* in vivo, *in the absence or in the presence of different concentrations VIP.* Mean values ± SE. Data number in parenthesis.

In other experiments (fig. 2), after some control absorption periods, the lumen was exposed for 5 min to a saline solution with 10^{-8} or 10^{-7} M VIP, and the D-galactose absorption with the same VIP concentration was then measured. The jejunum was then thoroughly washed with saline solution (150 mM NaCl) and finally D-galactose absorption from a VIP-free solution was determined. These

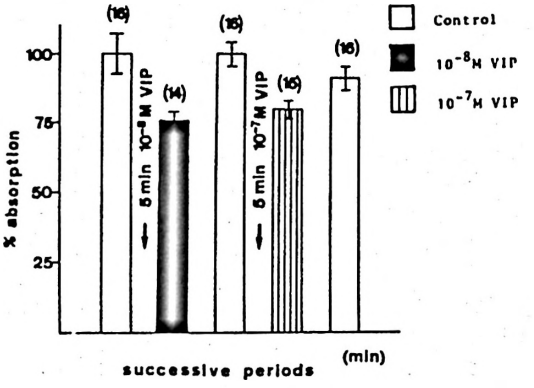

Fig. 2. *Effects of VIP on 1 mM D-galactose absorption by rabbit jejunum* in vivo. Means values ± SE, given as percent of control D-galactose absorption. Data number in parenthesis.

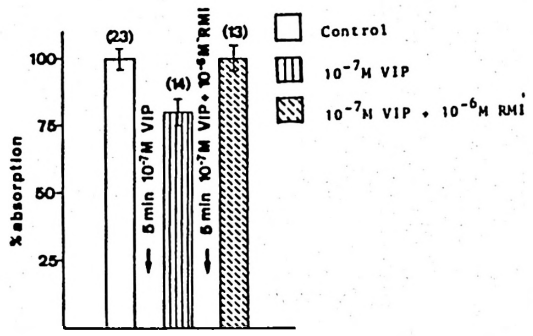

Fig. 3. *Absorption of 1 mM D-galactose by rabbit jejunum* in vivo *in the absence or in the presence of* 10^{-7}*M VIP,* 10^{-6}*M RMI 12330A.*
Mean values ± SE, given as percent of control D-galactose absorption. Data number in parenthesis.

experiments show that, when the mucosa had previously been exposed to the hormone, VIP significantly ($p < 0.001$) reduced D-galactose absorption and the inhibitory effect reached 21 %. On the other hand, washing with saline solution reversed the VIP inhibition, restoring D-galactose absorption to its control values. These results suggest that VIP takes some time to exert its inhibitory influence on D-galactose absorption.

Further experiments were carried out with other groups of animals. After some periods of sugar absorption under control conditions, the mucosa was exposed to 10^{-7} M VIP for 5 min and D-galactose absorption with the same VIP concentration was then measured. The jejunum segment was immediately washed with saline solution, followed by the mucosa being exposed to 10^{-7} M VIP and 10^{-6} M RMI 12330A (which has been described as an adenyl cyclase inhibitor) for 5 min, and then the galactose absorption with the same VIP and RMI 12330A concentration was measured (fig. 3).

RMI 12330A completely reversed the VIP inhibition, restoring D-galactose absorption to its control values. The results suggest that inhibition of intestinal sugar transport by VIP, probably involves intracellular action.

Discussion

Luminal VIP at 10^{-7} or 10^{-8} M concentration decreased D-galactose absorption in rabbit jejunum *in vivo*, which is in agreement with previous observations for absorption in pigs (13).

The inhibitory effect of VIP was only observed when the intestinal mucosa had previously been exposed to the hormone for 5 min and disappeared afterwards through washing with saline solution. These results suggest that it takes some time for VIP to exert its effect and that this action does not damage the intestinal mucosa.

These results could suggest that the VIP inhibitory action on D-galactose intestinal absorption is due to the binding of VIP to receptors on enterocyte membranes and to a consequent increase in intracellular cAMP (1, 2, 5, 13, 14). This action of VIP could be blocked by RMI 12330A (7, 15).

The increase in intracellular cAMP produces an inhibitory effect on the basolateral intestinal outflux of D-galactose as observed in rat and guinea pig *in vitro* under theophylline action (8). The inhibition of the basolateral exit could explain the inhibition of intestinal absorption.

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Resumen

La absorción de D-galactosa a través del yeyuno de conejo en periodos de perfusión de 1 min, no se altera por la presencia de VIP 10^{-7} y 10^{-8} M en la

solución del azúcar. Si se expone la mucosa al VIP durante 5 min, las absorciones sucesivas quedan inhibidas. Esta inhibición es reversible por lavado con solución salina. El efecto del VIP desaparece cuando se añade RMI 12330A 10^{-6} M a la solución junto con VIP 10^{-7} M y D-galactosa 1 mM. Estos resultados sugieren la existencia de receptores del VIP en el borde en cepillo de la membrana y su acción podría estar mediada a través del AMPc.

Palabras clave: Absorción de galactosa, VIP, Yeyuno de conejo.

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