

Effect of serotonin on D-galactose transport across the rabbit jejunum

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Summary — The patterns of storage and release of serotonin found in the enterochromaffin cells of the intestinal mucosa suggest that this hormone may be an important modulator of intestinal functions. Serotonin has been shown to produce secretion of water and electrolytes in rabbit ileum, but the hormone does not appear to interact significantly with other transport processes. The aim of the present study was to determine the effect of serotonin on D-galactose absorption in rabbit jejunum. The results obtained show that serotonin (10^{-8} and 10^{-6} M) partially reduced (by 20 and 40 % respectively) D-galactose uptake across the mucosal border. This effect was concentration-dependent, and it seemed to be caused by the inhibition of Na^+ -dependent sugar transport. Methysergide, an antagonist of serotonin which binds with receptor 2 of serotonin, blocked the effect of serotonin. These findings suggest that serotonin may act as a regulator of sugar intestinal absorption, and that this serotonin regulation could be mediated by a direct or indirect action of the complex serotonin—receptor, which may inhibit the Na^+ -dependent transport system of sugars located in the brush-border membrane.

serotonin — D-galactose — intestinal transport — rabbit

Résumé — **Effet de la sérotonine sur le transport du D-galactose dans le jéjunum du lapin.**

La sérotonine est une hormone gastro-intestinale qui se trouve principalement dans les cellules entérochromaffines de la muqueuse intestinale. L'étude du stockage comme de la libération de la sérotonine dans le tractus gastro-intestinal, a suggéré que la sérotonine peut agir comme modulateur des fonctions intestinales. La sérotonine produit la sécrétion d'eau ainsi que des électrolytes dans l'iléum du lapin, mais cette hormone n'a montré aucune interaction significative avec les autres processus de transport intestinal. Le but de ce travail a été de déterminer l'effet de la sérotonine sur l'absorption du D-galactose dans le jéjunum du lapin. Les résultats obtenus montrent que la sérotonine (10^{-8} et 10^{-6} M) a diminué partiellement (20 et 40 % respectivement) l'entrée du D-galactose dans la muqueuse. Cet effet dépend du degré de concentration de la sérotonine et il nous semble qu'il pourrait être une inhibition du transport des sucres dépendant du Na^+ . La métysergide, un antagoniste de la sérotonine qui se lie au récepteur 2 de la sérotonine, a inhibé l'effet de la sérotonine. Ces résultats suggèrent que la sérotonine pourrait jouer une fonction régulatrice sur l'absorption intestinale des sucres et que cette régulation pourrait aussi être médiée par une action directe ou indirecte du complexe récepteur-sérotonine inhibé par le système de transport des sucres dépendant du Na^+ situé dans la membrane de la bordure en brosse.

sérotonine — D-galactose — transport intestinal — lapin

INTRODUCTION

Serotonin (5-hydroxytryptamine) (5 HT) has been found throughout the gastrointestinal tract, mainly in the mucosa and in significant amounts in the myenteric plexus. The major locus of serotonin in the body appears to be the enterochromaffin cells (EC) which are located in the base of the crypts and villi (Nemoto *et al.*, 1982) of the intestinal mucosa.

It would be appear that serotonin is released from the enterochromaffin cells into both the circulation and the intestinal lumen (Ahlman *et al.*, 1981; Gronstad *et al.*, 1984) and the mechanisms for these releases have been shown to be independent in both cases (Money *et al.*, 1988). Furthermore, the release of serotonin seems to be regulated by either cholinergic, adrenergic and/or peptidergic nerves as well as by intraluminal stimuli (Ferrara *et al.*, 1987; Gronstad *et al.*, 1987). These observations suggest that the local paracrine/neurocrine actions mediated by serotonin may be important modulators of intestinal functions (Ferrara *et al.*, 1987; Money *et al.*, 1988).

Luminal administration of 5 HT has a number of effects on the gastrointestinal tract, including increased motility (Björk *et al.*, 1988), altered mesenteric circulation (Biber *et al.*, 1973; Fondacaro, 1984; Gronstad *et al.*, 1986) and an increase in the production of protective mucus (Black *et al.*, 1979). It is also a well known fact that serotonin causes net water and electrolyte secretion in the small intestine *in vivo* (Donowitz *et al.*, 1977; McFadden *et al.*, 1986) and *in vitro* (Donowitz *et al.*, 1980a).

However, as some secretagogues like theophylline (Randles et Kimmich, 1978; Ilundain *et al.*, 1985) and gastrointestinal hormones such as somatostatin (Sorribas

et al., 1987) or VIP (Rodriguez-Yoldi *et al.*, 1988) have interfered with intestinal sugar transport, the aim of the present study was to investigate whether serotonin has an effect on D-galactose absorption in jejunum of rabbits; and if so, to determine the causes.

MATERIALS AND METHODS

Animals and incubation solutions

The study was performed on male New Zealand rabbits weighing 2.0–2.5 kg. The animals were maintained on a standard rabbit diet with free access to water. After killing by a blow on the head, the proximal jejunum (5 cm distal to the ligament of Treitz) 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. The Ringer's solution contained in mM : 140 NaCl, 10 KHCO₃, 0.4 KH₂PO₄, 2.4 K₂HPO₄, 1.2 CaCl₂ and 1.2 MgCl₂, and was continuously bubbled with 95 % O₂/5 % CO₂. In experiments where Na⁺- free conditions were required, NaCl was omitted and replaced with 140 mM choline chloride.

Extracellular space determinations

Pieces of jejunum were incubated in Ringer's solution at 37 °C containing 0.02 µCi/ml¹⁴C-labelled poly(ethylene glycol) (PEG) (m. w. 4000, PEG 4000, Amersham) for 30 min. The pieces of mucosa were then gently blotted on filter paper and weighed, then extracted in 1 ml 0.1 N HNO₃ overnight. Aliquots of the extracts were counted with aliquots of the bathing solutions. Following extraction, the tissues were dried at 80 °C for 24 h, then reweighed. Tissue water was calculated as the difference between wet and dry weights.

The serotonin at two concentrations (10^{-6} and 10^{-8} M) was present in the bathing solution from the start of the incubation and had no significant effect either on the extracellular space (ranging from 0.07 ± 0.005 — 0.066 ± 0.004 ml/g wet weight), tissue water fraction (ranging from 0.86 ± 0.002 — 0.87 ± 0.002 ml/g wet weight) or cell water fraction (ranging from 0.79 ± 0.004 — 0.80 ± 0.004 ml/g wet weight).

Sugar uptake measurements

Pieces of jejunum weighing ≈ 100 mg were incubated at 37°C in Ringer's solution containing D-galactose labelled with ^{14}C for 30 min. Previous results obtained in our laboratory have shown that the steady-state is reached when tissues are incubated for 30 min. At the end of the experiment the tissues were washed with gentle shaking in ice-cold Ringer's solution and blotted carefully on both sides to remove excess moisture. The tissue was weighed wet and extracted by shaking for 15 h in 0.5 ml 0.1 N HNO_3 . Samples were taken from the bathing solutions and from the extracts of the tissues for radioactivity counting. All the modifiers were added to the incubation solution at the beginning of the incubation period. The D-galactose uptake results were expressed as the ratio between the concentration of sugar in cell water (C) after correction for extracellular space, and the medium sugar concentration (M), C/M.

Transepithelial flux measurements

The stripped mucosa was mounted as a flat sheet in Ussing-type chambers. The bathing solutions on the mucosal and surfaces of the tissue were maintained at 37°C using a circulating water bath. Both solutions contained D-galactose at the same concentration. Mucosal to serosal sugar fluxes were measured by placing the ^{14}C -labelled galactose in the mucosal side, and serosal to mucosal fluxes by placing the ^{14}C -labelled galactose in the serosal side. Samples were removed from the

non-radioactively labelled side at 20-min intervals for 80 min, after a 60-min preincubation period. One sample only was taken for counting from the radioactively labelled side. Samples of the radioactive solution were counted using a liquid scintillation counter.

Materials

D-galactose and serotonin were obtained from Sigma (St. Louis, MO). Methysergide was supplied by Sandoz. PEG was obtained from Merck.

Statistics

Results are expressed as mean \pm SE. Statistical significance was evaluated by the analysis of variance (ANOVA) and the Sheffe's test (Sheffe, 1953).

RESULTS

Effect of serotonin on steady-state cell water D-galactose accumulation

In control conditions (in the absence of exogenous modifiers) the C/M ratio (cell water free sugar concentration/sugar concentration in the incubation solutions) was ≈ 3.26 . When serotonin was present in the medium at a concentration of 10^{-8} or 10^{-6} M, the C/M ratios were 2.56 and 1.76 respectively. Serotonin at both concentrations (10^{-8} or 10^{-6} M) was found to reduce the D-galactose accumulation in rabbit jejunum by 20 and 40 % respectively (see Table I).

Table I. Effect of different concentrations of serotonin (5HT) on the cell to medium sugar concentration ratio (C/M).

[5 HT] M	C/M		
	0	10^{-8}	10^{-6}
	3.42 ± 0.05 (8)	$2.56 \pm 0.06^*$ (8)	$1.76 \pm 0.06^*$ (8)

Concentration of sugar in the incubation solution was 0.5 mM. The values presented are the means \pm SE. The figures in brackets indicate the number of rabbits.

* $P < 0.001$ compared with 5 HT = 0 M.

Effect of serotonin on transmural mucosal to serosal (Jms), serosal to mucosal (Jsm) and net (Jms—Jsm) D-galactose fluxes

The results summarized in Table II show that serotonin at both 10^{-8} and 10^{-6} M concentrations significantly reduced

mucosal to serosal D-galactose flux, and this reduction was greater at 10^{-6} M serotonin. Neither 10^{-6} nor 10^{-8} M modified serosal to mucosal D-galactose fluxes. Consequently net fluxes (Jms—Jsm) significantly diminished when serotonin (10^{-6} or 10^{-8} M) was present in the medium.

Table II. Effect of serotonin (5 HT) on transmural mucosal to serosal (Jms); serosal to mucosal (Jsm) and net (Jms—Jsm) fluxes across sheets of rabbit jejunum.

[5 HT] M	$\mu\text{mol D-galactose} \times \text{cm}^{-2} \times \text{h}^{-1}$		
	0	10^{-8}	10^{-6}
Jms	0.263 ± 0.013 (15)	$0.176 \pm 0.011^*$ (14)	$0.161 \pm 0.011^*$ (14)
Jsm	0.064 ± 0.004 (12)	0.073 ± 0.003 (12)	0.073 ± 0.003 (12)
Jnet	0.199 ± 0.013 (12)	$0.103 \pm 0.011^*$ (12)	$0.087 \pm 0.01^*$ (12)

Sugar concentration in the bathing solution was 4 mM. The values presented are the means \pm SE of the number of rabbits (indicated in brackets).

* $P < 0.001$ compared with 0 M 5 HT.

Effect of serotonin on D-galactose Na⁺-dependent transport

The D-galactose concentration assayed was 20 mM to guarantee that both Na⁺-dependent transport and passive diffusion worked in the experiments. When Na⁺ was removed from the bath solution serotonin did not modify either D-galactose accumulation or D-galactose mucosal to serosal flux (see Table III).

Effect of methysergide on serotonin-induced reduction of D-galactose transport in rabbit jejunum

Methysergide is a derivative of LSD and acts as an inhibitor of the peripheral effects of serotonin (Leff and Martin, 1988). When methysergide and serotonin were both in the bath solution D-galactose accumulation reached the control values, so methysergide reduced the effect of serotonin on D-galactose transport. Methysergide alone did not modify the D-galactose accumulation in rabbit jejunum (Table IV).

DISCUSSION

Serotonin (5 HT) has been found throughout the gastrointestinal tract and several authors have claimed that serotonin plays an important physiological part in the intestinal functions by acting on several intestinal processes.

Some studies have shown that serotonin alters electrolyte transport in rabbit ileum by inhibiting neutral NaCl absorption but does not seem to interact significantly with other ileal transport processes, such as D-glucose intestinal absorption (Donowitz *et al.*, 1980a).

The results obtained in our laboratory show that serotonin partially diminishes both steady-state cell water D-galactose accumulation and mucosal to serosal (Jms) transmural D-galactose fluxes in rabbit jejunum (Tables I and II). These findings indicate that serotonin reduces the D-galactose uptake across the brush border of the jejunal mucosa and that 5 HT could act on the transport system of monosaccharide located on the brush border.

However, as D-galactose transport across the jejunal brush border is mainly

Table III. Effect of serotonin (5 HT) on D-galactose transport in the absence of Na⁺ in the medium.

[5 HT] M	C/M			Jms		
	0	10 ⁻⁸	10 ⁻⁶	0	10 ⁻⁸	10 ⁻⁶
[Na ⁺] 140 mM	1.0 ± 0.09 (5)	0.74 ± 0.07 ** (5)	0.61 ± 0.06 * (5)	1.02 ± 0.05 (10)	0.88 ± 0.03 (10)	0.73 ± 0.06 * (10)
[Na ⁺] 0 mM	0.51 ± 0.05 (5)	0.47 ± 0.02 (5)	0.47 ± 0.04 (5)	0.48 ± 0.04 (10)	0.49 ± 0.03 (9)	0.55 ± 0.04 (10)

D-galactose concentration in C/M and Jms experiments was 20 mM. C/M is the cell to medium sugar concentration ratio. Jms is expressed in $\mu\text{mol D-galactose} \times \text{cm}^{-2} \times \text{h}^{-1}$. The values are the means \pm SE. The figures in brackets indicate the number of rabbits.

* $P < 0.001$ compared with 0 M 5 HT; ** $0.001 < P < 0.05$ compared with 0 M 5 HT.

Table IV. Effect of methysergide on serotonin induced reduction of D-galactose transport in rabbit jejunum.

C/M			
Cont.	Met	5 HT	5 HT + Met
3.12 ± 0.14 (10)	3.08 ± 0.21 (10)	1.88 ± 0.08 * (10)	3.60 ± 0.33 # (10)

The values presented are the means ± SE; the figures in brackets indicate the number of rabbits. D-galactose concentration : 0.5 mM. Cont : tissues incubated in the absence of serotonin and methysergide; 5 HT : tissues incubated in presence of serotonin 10⁻⁶ M; 5 HT + Met; tissues incubated with serotonin 10⁻⁶ M and methysergide 10⁻⁶ M.

* $P < 0.001$ compared with Cont; # $P < 0.001$ compared with 5 HT.

the result of two mechanisms, Na⁺-dependent and passive transport, it was worth determining whether serotonin acts on only one or on both transport systems.

To this end, the effects of serotonin on D-galactose accumulation and on mucosal to serosal transmural fluxes were measured when Na⁺ had been removed from the bath solution. The results in Table III show that serotonin did not modify either sugar accumulation or Jms sugar fluxes in the absence of Na⁺. These findings suggest that serotonin might have a purely local action, principally located on the Na⁺-dependent transport.

In order to study the effect of serotonin in depth, the action of methysergide was assayed. Methysergide behaves as a competitive inhibitor of serotonin by interacting with serotonin peripheral receptors (Leff and Martin, 1988). Serotonin receptors have been localized in the gastrointestinal tract in two separate sites : the myenteric plexus and the mucosal-submucosal interface (Gershon *et al.*, 1985).

The results gathered in Table IV show that methysergide blocked the inhibition

produced by serotonin on D-galactose absorption but methysergide alone did not alter this process. These effects are in partial agreement with the results obtained from studies of the intestinal secretion produced by serotonin, in which two antagonists of serotonin that bind with type 2 serotonin receptors, methysergide (Donowitz *et al.*, 1977) and cisapride (Moriarty *et al.*, 1987), inhibit the secretory action of serotonin.

CONCLUSION

To sum up, the present study shows that serotonin produces a reduction of D-galactose absorption across rabbit jejunum, and that this effect seems to be located on Na⁺-dependent uptake across the mucosal border. This effect could be caused by a local effect of the hormone, mediated by the binding to receptors located in intestinal mucosa. Several authors have postulated the role of calcium in modulating the serotonin gastrointestinal effects (Donowitz *et al.*,

1980b; Beubler *et al.*, 1986; Zinner *et al.*, 1986; Money *et al.*, 1988). Further studies should attempt to determine whether calcium or other physiological substances are responsible for the effects of serotonin on sugar intestinal transport.

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REFERENCES

- Ahlman H., DeMagistris L., Zinner M. & Jaffe B.M. (1981) Release of immunoreactive serotonin into the lumen of the feline gut in response to vagal nerve stimulation. *Science* 213, 1 254-1 255
- Beubler E., Bukhave K. & Rask-Madsen J. (1986) Significance of calcium for the prostaglandin E₂-mediated secretory response to 5-hydroxytryptamine in the small intestine of the rat *in vivo*. *Gastroenterology* 90, 1972-1977
- Biber B., Fara J. & Lundgren O. (1973) Intestinal vascular responses to 5-hydroxytryptamine. *Acta Physiol. Scand.* 87, 526-534
- Black J.W., Bradbury J.E. & Wyllie J.H. (1979) Stimulation of colonic mucus output in the rat. *Br. J. Pharmacol.* 66, 456P-457P
- Björk S., Ahlman H., Dahlstrom A., Phillips S.F. & Kelli K.A. (1988) Serotonergic regulation of canine enteric motility (measured as electrical activity) and absorption: physiologic and morphologic evidence. *Acta Physiol. Scand.* 133, 247-256
- Donowitz M., Charney A.N. & Heffernan J.M. (1977) Effect of serotonin treatment on intestinal transport in the rabbit. *Am. J. Physiol.* 232, E85-E94
- Donowitz M., Tai Y.H. & Asarkov N. (1980a) Effect of serotonin on active electrolyte transport in rabbit ileum, gallbladder and colon. *Am. J. Physiol.* 239, G463-G472
- Donowitz M., Asarkov N. & Pike G. (1980b) Calcium dependence of serotonin-induced changes in rabbit ileal electrolyte transport. *J. Clin. Invest.* 66, 341-352
- Ferrara A., Zinner M.J. & Jaffe B.M. (1987) Intraluminal release of serotonin, Substance P, and gastrin in the canine small intestine. *Dig. Dis. Sci.* 32, 289-294
- Fondacaro J.D. (1984) Intestinal blood flow and motility. In: *Physiology of the Intestinal Circulation* (Shepherd A.P. & Granger D.N., eds.) N.Y. Raven Press, pp. 107-120
- Gershon M.D., Takaki M., Tamir H. & Branchek T. (1985) The enteric neural receptor for 5-hydroxytryptamine. *Experientia* 41, 863-868
- Gronstad K., Ahlman H., Dahlstrom A., Jaffe B.M. & Zinner M.J. (1984) The effects of vagal nerve stimulation on levels of serotonin and substance P in the portal vein of cat. *Biog. Amines* 1, 303-306
- Gronstad K., Dahlstrom A., Jaffe B.M. & Ahlman H. (1986) Regional and selective changes in blood flow of feline small intestine induced by endoluminal serotonin. *Acta Physiol. Scand.* 127, 207-213
- Gronstad K., Dahlstrom A., Florence M.J., Zinner M.J., Ahlman H. & Jaffe B.M. (1987) Regulatory mechanisms in endoluminal release of serotonin and substance P from feline jejunum. *Dig. Dis. Sci.* 32, 393-400
- Ilundain A., Alcalde A.I., Barcina Y. & Larralde J. (1985) Calcium dependence of sugar transport in rat small intestine. *Biochim. Biophys. Acta* 818, 67-72
- Leff P. & Martin G.R. (1988) The classification of 5-hydroxytryptamine receptors. *Med. Res. Rev.* 8, 187-202
- McFadden D., Jaffe B.M. & Zinner M.J. (1986) The effects of luminally administered serotonin and substance P on jejunal handling water and electrolytes in awake dogs. *Am. J. Surg.* 151, 81-86
- Money S.R., Kimura K., Patroianu A. & Jaffe B.M. (1988) Effects of intravenous calcium on release of serotonin into jejunal lumen and portal circulation. *Dig. Dis. Sci.* 33, 977-981

- Moriarty K.J., Higgs N.B., Woodford M., Warhurst G. & Turnberg L.A. (1987) Inhibition of the effect of serotonin on rat ileal transport by cisapride : evidence in favour of the involvement of 5 HT₂ receptors. *Gut* 28, 844-848
- Nemoto N., Kawadi A. & Shikata T. (1982) Immunohistochemical demonstration of serotonin (5-hydroxytryptamine) containing cells in the human and rat intestines. *Biomed. Res.* 3, 181-187
- Randles J. & Kimmich G.A. (1978) Effects of phloretin and theophylline on 3-O-methylglucose transport by intestinal epithelial cells. *Am. J. Physiol.* 234, C64-C73
- Rodriguez-Yoldi M.J., Arruebo P., Alcalde A.I. & Murillo M.D. (1988) Influence of VIP on D-galactose transport across rabbit jejunum *in vivo*. *Rev. Esp. Fisiol.* 44, 127-130
- Sheffe H. (1953) A method for judging all contrasts in the analysis of variance. *Biometrika* 40, 87-104
- Sorribas V., Arruebo P., Murillo D. & Alcalde A.I. (1987) Effect of somatostatin on D-galactose transport across the small intestine of rats. *Comp. Biochem. Physiol.* 86, 63-66
- Zinner M.J., McFadden D., Sherlock D. & Jaffe B.M. (1986) Verapamil reversal of serotonin-induced jejunal secretion of water and electrolytes in awake dogs. *Gastroenterology* 90, 515-519