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Effect of Different β-Adrenergic Agonists on the Intestinal Absorption of Galactose and Phenylalanine

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Abstract

Nutrient transport across the mammalian small intestine is regulated by several factors, including intrinsic and extrinsic neural pathways, paracrine modulators, circulating hormones and luminal agents. Because β-adrenoceptors seem to regulate gastrointestinal functions such as bicarbonate and acid secretion, intestinal motility and gastrointestinal mucosal blood flow, we have investigated the effects of different β-adrenergic agonists on nutrient absorption by the rat jejunum in-vitro.

When intestinal everted sacs were used the β_2-agonist salbutamol had no effect either on galactose uptake by the tissue or mucosal-to-serosal flux whereas mixed β_1- and β_2-agonists (isoproterenol and orciprenaline) and β_3-agonists (BRL 35135, Trecadrine, ICI 198157 and ZD 7114) inhibited galactose uptake and transfer of D-galactose from the mucosal-to-serosal media across the intestinal wall (although the inhibiting effects of isoproterenol and Trecadrine were not statistically significant). In intestinal everted rings both Trecadrine and BRL 35135 clearly reduced galactose uptake, the effect being a result of inhibition of the phlorizin-sensitive component. Total uptake of phenylalanine by the intestinal rings was also reduced by those β_3-adrenergic agonists.

These results suggest that β_1- and β_3-adrenergic receptors could be involved in the regulation of intestinal active transport of sugars and amino acids.

Transport of water, electrolytes and nutrients across the mammalian small intestine is regulated by different mechanisms, for example intrinsic and extrinsic neural pathways, paracrine modulators, circulating hormones and luminal agents. Neural modulation of transport is altered by adrenergic, cholinergic or peptidergic influences (Barry et al 1994).

β-Adrenergic receptors are members of a protein family that interacts with guanine nucleotide binding proteins (Emorine et al 1991) and in the presence of pharmacological agonists elicits a signal that results in an increase in intracellular cAMP levels (Caron et al 1985). An increase in intracellular cAMP leads to activation of protein kinase A, which results in protein phosphorylation and, therefore, in the physiological or pharmacological response (Lefkowitz & Caron 1988). The initial classification of β-adrenoceptors into β_1 and β_2 subgroups was based on the activity of different β-agonists as cardiac stimulants or bronchodilators respectively (Lands et al 1967). Further pharmacological experiments have shown the occurrence of distinct adrenoceptors among the α and β subtypes (Bond et al 1986; Bond & Clarke 1988). Thus, the atypical β_3-adrenoceptor is present in a wide variety of tissues, including brown and white adipose tissue, skeletal muscle, the brain, the gall bladder and several regions of the gastrointestinal tract, for example the oesophagus, gastric fundus, jejunum, ileum and colon (Manara et al 1995).

β-Adrenoceptors play important functions in the gastrointestinal tract. They mediate the stimulation of bicarbonate secretion in rat caecum (Canfield & Abdul-Ghaffar 1992) and acid secretion in the rat stomach (Canfield & Paraskeva 1992). In-vitro studies have shown that isoproterenol, salbutamol, SR 58611A and BRL 37344 stimulated bicarbonate secretion, and that SR 58611A and BRL 37344 also enhanced acid secretion in a concentration-dependent manner. It has been proved that β_3-adrenoceptors can play an important role in the regulation of intestinal motility (Thollander et al 1996) and are potent inhibitors of experimental gastrointestinal ulceration; it has been suggested that the

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mechanism of protection involves accentuation of gastrointestinal mucosal blood flow (Anthony 1996). Autoradiographic experiments using (−)-[^{125}I]cyanopindolol, a radioactive ligand specific for β_3-adrenoceptors, revealed atypical binding to the ileal mucosa of the rat, implying that these receptors could have a regulatory role in intestinal function (Roberts et al 1995).

In in-vitro experiments with rat intestine an increase of mucosal-to-serosal 3-*O*-methylglucose flux has been observed after mucosal perfusion with adrenaline, noradrenaline or several adrenergic agonists. The effect seems to be mediated by β_2-adrenoceptors and subsequent protein kinase A activation (Ishikawa et al 1997). Results obtained in our laboratory indicate that treatment of rats for 35 days with the β_3-agonist Trecadrine (Barrionuevo et al 1996) results in inhibition of galactose intestinal transport without modification of brush-border enzyme activity (Díez-Sampedro et al 1997).

The current experiments were performed to investigate the effect of different β-adrenergic agonists on in-vitro nutrient transport in the rat jejunum. The β-agonists tested were salbutamol, a β_2-agonist, the mixed β_1- and β_2-agonists isoproterenol and orciprenaline, and the β_3-agonists BRL 35135, Trecadrine, ICI 198157 and ZD 7114 (Howe 1993).

Materials and Methods

Animals

Experiments were performed on male Wistar rats, 180–200 g, supplied by the Centre of Applied Pharmacology and kept under good laboratory practice conditions. The rats were fasted for 24 h and anaesthetised by subcutaneous injection of 60 mg kg^{-1} sodium pentobarbital. A midline incision was made in the abdomen and the small intestine was revealed. The entire length of the jejunum was then carefully removed and placed immediately in ice-cold saline solution.

Intestinal everted sacs

The jejunum was rinsed with saline solution and everted jejunum sacs approximately 3 cm long were prepared (Pérez et al 1996). The sacs were filled with physiological solution containing the agonist and incubated for 30 min in saline solution (10 mL) containing D-galactose (2 mM) with [1-^{14}C]galactose (0·25 μCi) and the agonist. The composition of physiological solution was (mM): 140·0 NaCl, 5·6 KCl, 3·0 $CaCl_2$, 2·8 KH_2PO_4, 2·8 $MgSO_4$, 6·1 Tris and 4·9 HCl (pH 7·4). Incubation was performed with continuous oxygenation (95% O_2–5% CO_2) and different β-agonists were added directly to the incubation medium at final concentrations of 0·01 or 0·1 mM. At the end of the incubation period the sacs were removed, opened and emptied for assay of mucosal-to-serosal galactose flux. The tissue was washed in ice-cold saline solution and cut into two segments which were weighed and extracted individually for 24 h in nitric acid (0·1 M; 1 mL). Each segment was then dried for 48 h at 110°C and weighed to obtain the dry weight for calculation of the water content of the tissue. Samples were taken from the serosal medium, the incubation solution and the tissue extracts and the radioactivity was measured by liquid scintillation. Results are expressed as μmol galactose transferred to the serosal side g^{-1} wet weight and the sugar contained in the tissue in μmol g^{-1} wet weight or in mM concentration in the intracellular water.

Intestinal everted rings

Briefly, rat jejunum was everted and cut into small rings and four rings randomly taken were incubated in physiological solution (10 mL) containing D-galactose (2 mM) with [1-^{14}C]-galactose (0·25 μCi) or L-phenylalanine (1 mM) with [1-^{14}C]phenylalanine (0·25 μCi). Incubation was performed for 30 min at 37°C with continuous oxygenation (95% O_2–5% CO_2). The rings were then removed, washed with ice-cold saline solution, weighed and extracted for 24 h with nitric acid (0·1 M; 0·5 mL). Samples were taken from the incubation solution and from the tissue extracts and radioactivity was measured by liquid scintillation counting (Lugea et al 1994). To determine the passive uptake of galactose by the tissue, phlorizin (1 mM) was added to the saline solution. Trecadrine or BRL 35135 were added directly to the incubation medium at a final concentration of 0·01 or 0·1 mM.

Statistical methods

All results are expressed as mean ± s.e. (number of data). Means were compared by use of the non-paired Student's *t*-test. Differences were considered statistically significant if $P < 0·05$.

Results

The effect of different β-adrenergic agonists on transmural transport of galactose and on sugar uptake by intestinal everted sacs has been studied (Table 1). Although the β_2-agonist salbutamol did not modify either galactose uptake by the tissue or mucosal-to-serosal flux, the mixed β-agonists isoproterenol and orciprenaline and the β_3-agonists BRL 35135, ZD 7114, ICI 198157 and Trecadrine inhibited galactose uptake by the intestinal tissue. Transmural mucosal-to-serosal flux of sugar across

Table 1. Effect of β-adrenergic agonists on mucosal-to-serosal flux and on intestinal uptake of 2 mM galactose after 30 min incubation.

Treatment	Mucosal-to-serosal flux (μmol galactose g^{-1} wet weight)	Galactose uptake (μmol galactose g^{-1} wet weight)	Galactose uptake (mmol L^{-1})
Control	10·11 ± 0·41 (13)	4·17 ± 0·13 (29)	5·40 ± 0·16 (27)
Salbutamol	10·20 ± 0·51 (13)	3·95 ± 0·17 (30)	5·27 ± 0·23 (29)
Control	11·51 ± 0·43 (10)	5·16 ± 0·21 (23)	6·96 ± 0·27 (22)
Isoproterenol	10·81 ± 0·53 (12)	4·53 ± 0·12 (23)*	6·19 ± 0·17 (23)*
Control	11·24 ± 0·75 (9)	4·59 ± 0·27 (24)	6·67 ± 0·34 (23)
Orciprenaline	8·05 ± 0·87 (9)*	3·29 ± 0·29 (23)‡	4·27 ± 0·35 (22)‡
Control	10·26 ± 0·60 (9)	4·11 ± 0·15 (20)	5·52 ± 0·20 (20)
BRL 35135	7·75 ± 0·34 (9)†	3·11 ± 0·12 (20)‡	4·12 ± 0·14 (21)‡
Control	11·47 ± 0·37 (8)	5·20 ± 0·14 (21)	7·12 ± 0·20 (22)
Trecadrine	11·09 ± 0·86 (9)	4·53 ± 0·14 (22)†	6·09 ± 0·49 (20)‡
Control	9·35 ± 0·61 (14)	4·87 ± 0·17 (30)	6·31 ± 0·19 (26)
ZD 7114	7·40 ± 0·44 (14)*	4·06 ± 0·13 (27)‡	5·47 ± 0·18 (28)†
Control	10·33 ± 0·21 (13)	4·89 ± 0·15 (28)	6·67 ± 0·19 (27)
ICI 198157	8·12 ± 0·33 (13)‡	4·17 ± 0·53 (30)‡	5·69 ± 0·68 (29)‡

Agonists were tested at 0·01 mM (Trecadrine, ZD 7114 and ICI 198157) or 0·1 mM. Results are means ± s.e. The number of determinations is given in parentheses. *$P < 0·05$, †$P < 0·01$, ‡$P < 0·001$, significantly different from control values.

the intestinal wall was reduced by the presence of orciprenaline, ZD 7114, ICI 198157 or BRL 35135; the effects of isoproterenol and Trecadrine were not significant (Table 1). Uptake by the tissue was more sensitive than mucosal-to-serosal flux to the presence of the agonists. Thus some β-agonists which have no effect on galactose flux significantly inhibit sugar uptake.

Because all β_3-agonists clearly inhibit sugar transport the second part of the study was focused on them; the effect of BRL35135 (0·1 mM) and Trecadrine (0·1 or 0·01 mM) on nutrient uptake by everted intestinal rings was studied. Results confirmed that both compounds clearly reduced total uptake of galactose after 30 min incubation. When sugar transport was inhibited with 1 mM phlorizin the same galactose uptake value was obtained under all conditions: control (0·85 ± 0·03, n = 58), 0·01 mM Trecadrine (0·82 ± 0·03, n = 38), 0·1 mM Trecadrine (0·82 ± 0·02, n = 40) or 0·1 mM BRL 35135 (0·97 ± 0·08, n = 28). These values represent about the 10–15% of the value obtained in the absence of phlorizin and the agonists, implying that phlorizin-resistant passive component was not affected by β_3-agonists.

Also total uptake of phenylalanine by intestinal rings was clearly reduced by the presence of the β_3-agonists (Table 2), inhibition by BRL 35135 being higher than that by Trecadrine.

Discussion

The in-vitro experiments showed that different β-agonists inhibit intestinal transport of galactose. Among the products tested only salbutamol (0·1 mM), a specific β_2-adrenergic agonist, did not modify sugar absorption, which could indicate that β_2-adrenoceptors are not involved. However, the mixed β_1- and β_2-agonists isoproterenol and orciprenaline, induced a clear reduction in galactose uptake by the intestinal tissue. By using a double perfusion technique in the rat small intestine in-vitro, a stimulatory effect on transmural transport of glucose has been obtained when adrenaline and also noradrenaline are perfused into the luminal side. The same effect was also observed with

Table 2. Effect of Trecadrine and BRL 35135 on phenylalanine uptake by intestinal rings after 30 min incubation.

Treatment	μmol phenylalanine g^{-1} wet weight		Inhibition (%)
	With agonist	Control	
Trecadrine 0·01 mM	3·02 ± 0·15 (38)*	4·64 ± 0·11 (20)	34·9
Trecadrine 0·1 mM	3·09 ± 0·12 (32)*	4·47 ± 0·20 (20)	31·5
BRL 35135 0·1 mM	1·48 ± 0·11 (32)*	2·99 ± 0·14 (20)	50·7

Results are means ± s.e. The number of determinations is given in parentheses. *$P < 0·001$, significantly different from control values.

0·01 mM isoproterenol or procaterol and has been explained as a consequence of the occurrence of β_2-adrenoceptors in the intestinal tissue (Ishikawa et al 1997).

With an in-vivo canine model of ileal absorption, the intraluminal perfusion of noradrenaline did not modify glucose absorption, whereas absorption was reduced by the α_2-adrenergic agonist clonidine, suggesting the participation of this receptor in Na^+-coupled nutrient transport (Barry et al 1993, 1994). It has also been shown that α_1-adrenergic receptor activation results in a proabsorptive response for water and ions, whereas α_2- and β-receptor activation induces a prosecretory response (Barry et al 1994). In this sense, α_2-adrenoceptors are preferentially expressed in the intestinal crypts (Paris et al 1990).

The four β_3-adrenergic agonists tested, BRL 35135, Trecadrine, ZD 7114 and ICI 198157 clearly inhibited both sugar uptake by the intestinal tissue and transmural transport of galactose. It can be assumed that the activation of β_3-adrenoceptors by their corresponding agonists results in a reduction in galactose transport in rat small intestine.

Furthermore, by use of rat intestinal rings it can be shown that Trecadrine (0·01 and 0·1 mM) and BRL 35135 (0·1 mM) both reduce the active-transport component of sugar absorption, because when phlorizin is present in the incubation medium the inhibitory effect on sugar uptake is not observed. Trecadrine at 0·01 mM inhibited 20% of galactose transport; at 0·1 mM inhibition was 28%. Inhibition by BRL 35135 is quite similar, reaching 32% at 0·1 mM.

It has been reported that β_3-adrenoceptors play a significant role in the increase of glucose uptake in response to adrenergic stimulation in brown adipocytes (Nikami et al 1996). Although glucose transport in adipocytes and enterocytes is by different mechanisms, this supports the belief that β_3-adrenoceptors are important in the regulation of sugar transport.

Phenylalanine uptake by jejunum intestinal rings is also reduced by Trecadrine and BRL 35135. Inhibition is similar (34%) irrespective of whether the concentration of Trecadrine is 0·01 or 0·1 mM, which seems to indicate that the effect is maximum at the lower concentration. The inhibitory effect of BRL 35135 on phenylalanine absorption is higher than that of Trecadrine; the uptake is reduced by 50%. The effect resulting from activation of β_3-adrenoceptors seems to be greater for phenylalanine uptake than for uptake of galactose.

Because our experiments were performed in-vitro, indirect effects of β_3-agonists on motility or intestinal blood flow can be excluded (Anthony 1996). Thus inhibition of galactose or phenylalanine transport could be related to a direct effect on enterocyte transporters.

The concentrations of the agonists used are higher than physiological adrenaline serum concentrations, as is usually reported for in-vitro assays; they were chosen according to the reported effects of Trecadrine on glycerol release (Martínez et al 1996). It has been reported that high levels of noradrenaline can be achieved in the extracellular fluid around enterocytes (Paris et al 1990; Hilde-brand & Brown 1992). The potential role of β_3-adrenoceptors on modulation of intestinal transport is also supported by our previous results in ex-vivo experiments. After 35 days treatment with Trecadrine (1 mg kg^{-1} day^{-1}, i.p.) sugar uptake by rat intestinal rings was clearly inhibited without changes in sucrase or maltase enzymatic activity (Díez-Sampedro et al 1997). This inhibition of intestinal absorption of nutrients by β_3-agonists could be related to the anti-obesity effect of these drugs (Howe 1993).

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