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CENTRAL NERVOUS STIMULI INCREASE DUODENAL BICARBONATE SECRETION BY RELEASE OF MUCOSAL MELATONIN

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A number of common diseases in humans, including gastroduodenal ulcer and irritable bowel syndrome, show circadian rhythms in pain and discomfort. The neurohormone melatonin is released from enterochromaffin cells in the intestinal mucosa and from the pineal gland but its role in gastrointestinal function is largely unknown. We have studied the involvement of melatonin in stimulation of the mucosa-protective alkaline secretion by the duodenal mucosa. A 12-mm segment of proximal duodenum with an intact blood supply was cannulated in situ in anesthetized rats and duodenal HCO₃⁻ secretion titrated by pH-stat. Duodenal close intra-arterial infusion of melatonin or the full agonist 2-iodo-N-butanoyl-5-methoxytryptamine significantly increased the secretion and pretreatment with the melatonin (predominantly MT₂-receptor specific) antagonist luzindole almost abolished the response. Intracerebroventricular (i.c.v.) infusion of the alpha₁-adrenoceptor agonist phenylephrine (12.2 μg kg⁻¹ h⁻¹) caused an up to fivefold increased in the alkaline secretion and the melatonin antagonist luzindole or cutting all peri-carotid nerves abolished the duodenal secretory response to i.c.v. phenylephrine. Peripheral melatonin thus stimulates duodenal mucosal HCO₃⁻ secretion and endogenous melatonin, very likely released from mucosal enterochromaffin cells, is involved in mediating neural stimulation of the secretion.

Key words: Bicarbonate secretion, duodenum, enterochromaffin cells, luzindole, phenylephrine

INTRODUCTION

The duodenal epithelium secretes HCO₃⁻ at higher rates than does the stomach (or more distal small intestine) and the duodenal secretion is currently accepted as the most important defence mechanism against acid discharged from the stomach (1, 2). HCO₃⁻ entering the continuous layer of visco-elastic mucus gel on top of the epithelial surface maintains pH in its cell-facing portion at neutrality at acidities encountered in the healthy duodenum.

The mucosal HCO₃⁻ secretion is under central as well as local intestinal neurohumoral influence. This made it of interest to study effects of the
neurohormone melatonin on mucosal $\text{HCO}_3^-$ secretion (3, 4). Melatonin is synthesized and released from the pineal gland in a circadian fashion and exerts its action within the central nervous system as well as in peripheral tissues. Circadian rhythms in pain and discomfort are frequently observed in duodenal ulcer and other gastrointestinal diseases. The incidence of gastroduodenal ulcer reportedly peaks at certain periods of the year (5). Knowledge about the neurohumoral mediation of these variations may provide interesting information about intestinal pathophysiology and provide clues to future therapy. Importantly, melatonin is also produced by the enterochromaffin cells (EC-cells) of the intestinal mucosa (6) and some studies suggest that nutrients in the lumen influences the intestinal release of melatonin and other EC-cell products.

METHODS

This study was approved by the Uppsala University Ethical Committee for Experiments with Animals. Male outbred Sprague-Dawley rats (Mølegenaaard Breeding Center, Ejby, Denmark) weighing 190—260 g or $F_1$-hybrids of Lewis x Dark Agouti rats (Animal Department, Biomedical Center, Uppsala, Sweden), weighing 200—260 g were placed in a conditioning unit under standardized temperature and light conditions (21—22°C, 12:12 h light-dark

![Diagram](image.png)

Fig. 1. The hepatic artery was cannulated, tied 3—4 mm proximal to its entrance into the liver (indicated by arrow), and perfused in the retrograde direction at 17 µl/min. The perfusion results in distribution of the perfusate mainly to the duodenum (via the cranial pancreatico-duodenal artery), enabling the use of very low doses of secretagogues and avoiding the possibility of central nervous actions of infused compounds.
cycle) for at least four days after purchase. The rats were kept in cages in groups of two or more and had access to tap water and pelleted food (Ewos, Södertälje, Sweden) *ad libitum*.

Animals were deprived of food for 20—24 h before the experiments, but had free access to drinking water. Experiments were started by anaesthetizing the animal close to 9 am with 5-ethyl-5-(1'-methyl-propyl)-2-thiobarbiturate (Inactin®, RBI, Natick, MA), 120 mg/kg body weight intraperitoneally. Anesthesia was performed within the Animal Department by the person who had previously handled the animals. Subsequently, the rats were tracheotomized with a tracheal tube to facilitate respiration and body temperature was maintained at 37—38°C throughout experiments by a heating pad controlled by a rectal thermistor probe. The surgical and experimental procedures have been described previously (3, 7, 8). A brief summary is given and some modifications, including close intra-arterial (i.a.) infusion to the duodenum, are described here. A femoral artery and vein were catheterized with PE-50 polyethylene catheters (Becton-Dickinson,

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**Fig. 2.** Melatonin and the full melatonin receptor agonist 2-IbMT administered to the duodenum by close intra-arterial infusion significantly (*P* < 0.05) increased mucosal HCO$_3^-$ secretion. Means ± SE of HCO$_3^-$ secretion and mean arterial blood pressure (BP) are shown (*n* = 6 in both groups).
Parsippany, NJ). For continuous recordings of systemic arterial blood pressure, the arterial catheter containing 20 IU/ml of heparin isotonic saline, was connected to a transducer operating a PowerLab system (AD Instr., Hastings, UK). The vein was used for injection of some of the drugs as well as for continuous infusion of Ringer solution at a rate of 1.0 ml/h. The latter was made to compensate for fluid loss and to avoid acid-base changes during the experiments. The duodenal transmucosal electrical potential difference (PD) was measured in some experiments and recorded between the duodenal mucosa and posterior vena cava with a high-input impedance voltmeter via matched calomel half-cells. The half-cells were connected to the animal by means of agar bridges (2 M KCl) with their distal ends located in the luminal solution and the posterior vena cava, respectively.

The abdomen was opened by a midline incision and the gastric pylorus was ligated with a suture. For measurement of duodenal mucosal HCO$_3^-$ secretion, a 12 mm segment of duodenum with its blood supply intact, starting 10—12 mm distal to the pylorus was cannulated in situ between two glass tubes connected to a reservoir. Fluid (10 ml of 154 mM NaCl), maintained at 37°C

**Fig. 3.** Intracerebroventricular (i.c.v.) infusion of melatonin (20 nmol/kg,h) did not affect the HCO$_3^-$ secretion by the duodenal mucosa. Rates of secretion in melatonin-infused animals was not different (P > 0.05) from that in controls infused with vehicle (artificial cerebrospinal fluid) alone (n = 6 in both groups).
by a water jacket, was rapidly circulated by a gas lift of 100% oxygen. HCO$_3^-$
secretion into the luminal perfusate was continuously titrated with 50 mM HCl
at pH 7.4 under automatic control of a pH-stat system (Radiometer, Copenhagen,
Denmark).

To study effects elicited in the duodenal segment and minimizing possible
central nervous actions, compounds were administered to the duodenum by
close i.a. infusion (Fig. 1). The hepatic artery was cannulated, tied 3—4 mm
proximal to its entrance into the liver, and perfused in the retrograde direction
at 17 µl/min. This perfusion results in distribution of the perfusate mainly to
the duodenum (via the cranial pancreatico-duodenal artery) and pancreas. The
distribution was checked visually at the start of experiments by i.a. injection of
a small amount (~0.1 ml) of a marker dye (Evans Blue, 2% solution in saline).

I.c.v. infusions were made to study duodenal secretory stimulation elicited
in the central nervous system. A metal cannula was inserted into the right
lateral cerebral ventricle by using a stereotaxic instrument (Model 900; Kopf
Instr., Tujunga, CA). A skin incision was made over the right parietal bone,
and a 1 mm hole was drilled through the bone, 0.8 mm posterior to the bregma
and 1.5 mm lateral to the midsagittal suture. A stainless steel cannula was
inserted stereotactically and cemented to the skull with cement (Fuji type II,
GC Corp., Tokyo, Japan). Artificial cerebrospinal fluid [(in mM) 151.5 Na$^+$,
3.0 K$^+$, 1.2 Ca$^{2+}$, 0.8 Mg$^{2+}$, 132.8 Cl$^-$, 25 HCO$_3^-$, 0.5 phosphate; pH 7.4] was
infused through this cannula at a rate of 30 µl/h. All agents infused i.c.v. had
been dissolved in artificial cerebrospinal fluid. The location of the end of the
cannula within the i.c.v. space was tested at the end of most experiments by
adding Evans blue solution to infusate, followed by dissection of the brain.

Data analyses

Descriptive statistics are expressed as means ± SE, with the number of
experiments given in parentheses. Rates of alkaline secretion by the duodenum are
expressed as microequivalents of base (HCO$_3^-$) per centimeter of intestine per hour
(Eq·cm$^{-1}$·h$^{-1}$). The secretion and mean arterial blood pressure (BP) were recorded
continuously and registered at 10-min intervals. The statistical significance of data
was tested by repeated-measures analysis of variance, using ANOVA.

Chemicals and drugs

L-phenylephrine hydrochloride and prostaglandin E$_2$ (PGE$_2$) were purchased
from Sigma Chemicals (St. Louis, MO). Melatonin and 5-ethyl-5-(1'-methyl-
propyl)-2-thiobarbiturate (Inactin®) were from Research Biochemicals International
(Natick, MA). Prazosin hydrochloride, the melatonin receptor agonist
2-iodo-N-butanoyl-5-methoxytryptamine (2-IbMT), and the antagonist luzindole were obtained from Tocris Cookson Ltd. (Avonmouth, Bristol, UK).

RESULTS

Stimulation of duodenal $\text{HCO}_3^-$ secretion by close intra-arterial melatonin

Close i.a. infusion of melatonin (20, 200 and 2000 nmolkg$^{-1}$h$^{-1}$) to the duodenum significantly increased mucosal $\text{HCO}_3^-$ secretion (Fig. 2) but did not affect the transmucosal PD (p < 0.05, not shown). This secretion started to increase 20 min after the start of infusion of the lowest dose tested (20 nmolkg$^{-1}$h$^{-1}$) and peaked after 60 min. The rise was from 10.6 ± 1.1 to 20.0 ± 2.3 µEqcm$^{-1}$h$^{-1}$ (P < 0.05); secretion did not increase further with the higher doses. In contrast, $\text{HCO}_3^-$ secretion tended to decline with 200 and 2000 nmolkg$^{-1}$h$^{-1}$, but was always, significantly greater (P < 0.05) than the basal secretion. PGE$_2$ (20 µM luminally) added at the end of experiments as a test of the viability of the preparation (not shown) increased the $\text{HCO}_3^-$ secretion and also caused a rise in transmucosal PD (from 1.2 ± 0.6 to 2.4 ± 0.6 mV, lumen negative). As found with melatonin, close i.a. infusion of the receptor agonist 2-iodo-N-butanoyl-5-methoxytryptamine (2-IbMT) significantly stimulated (P<0.05) the $\text{HCO}_3^-$ secretion (Fig. 2). Neither melatonin (Fig. 2) nor 2-IbMT affected the mean arterial blood pressure.

The melatonin MT$_1$/MT$_2$ (MT$_2$ > MT$_1$) receptor antagonists luzindole (600 nmol/kg, n = 6) did not affect spontaneous (basal) $\text{HCO}_3^-$ secretion but abolished the response to melatonin. In contrast, prazosin, which is an antagonist at melatonin MT$_3$ receptors, did not affect the rise in secretion in response to melatonin (n = 6), and neither luzindole nor prazosin inhibited the response to prostaglandin E$_2$.

Intracerebroventricular melatonin

Effects of central nervous administration of melatonin were tested by intracerebroventricular (i.c.v.) infusion of the compound (Fig. 3). Infusion of 20 nmolkg$^{-1}$h$^{-1}$ of melatonin for 110 min into a lateral brain ventricle affected neither the duodenal $\text{HCO}_3^-$ secretion nor the mean arterial blood pressure.

DISCUSSION

The mucosal $\text{HCO}_3^-$ secretion is under central as well as local intestinal neurohumoral influence. A number of common diseases in humans, including
gastroduodenal ulcer and irritable bowel syndrome, are well known for showing circadian rhythms in pain and discomfort. Further, studies in fasting animals have demonstrated that the gastric secretions of HCO$_3^-$ and mucus, both important in mucosal protection, shows day and night rhythms with peak times different from that of the mucosa-aggressive H$^+$ secretion (9). This phase shift in secretory rhythms may, in theory, result in circadian variations in mucosal vulnerability to acid injury. Fuller information about circadian rhythms and, in particular, about the neurohumoral pathways from internal clocks to tissues and ion transporting cells, should provide relevant information about intestinal pathophysiology and may provide clues to future therapy. It is thus of interest to study the effects the pineal gland neurohormone melatonin on the mucosa-protective HCO$_3^-$ secretion in the duodenum. In humans and other mammals, including rodents, melatonin secretion from the pineal gland peaks at darkness (night), independently of species differences in day or night activity (10). Melatonin is synthesized from tryptophan, with serotonin as an intermediate, and is released from the pineal gland in mammals into the circulation. Being a non-polar and lipid-soluble indoleamine, melatonin crosses the blood-brain barrier and acts at melatonin-specific receptors in the central nervous system as well as such receptors in peripheral tissues. Three G-protein coupled melatonin receptor subtypes are reported and the mammalian MT$_1$ and MT$_2$ subtypes, but not yet the MT$_3$ subtype, have been cloned (10).

Importantly, melatonin is also one of the transmitters produced by the enterochromaffin cells of the intestinal mucosa (6) and the total amount of melatonin in the alimentary tract is considerably higher than that in the central nervous system (11). A physiological role of this source of melatonin has not been clarified before, nor has any interaction between pineal gland and intestinal mucosal melatonin production. The results of the present work demonstrates that melatonin stimulates duodenal mucosal secretion of HCO$_3^-$, very likely via action at enterocyte MT$_2$-receptors, and that stimulation occurs without significant changes in the transepithelial electrical potential difference. The highest concentration of 2-iodomelatonin binding sites are, as proposed for the electroneutral HCO$_3^-$ exporter, located in the villi of the rat small intestine (12). Melatonin may thus stimulate the mucosa-protective HCO$_3^-$ secretion in the most acid-exposed (villus) portion of the duodenal epithelium.

The MT$_1$/MT$_2$ (MT$_2$ > MT$_1$) receptor antagonist luzindole inhibited the response to i.a. melatonin but did also abolish the marked rise in HCO$_3^-$ secretion induced by central nervous (i.c.v.) infusion of the adrenoceptor agonist phenylephrine (3). This centrally elicited response was similarly prevented by sub-laryngeal ligation of all nerves around the carotid arteries (3). Further, the stimulation by i.c.v. phenylephrine in pineal gland ectomized animals was the same as that in intact animals, making it very unlikely that central nervous melatonin is involved in mediating the response. The combined
results thus strongly suggest that intestinal (and not central nervous) sources of melatonin are involved in mediating central nervous influence on duodenal epithelial HCO$_3^-$ secretion and mucosal protection.

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