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GASTRIC CYTOPROTECTIVE ACTIVITY OF ENDOGENOUS 5-HT

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The role of endogenous serotonin in the formation of gastric damage was studied in rats. Stress ulcers were induced by ultrasounds, immobilization and immobilization plus cold. The damage of gastric mucosa was estimated (arbitrary scale) and serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in this tissue measured. In all examined groups of animals with gastric mucosal damages the lower levels of 5-HT and 5-HIAA in gastric mucosa were observed. In some experimental groups animals were treated with serotonergic receptor antagonists 30 min. before stress. The administration of ICS 205—930 (80 µg/kg), 5-HT3 receptor antagonist, and DAU-62855 (80 µg/kg), 5-HT4/5-HT3 receptors antagonist, reduced the intensity of stress gastric injuries. In contrast the administration of methysergide (8 mg/kg), 5-HT1/5-HT2 receptors antagonist, enhanced the stress gastric mucosa damage. 16, 16 dimethyl PGE2 (10 µg/kg) protected stomach against stress stimuli and accompanied increase of serotonin and 5-HIAA concentration in gastric mucosa was observed. Both 5-HT3/5-HT4 receptor antagonist had an additive cytoprotective effect when given in combination with PGE2 analog. In the presence of methysergide gastroprotective effect of PGE2 was abolished. The present studies demonstrate that cytoprotective effect of endogenous serotonin depends on 5-HT1 and 5-HT2 receptors stimulation in the gastric mucosa and the protective effect of prostaglandins depends partly on the regulation of serotonin metabolism.

Key words: serotonin, serotonin receptors, gastric cytoprotection, prostaglandins, rat stomach, stress ulcers.

INTRODUCTION

The endogenous serotonin (5-hydroxytryptamine, 5-HT) is present in high concentrations in the gastrointestinal tract and located in the enterochromaffin cells, mast cells and enteric neurons (1, 2). The stomach, along with the ascending colon, contain the highest concentrations of this biogenic amine (3, 4). The release of 5-HT from the intestinal and gastric mucosa is regulated by complex mechanism of neuronal and humoral inputs to the

enterochromaffin cells (5). Many effects are induced by release of serotonin into the portal blood (6, 7) or the gastric and small intestinal lumen (8, 9). 5-HT has both excitatory and inhibitory effects on motor function of stomach and intestine (10—12), alters gastrointestinal blood flow (13, 14), inhibits gastric acid secretion induced by different secretory stimuli (15—17), induces intestinal secretion (18), stimulates output of gastric mucus (13). It may be considered as an enteric neurotransmitter or as an enterogastrone. The effect of released amine in the digestive tract depends on the site and the type of receptor involved. 5-HT receptors are divided into seven major types: 5-HT$_1$ — 5-HT$_7$ (19, 20).

Through receptors serotonin stimulates or inhibits nerves and smooth muscles and the final effect depends on its direct and indirect action (12). The role of serotonin in gastric stress ulcer formation remains unclear. In rat, exogenous serotonin produces gastric mucosal ischaemia and injury, which is indistinguishable from restraint-induced injury (21, 22). Blackman et al. (23) reported that serotonin released by reserpine may be involved in pathomechanism of gastric mucosal injury induced by this drug. In contrast, it has been demonstrated that serotonin may even protect gastric mucosa against harmful stimuli (14, 24). The connection between gastric damage and a disturbed metabolism of prostaglandins (PGs) is well established (25, 26) and it has been suggested that serotonin induced gastric cytoprotection depends on an enhanced PGs production (15, 18). The present studies were undertaken to examine the role of 5-HT in the mechanism of stress-induced acute gastric mucosal injury in rats.

**MATERIALS AND METHODS**

The male Wistar rats, 180—200 g were used. The animals were fasted for 24 h before experiments with free access to water. The gastric lesions were induced by ultrasounds, immobilization and immobilization plus cold. The animals were separately kept in the boxes for 24 h and exposed to the action of ultrasounds with a frequency in the air 17—24 KHz (WC-US generator). Immobilization was induced by placing animals in the plexiglass boxes for 4 h at the room temperature. The low temperature (−1, +2°C) was applied for 3 h (refrigerator). Serotonergic receptor (5-HT$_3$, ICS 205-930; 5-HT$_4$, DAU 6285 and 5-HT$_1$/5-HT$_2$, methysergide) antagonists and 16,16 dimethyl PGE$_2$ or their combination were given 30 min before exposure of animals to stress. ICS 205-930 (80 µg/kg), methysergide (8 mg/kg) were given i.p., while 16,16 dimethyl PGE$_2$ (10 µg/kg) p.o.

Animals were sacrificed by decapitation. The stomachs were quickly removed, washed and opened along the great curvature. After the stomachs had been spread on the preparation plate, the gastric mucosal injury was estimated according to the arbitrary scale (0—5 scores). Concentration of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were determined by spectrofluorimetric method of Curzon (27).
Drugs

The following drugs were used: 5-hydroxytryptamine-creatine sulfate, 5-hydroxy-3 indolyl acetic acid, cysteine, o-phtalaldehyde (Sigma), methysergide, ICS 205-930 (Sandoz AG, Switzerland), DAU 6285 (Boehringer Ingelheim, Italy), 16,16 dimethyl PGE2 (Syntex USA). Drugs were dissolved in distilled water.

Statistical analysis

All data are expressed as mean ± SEM. Comparisons between group of parametric data were made by Student's t test. Comparisons between groups of nonparametric data (injury score) were made by Mann-Whitney U-test.

RESULTS

In all examined groups of animals (except controls) the gastric mucosal damage was found: ultrasounds (Ult) produced the least effect, immobilization (I) — moderate and immobilization plus cold (I + cold) — the strongest one (Fig. 1). Concentrations of 5-HT and 5-HIAA in the gastric mucosa of rats

![Graph showing injury scores](image_url)

Fig. 1. The damage of gastric mucosa induced by stress ultrasounds, immobilization, immobilization and cold C — control, Ult — ultrasounds 24 h, I — immobilization 4 h, I + cold — immobilization 3 h and cold *** p < 0.005
Fig. 2. Serotonin in the gastric mucosa in rats after stress induced by ultrasounds, immobilization, immobilization and cold. C — control, Ult — ultrasounds 24 h, I — immobilization 4 h, I + cold — immobilization 3 h and cold. n — number of rats. X ± SEM ** p < 0.01 *** p < 0.001

following Ult, I and I + cold stress are showed in Fig. 2 and 3, respectively. The concentration of 5-HT dropped in all stress groups as compared with the controls (7.03 ± 0.14 μg/g). Accordingly, also 5-HIAA concentration decreased.

The administration of ICS 205-930 (5-HT₃ receptor antagonist) protected the gastric mucosa against stress as compared with respective controls (Fig. 4). The levels of serotonin and 5-HIAA were not changed (Fig. 5). DAU — 6285 (5-HT₄ and 5-HT₃ receptors antagonist) decreased the incidence of stress damage (Fig. 6). Administration of methysergide (5-HT₁ and 5-HT₂ receptors antagonist) increased the gastric mucosal injures (Fig. 7).

Pretreatment of rats with prostaglandins has protective effect against all kinds of stress stimuli as evidenced by data in Fig. 8. After the administration of 16,16 dimethyl PGE₂ the serotonin concentration in the gastric mucosa increased in comparison with the untreated stress groups (Fig. 9).
Fig. 3. 5-HIAA in the gastric mucosa in rats after stress induced by ultrasounds, immobilization, immobilization and cold C — control Ult — ultrasounds 24 h I — immobilization 4 h I + cold — immobilization 3 h and cold n — number of rats X±SEM *** p < 0.001

There was a parallel increase in 5-HIAA concentration (Fig. 10). The additive, cytoprotective effect has been observed when ICS 205-930 and 16,16 dimethyl PGE₂ were given in combination. No stress ulcers were seen. Methysergide given together with PGE₂ abolished its cytoprotective effect (Fig. 11).

DISCUSSION

The role of serotonin in the pathogenesis of gastric damage remains largely unclear. Exogenous 5-hydroxytryptamine produces gastric mucosal ischaemia and injury but has no effect on neuronal activity in rat's isolated forestomach (28) or in the human isolated and intact gastrointestinal tract (29). Its rapid catabolism and poor ability to cross anatomical and functional barriers cause that only endogenous amine released at site of action, quickly binds to receptors (10).

Endogenous 5-HT directly stimulates smooth muscles in the gastrointestinal tract. This effect may be indirectly modulated by stimulatory or inhibitory action of serotonin on cholinergic neurotransmission (12, 30).
Stressful stimuli have been shown to disturb the metabolism of the amine. Arka (22) reported the elevation of antral serotonin following restraint stress in the rat. Indirect experiments showed a decrease in number of enterochromaffin cells (EC) containing 5-HT and 100% increase of 5-HIAA in urine after restraint stress (31, 32). Studies on the effect of stressors have noted decrease in the concentration of brain 5-HT, which appeared to be related to increased serotonin release and catabolism (33, 34). The decrease of amine in brain was accompanied by lower level of serotonin in gastrointestinal tract (35). Also reserpine-induced, acute gastric injuries in rat were concomitant with decreased levels of serotonin in the mucosa (14). Pretreatment with $5\text{-HT}_1$, $5\text{-HT}_2$ receptor antagonist — methysergide intensified methysergide damages and
Fig. 5. The influence of ICS-205—930 80 μg/kg on serotonin levels (Fig A) and 5-HIAA (Fig B) in the gastric mucosa in rats, after stress induced by: ultrasounds, immobilization, immobilization and cold C — control UIt — ultrasounds 24 h I — immobilization 4 h I+cold — immobilization 3 h and cold n — number of rats
Fig. 6. The influence of DAU — 6285 80 μg/kg on gastric mucosa damage induced by stress ultra-sounds, immobilization, immobilization and cold. C — controls, Ult — ultrasounds 24 h, I — immobilization 4 h, I + cold — immobilization 3 h and cold, n — number of rats. ***p < 0.005

Fig. 7. The influence of methysergide 8 mg/kg on gastric mucosa damage induced by: ultrasounds, immobilization, immobilization and cold. C — controls, Ult — ultrasounds 24 h, I — immobilization 4 h, I + cold — immobilization 3 h and cold, n — number of rats. **p < 0.01, ***p < 0.005
Fig. 8. Effects of 16,16 dimethyl PGE₂ on gastric damage induced by: ultrasounds, immobilization, immobilization and cold. C — controls, Ult — ultrasounds 24h, I — immobilization 24h, I+cold — immobilization 4h and cold, n — number of rats. *** p < 0.005

Fig. 9. Effects of 16,16 dimethyl PGE₂ on serotonin levels in the gastric mucosa in rats, after stress induced by: ultrasounds, immobilization, immobilization and cold. C — controls, Ult — ultrasounds 24h, I — immobilization 24h, I+cold — immobilization 4h and cold, n — number of rats. ±SEM. ** p<0.001 *** p < 0.005
Fig. 10. Effects of 16,16 dimethyl PGE₂ on 5-HIAA levels in the gastric mucosa in rats, after stress induced by: ultrasounds, immobilization and cold. C — controls. Ultrasound I — immobilization. I + Cold — immobilization 3h and cold. n — numbers of rats. X±SEM * p < 0.05 ** p < 0.001 *** p < 0.001

Fig. 11. Effects of 16,16 dimethyl PGE₂ methysergide on damage of gastric mucosa induced by: ultrasounds, immobilization. C — controls. Ultrasound I — immobilization. I + cold — immobilization 3h and cold. n — numbers of rats. *** p < 0.005
supported the view on protective action of endogenous serotonin. We have confirmed this observation in experiments where gastric mucosal injury in rat was induced by anti-inflammatory drugs. The gastric damage was reciprocally related to the serotonin levels in the mucosa (36).

In the present studies in all groups of animals with induced gastric ulcers, the levels of serotonin and its principle metabolite 5-HIAA were decreased. Mechanism of enhanced release of 5-HT is believed to be mediated via release of stimulatory neurotransmitter. Cholinergic release of amine was observed in the gastrointestinal tract under physiological and pathological conditions (5, 30, 37). The potent gastric 5 HT release was induced by thyrotropin — releasing hormone (TRH) and its stable analogue RX 77 368. It suggested a modulatory role of TRH in vagal gastric 5-HT release (8, 39, 40). Endogenous serotonin released from GI stores produces an inhibitory tone on vagally stimulated gastric secretory and motor function (41).

In stress ulcers the decreased mucosal blood flow (14, 42) increased gastric motility (43, 44), decreased gastric alkaline secretion (25) are frequently reported. Opposite effects are observed when exogenous serotonin is injected (13).

The administration of ICS 205-930 (5-HT₃ receptor antagonist) caused the decrease of damage of the gastric mucosa in all experimental groups. Functional 5-HT₃ receptors have been extensively characterized in mammalian peripheral neurons. They are present on postganglionic autonomic neurones of the sympathetic and parasympathetic nerves of various tissues (45). The role of the 5-HT₃ receptor remains incompletely understood, but it is probably involved in the modulation of gastrointestinal motility (46, 47) and delayed emesis (37, 48). The effects of 5-HT₃ receptor stimulation are somewhat controversial.

The second 5-HT₃ receptor antagonist DAU 6285 protected gastric mucosa against stress ulcers. Its pharmacological activity combines blockade of both: 5-HT₃ and 5-HT₄ receptors, the later being 5 times more potent than ICS 205-930, which blocks 5-HT₄ receptors in micromolar doses (50, 51). 5-HT₄ receptors are thought to stimulate gastrointestinal motility through modulation of intramural cholinergic nerve pathways subserving peristalsis (46, 49). It seems established that gastric mucosa protection by serotonin 5-HT₃ and 5-HT₄ antagonists reflects their inhibitory activity on the stomach motility.

In contrast the administration of 5-HT₁ and 5-HT₂ receptors antagonist methysergide caused the increase of gastric mucosa stress injures. In human and dogs methysergide treatment does not change (17) or causes the increase of basal and pentagastrin-stimulated gastric acid secretion (16). This drug produced a dose-dependent increase in the reserpine-induced acute gastric mucosal injury (14). Methylsergide has been shown to stimulate
gastrointestinal motility (16, 52). The results of its effect are logical consequence of inhibiting 5-HT$_1$/5-HT$_2$ receptors. These receptors are distributed on both the nerve and muscular structures and also on the vascular smooth muscle (53, 54). 5-HT causes nerve-mediated contractions primarily due to acetylcholine release from cholinergic motor neurons but also causes a direct moderate excitation of smooth muscle contractility through activation of ileal 5-HT$_2$ and gastric 5-HT$_2/1_C$ receptors (49, 55). 5-HT $-$ induced vasodilatation is attributed to stimulation of 5-HT$_1$ $-$ like receptors, which are located primarily on resistance vessels. The contribution of 5-HT$_2$ and 5-HT$_3$ receptors to vasodilatation has been also reported (54). Serotonin-induced vasoconstriction occurred in most cases in large arteries and was due mainly to stimulation of 5-HT$_2$ receptors although 5-HT$_1$-like receptors were probably also involved (56, 57).

5-HT$_{1A}$ receptor antagonists decreased blood pressure by a reduction in total peripheral resistance. This vasodilatation seems to be widespread (58). Some 5-HT$_1$-like receptor agonists have differential effects depending on the animal species used. Methysergide produced an increase in the magnitude of the submucosal and mucosal vasoconstriction relative to that achieved by reserpine alone (14). The decrease of serotonin in the gastric wall potentiated the motility effects of cholinergic stimulation (41). In the present studies 5-HT$_1$/5-HT$_2$ antagonist had double undesired activity in stomach: eliminated the serotonin dependent increase of mucosal blood flow and blocked inhibiting motility 5-HT$_1$ receptors.

Our results confirmed the cytoprotective effect of prostaglandins (59—62) and showed that it was accompanied by an increase of serotonin and 5-HIAA concentrations in the gastric mucosa. It suggests that amine is involved in PGs gastroprotection in rat.

The combined administration of prostaglandins and serotonergic 5-HT$_3$, 5-HT$_4$ receptor antagonists fully protected gastric mucosa against stress injuries while the administration of 16,16 dimethyl PGE$_2$ with 5-HT$_1$/5-HT$_2$ receptor antagonist was ineffective.

The disturbance of mucosal blood flow playing an essential role in the pathomechanism of gastric mucosal injury (14, 24, 63) is normalized by both prostaglandins and serotonin. PGs appear to maintain the mucosal blood flow (26, 60) probably in part by the serotonin turnover increase. The modulatory effect of 5-HT on the vascular tone has to be considered. Haddly (64) reported that when small vessels are already dilated amine cannot dilate them further, but continues to constrict large vessels. When arterioles are constricted serotonin dilates them more than it constricts large vessels. Thus 5-HT in similar way as prostaglandins normalizes the disturbed gastric mucosal blood flow (GMBF). This action of 5-HT on small blood vessels is determined probably by the magnitude of adrenoceptor stimulation (65). Serotonin acts as
a vasodilator with high input and a vasoconstrictor with low input of adrenoreceptor stimulation (56). Protective action of 5-hydroxytryptamine depending on the maintenance of GMBF was demonstrated (24).

The additional cytoprotective actions of 5-HT can not be excluded. Serotonin enhances the platelet-derived epidermal growth factor-induced DNA synthesis (66) and may act as an important growth regulator for vascular smooth muscle cells. The way by which PGs influence gastric 5-HT turnover may involve secondary to gastric acid inhibition, release of gastrin. Stimulation of histidine decarboxylase by gastrin is well documented (67) and it is suggested that gastrin may exert the same effect on decarboxylase responsible for serotonin synthesis.

It may be concluded that the physiological role of serotonin in the gastric mucosa is not clear yet. The further studies with selective agonists and antagonists of respective 5-HT receptors are needed. The present studies showed that: 1. In rat serotonin metabolism is disturbed in stress-induced acute gastric mucosal injuries. 2. Cytoprotective action of endogenous 5-HT is exerted through 5-HT<sub>1</sub> and 5-HT<sub>2</sub> receptors. 3. 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptor stimulation favors the generation of gastric mucosa damage.

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