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RAPID COMMUNICATION

Protective effects of amphetamine on gastric ulcerations induced by indomethacin in rats

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Abstract

AIM: To study the effects of amphetamine, an indirect-acting adrenomimetic compound on the indomethacin-induced gastric ulcerations in rats.

METHODS: Male Wistar-Bratislava rats were randomly divided into four groups: Group 1 (control), received an ulcerogenic dose of indomethacin (50 μmol/kg) and Groups 2, 3 and 4, treated with amphetamine (10, 25 and 50 μmol/kg). The drug was administered simultaneously with indomethacin and once again 4 h later. The animals were sacrificed 8 h after indomethacin treatment. The stomachs were opened and the incidence, the number of lesions and their severity were evaluated. The results were expressed as percentage and as mean ± standard error (mean ± SE).

RESULTS: The incidence of ulceration in the control group was 100%. Amphetamine, at doses of 10, 25 and 50 μmol/kg, lowered the incidence to 88.89%, 77.78% and 37.5% respectively. The protection ratio was positive: 24.14%, 55.17% and 80.6% respectively. The total number of ulcerations/rat was 12.44 ± 3.69 in the control group. It decreased to 7.33 ± 1.89, 5.33 ± 2.38 and 2.25 ± 1.97 under the effects of the above-mentioned doses of amphetamine.

CONCLUSION: Amphetamine affords a significant dose-dependent protection against the indomethacin-induced gastric ulcerations in rats. It is suggested that the adrenergic system is involved in the gastric mucosa protection.

Key words: Amphetamine; Gastric ulceration; Indomethacin; Rats

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INTRODUCTION

The involvement of the adrenergic system in some models of gastric ulcerations is well documented[1-4]. Our previous investigations have shown that amphetamine, an indirectly acting sympathomimetic amine has a protective activity on gastric ulcerations induced by reserpine and immobilization in rats[5]. These findings were interpreted as an evidence of a protective activity of the adrenergic mechanisms on the integrity of the gastric mucosa[3,4,6].

Other models of gastric ulcers imply the administration of some nonsteroidal anti-inflammatory agents such as acetylsalicylic acid[7], phenylbutazone[8] or indomethacin[9]. The genesis of these ulcerations arises from both the local depression of prostanoid biosynthesis[10-12] and a local injury[13]. However, the participation of the adrenergic system in this experimental setting is not excluded[14,15].

In this paper, we studied the effect of amphetamine on the indomethacin-induced gastric ulcerations in albino rats.

MATERIALS AND METHODS

Animals and ulcer model

The experiments were carried out on male Wistar albino rats, weighing 144-255 g (from the Animal Center of University of Medicine and Pharmacy Iuliu Haţieganu, Cluj-Napoca). The animals were kept in a room with a constant temperature (20-22℃) and humidity, under a natural light-dark regime. They were fed common rat chow and received water ad libitum. The food was withdrawn 12 h prior to administration of the ulcerogenic agent. The rats were randomly divided in four groups.

Control group (Group Ⅰ) was administered intraperitoneally (ip) with indomethacin 50 μmol/kg. Groups Ⅱ, Ⅲ and Ⅳ were simultaneously ip injected with

Table 1 Effect of amphetamine on incidence and severity of indomethacin-induced gastric ulcerations (%)

Groups		TU	LU	PR
I	I_{50}	100	100	
II	$I_{50} + A_{10}$	88.89	77.778	(+) 24.138
III	$I_{50} + A_{25}$	77.78	66.667	(+) 55.172[1]
V	$I_{50} + A_{50}$	37.5[a]	12.5[a]	(+) 80.603[1]

The subscript indices represent the doses expressed as μmol/kg. I : Indomethacin; A: Amphetamine; TU: Total number of ulcerations; LU: Large ulcerations (> 1 mm); PR: Protection ratio. [a]$P < 0.05$ *vs* group I (Fischer's exact probability and median's test); [1]Significant at PR > (+) 33.33%.

Table 2 Effect of amphetamine on number and severity of indomethacin-induced gastric ulcerations (mean ± SE)

Groups		Number of ulcerations/stomach		Severity
		TU	LU	UI
I (9)	I_{50}	12.44 ± 3.69	10.11 ± 3.74	1.61 ± 0.31
II (9)	$I_{50} + A_{10}$	7.33 ± 1.89	5.44 ± 1.52	1.22 ± 0.25
III (9)	$I_{50} + A_{25}$	5.33 ± 2.38	3.11 ± 1.9	0.72 ± 0.21[a]
IV (8)	$I_{50} + A_{50}$	2.25 ± 1.91[c]	1.0 ± 1.0[c]	0.31 ± 0.19[b]

The subscript indices represent the doses expressed as μmol/kg. I: Indomethacin; A: Amphetamine. TU: Total number of ulcerations; LU: Large ulcerations (> 1 mm); UI: Ulcer index. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.05$ *vs* group I .

indomethacin and amphetamine sulphate, and 4 h later, only with amphetamine. The doses of amphetamine were 10, 25 and 50 μmol/kg , respectively.

During the ulcerogenesis period, the rats were fasted, but water was allowed ad libitum. Eight hours after indomethacin administration, the animals were sacrificed; the stomachs were removed, opened along the curvatura maior and rinsed with saline. They were examined immediately, with a magnifying glass. The following parameters were recorded: the body weight (g), the incidence of ulcerations/group, the total number of ulcerations (TU), the number of large ulcerations (LU) i.e. ulcerations larger than 1 mm and the severity of ulcerations estimated according to a scale between 0 and 4 (ulcer index-UI)[3].

Drugs

Indomethacin: Powder supplied by Terapia SA, Cluj-Napoca, Romania, was suspended in a mixture of 2% methylcellulose and 1% glycerin, ten times diluted with saline. The concentration was 0.025 mol/L (8.945 g/L) and the dose, 50 μmol (17.89 mg)/kg.

Amphetamine sulphate (racemate): It was prepared as an aqueous solution 0.025 mol/L (9.21 g/L) for the dose of 50 μmol (18.425 mg)/kg. Doses of 10 μmol (3.685 mg)/kg and 25 μmol (9.2105 mg)/kg were adapted after dilution. All drugs were ip administered in a volume of 2 mL/kg.

Ethical issues

The study was conducted in accordance with the Helsinki's Declaration on Animal's Studies and approved by the local ethics committee.

Statistical analysis

All values are expressed as means and standard errors (mean ± SE). The indicators of ulcerogenesis were analyzed initially by a one-way ANOVA test. The significance of differences between groups was observed by the bilateral Student's *t* test and by multiple comparison tests of Newman-Keuls and Scheffé. This last test allows comparisons between blocks of groups. These procedures were followed by non-parametric Kruskal-Wallis, Mann-Whitney, median's and Fischer's exact probability tests[16,17]. The statistical significance of the differences was admitted if $P < 0.05$.

The protection ratio (PR) was calculated according to the formula:

$$PR\% = (UI_{control} - UI_{treated}) / UI_{control} \times 100$$

Statistical significance for PR was considered for values outside (-) 33% → (+) 33% range[3].

RESULTS

The body weight of the rats was a homogenous parameter, i.e. there were no significant differences between groups (data not included in Tables). The doses of 25 and 50 μmol/kg of amphetamine induced a stereotyped behavior. It was not affected by indomethacin.

Indomethacin induced ulcerations in 100% of the rats. The total number of ulcerations was 12.4 ± 3.69, most of them (10.1 ± 3.74) were larger than 1 mm. The UI was 1.61. When amphetamine was administered, all the parameters diminished in a dose-dependent manner (Tables 1 and 2). In addition, a dose-dependent increase of the PR was found (Table 1).

Significant differences between the control group and the groups treated with 25.0 μmol/kg and 50.0 μmol/kg of amphetamine were found in most tests. Some tests showed significant differences between the 10.0 μmol/kg amphetamine administered group and the group that received 50.0 μmol/kg (Tables 1 and 2). For the parameters of ulcerogenesis (TU, LU, and UI), an increase of significance was found in the order: total ulcerations (TU) < ulcerations larger than 1 mm (LU) < ulcer index (UI).

DISCUSSION

These results seem at first glance rather surprising, since, according to most authors, the ulcerations produced by nonsteroidal anti-inflammatory agents are due to an impaired prostanoids biosynthesis in the gastric mucosa[10,18,19], and to a local injury as well[13].

It is possible that other mechanisms are involved in the ulcerogenesis caused by indomethacin: the direct cytotoxic effect[20], the promotion of apoptosis of gastric mucosal cells[21-23] as well as the induction of interactions between polymorphonuclear leukocytes and endothelium of gastric mucosa vessels[24-28]. The effects of amphetamine may be related to any of these mechanisms. However, only the links between prostaglandins and adrenergic system are firmly established[29]. Prostaglandins, particularly those

from E series inhibit catecholamines release[30,31]. On the other hand, adrenergic stimulation evokes an increase of prostaglandin release[32,33].

It is well known that amphetamine acts mainly via the release of dopamine and noradrenaline from both central and peripheral sites[34,35]. The question arises whether dopamine or noradrenaline or both are involved in the protection of gastric ulcerations afforded by amphetamine. Our previous results have shown that dopaminergic mechanisms were not involved in the protective activity of amphetamine and catecholamines in restraint and reserpine-induced gastric ulcerations[3,5,7]. We did not investigate the involvement of dopamine receptors in indomethacin-ulcerations.

On the other hand, there are data in the literature indicating that the dopaminergic system may be also involved in the genesis of the ulcerations induced by nonsteroidal anti-inflammatory agents[36,37]. So, dopaminergic agonists, such as apomorphine and bromocriptine, diminished significantly the incidence and the severity of the ulcerations evoked by indomethacin in albino rats. The protective activity of apomorphine was antagonized by haloperidol[36]. This agent and other dopaminergic antagonists aggravated gastric ulcerations induced by indomethacin[36], reserpine[37] and stress[38,39]. These findings suggest that dopamine would have a protective activity in various models of experimental ulcers[37,40-42]. It was also shown that gastric pentadecapeptide BPC 157 markedly affects the central and peripheral dopaminergic system. It blocks the acute stereotype??? elicited by amphetamine[43]. At the same time, it completely prevents the gastric ulcerations induced by haloperidol[37,44].

More recently, a cocaine- and amphetamine-regulated transcript (CART) messenger RNA was isolated from the brain[45,46], peripheral nerves[47] and tissues[48,49]. Its corresponding polypeptide inhibits after central injection, food intake, the gastric emptying and gastric acid secretion in 24 h fasted rats. However, CART polypeptide, ip injection does not have the same effect. It was also shown that the intracisternal injection of CART greatly reduces the gastric acid output elevated by subcutaneous indomethacin administration[50]. These data are consonant with the view that the adrenergic system has a protective action on gastric mucosa.

In conclusion, amphetamine protects gastric mucosa against the damaging effect of indomethacin. The mechanism of action is still obscure, it might involve both the central and peripheral segments of the adrenergic system.

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