INVESTIGATION ON THE MECHANISM INVOLVED IN THE EFFECTS OF AGMATINE ON ETHANOL-INDUCED GASTRIC MUCOSAL INJURY IN RATS

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Summary

Effects of agmatine, an endogenous metabolite formed by decarboxylation of L-arginine, on ethanol-induced gastric mucosal injury were investigated in rats. Agmatine at 1 and 10 mg/kg i.p doses significantly increased ethanol-induced gastric mucosal injury. This effect of agmatine was abolished completely by pretreatment with idazoxan, an imidazoline receptor-antagonist and α2 receptor- antagonist, (0.5 mg/kg i.p), partly by yohimbine, an α2 receptor- antagonist, (1 mg/kg i.p) but not by L-arginine, a precursor of nitric oxide, (500 mg/kg i.p). Our results suggest that agmatine had a potent ulcerogenic effect mediated, at least in part, by both α2- adrenoceptors and imidazoline receptors.

Key Words: agmatine, ethanol ulcer, α2-adrenoceptors, imidazoline receptors

Agmatine, an amine recently identified as an endogenous clonidine-displacing substance in mammalian brain, is generated by the decarboxylation of L-arginine by the enzyme arginine decarboxylase (1,2,3). It is a biologically active substance (4) but the mode and sites of action have not been fully defined. It binds with high affinity to both α2- adrenoceptors of all subclasses and to the imidazoline receptors of the I1 and I2 subclasses (5). It is widely distributed in mammalian tissue, such as, brain, stomach, intestine and aorta; thus agmatine may act as a neurotransmitter (1,3).

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In some recent studies it has been suggested that agmatine inhibited nitric oxide synthase in isolated aorta and rat brain (6). It is well known that endothelial cells release a labile vasodilator substance (7) now characterized as a nitric oxide (NO) biosynthesized from L-arginine by a constitutive NO synthase (NOS) (8). NO is important in the regulation of gastric mucosal blood flow, gastric mucus (9,10), acid (11) and alkaline secretion (12) and is involved in the defense of the gastric mucosal integrity (13). More recently, several studies have indicated that inhibition of NOS by various NOS inhibitors such as N\(^\circ\)-nitro L-arginine aggravated gastric mucosal injury caused by ethanol in rat (14).

Recently it has been demonstrated that agmatine has potent ulcerogenic actions on rat stomach after stress challenge (15) but the mechanisms underlying the mucosal injury of stomach are not fully understood. Since \(\alpha_2\)-adrenoceptors or imidazoline receptors mediated functions of agmatine have been uncertain, in this study, we investigated whether imidazoline receptors and/or \(\alpha_2\)-adrenoceptors are involved in the effects of agmatine on ethanol-induced gastric mucosal injury in rat stomach using idazoxan, an imidazoline receptor antagonist and \(\alpha_2\)-adrenoceptor antagonist and yohimbine, an \(\alpha_2\)-adrenoceptor antagonist. Moreover, we also examined the role of nitric oxide on this phenomenon.

**Methods**

Wistar albino rats of either sex (200-250 g body weight) were housed in a room with constant temperature (25°C) and photoperiod (12 h light, 12 h dark), and were supplied with standard rat chow and tap water ad libitum. Rats were deprived of food but not water for 24 hours prior to an experiment. Ethical approval was guaranteed by the Kocaeli University of Ethics Committee (Kocaeli, Turkey).

Vehicle (distilled water, n=14) or agmatine ip. at doses of 0.5, 1, 10, 50 mg/kg (n=8 rats in each dosage group) was given 10 minutes before 50% ethanol (1 ml to each rat, intragastrically using orogastric tube, No 2, Rusch) (16). Other groups of animals received idazoxan (0.5 mg/kg ip), an \(\alpha_2\)-adrenoceptor antagonist that also interacts with imidazoline receptors, yohimbine (1 mg/kg ip), an \(\alpha_2\)-adrenoceptor antagonist; or L-arginine (500 mg/kg ip), a nitric oxide synthase precursor, 10 minutes prior to ethanol. In other series of experiments, these antagonists were given 10 minutes prior to ip 1 mg/kg agmatine and then ethanol was given 10 minutes after (n=6-9 rats in each group). Two hours later, animals were killed by cervical dislocation, and the stomachs were removed, opened along the greater curvature, rinsed out gently to recover gastric contents and examined for measurement of mucosal lesions. Each lesion was measured along its greatest diameter (mm). When assessing the size of petechiae, five such lesions were considered equivalent to 1 mm ulcer. The sum of the lesions' lengths in each group was divided by the number of rats in that group and expressed as the mean ulcer index (17).

Mucosal damage was evaluated microscopically as reported previously (18). Samples of the entire upper corpus of the stomach, 5 mm below the limiting ridge, were excised for histological analysis. The tissue was fixed in 10% formalin, embedded in paraffin, and sectioned into 5 \(\mu\)m-thick pieces. The sections were then stained with haematoxylin and eosin and examined under a light microscope. Each section was assessed on a scale of 1-5 as follows: normal appearance, 0; surface epithelial cell damage, 1; vasocongestion, hemorrhage, and/or edema in the upper mucosa, 2;
vasocongestion, hemorrhage, and/or edema in the mid to lower mucosa, 3; glandular disruption or necrosis in the upper mucosa, 4; and deep necrosis or ulceration, 5. Each section was coded to eliminate observer bias and was evaluated on a cumulative basis to give a histological index with a maximum score of 15.

Agmatine, idazoxan, yohimbine and L-arginine were purchased from Sigma Chemicals (St. Louis, MO, USA). All drugs were dissolved in distilled water and administered intraperitoneally in a volume of 1 ml/kg.

All data are expressed as mean ± SEM. Statistical significance of differences between values was analysed with one way analysis of variance (ANOVA) followed by Student Newman-Keul’s post hoc test. The score of histological damage was assessed by the Kruskal-Wallis test. A probability (P value) of 0.05 or less was taken to indicate statistical significance.

Results

Rats administered distilled water were served as controls in all experiments since ethanol-induced lesion indexes were the same in all. Agmatine (1 mg/kg i.p) given alone had no effect on gastric mucosa in naive rats (data not shown).

![Fig 1](image)

Effect of agmatine on gross ethanol-induced gastric mucosal hemorrhagic damage in rats. Agmatine (0.5, 1, 10, 50 mg/kg) was administered intraperitoneally 10 min before intragastric administration of 1 ml of 50% ethanol. Gastric mucosal damage was measured 2 hours after intragastric administration. Values are expressed as mean ± SEM of 8 rats. Significantly different from control (14 rats) according to one-way ANOVA followed by Student Newman-Keul’s test.

*p<0.01

#p<0.05
Intragastric administration of 50% ethanol caused macroscopic hemorrhagic lesions. Intragastric administration of agmatine at doses of 1 and 10 mg/kg significantly exacerbated gastric mucosal damage (p<0.01, p<0.05, respectively) caused by ethanol. The 0.5 and 50 mg/kg doses of agmatine had no significant effect on ethanol-induced gastric lesions (Fig 1). This increase in mucosal damage caused by agmatine was reduced by pretreatment with yohimbine (1 mg/kg) and completely abolished by pretreatment with idazoxan (0.5 mg/kg). However L-arginine (500 mg/kg) did not affect aggravated mucosal injury caused by agmatine (Fig 2).

Intragastric administration of yohimbine (1 mg/kg), idazoxan (0.5 mg/kg) or L-arginine (500 mg/kg) given alone had no significant effect on hemorrhagic damage induced by ethanol (Fig 2).

Effect of idazoxan (Ida), yohimbine (Yoh) and L-arginine (L-Arg) on the actions of agmatine (Ag) on gross ethanol-induced gastric mucosal injury in rats. Idazoxan, yohimbine or L-arginine was given 10 min prior to ethanol or was given 10 min prior to agmatine and then ethanol was given 10 min after. Rats were divided into eight groups of 6-14 rats each as follows: Control group (Control, n=14): distilled water i.p and 50% ethanol i.g; agmatine group (Ag, n=8): agmatine (1 mg/kg i.p) and 50% ethanol i.g; idazoxan group (Ida, n=7): idazoxan (0.5 mg/kg i.p) and 50% ethanol i.g; idazoxan plus agmatine group (Ida+Ag, n=6): idazoxan (0.5 mg/kg i.p) plus agmatine (1 mg/kg i.p) and 50% ethanol i.g; yohimbine group (Yoh, n=6): yohimbine (1 mg/kg i.p) and 50% ethanol i.g; yohimbine plus agmatine group (Yoh+Ag, n=9): yohimbine (1 mg/kg i.p) plus agmatine (1 mg/kg i.p) and 50% ethanol i.g; L-arginine group (L-Arg, n=6): L-arginine (500 mg/kg i.p); L-arginine plus agmatine group (L-Arg+Ag, n=8): L-arginine (500 mg/kg i.p) plus agmatine (1 mg/kg i.p) and 50% ethanol i.g.

Values are means± SEM. n, number of animals.
* p<0.001, control vs agmatine group.
** p<0.01, control vs yohimbine+agmatine group
*** p<0.001 control vs L-arginine+agmatine group
# p<0.01, agmatine vs idazoxan+agmatine group
# # p<0.05, agmatine vs yohimbine+agmatine group
The histologic score of mucosal damage induced by intragastric administration of 50% ethanol was 3.70±0.6 (Fig 3). Pretreatment with agmatine (1 mg/kg, i.p) increased values significantly about threefold (p<0.001). Pretreatment with idazoxan (0.5 mg/kg i.p ) inhibited the mucosal damage induced by agmatine (1 mg/kg i.p), bringing the histologic score down to control levels (Fig 3). Pretreatment with yohimbine (1 mg/kg, i.p) partly reduced the score (p<0.05) but it did not return to control values (Fig 3). However, pretreatment with idazoxan (0.5 mg/kg i.p) or yohimbine (1 mg/kg, i.p) had no significant effect on the histologic score caused by ethanol (Fig 3).

**Fig. 3**

Effect of agmatine on histological score of 50% ethanol-induced gastric mucosal injury in rats. Rats were divided into three groups as follows: Control group (Control, n=14): distilled water i.p and 50% ethanol i.g; agmatine group (Ag, n=6): agmatine (1 mg/kg i.p) and 50% ethanol i.g; idazoxan plus agmatine group (Ida+ag, n=7): idazoxan (0.5 mg/kg i.p) plus agmatine (1 mg/kg i.p) and 50% ethanol i.g; idazoxan group (Ida, n=8): idazoxan (0.5 mg/kg i.p) and 50% ethanol i.g; yohimbine group (Yoh, n=6): yohimbine (1mg/kg i.p) and 50% ethanol i.g, yohimbine plus agmatine group (Yoh+Ag, n=9): yohimbine (1 mg/kg i.p) plus agmatine (1 mg/kg i.p) and 50% ethanol i.g; Values are means± SEM.

**p<0.001, control vs agmatine group**

* *p<0.01, control vs yohimbine+agmatine group

##p<0.001, agmatine vs idazoxan+agmatine group

###p<0.001, agmatine vs yohimbine+agmatine group

n, number of animals.
Discussion

The present study demonstrates that agmatine, an arginine metabolite, administrated before ethanol significantly aggravated macroscopic and microscopic mucosal injury caused by ethanol at doses of 1.0 and 10 mg/kg i.p. This effect of agmatine may be related to one of several mechanisms.

First, it has been shown that NO has an important modulatory role in the regulation of gastrointestinal integrity (13) and exogenously administrated agmatine might exert this effect via interaction with nitric oxide synthase. Since the common substrate arginine is used by both arginine decarboxylase and NO synthase, that agmatine might exert a modulatory effect on nitric oxide production could be speculated. It has been widely shown that NOS inhibitors significantly aggravate mucosal injury caused by chemical challenge in rats (14,19,20). Recently, agmatine has been shown to inhibit NOS activity in isolated aorta (6). However, we did not observe any significant change on increased mucosal injury after pretreatment with L-arginine. Thus, it is unlikely that inhibition of the NO/cGMP pathway explains the present results.

Secondly, this interaction may depend upon α2 adrenoceptors and/or imidazoline receptors. Agmatine binds to α2-adrenoceptors (5) and drugs such as clonidine which bind to these receptors also have been associated with both gastroprotection as well as with exacerbation of gastric pathology (21,22). Recently, Al-Bekari et al. (23) demonstrated that clonidine, an α2-adrenoceptor agonist exerted inconsistent effects in the gut, depending on the doses used. It exacerbated ethanol-induced gastric mucosal damage, but also decreased gastric acid secretion and enhanced gastric adherent mucus. However, in this study, yohimbine did not completely reverse exacerbated macroscopic and microscopic gastric mucosal damage caused by agmatine, suggesting that this effect of agmatine was not entirely mediated by α2-adrenoceptor stimulation. We confirm, at least in the rat, exacerbated mucosal damage by agmatine partly involves activation of the α2-adrenoceptors.

Alternatively, it has been reported that agmatine acts as an agonist at imidazoline receptors (5) and this action could be responsible for its ulcerative effects. In the present study idazoxan, an imidazoline receptor antagonist, that also interacts with α2-adrenoceptors, almost completely prevented the effects of agmatine. It is reported that moxonidine, an exogenous agonist of I2 imidazoline receptors, decreased the length of the lesions as well as the number of the lesions in an ethanol-induced model of gastric mucosal injury and significantly decreased acid secretion as well as pepsin output in pylorus-ligated rats (24). Conversely, agmatine, an endogenous imidazoline receptor agonist, significantly exacerbated stress-induced gastric mucosal injury and increased gastric secretion (15). Since agmatine exerts opposite effects to those of specific imidazoline agonists, Glavin et al. (24) suggested that it is one possible explanation that agmatine functions as an inverse agonist rather than true agonist at imidazoline sites mediating gastrointestinal secretion responses in rats, resulting in augmented gastric secretion and exacerbated gastric mucosal injury.

In naive rats, agmatine alone produced no observable change in gastric mucosal injury. This is unlikely to be related to the direct effects of agmatine itself.

In conclusion, since α2 or imidazoline receptors mediated functions of agmatine have been unclear, the potent ulcerogenic effect of agmatine in rat stomach was analysed in the presence of yohimbine, a pure α2-adrenoceptor antagonist, or idazoxan, an α2-adrenoceptor antagonist which also binds to imidazoline receptors, preferable of the I2 subtypes. Complete reversal of agmatine-induced increase in ethanol induced both macroscopic and microscopic gastric mucosal damage by idazoxan.
pretreatment suggests that the actions of agmatine might involve imidazoline receptors and as Glavin et al (24) suggest, agmatine may have function as an "inverse agonist" at central imidazoline receptors, resulting in exacerbated ethanol-induced gastric mucosal injury in rats.

References