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Research report

Evidence for the gastric cytoprotective effect of centrally injected agmatine

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ABSTRACT

Agmatine (decarboxylated arginine) exerts cytoprotective action in several tissues, such as in the brain, heart or kidneys, but there is still controversy over the effects of agmatine on the gastric mucosa. The aim of the present study was to reveal the potential gastroprotective action of agmatine by using an acid-independent ulcer model to clarify which receptors and peripheral factors are involved in it. Gastric mucosal damage was induced by acidified ethanol. Mucosal levels of calcitonin gene-related peptide (CGRP) and somatostatin were determined by radioimmunoassay. For analysis of gastric motor activity the rubber balloon method was used. It was found that agmatine given intracerebroventricularly (i.c.v., 0.044–220 nmol/rat) significantly inhibited the development of ethanol-induced mucosal damage, while in the case of intraperitoneal injection (0.001–50 mg/kg i.p.) it had only a minor effect. The central gastroprotective action of agmatine was completely antagonized by mixed alpha2-adrenoceptor and imidazoline I1 receptor antagonists (idazoxan, efaroxan), but only partially by yohimbine (selective alpha2-adrenoceptor antagonist) and AGN 192403 (selective I1 receptor ligand, putative antagonist). It was also inhibited by the non-selective opioid-receptor antagonist naloxone and the selective δ-opioid receptor antagonist naltrindole, but not by β-funaltrexamine and nor-Binaltorphimine (selective μ- and κ-opioid receptor antagonists, respectively). Furthermore, the effect of agmatine was antagonized by bilateral cervical vagotomy and by pretreatment with indomethacin and NG-nitro-L-arginine. Agmatine also reversed the ethanol-induced reduction of gastric mucosal CGRP and somatostatin content, but did not have any significant effect on gastric motor activity. These results indicate that agmatine given centrally induces gastric cytoprotection, which is mediated by central imidazoline I1 receptors, alpha$_2$-adrenoceptors and δ-opioid receptors. Activation of these receptors induces the release of different mucosal protective factors, such as NO, prostaglandins, CGRP and somatostatin by a vagal-dependent mechanism. Alterations of gastric motility are not likely to contribute to the observed protective effect.

1. Introduction

Agmatine, an endogenous aminoguanidine, has long been known in lower life forms as a metabolic intermediate in polyamine synthesis, but its biosynthesis in mammalian tissues has been recognized only in 1994 (Li et al., 1994). It is present in low (pico- and nanomolar) concentrations in many organs (Raasch et al., 1995), and originates from arginine by decarboxylation, although a significant portion is probably absorbed from the gastrointestinal (GI) tract as well (Li et al., 1994; Molderings et al., 2003).

It was originally described as an endogenous ligand of the imidazoline I_1 receptors, but subsequent studies revealed that agmatine has much wider actions. It is a neuromodulator and co-transmitter, which is capable to interact with multiple molecular targets, including several receptors (e.g. imidazoline I_1 and I_2, alpha$_2$-adrenergic, nicotinic Ach, $5HT_3$ or NMDA), ion channels (voltage-gated Ca^{2+} channels, ATP-sensitive K^+ channels) or enzymes (e.g. all isoforms of nitric oxide synthases) (recently reviewed by Molderings and Haenisch, 2012; Piletz et al., 2013). Furthermore, it possesses cytoprotective action by scavenging free radicals and protecting mitochondrial functions (Arndt et al., 2009). Accordingly, over the

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past two decades numerous effects, including neuro-, nephro- and cardioprotection have been attributed to agmatine and ample evidence has accumulated that it might have beneficial effect in the treatment of various diseases, like neuropsychiatric disorders, hypertension or diabetes mellitus (Molderings and Haenisch, 2012; Piletz et al., 2013; Moretti et al., 2014).

Both agmatine and imidazoline binding sites are localized in the GI tract (Houi et al., 1987; Molderings et al., 1999a; Raasch et al., 1995), and various studies have been conducted to assess whether agmatine has a role in the regulation of GI functions. However, there is still controversy over the effects of agmatine on the gastric mucosal integrity. Early investigations reported that it aggravates stress- and ethanol-induced gastric mucosal damage probably by acting on imidazoline receptors (Glavin et al., 1995; Utkan et al., 2000), but a recent study demonstrated that sub-chronic oral administration of agmatine in high dose (about 100 mg/kg) is safe, and does not lead to gastric mucosal damage (Gilad and Gilad, 2013). Moreover, Al Masri and El Eter (2012) found that agmatine has protective effect against ischemia reperfusion injury and it has also been raised that the presence of agmatine (a strong base) in the mucosa of the stomach may enhance mucosal defense against gastric acid (Steer, 2009). Furthermore, agmatine is able to interact with alpha$_2$-adrenergic receptors (Li et al., 1994; Piletz et al., 1995) and with the endogenous opioid system (Wu et al., 2008), which both have been implicated in mucosal protection (Gyires and Rónai, 2001; Gyires et al., 2000).

Many lines of evidence indicate that besides peripheral factors also central nervous system (CNS) has significant influence on the development of gastric erosions. Numerous neuropeptides induce gastroprotection after central administration (for reviews see Gyires, 2012; Tache, 2012), which is mediated (in most cases) by a common peripheral effector pathway, namely the activation of vagal efferents and the consequent release of prostaglandins, nitric oxide (NO) and CGRP (originating from the capsaicin sensitive primary afferent nerves).

Beside the dorsal vagal complex (DVC) the hypothalamus has also significant impact on GI functions and gastric mucosal defense (Tache, 2012; Gyires et al., 2013). It is of interest that agmatine is localized in both the DVC and hypothalamus (Otake et al., 1998), and the highest activity of both arginine decarboxylase and agmatinase (the enzymes responsible for the synthesis and degradation of agmatine) was found in the hypothalamus (Iyo et al., 2006; Sastre et al., 1996). These findings imply that agmatine, besides its role in the etiopathogenesis of various CNS disorders (Moretti et al., 2014; Uzbay, 2012) may also be involved in the central regulation of gastric mucosal integrity.

Therefore, the present study aimed to analyze the potential gastroprotective effect of centrally and peripherally injected agmatine on gastric mucosal defense and to investigate the involvement of imidazoline I_1 receptors, alpha$_2$-adrenergic receptors and the endogenous opioid system in the agmatine-induced action. In addition, we aimed to identify, which peripheral factors (e.g. release of local mediators or alterations in gastric motility) are involved in the gastroprotective effect of agmatine.

2. Materials and methods

2.1. Animals

For all experiments male Wistar rats were used. The animals were housed in a temperature- and humidity-controlled room at a 12-h light/dark cycle under conditions of animal housing and experimentation according to ethical guidelines issued by the Ethical Board of Semmelweis University, based on EC Directive 86/609/EEC.

After one week habituation rats were randomly divided into the experimental groups (5–8 rats/group). All procedures conformed to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes, and all efforts were made to minimize the suffering of animals. The experiments were approved by the Animal Ethics Committee of Semmelweis University, Budapest (permission number: 22.1/606/001/2010).

2.2. Experimental procedures

2.2.1. Gastric mucosal damage induced by acidified ethanol

Gastric mucosal lesions were induced by acidified ethanol (98 ml absolute ethanol + 2 ml concentrated HCl), which was given intragastrically in a volume of 0.5 ml/rat by an oral gavage using a stainless steel cannula. The experiments were performed on young 6 weeks old male Wistar rats (140–170 g), because our former study revealed that gastric mucosal susceptibility to ethanol and the gastroprotective effect of opioid peptides, capsaicin and prostaglandin E_2 is age-related, and mucosal defensive processes are more efficient in 6–8 weeks old rats than in elder ones (Gyires and Barna, 2002). Before the experiments rats were deprived of food for 24 h with free access to tap water. 60 min after the injection of ethanol the animals were sacrificed, the stomachs were excised, opened along the greater curvature, rinsed with saline and examined for lesions. Total number of mucosal lesions was assessed in blinded manner by calculation of the lesion index based on a 0–4 scoring system described previously (Gyires, 1990). The lesion index was calculated as the total number of lesions multiplied by the respective severity factor.

In order to determine the effect of agmatine on ethanol-induced mucosal damage, in four consecutive experiments a total of 80 rats were randomly divided into 16 groups (5 rats/group) and agmatine was injected either into the lateral brain ventricle (intracerebroventricularly, i.c.v.) 10 min before the ethanol challenge in a volume of 10 μl, or intraperitoneally (i.p.) in a volume of 0.5 ml/100 g 20 min before the administration of ethanol, as described previously (Gyires et al., 2000), in the following doses: 0.044, 0.22, 0.88, 1.76, 4.4, 44 and 220 nmol/rat i.c.v. and 0.001, 0.005, 0.02, 0.1, 1, 10 and 50 mg/kg i.p.

To compare the effect of the applied doses and to establish the dose–response relationships, results were expressed as the percentage of the lesion indices of the respective control groups.

In another experiment agmatine was injected directly into the lateral hypothalamus (LH), in order to analyze the role of this nucleus in the gastroprotective action. 15 rats were anesthetized with pentobarbital (35 mg/kg i.p.), and guide cannulas (Bilaney Consultants, Düsseldorf, Germany) were implanted with stereotaxic surgery (Stoelting, IL, USA) and fixed with dental cement (Adhesor Cement, Spofa Dental, Jičín, Czech Republic). After 3 days recovery, rats were randomly divided into 2 groups (7–8 rats/group), and either saline or agmatine (0.88 nmol/rat) was injected into the LH in a volume of 1 μl, 10 min before the ethanol challenge. For the injection the following coordinates were used (relative to bregma): posterior 1.8 mm; lateral 2.0 mm; ventral 8 mm (Paxinos and Watson, 1986). The site of injection was subsequently confirmed histologically, and only the animals with appropriately placed injection sites were used for data analysis.

In additional experiments various antagonists were combined with agmatine. They were given either i.c.v. (together with i.c.v. injected agmatine, in a total volume of 10 μl), or intravenously (i.v.), subcutaneosly (s.c.) or orally (15 min, 20 min or 60 min before the i.c.v. injection of agmatine, respectively, in a volume of 0.5 ml/100 g) (Gyires et al., 2000, 2014). The applied doses of drugs were selected based partly on our preliminary results, partly on the literature data.