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Short communication

Effects of centrally or peripherally injected adrenomedullin on reserpine-induced gastric lesions

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Abstract

Adrenomedullin intracerebroventricularly administered (0.1 to 20 ng/rat i.c.v.), showed significant gastroprotective activity in a dose-dependent manner. When the peptide was intravenously administered (1 to 1000 ng/kg i.v.) it did not show significant gastroprotective activity in the same test. The gastroprotective effect of the peptide (10 ng/rat) was abolished by bilateral adrenalectomy, by pretreatment with the β-adrenoceptor antagonist, propranolol (1 mg/kg i.p.), or by a calcitonin gene-related peptide (CGRP) receptor antagonist, CGRP-(8-37) fragment (1 or 10 ng/rat i.c.v.). This study showed that adrenomedullin is protective against reserpine-induced gastric lesions, that the action involves sympathetic nerve activity, and moreover interferes with CGRP receptors.

Keywords: Adrenomedullin; Ulcer; Reserpine; Adrenalectomy; CGRP-(8-37) (calcitonin gene-related peptide-(8-37) fragment)

1. Introduction

Adrenomedullin is a 52-amino-acid peptide originally isolated from a human adrenal pheochromocytoma, using a detection system based on its ability to elevate platelet cAMP level (Kitamura et al., 1993). Immunoreactive adrenomedullin was detected by radioimmunoassay in many tissues, including normal adrenal medulla, heart, kidney, pancreas, intestine and plasma (Ichiki et al., 1994). Adrenomedullin is considered to be a member of the calcitonin gene-related peptide (CGRP) superfamily, including calcitonin, CGRP and amylin (Kitamura et al., 1995). Several studies showed that some peptides of this superfamily, after administration into the brain, can influence, through the autonomic nervous system, some gastrointestinal functions (Tachè, 1992; Guidobono et al., 1994; Clementi et al., 1996). The main biological effects of peripherally injected adrenomedullin seem to be vasodilation (Kitamura et al., 1995), bronchodilation (Kanazawa et al., 1994), and reduction of mean blood pressure (Ishiyama et al., 1993). It was suggested that some cardiovascular activities of the peptide can involve CGRP receptors (Nuki et al., 1993). However, evidence showed that centrally administered adrenomedullin also interfered with the autonomic nervous system, modifying some gastrointestinal functions. In fact it inhibits gastric emptying (Martinez et al., 1997) and prevents ethanol-induced gastric injury (Kaneko et al., 1998). Even though there are specific binding sites for adrenomedullin in some brain areas (Owji et al., 1995), it was suggested that some of its central effects could involve the CGRP receptors (Martinez et al., 1997).

The aim of the present study was to examine in conscious rats (1) whether different doses of intracerebroventricularly (i.c.v.) or intravenously (i.v.) administered adrenomedullin show gastroprotective activity on reserpine-induced gastric lesions, (2) to establish the mechanisms involved in this possible effect.

2. Materials and methods

2.1. General

Male Sprague–Dawley rats (230–250 g) were starved for 48 h before use but were allowed free access to tap

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water, which was removed 1 h before the experiment. The rats were kept separately in cages with raised mesh floors (to prevent coprophagy) during the fasting period. Room temperature was maintained at 22 ± 1°C; 65 ± 5% relative humidity; 12-h light/dark.

2.2. Drugs

Human adrenomedullin and CGRP-(8-37) fragment were purchased from Peninsula Laboratories Europe (UK), reserpine, propranolol hydrochloride and phentolamine hydrochloride were purchased from Sigma (Italy).

2.3. Surgery

One week prior to the experimental session, male rats were implanted with permanent plastic cannulae in the right lateral ventricle. The operation was performed according to the method described by Brakkee et al. (1979), under ether anesthesia. Adrenalectomy was performed 48 h before the experiments in fasted rats under ketamine hydrochloride anesthesia.

2.4. Reserpine-induced gastric lesions

The method of Lau and Ogle (1981) was used. Reserpine was administered intraperitoneally (i.p.) at the dose of 25 mg/kg of 0.5% acetic acid solution. Adrenomedullin was injected i.c.v. (0.1 to 20 ng/rat) or i.v. (1 to 1000 ng/kg) immediately before reserpine administration. Four hours later, the animals were killed by decapitation, the stomach was removed, opened along the greater curvature and examined under 3-fold magnification. The number and severity of lesions in the glandular mucosa were scored bind from 0 to 5 as follows:

0	no lesions
0.5	diffuse hyperemia
1	1 to 2 small ulcers
1.5	3 to 6 small ulcers
2	7 to 10 small ulcers
2.5	more than 10 small ulcers
3	1 marked ulcer plus 0 to 4 small ulcers
3.5	1 marked ulcers plus 5 or more small ulcers
4	2 marked ulcers plus 0 to 4 small ulcers
4.5	2 marked ulcers plus 5 or more small ulcers
5	3 or more marked ulcers

The anti-ulcer effect was expressed as percent protection vs. the control group. Separate experiments were carried out with adrenomedullin at the dose of 10 ng/rat i.c.v. after pretreatment with phentolamine (1 mg/kg i.p.), propranolol (1 mg/kg i.p.) or CGRP-(8-37) fragment (1 or 10 μg/rat) 15 min before reserpine. The controls received phentolamine, propranolol, CGRP-(8-37) fragment or saline plus reserpine at the same time.

2.5. Statistical analysis

The results are expressed as means ± S.E. The statistical significance of the difference between groups was determined using the analysis of variance (ANOVA) followed by Dunnett's *t*-test. Statistical significance was set at $P < 0.05$ (Dunnett, 1955).

3. Results

The effects of i.c.v. administration of adrenomedullin on reserpine-induced gastric lesions are presented in Fig. 1. The peptide exerted maximal activity (72%) at the dose of 10 ng/rat; the dose of 20 ng/rat produced a smaller effect (47%). This protective effect was inhibited after pretreatment with different doses of CGRP-(8-37) fragment, and this inhibition was already present at the dose of 1 μg/rat (Fig. 1). In contrast, the i.v. administration of different doses (1 to 1000 ng/kg) did not show significant gastroprotective activity. Adrenomedullin exerted its maximal activity (28%) at the dose of 100 ng/kg.

Bilateral adrenalectomy abolished the gastroprotective effect of the peptide compared with the effect in sham-operated rats (Table 1). Phentolamine (1 mg/kg i.p.) by itself showed gastroprotective activity (25%) vs. saline-treated animals, which was not statistically significant, but the gastroprotective activity of the peptide was not modified (Table 1). Pretreatment with propranolol (1 mg/kg

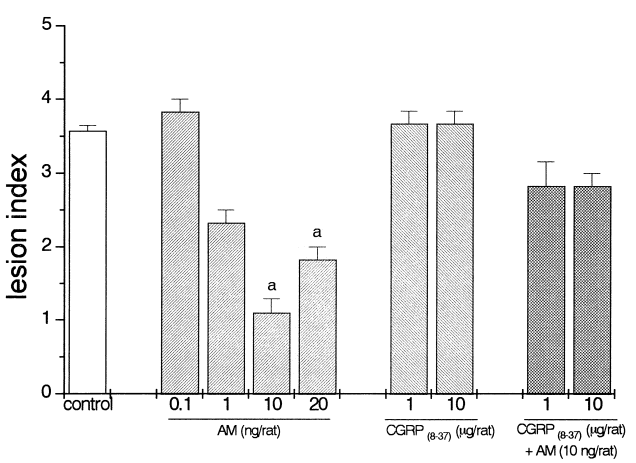

Fig. 1. Effect of different doses (0.1 to 20 ng/rat) of i.c.v. injected adrenomedullin (AM), alone or in association with CGRP-(8-37) fragment (1 or 10 ng/rat), on reserpine-induced gastric damage in rats ($n = 6$). [a] $P < 0.05$ vs. controls.

Table 1
Reversal by bilateral adrenalectomy, or adrenergic blockade with phentolamine or propranolol, of gastroprotective effect of i.c.v.-injected adrenomedullin (AM) on reserpin-induced gastric ulcers in rats ($n = 6$)

Experimental conditions	Ulcer index mean ± S.E.	Percent difference	P (Dunnett's test)
A. Adrenalectomy			
Sham operation + saline i.c.v.	3.67 ± 0.17	–	–
Sham operation + AM i.c.v.	1.25 ± 0.17	−66	< 0.05
Adrenalectomy + saline i.c.v.	4.92 ± 0.08	+34	< 0.05
Adrenalectomy + AM i.c.v.	4.83 ± 0.10	+32	< 0.05
B. Adrenergic blockade			
Saline i.p. + saline i.c.v.	3.57 ± 0.14	–	–
Saline i.p. + AM i.c.v.	1.08 ± 0.20	−70	< 0.05
Phentolamine i.p. + saline	2.67 ± 0.24	−25	–
Phentolamine i.p. + AM i.c.v.	1.25 ± 0.11	−65	< 0.05
Propranolol i.p. + saline i.c.v.	3.58 ± 0.24	–	–
Propranolol i.p. + AM i.c.v.	3.50 ± 0.22	–	–

Bilateral adrenalectomy was performed 48 h before the experiments. AM (10 ng) or saline (10 μl) was injected i.c.v. immediately before reserpine (25 mg/kg i.p.). Phentolamine (1 mg/kg), propranolol (1 mg/kg) or saline (10 ml/kg) was administered i.p. 15 min before AM. The ulcer index was evaluated 4 h after reserpine.

i.p.) blocked the i.c.v. adrenomedullin-induced inhibition of the gastric lesions produced by reserpine (Table 1).

4. Discussion

The results showed that i.c.v. injection of adrenomedullin (0.1 to 20 ng/rat) prevented reserpine-induced gastric ulcers in rats in a dose-dependent manner. When the peptide was given i.v. (1 to 1000 ng/kg), it did not modify the gastric mucosal damage induced by reserpine significantly. It is, therefore, most likely that adrenomedullin acts in the central nervous system.

It is known that the etiology of gastric ulceration is multifactorial and includes interaction among gastric acid secretion, intestinal motility and changes in gastric mucosal circulation. Reserpine induced gastric mucosal damage through various mechanisms. It produces constriction of the veins from the middle layer to the muscolaris mucosa and congestion with ischemia in the gastric mucosa, and later hypermotility (Kagoshima and Suguro, 1982). Moreover, Sandor and Cuparencu (1977), among others, have claimed that the main pathogenetic factor in reserpine-induced ulceration was depression of adrenergic activity with an increase of cholinergic tone, mainly at the central level. Since centrally administered adrenomedullin stimulates abdominal sympathetic nerve activity (Takahashi et al., 1994), and inhibits gastric emptying (Martinez et al., 1997), we suppose that the gastroprotective activity of adrenomedullin in reserpine-induced ulcers could be due to interference with abdominal sympathetic activity through adrenal-dependent β-adrenergic mechanisms. The peptide, including a decrease of gastric motor function, could also improve the blood microcirculation in the gastric mucosa. This hypothesis is supported by evidence that adrenalectomy, or treatment with the β-adrenergic blocker, propranolol, inhibited the gastroprotective effect of the peptide.

It was suggested that adrenomedullin exerts some central or peripheral effect involving CGRP receptors (Martinez et al., 1997; Nuki et al., 1993). Our results suggest that the gastroprotective activity shown by centrally administered adrenomedullin also does involve CGRP receptors. In fact, the i.c.v. injection of CGRP-(8-37) fragment, a CGRP receptor antagonist, abolished this effect of the peptide. This result is in agreement with the report by Martinez et al. (1997) that either adrenomedullin or CGRP inhibits gastric emptying in rats by acting on the autonomic nervous system through common mechanisms.

In summary, the present study showed that centrally administered adrenomedullin inhibits reserpine-induced gastric lesions, and the results suggest that this effect is due to interference with sympathetic nerve activity through adrenal-dependent β-adrenergic mechanisms. Moreover, our results indicate that adrenomedullin acts by interfering with CGRP receptors, but do not exclude the possibility that the peptide can also act on its own receptors.

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