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Effect of capsaicin and resiniferatoxin on gastrointestinal blood flow in rats

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Abstract

The effect of capsaicin and resiniferatoxin on gastrointestinal blood flow was studied in anaesthetized rats by laser Doppler flowmetry. Resiniferatoxin injected into the jugular vein (0.08–1.6 nmol/kg) produced a marked and dose-dependent increase in gastric blood flow, while the effect of capsaicin (0.33–19.6 nmol/kg) was transient, variable and accompanied by profound systemic blood pressure changes. After acute bilateral cervical vagotomy combined with sympathetic neurone blockade (guanethidine 16 μmol/kg) or α-adrenoceptor blockade (phentolamine 1.6 μmol/kg), the vasodilator response to injected resiniferatoxin was more pronounced, indicating that the resiniferatoxin-induced gastric vasodilatation is not due to reflexes via parasympathetic or sympathetic efferent fibres. Resiniferatoxin given i.v. (0.08–0.64 nmol/kg) evoked a similar increase in the blood flow of the jejunum. Capsaicin (0.33–33 μM) or resiniferatoxin (0.16–1.6 μM) applied topically to the serosal surface of the stomach or jejunum produced a pronounced and long-lasting increase in blood flow after vagotomy and guanethidine treatment. The blood flow and blood pressure responses to capsaicin and resiniferatoxin were absent in rats desensitized with systemic capsaicin pretreatment. These laser Doppler data provide the first evidence for the effect of resiniferatoxin on gastrointestinal microcirculation and indicate the advantages of this agent and technique to study the sensory-efferent function of capsaicin-sensitive fibres.

Keywords: Gastrointestinal microcirculation; Laser Doppler; Capsaicin; Resiniferatoxin

1. Introduction

Capsaicin, the pungent principle of hot peppers stimulates, and subsequently at large doses, desensitizes a subset of primary afferent neurones with C and A δ thin fibres (Szolcsányi, 1993). Capsaicin-sensitive sensory nerves are involved in modulating local defense mechanisms against gastric ulcer. Stimulation of sensory nerves with intragastric capsaicin in low concentrations prevented (Szolcsányi and Barthó, 1981; Holzer and Lippe, 1988; Szolcsányi, 1990; Holzer et al., 1989), while their selective ablation with neurotoxic doses of systemic capsaicin aggravated experimental gastric ulcers (Szolcsányi and Barthó, 1981; Holzer and Lippe, 1988). A similar protective role for capsaicin-sensitive sensory nerves was demonstrated in the intestine (Evangelista and Meli, 1989). Intragastric capsaicin increased gastric mucosal blood flow detected by different clearance techniques (Limlomwongse et al., 1979; Lippe et al., 1989; Holzer et al., 1991) and its submucosal application caused arteriolar dilatation (Chen et al., 1992). Enhancement of the local microcirculation is considered to be responsible for the anti-ulcer effect of capsaicin (Szolcsányi and Barthó, 1981; Holzer, 1991a). The microvasculature was shown to be essential for mucosal integrity, this being not only the initial site of damage by injurious agents such as ethanol, but also the site where protective agents, e.g. prostaglandins, exert their anti-ulcer effects (Guth et al., 1984; Trier et al., 1987).

Although blood flow studies with various techniques provided evidence for a vasodilator action of capsaicin in the gastric mucosa, systemic cardiovascular responses evoked by local or systemic (Longhurst et al., 1980; Donnerer and Lembeck, 1982) capsaicin application interfere with the gastric microcirculatory responses. Continuous measurement of blood pressure and microcirculatory changes by laser Doppler flowmetry (Kiel and Shepherd,

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1990) makes it possible to overcome these difficulties. Another approach is to use resiniferatoxin, a potent capsaicin analogue (Maggi et al., 1990; Szallasi and Blumberg, 1990; Szolcsányi et al., 1990; Winter et al., 1990) which is less prone to evoke acute cardiovascular reflexes. The agent possesses gastroprotective effects similar to those of capsaicin (Szolcsányi, 1990; Abdel-Salam et al., 1994, 1995).

The present paper reports on the first laser Doppler study to compare the effects of capsaicin and resiniferatoxin on gastrointestinal blood flow. Particular attention was paid to the disclosure of secondary effects of vagal and sympathetic reflexes as well as blood pressure changes.

2. Materials and methods

2.1. Animals

Male rats of the Sprague-Dawley strain (200–240 g) were used. The animals were deprived of food for 24 h prior to the experiments, but were allowed free access to tapwater.

2.2. Study design

2.2.1. Animal preparation

Anaesthesia was carried out with thiobutobarbital (Inactin), given i.p. in a dose of 100 mg/kg body weight. A cannula (Y-shaped) inserted into the trachea served for facilitation of spontaneous breathing and for occasional aspiration of secretions. Respiratory movements were monitored by a low pressure transducer, connected to one side of the cannula. A polyethylene tube filled with heparinized saline was inserted into the right common carotid artery and connected to a pressure transducer (Statham) for continuous monitoring of systemic arterial blood pressure and heart rate with a physiological chart recorder (Grass polygraph, Model 7). The left internal jugular vein was cannulated for drug administration. Body temperature, maintained at 37 ± 0.5°C by a heating pad and a heating lamp, was monitored with a rectal thermometer. The animals received 1.5 ml of physiological saline every 1 h through the internal jugular vein to avoid dehydration during the observations, and in addition haematocrit values were obtained before and after the experiment to check for adequate hydration of the animals.

2.2.2. Laser Doppler flowmetry

Under anaesthesia, the abdomen was opened through a midline incision, the gastrohepatic ligaments were cut, and the stomach was gently exteriorized. Since movements of the stomach and respiration during the experiment could cause changes in the probe position, the stomach was gently placed on a transparent four-legged table-like plastic slide and fixed with fine sutures to metal needles inserted at both sides of the stomach. With this method of fixation, motion artifacts were eliminated, since no jumps occurred in the recordings and respiratory movements caused no synchronous change in the tracings. MBF3D dual-channel laser Doppler flowmeter (Moor Instruments, UK) was used. The two probes (P5b type) supported by standard probe holders were placed perpendicularly to the outer surface of the stomach or the jejunum. Warm saline 37°C was poured continuously over the stomach to keep the tissues moist. The tips of the probes were just in contact with the serosal surface as checked from time to time with an operating microscope. Stable recordings could be obtained for long periods of time (1 h). I.v. injection of saline in a volume of 1 ml had no effect on the stability of the laser Doppler signal.

2.2.3. Experimental procedure

The effect of i.v. administration of capsaicin (0.33–19.6 nmol/kg) and resiniferatoxin (0.08–1.6 nmol/kg) on gastric blood flow was first studied in animals with intact vagus nerves. Capsaicin in a lower dose range was tested, in pilot experiments, however, it was without effect on gastric blood flow. Capsaicin was given both as i.v. bolus and slowly over 30–60 s, and resiniferatoxin was given slowly over 30–60 s, especially with higher doses. In one group of animals, the effect of capsaicin and resiniferatoxin was evaluated after pretreatment with i.v. propranolol (3.4 μmol/kg) and i.v. atropine (1.5 μmol/kg).

In another experimental series, the effect of capsaicin and resiniferatoxin was examined after acute bilateral cervical vagotomy. The vagus nerves were carefully separated and cut at the cervical region with an interval of 12–18 min between cutting the left and right sides. Thereafter, in order to achieve blockade of the adrenergic sympathetic fibres, guanethidine was given i.v. in a dose of 16 μmol/kg 35–40 min after cervical vagotomy. Capsaicin or resiniferatoxin was given i.v. in the same doses as described before. The dose of guanethidine was repeated during the experiment if the blood pressure returned to pretreatment levels. In some experiments the effect of guanethidine on gastric blood flow was measured for 60 min. In a further group of animals, the α-adrenoceptor blocking agent, phentolamine, was administered in boluses of 0.79 and 1.6 μmol/kg (over 3–5 min) after bilateral cervical vagotomy.

To compare the vasodilator effect of isoprenaline to that of resiniferatoxin, the drug was given as i.v. bolus injection to intact animals (2–32 nmol/kg within 5 s) and after bilateral cervical vagotomy (32 nmol/kg).

In a third series of experiments, the effect of i.v. capsaicin (0.3, 3 and 9.8 nmol/kg) and resiniferatoxin (0.08–0.64 nmol/kg) on intestinal blood flow was studied. Resiniferatoxin was given i.v. to intact animals and after bilateral cervical vagotomy and sympathetic neurone blockade with i.v. guanethidine (16 μmol/kg). Capsaicin was studied under the latter conditions only (not in intact

animals). Blood flow was measured with the laser probes placed against the jejunal surface. In addition, the effect of i.v. guanethidine on intestinal blood flow was also evaluated after bilateral cervical vagotomy for 30 min.

In another series of experiments, capsaicin or resiniferatoxin was applied locally to the serosal surface of the stomach or jejunum in different concentrations and after bilateral cervical vagotomy and i.v. guanethidine (16 μmol/kg) or i.v. phentolamine (1.6 μmol/kg) or after pretreatment with i.v. propranolol (3.4 μmol/kg) and i.v. atropine (1.5 μmol/kg) as before. Histamine chloride was given to compare its effect with that of capsaicin and resiniferatoxin. 30 μl from each solution was applied around each probe site with a micropipette.

In the last series of experiments, the effect of i.v. or local application of capsaicin or resiniferatoxin on gastric blood flow was measured after s.c. capsaicin pretreatment with a cumulative dose of 180 mg/kg. The pretreatment was given on 3 consecutive days (30 + 60 + 90 mg/kg). The last dose was given 1–2 days before the experiment.

2.3. Data analysis

Basal values were recorded before the experiment and before the administration of each dose of the drugs (at least at 5-min intervals). The maximum values of the responses were expressed as percentages of their respective controls. The number of animals used in different experiments is indicated in the text in parentheses. Blood flow changes are given as mean ± S.E.M. Statistical analysis of the results was performed using linear regression analysis or Student's t-test and P values of < 0.05 were considered significant. All changes in blood flow reported were significantly different from basal values.

2.4. Drugs

The following drugs were used: Inactin (BYK, Germany), isoproterenol (Isuprel HCl, Winthrop, New York, USA), phentolamine (Ciba, Switzerland), guanethidine sulfate (Sigma, USA), histamine chloride (histaminum bihydrochloricum, Peremin, Chinoin, Hungary), atropine (Egis, Hungary), propranolol hydrochloride (Inderal, ICI, UK), capsaicin (Sigma), resiniferatoxin (Sigma, LC Laboratories, USA). Stock solutions of capsaicin (1 mg/ml) or resiniferatoxin (0.1 mg/ml) contained 10% ethanol, 10% Tween 80, 80% saline solution. The drugs were freshly dissolved with isotonic NaCl immediately before the experiments to obtain the necessary doses for i.v. injection and the appropriate concentrations for topical application.

3. Results

3.1. Effect of i.v. capsaicin on gastric blood flow

In animals with intact vagal nerves ($n = 6$), i.v. injection of capsaicin over a dose range of 0.33–19.6 nmol/kg evoked a triphasic effect made up of an initial fall, followed by a transient increase then a decrease in arterial blood pressure. The initial hypotensive effect of i.v. capsaicin did not change, while the subsequent rise in arterial

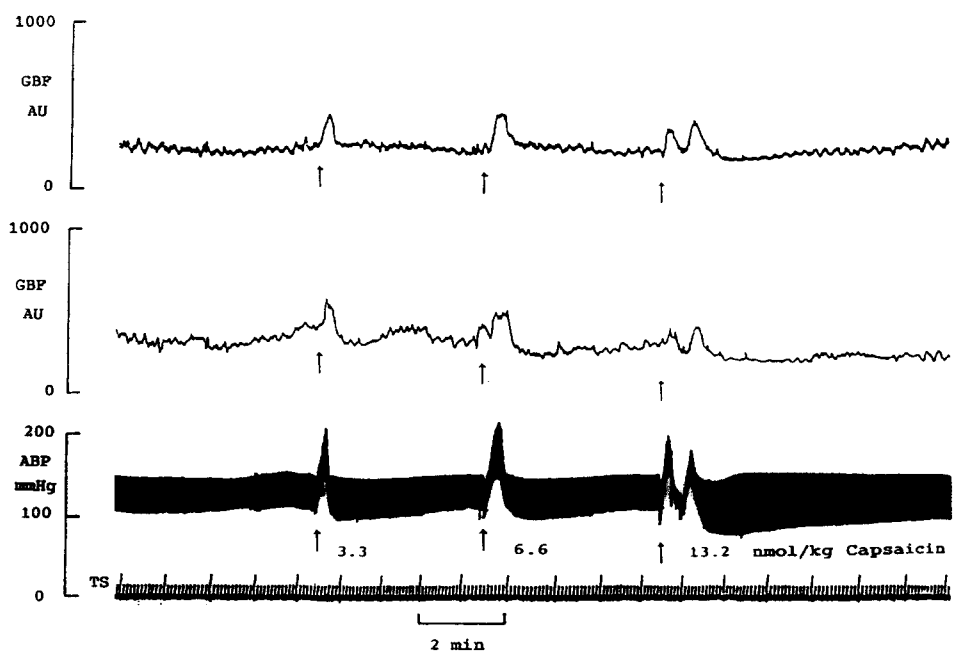

Fig. 1. Experimental tracing of laser Doppler flowmetry showing the effect of i.v. capsaicin on gastric blood flow (GBF; AU, arbitrary units) and arterial blood pressure (ABP; mm Hg) in intact rats. Capsaicin was injected in three subsequent doses of 3.3, 6.6 and 13.2 nmol/kg, respectively. Two laser probes were applied simultaneously on the serosal surface of the stomach.

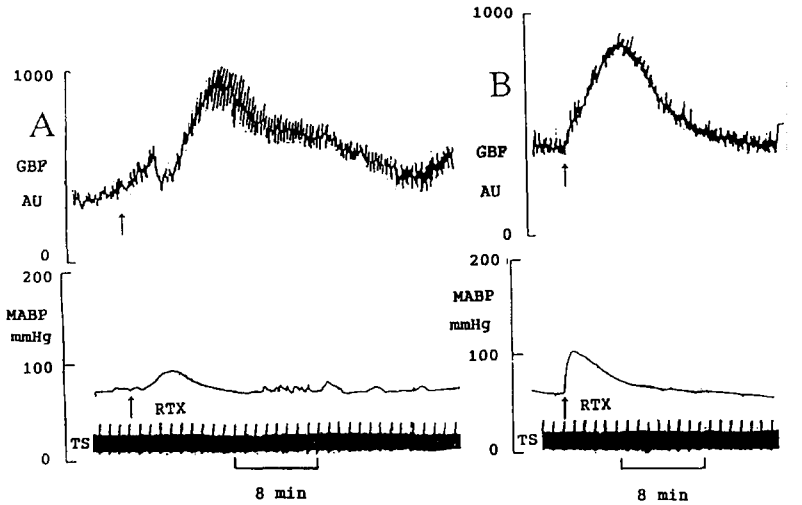

Fig. 2. Experimental tracings of laser Doppler flowmetry from two different animals showing the effect of i.v. resiniferatoxin (RTX) (0.64 nmol/kg) on gastric blood flow (GBF; AU, arbitrary units) and mean arterial blood pressure (MABP; mm Hg). (A) In an animal with intact vagus nerve. (B) in an animal after bilateral cervical vagotomy and subsequent sympathetic blockade with i.v. guanethidine in 16 μmol/kg.

blood pressure was enhanced following vagal transection combined with guanethidine (16 μmol/kg, $n = 5$) or phentolamine (1.6 μmol/kg, $n = 6$). A sharp and transient rise in gastric blood flow occurred which often coincided with the rise in arterial blood pressure following the initial hypotension evoked by the drug. Capsaicin, 3.3, 9.8 and 19.6 nmol/kg elicited a 15.8 ± 4.1, 16.6 ± 2.6 and 15.9 ± 1.3% increase in gastric blood flow, respectively ($n = 4$). No change in gastric blood flow or in blood pressure was obtained with the solvent ($n = 4$). Similar blood flow changes were observed when capsaicin was injected i.v. after cervical vagotomy combined with guanethidine or phentolamine. In the latter conditions capsaicin in doses of 0.33 and 3.3 nmol/kg caused 21.2 ± 4.5 and 26.6 ± 3.3% increases in gastric blood flow, respectively ($n = 4$). Similarly, after cervical vagotomy and guanethidine treatment, 22 ± 3 and 30 ± 4% increases were obtained with capsaicin 0.33 and 3.3 nmol/kg, respectively ($n = 4$). It should be mentioned that in 2 rats with vagotomy combined with phentolamine, capsaicin (0.33 nmol/kg) evoked an enhancement in gastric blood flow that lasted for several min and which was not related to blood pressure changes.

In experiments in which 3.3 and 9.8 nmol/kg capsaicin

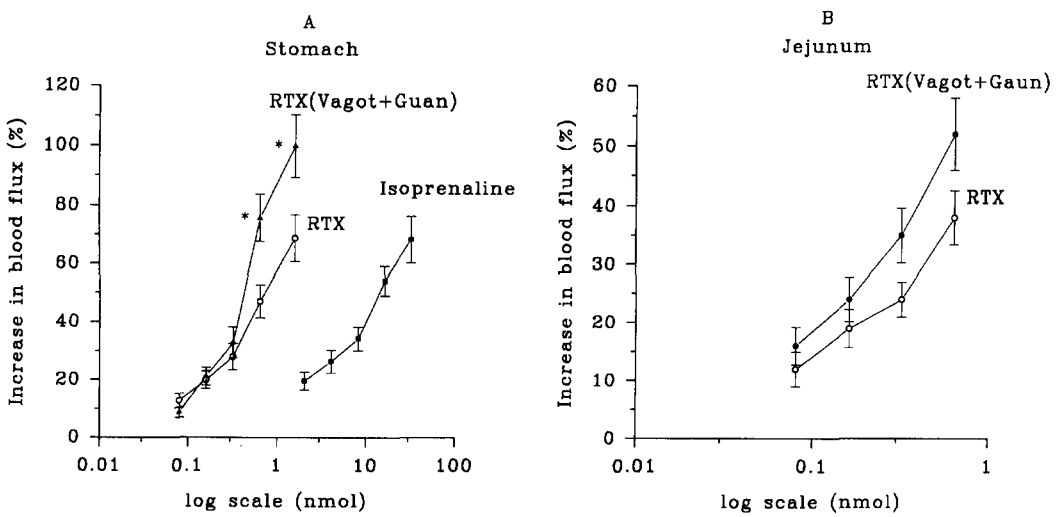

Fig. 3. (A) Log dose-response curves of the effect of i.v. resiniferatoxin (RTX) and isoprenaline (nmol/kg) on gastric blood flow. Resiniferatoxin was given to animals with intact vagal innervation ($n = 8$) and after cervical vagotomy combined with sympathetic neurone blockade with i.v. guanethidine (16 μmol/kg) (vagot + Guan) ($n = 10$). Isoprenaline was given to animals with intact vagus nerves ($n = 8$). (B) Log dose-response curves of the effect of i.v. resiniferatoxin (RTX) (nmol/kg) intestinal mucosal blood flow. Resiniferatoxin was administered to intact animals ($n = 5$) and after cervical vagotomy combined with guanethidine treatment ($n = 12$). Results are expressed as % changes from basal values before the administration of each dose of the drug and are means ± S.E.M. Statistical significance of the difference in blood flow values in the resiniferatoxin-treated groups (intact vs. vagot + Guan) is denoted by * $P < 0.05$.

was injected over a period of 30–60 s, a 22 ± 4.4 and 27 ± 5 increase in gastric blood flow and a rise in blood pressure of 21.4 ± 5.3 and 27 ± 6.6 mm Hg, respectively, were observed ($n = 4$). The correlation between the two responses was marked ($r = 0.88$ for the dose of 3.3 nmol/kg and $r = 0.82$ for 9.8 nmol/kg) and the time course of changes in gastric microcirculation and that of blood pressure was similar (Fig. 1).

A prolonged increase in gastric blood flow, which did not mirror the blood pressure changes was, however, observed with resiniferatoxin. Therefore, most of the experiments were done with this capsaicin-type agent.

3.2. Effect of i.v. resiniferatoxin on gastric blood flow

3.2.1. Experiments in intact animals

In animals with intact vagal innervation ($n = 8$), i.v. resiniferatoxin produced an increase in the gastric blood flow lasting for several minutes (Fig. 2A). The effect was dose-dependent (Fig. 3A) and reproducible at the low dose range of 0.08–0.32 nmol/kg. Administration of resiniferatoxin, 0.16 nmol/kg, repeated four times with 20-min intervals yielded consecutive increases of blood flow values by 33.8, 15.3, 22.5 and 29.3%, respectively ($n = 3$). With higher doses (0.64–1.6 nmol/kg) a tendency to desensitization was observed. The duration of blood flow changes produced by resiniferatoxin ranged between 4–8 min (4.8 ± 0.8 min) for the dose of 0.08 nmol/kg ($n = 5$), 4–12 min (6.9 ± 0.6 min) for 0.16–0.32 nmol/kg resiniferatoxin ($n = 10$), and up to 24–30 min (27.3 ± 1.1 min) for 0.64 nmol/kg resiniferatoxin ($n = 6$) and 30 min (30.0 ± 4.0 min) for resiniferatoxin, 1.6 nmol/kg ($n = 4$).

The maximum increase in arterial blood pressure in response to i.v. resiniferatoxin was 5–10 mm Hg with 0.08 nmol/kg resiniferatoxin ($n = 5$), 10–20 mm Hg with 0.16 nmol/kg resiniferatoxin ($n = 6$), 10–30 mm Hg with 0.32 nmol/kg resiniferatoxin ($n = 9$), 15–30 mm Hg with 0.64 nmol/kg resiniferatoxin ($n = 5$) and 35–40 mm Hg with

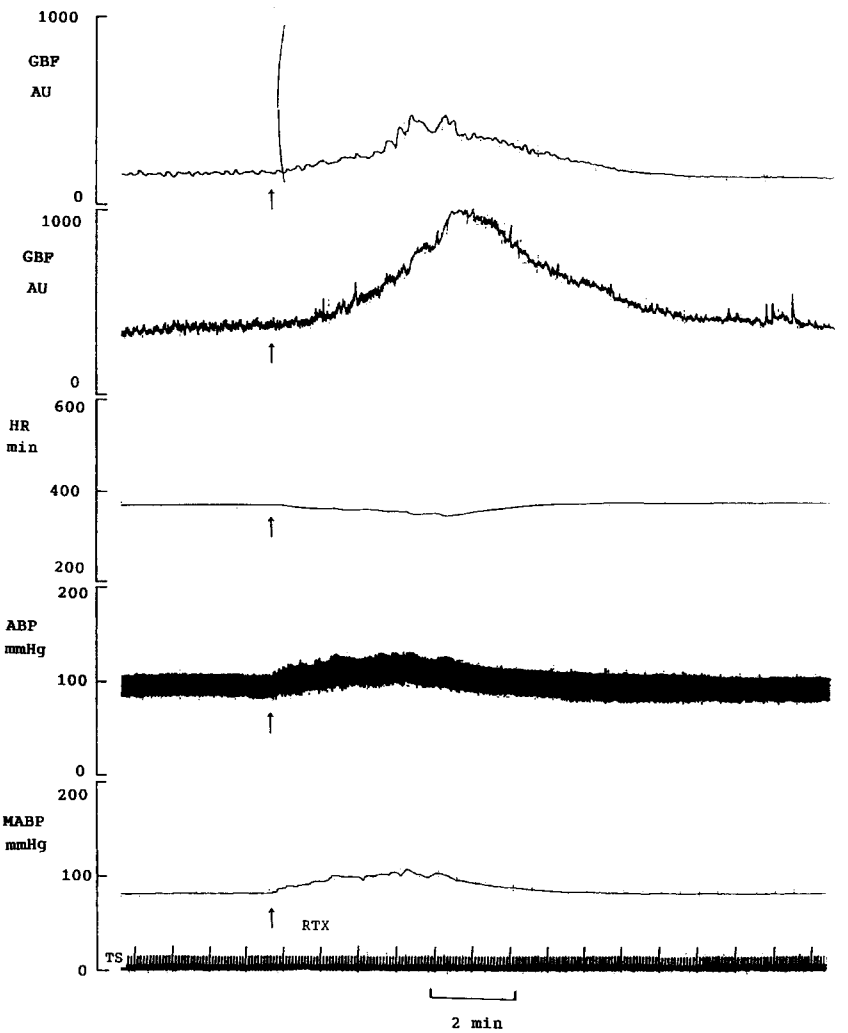

Fig. 4. Experimental tracing of laser Doppler flowmetry showing the effect of i.v. resiniferatoxin (RTX), 0.16 nmol/kg, on gastric blood flow (GBF; AU, arbitrary units) arterial blood pressure (ABP; mm Hg) and mean arterial blood pressure (MABP; mm Hg) after surgical vagotomy and α-adrenoceptor blockade with i.v. phentolamine (1.6 μmol/kg). Two laser probes were applied simultaneously on the serosal surface of the stomach.

1.6 nmol/kg resiniferatoxin ($n = 4$). These responses commenced at about 30 s, and the arterial blood pressure changes lasted from 1–6 min. The heart rate was only minimally changed after resiniferatoxin. Injection of the vehicle had no effect on the laser Doppler signal or blood pressure ($n = 4$).

3.2.2. Experiments after cervical vagotomy and guanethidine

Bilateral cervical vagotomy significantly reduced gastric blood flow by 34.4 ± 3.7% ($n = 23$) at about 30–35 min after cutting of the second vagus. Since, during this time, the blood pressure had remained at the same level, this effect of vagotomy on gastric blood flow could not be attributed to a decrease in perfusion pressure. Vagal transection resulted in only a brief (lasting for about 30 s) decrease in arterial blood pressure. Bilateral subdiaphragmal vagotomy done for comparison was found to reduce gastric blood flow by 20.1 ± 4% ($n = 8$).

In vagotomized rats, guanethidine given i.v. in a dose of 16 μmol/kg caused a further yet slight decrease in the laser Doppler flowmetry signal that lasted for about 60 min ($n = 8$). The maximal decrease in arterial blood pressure was 30–45 mm Hg, while that of blood flow was 20.2 ± 4% of its original level before guanethidine administration.

After acute cervical vagotomy combined with sympathetic blockade with guanethidine, the effect of resiniferatoxin on gastric blood flow persisted (Fig. 2B) and, in fact, was enhanced, particularly after higher doses of 0.64 and 1.6 nmol/kg ($n = 10$) (Fig. 3A). Note in the recording of Fig. 2B that the increase in blood pressure remained after guanethidine treatment and had a time course different from that of the enhancement in gastric blood flow. The increase in gastric blood flow lasted between 2–8 min (5.3 ± 1.8 min) for resiniferatoxin in 0.08 nmol/kg ($n = 5$), 4–12 min (8.2 ± 0.9 min) for 0.16–0.32 nmol/kg resiniferatoxin ($n = 15$ and 10, respectively), 4–26 min (13.5 ± 1.7 min) for 0.64 nmol/kg resiniferatoxin ($n = 11$), and 12–36 min (23.1 ± 2.6 min) for resiniferatoxin in the dose of 1.6 nmol/kg ($n = 11$). On the other hand the maximum rise in arterial blood pressure in response to 0.64 and 1.6 nmol/kg doses commenced before 30 s in all cases, and the duration was usually between 1–5 min. The maximum rise in arterial blood pressure observed was within the range of 10–15 mm Hg with 0.08 nmol/kg resiniferatoxin ($n = 5$), 10–30 mm Hg with 0.16–0.32 nmol/kg resiniferatoxin ($n = 15$ and 7, respectively), 15–60 mm Hg (in one case 75 mm Hg) with 0.64 nmol/kg resiniferatoxin ($n = 11$) and 30–60 mm Hg (in one case 95 mm Hg) with 1.6 nmol/kg resiniferatoxin ($n = 11$).

3.2.3. Experiments after vagotomy and α-adrenoceptor blockade

Phentolamine given i.v. in doses of 0.79 and 1.6 μmol/kg to surgically vagotomized rats did not produce an increase in gastric blood flow ($n = 11$). In most experiments, particularly when arterial blood pressure was markedly reduced, the gastric blood flow was also decreased. In these experiments, when arterial blood pressure was normalized again, the gastric blood flow returned to its pretreatment levels. The dose of 1.6 μmol/kg reduced gastric blood flow in 8/11 animals by an average of 26.2 ± 3.1% (range 9.2–48.8%) as compared to control values. The maximum decrease in arterial blood pressure observed with 0.79 and 1.6 μmol/kg phentolamine was within the range of 30–60 mm Hg ($n = 6$) and 40–75 mm Hg ($n = 11$), respectively.

When resiniferatoxin was given after vagotomy combined with phentolamine, the gastric blood flow responses to resiniferatoxin (Fig. 4) were more pronounced that those

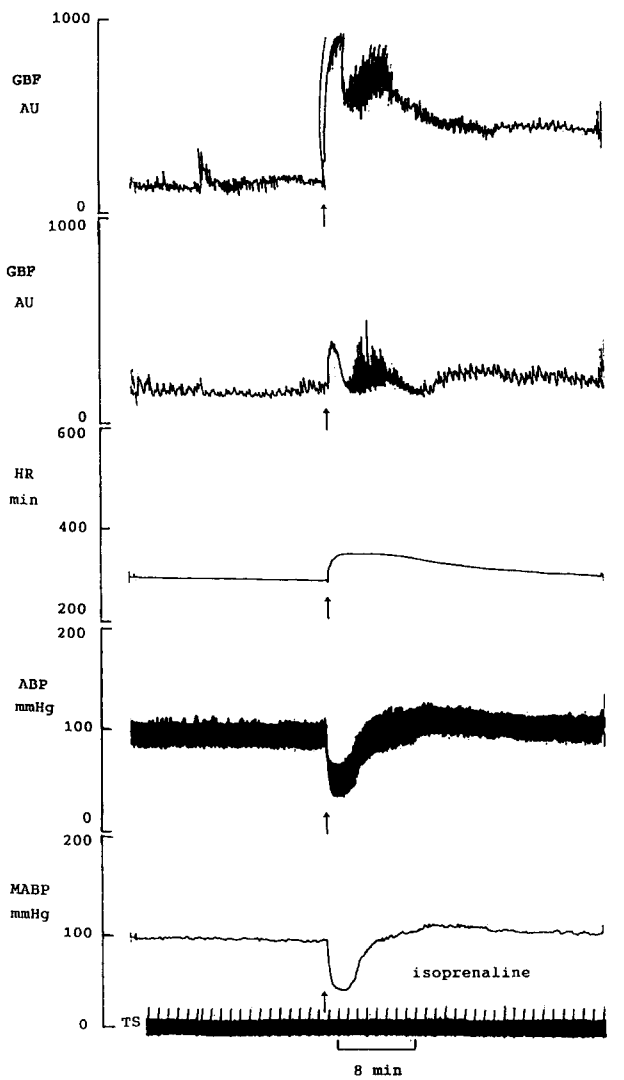

Fig. 5. Experimental tracing of laser Doppler flowmetry showing gastric blood flow (GBF; AU, arbitrary units), arterial blood pressure (ABP; mm Hg), mean arterial blood pressure (MABP; mm Hg), heart rate (HR; beats/min) following i.v. injection of isoprenaline in a dose of 32 nmol/kg. Two laser probes were applied simultaneously on the serosal surface of the stomach.

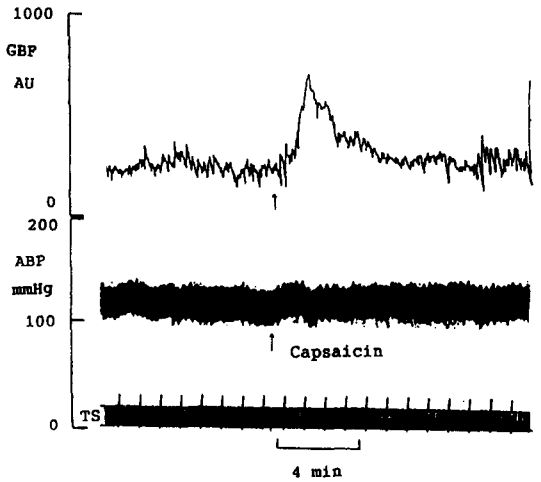

Fig. 6. Experimental tracing of laser Doppler flowmetry showing gastric blood flow (GBF; AU, arbitrary units), arterial blood pressure (ABP; mm Hg) following topical application of capsaicin (3.3 μM) on the serosal surface of the stomach after cervical vagotomy combined with α-adrenoceptor blockade with i.v. phentolamine (1.6 μmol/kg).

in the guanethidine-treated group. The corresponding elevations in gastric blood flow to 0.16 nmol/kg were 68.1 ± 12.8 and 21.2 ± 4%, respectively ($n = 7$) ($P < 0.01$). The duration of blood flow changes induced by resiniferatoxin, 0.16 nmol/kg, after cervical vagotomy, and combined with phentolamine treatment, ranged between 2.5 and 15 min (8.4 ± 1.0 min) ($n = 7$). In most cases the maximum rise in the arterial blood pressure response to i.v. resiniferatoxin was within the range of 15–30 mm Hg with 0.16 nmol/kg resinifertoxin ($n = 5/7$). Higher responses of 50 and 70 mm Hg were noted at 0.16 nmol/kg in 2/7 animals. The changes in arterial blood pressure lasted for 2–6 min, with an occasional response prolonged to 9 min in 2/7 animals.

3.3. Effect of i.v. isoprenaline

In order to compare the potency of resiniferatoxin to that of another vasodilator agent, the effect of the β-adrenoceptor agonist, isoprenaline, was tested. Isoprenaline produced a marked and dose-dependent increase in gastric blood flow ($n = 8$), although it was much less effective than resiniferatoxin (Fig. 3A, Fig. 5). The vasodilator response to isoprenaline was even more marked after cervical vagotomy as tested in 4 other animals at a 32 nmol/kg dose that produced a 97.9 ± 11% increase in gastric blood flux (vs. 68.3% in intact animals). Note that the laser Doppler flowmetry signal rose immediately, together with a decline in arterial blood pressure after the injection and usually fell at the end of hypotension (Fig. 5). The increase in blood flow lasted between 4–12 min (7.0 ± 2.5 min) with isoprenaline, 2 nmol/kg ($n = 4$), 6–20 min (12.8 ± 1.5) for 4–8 nmol/kg isoprenaline ($n =$

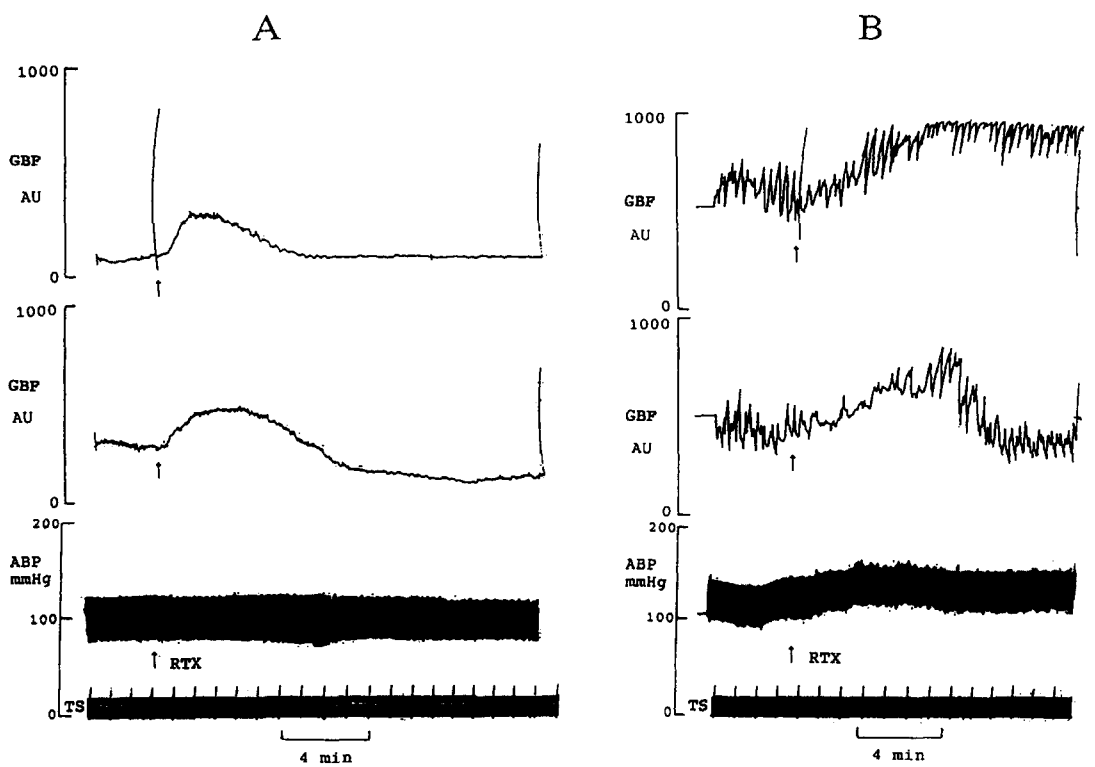

Fig. 7. Experimental tracing of laser Doppler flowmetry from two rats, showing gastric blood flow (GBF; AU, arbitrary units), arterial blood pressure (ABP; mm Hg) following (A) topical application of resiniferatoxin (RTX) (1.6 μM) on the serosal surface of the stomach after cervical vagotomy combined with guanethidine (16 μmol/kg i.v.) and (B) after atropine (1.5 μmol/kg i.v.) and propranolol (3.4 μmol/kg i.v.) treatment. Two laser probes were applied simultaneously on the serosal surface of the stomach.

8), 8–32 min (18.0 ± 2.7 min) for 16 nmol/kg ($n = 8$) and 12–38 min (26.7 ± 1.9 min) for an isoprenaline dose of 32 nmol/kg ($n = 8$).

3.4. Effect of i.v. capsaicin and resiniferatoxin on intestinal blood flow

Guanethidine administered i.v. in 16 μmol/kg after cervical vagotomy produced a reduction in intestinal blood flow throughout the measurement period (30 min). Blood flow was reduced by 25.6% (range 8.8–57%) of its control value at the end of the measurement period ($n = 9$). Capsaicin administered i.v. in doses of 0.3, 3 and 9.8 nmol/kg after vagotomy combined with guanethidine produced 14.2 ± 1.4, 29 ± 4.7 and 28.1 ± 4.9% increases in intestinal blood flow, respectively ($n = 6$). As in the stomach, the increases in intestinal blood flow evoked by i.v. capsaicin were transient and coincided with the transient and sharp phase of the blood pressure increase following injection of the drug, again making the interpretation of these results difficult. The effect of resiniferatoxin on intestinal blood flow was investigated in intact rats ($n = 5$) and after bilateral cervical vagotomy combined with guanethidine treatment ($n = 12$). The magnitude of the evoked increase in blood flow of the small intestine (Fig. 3B) was similar to that observed in the stomach (Fig. 3A).

3.5. Effect of locally applied capsaicin, resiniferatoxin and histamine on gastrointestinal blood flow

Capsaicin and resiniferatoxin applied topically to the serosa of the stomach evoked marked and sustained vasodilatation. In intact animals, local application of capsaicin at various concentrations (0.33, 3.3 and 33 μM) yielded consecutive increases in gastric blood flow of 29.1 ± 4.1% ($n = 7$), 60.9 ± 13% ($n = 6$) and 114.1 ± 19% ($n = 6$), respectively. Resiniferatoxin, 1.6 μM, evoked a 46.2 ± 3% ($n = 4$) increase in gastric blood flow. After treatment with i.v. atropine (1.5 μmol/kg) and i.v. propranolol (3.4 μmol/kg), capsaicin (3.3 μM) and resiniferatoxin (1.6 μM) yielded an increase in gastric blood flow of 51.4 ± 7.3% ($n = 4$) and 41.3 ± 5% ($n = 4$), respectively. After cervical vagotomy combined with phentolamine, capsaicin applied at 3.3 and 33 μM concentration yielded 59.9 ± 7% ($n = 6$) and 74.1 ± 11% ($n = 6$) increases in gastric blood flow, respectively (Fig. 6). Resiniferatoxin, 1.6 μM, elicited a 68.2 ± 8.3% ($n = 6$) increase in gastric blood flow. Similarly, after cervical vagotomy and guanethidine treatment, increases of 47.8 ± 7% ($n = 4$) and 48.6 ± 6% ($n = 6$) were obtained with capsaicin 3.3 μM and resiniferatoxin 1.6 μM, respectively (Fig. 7).

After cervical vagotomy and guanethidine treatment, capsaicin (3.3 μM) and resiniferatoxin (1.6 μM) applied to the jejunal surface evoked a 35.7 ± 6.1% ($n = 6$) and 32.7 ± 5.2% ($n = 5$) increase in blood flow, respectively. In comparison, histamine (5.4 mM) applied to the serosa of the stomach or jejunum produced 142.3 ± 27.4% ($n = 7$) and 40.6 ± 3.2% ($n = 6$) increases in blood flow in these sites, respectively. Serosal application of the solvent for capsaicin and resiniferatoxin had no effect on the laser Doppler signal ($n = 4$).

3.6. Experiments in capsaicin-desensitized rats

In order to decide whether the resiniferatoxin-induced vascular changes were due to an effect on capsaicin-sensitive nerve terminals, the experiments were repeated in rats desensitized by systemic capsaicin doses (cumulative dose 180 mg/kg s.c., last dose given 1–2 days before the experiment). In these rats capsaicin (3.3 and 9.8 nmol/kg) or resiniferatoxin (0.64 and 1.6 nmol/kg) injected i.v. or applied locally in a concentration of 3.3–33 μM to the serosal surface of the stomach did not evoke changes in gastric microcirculation or blood pressure ($n = 5$).

4. Discussion

The present study with laser Doppler flowmetry has provided evidence that capsaicin and resiniferatoxin produce an increase in gastrointestinal blood flow in the rat. Laser Doppler flowmetry has the advantage over methods presently employed for measuring gastrointestinal blood flow in that it provides continuous localized recordings with high sensitivity. The technique is particularly useful for recording the effect of drugs on the microcirculation under 'own control' conditions, since the recorded signals do not represent absolute values (Kiel and Shepherd, 1990). Numerous validation studies have shown that laser Doppler flowmetry measurements in gastrointestinal tissue correlate linearly with data obtained by other established blood flow measuring techniques (Shepherd and Riedel, 1982; Kiel et al., 1985).

In the present study systemic administration of capsaicin was found to result in short-lasting changes in gastrointestinal blood flow which coincided with the increase in arterial blood pressure changes evoked by the drug. Therefore, the enhancement in microcirculation was certainly at least partly due to the increase in perfusion pressure in the gastric mucosa. Injection of resiniferatoxin resulted in a pronounced and consistent increase in gastrointestinal blood flow. The observed vasodilator response to resiniferatoxin was not due to vagal or sympathetic reflexes since bilateral cervical vagotomy combined with α-adrenoceptor blockade with phentolamine or sympathetic blockade with guanethidine did not inhibit the response which, in fact, became more pronounced. This might have been due to the prevention of compensatory reflexes and to the reduction of basal blood flow observed in these conditions. The changes in arterial blood pressure evoked by resiniferatoxin were not due to a sympathetic

reflex either e.g. to nociceptive stimulation, since pretreatment with guanethidine or phentolamine did not inhibit these effects. Hence the action of resiniferatoxin is independent of autonomic nerves. The lack of blood flow and blood pressure changes in response to resiniferatoxin in rats desensitized with systemic capsaicin pretreatment is consistent with earlier data suggesting a highly selective site of action of the irritant on capsaicin-sensitive afferents (Szolcsányi et al., 1990).

Capsaicin is capable of evoking various cardiovascular responses in rats, cats and dogs (Longhurst et al., 1980; Donnerer and Lembeck, 1982; Szolcsányi, 1984; Holzer, 1991b). In the autoperfused canine stomach preparation, capsaicin injected into the left gastroepiploic artery evokes significant increases in systolic arterial blood pressure, heart rate and contractility, whereas its i.v. injection into the inferior vena cava evokes opposite responses (Longhurst et al., 1980). In the rat, i.v. capsaicin evokes a reflex fall in blood pressure, bradycardia and apnoea by exciting the chemosensitive receptors of the pulmonary vagal C-fibres (Coleridge and Coleridge, 1986) and in addition has a direct, short, vasoconstrictor action (Donnerer and Lembeck, 1982). Unlike capsaicin, i.v. injection of resiniferatoxin in rats (2 ng–5 μg/kg) does not elicit the pulmonary chemoreflex triad which is probably attributable to its slower time course for stimulation of sensory receptors (Szolcsányi et al., 1990).

The local action of capsaicin is mediated by peptidergic sensory fibres (Szolcsányi, 1984, 1993; Holzer, 1991a,b). Capsaicin-sensitive sensory fibres containing vasodilator peptides such as calcitonin gene-related peptide and tachykinins form a dense plexus around gastric submucosal blood vessels (Sternini et al., 1987; Green and Dockray, 1988). It is suggested that release of these sensory neuropeptides from capsaicin-sensitive sensory nerve endings is responsible for the vasodilatation and enhancement of microcirculation evoked by capsaicin or resiniferatoxin (Szolcsányi, 1984, 1988; Holzer, 1991b). In this same way, capsaicin enhances gastric mucosal blood flow (Limlomwongse et al., 1979; Lippe et al., 1989; Holzer et al., 1990), dilates gastric (Chen et al., 1992) and ileal (Vanner, 1993) submucosal arterioles and increases mesenteric blood flow (Rózsa et al., 1986).

Although the present laser Doppler recordings measured the microcirculation through the entire gastric or intestinal wall, the significance of mucosal responses is emphasized since the rat gastric wall has a thin muscular layer (20% by weight) in comparison to the mucosal-submucosal layer (80%) (Holm-Rutili and Berglindh, 1986).

In conclusion, the present study with the laser Doppler technique has provided firm evidence that both resiniferatoxin and capsaicin enhance the microcirculation in the stomach and jejunum of the rat. Therefore the results support the notion that enhancement of the microcirculation is an important mechanism that underlies the mucosaprotective effects of capsaicin-type agents administered intragastrically (Szolcsányi and Barthó, 1981; Holzer, 1991a).

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