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Gastric blood flow responses to autonomic nerve stimulation and related pharmacological studies in rats

S. YANO*, A. FUJIWARA, Y. OZAKI AND M. HARADA

Department of Drug Evaluation and Toxicological Sciences, Faculty of Pharmaceutical Sciences, Chiba University, 1–33, Yayoi-cho, Chiba 260, Japan

The effects of autonomic nerve stimulation on rat gastric blood flow (GBF) were studied using a cross thermocouple method. Stimulation of the periarterial nerve bundles along the left gastric artery produced a decrease in GBF which was antagonized by phenoxybenzamine (0·05 mg kg^{-1} i.v.), but not by propranolol (1 mg kg^{-1} i.v.). Stimulation of the vagus nerves elicited an increase in GBF, within a latency of 20 s, which was not apparently affected by atropine (0·15 and 1·5 mg kg^{-1} i.v.) but was completely blocked by hexamethonium (10 mg kg^{-1} i.v.). The GBF increase due to acetylcholine (0·25 μg rat^{-1} i.a.) was markedly blocked by atropine (0·15 mg kg^{-1} i.v.). Vagal stimulation also produced both the cholinergic excitation and non-cholinergic inhibition of gastric motility. The vagally induced GBF increase was little affected by any pretreatment with phentolamine, propranolol, indomethacin or aprotinin. These results suggest that sympathetic nerve stimulation decreases GBF through α-adrenoceptors and parasympathetic nerve stimulation increases GBF through a non-cholinergic mechanism in rats and that the GBF increase may result from a primary dilator effect of vagal stimulation on the blood vessels because of the immediate initiation of the response.

Gastric blood flow (GBF) is under control via extrinsic and intrinsic nervous activity in the stomach as well as via hormones and tissue metabolites in the circulation. Stimulation of the sympathetic nerves causes a decrease in canine GBF (Peter et al 1963), a decrease followed by an increase in cat GBF (Jansson et al 1966), and a constriction followed by a dilation of gastric submucosal arterioles in rats (Guth & Smith 1975a,b). Stimulation of the parasympathetic nerves causes an increase in GBF of dogs (Peter et al 1963) and cats (Martinson 1965a) and a dilation of gastric submucosal arterioles in rats (Guth & Smith 1975b). The GBF decrease induced by sympathetic stimulation is expected to be antagonized by α-adrenoceptor blockers, but there is no crucial pharmacological study on this point. The GBF increase elicited by parasympathetic stimulation is largely reduced by atropine in cats (Jansson et al 1970). There is, however, apparently no documentation regarding such antagonism in other species such as dogs and rats. In addition, the atropine-resistant vasodilator response to parasympathetic stimulation has been known in the salivary gland (Heidenhain 1872), pancreas (Hilton & Jones 1968) and colon (Hultén 1969).

The present study was carried out to monitor GBF changes during autonomic nerve stimulation in rats and to assess pharmacological effects of several drugs including autonomic blockers on the GBF response.

* Correspondence.

METHODS

Male Wistar rats, 240 and 320 g, were fasted for 18 h but were allowed free access to water before the experiment. The procedures for determining regional GBF by a cross thermocouple method were similar to those previously reported (Yano et al 1981). Briefly, the rat was anaesthetized with urethane, 1·5 g kg^{-1} i.p., a cannula inserted into the trachea to allow adequate ventilation, and the right femoral vein cannulated for the administration of drugs. The abdomen was opened, then the stomach exteriorized. A wire-typed cross thermocouple element (W-41, Unique Medical) was transversally implanted into the gastric wall of the glandular portion with a serosal access. Electric potential, which reflexes regional blood flow on the basis of heat clearance, was monitored on a recorder (056, Hitachi) via an amplifier (UM 2000, Unique Medical). The rectal temperature of the animal was maintained between 37 and 38 °C by radiant heat. For checking the GBF responsiveness in the experiment, tetragastrin was injected i.v. at a dose of 7·5 μg kg^{-1}. At the end of experiments, the animals were killed by infusion of KCl, which mostly caused a marked decrease in GBF. The above procedures

applied to all the animals; below are given the additional procedures for each group of experiments. Statistical significance was assessed using Student's *t*-test for paired and unpaired comparison.

Sympathetic nerve stimulation

The periarterial nerve bundles along the left gastric artery were dissected and severed. The peripheral end of the nerve bundles was placed on bipolar, platinum electrodes positioned in the mineral oil-filled well. Impulses were delivered to the electrodes by a square-wave stimulation (Electro-Stimulator 3F31, San-Ei). A repetitive stimulation (10 V, 1 ms, 20 Hz) was applied for 30 s five times at 10 min intervals. Such stimulation was demonstrated to induce relatively constant GBF responses in preliminary experiments.

Parasympathetic nerve stimulation

The bilateral vagi were exposed in the neck, separated from the carotid arteries, ligated and severed centrally. The peripheral end of the left vagus nerves was placed on bipolar, platinum electrodes and the adjacent skin was formed into a well containing mineral oil. A repetitive stimulation for 2 min which elicits constant GBF responses five times at 15-min intervals was 5 V, 0·5 ms and 8 Hz. These stimulus parameters were determined by varying duration (0·1–5 ms), voltage (1–15 V) and frequency (0·5–25 Hz), as presented in Results.

Measurement of gastric motility and blood pressure

Gastric motility was recorded by an intragastric balloon method. A balloon (2·5–3·0 ml) was introduced into the stomach through an incision made in the forestomach. A vinyl tube attached to the balloon was connected to a fluid-filled pressure transducer (LPU-0·1–350, San-Ei) and the balloon pressure was displayed on a chart recorder (056, Hitachi). Systemic blood pressure was monitored via a femoral artery by means of a pressure transducer (MPU–0·5–290, San-Ei).

Drugs and their administration

Drugs used were tetragastrin (Sana-Yakuhin), atropine sulphate (Wako), hexamethonium bromide (Yamanouchi), acetylcholine chloride, phenoxybenzamine hydrochloride (Tokyo-Kasei), indomethacin (Sigma), aprotinin (Trasylol, Bayer), and bradykinin (Nakarai). Drug doses were expressed in terms of the salt. All drugs except acetylcholine and bradykinin were administered via an i.v. route, while the latter were given by a close intra-arterial (i.a.) injection in the stomach according to the procedures described previously (Yano et al 1981).

RESULTS

Sympathetic nerve stimulation

Stimulation of the peripheral nerve bundles (10 V, 1 ms, 20 Hz) caused a decrease in GBF and its repetition tended to slightly reduce the response (Fig. 1A); weak stimulation (5 V, 0·5 ms, 8 Hz) or increasing frequency (10 V, 1 ms, 30 Hz) produced marked tachyphylaxis on its repetition. When stimulation was ceased, the decrease in GBF promptly returned to the resting level, frequently with a transient rebound increase.

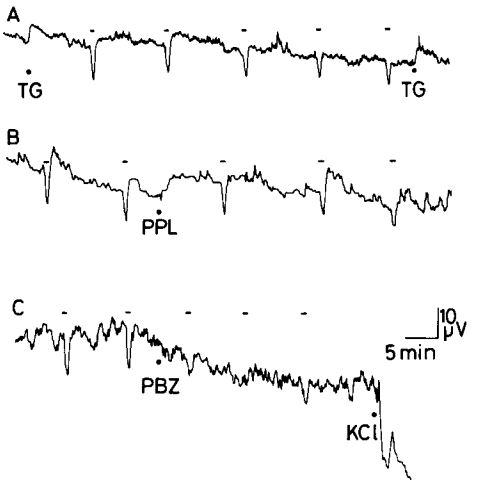

FIG. 1. Typical recordings of GBF decrease induced by periarterial nerve stimulation (10 V, 1 ms, 20 Hz for 30 s at 10-min intervals) in control (panel A) and effects of adrenoceptor blockers on the GBF response (panel B & C). The GBF decrease was not affected after propranolol 1 mg kg^{-1} i.v. but was depressed after phenoxybenzamine 0·05 mg kg^{-1} i.v. (n = 6). PPL: propranolol. PBZ: phenoxybenzamine. TG: tetragastrin (7·5 μg kg^{-1} i.v.). Horizontal bars: stimulation period.

The antagonism of the GBF response to nerve stimulation by adrenoceptor blockers is presented in Fig. 1. β-Adrenoceptor blockade by propranolol, 1 mg kg^{-1}, had no effect on the response, but α-adrenoceptor blockade by phenoxybenzamine, 0·05 mg kg^{-1}, apparently reduced the response; although phenoxybenzamine resulted in a small decrease in the resting GBF level, the subsequent infusion of KCl produced a much greater decrease in GBF than did nerve stimulation.

Parasympathetic nerve stimulation

Stimulation of the vagus nerves (5 V, 0·5 ms, 8 Hz) caused an increase in GBF, and its repetition

produced almost the same responses. The conditions for such repeatable stimulation were determined in experiments where one of three stimulus parameters varied in magnitude with the others kept constant at a given value (Fig. 2). The GBF response was augmented by increasing the magnitude of each parameter; the plateau response was seen at 10 V for voltage, at 1 ms for duration, and at 10 Hz for frequency. In some cases an initial small decrease appeared immediately before the GBF increase upon vagal stimulation, while in most cases an additional increase occurred transiently after the cessation of stimulation.

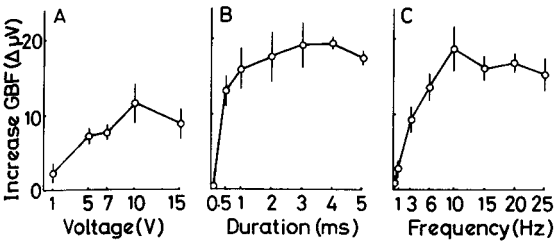

FIG. 2. Influences of varying voltage (panel A; 10 Hz, 2 ms), impulse duration (panel B; 10 Hz, 10 V), and frequency (panel C; 5 V, 2 ms) of vagus nerve stimulation on the GBF response. The GBF response was expressed as changes in electric potential (μV). The values represent the mean ± s.e.m. from 5 experiments.

Influences of vagal stimulation on GBF and gastric motility were compared and effects of atropine and hexamethonium on both the responses were studied (Figs 3 and 4). The GBF increase due to nerve stimulation was slightly reduced following atropine, 0·15 mg kg^{-1}; this change was not statistically significant. The subsequent administration of hexamethonium, 10 mg kg^{-1}, caused a sustained decrease in the resting GBF level and also exerted a complete inhibition of the GBF response to nerve stimulation. The response to tetragastrin, however, remained unchanged. Moreover, nerve stimulation produced a marked contraction of the stomach which persisted during stimulation. After the cessation of stimulation, there was a relaxation which slowly returned to the basal gastric tone. After treatment with atropine, nerve stimulation caused gastric relaxation, followed by a contraction occurring after the cessation of stimulation, i.e. a poststimulation contraction. All these responses during or after stimulation were suppressed by administration of hexamethonium.

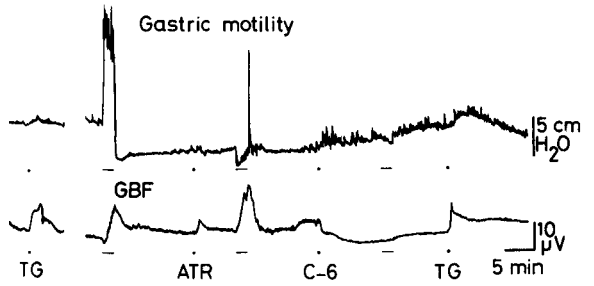

FIG. 3. Typical recordings of GBF (lower) and gastric motility (upper) changes in response to vagus nerve stimulation and effects of atropine and hexamethonium on these responses. Gastric contraction induced by nerve stimulation was abolished by atropine 0·15 mg kg^{-1} i.v., while the GBF increase induced by nerve stimulation was blocked by hexamethonium 10 mg kg^{-1} i.v., but not by atropine (n = 6). ATR: atropine. C-6: hexamethonium. TG: tetragastrin (7·5 μg kg^{-1} i.v.). Horizontal bars: stimulation period.

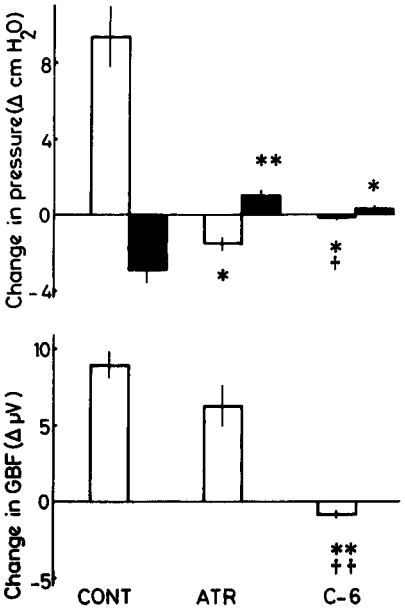

FIG. 4. Effects of atropine and hexamethonium on GBF (lower) and gastric motility (upper) changes in response to vagus nerve stimulation. Open columns: change during stimulation. Solid columns: change after the cessation of stimulation. CONT: control (n = 6). ATR; atropine (0·15 mg kg^{-1} i.v.; n = 6). C-6: hexamethonium (10 mg kg^{-1} i.v.; n = 6). *$P < 0·01$, **$P < 0·001$ compared with control. †$P < 0·05$, ††$P < 0·01$ compared with atropine. The values represent the mean ± s.e.m.

The effect of atropine on the GBF increase due to acetylcholine and to vagal stimulation was compared in the same animals (Fig. 5). Acetylcholine, 0·25 μg rat^{-1} i.a., and vagal stimulation produced a definite increase in GBF before treatment with atropine; the net changes were 15·6 ± 2·9 and 13·9 ± 2·5 μV (the mean ± s.e.m., n = 6), respectively. The GBF increase due to acetylcholine was markedly blocked after atropine, 0·15 mg kg^{-1}; con-

trol percentage of the response was 13·7 ± 7·2 (the mean ± s.e.m., n = 6; $P < 0.001$). On the other hand, the GBF increase due to vagal stimulation was not significantly reduced either by 0·15 mg kg^{-1} or by 1·5 mg kg^{-1} of atropine, although a somewhat greater inhibition was seen with increasing dosage of atropine; control percentage of the response was 82·2 ± 12·7 and 67·8 ± 12·3 (the mean ± s.e.m., n = 6), respectively.

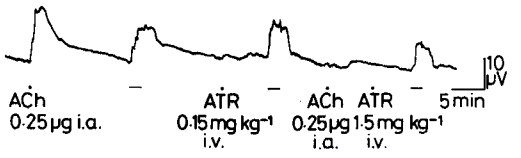

FIG. 5. Typical recordings of GBF increase in response to i.a. acetylcholine and vagus nerve stimulation and effects of atropine on these responses. The GBF increase due to acetylcholine 0·25 μg rat^{-1} i.a. was antagonized by atropine 0·15 mg kg^{-1} i.v., while the GBF increase due to nerve stimulation was not apparently blocked after atropine at 0·15 mg kg^{-1} and additionally at 1·5 mg kg^{-1} i.v. (n = 6). ACh: acetylcholine. ATR: atropine. Horizontal bars: stimulation period.

The effect of adrenoceptor blockers on the GBF response to nerve stimulation is shown in Fig. 6. Phentolamine, 1 mg kg^{-1}, produced a lowering of the resting GBF level together with a fall in blood pressure. In contrast, propranolol, 1 mg kg^{-1} caused a transient increase in GBF which coincided in elevation of blood pressure. The treatment with phentolamine or propranolol exerted no significant change in the GBF response to nerve stimulation; control percentage of the response was 106 ± 12 or 110 ± 7 (the mean ± s.e.m., n = 6), respectively.

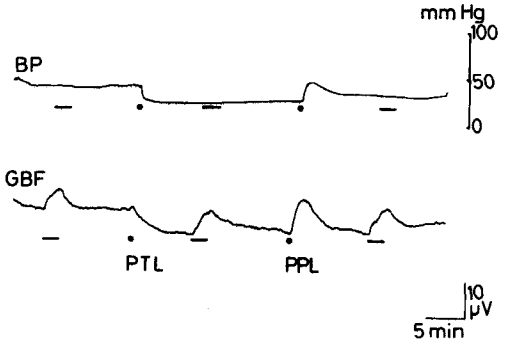

FIG. 6. Typical recordings of GBF (lower) and blood pressure (upper) responses to vagus nerve stimulation after adrenoceptor blockers. The GBF response to nerve stimulation was affected neither by phentolamine 1 mg kg^{-1} i.v. nor by propranolol 0·5 mg kg^{-1} i.v., although blood pressure was lastingly decreased by the former blocker and transiently increased by the latter blocker (n = 6). BP: mean blood pressure. PTL: phentolamine. PPL: propranolol. Horizontal bars: stimulation period.

Indomethacin, 10 mg kg^{-1}, which was used for inhibiting prostaglandin biosynthesis, caused a slight decrease in the resulting GBF level of the atropinized rat (Fig. 7). Stimulation at 5, 20, and 35 min after this treatment caused no prominent change compared with controls; control percentage of the response was 88 ± 15, 89 ± 14, and 108 ± 11 (the mean ± s.e.m., n = 5), respectively. The effect of aprotinin, which was used as a protease inhibitor that depresses the kinin releasing enzyme kallikrein, was explored (Fig. 7). The GBF increase due to nerve stimulation was not significantly affected 5 and 20 min after treatment with aprotinin, 10 000 KIU (kallikrein inhibitor units) rat^{-1} i.v.; control percentage of the response was 101 ± 11 and 91 ± 24 (the mean ± s.e.m., n = 6), respectively. The dilator response to bradykinin, 0·2 μg rat^{-1} i.a. remained unchanged before or after treatment with aprotinin (Fig. 7).

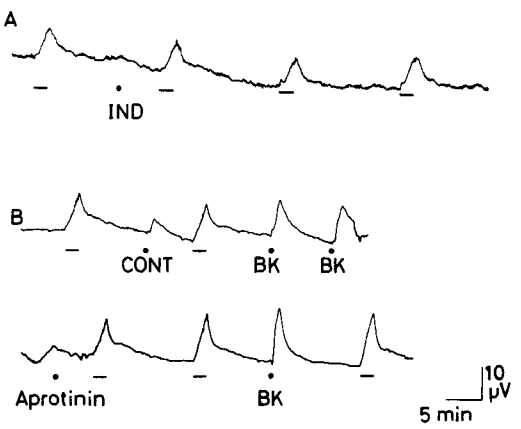

FIG. 7. Typical recordings of GBF response to vagus nerve stimulation after indomethacin 10 mg kg^{-1} i.v. (panel A) or after aprotinin 10 000 KIU rat^{-1} i.v. (panel B). Indomethacin did not affect the GBF increase induced by nerve stimulation in rats pretreated with atropine 0·15 mg kg^{-1} i.v. (n = 5). Aprotinin did not modify the GBF increase induced by nerve stimulation or by bradykinin 0·2 μg rat^{-1} i.a. (n = 6). CONT: saline control (20 μl rat^{-1} i.a.). IND: indomethacin. BK: bradykinin. Horizontal bars: stimulation period.

DISCUSSION

The blood vessels in the gastric mucosa and submucosa are adrenergically innervated (Jacobowitz 1965; Norberg 1967). The results of the present study with rats demonstrated that periarterial nerve stimulation produces a decrease in GBF which is antagonized by

α-adrenoceptor blockade. The GBF decrease is not accompanied by an 'autoregulatory escape' during stimulation which was reported to occur in cats (Jansson et al 1966) or in rats (Guth & Smith 1975b). It is conceivable that a stimulation period of 30 s applied in the present experiments is too short to exert such escape responses. Intra-arterial administration of noradrenaline produces a GBF decrease followed by a GBF increase in rats (Yano et al 1981) and in dogs (Zinner et al 1975). In rat GBF α-adrenoceptor blockade attenuated both the decrease and increase components, while in dog GBF the same blockade abolished only the decrease components and β-adrenoceptor blockade reduced the increase components. Thus, sympathetic nervous control of GBF in rats may be mediated predominantly through the α-adrenoceptors of the blood vessels.

In the present study, the GBF increasing response to i.a. acetylcholine was markedly blocked by atropine at the lower dose of 0·15 mg kg^{-1}, which was consistent with the findings of Yano et al (1981). In contrast, vagus nerve stimulation caused an increase in GBF which was not apparently affected by atropine even at the higher dose of 1·5 mg kg^{-1} but was completely inhibited by hexamethonium. Such atropine-resistant increase in blood flow has so far been reported in the salivary gland (Heidenhain 1872), pancreas (Hilton & Jones 1968) and colon (Hultén 1969). As regards the response of the stomach, atropine largely reduced the increase in cat GBF due to vagal stimulation, together with frequent observations of a small increase (15–30% of control) after the blockade (Martinson 1965b; Jansson et al 1970). The atropine-sensitive GBF increase was considered to be secondary to an augmented gastric secretion (Jansson et al 1970). In the present experiments, however, the GBF increase occurred with a short latency (less than 20 s) after the onset of vagal stimulation, which was consistent with a demonstration of the prompt vasodilation (less than 10 s) by Guth & Smith (1975b) who accordingly proposed a primary vasodilator effect of vagal stimulation in the rat stomach.

The effect of atropine on the vasodilator response to vagal stimulation was dependent on the stimulus frequency in the salivary gland; with stimulus frequencies of up to 5 Hz, the increase in blood flow was reduced by atropine but, at higher frequencies, it was only slightly reduced by the blockade (Darke & Smaje 1972). In contrast, the atropine-sensitive GBF increase in cats was elicited by vagal stimulation at a frequency as high as 8 Hz (Jansson et al 1970). In the present experiments, stimulation at a frequency of 8 Hz produced the atropine-resistant GBF increase in rats. From these facts, the effect of atropine on the vasodilator response to vagal stimulation at a given frequency seems to vary with either organs or animal species. In addition to the GBF response, vagal stimulation primarily excites, but may also inhibit, gastric motility (Martinson & Muren 1963). The relaxation elicited by vagal stimulation is mediated via a non-cholinergic mechanism (Campbell 1966). The results of rat gastric motility in the present experiments were demonstrated to be similar to the findings described above.

The transmission mechanism(s) involved in the atropine-resistant vasodilator response to vagal stimulation remains unknown. Administration of α- and β-adrenoceptor blockers produced no change in the GBF response, suggesting non-adrenergic features of the transmission. Treatment with indomethacin did not affect the GBF response to nerve stimulation in atropinized rats, which indicates no possible involvement of endogenous prostaglandins in the GBF response. Injection of aprotinin, thought to be a kallikrein inhibitor, which was reported to partly abolish the atropine-resistant vasodilator response to pelvic nerve stimulation in the cat colon (Fasth et al 1981), produced no effect on the GBF increase due to vagal stimulation in rats. Accordingly, this finding does not support the view that plasma kinins such as bradykinin play a physiological role in the atropine-resistant GBF increase in rats. Recently, vasoactive intestinal polypeptide (VIP) has been considered as a possible candidate for the transmitter of the atropine-resistant vasodilation in the salivary gland (Bloom & Edwards 1980). Moreover, adenosine triphosphate (ATP) and its related nucleotides, some of which have a vasodilator effect, are supposed to be transmitters in non-adrenergic and non-cholinergic responses of the gastrointestinal tract (Burnstock 1972). We also have observed that both VIP and ATP cause a definite increase in rat GBF (Yano et al, submitted for publication). A possible involvement of these transmitter candidates in the atropine-resistant GBF response remains to be established.

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