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VASODILATATION BY PROSTAGLANDIN $F_{2\alpha}$ IN THE CANINE TONGUE THROUGH A PARASYMPATHETIC MECHANISM

T. SHIMIZU & N. TAIRA

Department of Pharmacology, Tohoku University School of Medicine, Sendai 980, Japan

1 The vascular bed of the tongue *in situ* was perfused with blood through the lingual arteries at a constant pressure in anaesthetized dogs. All drugs except for SQ 14,225 were administered intra-arterially.

2 Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) produced a dose-dependent increase in blood flow through the lingual arteries (vasodilatation).

3 Marked desensitization was observed on the vasodilator responses to repeated administration of $PGF_{2\alpha}$.

4 The vasodilator response to $PGF_{2\alpha}$ was abolished by tetrodotoxin in doses that abolished the vasodilator response to electrical stimulation of the lingual nerve.

5 The vasodilator response to $PGF_{2\alpha}$ was not affected by hexamethonium in doses that almost abolished the vasodilator response to lingual nerve stimulation.

6 The vasodilator responses to $PGF_{2\alpha}$ and to lingual nerve stimulation were scarcely modified by (−)-hyoscyamine in doses that fully antagonized the vasodilator response to acetylcholine.

7 Electrical stimulation of the vago-sympathetic trunk and noradrenaline produced a decrease in blood flow through the lingual arteries.

8 These results indicate that the vasodilator response of the tongue to $PGF_{2\alpha}$ is due exclusively to excitation of parasympathetic postganglionic neurones and that neuronal receptors involved are quite distinct from nicotinic receptors.

9 Intravenous administration of SQ 14,225, an inhibitor of angiotensin I converting enzyme or kininase II, augmented the vasodilator responses to bradykinin and kallikrein but not that to lingual nerve stimulation.

10 The results suggest that neither kallikrein nor kinin (including bradykinin) is responsible for the parasympathetically induced vasodilatation in the tongue.

Introduction

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a weak vasoconstrictor in the dog (Nakano & McCurdy, 1968; Nakano, Perry & Denton, 1968; Greenberg & Sparks, 1969; Nakano & Cole, 1969; Shehadah, Price & Jacobson, 1969). However, Taira & Satoh (1973) and Taira, Narimatsu & Satoh (1975) have demonstrated that in the canine submandibular gland, $PGF_{2\alpha}$ given intra-arterially produces vasodilatation accompanying salivation. Since the vasodilatation in response to $PGF_{2\alpha}$ is abolished completely by tetrodotoxin (Taira & Satoh, 1973) but remains unaffected by hexamethonium (Taira *et al.*, 1975), these authors concluded that in the submandibular gland, $PGF_{2\alpha}$ causes vasodilatation by excitation of parasympathetic postganglionic neurones and that the neuronal receptors involved differ distinctly from nicotinic receptors. The vasodilatation in response to $PGF_{2\alpha}$, like vasodilatation produced by electrical stimulation of the chorda-lingual nerve, is atropine-resistant (Taira *et al.*, 1975) and therefore the nature of the transmitter still remains to be elucidated.

It has also been shown that in the mammalian tongue there are numerous parasympathetic ganglia derived from the chorda-lingual nerve (Okamura, 1936; Fitzgerald & Alexander, 1969) and that electrical stimulation of the chorda-lingual nerve causes vasodilatation in the tongue as well as in the submandibular gland (Heidenhain, 1883; Hilton & Lewis, 1958). Thus, it was of interest to know whether $PGF_{2\alpha}$ would produce vasodilatation in the tongue as well as in the submandibular gland. We performed the present experiments to elucidate this point. Since involvement of bradykinin or a related polypeptide has been suggested for the atropine-resistant vasodila-

tation of the tongue in response to chorda-lingual stimulation (Hilton & Lewis, 1958), we also examined the effect of SQ 14,225, an inhibitor of angiotensin I converting enzyme (Cushman, Cheung & Ondetti, 1977), on the vasodilatation of the tongue caused by chorda-lingual stimulation. SQ 14,225 potentiates the vasodepressor effect of bradykinin (Cushman *et al.*, 1977).

Methods

Experiments were performed on 29 adult mongrel dogs of either sex, weighing 11 to 19 kg. Anaesthesia was induced by a single intravenous injection of pentobarbitone sodium 30 mg/kg and hourly intravenous injections of the same anaesthetic 4 to 5 mg/kg. The upper cervical region was incised in the midline, and the left and right lingual arteries were exposed. Two arms of a Y-shaped cannula were inserted into the left and the right lingual artery at points about 3 cm distal to their origin. Arterial blood from the right femoral artery was conducted to the Y-shaped cannula by means of a peristaltic pump (Harvard Apparatus, Model 1215). Constant pressure perfusion was accomplished by shunting a fraction of blood through a Starling pneumatic resistance to the right femoral vein. The perfusion pressure was adjusted initially to approximate to the mean systemic blood pressure. The perfusion pressure was monitored from a side arm of the perfusion circuit and the systemic blood pressure was measured from the left femoral artery by pressure transducers (Nihon Kohden, MPU-0.5). Blood flow through the arteries was measured by an electromagnetic flowmeter (Nihon Kohden, MF-46-3) situated just proximal to the Y-shaped cannula. Coagulation of blood was prevented by an initial intravenous injection of heparin sodium 500 units/kg and by hourly intravenous injections of 100 units/kg. All recordings were made on an ink-writing rectigraph (San-ei Instrument, Rectiholy 8S).

The left and right lingual nerves were cut and the distal end of either cut nerve was stimulated with square electric pulses of submaximum intensity (6 to 8 V) and of 0.1 ms duration at a frequency of 10 Hz for 30 seconds. In 5 animals the frequency was varied from 1 to 30 hertz. In 5 animals the left vago-sympathetic trunk was cut at the mid cervical level and the central end of the cut trunk was stimulated by square electric pulses of 20 to 25 V and 2 ms duration at 1, 3, 10 and 30 Hz for 15 seconds.

In 5 dogs at the conclusion of experiment, 0.4% w/v indigo carmine in 0.9% w/v NaCl solution (saline) was injected intra-arterially and the area perfused was checked. The whole portion of the tongue was found to be clearly stained by the dye.

The drugs used in the present experiments were acetylcholine chloride (Daiichi), bradykinin (Peptide Research Institute), hexamethonium bromide (Yamanouchi), (−)-hyoscyamine sulphate (Alps Yakuhin), kallikrein (Bayer), 1-(D-3-mercapto-2-methyl-1-oxopropyl)-L-proline (SQ 14,225) (Squibb), (−)-noradrenaline base (Fluka), prostaglandin $F_{2\alpha}$ tromethamine (Upjohn) and tetrodotoxin (Sankyo, each ampoule also contains citric acid and sodium citrate). All drugs except for SQ 14,225 and noradrenaline were dissolved in saline. Noradrenaline was dissolved in 0.01 N HCl. Just before use, SQ 14,225 was dissolved in 100 mM phosphate buffer solution made from redistilled water and equilibrated with nitrogen gas. All drug solutions except for the SQ 14,225 solution were diluted with saline to the desired concentrations. Agonist solutions in volumes of 10 or 30 µl were injected (in 4 s) by the use of individual microsyringes into rubber tubing just proximal to the Y-shaped cannula. Antagonist solutions were infused intra-arterially at a rate of 0.1 or 0.2 ml/min by the use of an infusion pump (Harvard Apparatus, Model 600-900). SQ 14,225 was injected into the femoral vein.

Values in the text are arithmetic means ± s.e. (unless otherwise stated). The difference between mean values was analysed by Student's t test and judged to be significant when P values < 0.05.

Results

Basal values of main parameters under resting conditions

The mean systemic blood pressure of all 29 dogs used was 110 ± 2 mmHg. In 24 preparations in which the lingual nerves were cut bilaterally but the sympathetic nerve supply to the tongue was left intact, the retrograde pressure measured by clamping the tubing just proximal to the side arm to the pressure transducer was 59 ± 3 mmHg. In these preparations the mean blood flow through the lingual arteries was 20 ± 2 ml/min at a constant perfusion pressure of 106 ± 11 (s.d.) mmHg. In 5 preparations in which the lingual nerves were cut bilaterally and the left vago-sympathetic trunk was sectioned, the retrograde pressure measured in a similar way was 52 ± 3 mmHg. In these preparations the mean blood flow through the lingual arteries was 27 ± 7 ml/min at a constant perfusion pressure of 105 ± 7 (s.d.) mmHg.

Effects of prostaglandin $F_{2\alpha}$ on blood flow through the lingual arteries

Single injections of $PGF_{2\alpha}$ (0.1 to 10 nmol) and acetylcholine (0.03 to 3 nmol) into the lingual arteries

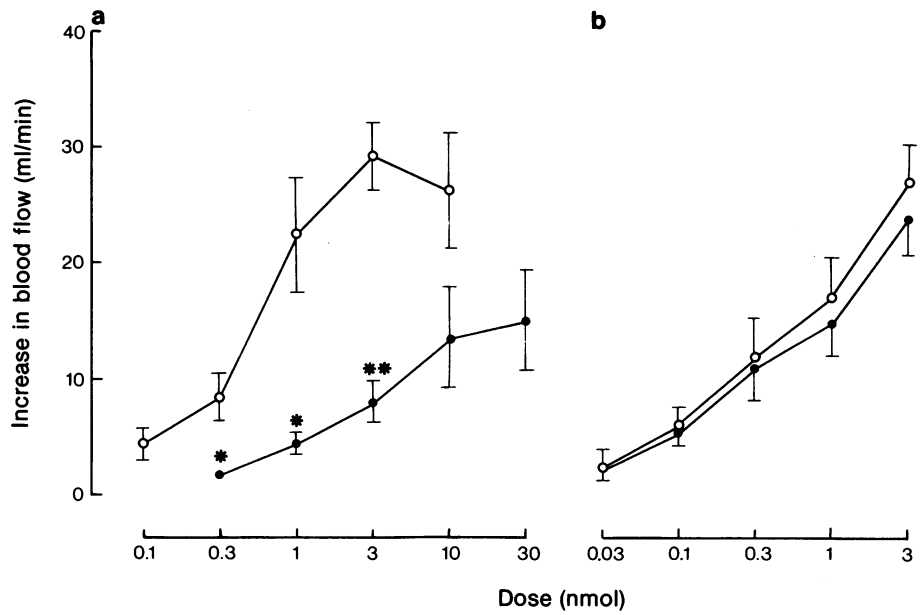

Figure 1 Dose-response curves for increase in blood flow through the lingual arteries to intra-arterial prostaglandin $F_{2\alpha}$ (a) and acetylcholine (b). (○) denotes the first and (●) the second dose-response curves. Each point represents the mean of 6 values from 6 animals. Vertical bars show s.e. mean. * $P < 0.05$ and ** $P < 0.01$ compared with corresponding values of the first dose-response curves.

caused a dose-dependent increase in blood flow (Figure 1). Blood flow responses to $PGF_{2\alpha}$ were slower to develop and longer in duration than those to acetylcholine. Blood flow responses to $PGF_{2\alpha}$ were diminished when intervals between injections were short. Therefore, upon determination of a dose-response curve to $PGF_{2\alpha}$ at least 15 min were allowed to elapse before the next dose was given. In spite of this precaution the second dose-response curve to $PGF_{2\alpha}$ (0.3 to 30 nmol) was situated to the right of the first curve by about 1 log unit (in doses increasing blood flow by 10 ml/min) and was much flatter than the first curve (Figure 1). Therefore, in the experiments described in the following sections only one dose-response curve to $PGF_{2\alpha}$ was obtained from one animal under the action of a given antagonist, and the first dose-response curve to $PGF_{2\alpha}$ presented in this section served as control. The first and second dose-response curves to acetylcholine (0.03–3 nmol), unlike those to $PGF_{2\alpha}$, were not significantly different (Figure 1).

Effects of tetrodotoxin on blood flow responses to prostaglandin $F_{2\alpha}$

Before infusion of tetrodotoxin into the lingual arteries in 5 animals, submaximum electrical stimulation (6 V, 0.1 ms, 10 Hz and for 30 s) of the lingual nerve increased the blood flow by 26.6 ± 3.6 ml/min at the peak. Infusions of tetrodotoxin into the lingual arteries at a rate of 1.5 ± 0.6 (s.d.) nmol/min caused virtually no change in basal blood flow. However, the infusion almost abolished an increase in blood flow in response to electrical stimulation of the lingual nerve (2.3 ± 0.3 ml/min) in 10 to 15 min after the start of infusion (Figure 2). During blockade of neural excitation thus attained, $PGF_{2\alpha}$ (0.1 to 30 nmol) failed to produce an increase in blood flow (Figure 2). In contrast, blood flow responses to acetylcholine (0.03 to 3 nmol) were not significantly different before and during infusion of tetrodotoxin (Figure 2).

Effects of hexamethonium on blood flow responses to prostaglandin $F_{2\alpha}$

In 5 animals hexamethonium was infused into the lingual arteries at a rate of 7.0 ± 3.5 (s.d.) μmol/minute. With this infusion, blood flow through the lingual arteries increased initially by 7.3 ± 2.8 ml/min, but returned gradually to the basal level within about 10 minutes. Blood flow responses (30.1 ± 3.6 ml/min) to electrical stimulation (6 V, 0.1 ms, 10 Hz and for 30 s) of the lingual nerve were

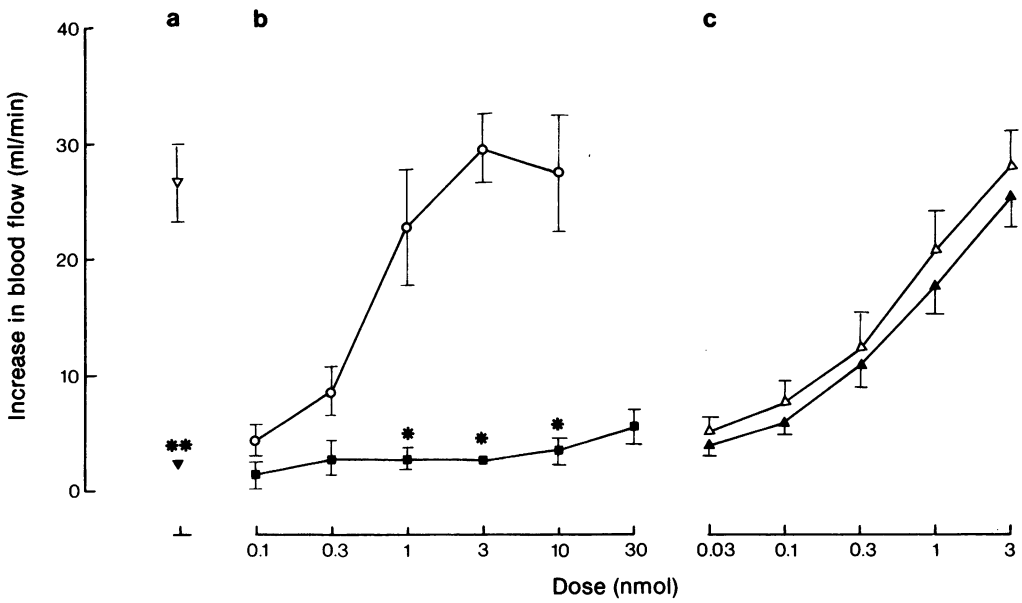

Figure 2 The mean increase in blood flow through the lingual arteries in response to lingual nerve stimulation (a) (6 V, 0.1 ms, 10 Hz and for 30 s) and dose-response curves for increase in blood flow through the lingual arteries to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (b) and acetylcholine (c). Open and filled symbols refer to responses before (control) and during infusion of tetrodotoxin (1.5 ± 0.6 (s.d.) nmol/min), respectively. Each point on the curves, except for the control curve for $PGF_{2\alpha}$, represents the mean of 5 values of 5 animals. It should be noted that the control dose-response curve to $PGF_{2\alpha}$ (○) is the same curve that is shown in Figure 1 ($n = 6$). Vertical bars show s.e. mean. $^*P < 0.05$ and $^{**}P < 0.01$ compared with corresponding control values.

reduced to a greater extent (3.2 ± 0.4 ml/min) in about 20 min after the start of infusion (Figure 3). During blockade by hexamethonium of ganglionic transmission via nicotinic receptors, the dose-response curve to $PGF_{2\alpha}$ for increase in blood flow was not significantly different from the control curve (Figure 3). The dose-response curves to acetylcholine for increase in blood flow were not significantly different before and during blockade of nicotinic receptors (Figure 3).

Effects of (−)-hyoscyamine on blood flow responses to prostaglandin $F_{2\alpha}$

In 6 dogs infusion of (−)-hyoscyamine (60.0 ± 19.0 (s.d.) nmol/min) into the lingual arteries almost abolished an increase in blood flow in response to acetylcholine (0.03 to 10 nmol) (Figure 4). During blockade of muscarinic receptors thus attained, submaximum electrical stimulation (6 to 8 V, 0.1 ms, 10 Hz and for 30 s) of the lingual nerve produced an increase in blood flow of 23.0 ± 3.5 ml/min, which was not significantly different from the increase of 24.3 ± 2.9 ml/min before (−)-hyoscyamine (Figure 4). Under these conditions $PGF_{2\alpha}$ (0.1 to 10 nmol) produced an increase in blood flow which was not statistically different from that in control animals (Figure 4). (−)-Hyoscyamine itself caused no change in basal blood flow.

Effects of electrical stimulation of the vago-sympathetic trunk and of noradrenaline on blood flow through the lingual arteries

Electrical stimulation of the left vago-sympathetic trunk (20 to 25 V, 2 ms at 1, 3, 10 and 30 Hz for 15 s) in 5 animals produced a frequency-dependent decrease in blood flow through the lingual arteries (Figure 5). Intra-arterial injections of noradrenaline (0.03 to 3 nmol) in these 5 animals also produced a dose-dependent decrease in blood flow through the lingual arteries (Figure 5).

Effects of SQ 14,225 on blood flow responses to parasympathetic nerve stimulation, bradykinin, kallikrein and acetylcholine

In 5 animals submaximum electrical stimulation (6 V, 0.1 ms at 1, 3, 10 and 30 Hz for 30 s) of the

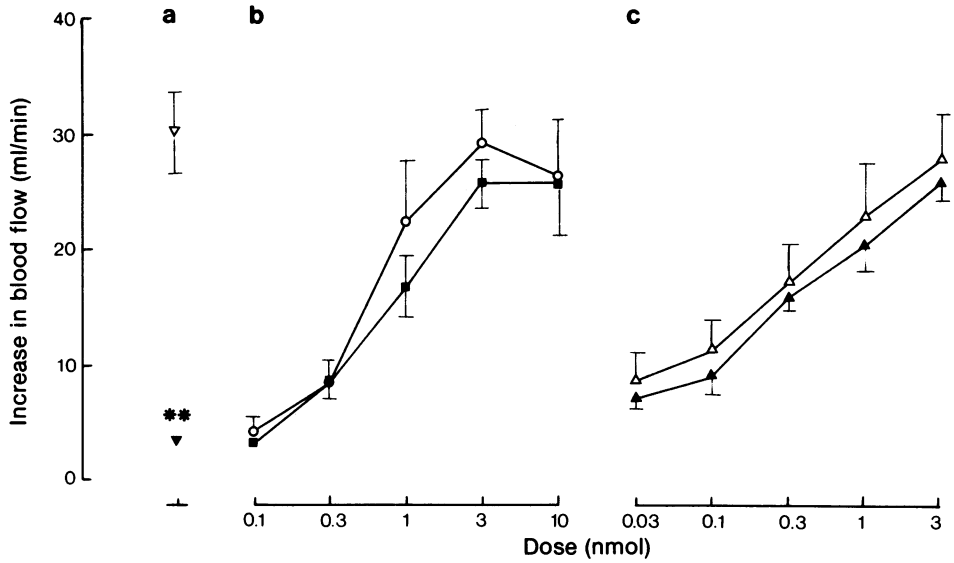

Figure 3 The mean increase in blood flow through the lingual arteries in response to lingual nerve stimulation (a) (6 V, 0.1 ms, 10 Hz and for 30 s) and dose-response curves for increase in blood flow through the lingual arteries to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (b) and acetylcholine (c). Open and filled symbols refer to responses before (control) and during infusion of hexamethonium (7.0 ± 3.5 (s.d.) μmol/min), respectively. Each point on the curves, except for the control curve for $PGF_{2\alpha}$, represents the mean of 5 values from 5 animals. The control dose-response curve to $PGF_{2\alpha}$ (○) is the same curve as that shown in Figure 1 (n = 6). Vertical bars show s.e. mean. $^{**}P < 0.01$ compared with corresponding control values.

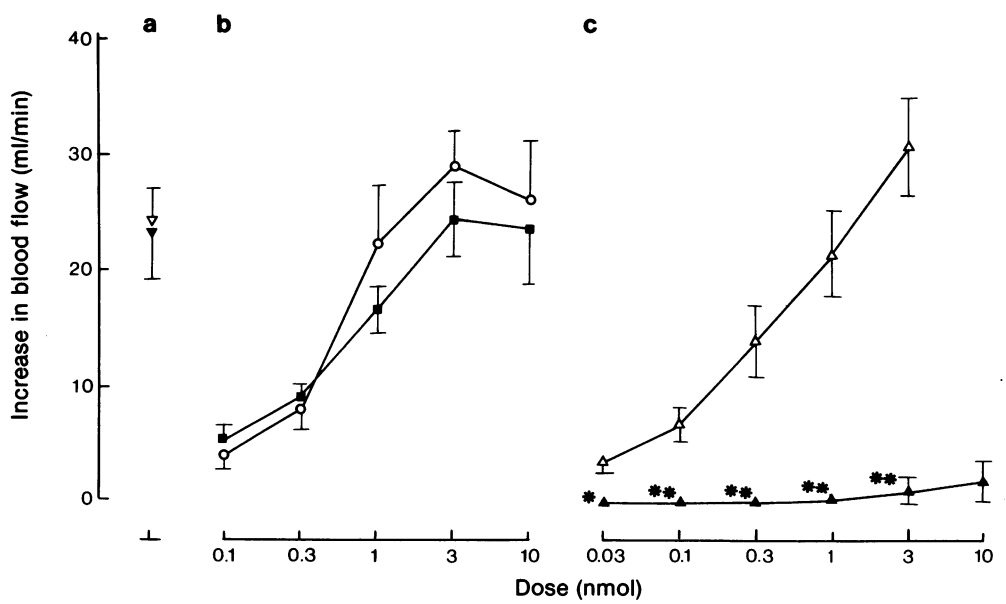

Figure 4 The mean increase in blood flow through the lingual arteries in response to lingual nerve stimulation (a) (6–8 V, 0.1 ms, 10 Hz and for 30 s) and dose-response curves for increase in blood flow through the lingual arteries to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (b) and acetylcholine (c). Open and filled symbols refer to responses before (control) and during infusion of (−)-hyoscyamine (60.0 ± 19.0 (s.d.) nmol/min), respectively. The control dose-response curve to $PGF_{2\alpha}$ (○) is the same curve as that shown in Figure 1. Vertical bars show s.e. mean (n = 6). $^{*}P < 0.05$ and $^{**}P < 0.01$ against corresponding control values.

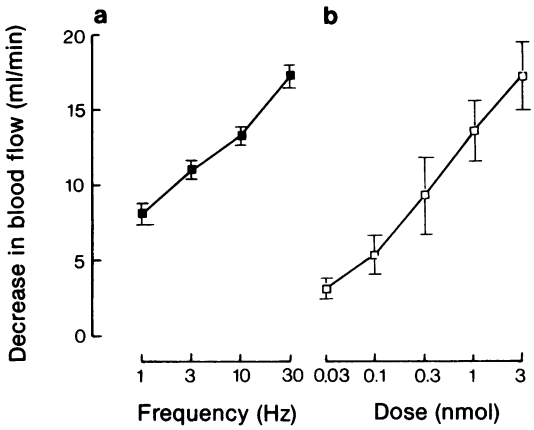

Figure 5 Frequency-response curve to electrical stimulation of the left vago-sympathetic trunk (a) (20–25 V, 2 ms and for 15 s) (■) and dose-response curve to noradrenaline (b) (□) for decrease in blood flow through the lingual arteries. Vertical bars show s.e. mean (n = 5).

lingual nerve produced an increase in blood flow through the lingual arteries in a frequency-dependent manner (Figure 6). In these animals injections of bradykinin (0.1 to 10 pmol), kallikrein (3×10^{-4} to 3×10^{-2} biological units), or acetylcholine (0.03 to 3 nmol) into the lingual arteries produced a dose-dependent increase in blood flow (Figure 6). An intravenous injection of SQ 14,225 shifted the dose-response curves for bradykinin or kallikrein to the left by about 0.6 log units (Figure 6). In contrast, the dose-response curve for acetylcholine and the frequency-response curve for stimulation of the lingual nerve were not significantly changed by SQ 14,225 (Figure 6). A few minutes after the intravenous injection of SQ 14,225 the blood flow through the lingual arteries increased by 7.2 ± 2.5 ml/min and then decreased gradually, but remained slightly higher than the pre-drug level.

Discussion

The area stained by indigo carmine injected into the lingual arteries at the conclusion of the present experiments was confined mainly to the tongue, and the sublingual glands were spared. Thus, it is reasonable to conclude that the perfused area was mainly the tongue and blood flow responses to electrical stimulation of the lingual nerve or the vago-sympathetic trunk and to drugs injected intra-arterially reflected responses of the vascular bed of the tongue.

$PGF_{2\alpha}$ injected into the lingual arteries produced a marked vasodilatation. Since the vasodilator response to $PGF_{2\alpha}$ was abolished by tetrodotoxin in doses which just abolished that to lingual nerve stimulation, it is highly probable that the vasodila-

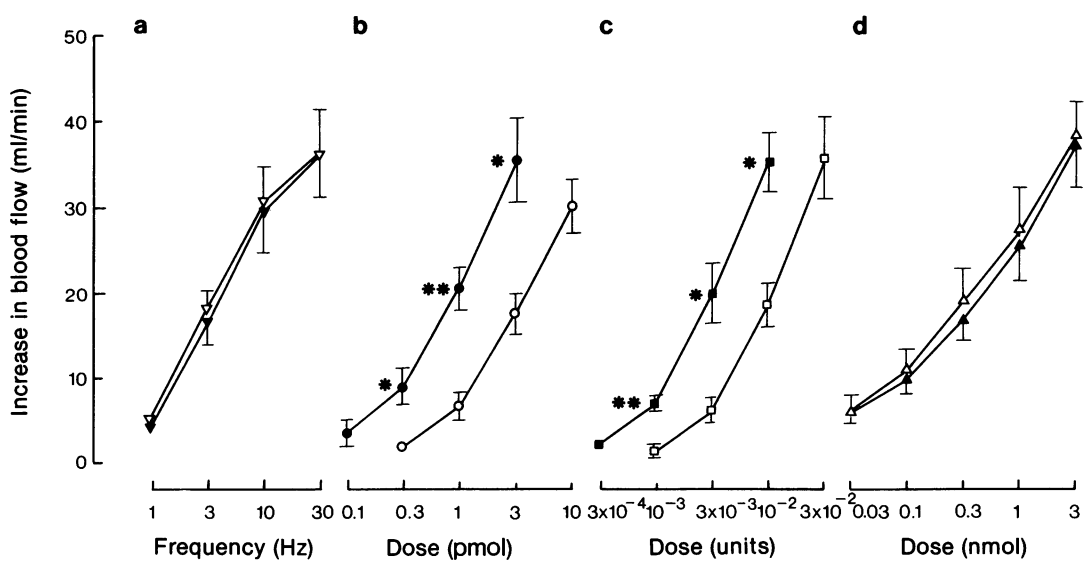

Figure 6 Frequency-response curves to lingual nerve stimulation (a) (6 V, 0.1 ms and for 30 s) and dose-response curves to bradykinin (b), kallikrein (c) and acetylcholine (d) for increase in blood flow through the lingual arteries. Open and filled symbols refer to responses before (control) and after a single injection of SQ 14,225 (1 mg/kg i.v.), respectively. Vertical bars show s.e. mean (n = 5). $^{*}P < 0.05$ and $^{**}P < 0.01$ against corresponding control values.

tation was produced by excitation of parasympathetic nerves. Possible involvement of sympathetic nerves in the vasodilator response can be ruled out by the findings that electrical stimulation of the central stump of the cut vago-sympathetic trunk i.e. the peripheral stump of the cervical sympathetic nerve or intra-arterial noradrenaline produced only vasoconstriction. Persistence of the vasodilator response to $PGF_{2\alpha}$ during blockade by hexamethonium of ganglionic transmission via nicotinic receptors as shown by great reduction of the vasodilator response to lingual nerve stimulation suggests that a site of action of $PGF_{2\alpha}$ is the parasympathetic postganglionic neurone and that the receptors involved differ distinctly from nicotinic receptors there. Anatomical studies have shown that in the mammalian tongue there are numerous ganglia derived from the chorda-lingual nerves (Okamura, 1936; Fitzgerald & Alexander, 1969). It is well known that vasodilatation of the tongue in response to electrical stimulation of the chorda-lingual nerve is atropine-resistant (Erici & Uvnäs, 1952). Like vasodilatation caused by lingual nerve stimulation, the vasodilator response to $PGF_{2\alpha}$ was scarcely affected by (−)-hyoscyamine in doses which fully antagonized the vasodilator effect of acetylcholine. This finding supports the conclusion drawn above that the vasodilatation produced by $PGF_{2\alpha}$ is due entirely to its excitant action on parasympathetic postganglionic neurones. Thus, the present results are in line with the results obtained in the canine submandibular glands (Taira & Satoh, 1973; Taira *et al.*, 1975). The present results also indicate that $PGF_{2\alpha}$ has no direct effect on the vascular bed of the tongue. This is of interest in view of the observations that in most arterial beds of the dog, $PGF_{2\alpha}$ has a direct vasoconstrictor action (Nakano & McCurdy, 1968; Nakano *et al.*, 1968; Greenberg & Sparks, 1969; Nakano & Cole, 1969; Shehadah *et al.*, 1969) and in the vascular bed of the dog urinary bladder it has a direct vasodilator action (Taira, 1974).

The vasodilator responses to lingual nerve stimulation and to $PGF_{2\alpha}$ were both atropine-resistant. Thus, possible mechanisms for the atropine-resistance of these parasympathetically induced vasodilatations should be inferred. Hilton & Lewis (1958) have postulated that secretomotor fibres to minor salivary glands in the tongue are excited upon stimulation of the chorda-lingual nerve and that kallikrein is released from the glandular cells during activity to form bradykinin or a related polypeptide which is responsible for the atropine-resistant vasodilatation. In the present experiments SQ 14,225, the inhibitor of angiotensin I converting enzyme (Cushman *et al.*, 1977), at a dose which greatly potentiated the vasodilator responses to bradykinin and kallikrein, failed to augment the vasodilator response to lingual nerve stimulation. Since kininase II which inactivates bradykinin is the same enzyme that converts angiotensin I to II (Yang, Erdös & Levin, 1971), it is to be expected that SQ 14,225 would potentiate the action of bradykinin. The failure of potentiation of the vasodilator response to lingual nerve stimulation by SQ 14,225 rules out the possibility that kallikrein and kinins play an essential role in the atropine-resistant vasodilatation. Using bradykinin potentiating peptides in the submandibular gland of the dog, Ferreira & Smaje (1976) reached a conclusion similar to ours.

As opposed to the kallikrein-kinin hypothesis, physiological evidence has been gained to indicate the existence of vasodilator nerve fibres ending close to the smooth muscle cells of blood vessels in the submandibular gland (Karpinski, Barton & Schachter, 1971). In the human tongue a histochemical study (El-Rakhawy & Bourne, 1961) has also shown direct innervation of arteries and arterioles by parasympathetic nerve fibres. However, the presence of acetylcholinesterase in these nerve fibres is difficult to reconcile with the atropine-resistance of the parasympathetically induced vasodilatation. Possibly a novel parasympathetic transmitter substance is involved.

In the present experiments the vasodilator response to $PGF_{2\alpha}$ showed marked desensitization. As discussed in the preceding paragraph, the vasodilator response to $PGF_{2\alpha}$ is due entirely to the excitatory action of $PGF_{2\alpha}$ on parasympathetic postganglionic neurones. Marked desensitization has also been observed in the excitatory responses of brain stem neurones (Avanzino, Bradley & Wolstencroft, 1966). Thus, desensitization or tachyphylaxis seems to be a common feature of neuronal excitatory responses to $PGF_{2\alpha}$. The absence of such marked desensitization in the responses to $PGF_{2\alpha}$ of parasympathetic postganglionic neurones in the canine submandibular gland as observed in the previous experiments (Taira *et al.*, 1975) can be ascribed to the relatively small doses of $PGF_{2\alpha}$ used in those experiments.

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