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ASSESSMENT OF THE EFFECTS OF VASOACTIVE INTESTINAL PEPTIDE (VIP) ON BLOOD FLOW THROUGH AND SALIVATION OF THE DOG SALIVARY GLAND IN COMPARISON WITH THOSE OF SECRETIN, GLUCAGON AND ACETYLCHOLINE

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1 The vascular bed of the submandibular gland *in situ* was perfused with blood through the glandular artery at a constant pressure in anaesthetized dogs. All drugs were administered intra-arterially.

2 Vasoactive intestinal peptide (VIP), secretin and acetylcholine produced a dose-dependent increase in blood flow through the artery (vasodilatation) but glucagon was almost ineffective.

3 Dose-blood flow response curves for VIP and secretin were parallel, and VIP was about 100 times as potent as secretin on a molar basis. Dose-blood flow response curves for acetylcholine were flatter than those for VIP and secretin. Acetylcholine was approximately as potent as secretin on a molar basis.

4 No tachyphylaxis developed to the vasodilator action of VIP.

5 The vasodilator responses to VIP and to electrical stimulation of the chordolingual nerve were scarcely modified by (−)-hyoscyamine in doses that fully antagonized the vasodilator response to acetylcholine.

6 VIP, secretin and glucagon were ineffective in eliciting salivary secretion.

7 The possibility that VIP is released from parasympathetic vasodilator nerves and mediates the atropine-resistant vasodilatation in the dog submandibular gland is discussed.

Introduction

Vasoactive intestinal peptide (VIP) isolated by Said & Mutt (1970; 1972) from the hog small intestine is now widely investigated because of its very potent biological activities (Said & Mutt, 1970; 1972) and its presence not only in endocrine cells in the gastrointestinal tract (Polak, Pearse, Garaud & Bloom, 1974; Bryant, Bloom, Polak, Albuquerque, Modlin & Pearse, 1976) but also in neurones in the autonomic and central nervous system (Bryant *et al.*, 1976; Said & Rosenberg, 1976). The presence of VIP in neurones led to the supposition that VIP may play a physiological role as a neurotransmitter or a neuromodulator (Bryant *et al.*, 1976). Indeed, Schaffalitzky de Muckadell, Fahrenkrug & Holst (1977) demonstrated the release of VIP upon electrical stimulation of the vagus nerve in anaesthetized hogs and suggested that VIP is possibly involved in the atropine-resistant pancreatic vasodilatation and exocrine secretion produced by vagal nerve stimulation in hogs.

The atropine-resistance of vasodilatation of the salivary gland produced by electrical stimulation of the chordolingual nerve is a century-old problem (Heidenhain, 1872). The vasodilatation of the tongue produced by chordolingual nerve stimulation is also atropine-resistant (Erici & Uvnäs, 1952; Hilton & Lewis, 1958). Although attempts to interpret the atropine-resistance by a special cholinoceptive mechanism (Dale & Gaddum, 1930; Schachter, 1967) have been made, other possibilities have also been sought. These include the involvement of the kallikrein-kinin system (Hilton & Lewis, 1955; 1958) and the suggestion of the possible release of novel transmitters (Taira, Narimatsu & Satoh, 1975; Shimizu & Taira, 1978). Studies on the salivary gland (Ferreira & Smaje, 1976) and the tongue (Shimizu & Taira, 1978) of the dog using bradykinin-potentiating substances ruled out the possible involvement of the kallikrein-kinin system. Bryant *et al.* (1976) have demonstrated that in rats, hogs and man, nerve fibres in the salivary glands contain high concentrations of VIP. This tempted us to speculate that VIP might be responsible for the parasympathetically induced atropine-resistant vasodilatation there. In this respect it was of interest to investigate how potent VIP is in producing vasodilatation in the dog salivary gland and whether tachyphylaxis develops to its vasodilator action. VIP has been

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shown to be very potent in producing vasodilatation or hypotension (Said & Mutt, 1970). Since VIP has been found to have an amino acid sequence related to that of secretin and glucagon (Mutt & Said, 1974), the effects of the latter two polypeptides were also investigated.

Methods

Experiments were performed on 16 dogs of either sex, weighing 12 to 25 kg. The animals were anaesthetized with pentobarbitone sodium initially at a dose of 30 mg/kg intravenously and subsequently with hourly supplemental intravenous doses of 4 to 5 mg/kg. The duct of the submandibular gland, the chordolingual nerve and the external carotid, maxillary, facial and glandular arteries were exposed on either side. The duct was cannulated with polyvinyl tubing and the saliva passed into a water-filled bottle displacing water which overflowed on to an electronic drop counter (Data-Graph, HT 21). One drop was approximately 15 μl. After the animal had been given heparin sodium (500 units/kg i.v.), the external carotid and maxillary arteries were ligated and a polyethylene cannula was introduced into the facial artery through a cut made in the cranial end of the external carotid artery. Then, the facial artery was ligated just distally to the origin of the glandular artery. Small branches to muscles were all ligated. Blood from the femoral artery was delivered to the cannula placed in the facial artery by means of a peristaltic pump (Harvard Apparatus, Model 1210). A Starling pneumatic resistance was placed distally to the pump to shunt a fraction of blood to the femoral vein. Thus, the glandular vascular bed was perfused selectively with blood at a constant pressure. Perfusion pressure was set initially to approximately the level of the mean systemic blood pressure and kept constant throughout the experiment. Perfusion pressure was monitored from the side arm of the perfusion circuit and systemic blood pressure at the external carotid artery with pressure transducers (Nihon Kohden, MPU-0.5). Blood flow through the glandular artery was measured with an electromagnetic flow meter (Nihon Kohden, MF-46), a flow probe of which was placed just proximally to the cannula introduced into the facial artery. The chordolingual nerve was cut in all experiments and the distal stump of the cut nerve was stimulated electrically, when necessary. Stimulus parameters were 6 V, 0.1 ms, 10 Hz and for 30 s. Details of the preparation have been given previously (Satoh, Takeuchi & Hashimoto, 1972).

Drugs used were highly purified porcine VIP (a generous gift from Professor Victor Mutt), highly purified porcine secretin (Eisai; 16000 units/mg), glucagon (Novo Industri), acetylcholine chloride (Daiichi Seiyaku) and (−)-hyoscyamine sulphate (Alps Yakuhin). VIP was dissolved in 100 mM phosphate buffer solution and other drugs in 0.9% w/v NaCl solution (saline). Agonist solutions in volumes of 10 or 30 μl were injected (in 4s) by the use of individual syringes into rubber tubing just proximal to the cannula placed in the facial artery. Antagonist solutions were infused intra-arterially at a rate of 0.1 ml/min by the use of an infusion pump (Harvard Apparatus, Model 600-900).

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 were less than 0.05.

Results

Basal values of main parameters under resting conditions

Submandibular glands were all acutely parasympathetically decentralized but the sympathetic nerve supply to the gland was left intact. In 16 such glands, spontaneous salivary secretion was 5.4 ± 0.7 drops/10 min or 8.1 ± 1.1 μl/min and the mean blood flow through the glandular artery was 5.5 ± 0.7 ml/min at a constant perfusion pressure of 121 ± 16 (s.d.) mmHg. The mean systemic blood pressure of the 16 dogs was 121 ± 3 mmHg and the retrograde pressure at the glandular artery measured by clamping the tubing just proximal to the side arm to the pressure transducer was 57 ± 7 mmHg.

Effects of VIP, secretin, glucagon and acetylcholine on blood flow through and salivary secretion of the submandibular gland

VIP was given to 5 and secretin to 6 of the 16 glands, and glucagon to the remaining 5 glands. Acetylcholine was given to all 16 glands. Single injections of VIP (0.3 to 100 pmol), secretin (0.1 to 10 nmol) and acetylcholine (0.1 to 30 nmol) into the glandular artery produced a dose-dependent increase in blood flow. A near maximum increase in blood flow was produced by 100 pmol of VIP or 10 nmol of secretin. However, glucagon even in a dose as large as 30 nmol produced only a slight increase in blood flow, although the glandular vascular bed of the 5 glands responded to acetylcholine in the same way as the remaining 11 glands. Typical experiments are shown in Figure 1 and dose-response curves for peak increase in blood flow to the three polypeptides are shown in Figure 2, and dose-blood flow response curves for acetylcholine in Figure 3. The dose-blood flow response curves for VIP and secretin were parallel, and VIP was about 100 times as potent as secretin on a molar basis. The dose-blood flow re-

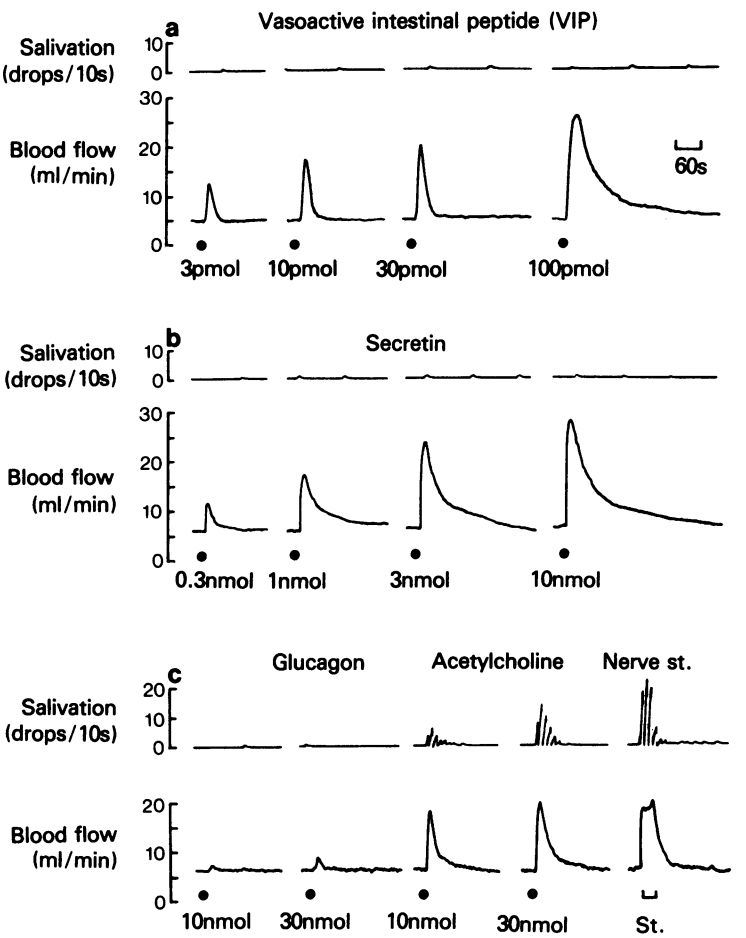

Figure 1 Salivary and blood flow responses of dog submandibular glands *in situ* to vasoactive intestinal peptide (VIP), secretin, glucagon and acetylcholine and to electrical stimulation of the chordolingual nerve (6 V, 0.1 ms, 10 Hz and for 30 s) (Nerve st.). Records of responses to glucagon, acetylcholine and nerve stimulation were obtained from the same gland (c). Records of responses to VIP (a) and to secretin (b) were obtained from two other glands. One drop of saliva had a volume of approximately 15 μl.

sponse curve to acetylcholine was flatter than that to VIP or secretin. Secretin was approximately equipotent to acetylcholine. The three polypeptides were almost ineffective in eliciting salivary secretion. Acetylcholine produced salivary secretion.

The second dose-blood flow response curves to VIP obtained in two glands in the absence of (−)-hyoscyamine appeared similar to the first curves, suggesting that there is no tachyphylaxis to its action. This was substantiated by the experiments described in the following section.

Absence of modification of blood flow responses to VIP by (−)-hyoscyamine

The experiments were done on the same 5 glands that received VIP. Single injections of acetylcholine (0.1 to 30 nmol) produced an increase in blood flow and salivary secretion in a dose-dependent manner. Electrical stimulation (6 V, 0.1 ms, 10 Hz and for 30 s) of the chordolingual nerve produced an increase in blood flow (13.3 ± 0.9 ml/min, $n = 5$) comparable to that produced by 3 to 10 nmol of acetylcholine at the peak effect. The stimulation also elicited salivary secretion (1377 ± 236 μl/response, $n = 5$) greater in amount than that caused by 30 nmol of acetylcholine. Infusion of (−)-hyoscyamine at a rate of 24.0 ± 8.2 (s.d.) nmol/min into the glandular artery greatly reduced the increase in blood flow in response to acetylcholine (0.1 to 30 nmol) about 3 min after the start of infusion (Figure 3). The salivary responses to acetylcholine (1 to 30 nmol) and to electrical stimulation of the chordolingual nerve were abolished by the (−)-hyoscyamine infusion. With the blockade of

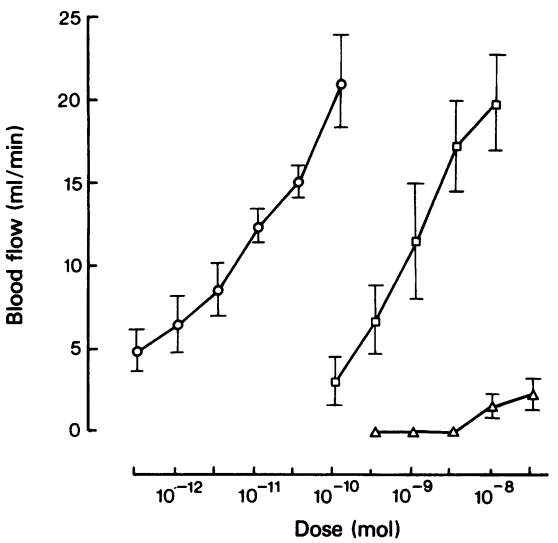

Figure 2 Dose-response curves to vasoactive intestinal peptide (VIP, ○, $n = 5$), secretin (□, $n = 6$) and glucagon (△, $n = 5$) for peak increase in blood flow through the glandular artery. Each point represents the mean value and vertical bars show s.e. mean.

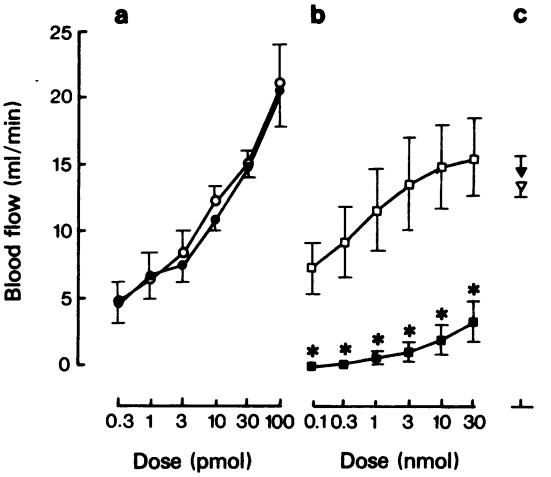

Figure 3 Dose-response curves to vasoactive intestinal peptide (VIP) (a) and acetylcholine (b) for increase in blood flow through the glandular artery and the mean increase in blood flow through the same artery to chordolingual nerve stimulation (6 V, 0.1 ms, 10 Hz and for 30 s) (c). Open and filled symbols refer to responses before (control) and during intra-arterial infusion of (−)-hyoscyamine (24.0 ± 8.2 (s.d.) nmol/min). The control dose-response curve to VIP is the same as that shown in Figure 2. Each point represents the mean of 5 values from the same 5 glands. Vertical bars show s.e. mean. $^*P < 0.01$ compared with corresponding control values.

muscarinic receptors thus attained, VIP (0.3 to 100 pmol) produced an increase in blood flow which was not significantly different from that in the control (Figure 3). The vasodilator responses to nerve stimulation were also unaffected by (−)-hyoscyamine (14.3 ± 1.2 ml/min against 13.3 ± 0.9 ml/min in control, $n = 5$) (Figure 3).

Discussion

In the present experiments, VIP injected into the glandular artery of the submandibular gland of dogs increased blood flow without eliciting salivary secretion. The vasodilator response to VIP was not modified by the blocking action of (−)-hyoscyamine, the active moiety of atropine. Secretin injected into the glandular artery also produced vasodilatation without stimulating salivary secretion. The dose-blood flow response curves for the two peptides were parallel and the two peptides were able to produce maximum vasodilatation. In other words, the peptides behaved as full agonists and on a molar basis VIP was about 100 times as potent as secretin. The dose-blood flow-response curve to acetylcholine was flatter than those for the two peptides. Thus, strict comparison of the vasodilator potencies of VIP and secretin with that of acetylcholine is difficult. In a rough comparison, secretin and acetylcholine were almost equipotent, and consequently VIP was about 100 times as potent as acetylcholine. Unlike VIP and secretin, glucagon produced only slight vasodilatation in large doses. Thus, the dose-blood flow response curve for glucagon was far flatter than those for VIP and secretin. This can be understood if the following is taken into account. VIP is more closely related in chemical structure to secretin than to glucagon (Mutt & Said, 1974); VIP has nine amino acids in the same position as in secretin as opposed to six amino acids in the same position as in glucagon. In guinea-pig exocrine pancreatic cells VIP and secretin have been shown to interact with the same receptors but glucagon does not (Klaeveman, Conlon & Gardner, 1975). Similarly, in vascular smooth muscle of the dog submandibular gland, VIP and secretin may stimulate the same receptor. Unlike vascular smooth muscle, secretory cells or myoepithelial cells do not appear to be endowed with such receptors.

As shown in the present experiments, the vasodilator potency of VIP in the glandular vascular bed is extremely high, being approximately 100 times that of acetylcholine; it appears to be matched only by that of bradykinin (Satoh *et al.*, 1972; unpublished observations). The high vasodilator potency of bradykinin together with the detection of kallikrein in perfusates of the salivary gland (Hilton & Lewis, 1955) and of the tongue (Hilton & Lewis, 1958) upon

chordolingual nerve stimulation led to the supposition that the kallikrein-kinin system mediates the atropine-resistant vasodilatation of these tissues in response to chordolingual nerve stimulation (Hilton & Lewis, 1955; 1958). However, the failure of bradykinin-potentiating substances to potentiate the vasodilator responses of the salivary gland (Ferreira & Smaje, 1976) and of the tongue (Shimizu & Taira, 1978) of dogs is hard to reconcile with the kallikrein-kinin hypothesis (Hilton & Lewis, 1955; 1958). Alternatively, the high vasodilator potency of VIP and the lack of tachyphylaxis to its vasodilator action tempt us to speculate that VIP may mediate the parasympathetically induced atropine-resistant vasodilatation of the salivary gland and tongue. The demonstration by an immunochemical method of the presence of VIP in nerve fibres in the salivary glands in rats, hogs and man (Bryant *et al.*, 1976) may favour our supposition. However, the presence of VIP in nerve fibres in the dog submandibular gland has not been shown as yet. In the study by Bryant *et al.* (1976) it was not clear whether immunochemically stained VIP nerve fibres were sympathetic or parasympathetic. Said & Rosenberg (1976) have claimed that the distribution of VIP resembles the tissue distribution of noradrenaline. Thus, the consolidation of the VIP hypothesis awaits further study.

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