J. Physiol. (1968), **195**, *pp.* 505–519
With 5 text-figures
Printed in Great Britain

KININS, BETA-ADRENERGIC RECEPTORS AND FUNCTIONAL VASODILATATION IN THE SUBMAXILLARY GLAND OF THE CAT

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(*Received* 8 *September* 1966)

SUMMARY

1. The close arterial infusion of bradykinin into the submaxillary gland of the cat produced a pronounced hyperaemia that could be blocked by simultaneous perfusion of the gland with blood containing carboxypeptidase B. Carboxypeptidase B, however, failed to reduce the vasodilatation of chorda tympani nerve stimulation suggesting that the kinins are not involved in the regulation of submaxillary gland blood flow.

2. Isoproterenol injections produced pronounced salivary gland vasodilatation. Beta-adrenergic blocking drugs reduced or abolished the hyperaemia of isoproterenol and reduced that of chorda tympani nerve stimulation. The combination of beta-blocking drugs and atropine could abolish or reduce further this nerve induced hyperaemia.

3. The above results suggest that stimulation of cholinergic and beta-adrenergic receptors could account for the chorda tympani induced hyperaemia. Conclusive proof of this possibility remains to be determined.

INTRODUCTION

While stimulation of the chorda tympani nerve is known to result in an increase of submaxillary gland blood flow, there is no unanimity of opinion regarding the cause of this hyperaemia. The view that true vasodilator fibres are responsible was first challenged by Barcroft (1914), who showed that oxygen consumption was increased when vasodilatation was produced by chorda or sympathetic stimulation. He suggested that the dilatation may occur as a result of metabolic products and not by special vasodilator nerve fibres. However, he did not exclude a possible role of

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vasodilator fibres as he stated: 'It is not impossible that under normal circumstances dilatation may be instituted by dilator fibres and maintained by metabolic products.' Additional support for the predominant if not total role of metabolic products in controlling salivary gland blood flow has been presented by Hilton & Lewis (1955*a*, *b*, 1956), who concluded that this hyperaemia is the result of the release of a stable vasodilator substance (kallikrein) produced by the gland cell. In contrast, Bayliss (1923) and Terroux, Sekelj & Burgen (1959) noted that the metabolic and vasodilator activity could be dissociated and concluded that the hyperaemia was not secondary to secretory metabolism. Also, the experiments conducted by Bhoola, Morley, Schachter & Smaje (1965) suggested that kallikrein does not mediate chorda tympani induced vasodilatation and these authors concluded that the atropine-resistant vasodilatation is merely a reflection of variation in sensitivity of cholinergic receptors to atropine.

In the present study, the relative contribution made by the kinins, and by cholinergic and beta-adrenergic receptors to the vasodilatation induced by chorda tympani nerve stimulation has been investigated in the submaxillary gland of the cat. A preliminary report of these data has appeared (Webster, 1966).

METHODS

Cats of both sexes, weighing 1·0–3·0 kg, were anaesthetized with pentobarbital sodium (25 mg/kg) injected intraperitoneally. The femoral vein was cannulated with polyethylene tubing. A mid-line incision was made that began at the mandible and ended at the mid tracheal level. After insertion of a tracheostomy tube, the submaxillary gland and the vein draining it were identified. This vein was not disturbed while all other branches of the external jugular vein were ligated. All branches of the ipsilateral external carotid artery were tied with the exception of the artery to the submaxillary gland. Heparin sodium (Upjohn; 2000 u.) was given intravenously to prevent clotting. A polyethylene cannula was inserted into the lingual artery until it barely extended into the lumen of the carotid artery.

The opposite carotid artery was cannulated with polyethylene tubing and the end-pressure was recorded using a Statham P23Db transducer. The ipsilateral jugular vein was cannulated at the mid-cervical level; blood flow through this cannula then represented only that which flowed through the gland and was measured by a drop rate-meter similar to that described by Goldschmidt & Lindgren (1962). The venous outflow from the gland was returned to the cat via the femoral vein in 5·0 ml. aliquots except during and immediately following the injection of drugs. In the latter case, the blood loss was replaced with donor blood so that the rate of infusion approximated the venous outflow. Arterial blood pressures and blood flow were recorded continuously on a direct writing Sanborn oscillograph.

To 50% of the cats, 10 mg of diphenylhydramine HCl (Benadryl®, Parke-Davis) was given intravenously (femoral vein) in order to prevent or minimize transfusion reactions. Neither this compound nor the infusion of donor blood detectably altered the vascular response of the salivary gland to a given level of chorda tympani nerve stimulation.

Bipolar stimulating electrodes were placed under both the chorda tympani nerve and the submaxillary duct near the hilum of the gland. In three animals, the chorda lingual nerve was stimulated after all branches of the lingual nerve had been sectioned with the exception

of the chorda tympani nerve. The lingual nerve was sectioned proximally. A Grass (model S4) impulse generator was used. Intensity ranged from 2 to 20 V; impulse frequency ranged from 0·3 to 20/sec and impulse duration from 0·5 to 1·0 msec. In some experiments the impulse frequency and duration were held constant while the voltage was increased. In others, only the frequency was changed. Increases in either one of these parameters resulted in an increase in blood flow.

Materials. Synthetic bradykinin obtained through the courtesy of E. D. Nicolaides (Parke-Davis and Co., Ann Arbor, Michigan, U.S.A.) was dissolved in sterile 0·25% casein (Hammersten), pH 7·5, and infused into the cannulated lingual artery at 1·5–4·0 μg/min (infusion rates 0·03–0·07 ml./min). Since the casein *per se* usually produced some vasodilatation, the hyperaemia produced by infusing 0·25% casein solution was determined in each experiment and subtracted from the increase in blood flow associated with infusion of the casein-bradykinin mixture.

Carboxypeptidase B was prepared from hog pancreas as described previously (Webster, Skinner & Powell, 1967) and an amount of solution equivalent to 10–20 mg of pure carboxypeptidase B was injected intravenously (femoral vein). Owing to the rapid excretion or metabolism of this enzyme by the kidney (Webster *et al.* 1967), one half of the initial concentration was injected every 20 min so that relatively constant blood levels were maintained. The average blood level (80–170 μg/ml. of pure carboxypeptidase B) was determined by measuring the concentration of the enzyme in the collected venous plasma using the method of Folk, Piez, Carroll & Gladner (1960). Normal cat plasma was essentially incapable of digesting hippuryl-L-arginine under these conditions, values of carboxypeptidase B being less than 2·5 μg/ml.

Isoproterenol HCl (Winthrop Labs) was given by close arterial injection (lingual artery) in 2–20 μg doses (volumes 0·1–0·2 ml.). Dichloroisoproterenol (DCI) and propranolol (Ayerst) were given in variable doses from 1 to 10 mg (1·0 ml. volume or less) by close arterial injection or by slow intra-arterial infusion. Atropine sulphate was injected intravenously (0·5–1·0 mg/kg via femoral vein) or by close arterial injections (0·15–0·40 mg). Acetylcholine (0·34–0·68 μg/min) was given by close arterial infusion.

RESULTS

Carboxypeptidase B, chorda tympani nerve stimulation and salivary gland blood flow. Carboxypeptidase B, an enzyme capable of abolishing or reducing the vasodilator effects of kinins *in vivo* (Erdos, Wohler & Levine, 1963; Webster *et al.* 1967) was used to evaluate the role of kinins in producing the hyperaemia of chorda tympani nerve stimulation. It was reasoned that this enzyme should reduce or abolish the hyperaemia of chorda stimulation if the kinins are responsible for this vasodilatation. Nine cats were studied in the following manner. Synthetic bradykinin was infused close arterially (1·5–4·0 μg/min; volume 0·03–0·07 ml./min) for 60 sec, and the effect on salivary gland blood flow was recorded. Upon return of blood flow to control values, the chorda tympani nerve was stimulated, usually at three intensities, during which blood flow from the gland was recorded. The durations of stimulation varied from 10 to 60 sec with the majority being for 1 min. Carboxypeptidase B was then injected intravenously and the bradykinin infusion and nerve stimulation repeated. Since the bradykinin that was infused was dissolved in a solution of casein

(0·25 %), the vasodilator effect of infused casein was also determined. Thus, the degree of vasodilatation induced by the diluent could be estimated.

Carboxypeptidase B was found to abolish or reduce markedly the pronounced vasodilatation associated with bradykinin infusion but failed to alter the hyperaemia induced by nerve stimulation even when less than maximal vasodilatation was produced by low levels of stimulation. Figure 1 depicts the results from one experiment. In this figure, salivary gland vascular–resistance is plotted (arterial pressure in mm Hg/flow, i.e. mm

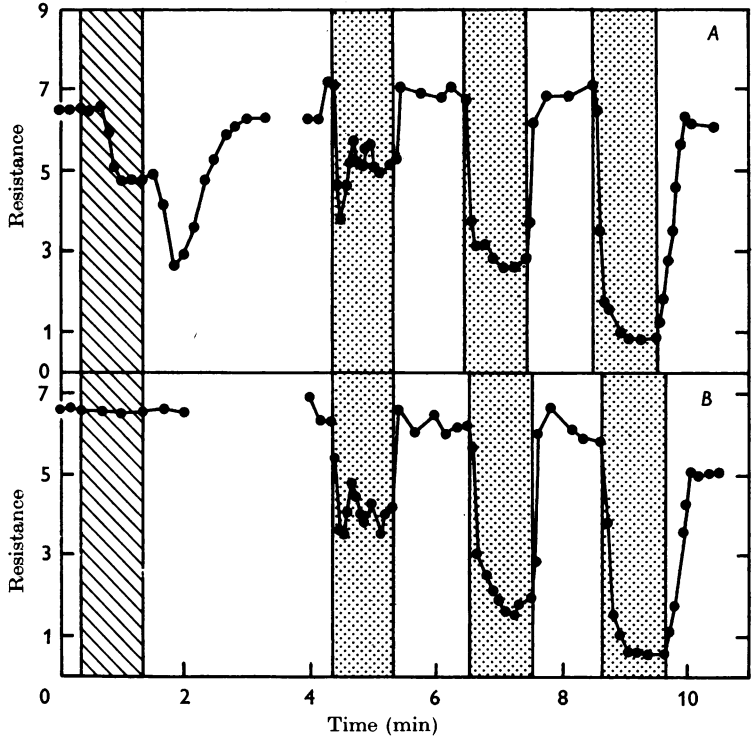

Fig. 1. Effect of carboxypeptidase B on vasodilatation induced by infusion of bradykinin and chorda tympani nerve stimulation. Resistance (mm Hg.ml. flow)$^{-1}$ 100 g^{-1}.min^{-1}) plotted against time. The 60 sec periods of bradykinin infusion at 2·7 μg/min (left; hatched areas) and nerve stimulation (right; stippled areas) are shown. Stimulation voltage, 2·0 V; impulse duration, 0·5 msec; and stimulation frequencies, 2/sec, 3/sec, 5/sec, respectively. Panel *A* = without carboxypeptidase B. Panel *B* = with carboxypeptidase B (130 μg/ml. blood).

Hg.ml.$^{-1}$ 100 g^{-1} min^{-1}). Panel *A* of this figure shows the changes in vascular resistance induced by bradykinin (left) and by three levels of nerve stimulation (right). As shown, increasing the frequency of stimulation resulted in greater reductions in vascular resistance. After admini-

stration of carboxypeptidase B (panel B) the decrease in vascular resistance produced by bradykinin was abolished but that which occurred with nerve stimulation was unchanged. Prolonged perfusion of the gland (40 min) with blood containing carboxypeptidase B did not affect chorda tympani induced hyperaemia. Table 1 and Fig. 2 summarize the results

TABLE 1. The effect of carboxypeptidase B, atropine and beta-blocking drugs on blood pressure and submaxillary gland blood flow before and during chorda tympani nerve stimulation. The mean and standard error of the integrated systemic blood pressure and submaxillary gland blood flow are shown for the non-stimulated and stimulated gland before and following the administration of carboxypeptidase-B (Cbx-B), atropine and the combination of atropine and beta-adrenergic blocking agents. Pressure is in mm Hg and flow in ml /100 g.min

No. of animals	No. of observations	Stimulation: Volts / Frequency (sec^{-1})	Stimulation: Duration (sec)	Control: Pressure	Control: Flow	Stimulation: Pressure	Stimulation: Flow	Mean flow increase	
9	21	6·6 / 2·8	44·3	133·2 ± 5·4	34·0 ± 8·6	130·6 ± 5·3	88·0 ± 11·0	54·0	Cbx-B alone
9	21	6·6 / 2·8	44·3	132·5 ± 5·8	33·0 ± 8·6	129·6 ± 5·8	87·0 ± 11·8	54·0	
8	20	7·2 / 5·9	41·3	116·7 ± 4·3	24·0 ± 3·0	115·3 ± 4·2	86·0 ± 13·0	62·0	Atropine alone
8	20	7·2 / 5·9	41·3	115·9 ± 5·7	25·0 ± 2·5	115·1 ± 5·6	46·0 ± 2·7	21·0	
9	20	3·4 / 2·9	60·0	127·0 ± 6·1	34·0 ± 2·3	124·9 ± 6·2	98·0 ± 13·5	64·0	Atropine plus beta blocking drugs
9	20	3·4 / 2·9	60·0	122·9 ± 6·4	30·0 ± 1·8	121·1 ± 6·4	37·0 ± 2·7	7·0	

from these experiments. These data represent the mean changes in blood flow and pressure produced by the various levels of nerve stimulation. Mean blood flow during each period of stimulation was obtained by integrating the flow recording. As illustrated in Panel *A* nerve stimulation increased blood flow from a control of $34{\cdot}0 \pm 8{\cdot}6$–$88{\cdot}0 \pm 11{\cdot}0$ ml./100 g.min. After carboxypeptidase B was given in amounts sufficient to elevate the blood concentration to 80–170 μg/ml., the corresponding values were $33{\cdot}0 \pm 8{\cdot}6$–$87{\cdot}0 \pm 11{\cdot}8$ ml./100 g.min. Systemic blood pressures were comparable both before and after carboxypeptidase B. In none of the nine animals was the chorda induced hyperaemia reduced by carboxypeptidase B. Also carboxypeptidase B did not reduce the secretion rate of saliva in two animals studied.

While the levels of carboxypeptidase B that were achieved abolished the vasodilatation induced by infusing bradykinin, no attempt was made to determine the concentration of kinin needed to exceed the destructive capacity of the enzyme. However, the hyperaemia induced by bradykinin infusion sometimes exceeded that produced by the highest intensity of

nerve stimulation. Infusion of carboxypeptidase B abolished this vasodilatation but was not associated with any reduction in chorda induced hyperaemia.

Atropine, chorda tympani nerve stimulation and blood flow. Unlike carboxypeptidase B, atropine was effective in reducing the hyperaemia produced by low levels of nerve stimulation. With the exception of one cat in which atropine was effective in abolishing chorda induced hyperaemia, an atropine resistant vasodilatation persisted in the eight animals

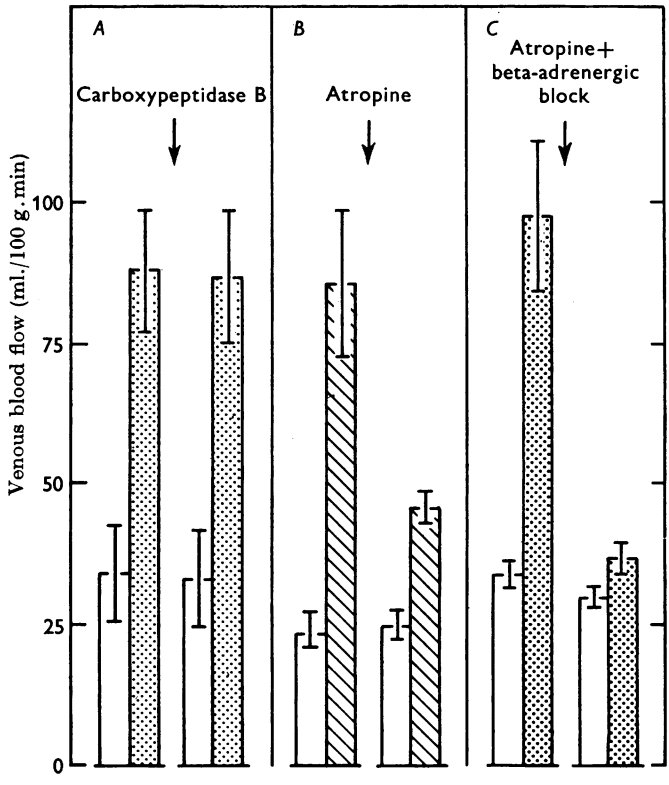

Fig. 2. Effect of carboxypeptidase B (panel *A*), atropine (panel *B*), and atropine + beta-adrenergic blocking drugs (panel *C*) on submaxillary gland blood flow during stimulation of the chorda tympani nerve of the cat. The mean values ± 1 S.E. are plotted. Control flow = open bars; stimulation flow = shaded bars.

studied. Atropine was given either by intravenous injection (0·5–1·0 mg/kg) or by close arterial injection (0·15–0·04 mg). When given via the arterial route, the gland was exposed to the drug only during the time of transit, since the venous blood was not returned to the animal and recirculation did not occur. It was found that the maximum reduction of hyperaemia

with chorda tympani stimulation occurred following the first injection of these amounts of atropine and subsequent doses failed to alter further the vascular response. Figure 3 illustrates the effect of atropine on blood flow

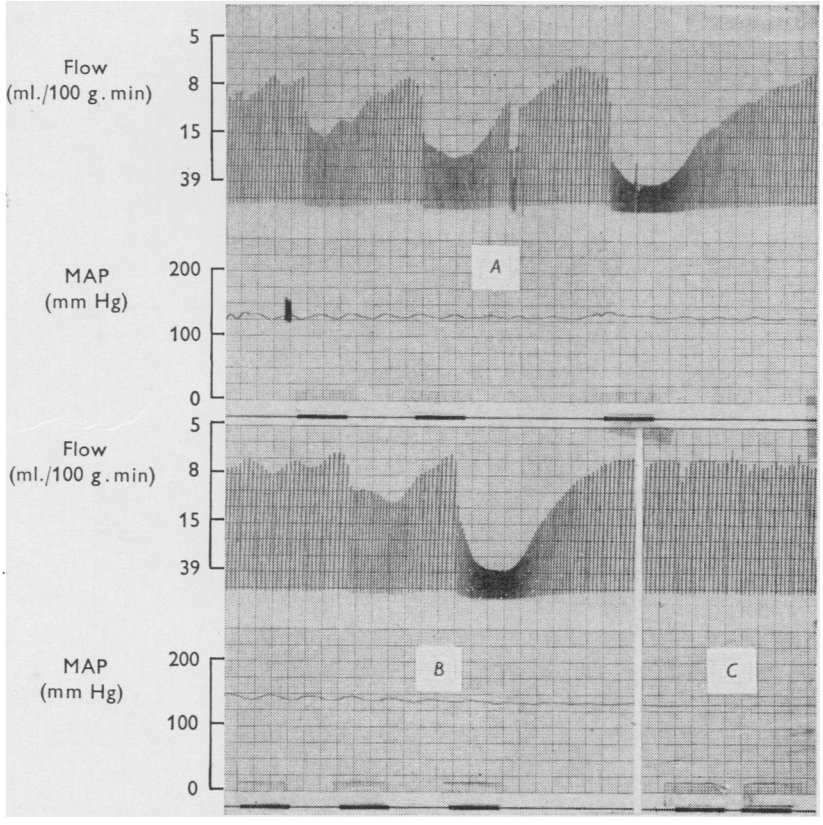

Fig. 3. Record showing the abolition of chorda tympani induced hyperaemia by the combination of atropine and propranolol. MAP = mean arterial pressure. Stimulation frequency, 20/sec and impulse duration, 0·5 msec. Panel *A*, chorda tympani nerve stimulation at 3 V, 5 V and 10 V, respectively. Panel *B*, response to same levels of stimulation after intravenous administration of atropine (0·5 mg/kg). Panel *C*, response to stimulation at 10 V and 15 V following the close arterial injection of propranolol (4·5 mg). Each 60-sec period of stimulation (3 large squares) is indicated by the black bars at the bottom of each panel.

through the gland with low intensities of chorda tympani stimulation. As is shown in panel *A*, stimulation of this gland at 3, 5 and 10 V at a constant frequency of 20/sec produced a graded increase in the hyperaemia. Following intravenous injection of atropine (0·5 mg/kg), nerve stimulation at 3

and 5 V resulted in reduced hyperaemia. However, little or no change was observed in the vasodilatation resulting from chorda stimulation at 10 V (compare panel *A* with panel *B*). In this manner atropine reduced the hyperaemia associated with the lowest levels of stimulation but had little effect on that accompanying the highest level of stimulation. Table 1 and Fig. 2 summarize the changes in blood flow and pressure produced by chorda tympani nerve stimulation before and following administration of atropine. Again mean values for flow and pressure were obtained by integrating the flow and pressure curves during the control and stimulation periods. Before administration of atropine, chorda stimulation increased blood flow from a mean control of $24 \pm 3{\cdot}0$–$86{\cdot}0 \pm 13{\cdot}0$ ml./100 g.min. Following atropine the control blood flow was essentially unchanged at $25 \pm 2{\cdot}5$ ml./100 g.min but increased with chorda stimulation to only $46 \pm 2{\cdot}7$ ml./100 g.min, illustrating the effectiveness of atropine in reducing but not abolishing the hyperaemia associated with chorda tympani stimulation. Systemic blood pressure was constant during these experiments.

Beta-adrenergic blocking drugs, chorda tympani nerve stimulation and blood flow. Beta-adrenergic receptors have been proposed recently (Bhoola, *et al.* 1965; Davey, Davies, Reinert & Scholfield, 1965) to explain the after dilatation associated with stimulation of the sympathetic nerves to the cat submaxillary gland. In the present study single, close arterial injections of 2–20 μg isoproterenol (0·1–0·2 ml.) were injected in nine cats and in every instance a marked hyperaemia resulted. It was also shown that dichloroisoproterenol (DCI) or propranolol when given in doses of 1·0–10·0 mg (volumes 1·0 ml. or less, close arterially) reduced substantially or abolished the vasodilatation associated with isoproterenol injection. A marked hyperaemia was observed consistently during and for 2–10 min after the initial infusions of the beta-blocking drugs and was also seen with repeated injections. The blood flow response to isoproterenol and nerve stimulation was evaluated when flow returned to the level that existed before the infusion of the beta-blocking drugs (Figs. 2–5). In order to avoid systemic effects, the venous blood was not recirculated during and immediately after the injection of the beta-blocking drugs. The gland, therefore, was exposed to the drug for only a short period of time. The amount of blocking drug actually accepted by the gland was not determined, but only a small portion of these relatively large doses probably remained in the gland, since in three animals the venous effluent collected during the administration of either DCI or propranolol was reinfused and resulted in reductions in both blood pressure and heart rate.

Chorda tympani induced hyperaemia was evaluated before and after beta-adrenergic blocking drugs were given. As shown in Fig. 4, which is

representative of the response seen in the majority of the nine cats studied, the close arterial infusion of propranolol (1·5 mg) reduced moderately the hyperaemia induced by stimulation of the chorda tympani nerve at 4 impulses/sec and 3 V. A second dose of propranolol (1·5 mg) caused a further reduction in this chorda induced hyperaemia. Blood pressure varied only slightly in these experiments. In one cat, propranolol alone

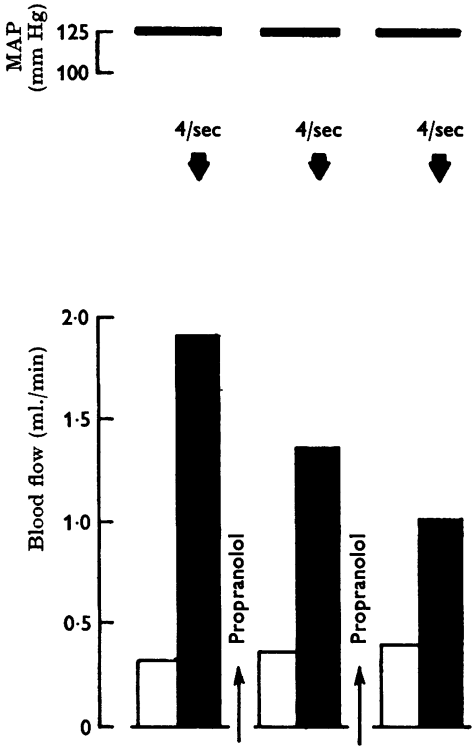

Fig. 4. Graph showing the effect of chorda stimulation on submaxillary gland blood flow before and following the close arterial injection of propranolol. Stimulation criteria were constant at 3 V 0·5 msec duration, 4/sec frequency. The duration of stimulation was 60 sec. MAP = mean arterial pressure. The open bars represent control blood flow through the submaxillary gland, while the closed bars indicate mean blood flow during chorda stimulation. Propranolol was infused close arterially for 60 sec in total doses of 1·5 mg each.

completely blocked the vasodilatation produced by chorda tympani stimulation at 2 V and 0·5, 1 and 2 impulses/sec. In two animals, beta-blocking drugs produced only slight reductions in chorda induced hyperaemia. Atropine reduced markedly the hyperaemia in one of these cats.

The possible atropine-like effect of propranolol was investigated in two cats. In each, acetylcholine was infused for 60 sec close arterially at 0·34

and 0·68 μg/min. Propranolol was infused close arterially in repeated doses (total for each cat 8·0 and 6·5 mg, respectively) following which the acetylcholine infusions were repeated. Propranolol failed to alter the vasodilatation induced by these amounts of acetylcholine while atropine (0·5 mg/kg intravenously) abolished the hyperaemia.

Combination of atropine and beta-adrenergic blocking drugs. The combination of atropine and beta-blocking drugs was most effective in reducing the hyperaemia produced by chorda tympani nerve stimulation. Of the nine cats studied, in which hilar nerve stimulation was used, chorda tympani induced hyperaemia was abolished in three cats, reduced markedly in four and only minimally in two. Figure 3, panel *C* illustrates the total abolition of the atropine resistant hyperaemia by beta-blocking drugs. Atropine had previously reduced partially the vasodilatation induced by chorda stimulation at 3 V, 20/sec and 5 V, 20/sec but left essentially unchanged the hyperaemia response to 10 V, 20/sec. Following the close arterial injection of 4·5 mg propranolol, the hyperaemic response to all previous levels of stimulation was abolished. Even stimulation of the nerve at 15 V, 20/sec failed to produce vasodilatation. The results of these studies are summarized in Table 1 and Fig. 2. As is shown, arterial pressure was essentially unaltered either before or after the dual blockade. Before cholinergic and beta-adrenergic block, chorda stimulation increased mean salivary gland blood flow from $34{\cdot}0 \pm 2{\cdot}3$ to $98{\cdot}0 \pm 13{\cdot}5$ ml./100 g.min. After both atropine and propranolol were given blood flow increased from $30{\cdot}0 \pm 1{\cdot}8$ to only $37{\cdot}0 \pm 2{\cdot}7$ ml./100 g.min.

In three additional animals, the combination of atropine and propranolol was also effective in abolishing completely the hyperaemia produced by chorda lingual nerve stimulation. Figure 5 illustrates one such experiment. As is shown, atropine (0·5 mg/kg intravenously) reduced substantially the vasodilatation of chorda lingual stimulation at 4 V, 2/sec. However, stimulation at 4 V, 5/sec produced an increase in blood flow which could be almost completely blocked by the close arterial injection of 1·18 mg propranolol. Following a second injection of propranolol (2·96 mg) stimulation at 5/sec still produced this small residual hyperaemia and stimulation at 10/sec produced a pronounced increase in salivary gland blood flow. Two additional close-arterial injections of propranolol (2·96, 3·49 mg) were required to essentially abolish this residual chorda induced hyperaemia. This figure also illustrates that a given amount of beta-blocking drug may be effective in abolishing the atropine resistant hyperaemia induced by one intensity of chorda stimulation but not that of higher intensities. There was not always an exact parallelism between suppression of vasodilatation produced by injection of isoproterenol and suppression of chorda tympani induced hyperaemia.

Although this effect was not quantitatively evaluated, preliminary data suggested that a given dose of propranolol could more readily block the vasodilator response to close arterial injection of isoproterenol than that to chorda tympani stimulation. Presumably the concentration of catecholamine in proximity to resistance vessels would be greater with release of these amines from tissues as opposed to their intra-arterial infusion. Nevertheless, isoproterenol (2–20 μg) failed to elicit vasodilatation in the animals in which atropine and beta-blocking drugs had completely abolished chorda hyperaemia and continued to produce a hyperaemic response, although reduced, when this combination was not effective in completely preventing the vasodilatation.

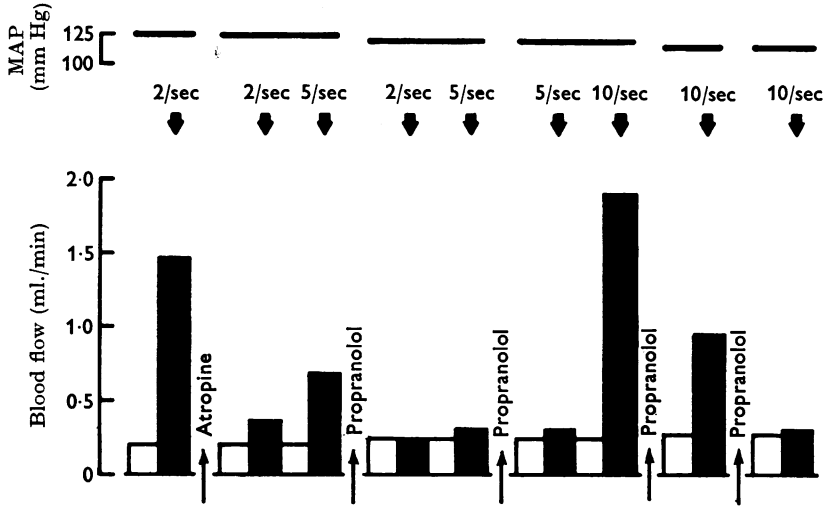

Fig. 5. Graph showing the effect of chorda stimulation on submaxillary gland blood flow following injection of atropine and propranolol. Voltage = 4·0 V and impulse duration, 0·5 msec. Frequency of stimulation as shown. MAP = mean arterial pressure. Atropine was injected intravenously (0·5 mg/kg). Propranolol was infused close arterially for the following total doses in the order shown: 1·18, 2·96, 2·96 and 3·49 mg. Each period of nerve stimulation lasted 60 sec.

Although injury to the gland vasculature might account for the lack of vasodilatation associated with chorda stimulation when atropine and beta-blocking drugs were used, several observations made this possibility unlikely. First, as shown in Figs. 3 and 5, control blood flow was not changed markedly before and following drug administration. Secondly, even though isoproterenol (2–20 μg) injected close arterially did not produce vasodilatation, sodium pentobarbital (1–6 mg) given either intravenously or close arterially always resulted in a hyperaemic response, sometimes exceeding that produced by chorda stimulation before atropine

and beta-blocking drugs were given. Finally, bradykinin (2·4 μg/min), infused close arterially in one cat, increased blood flow even though isoproterenol and nerve stimulation failed to change vascular resistance.

DISCUSSION

That the kallikreins are present in glandular tissue, that their endogenous release can result in local formation of kinins, and that these kinins are potent vasodilating substances is well established (Erdos, 1966). It would seem, therefore, as suggested by Hilton & Lewis (1956) that these polypeptides could play a role in the regulation of glandular blood flow. These authors demonstrated that sympathetic and parasympathetic stimulation of the cat submaxillary gland, during perfusion of the gland with Locke solution, can cause an increased release of salivary kallikrein in the venous effluent. They suggested that nerve stimulation released a kallikrein from the secretory gland cells and that this enzyme diffused into the interstitial fluid where it released a vasoactive polypeptide from kininogen. Their data from the submaxillary gland, together with analogous data obtained by stimulation of the sweat glands, pancreatic glands, and glandular elements of the tongue (Hilton, 1963) have led these authors to propose that the kinins act as physiological mediators of functional vasodilatation in glands. If a substance is a physiological mediator, however, its biological effects should be reduced in the presence of inactivating or blocking agents. As found in these studies, carboxypeptidase B, an enzyme which inactivates the kinins by removal of the COOH-terminal arginine, reduced markedly the hyperaemia produced by infusion of bradykinin but failed to alter the increase in blood flow induced by chorda tympani nerve stimulation.

Since previous studies have shown that carboxypeptidase B (Webster *et al.* 1967) and dextrans of comparable molecular weight (Arturson & Kjellmer, 1964) appear in lymph after systemic administration, it is reasonable to assume that carboxypeptidase B enters the interstitial fluid of salivary glands where inactivation of endogenously released kinin could occur. The concentration of carboxypeptidase B in interstitial fluid was not determined and, consequently, no statement can be made relative to the amount of bradykinin that could be destroyed. It was for this reason that multiple levels of chorda stimulation were used. Thus, when less than maximal vasodilatation was produced, any effect of carboxypeptidase B would be anticipated to reduce the vasodilatation if the kallikrein-kininogen-kinin system is operative in producing chorda induced hyperaemia. In no experiment did carboxypeptidase B alter vasodilatation of chorda tympani nerve stimulation.

These data are in agreement with those of Bhoola *et al.* (1965), Beilenson, Schachter & Smaje (1965) and Morley, Schachter & Smaje (1966), who, using different experimental procedures, were also unable to support the hypothesis that the kinins produce chorda tympani induced hyperaemia. These authors showed that the vasodilatation produced by injection of dialysed cat saliva does not mimic that of nerve stimulation. Further, vasodilatation still accompanied nerve stimulation when the gland had been depleted of its kallikrein or during perfusion of the gland with horse serum, a substrate from which cat salivary kallikrein cannot release kinin. Also, desensitization of the gland to bradykinin had little effect on nerve induced hyperaemia. Finally, atropine completely blocks chorda induced vasodilatation in the rabbit, even though the submaxillary gland of the rabbit contains a kallikrein. While this evidence argues against the kinins being physiological mediators of functional vasodilatation, the increased amounts of kallikrein found in the venous effluent (Hilton & Lewis, 1956) and in saliva (Beilenson *et al.* 1965) following nerve stimulation may have physiological significance other than in controlling blood flow.

That beta-adrenergic receptors are present in submaxillary glands is supported by the data in this paper as well as in those of others (Emmelin, Holmberg & Ohlin, 1965; Bhoola *et al.* 1965; Davey *et al.* 1965). In the present studies isoproterenol injected close arterially in cats produced consistently a marked hyperaemia which could be reduced or abolished by beta adrenergic blocking drugs. Close arterial injection of these beta-adrenergic blocking drugs was also found to suppress partially the vasodilatation following chorda tympani nerve stimulation, suggesting that stimulation of beta receptors might contribute to chorda induced hyperaemia. Further studies showed that the combination of beta-adrenergic blocking drugs and atropine was effective in reducing or abolishing the vasodilatation resulting from chorda tympani nerve stimulation. In addition a parallelism existed between the vasodilatation induced by isoproterenol and that of the atropine resistant chorda stimulation. In those cats in which the dual blockade with atropine and beta-adrenergic blocking drugs had abolished the nerve induced hyperaemia, the hyperaemia induced by isoproterenol was also abolished. Similarly, in those animals in which some residual vasodilatation could be induced after the dual blockade, the gland still responded to increased levels of isoproterenol. In view, however, of the relatively large amounts of beta-adrenergic blocking drugs needed to achieve this blockade, it appeared possible that damage to the vasculature of the gland could have accounted for the reduced hyperaemia. No evidence for this possibility could be found, since acetylcholine, pentobarbital and bradykinin were all capable of producing vasodilatation after propranolol blockade. In addition, it was apparent

that competition was taking place between the beta-adrenergic blocking drug given close arterially and isoproterenol and/or chorda tympani nerve stimulation. A given level of propranolol could block the vasodilatation produced by small amounts of isoproterenol or low levels of chorda stimulation but had little or no effect when greater concentrations or higher levels were employed.

While the above data suggest that stimulation of cholinergic and beta-adrenergic receptors could account for chorda tympani induced hyperaemia, they must be interpreted with caution since relatively large doses of beta-blocking drugs were required and the possibility of a non-specific effect exists. Also, other authors (Davey *et al.* 1965) have shown that cats treated with reserpine 24 hr earlier exhibited normal vasodilatation in response to chorda tympani stimulation even though the vascular response to sympathetic stimulation had been abolished. These authors concluded that the physiological mechanism for this atropine resistant vasodilatation was not adrenergic, a view shared by Schachter & Beilenson (1967). Nevertheless, the amount of catecholamines in the submaxillary glands from cats treated with reserpine was not determined, and it is possible that the various stores of catecholamines were not depleted completely by reserpine. It is apparent that further studies will be required to elucidate the role of beta-adrenergic receptors in chorda induced, atropine resistant hyperaemia in the sub-maxillary gland of the cat.

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