J. Physiol. (1973), **228**, *pp*. 361–376
With 5 text-figures
Printed in Great Britain

THE EFFECT OF CHRONIC DUCT LIGATION ON THE VASCULAR AND SECRETORY RESPONSES OF THE CAT'S SUBMAXILLARY GLAND

BY A. C. DARKE* AND L. H. SMAJE

From the Department of Physiology, University College London, Gower Street, London WC1E 6BT

(*Received 19 June 1972*)

SUMMARY

1. The object of the present experiments was to re-investigate the role of kallikrein in functional hyperaemia in the submaxillary gland of the cat. Kallikrein concentration in the gland and saliva was reduced by ligation of the duct for 2–3 days before the acute experiment, at which time the duct was cannulated proximal to the ligation and blood flow and salivation measured.

2. Despite the fall in kallikrein concentration, a normal two-phase vascular response was obtained on continuous stimulation of the chorda-lingual nerve at 1–5 Hz and the progressive increase in blood flow to a constant level characteristic of 10 and 20 Hz stimulation was also seen. Quantitatively, the increase in blood flow following a 10 sec stimulation was reduced to about 75 % of control values at all stimulation frequencies used and the maintained vasodilatation was reduced when using continuous stimulation at 1 Hz but not at 10 and 20 Hz.

3. The supersensitivity to the vasodilator properties of bradykinin, previously reported to follow duct ligation, was confirmed.

4. The increased blood flow produced by chorda-lingual nerve stimulation could be delayed by circulatory arrest. Evidence was obtained suggesting that the vascular response to chorda stimulation delayed by arterial occlusion was not mediated by kallikrein alone.

5. It was concluded that kallikrein was not the sole mediator of functional hyperaemia in the cat submaxillary gland but that a combination of vasodilator nerves and kallikrein release explained more phenomena than either hypothesis alone.

* M.R.C. Scholar. Present address: Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada.

INTRODUCTION

A hypotensive substance, kallikrein, was found in saliva by Ungar & Parrot (1936) who suggested that it might be responsible for mediation of functional vasodilatation in the salivary gland. It has since been established that kallikrein acts by releasing a potent vasodilator peptide, almost identical to bradykinin, from an α_2-globulin substrate in plasma (Werle, 1955). The exact role of kallikrein in functional vasodilatation is, however, controversial. Hilton and his collaborators (Hilton & Lewis, 1955*a*, *b*; 1956; Hilton & Torres, 1970; Gautvik, Hilton & Torres, 1970) have implicated kallikrein as the main agent responsible for the blood flow increase whereas Schachter and his colleagues have denied this possibility maintaining that acetylcholine released from vasodilator nerves is the transmitter (Bhoola, Morley, Schachter & Smaje, 1965; Morley, Schachter & Smaje, 1966; Beilenson, Schachter & Smaje, 1968; Schachter & Beilenson, 1968; Karpinski, Barton & Schachter, 1971). Some other groups also inclined to the vasodilator nerve hypothesis (Terroux, Sekelj & Burgen, 1959; Skinner & Webster, 1968) while Gautvik (1970*a*, *b*, *c*) has suggested that two phases might be involved, vasodilator nerves initiating the flow increase which is then maintained by the release of kallikrein.

None of the authors mentioned, however, has studied the response to stimulation at frequencies other than 10 or 20 Hz. In a previous paper (Darke & Smaje, 1972) the increased blood flow in the cat's submaxillary gland resulting from continuous stimulation of the chorda-lingual nerve at low frequencies (1–5 Hz) was shown to have two phases. A transient substantial flow increase was followed by a decline to a lower level which was maintained as long as stimulation continued. At higher frequencies (10–20 Hz), by contrast, the initial sharp increase in blood flow was not followed by a decline but by a further rise to a constant level. Neither salivation alone nor the release of potassium by the gland cells was closely correlated with these vascular responses. Atropine depressed both the transient and the maintained response to low frequency stimulation, but at 10–20 Hz the maintained vasodilatation was unaffected by the drug.

One of the techniques used to investigate the role of kallikrein in functional vasodilatation in the submaxillary gland has been chronic duct ligation, which leads to a reduction in kallikrein concentration in both gland and saliva (Beilenson *et al.* 1965, 1968; Hilton & Torres, 1967, 1970). In the present paper, experiments are described in which this technique was used to reduce salivary kallikrein concentration and the vascular and secretory responses to chorda-lingual nerve stimulation at 1–20 Hz were subsequently measured. A further line of evidence presented for the proposed role of kallikrein as the mediator of functional vasodilatation is that

the increase in glandular blood flow produced by chorda stimulation can be delayed by circulatory arrest (Hilton & Lewis, 1955*a*). The stability of such delayed vasodilatation has been further investigated.

METHODS

Animals and anaesthesia

Experiments were performed on forty-one cats of both sexes, their weights ranging from 1·9 to 4·5 kg. In acute experiments anaesthesia was induced with sodium pentobarbitone (35 mg/kg I.P., 'Nembutal', Abbott Laboratories Ltd.) and usually maintained with intravenous infusions of chloralose (10 mg/ml. in 0·9 % NaCl) as required. In some of the experiments anaesthesia was maintained with pentobarbitone (6 mg/ml. in 0·9 % NaCl) or a mixture of chloralose and urethane (20 mg/ml. and 50 mg/ml. respectively, in 0·9 % NaCl). No differences in the results were observed using different anaesthetics. In recovery experiments the cats were anaesthetized with a solution of pentobarbitone (35 mg/kg I.P.) made up on the day of operation from sterile powder and sterile water (Martindale Samoore Ltd.) and aseptic precautions were taken throughout the operations.

Dissection and recording techniques. The procedures used for recording submaxillary gland blood flow, salivation and arterial blood pressure and for stimulating the chorda-lingual nerve have been described previously (Darke & Smaje, 1972). The chorda-lingual nerve was stimulated with square waves of 1 msec duration, supramaximal voltage (7·5–12·5 V) and frequency 1–20 Hz.

Close-arterial injections into the submaxillary gland were made by means of a motor-driven syringe delivering 0·87 ml./min via cannulae ('blue' or 'green' Portex, Portland Plastics Co. Ltd.) in the lingual or external maxillary arteries, all other branches of the common carotid and external carotid arteries except that to the submaxillary gland having been previously ligated. An identical preparation was used in experiments in which the arterial blood supply was to be occluded by clamping the common carotid artery.

Depletion of glandular kallikrein. The submaxillary duct was ligated as described by Beilenson *et al.* (1968). At the beginning of the acute experiment 2 to 3 days later the sympathetic supply to the gland was stimulated intermittently for 15 min as this further reduces the kallikrein concentration (Beilenson *et al.* 1968).

Kallikrein assay. Kallikrein in saliva samples and gland extracts was assayed by its ability to release kinin from a plasma protein substrate. The kinin released was assayed on the isolated rat uterus.

Sample preparation. Saliva samples were stored at $-30°$ C until the day of assay when they were thawed and kept at 4° C until required. Right and left glands were removed at the end of the experiments and quickly frozen in a mixture of solid CO_2 and acetone. The frozen glands were stored at $-30°$ C and subsequently freeze-dried and ground to a powder. Samples of the powder were homogenized in de Jalon's solution (NaCl, 154 mM; $NaHCO_3$, 5·95 mM; $CaCl_2$, 2·7 mM; KCl, 5·6 mM; glucose, 2·8 mM), centrifuged and the supernatant stored at $-30°$ C until assayed.

Rat uterus. Rats were given 0·01 mg stilboestrol in arachis oil (0·1 mg/ml. I.P.) approximately 20 hr before use. The rats were killed and 2–3 cm of one of the split uterine horns suspended in a 5 ml. organ-bath containing de Jalon's solution with the addition of atropine and mepyramine (2×10^{-5} g/l. for each). The bath was aerated with 95 % O_2, 5 % CO_2 and maintained at 32° C. Contractions of the uterus were recorded on a Devices two-channel pen-recorder using the isotonic transducer described by Jewell, Kretzschmar & Woledge (1967). The load on the uterus was 0·2 g.

Standard. A kallikrein standard was prepared by making an aqueous extract of eighteen freeze-dried normal unstimulated cat submaxillary glands. The homogenate was centrifuged and the supernatant stored in desiccators until required.

Substrate and assay conditions. The substrate for kallikrein was prepared from fresh human plasma according to the method of Amundsen, Nusted & Waaler (1963). In preliminary trials, the maximum kinin release from this substate was obtained when kallikrein samples were incubated with 0·3 ml. substrate at 32° C for 2 min and these conditions were used in all subsequent assays. The volume of the mixture to be incubated was always made up to 1 ml. and this was the volume injected into the organ bath. The dose cycle was 4 min. The kallikrein standard was assayed against synthetic bradykinin and 1 μg standard was found to be equipotent with 8 ng bradykinin. For comparative purposes the kallikrein concentrations of the saliva samples and gland extracts have been expressed in terms of the amount of bradykinin released.

Vascular responses in circulatory arrest experiments (see Results 5)

A 'stable vasodilator response' was defined as the difference, in drops, between the increased blood flow measured after release of the arterial occlusion alone, and the increased blood flow found when the chorda-lingual nerve was also stimulated for 10 sec at 10 Hz at the beginning of the occlusion. The variation between animals and in results from one animal during an experiment was minimized when the stable vasodilator response was expressed as a percentage of the response to a straightforward 10 sec chorda stimulation at 10 Hz performed before the arterial occlusion. The effects of drug injections were measured in a similar manner. The difference between the response following close-arterial injections of kallikrein or bradykinin during arterial occlusion and the response to the injection of an equivalent volume of 0·9 % NaCl during the occlusion was expressed as a percentage of the response to the drug alone. The response to the drug, in turn, was taken as the number of drops above the basal flow resulting from the injection minus any extra drops produced by an equal volume of 0·9 % NaCl.

Drugs

The following drugs were used during the experiments.
Acetylcholine chloride ('Acecoline', Laboratories Lematte et Boinot).
Bradykinin ('B.R.S. 640', Sandoz Ltd.).
Mepyramine maleate ('Anthisan', May & Baker Ltd.).
Stilboestrol (B.D.H. Ltd.).
In the case of salts, doses are expressed in terms of the salt.

RESULTS

1. *Depletion of salivary and glandular kallikrein by chronic duct ligation*

A combination of 2–3 days ligation of the submaxillary duct and stimulation of the sympathetic nerve supply to the gland resulted in a marked reduction in glandular and salivary kallikrein concentrations. As shown in the Table, the mean kallikrein concentration of the ligated glands was 0·2 % of that of the contralateral control glands, and the mean salivary concentration was approximately 1 % of that in saliva collected from unligated glands. In three of the fifty-seven saliva samples and one of the

twelve glands from the duct-ligated animals, kallikrein could not be detected and in these cases the maximum possible kallikrein concentration was estimated by adding standard kallikrein to the incubation mixture of substrate and extract. The minimum amount of standard causing detectable kallikrein release divided by the amount of ligated gland saliva or extract present gave an indication of the maximum possible kallikrein concentration in the material. These figures were used in the statistical calculations.

TABLE 1. Kallikrein concentration in saliva and submaxillary gland extracts from normal cats and from cats with chronically ligated submaxillary gland ducts. Concentration in saliva is expressed as the amount of bradykinin (ng) released per ml. of saliva and glandular concentration as the amount released per mg of freeze-dried gland

		Saliva Frequency of stimulation (Hz)					Gland	
		1	2	5	10	20	R	L
Control	Mean	20,946	9282	15,579	45,153	19,298	5365	6960
	S.E. of mean	12,673	3617	10,760	41,021	14,417	1756	1767
	n	6	8	8	8	8	3	3
Chronic ligation of R duct	Mean	232	192	60	161	279	8·9	3987
	S.E. of of mean	183	71	26	75	124	2·6	1122
	n	6	12	13	13	13	12	12
	% of control	1·3	2·1	0·4	0·4	1·4	0·2	—

2. *The effect of chronic duct ligation on the vascular response to chorda stimulation*

Qualitatively, the vasodilatation in the submaxillary gland produced by chorda stimulation was unaffected by duct ligation. The two-phase blood flow response obtained at low frequencies of stimulation (1–5 Hz) and the increase characteristic of 10 and 20 Hz stimulation (see Darke & Smaje, 1972) were still present (see Fig. 1). To make quantitative comparisons of the magnitude of the vascular response it was necessary to account for the variation in flow rate during the stimulation period. An index of the initial phase of the response was obtained by measuring the extra drops of blood above the basal level as a result of a 10 sec chorda stimulus. The plateau phase of the response was measured by taking the steady part of the increased flow rate produced by continuous stimulation for 1–3 min. This somewhat arbitrary method was chosen because the low-frequency transient response lasted different periods at different stimulation frequencies and the response found in the first 10 or 15 sec of continuous stimulation gave less reproducible results than the technique described

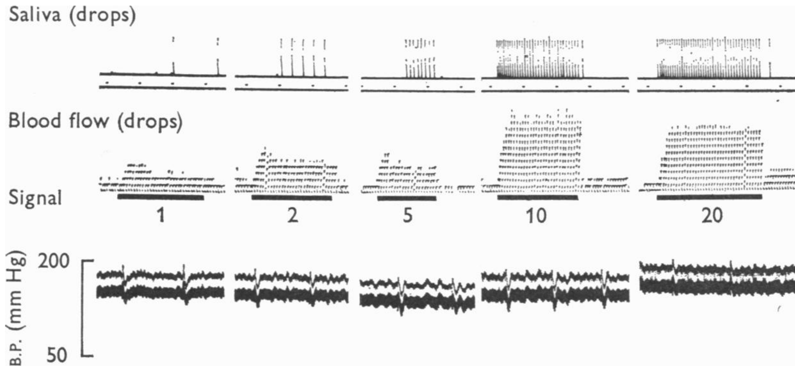

Fig. 1. The secretory and vascular response to chorda-lingual nerve stimulation, at the frequencies shown, in a submaxillary gland whose duct had been ligated 3 days previously. The blood flow integrator was reset every 5 sec. Stimuli were applied in a random order during the experiment but are rearranged here for illustrative purposes.

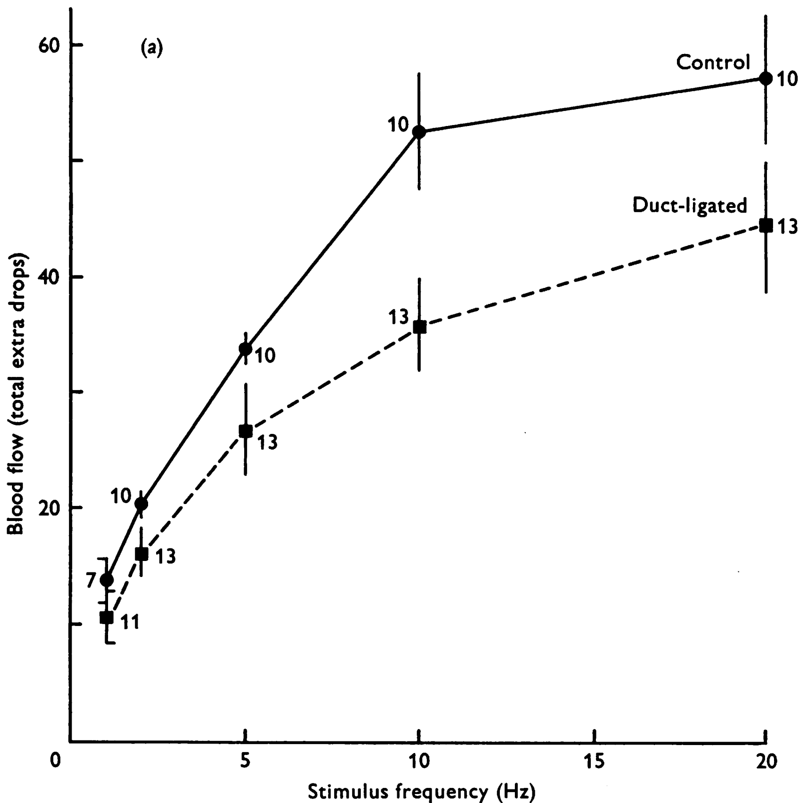

Fig. 2*a*. For legend see facing page.

above. Following duct ligation a 10 sec stimulus led to a smaller increase in blood flow than in control animals at all frequencies of chorda stimulation used (see Fig. 2*a*); the percentage depression of the response was approximately the same for each frequency. Continuous stimulation, on the other hand, produced a maintained vasodilatation which was reduced only at frequencies below 10 Hz, the maximum blood flow obtained being unaffected by chronic duct ligation (see Fig. 2*b*). In this case the percentage depression of the response fell with increasing frequency.

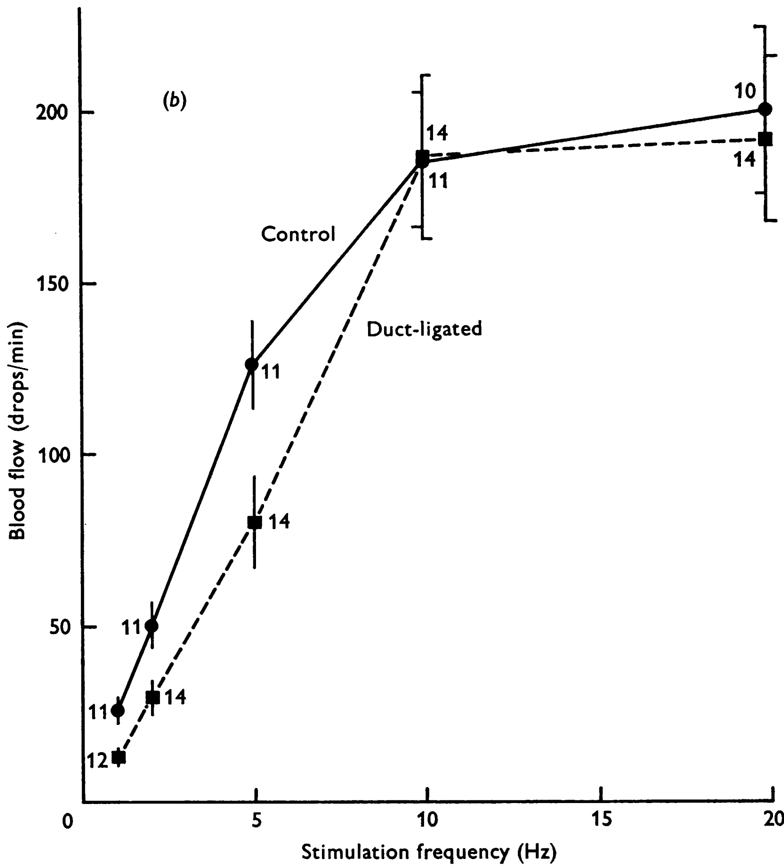

Fig. 2*b*

Fig. 2. Relationship between the vascular response and the frequency of stimulation of the chorda-lingual nerve in control (●—●) and duct-ligated (■ . . . ■) cats. *a*, extra drops flowing as a result of a 10 sec stimulus. *b*, maintained rate of blood flow (above basal) during continuous stimulation. Mean values ± S.E. of mean are given. The number adjacent to each point is the number of experiments performed.

3. *The effect of chronic duct ligation on the secretory response to chorda stimulation*

In order to determine whether other aspects of glandular activity were normal following chronic duct ligation, the salivation rate produced by chorda stimulation at different frequencies was also studied in control and

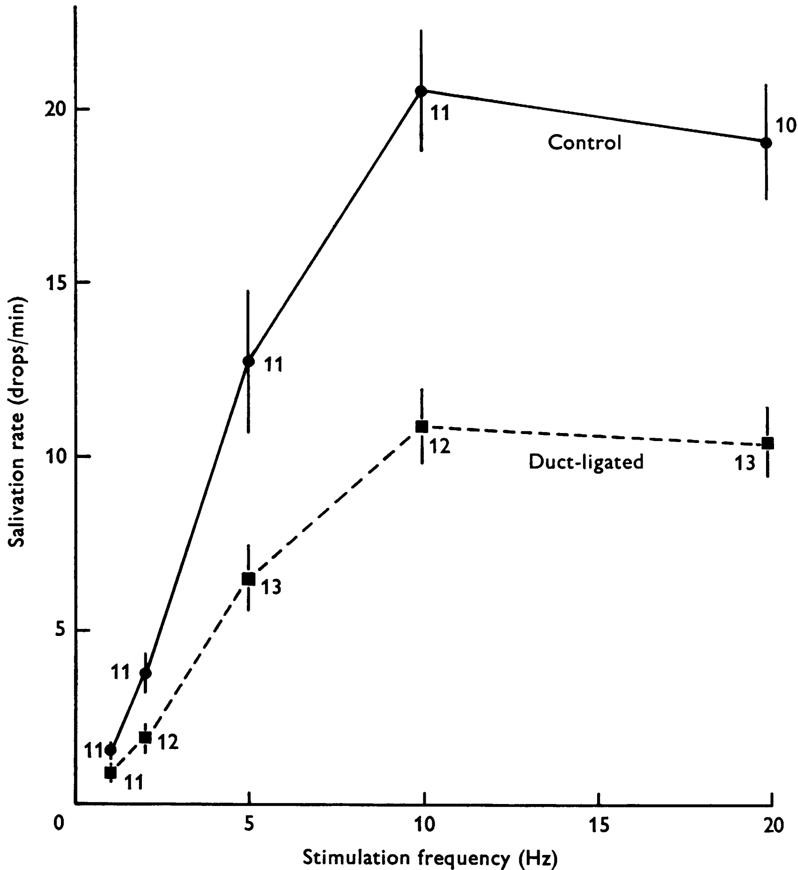

Fig. 3. Relationship between salivation rate and the frequency of stimulation of the chorda-lingual nerve in control (●—●) and duct-ligated (■ . . . ■) cats. Mean values ± S.E. of mean are given. The number adjacent to each point is the number of experiments.

duct-ligated animals. In control preparations during continuous stimulation salivation was not constant but, especially at high stimulus frequencies, tended to fall off with time (Darke & Smaje, 1972). Following duct ligation, salivation occurred at a steady rate.

A basis for comparing one preparation with another was obtained by

expressing salivation rate as the number of drops of saliva produced during a 1 min period which started 30 sec after beginning the stimulation. Following duct ligation the salivation rate was markedly reduced (see Fig. 3). The mean decrease in the response for the stimulation frequencies used was 47 % of the control value.

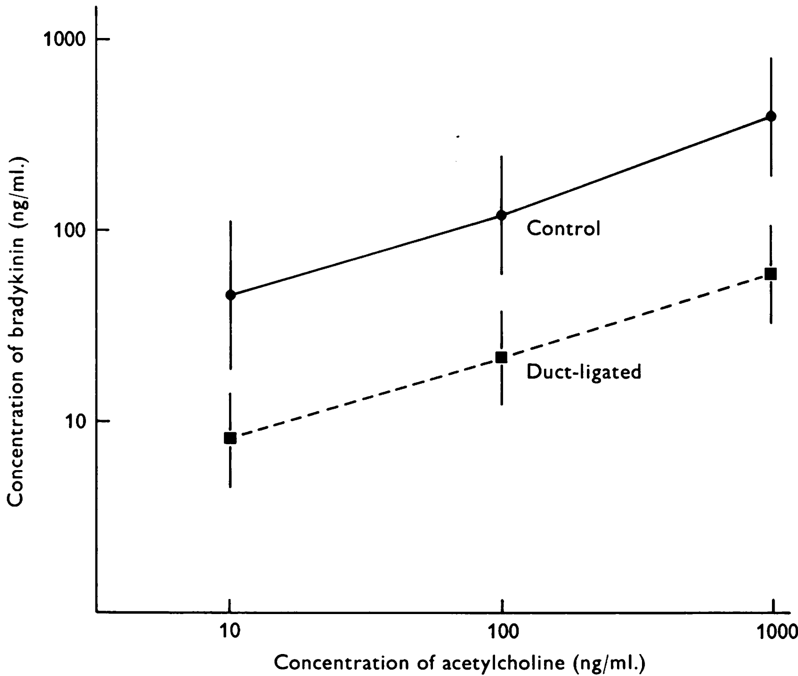

Fig. 4. Equipotent vasodilator concentrations of bradykinin and acetylcholine are plotted for control (●—●) and duct-ligated (■... ■) cats. For further details, see text. Mean values ± S.E. of mean are given. $n = 5$.

4. *The effect of chronic duct ligation on the vascular responses to bradykinin, acetylcholine and kallikrein*

Following duct ligation Hilton & Torres (1967) observed an increased vascular sensitivity in the submaxillary gland to bradykinin, but not to acetylcholine. They suggested that the low concentration of salivary kallikrein after duct ligation could still be responsible for the functional vasodilatation because of the supersensitivity to bradykin. Similar experiments have been repeated here.

Using the motor-driven syringe (see Methods) preliminary trials showed that a maximum response for a given concentration of acetylcholine or bradykinin was obtained with an infusion of 15 sec duration; with longer-lasting infusions the blood flow increase was reduced. There were sub-

stantial variations in the responses in any one animal and between animals and the consequent rather large standard errors of the mean made interpretation rather difficult. It appeared, however, that while the response to acetylcholine after duct ligation was not consistently greater than that before ligation, the response to bradykinin after duct ligation was increased over most of the dose range. Inter-animal variation was minimized by plotting equipotent concentrations of bradykinin and acetylcholine against one another (Fig. 4). When expressed in this way the results show that over a hundredfold range of acetylcholine concentration (10^{-8}–10^{-6} g/ml.) the dose of bradykinin required to produce the same response as the dose of acetylcholine was six times greater in control cats than in duct-ligated animals. The increase in bradykinin sensitivity calculated from the raw data varied from 5 to 10.

5. *The vascular response following chorda-lingual nerve stimulation during a period of arterial occlusion*

A short period of chorda stimulation (10 sec) at the beginning of a 30–90 sec arterial occlusion resulted, on release of the occlusion, in an hyperaemia larger than that after an equivalent period of occlusion alone. This confirms the findings of Hilton & Lewis (1955*a*). If the delayed hyperaemia were due to kallikrein release one might expect close-arterial injection of kallikrein or bradykinin at the beginning of the period of occlusion to mimic the delayed response to chorda stimulation. By varying the length of the occlusion the relative stabilities of bradykinin and kallikrein, and the stable vasodilator substance produced by chorda stimulation, could be made.

Details of the methods used to delineate the vascular responses to chorda-lingual nerve stimulation and drug injection are described in the Methods section.

The doses of bradykinin and kallikrein were chosen to produce approximately the same increase in blood flow on close-arterial injection as did the chorda stimulation. Despite the similar effect in the non-occluded gland, the vasodilator responses to bradykinin and kallikrein were significantly greater than that produced by chorda stimulation when the arterial inflow was also occluded for 30 sec (see Fig. 5). For 90 sec occlusions, however, the effect of bradykinin or kallikrein was reduced and the apparent rate of decay of the response suggested that for occlusions of 2 min duration there would be no difference between the response to close-arterial injection during occlusion and to occlusion alone (see Fig. 5). Only two occlusion durations were used because repeated injections of bradykinin and kallikrein into a gland with an occluded blood supply had a deleterious effect on the vascular response to these agents. The post-occlusive vaso-

dilator response to an initial chorda stimulation thus appeared to be more stable than that to injection of kallikrein or bradykinin.

If kallikrein were responsible for the delayed hyperaemia following chorda stimulation then reduction of the kallikrein concentration by duct ligation might be expected to have some effect. In seventeen controls and seven duct-ligated cats, hyperaemia due to chorda stimulation at 10 Hz

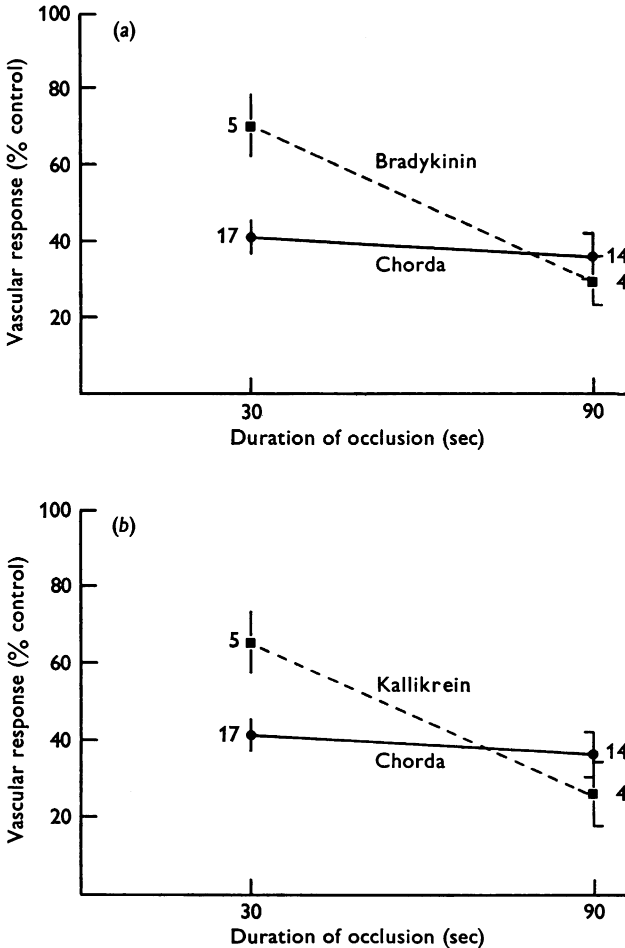

Fig. 5. Relationship between the delayed blood flow increase produced on release of arterial occlusion of the submaxillary gland and the duration of the occlusion. *a*, The response to chorda-lingual nerve stimulation for the first 10 sec of the occlusion is compared with close-arterial injection of bradykinin given at the start of the occlusion. *b*, Chorda-lingual nerve stimulation is compared with close-arterial injection of kallikrein. Mean values ± S.E. of mean are given. Number adjacent to each point is the number of experiments. For further details, see text.

for 10 sec was delayed by arterial occlusion lasting for 30–120 sec. Since the response to a 10 sec stimulus by itself was reduced by duct ligation (see Fig. 2*a*) the stable vasodilator response was used alone and not compared with the chorda stimulus. There was no significant difference between control responses and those in duct-ligated cats for any duration of arterial occlusion ($P > 0{\cdot}1$ for all occlusion durations) even though the mean glandular kallikrein concentration was 0·7 % of the contralateral control level.

DISCUSSION

In confirmation of previous reports (Beilenson *et al.* 1965, 1968; Hilton & Torres, 1967, 1970) a few days ligation of the submaxillary duct resulted in a marked reduction in kallikrein concentration in the gland and in the saliva produced by chorda-lingual nerve stimulation. The mean concentration in saliva produced by stimulation at 20 Hz was equivalent to 0·3 μg bradykinin/ml. which was 1·4 % of the control value. In the experiments of Beilenson *et al.* (1965, 1968) in which a less sensitive assay system was used, kallikrein was usually undetectable, whereas Hilton & Torres (1967, 1970) found the average salivary kallikrein concentration after duct ligation to be equivalent to 11·2 μg bradykinin/ml. which was 3·5 % of the control value. The present results confirm the presence of kallikrein in gland extracts and saliva in the duct-ligated cats but we obtained greater percentage reductions than Hilton & Torres. In the experiments of these authors the control salivary kallikrein concentrations appear to be some fifteenfold greater than in those of both Beilenson *et al.* and the present authors. Although Hilton & Torres used a purer substrate and there is a very wide variation in kallikrein concentration between different cats (e.g. see Table 1), such a difference is difficult to explain. In any event, the changes in concentration are of greater importance and in general all authors are in agreement in the direction of the changes.

The qualitatively normal vasodilator response in the face of a greatly reduced salivary kallikrein concentration (see Fig. 1) does not suggest that either phase of the normal two-phase response at 1–5 Hz is solely caused by kallikrein release. On the other hand, the fact that the total response to a 10 sec stimulus was reduced to about 75 % of control at all frequencies used and the observation that maintained vasodilatation at 1–5 Hz was reduced by up to 50 % suggest that kallikrein might be involved. Against this must be set the observation that the maximum vasodilatation ever obtained in normal glands (that produced by 10 and 20 Hz stimulation) was not significantly affected by the duct ligation. The apparent discrepancy between the present results and those of Beilenson *et al.* (1965, 1968) who reported that the vascular response was unaffected by duct

ligation is thus explained by the fact that these authors used a frequency of stimulation of 20 Hz. Under such circumstances the present results agree that there would be no difference between control and duct-ligated preparations. Hilton & Torres (1970) expressed the response as the total number of drops of blood flowing during the minute commencing with the onset of stimulation which lasted for 5, 10 or 15 sec. Since basal flow is unaffected by duct ligation it would have been expected on the basis of the present results (see Fig. 2*a*) that an effect of duct ligation would have been observed, but no differences were reported. It is possible that the lower absolute kallikrein concentrations obtained in the present experiments might be relevant. Whatever the reason, it seems clear that the combined effect of duct ligation and cervical sympathetic nerve stimulation was to cause a reduction in the vasodilator response to chorda-lingual nerve stimulation at frequencies less than 10 Hz.

It is pertinent to ask if the reduction in vascular response following duct ligation is a specific effect of kallikrein depletion. In answering this point it is relevant to consider the effect of duct ligation on the secretory response of the submaxillary gland to chorda stimulation. The salivation rate at all frequencies of chorda stimulation was reduced to 47 % of control after duct ligation. This observation confirms that of Beilenson *et al.* (1968) who, with a smaller series of animals, reported a reduction in saliva flow to 30–90 % (mean 62 %) of control. This effect is not likely to be directly due to kallikrein depletion as kallikrein does not provoke salivation by itself (Burgen & Emmelin, 1961).

Other changes in the ligated glands must therefore be sought. There are several reports of biochemical and histological changes occurring in the submaxillary gland after duct ligation. Both Garrett (1966*a*, *b*) and Tamarin (1967) comment on the histological changes occurring within a short time after duct ligation. In Tamarin's studies in the rat, changes in the architecture of the acinar cells were evident after one day's ligation and over the next 10 days or so there was a gradual disappearance of stored secretory material. These findings agree with the results of biochemical and histochemical studies on the mouse (Junquiera, 1951) which showed that after 15–30 days ligation there was a marked decrease in the amount of amylase and protease in the gland, the number of mitochondria also decreased and the levels of various intracellular enzymes were reduced. The periods of duct ligation in these studies were much longer than the two or three days used in the present experiments but Werle & Trautschold (1963) were able to demonstrate a marked reduction in the activity of glutamic-pyruvic and glutamic-oxaloacetic transaminase after ligation of the rat submaxillary gland for only 1 day. In view of these other changes in the submaxillary gland following duct ligation it is not justifiable to

attribute the depression of the vasodilator response to a specific effect of kallikrein depletion.

The circulatory arrest experiments also do not support the contention that kallikrein is the 'stable vasodilator substance', as duct ligation has no effect on the response and kallikrein does not appear to be sufficiently stable (Fig. 5). Since the response to a 10 sec chorda stimulus was reduced by duct ligation (Fig. 2*a*), the fact that the stable vasodilator response was not suggests that the delayed vasodilatation is due neither to kallikrein release alone nor to a delay in the appearance of the normal chorda vasodilatation. Although these results do not exclude the possibility that kallikrein makes some contribution to the delayed vasodilatation, it is necessary to consider other mechanisms which could account for this phenomenon. The simplest explanation consistent with these observations is that the 'stable vasodilator response' is due to an exaggerated reactive hyperaemia: it would be reasonable to suppose that the substance, or substances, responsible for reactive hyperaemia would be produced at a greater rate when the metabolism of the gland was increased by chorda stimulation. Thus when chorda stimulation accompanied occlusion the 'metabolite' would accumulate at an accelerated rate and be manifested in a greater than normal vasodilatation on release of the occlusion.

In some respects the present results raise more problems than they solve. Confirmation of the bradykinin supersensitivity following duct ligation previously found by Hilton & Torres (1970) is interesting but it is not possible at present to assess the physiological significance of this finding.

In view of Gautvik's work (1970*a*, *b*, *c*) one might have predicted that the early phase of the vascular response, largely nervous in origin according to this author, would be unaffected by kallikrein depletion, but on the contrary, this phase is affected most. Arguing from the same work, the late phase maintained vasodilatation should be most affected, since this was thought to be secondary to kallikrein release, but at stimulation frequencies of 10 and 20 Hz no reduction in response was noted at all. This author's interesting experiments should be interpreted with caution, however, as he used perfused glands whose behaviour did not always accord with that found in glands with natural circulation. The vascular response to acetylcholine, for instance, was not easily blocked by atropine whereas this drug readily blocks the increased blood flow caused by acetylcholine in the normal gland. Extrapolation to the present results is also difficult in that Gautvik used only 20 Hz stimuli and our results make it clear that the response obtained is frequency dependent.

From a previous paper it appeared that vasodilatation and salivation were, in part, correlated (Darke & Smaje, 1972). The relationship was not

a simple one as atropine blocked salivation yet only reduced the vascular response to chorda-lingual nerve stimulation. The present results do not lend strong support to the view that salivary kallikrein mediates functional vasodilatation in the gland although it is admitted that they provide little to favour positively the vasodilator nerve hypothesis either. Fig. 2 suggests one possible explanation for the two-phase vascular response and the differential sensitivity to duct ligation. A rapidly exhausted kallikrein release could be responsible for the initial phase of increased blood flow and the maintained response might be secondary to vasodilator nerves releasing acetylcholine. There is evidence that kallikrein concentration in saliva falls rapidly on continued stimulation (Beilenson *et al.* 1968) and this together with the greater reduction of the initial phase of the response by duct ligation is consistent with this suggestion. It is more difficult for the hypothesis to accommodate Gautvik's work and our own observations that the initial phase of the response is also more sensitive to atropine (Darke & Smaje, 1972). Furthermore, the response in the rabbit, which is qualitatively similar to that in the cat (A. C. Darke & L. H. Smaje, unpublished), is blocked in its entirety by small doses of atropine (Morley *et al.* 1966). Involvement of both vasodilator nerves and the release of kallikrein in some unknown combination would appear to explain more results than either hypothesis alone but full interpretation of these results must await a better understanding of all the consequences of duct ligation.

We are indebted to the Medical Research Council for an equipment grant to L. H. Smaje and wish to thank Mrs G. Davis for technical assistance.

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