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TWO PHASES OF CHORDA-LINGUAL INDUCED VASODILATATION IN THE CAT'S SUBMANDIBULAR GLAND DURING PROLONGED PERFUSION WITH LOCKE SOLUTION

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SUMMARY

1. The effect of stimulation of the chorda-lingual nerve on the venous flow has been studied in cat submandibular glands perfused with Locke solution for 2–4 hr.

2. When trains of pulses at 25 Hz were given for 1–5 sec, two distinct phases of vasodilatation were observed: a rapid initial phase of high amplitude and a slower developing more prolonged phase of smaller amplitude.

3. Repeated stimulations did not lead to a reduction of the vasodilatory response.

4. A close relationship was found between the duration and magnitude of the second phase of vasodilatation and the duration and magnitude of the post-stimulatory, active reuptake of potassium.

5. When the active reuptake of potassium was prevented either by ouabain (which inhibits active transport) or by atropine (which abolishes the stimulation induced loss of potassium) the second phase of vasodilatation was severely reduced, while the initial phase remained virtually normal.

6. It is concluded that the initial phase of vasodilatation probably is mediated by vasodilator nerve fibres. The second phase is perhaps causally related to the post-stimulatory, active transport of cations. An involvement of bradykinin formation is highly unlikely under the given experimental conditions.

INTRODUCTION

The mechanism of the functional vasodilatation in salivary glands has been the subject of much discussion. On the one hand it has been suggested that activation of the cat submandibular gland by parasympathetic or sympathetic stimulation caused a release of a bradykinin-forming enzyme

(kallikrein), and that bradykinin formed from precursors in the plasma is responsible for the accompanying vasodilatation (Hilton & Lewis, 1955*a*, *b*; 1956; Gautvik, Hilton & Torres, 1970). On the other hand the view was taken that functional vasodilatation was due to the action of vasodilator nerve fibres (Bhoola, Morley, Schachter & Smaje, 1965; Morley, Schachter & Smaje, 1966; Beilenson, Schachter & Smaje, 1968; Schachter, Barton & Karpinski, 1973). Gautvik (1970*a*, *b*, *c*) demonstrated that the chorda-lingual induced vasodilatation in the cat submandibular gland was composed of two phases: a quickly rising primary phase, which at cessation of stimulation continued in a second, slowly developing, but more prolonged phase. The hypothesis was proposed that the former was caused by vasodilator nerve fibres, while the latter was due to formation of bradykinin. However, Gautvik's unifying hypothesis has been subject to criticism by Darke & Smaje (1973). These authors concluded that if bradykinin formation did play any role at all in the functional vasodilatation, it was more likely to be involved in the first phase, while the maintained response was thought to be due to liberation of acetylcholine from vasodilator nerve fibres.

It has been the purpose of the present work to study the vascular response to stimulation of the chorda-lingual nerve during kininogen-free conditions in order to investigate the existence of vasodilator mechanisms different from bradykinin-formation. It has been demonstrated that two distinct phases of vasodilatation exist in the cat submandibular gland during prolonged perfusion with (kininogen-free) Locke solution. An involvement of bradykinin-formation appears extremely unlikely under these conditions. A preliminary communication of the observations reported here has been given (Poulsen, 1974*b*).

METHODS

Cats weighing 2·3–3·5 kg anaesthetized with chloralose (80 mg/kg I.P.) were used for the experiments.

Surgical procedures

The rectal temperature was maintained at 37° C. A tracheostomy tube was inserted through a mid line incision. Supplementary oxygen was given through a catheter inserted in the tube. A catheter was inserted in the right femoral vein. The left submandibular main duct was cannulated with a thin polyethylene tube (outer diameter 1·1 mm). The chorda-lingual nerve was cut, and the peripheral end was ligated. All branches from the left common carotid artery (except the one supplying the submandibular (and generally the sublingual) gland) were ligated twice and divided between the ligatures. This procedure was repeated on the external jugular vein. For technical reasons only one or two of the biggest veins draining the submandibular gland were left intact, while the others (smaller) branches were ligated

and cut. However, the glands were probably not damaged, since the secretory rate was not reduced (680 μl./min, S.E. of mean = 36 μl./min, $n = 21$, during perfusion with Locke solution after isolation, compared by 620 μl./min, S.E. of mean = 36 μl./min, $n = 21$, during autoperfusion before vascular surgery), neither did the isolated glands secrete saliva spontaneously. The submandibular gland with its duct and nerve (and the sublingual gland) was freed from the surroundings, only being connected to the cat through the vessels. After heparinization of the cat (7–10 mg/kg) the common carotid artery and the external jugular vein were cannulated for perfusion.

Perfusion procedure

The gland was perfused through the carotid cannula under a non-pulsative pressure (hydrostatic) of about 90 mmHg (slightly more initially and slightly less finally due to emptying of the reservoirs). In an exceptionally long experiment with a high flow of venous perfusion fluid the perfusion pressure reduction was by about 10 mmHg. However, in most experiments the reduction was less than 5 mmHg. When a direct comparison was made, e.g. tests done before and after ouabain or atropine (Figs. 5, 6) the reduction in perfusion pressure did not exceed 2 mmHg. The composition of the Locke's solution was: (mM) NaCl 140, KCl 4·0, Na_2HPO_4 2·4, NaH_2PO_4 0·6, $CaCl_2$ 1·5, $MgCl_2$ 1·0, and glucose 5·5. The perfusion fluid was equilibrated with oxygen at 37–38° C in the reservoir. When stated ouabain (g-Strophanthin, Fluka) was present in the perfusion fluid in a concentration of 2×10^{-5} M. Atropine (Alfred Benzon) was given in concentrations of $3{\cdot}6 \times 10^{-8}$–$1{\cdot}4 \times 10^{-7}$ M when stated.

Immediately after perfusion of the gland was established, the gland was transferred from the cat to a chamber maintained at 37° C. In some experiments a thermocouple (Ellab) was placed on the surface of the gland enabling continuous registration of the gland temperature. The chorda-lingual nerve was placed on two platinum electrodes for stimulation.

Technique of stimulation

The chorda-lingual nerve was stimulated by use of a Grass S 4 stimulator. The stimulator was connected to an electronic counter, whereby it was possible to give preset number of pulses. To ensure supramaximal stimulation rectangular pulses (10 V, 25 Hz, 1 msec) were given in all experiments (but very similar results could be obtained by using 10 Hz and even at 5 and 2 Hz).

Collection and analysis of perfusate

The venous perfusion fluid leaving the gland through the catheter in the external jugular vein passed a drop counter and was collected in test tubes by an automatic sample changer (Meditronic). The duration of the collection periods was 5, 10, 20, 30 or 60 sec. The potassium concentration of each sample of perfusion fluid was measured by using an Eppendorf flame photometer.

Salivary secretion

The amount of saliva secreted upon stimulation was measured either volumetrically, using a tuberculin syringe, or gravimetrically.

Calculations

All calculations were done by use of a Univac 1110 computer. The flow of venous perfusion fluid was calculated from the number of drops, the duration of the collection and the drop size. However, the latter was found to vary systematically with the drop frequency. Therefore, the dependence of the drop size on the drop frequency was established in preliminary experiments. In 95% of the samples the measured volume differed by less than 10% from the calculated value.

The total extra volume of venous perfusion fluid was calculated as the sum of the volumes of all samples involved in the particular phase, minus the product of the duration of the phase and the preceding resting flow rate (taken as the mean of the flow of the three samples immediately preceding the stimulation). Since salivary secretion was temporally dissociated from the post-stimulatory, active net uptake of potassium, the amount of the latter could simply be calculated as the sum of the products of sample volume and difference between arterial and venous potassium concentration, from the samples in which the potassium concentration was below the arterial level.

The curves describing single experiments have been redrawn from originals produced by the computer.

RESULTS

Reproducibility of vasodilatation

Fig. 1 demonstrates the reproducibility of the vasodilatory response to trains of stimulation pulses (1500 pulses, 60 sec) applied to the chorda-lingual nerve 7 times during a single experiment. In addition to demonstrating the reproducibility the Figure shows the features of the chorda-lingual induced vasodilatation in glands perfused with Locke solution. The flow increased (within 5 sec) to reach a more or less pronounced plateau (most readily seen on Fig. 1 at 80, 96, 112, 142, and 157 min of perfusion whereafter a further more slowly developing small increase occurred. After cessation of stimulation the flow decreased relatively slowly. The decline was apparently not monoexponential. The slight, but steady increase in the resting flow seen in Fig. 1 was observed in most experiments. In a series of six experiments the maximal flow obtained by stimulation of the chorda-lingual nerve (1500 pulses, 60 sec) was 12·4 (S.E. ± 1·7) ml./min. at the first stimulation after 65–91 min of perfusion and 13·3 (S.E. ± 1·2) ml./min at the last stimulation after 127–225 min of perfusion. The corresponding total extra volume of fluid perfused through the glands (in excess of the resting flow) were in the same situations 34·7 (S.E. ± 8·7) and 32·8 (S.E. ± 7·8) ml. respectively. The rates of salivary secretion were 823 (S.E. ± 60) μl./min, 742 (S.E. ± 59) μl./min, and the resting flow rates of perfusion fluid were 2·07 (S.E. ± 0·55) ml./min and 2·86 (S.E. ± 0·51) ml./min.

Dependence of vasodilatation on duration of stimulation

Fig. 2 shows the dependence of the vasodilatory response on the duration of stimulation of the chorda-lingual nerve. Since a constant frequency of pulses of stimulation was used (25 Hz) the figure also demonstrates the dependence on the number of pulses given. Brief trains of pulses (25–125 pulses, 1–5 sec) produced two distinct phases of vasodilatation. The initial phase was rapid, reaching its peak value within 5 sec and outlasting the period of stimulation by only 10–20 sec. At 25 pulses (1 sec) the initial phase of vasodilatation was followed by a phase of vasoconstriction lasting

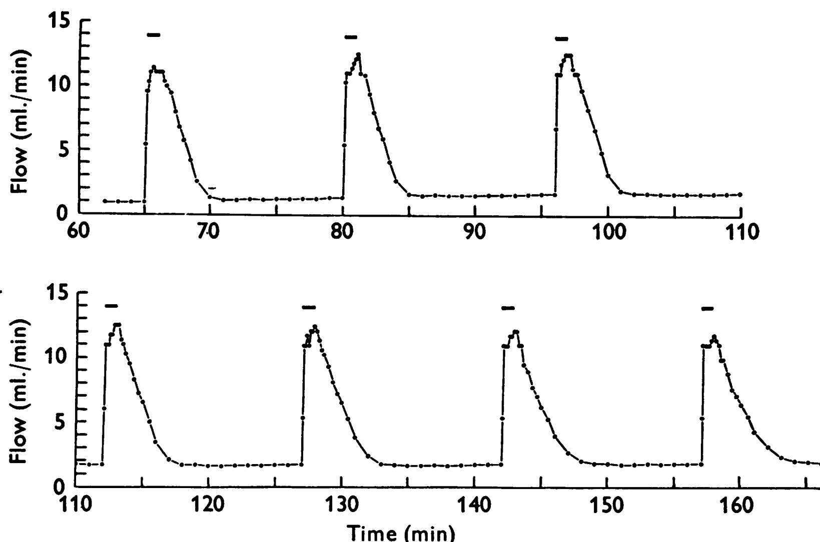

Fig. 1. Reproducibility of flow of venous perfusion fluid in a single experiment. Each horizontal bar indicates stimulation of the chorda-lingual nerve (1500 pulses, 60 sec). Time indicates duration of perfusion with Locke solution. The secretory rates obtained during the seven periods of stimulation were: 890, 890, 880, 890, 880, 860 and 860 μl./min, respectively.

10–15 sec. Thereafter a slowly developing, relatively small, but rather prolonged second phase of vasodilatation occurred. A similar pattern was observed at 125 pulses (5 sec) with the exception that there was no period of flow below the resting level between the two phases of vasodilatation and the magnitude of the second phase was bigger than at 25 pulses. The peak value of the rapid, initial phase was similar to that obtained at 25 pulses, but the duration was longer.

When longer trains of pulses were given, no clear distinction could be made between the two phases of vasodilatation, although the shape of the

curves makes it likely that the response consisted of two overlapping phases.

Table 1 demonstrates that the maximal flows obtained by 25, 125 and 1500 pulses were virtually equal. The table further shows the total extra volumes of perfusion fluid passing through the glands during the different phases of the vascular response to brief trains of pulses (25–125 pulses). While the maximal flow of the second phase of vasodilatation was rather small (see Fig. 2) the total extra volume of perfusion fluid of this phase was comparable to that of the initial phase.

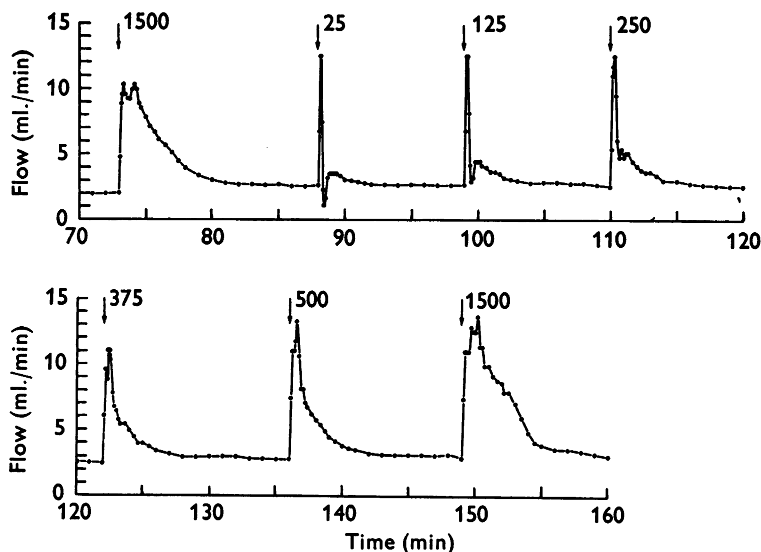

Fig. 2. Vasodilatory responses to different trains of pulses of stimulation. The arrows indicate the beginning of stimulation. The numbers behind the arrows indicate the number of pulses given. The frequency of stimulation pulses was 25 Hz throughout the experiment.

Vasodilatation and potassium transport

Fig. 3 shows the effect of stimulation of the chorda-lingual nerve on the flow of venous perfusion fluid and the venous potassium concentration. The concentration curve reveals typical 'potassium transients' (Burgen, 1956): a stimulation-induced initial steep increase in the venous potassium concentration, caused by a passive loss of potassium from the glandular cells, succeeded by a gradual decrease, below the arterial level, due to an active uptake of potassium by the cells (Petersen, 1970). It should be noticed that the flow and the potassium concentration returned to their resting levels almost simultaneously.

TABLE 1. Total extra volumes of venous perfusion fluid of the subsequent phases of the vascular response to trains of pulses (negative signs indicate phases with flow below the resting value). The maximal flows of venous perfusion fluid are also shown

	(25 pulses, 1 sec)				(125 pulses, 5 sec)				(1500 pulses, 60 sec)
Expt. no.	$Vol._1$ (ml.)	$Vol._{neg.}$ (ml.)	$Vol._2$ (ml.)	Max. flow (ml./min)	$Vol._1$ (ml.)	$Vol._{neg.}$ (ml.)	$Vol._2$ (ml.)	Max. flow (ml./min)	Max. flow (ml./min)
388	1·50	−0·20	0·87	8·86	2·15	−0·11	1·72	9·58	9·22
392	1·85	−0·19	1·16	8·86	2·95	0·00	2·02	8·15	9·22
393	2·32	−0·48	1·45	14·09	3·64	−0·02	3·61	15·28	16·68
394	1·59	−0·22	2·36	12·55	2·58	0·00	4·17	12·55	10·30
Mean	1·82	−0·27	1·46	11·09	2·83	−0·03	2·88	11·39	11·36

Fig. 4 shows that the total extra volume of fluid perfused through the glands (in excess of the resting flow) during the second phase of vasodilatation was positively correlated ($r = 0{\cdot}85$) to the amount of potassium taken up actively by the glandular cells.

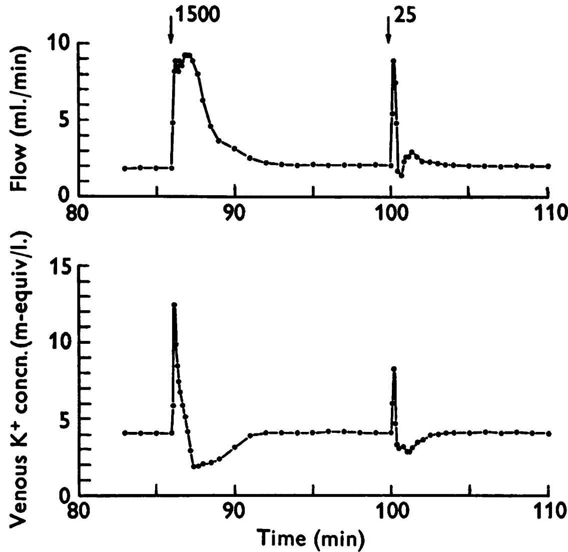

Fig. 3. Venous potassium concentration and flow of venous perfusion fluid in a single experiment. Symbols as in Fig. 2.

Effect of ouabain on vasodilatation and potassium transport

In a series of five experiments the effect of ouabain on the vascular response to nerve stimulation was studied. All five experiments exhibited the same pattern as the typical one shown in Fig. 5. The characteristic findings were: (1) an almost immediate slight increase in the venous potassium concentration, indicating a net loss of potassium from the glandular cells due to inhibition of the sodium-potassium pump; (2) abolition of the post-stimulatory reduction in venous potassium concentration, indicating a failing ability of the cells to perform a net uptake of potassium; (3) an almost normal (in the shown experiment even slightly enhanced) initial phase of vasodilatation; (4) a virtually abolished second phase of vasodilatation; (5) a progressive increase in the 'resting' post-stimulatory flow, which did not return to previous resting level; (6) stimulation-induced salivary secretion remained uninfluenced. Quantitative details are given in Table 2.

Effect of atropine on vasodilatation and potassium transport

In a series of five experiments the effect of atropine was studied. When using appropriate concentrations ($3{\cdot}6 \times 10^{-8}$–$1{\cdot}4 \times 10^{-7}$ M) stimulation-induced potassium release and salivary secretion could be severely reduced or entirely abolished, while the initial phase of vasodilatation remained uninfluenced or was slightly reduced. The second phase of vasodilatation was severely reduced, completely abolished or even inverted to a vaso-constriction. Fig. 6 shows how the response to stimulation depends on the

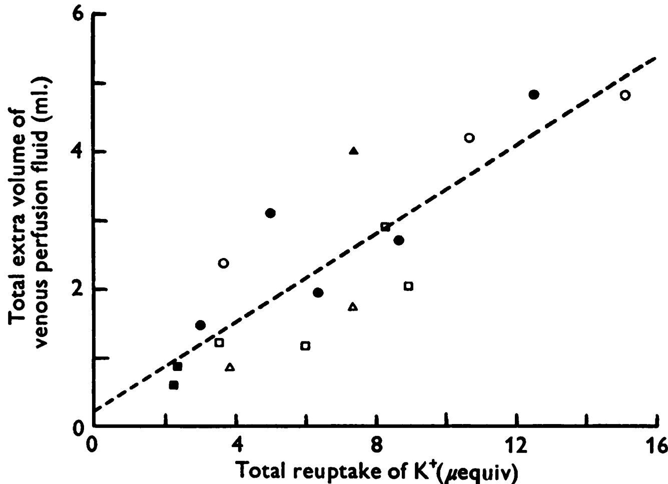

Fig. 4. The relationship between the second phase of vasodilatation and the amount of potassium taken up actively by the glandular cells during the post-stimulatory period of restitution. The ordinate is the time integral during the second phase of vasodilatation of the actual flow of venous perfusion fluid minus the preceding resting flow. The different symbols indicate results from different experiments. The calculated regression line is drawn.

concentration of atropine. Table 3 gives a quantitative description of the effect of atropine on the different phases of the vascular response to stimulation and on the active, post-stimulatory uptake of potassium. When the glands were stimulated for longer periods (60 sec), the flow returned faster to the resting value in the presence of atropine.

DISCUSSION

Three important observations have been made on cat submandibular glands perfused for prolonged periods with kininogen-free, Locke solution: (1) the remarkable reproducibility of the chorda-lingual induced vaso-dilatation; (2) the presence of two distinct phases of vasodilatation; (3) the

existence of a correlation between the second phase of vasodilatation and the post-stimulatory, active transport of cations.

It has been demonstrated that the vasodilatory response to stimulation of the chorda-lingual nerve after prolonged (3 hr) perfusion with Locke

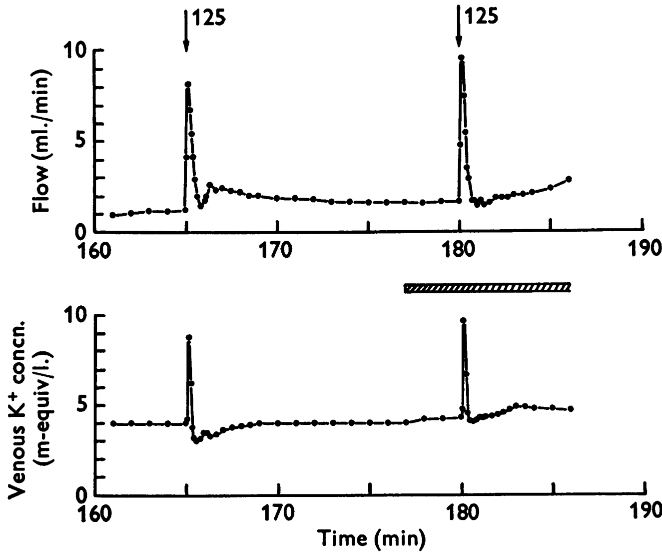

Fig. 5. The effect of the presence of ouabain (2×10^{-5} M), indicated by hatched horizontal bar, on flow of venous perfusion fluid and venous potassium concentration. Symbols as in Fig. 2.

TABLE 2. The effect of ouabain (2×10^{-5} M) on flow of venous perfusion fluid, potassium transport and secretion in five experiments. 'Δ flow max.$_1$' means the maximal increase of venous flow (above resting flow) during the initial phase of vasodilatation. 'Δ flow max.$_2$' is the corresponding value of the second phase of vasodilatation. 'Rate K^+-loss' is the maximal rate of the chorda-lingual induced passive loss of potassium from the glands to the perfusion fluid. 'Rate K^+-uptake' is the maximal rate of the corresponding post-stimulatory, active reuptake of potassium

		Rest. flow (ml./min)	Δ flow max.$_1$ (ml./min)	Rate K^+ loss (μequiv/min)	Rate K^+ uptake (μequiv/min)	Δ flow max.$_2$ (ml./min)	Secretion (mg)
Control	Mean	2·71	5·87	44·3	6·28	1·14	42
	S.E. of mean	0·76	0·55	5·9	1·53	0·14	9·6
Ouabain	Mean	2·61	5·17	45·9	−0·02	−0·02	43
	S.E. of mean	0·61	0·78	3·1	0·81	0·37	11·6

solution was not smaller than the response obtained after shorter periods of perfusion (1 hr). In individual experiments it was possible to produce several, almost identical vasodilatations (Fig. 1). These observations seem directly to indicate that kinin-formation from precursors present in normal cat plasma cannot play a major role under the given experimental conditions. This conclusion is supported by Gautvik's (1970*b*) demonstration that the phase of vasodilatation which he ascribed to kinin-formation

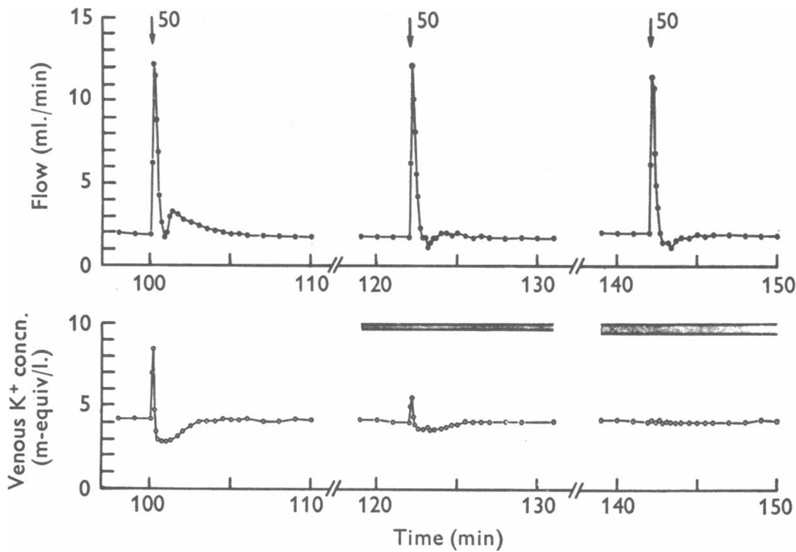

Fig. 6. The effect of atropine on flow of venous perfusion fluid and venous potassium concentration. The thin and thick shaded horizontal bars indicate the presence of atropine in concentrations of $3{\cdot}6 \times 10^{-8}$ and $7{\cdot}2 \times 10^{-8}$ M, respectively. The amounts of saliva secreted in response to the three periods of stimulation were: 29·5, 5·0 and 0·5 mg, respectively. Symbols as in Fig. 2.

could not be evoked after 5–15 min of kininogen-free perfusion, while the glands in the present work were perfused with Locke solution for about 1 hr before the first stimulation. Since kininogens may be present in the glandular interstitial fluid at the beginning of the perfusions, it would be pertinent to estimate the amount of kininogen in the glands at the beginning of the experiments. Using the value found by Gautvik *et al.* (1970) for the concentration of kininogen 2 (the substrate of glandular kallikrein) in normal cat plasma, and the inulin distribution space from the rat submandibular gland (Schneyer & Schneyer, 1960) as well as the assumption that the concentration of kininogen 2 of the interstitial fluid equals that of the plasma, an upper limit for the glandular content

TABLE 3. The effect of atropine ($7\cdot2\times10^{-8}$–$1\cdot4\times10^{-7}$ M) on vascular response and glandular reuptake of potassium. Stimulation for 1–5 sec (25–125 pulses) was used

	Control						Atropine					
Expt. no.	$Vol._1$ (ml.)	$Vol._{neg.}$ (ml.)	$Vol._2$ (ml.)	K^+_{uptake} (μequiv)	Rest. flow (ml./min)	Max. flow (ml./min)	$Vol._1$ (ml.)	$Vol._{neg.}$ (ml.)	$Vol._2$ (ml.)	K^+_{uptake} (μequiv)	Rest. flow (ml./min)	Max. flow (ml./min)
424	2·93	−0·02	3·19	7·50	4·47	16·96	1·79	−0·01	0·09	0·27	4·28	12·13
426	3·30	−0·04	1·73	7·53	1·91	12·13	2·65	−0·94	0·00	0·26	2·00	11·46
430	1·47	−0·04	0·78	2·66	1·06	4·88	0·84	−0·35	0·02	0·14	1·35	4·21
435	3·60	0·00	1·82	4·32	2·40	14·18	2·84	0·00	0·96	0·07	2·47	14·18
436	3·07	−0·14	1·66	6·75	2·83	14·18	2·74	−0·40	0·65	1·31	2·40	13·49
Mean	2·87	−0·05	1·84	5·75	2·54	12·47	2·17	−0·40	0·34	0·41	2·50	11·09

of kininogen 2 at the beginning of perfusion with Locke solution can be calculated to about 0·3 μg (of bradykinin). From the results of Gautvik *et al.* (1970) the total consumption of kininogen 2 (during and after 1 min of stimulation) can be estimated to about 120 ng (of bradykinin formed). This means that even if it was assumed that kininogen 2 was neither washed out nor consumed during the one hour of perfusion preceding the first stimulation of the chorda-lingual nerve, a normal kinin-formation could maximally be evoked 2–3 times. However, it must be pointed out that it is very likely that a considerable fraction of the initial glandular content of kininogen 2 was washed out, since Hilton & Lewis (1956) concluded that the permeability of the glandular vessels probably was increased during perfusion with Locke solution. Furthermore, the initial concentration of kininogen 2 in the interstitial fluid would be expected to be smaller than that of the plasma. Thus it seems justified to conclude that kinin-formation cannot play a significant role in the chorda-lingual induced vasodilatation observed in the present experiments.

The existence of two phases of vasodilatation in the vascular response to stimulation of the chorda-lingual nerve has been described previously by Gautvik (1970*a*, *b*, *c*) and Darke & Smaje (1972, 1973). However, the present finding that the two phases can be completely separated by a period of resting flow (and even vasoconstriction) was not observed previously, since it requires rather brief periods of stimulation (1–10 sec) which has not been generally used. The effect of increasing the duration of stimulation (number of pulses) (Fig. 2) seems to indicate that the vasodilatory response to longer periods of stimulation represents the sum of two superimposed phases. The results obtained by stimulation for 20–60 sec are in very good agreement with those of Gautvik (1970*a*, *b*, *c*) and Darke & Smaje (1972). In spite of these similarities, it appears that some differences may exist between the underlying mechanisms. On the one hand the second phase of vasodilatation in Gautvik's (1970*b*) studies disappeared rapidly in the absence of bradykininogen in the perfusate, which was obviously not the case in the present work. On the other hand Darke & Smaje (1972) found that at high frequencies of stimulation (10–20 Hz) the initial phase of vasodilatation was reduced in the presence of atropine, while the second phase ('maintained response') remained almost unchanged. In contrast in the present study the second phase of vasodilatation was more reduced by atropine than the first phase (Fig. 6, Table 3). This apparent discrepancy may in part be explained by the fact that Darke & Smaje used, as a measure for the 'initial response', the total number of extra drops above basal as a result of 10 sec of stimulation. In this way both the first and the second phases described in the present work would have been combined in Darke & Smaje's 'initial response'.

However, even in the work by Darke & Smaje (1972, Fig. 3*b*) it can be seen that the very first part of the 'initial response' to stimulation at 20 Hz was in no way reduced in the presence of atropine. Furthermore, it must be pointed out that the 'maintained response' described by Darke & Smaje does not correspond to the second phase of vasodilatation in the present work, since the former was measured during stimulation of the chorda-lingual nerve, whereas the latter was measured after stimulation had ceased. However an excellent agreement exists between the first phase of vasodilatation in Gautvik's (1970*a*, *b*, *c*) experiments and in the present work. Gautvik concluded that this phase was due to the action of true vasodilator nerve fibres because of the rapidity of the response and its independence of the presence of bradykininogen. Since the first phase of vasodilatation in the present study has the same properties, it seems reasonable to adopt Gautvik's view. The tentative suggestion by Darke & Smaje (1973) that the initial phase of vasodilatation perhaps could be related to a rapidly exhausted kallikrein release (causing bradykinin formation) appears rather unlikely on the basis of the present findings. The observation that atropine only has a slight effect on the first phase of vasodilatation while the potassium release is abolished (Fig. 6, Table 3) seems to indicate that an increased potassium concentration does not contribute significantly. In contrast the high venous potassium concentration (10–13 m-equiv/l.) seen at more prolonged stimulation may in fact cause a slight vasoconstriction, as is the case in the rat aorta (Biamino & Wessel, 1973). Upon stimulation with brief trains of pulses the short period of reduced flow succeeding the initial phase of vasodilatation (Figs. 2, 3, Table 1) may perhaps be regarded as a sort of inverted 'reactive hyperaemia', i.e. a vasoconstriction following a period of relative hyperperfusion. This view is supported by the finding that the vasoconstriction is more pronounced when salivary secretion and post-stimulatory, active transport of cations are prevented by atropine (Fig. 6, Table 3).

The mechanism responsible for the second phase of vasodilatation in the present experiments is not clear. However, the observation that a similarity exists between the time course of the second phase of vasodilatation and the post-stimulatory active transport of potassium (and sodium) (Poulsen, 1974*a*) could suggest some kind of relationship between the two phenomena (Fig. 3). This view is supported by the finding that the total extra flow of perfusion fluid (in excess of the resting flow) during the second phase of vasodilatation is positively correlated to the amount of potassium taken up actively by the glandular cells (Fig. 4). Furthermore the observation that inhibition of active uptake of potassium by ouabain (Petersen, 1971) is accompanied by a marked reduction of the second phase of vasodilatation (while the first phase is not reduced) (Fig. 5) is in

accordance with the idea that the second phase is a consequence of the post-stimulatory, active transport of cations (Fig. 5). Finally the finding that administration of atropine is able to abolish both potassium transport and the second phase of vasodilatation (Fig. 6) gives support to the proposed idea. However, it cannot be excluded that the second phase of vasodilatation and the active uptake of potassium could be concomitant variables, perhaps both caused by some third unknown factor.

While a connexion has been established between the second phase of vasodilatation and the post-stimulatory, active transport of cations, the direct stimulus causing the vascular smooth muscles to relax is unknown. Preliminary experiments indicate that an increase in tissue osmolality which recently has been suggested to be involved in glandular vasodilatation (Lundvall & Holmberg, 1974) does not occur during the second phase of vasodilatation. A reduction in pH and P_{O_2} may contribute. The two latter possibilities are attractive, since they would be more pronounced during perfusion with Locke solution than during perfusion with blood, whereby it could be explained why the second phase of vasodilatation observed in the present work has not been described previously. However, there may also be other reasons for this discrepancy. In Gautvik's (1970*a*, *b*) experiments the glands were perfused at constant flows of about 0·5 ml./min. The perfusates generally had haematocrit values around 30 %, which means that the flow of fluid through the glands upon stimulation was about 30 times smaller than in the present experiments. Since the rate of the stimulation-induced potassium loss is dependent on the flow (L. P. Laugesen, J. O. D. Nielsen & J. H. Poulsen, unpublished) the amount of potassium lost to the perfusate and subsequently actively taken up, has undoubtedly been much smaller in Gautvik's experiments than in the present. According to the proposed idea the reduction in the potassium transport would lead to a reduction in the second phase of vasodilatation, which in that case could be difficult to detect with the technique used by Gautvik (1970*a*, *b*, *c*).

While it has been demonstrated that two different vasodilator mechanisms exist in the submandibular gland of the cat in the absence of kininogens, it cannot be excluded that bradykinin-formation may contribute to the functional vasodilatation during more physiological conditions.

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