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SOME FACTORS INFLUENCING STIMULATION-INDUCED RELEASE OF POTASSIUM FROM THE CAT SUBMANDIBULAR GLAND TO FLUID PERFUSED THROUGH THE GLAND

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SUMMARY

1. The release of K from the cat submandibular gland to the extracellular fluid (ECF) after stimulation with acetylcholine (ACh) and the subsequent uptake of K from the ECF was studied in glands perfused artificially with Locke solutions.

2. The first injection of ACh after shift of the perfusion fluid from control to K-free Locke solution evoked a normal loss of K and a normal secretion of saliva. The second injection only evoked a small release of K and a reduced secretion.

3. Perfusion with dinitrophenol (DNP) (10^{-4} M) containing solutions, Na-free Li Locke solutions and chloride-free nitrate Locke solutions inhibited salivary secretion and the uptake of K. The first injection of ACh after shift of the perfusion fluid from control to test solution gave a normal K loss, but thereafter the ACh-induced K-loss declined.

4. Perfusion with g-strophanthin (10^{-5}–10^{-4} M) always inhibited K uptake whereas K release was not affected primarily. The sensitivity of the secretory mechanism of different glands to strophanthin varied considerably.

5. Perfusion with tetraethylammonium Locke solution inhibited secretion, K uptake and release of K.

6. It is suggested that the release of K from salivary glands to the ECF after stimulation with ACh can be explained by diffusion as a consequence of an enhanced permeability of the cell membranes to K. Concomitantly with the release of K, Na is taken up. It is suggested that the subsequent uptake of K and extrusion of Na is due to active transport processes probably involving a Na–K activated ATP-ase.

INTRODUCTION

When a salivary gland is stimulated to secrete, intracellular K is lost both to the saliva and to the blood perfusing the gland (Burgen, 1956). A short time after the start of K release the gland begins to accumulate K (Burgen, 1956).

In the present work only the loss of K to the blood will be considered, as it is only possible to study the influence of changes in extracellular ion composition on this K transport.

Many different hypotheses have been proposed to account for the loss of K after stimulation with ACh, e.g. diffusion (Imai, 1965*b*; Schneyer, 1967), active transport (Burgen, 1967) and solvent drag exerted by the water flow through the acinus during the secretory process (Lundberg, 1958; Petersen & Poulsen, 1967, 1969). The aim of the present work was to exclude some of these possibilities. The fact that the metabolic inhibitor DNP, in doses which inhibit secretion severely, has no or at most a very small influence on the K loss, and the many discrepancies found between the effect of certain ion substitutions on secretion and K loss seem to exclude the last two possibilities, leaving the diffusion hypothesis as the most reasonable at the present time. Some of the findings have been briefly reported previously (Petersen, 1969).

METHODS

Cats (2–4 kg) were anaesthetized with chloralose (80 mg/kg, I.P.). The left submandibular duct was cannulated and all branches from the left carotid artery except the one supplying the submandibular gland were ligated. All tributaries to the external jugular vein except the one draining the submandibular gland were also ligated. The external jugular vein and thereafter the common carotid artery were cannulated. Through the carotid cannula the gland was perfused with modified Locke solutions of different composition (Table 1) under a hydrostatic pressure of 80 mm Hg at room temperature (19–23° C). The perfusion fluids were equilibrated with oxygen before use. The volume of perfusion fluid coming out of the jugular vein cannula per unit time was taken as the glandular perfusion fluid flow. The concentration of K in the fluid coming out of the jugular vein cannula was measured using an Eppendorff flame photometer. The sample size was 1–2 ml. Through the cannula inserted into the carotid artery short-lasting injections of ACh (0·1–10 μg) or adrenaline (1 μg) were given. The first 3 min after an injection of ACh all the perfusion fluid from the jugular vein was collected and analysed for K, samples being taken every 20 sec. During the other periods samples were collected every minute. The loss of K from the gland after an injection of ACh and the subsequent uptake were calculated from the difference between the amount of K flowing into and out of the gland in the same time period. The final result pooled from more experiments was expressed as the mean $\pm$ S.E. (no. of observations). After each injection of ACh the salivary secretion was recorded by counting the number of drops of saliva coming from the submandibular duct cannula (1 drop = 35 μl. saliva).

TABLE 1. Composition of modified Locke solutions (mM)

	Control (1)	K-free	TEA	Control (2)	Li	Control (3)	NO_3
NaCl	140	144	—	142	—	140	—
KCl	4	—	4	—	—	—	—
Na_2HPO_4	2·4	2·4	2·4	—	—	2·4	2·4
NaH_2PO_4	0·6	0·6	0·6	—	—	0·6	0·6
K_2HPO_4	—	—	—	1·8	1·8	—	—
KH_2PO_4	—	—	—	0·45	0·45	—	—
$CaCl_2$	1·5	1·5	1·5	1·5	1·5	—	—
$Ca(NO_3)_2$	—	—	—	—	—	1·5	1·5
$MgCl_2$	1·0	1·0	1·0	1·0	1·0	—	—
$MgSO_4$	—	—	—	—	—	1·0	1·0
$NaNO_3$	—	—	—	—	—	—	140
K_2SO_4	—	—	—	—	—	2	2
LiCl	—	—	—	—	142	—	—
TEACl	—	—	140	—	—	—	—
Glucose	5·5	5·5	5·5	5·5	5·5	5·5	5·5

RESULTS

K loss after stimulation with adrenaline

In all experiments where injections of adrenaline were given, it evoked a loss of K followed by a re-uptake (Fig. 1). The amount of K released varied, however, from gland to gland. In three different glands injections of 1 μg ACh and adrenaline evoked a mean K loss of 8·0 μ-equiv $\pm$ 1·7 (S.E.) (n = 5) and 7·1 μ-equiv $\pm$ 2·8 (n = 5) respectively. The subsequent K uptake in the same experiments was 13·8 μ-equiv $\pm$ 2·0 (n = 5) and 10·7 μ-equiv $\pm$ 2·8 (n = 5) respectively.

The effect of perfusion with a K-free solution

The first injection of ACh after shift to a K-free fluid always evoked a normal loss of K whereas the second injection only evoked a reduced loss (Fig. 2). In five different glands injections of 10 μg ACh during the control periods, the periods of K-free perfusion (the first injection) and the control periods after perfusion with K-free solution evoked a mean K loss of 11·1 μ-equiv $\pm$ 1·2 (n = 5), 10·7 μ-equiv $\pm$ 1·0 (n = 5) and 18·5 μ-equiv $\pm$ 1·7 (n = 5) respectively.

The effect of perfusion with DNP (10^{-4} M)

Fifteen min after start of perfusion with DNP (10^{-4} M) the loss of K after injection of ACh was unaltered compared with the control periods whereas the secretion was severely reduced. The uptake of K after the loss was also

severely reduced. After reintroduction of the control Locke solution the ability to take up K was completely restored and the secretion was better than during the period of inhibition (Fig. 3). A complete restoration of secretion could only be achieved after the second stimulation by ACh following the return to normal Locke solution. In four different glands

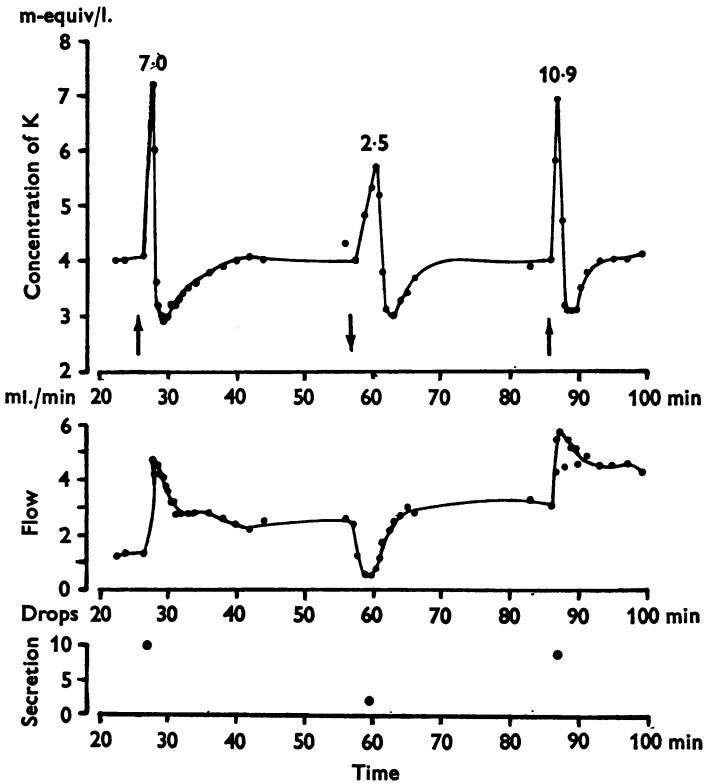

Fig. 1. The effect of ACh (1 μg) (↑) and adrenaline (1 μg) (↓) on K transport and secretion in a single experiment. The Figure shows the concentration of K in the perfusion fluid coming out of the gland vein, the glandular perfusion fluid flow and the number of drops of saliva coming out of the submandibular duct after each injection of either ACh or adrenaline. The figures above the upper curve give the amount of K (in μ-equiv) lost to the perfusion fluid after each injection.

perfused with control medium, then with DNP-containing medium and finally again with control medium injections of 10 μg ACh evoked a mean K loss of 11·2 μ-equiv $\pm$ 1·8 (n = 4), 10·3 μ-equiv $\pm$ 1·3 (n = 4) and 12·4 μ-equiv $\pm$ 2·0 (n = 4) respectively. The subsequent uptake of K in the same experiments was 20·0 μ-equiv $\pm$ 2·1 (n = 4), 2·8 μ-equiv $\pm$ 0·7 (n = 4) and 18·7 μ-equiv $\pm$ 4·3 (n = 4) respectively. The K uptake during perfusion with DNP-containing solution was significantly ($P < 0{\cdot}01$) smaller

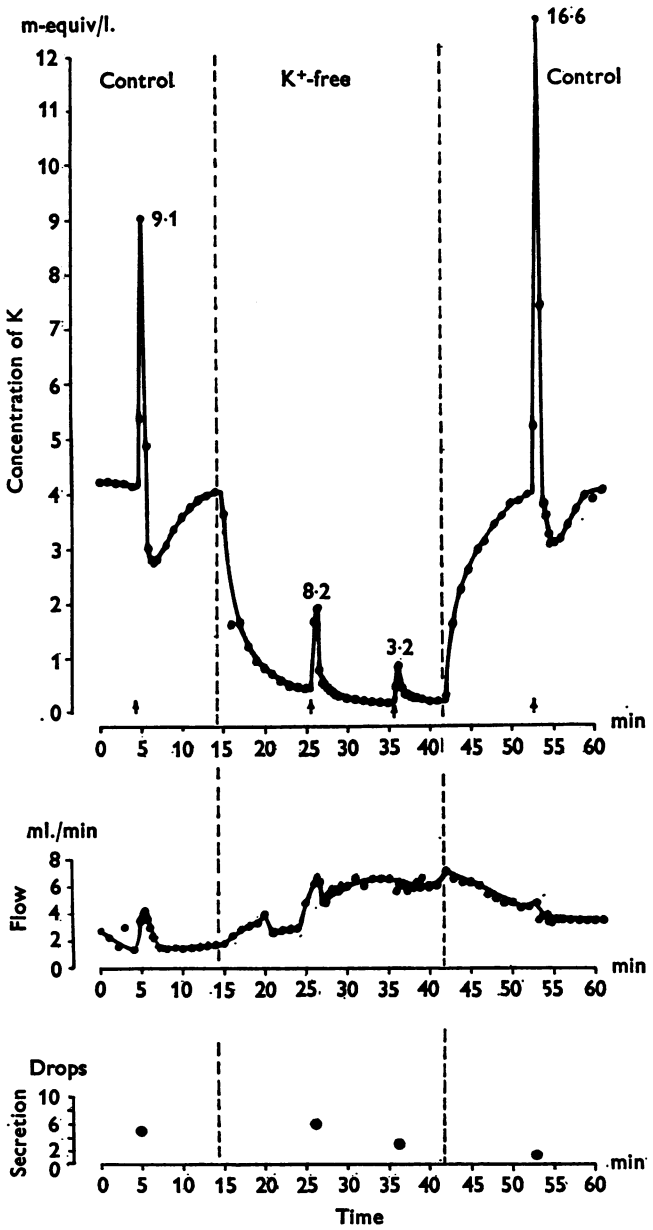

Fig. 2. The effect of perfusion with K-free Locke solution on K transport and secretion in a single experiment. ↑ denotes an injection of 10 μg ACh.

than the uptake in the control period. In three similar experiments where injections of 0·1 μg ACh were employed the following amounts of K were released in the above mentioned periods: 6·4 μ-equiv $\pm$ 1·8 (n = 3), 5·0 μ-equiv $\pm$ 1·0 (n = 3) and 5·0 μ-equiv $\pm$ 1·5 (n = 3) respectively. The

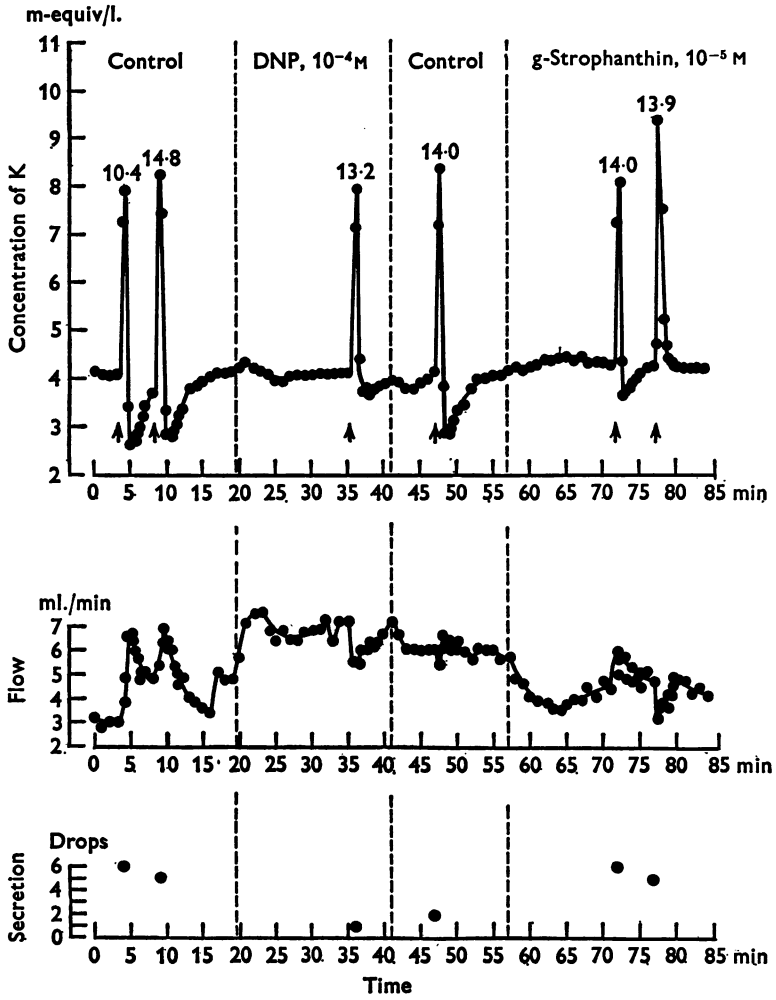

Fig. 3. The effect of DNP and g-strophanthin on K transport and secretion in a single experiment. ↑ denotes injection of 10 μg ACh.

uptake in the same experiments was 5·3 μ-equiv $\pm$ 1·9 (n = 3), 1·0 μ-equiv $\pm$ 0·6 (n = 3) and 6·0 μ-equiv $\pm$ 2·1 (n = 3) respectively. The apparent lack of effect of DNP on the K loss could possibly be explained by assuming that DNP inhibited both the one way flux of K out of the gland and the one way flux of K into the gland, and by assuming that these two

effects were balanced in such a way that the net loss of K was unaltered compared to control conditions. To investigate whether DNP had any inhibitory effect on the one way flux of K from the gland to the perfusion fluid, two experiments investigating the effect of DNP on K loss during perfusion with K-free Locke solution were carried out. Both glands were perfused with DNP containing solution for 15 min and thereafter the perfusion fluid was shifted to a K-free solution. Two minutes after introduction of the K-free fluid, ACh was injected. This resulted in a K loss that was uninhibited compared to the control period, whereas the secretion was reduced. On return to control Locke solution the secretion was restored and the K loss enhanced. The K loss in an additional K-free perfusion period was of the same size as in the preceding control period (Fig. 4).

The effect of perfusion with g-strophanthin (10^{-4}–10^{-5} M)

In four different glands injections of 10 μg ACh evoked a mean K loss of 9·5 μ-equiv $\pm$ 1·8 (n = 7) during the control periods and 10·7 μ-equiv $\pm$ 1·9 (n = 4) during the periods of perfusion with g-strophanthin (the first injection). In the same periods the K uptake was 14·6 μ-equiv $\pm$ 2·7 (n = 7) and 2·9 μ-equiv $\pm$ 0·6 (n = 4) respectively. The K uptake during the period of perfusion with g-strophanthin was significantly smaller ($P < 0{\cdot}01$) than the uptake during the control period. In two of the glands secretion was reduced during perfusion with g-strophanthin; in the remaining two, no effect on the secretion could be observed (Fig. 3). In all four experiments accumulation of K after ACh-induced K loss was severely depressed (Fig. 3). The experiment shown in Fig. 3 is the only one in which g-strophanthin was given after the gland had been influenced by DNP. In the other experiments the glands were perfused only with control Locke solution before the test period with g-strophanthin.

The effect of perfusion with a Na-free Li Locke solution

The first injection of ACh after shift of the perfusion fluid from control to Li Locke solution resulted in a normal loss of K, whereas the secretion was severely reduced as was the uptake of K after the loss. After repeated stimulations with ACh the K loss declined. Immediately after returning to the control Locke solution the gland started accumulating K. After this accumulation period had ceased the K loss after ACh was normal whereas the secretion was only partially restored (Fig. 5). The inhibitory effect of perfusing with a Na-free Li Locke solution on the K uptake was an immediate one as could be visualized by shifting the perfusion fluid from control to Li Locke solution in a period of K accumulation (Fig. 5). In three different glands the mean value of the K loss evoked by 10 μg ACh during control periods, periods of Li perfusion (the first injection) and

control periods following after Li perfusion was 13·2 μ-equiv $\pm$ 2·4 (n = 3), 16·1 μ-equiv $\pm$ 3·6 (n = 3) and 19·9 μ-equiv $\pm$ 2·6 (n = 4) respectively. The subsequent K uptake in the same glands was 24·0 μ-equiv $\pm$ 7·0 (n = 3), 3·1 μ-equiv $\pm$ 1·5 (n = 3) and 21·9 μ-equiv $\pm$ 2·8 (n = 4) respectively. The K uptake during perfusion with Li Locke solution was significantly smaller (P < 0·01) than during the control period.

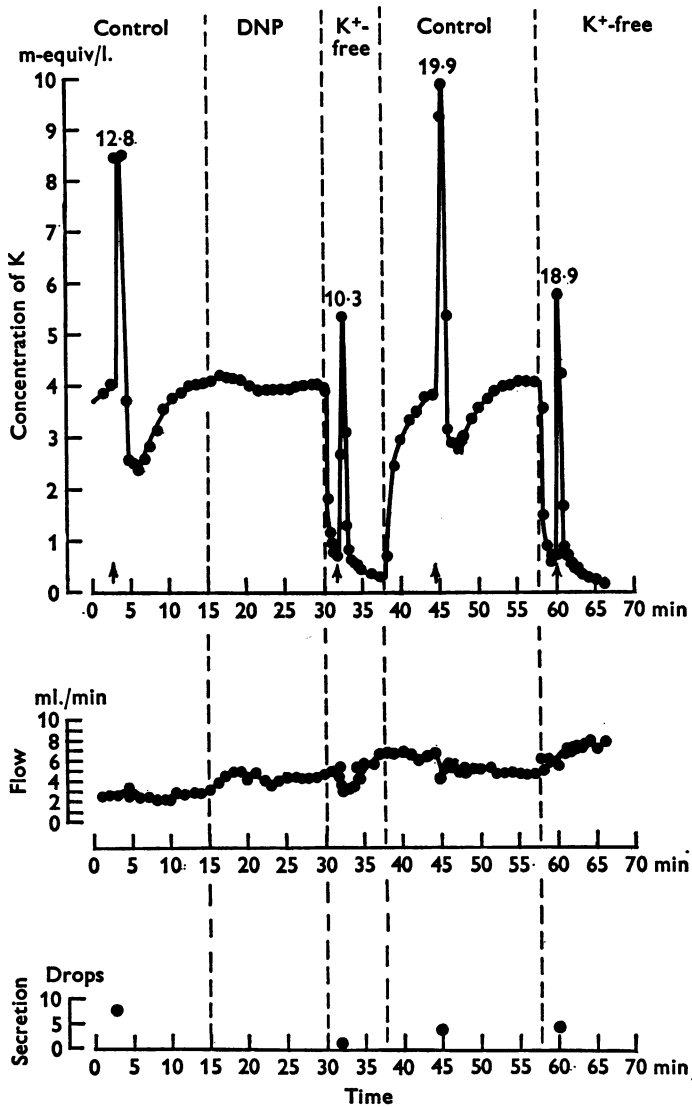

Fig. 4. The effect of DNP on K transport and secretion during perfusion with K-free Locke solution in a single experiment. ↑ denotes injection of 10 μg ACh.

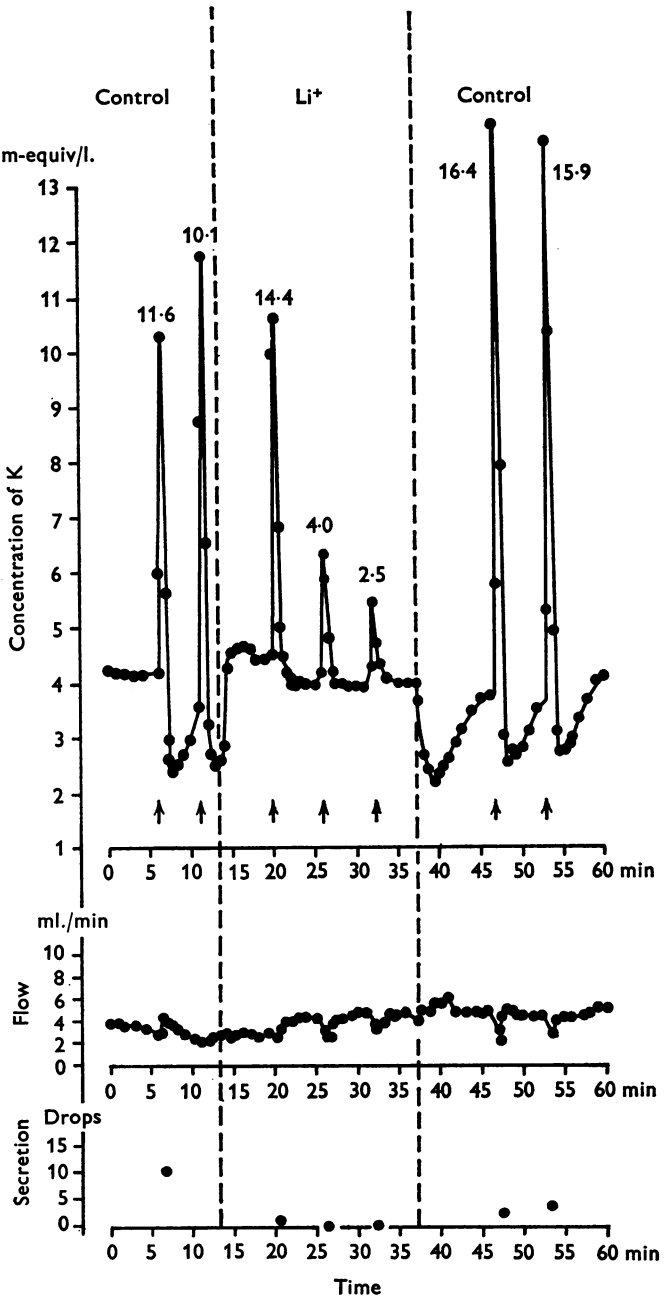

Fig. 5. The effect of perfusion with Li Locke solution on K transport and secretion in a single experiment. ↑ denotes injection of 10 μg ACh.

The effect of perfusion with a Na-free TEA Locke solution

The K loss after the first injection of ACh after shift of the perfusion fluid from control to TEA Locke solution was severely inhibited, while both secretion and K uptake were abolished (Fig. 6). In three experiments

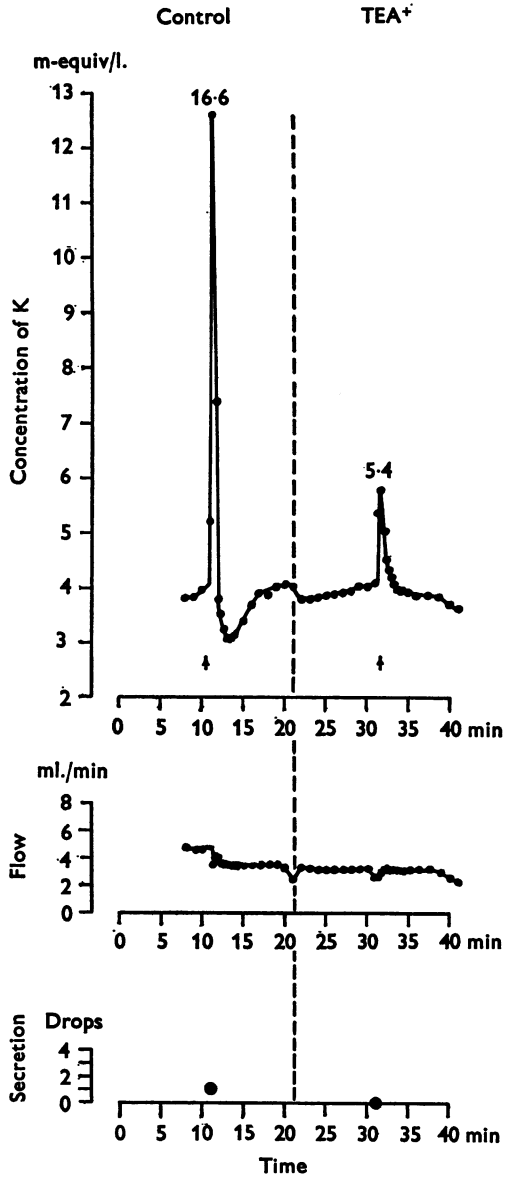

Fig. 6. The effect of perfusion with TEA Locke solution on K transport and secretion in a single experiment. ↑ denotes injection of 10 μg ACh.

injections of 10 μg ACh evoked a mean K loss of 12·2 μ-equiv $\pm$ 2·9 (n = 3) (control) and 3·0 μ-equiv $\pm$ 1·0 (n = 3) (TEA), respectively. The subsequent K uptake in these experiments was 13·8 μ-equiv $\pm$ 1·7 (n = 3) and 1·3 μ-equiv $\pm$ 0·8 (n = 3) respectively. The last value obtained during perfusion with TEA Locke solution is significantly lower ($P < 0{\cdot}01$) than the control value.

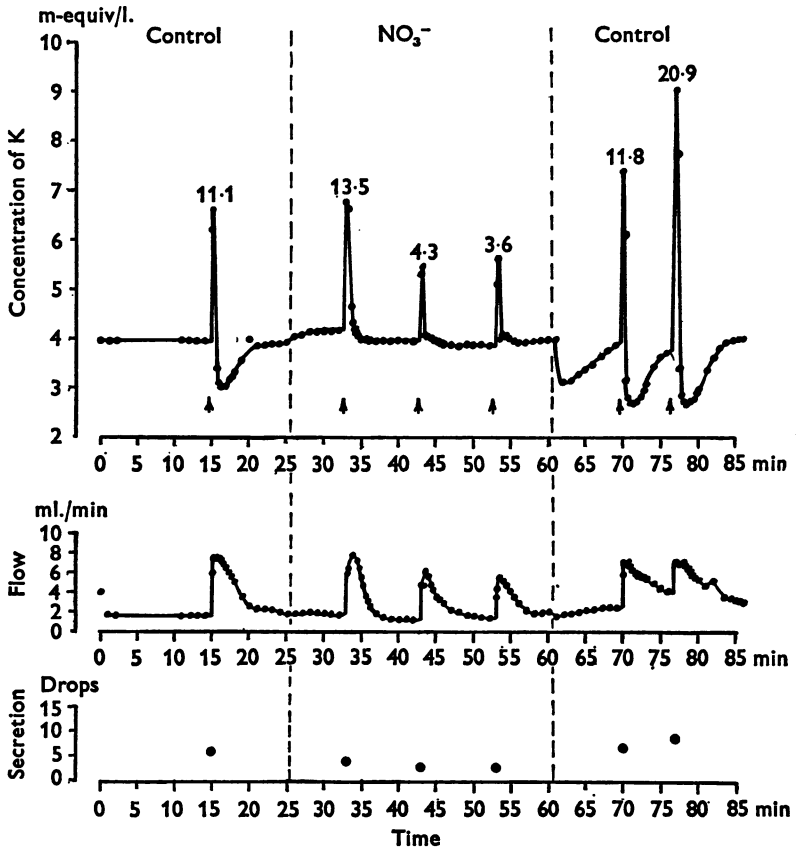

Fig. 7. The effect of perfusion with NO_3 Locke solution on K transport and secretion in a single experiment. ↑ denotes injection of 10 μg ACh.

In the experiment shown in Fig. 6 the secretion in the control period was very low. The inhibitory effect of TEA on secretion was, however, a real one. In five other experiments, in which the K transport was not always measured, the secretion in the control period was 6·8 drops of saliva $\pm$ 1·7 (n = 5) whereas the first injection of ACh in the TEA period only evoked 0·6 drops of saliva $\pm$ 0·5 (n = 5).

The effect of perfusion with a Cl-free NO_3 Locke solution

The K loss after the first injection of ACh after shift of the perfusion fluid to NO_3 Locke solution was normal. The following stimulations gave rise to somewhat smaller K losses. The secretion was inhibited and the uptake severely inhibited though not abolished. After reintroduction of control Locke solution the gland started accumulating K and thereafter both K loss, K uptake and secretion were normal (Fig. 7). The mean values of the K loss evoked after injections of 10 μg ACh during control periods, periods of NO_3 perfusion (the first injection) and final control periods were in four experiments: 7·7 μ-equiv $\pm$ 2·1 (n = 4), 6·2 μ-equiv $\pm$ 3·0 (n = 4) and 11·6 μ-equiv $\pm$ 3·4 (n = 4) respectively. The subsequent K uptake in these periods was 11·0 μ-equiv $\pm$ 2·3 (n = 4), 1·8 μ-equiv $\pm$ 0·8 (n = 4) and 15·7 μ-equiv $\pm$ 2·8 (n = 4) respectively. The K uptake during perfusion with NO_3 Locke solution was significantly lower ($P < 0{\cdot}01$) than during the control period.

DISCUSSION

In the present experiments it has been shown that both ACh and adrenaline injected into the artery of a resting gland cause a release of K to the perfusion fluid followed by a re-uptake. This is another example of a synergism between adrenaline and ACh in the salivary glands.

The cat submandibular gland is mainly composed of acinar cells, duct cells and myoepithelial cells. No information is available in the literature about the relationship between the volumes of these different cell types. From electrophysiological studies in this gland it is known, however, that by far the most frequent micro-electrode response obtained is a resting membrane potential of about -20 mV which increases to about -50 mV upon maximal stimulation with either ACh or adrenaline (type I response, Lundberg, 1955). In the dog submandibular, cat parotid and hamster submandibular glands it has been shown that this response originated from acinar cells (Imai, 1965*a*; Fritz & Botelho, 1969; Henriques & Sperling, 1966). In the cat sublingual gland only one micro-electrode response, which is similar to the type I response, can be obtained (Lundberg, 1957). This gland also loses K to both the saliva and the blood. The loss to the blood side per unit weight is even greater than in the submandibular gland (Lundberg, 1958). Because of these experimental facts, it is tempting to assume that a major part of the cat submandibular gland is made up of acinar cells with a low resting membrane potential and that a major part of the K loss originates from these cells.

The intracellular K concentration in the submandibular gland cells is not known, but assuming that the K content of the whole gland is evenly

distributed in the different cell types, the intracellular K concentration is about 115 m-equiv/l. (Burgen & Emmelin, 1961). The equilibrium potential for K across the basal cell membranes is thus: $E_K = RT/F \ln 4/115$ = (at a temperature of 20° C) $58 \log 4/115$ mV = -82 mV. This potential, by far, exceeds the acinar membrane potential of both the resting and secreting gland. This means that K is not in thermodynamic equilibrium across the basal cell membrane of the acinus. To account for the high intracellular K concentration we must assume the existence of a K accumulating mechanism and a low permeability of the cell membrane to K. As the resting membrane potential is relatively insensitive to changes in extracellular K concentration (Petersen & Poulsen, 1967) the permeability to K must be low, and as incubation of rat submandibular gland slices with g-strophanthin (5×10^{-4} M) decreases the intracellular K concentration (Schneyer & Schneyer, 1965) and g-strophanthin (10^{-7} M), iodoacetate (10^{-3} M) and DNP (10^{-4} M) decrease the influx of ^{42}K into dog submandibular gland slices (Siegel, 1969) there must exist an active mechanism transporting K into the acinar cells.

However, in the submandibular gland Lundberg (1955) described a type III micro-electrode response probably originating from the striated duct cells. This consisted of a large resting potential of -80 to -90 mV which was depolarized to about -20 mV during stimulation. Thus, during stimulation there is a very large electrochemical gradient for K transport out of the striated duct cells into the blood, much greater than the gradient for K transport out of the acinar cells. Even if the volume of the striated duct cells is much smaller than the volume of the acinar cells, they may contribute considerably to the loss of K to the blood side after injection of ACh. Finally, it should be remembered that there are many myoepithelial cells in the salivary glands, which might also contribute to the stimulation-induced K loss.

The loss of K from the gland after an injection of ACh could be explained either by a depression of the active uptake (influx) or by an enhanced efflux. The latter possibility must be correct since the K loss from a cat submandibular gland perfused with a K-free solution is accelerated by ACh (Fig. 2). Schneyer (1967) found that the efflux of ^{42}K from rat submandibular gland slices increased very much after addition of ACh to the bathing medium.

As outlined in the introduction three explanations of the increased K efflux after ACh could be given: (1) ACh activates a pump, transporting K out of the cells; (2) K moves as a consequence of a solvent drag effect exerted by the fluid transport through the cells during the period of salivary secretion; (3) ACh makes the basal cell membranes more permeable to K.

The findings in the present work that neither DNP nor g-strophanthin, in doses sufficiently high to inhibit secretion of saliva and K uptake severely, had any detectable effect on the K loss after ACh injection do not support the first hypothesis.

In the light of the present work the conclusion reached in a previous paper (Petersen & Poulsen, 1969) that DNP inhibits the K loss after ACh stimulation does not seem tenable. In that paper an inhibition of the K loss after several stimulations with ACh was demonstrated. The K loss after the first injection of ACh after introduction of the DNP solution was, as also shown in the present work, normal. As DNP strongly depresses the K uptake (Fig. 3), the inhibition of the K loss after several stimulations could be explained by depletion of the glandular K. It would of course be desirable to know how much the intracellular K concentration in the cells giving rise to the K loss was lowered by a single injection of ACh. As can be understood from what is stated above, this is impossible because we do not know how great a part of the gland is involved in the K loss. In the present work it is shown, however, that when a gland has been stimulated once in a medium where no K accumulation can occur the next injection of ACh evokes only a reduced loss of K (Figs. 2, 5 and 7).

According to the second hypothesis, suggested by Lundberg (1958) and supported by Petersen & Poulsen (1967, 1969), one would expect that the amount of K released from the gland after an injection of ACh should be related to the amount of saliva secreted, as demonstrated by Petersen & Poulsen (1969). In the present work it is shown, however, that some procedures inhibiting the amount of saliva produced after an injection of ACh are not accompanied by similar reductions in the amount of K released (Figs. 3, 4 and 5). The third hypothesis seems at the moment to account best for the release of K from salivary gland cells after they have been stimulated to secrete. It is also in accord with the well known effects of ACh on other post-synaptic membranes, e.g. the motor end-plate, some invertebrate inhibitory synapses and the sino-atrial node of the heart (Eccles, 1964). The effect of ACh on the gland cell membrane could be to increase the membrane permeability to both K and Na as is the case in the motor end-plate (Eccles, 1964), or to increase the permeability to both K and Cl as is probably the case in some invertebrate inhibitory synapses (Eccles, 1964). If the effect of ACh was to increase the permeability to K and Na this would result in an uptake of Na into the gland cells concomitant with the release of K since the equilibrium potential for Na across the basal cell membranes is about +74 mV (Burgen, 1967). The estimations of the intracellular Na, K and Cl concentrations made in the dog submandibular gland during resting conditions and after 5 min of continuous stimulation of the chorda lingual nerve (Imai, 1965*b*) are in

complete agreement with such a hypothesis. Imai showed that the gland cells took up as much Na as they had lost of K during the period of stimulation, whereas the intracellular Cl concentration did not change markedly from the resting condition to the stimulated condition. The only procedure by which it was possible to inhibit the loss of K after ACh without having previously depleted the gland of K was by perfusion with TEA Locke solution (Fig. 6). This finding can probably be explained by the failing ability of the gland cells to take up cations in exchange for K during this condition, whereas this works well when Na is replaced by Li.

Both perfusion with Li and NO_3 Locke solution severely inhibited K uptake, an inhibition which was readily reversible when returning to control Locke solution (Figs. 5 and 7). The nature of this inhibition is obscure. The fact that g-strophanthin (10^{-5}–10^{-4} M) severely inhibited K uptake, in some cases even without affecting the transport process forming the secretion (Fig. 3), seems to indicate that this K transport is mediated by a Na–K-activated ATP-ase (Skou, 1965). The existence of Na–K-activated ATP-ase in the dog parotid and rat submandibular gland has been demonstrated by Schwartz, Laseter & Kraintz (1963) and Schwartz & Moore (1968). The stimulus for the K pump would seem to be an increased intracellular Na concentration. During perfusion with Li Locke solution, however, no Na can enter the gland cells during ACh stimulation and as Li exerts no stimulatory effect at the Na site of the ATP-ase (Schwartz & Moore, 1968) no K uptake can occur. If this idea were correct one would expect Li to accumulate in the gland cells in the same manner as in the frog skin (Zerahn, 1955). Burgen (1958) showed that there was no significant accumulation of Li in either the submandibular or the parotid gland of the mongrel dog with plasma Li concentration up to 20 m-equiv/l. The situation may, however, be different when all Na in the ECF is replaced by Li. However, the immediate inhibition of K uptake exerted by the introduction of Na-free Li Locke solution, seen when shifting from control to Li Locke solution during a period of accumulation (Fig. 5), indicates that Li directly inhibits K uptake.

Previously it was suggested (Petersen & Poulsen, 1969) that the immediate inhibition of the K uptake exerted by SO_4 perfusion could be explained by the hypothesis that K was transported together with chloride, and as SO_4 probably only passes the cell membranes slowly, this would tend to inhibit the K uptake markedly. In the present work it is shown that perfusion with Cl-free NO_3 Locke solution also inhibits K uptake. This together with the above-mentioned arguments in favour of the hypothesis, that Na is taken up concomitantly with the release of K and is therefore also extruded when K is accumulated indicates that the previous hypothesis is less likely. Possibly either the lack of Cl ions extracellularly

or the presence of high extracellular concentrations of either NO_3 or SO_4 directly inhibit the K uptake.

The finding in the present work that the transport of K into and out of the gland cells at the base is uninfluenced by the very large transcellular water transports occurring during the periods of secretion suggests that the secretion process is anatomically separated from the K release and uptake site. That these two processes occur in two different cell types is one possibility. If, however, both K release and the secretion process occur in the same cell type this would imply that the transcellular water transport associated with the secretion does not move freely through the cytoplasm. Recently a volume transport hypothesis has been advanced to explain the isosmotic water transport across the gall bladder epithelium (Frederiksen & Leyssac, 1969). It is part of this hypothesis that the water transport proceeds through special intracellular structures so that the water transport is separated from the cytoplasmatic pool. Such a mechanism could possibly operate in the acinar cells of the salivary glands.

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