The Importance of Extracellular Calcium for Acetylcholine-Evoked Salivary Secretion

DOUGLAS and POISNER[1] showed that salivary secretion was severely impaired in the absence of extracellular Ca^{2+}. They were unable to distinguish between different possibilities for the mechanism of action of Ca^{2+} in the secretory process, mainly because, at that time, little was known about the descrete steps involved in the formation of this fluid.

In 1966 MARTINEZ, HOLZGREVE and FRICK[2] showed by using micropuncture technique that the primary acinar secretion was isotonic with plasma with plasma-like Na^+ and Cl^- concentrations. This has since been verified repeatedly by other groups[3]. PETERSEN[4] has worked out a model for the acinar secretory process: The immediate effect of acetylcholine (ACh) on the basal acinar cell membrane is suggested to be an increase in the permeability to Na^+ and K^+, with subsequent Na^+ influx and K^+ efflux. The Na^+ influx, possibly with the help of other factors (including Ca^{2+}), stimulates an ethacrynic acid sensitive Na^+ (NaCl) pump at the lateral membranes transporting solute into the intercellular spaces, water following passively thereafter.

The present work was undertaken to clarify whether Ca^{2+} influx from the interstitium to the acinar cytoplasm is of importance in triggering the salivary secretory response to acetylcholine.

Methods. Cats anaesthetized with chloralose (80 mg/kg i.p.) were used. The preparation of the submandibular gland for artificial perfusion has been described previously[5]. The composition of the perfusion fluids, which were equilibrated with oxygen before use, is given in Table I.

Table I. Composition of the perfusion fluids (m*M*)

	Control	Ca^{2+}-free	Low Na^+ Sucrose	Na^+-free Li^+
NaCl	140	140	—	—
KCl	4	4	—	—
Na_2HPO_4	2.4	2.4	2.4	—
NaH_2PO_4	0.6	0.6	0.6	—
K_2HPO_4	—	—	—	1.8
KH_2PO_4	—	—	—	0.45
$CaCl_2$	2.2	—	2.2	2.2
$MgCl_2$	1.0	1.0	—	—
$MgSO_4$	—	—	1.0	1.0
K_2SO_4	—	—	2.0	—
LiCl	—	—	—	142
Glucose	5.5	5.5	5.5	5.5
Sucrose	—	—	269	—

Perfusion was carried out at room temperatue (23–25 °C). The glands were stimulated to secrete by a 1 min close intra-arterial infusion of acetylcholine chloride (50 µg/min). The salivary secretory rate was measured in 1 min periods by collecting the saliva coming out of the cannulated submandibular duct into tuberculin syringes. All 6 experiments were carried out as shown in the Figure. At the appropriate times in the experiment, the effluent from the cannulated gland vein was collected and analyzed for Na^+ and Ca^{2+} by flame photometry (Eppendorf) and by atomic absorption spectrophotometry (Perkin-Elmer, model 290). In some of the experiments the saliva was also analyzed for Ca^{2+}.

Results. The Figure shows the course of experiment No. 3. It is clearly seen that in the first min of secretion there is no difference between the secretory rate measured in the control periods and the Ca^{2+}-free period, whereas secretion was abolished during perfusion with the low Na^+ solution. It is also seen that the secretory response to the 1 min lasting ACh infusion was much shorter in the Ca^{2+}-free period than in the control periods. In Table II are shown the values for secretory rates in the first min periods from all 6 experiments. The measured values for the Ca^{2+} concentration in the effluent at the time of stimulation in the Ca^{2+}-free periods, and the Na^+ concentration at the time of stimulation in the Na^+-free periods are also included. The secretory rate in the first min of secretion during the periods of perfusion with the Ca^{2+}-free fluid was 101% (ranging from 74-140%) of the secretory rate in the control periods preceding and succeeding the Ca^{2+}-free periods. The concentration of Ca^{2+} in the effluent from the gland during the first min of secretion in the Ca^{2+}-free periods was 43 µmol/l ± 9 (S.E.) ($n = 6$). The flow of perfusion fluid through the gland during the infusion of ACh was 5.9 ml/min ± 0.6 ($n = 6$) during perfusion with the Ca^{2+}-free fluid and 5.3 ml/min ± 0.2 ($n = 12$) during perfusion with the control fluid.

In experiments No. 1 and 3 the low Na^+ solution was a sucrose solution (see Table I). In these experiments stimulation did not evoke even a single drop of saliva (Table II). In the last 3 experiments the low Na^+ solution

[1] W. W. DOUGLAS and A. M. POISNER, J. Physiol., Lond. *165*, 528 (1963).
[2] J. R. MARTINEZ, H. HOLZGREVE and A. FRICK, Pflügers Arch. ges. Physiol. *290*, 124 (1966).
[3] J. A. YOUNG and C. J. MARTIN, Pflügers Arch. ges. Physiol. *327*, 285 (1971).
[4] O. H. PETERSEN, Phil. Trans. R. Soc. Lond. B., *262*, 307 (1971).
[5] O. H. PETERSEN, J. Physiol., Lond. *208*, 431 (1970).

Table II. Salivary secretory rate (µl/min) during the first min after start of ACh infusion

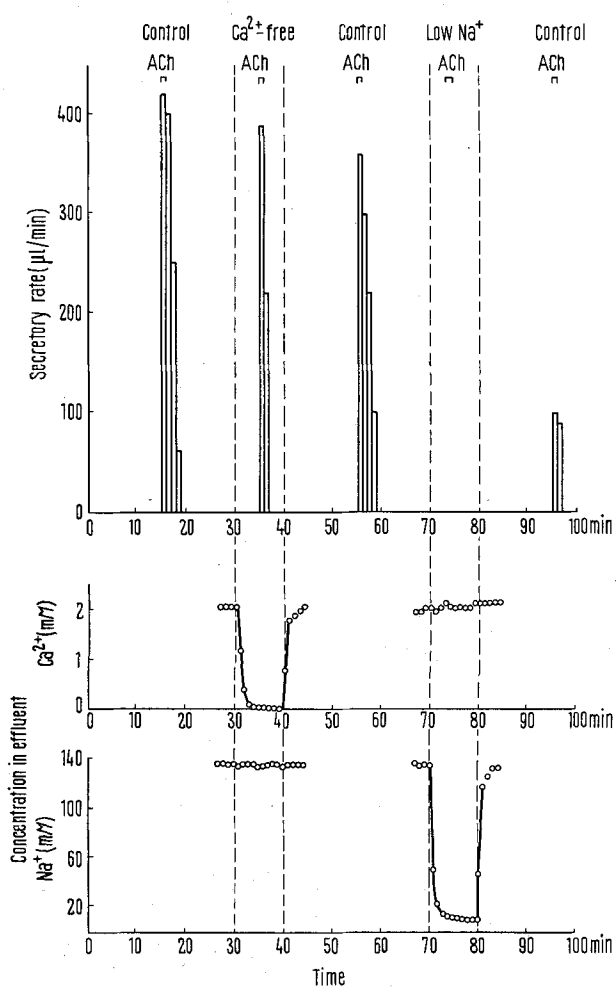
Exp. No.	Control	Ca^{2+}-free	$[Ca^{2+}]$ (µ*M*)[a]	Control	Low-Na^+[b]	$[Na^+]$ (m*M*)[c]	Control
1	320	400	30	250	0	9.5	70
2	330	280	40	280	—	—	—
3	420	390	60	360	0	12	100
4	320	210	43	250	10	10	110
5	410	400	9	330	80	6	220
6	250	230	75	250	20	15	160

[a] Ca^{2+} concentration in the effluent at the time of stimulation during the Ca-free period. [b] In experiment 1 and 3 this was a sucrose solution, in experiment 4–6 it was a lithium solution (see Table I). [c] Na^+ concentration in the effluent at the time of stimulation during the low-Na period.

was a Li^+ solution (see Table I). In these experiments the secretory rate in the first min after start of stimulation during the Na^+-free period was 15% (ranging from 6–29%) of the secretory rate in the control periods preceding and succeeding the Na^+-free period. The Na^+ concentration in the effluent from the gland during the first min of secretion in the Na^+-free period was 10.6 mmol/l $\pm$ 1.5 ($n = 5$). The flow of perfusion fluid through the gland during the infusion of ACh was 4.8 ml/min $\pm$ 0.6 ($n = 5$) during perfusion with the low Na^+ solutions and 4.5 ml/min $\pm$ 0.4 ($n = 10$) during perfusion with the control fluid.

The mean value of the concentration of Ca^{2+} in the saliva secreted during the first min period after start of stimulation was 1.2 mmol/l $\pm$ 0.1 ($n = 6$) in the control periods and 0.6 mmol/l $\pm$ 0.1 ($n = 3$) in the Ca^{2+}-free periods, whereas in the second min periods the mean values were 0.9 mmol/l $\pm$ 0.1 ($n = 6$) and 0.4 mmol/l (0.5 and 0.3) ($n = 2$), respectively.

Discussion. DOUGLAS and POISNER[1] discussed the different roles that Ca^{2+} might play in the secretory process of the salivary glands. One of the possibilities was that Ca^{2+} influx from the interstitium to the acinar cytoplasm could be the triggering mechanism for the secretory process, by analogy with what has been proposed for some endocrine glands[6–8]. If this were true we would expect the salivary secretory rate to depend on the extracellular Ca^{2+} concentration. However, 5 min after starting the perfusion with a Ca^{2+}-free fluid, the secretory rate in the first min following ACh stimulation was unimpaired (Figure). It might be argued that diffusion equilibrium between the interstitial fluid and the perfusion fluid can hardly be achieved in these 5 min. There is, however, evidence from the same gland indicating that diffusion equilibrium for other ions is rapidly attained. It has been shown[5] that replacement of a Locke solution by a Na^+-free Li^+ solution during a period of K^+ accumulation abolished K^+ uptake within 2 min. Also when the control solution was reintroduced K^+ accumulation started within 20 sec and reached the maximal rate within 3 min. From the Figure it is seen that 5 min after introduction of a low Na^+, Ca^{2+}-containing sucrose solution salivary secretion was abolished as has been shown previously[9]. Our result that salivary secretion is primarily uninhibited after introduction of Ca^{2+}-free fluid seems to indicate that ACh-induced Ca^{2+} influx, if it exists, is not the triggering mechanism for the initiation of the secretory process. There is no doubt, however, that extracellular Ca^{2+} is needed for the continuation of the secretory response to ACh. The results of the present work do not exclude the possibility that Ca^{2+} might be involved in stimulus-secretion coupling. By analogy with what happens in striated muscle[10] it could be proposed that salivary secretion is initiated as a consequence of an enhanced concentration of Ca^{2+} in the cytoplasm due to a release of Ca bound in the microsomes[11]. Such a release might be stimulated by the enhanced intracellular Na^+ concentration occurring as a consequence of the ACh-induced Na-influx from the interstitial fluid[4].



The course of a typical experiment (No. 3) showing the secretory rate and the concentration of Ca^{2+} and Na^+ in the effluent from the gland as a function of time after start of perfusion. The low Na^+ fluid was a sucrose solution (see Table I).

Zusammenfassung. 5 min nach Beginn der Perfusion der Submandibularisdrüse der Katze mit einer kalziumfreien Flüssigkeit ist die Acetylcholin-induzierte Sekretionsrate nicht reduziert, während dieselbe 5 min nach Einführen einer Na-freien Saccharoselösung blockiert ist. Dies zeigt, dass der Sekretionsprozess nicht durch Einströmen von Ca aus dem Extrazellulär- in den Intrazellulärraum ausgelöst wird.

J.R. MARTINEZ[12] and O.H. PETERSEN[13]

Institute of Medical Physiology C, University of Copenhagen (Denmark), 6 September 1971.

[6] P. BANKS, in *Calcium and Cellular Function* (Ed. A. W. CUTHBERT; Macmillan, London 1970), p. 148.
[7] W. W. DOUGLAS, T. KANNO and S. R. SAMPSON, J. Physiol., Lond. *191*, 107 (1967).
[8] E. K. MATTHEWS and P. M. DEAN, in *The Structure and Metabolism of the Pancreatic Islets* (Eds. S. FALKMER, B. HELLMAN and I. B. TÄLJEDAL; Pergamon Press, Oxford and New York 1970), p. 305.
[9] O. H. PETERSEN, Experientia *26*, 1103 (1970).
[10] H. DAVSON, *A Textbook of General Physiology* (Churchill, London 1970), p. 1403.
[11] Z. SELINGER, E. NAIM and M. LASSER, Biochim. biophys. Acta *203*, 326 (1970).
[12] Present address: Departamento de Fisiologia y Farmacologia, Facultad de Medicina, Universidad de El Salvador, San Salvador (El Salvador).
[13] The technical assistance of B. LYNDERUP and G. PEDERSEN is gratefully acknowledged.