The Importance of Extracellular Sodium and Potassium for Acetylcholine-Evoked Salivary Secretion

An immediate action of acetylcholine (ACh) on the basal acinar cell membrane is to increase the membrane potential (secretory potential)[1]. Evidence has been provided in favour of the hypothesis that the underlying mechanism of the secretory potential is a passive outward potassium current being partly short-circuited by an inward passive sodium current[2,3]. The present work was undertaken to test whether these passive currents are of importance for the salivary secretion process. The results showing that the salivary secretory rate was very sensitive to changes in external sodium concentration, and that the secretory rate was enhanced during perfusion with a potassium-free Locke solution and reduced when the external potassium concentration was augmented, indicate that the ACh-induced passive entry of sodium ions may have a special function in the activation of the secretory mechanism.

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[4]. The gland was stimulated to secrete by a continuous intra-arterial infusion of ACh (50 µg/min). The volume of saliva secreted during the first minute of stimulation was taken as the secretory rate. The gland was stimulated for 1 min with intervals of 10 min (Figure 1). The secretory rate in the test solution was expressed in per cent of the mean values of the secretory rates in the control periods immediately preceding and following the test period. The secretory rate was always measured 5 min after introduction of the test solution. The control Locke solution contained (m*M*): 140 NaCl, 4.0 KCl, 2.4 Na_2HPO_4, 0.6 NaH_2PO_4, 1.5 $CaCl_2$ 1.0 $MgCl_2$, 5.5 glucose. In the modified Locke solutions without potassium and with potassium concentrations of 10 and 20 meq/l, corresponding changes in sodium concentration were made to ensure constant osmolarity. In the modified Locke solutions with reduced sodium chloride concentration sucrose was added to maintain isosmolarity with the control Locke solution. In each experiment 3 different perfusion fluids were used (Figure 1).

Results. The effect of varying the external sodium chloride concentration. Reduction of the perfusate sodium concentration invariably reduced the secretory rate. This reduction was reversible when returning to control Locke solution (Figure 1). Figure 2 shows the results from the whole material (6 experiments on 6 cats).

The effect of varying the external potassium concentration. When potassium was omitted from the perfusion fluid the secretory rate was invariably enhanced, whereas the secretory rate was reduced when the perfusate potassium concentration was augmented. These effects were reversible. In Figure 3 is shown the total material from 7 experiments on 7 cats.

Discussion. The possibility that sucrose might have an inhibitory effect on the salivary secretory rate is unlikely since replacement of 50% of the perfusate sodium with lithium[5] had about the same effect as replacement of 50% of the perfusate sodium chloride with sucrose. When the perfusate sodium chloride concentration was reduced to 50% of the control value, the secretory rate was reduced to about 50% of the control level (Figure 2). When 50% of the perfusate chloride was replaced by either nitrate or sulphate, the salivary secretory rate was hardly affected[5,6]. This shows that it is sodium and not chloride that is especially important for the secretion process, in spite of the fact that the primary acinar secretion can be roughly described as an isotonic sodium chloride solution[7]. The reduction in secretory rate seen when reducing perfusate sodium concentration cannot therefore be due to lack of sodium for the secretory mechanism proper, because then reduction in perfusate chloride concentration should affect the secretory rate in the same manner as a reduction in perfusate sodium concentration. It could, however, be explained by some activating function of intracellular sodium on the secretory mechanism. The effect of varying the external potassium concentration on the secretory rate was very similar to the effect on the size of the secretory potentials[2,3]. However, the fact that reduction of perfusate sodium concentration leads to an increase in size of the secretory potential[2], while the secretion is reduced, indicates that

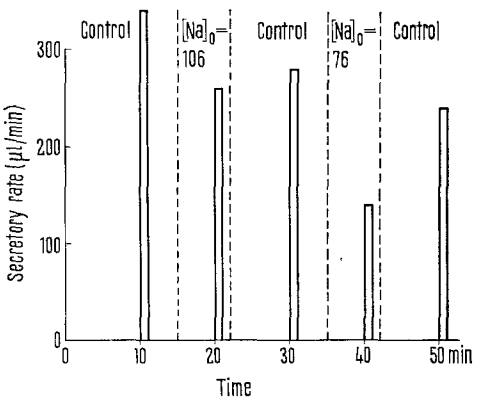

Fig. 1. The salivary secretory rate during perfusion with control Locke solution and Locke solutions with reduced sodium concentrations in experiment No. 68.

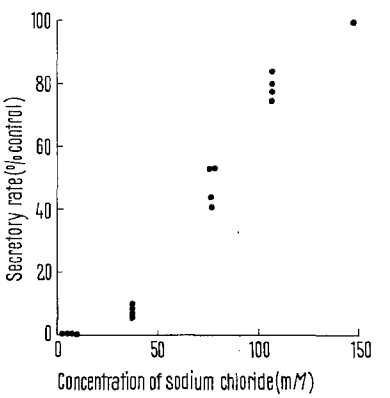

Fig. 2. The salivary secretory rate as a function of the sodium chloride concentration in the perfusion fluid.

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the rate of salivary secretion is not correlated to the size of the secretory potential but rather to the size of the inward passive sodium current. The size of the inward sodium current should depend on the size of the secretory potential, the electrochemical gradient favouring sodium entry being greater at higher membrane potentials. The effect of varying the perfusate potassium concentration on the secretory rate may thus be explained by the varying sizes of the secretory potentials influencing the sizes of the sodium currents.

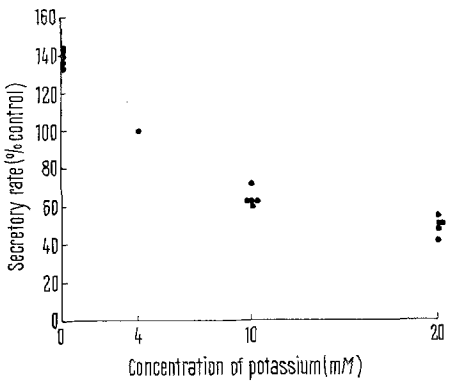

Fig. 3. The salivary secretory rate as a function of the potassium concentration in the perfusion fluid.

Zusammenfassung. Es wird gezeigt, dass die Speichelsekretionsrate der perfundierten Submandibulardrüse der Katze stark von der Natriumkonzentration im Perfusat abhängt. Die Sekretionsrate wird erhöht bei Perfusion mit kaliumfreier Lösung und reduziert während der Perfusion mit Lösungen erhöhter Kaliumkonzentrationen. Es ist möglich, dass ein durch Acetylcholin induzierter Natriumstrom in die Azinarzellen hinein den Sekretionsmechanismus aktiviert.

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Neuroanatomical Regions Relevant to Production and Analysis of Vocalization within the Avian *Torus semicircularis*

Vocalizations have been elicited by electrical stimulation of the *torus semicircularis* (ToS) of the Japanese quail, *Coturnix coturnix japonica*[1]. Comparison between elicited vocalizations and natural calls, using sound spectographic analysis, shows that elicited vocalizations closely resembled examples of 8 of the 15 natural calls emitted by adults of this species[1]. Vocalization has also been elicited from other avian species by stimulation of this neuroanatomical region[2–4]. Large lesions within the ToS result in long-lasting loss of 'alarm' calls in the redwing blackbird, *Agelaius phoeniceus*[5]. Both evoked potentials[6] and changes in single unit activity[7] in response to acoustic stimulation have been recorded in the large celled region of the ToS, the *nucleus mesencephalicus lateralis pars dorsalis* (Mld). Two experiments reported in this paper indicate that the region within the ToS containing low threshold sites for eliciting vocalization is spatially separate from the area responsibe to acoustic stimulation.

In the first experiment, tungsten microelectrode exploration of the right optic lobe was conducted in urethane anesthetized Japanese quail, *Coturnix coturnix japonica*, after removal of the overlying forebrain. As the microelectrode was advanced the bird was stimulated with repetitive 100 msec bursts of white noise. An attempt was made to ascertain the most effective frequency for stimulating each single unit encountered using 'pure' tones from 250–6000 Hz.

Tracks were run at 0.4 mm intervals in the sagital and anterior-posterior planes. Marker lesions were made at least 0.2 mm below and above the depths of the first and last 'acoustic' units or at a fixed depth if no acoustic units were encountered.

The results from 8 male quail are contained in Figure 1, A. All 30 tracks that penetrated the MLd located acoustic units within the MLd. None of the 17 tracks that penetrated the adjacent parvocellular region of the ToS, the *nucleus intercollicularis* (ICo), produced evidence of auditory activity within the ICo. 6 tracks that penetrated the ICo located single units responsive to auditory stimulation within the *formatio reticularis lateralis* (ventral to the ICo) as did one track that penetrated the medial portion of the MLd. This is in accord with neuroanatomical work which indicates that many fibers afferent and efferent to the MLd run through this region[8]. No acoustic units were recorded in 6 tracks that penetrated lateral to the ICo (not shown in Figure 1, A).

Tonotopic organization in the vertical plane was found within the MLd. The 'best frequency' of single units increased with distance from the dorsal border of the MLd.

Localization of acoustic units within the MLd supports previous neuroanatomical[8] and neurophysiological[6,7] evidence indicating that the MLd is homologous with the mammalian inferior colliculus. Thus the MLd probably plays a role in analysis of natural calls whereas the data reported above do not indicate such a role for the ICo.

In the second experiment, the right optic lobe of locally anesthetized birds was explored to determine thresholds for eliciting vocalization by electrical stimulation. A concentric electrode (inner core diameter 0.13 mm, outer electrode 29-guage stainless steel tubing) was lowered in 0.3 mm steps. At each step the animal was stimulated with biphasic pulses delivered at 60 pulses/sec, maximum current of negative phase being 200 μA. Once vocalization was elicited, the electrode was lowered in 0.2 mm steps and a threshold was determined at each depth by lowering current strength in a standardized sequence until no vocalization was elicited. A marker lesion was made at

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