pattern of the toxin reaction and binding is more complex than the formation of the simple ionic pair of quaternal agents like DTC or acetylcholine.

It is highly probable that other types of linkage like hydrophobic, hydrogen bonds or van der Waals forces take place at the moment of functional failure of the cholinergic transmembrane system caused by contact with Naja TX.

Zusammenfassung. Die Abhängigkeit der blockierenden Wirkung des Najatoxins von der Temperatur zeigt, dass es bei seiner Einwirkung auf das cholinergische System nicht zur Bildung einer Ionenbindung kommt. Die Aktivierungsenergie der blockierenden Wirkung des Najatoxins war 3.4 kcal. mol.

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Membrane Potential Measurement in Mouse Salivary Gland Cells

Membrane potentials in acinar cells have been measured in a number of different salivary glands from different species[1]. It has generally been found that the resting membrane potential (RP) is about –20 to –30 mV and that stimulation of the parasympathetic or sympathetic nerves to the gland causes a hyperpolarization. This stimulation-induced membrane potential change has been named the secretory potential[2]. The mechanism underlying the secretory potential has not been fully elucidated but different hypotheses have been proposed[2–4]. Since membrane potentials have not previously been measured in mouse salivary glands, this was attempted as a matter of routine, but since the results were surprising and illuminate some new aspects of salivary gland electrophysiology they are reported here.

Methods. The submaxillary or the parotid gland from young female mice was quickly removed after killing the animals and part of the gland was mounted in a perspex tissue bath through which a Krebs-Henseleit solution (37 °C, oxygenated) was pumped at a constant rate, as previously described for pancreatic preparations[5]. Membrane potentials were measured according to methods described by MATTHEWS[6] employing high resistance K-citrate filled micro-electrodes.

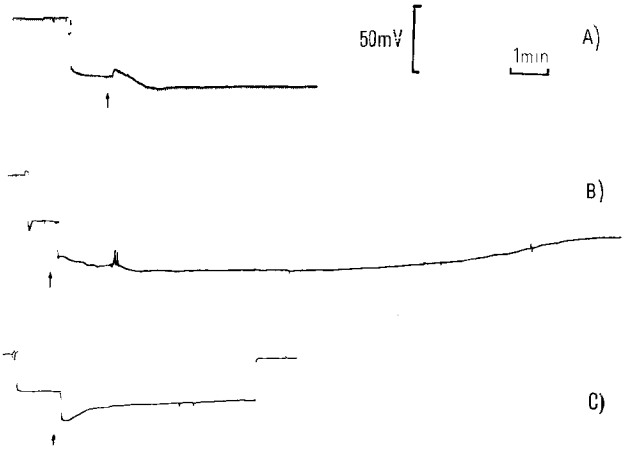

Fig. 1. Membrane potential measurement in mouse submaxillary gland. Downward deflections represent increased negativity of the micro-electrode. The sudden jump in potential seen in the left part of all 3 tracings corresponds in time to the insertion of the micro-electrode into the acinus. The arrows denote additions of ACh to the tissue bath to obtain for a short period a maximum concentration of 10^{-6} g/ml ($5.5 \times 10^{-6} M$). A and B were obtained during exposure of the gland to a normal Krebs-Henseleit solution while C was obtained during exposure to Strophanthin-G ($10^{-3} M$)-containing solution. B and C are from the same preparation.

Results. The resting membrane potentials in the mouse submaxillary gland ranged widely from –20 to –70 mV with a mean value of –47.0 mV ($n = 105$). In the parotid gland the mean value was –61.0 mV (52–70 mV) ($n = 20$). The micro-electrode was always inserted into cells situated just beneath the exposed surface of the gland. Figure 1 shows tracings of typical membrane potential recordings from the submaxillary gland. It is seen that acetylcholine (ACh) evoked biphasic secretory potentials. Sometimes a rapid shortlasting hyperpolarization was followed by a delayed longlasting hyperpolarization. At other times a rapid shortlasting depolarization was followed by a delayed longlasting hyperpolarization. When atropine sulphate ($1.4 \times 10^{-6} M$) was added to the superfusion fluid ACh failed to evoke any change in the membrane potential. In Figure 2 the dependence of the amplitude and polarity of the two phases of the secretory potential on the level of the resting membrane potential is shown. It is seen that the reversal potential for the rapid phase of the secretory potential was about –50 mV. During superfusion with a Strophanthin-G ($10^{-3} M$)-containing solution ACh always evoked monophasic hyperpolarizing secretory potentials of a relatively short duration (Figure 1). The mean value of the resting membrane potential (15–60 min after start of exposure to the drug) was –33.2 mV which was significantly ($P < 0.05$) lower than the resting membrane potential in the control periods of the same experiments.

Discussion. The very wide range of the magnitude of the resting membrane potentials in the mouse submaxillary gland is surprising but corresponds to a very recent finding in the cat submaxillary gland[7]. The fact that only cells very near to the surface were studied makes it highly unlikely that striated duct cells have been impaled. The secretory potentials obtained were similar to those seen in the acinar cells from the rat submaxillary gland[8]. It seems very likely, therefore, that the majority of the cells impaled were acinar, although high resting membrane potentials in salivary glands have previously been associated

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with granulated tubule cells[9] or striated duct cells[2]. It is interesting that the mean membrane potential in the mouse parotid gland, where there are no granulated tubules, was even higher than in the mouse submaxillary gland. The demonstration for the first time of a reversal potential for the initial phase of the secretory potential is consistent with the hypothesis previously put forward by PETERSEN[4] that ACh increases the permeability to K^+ and Na^+. Recently NISHIYAMA and KAGAYAMA[7] have reported that the magnitude of the initial depolarizing phase of the biphasic secretory potential in the cat submaxillary gland is dependent on the resting membrane potential in such a way that it increases with increasing resting potential. The slow and delayed hyperpolarization, reported in this paper, may be caused by a delayed increase in K^+ permeability, but it seems more likely that it is caused by an electrogenic pump, since it is abolished by Strophanthin-G. Such an electrogenic pump could be the Na^+-K^+ exchange pump of the acinar cells or the Na^+ pump directly responsible for the formation of the saliva[10]. More work is clearly needed to define the nature of the delayed hyperpolarization.

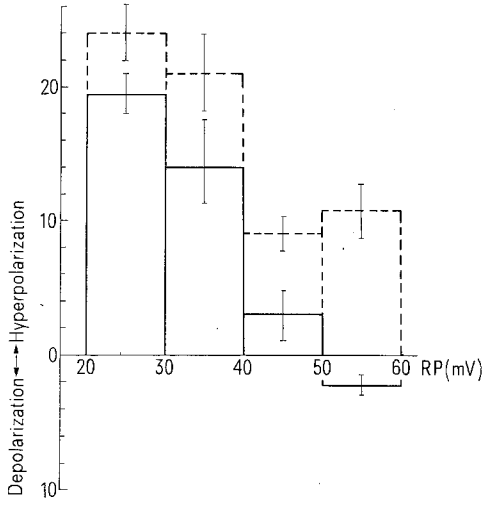

Fig. 2. Mouse submaxillary gland: Histogram showing the amplitude and polarity of the ACh-induced potential changes at different resting membrane potentials (RP). The fully drawn columns represent the first phase of the secretory potential while the columns outlined by the broken lines represent the second or delayed phase of the secretory potential.

Zusammenfassung. Acetylcholin erzeugt auf das Membranpotential von Azinuszellen der Glandula submandibularis der Maus eine diphasische Veränderung des Membranpotentials (rasche Hyperpolarisation und langsame, verzögerte Depolarisation oder rasche Depolarisation und langsame Hyperpolarisation). Hinweise sprechen dafür, dass Acetylcholin die Permeabilität für Na^+- und K^+-Ionen erhöht.

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Biphasic Secretory Potentials in Cat and Rabbit Submaxillary Glands

LUNDBERG[1] was the first to record transmembrane potentials in salivary glands. Stimulation of the secretory nerves to the submaxillary gland in the cat produced three types of potential changes (secretory potentials). The type I response to parasympathetic or sympathetic stimulation was a membrane hyperpolarization. LUNDBERG[2] suggested that the type I response was derived from the acinar cells and proposed that it was caused by an active transport of chloride ions through the contraluminal membrane from the interstitial fluid to the cytoplasm. Recently, YOSHIMURA and IMAI[3] and PETERSEN[4] have shown that the type I secretory potentials obtained from submaxillary glands in cat or dog are of normal size during perfusion with chloride-free solutions. PETERSEN[5–7] has suggested that the mechanism of action of acetylcholine on the contraluminal acinar cell membrane is to increase the permeability to both potassium and sodium ions. In the following some new and unexpected features of salivary gland electrophysiology will be reported.

Methods. Cats and rabbits anesthetized by chloralose (60–80 mg/kg) and urethane (1.5 g/kg), respectively, were employed. Membrane potentials were measured in the exposed submaxillary glands in vivo by using glass microelectrodes filled with $3M$ KCl, having resistances of $20–50\,M\Omega$. An indifferent electrode was placed under the neck skin opposite to the gland under study. The preganglionic lingual nerve fibres (parasympathetic) were stimulated at 0.2 or 20 c/sec through conventional stimulating electrodes.

Results. Single shock stimulation in both the cat and the rabbit resulted in either biphasic secretory potentials (type IB) (depolarization followed by hyperpolarization) or monophasic hyperpolarizing secretory potentials (type IM) (Figure 1).

In the cat submaxillary gland the mean latency of the type IB response was 176 msec $\pm$ 17 (80–300 msec) and that of the type IM was 291 msec $\pm$ 11 (100–500 msec). The resting membrane potentials for the cells displaying type IB responses were higher (-40.4 mV $\pm$ 0.4) than for those displaying type IM responses (-31.0 mV $\pm$ 0.8). Each histogram showing the frequency distribution of the resting potentials for these 2 groups exhibited

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