mg/kg; and atropine sulphate, 1–10 μg/kg, were given i.v. during the course of experiments.

Results. Degeneration secretion occurred in all the animals, but only in one of them bilaterally. The highest secretory rate, 1–2 drops/min, was obtained in the gland which had been previously sensitized by division of the tympanic nerve 10 days before the actual experiment. The other glands only produced 1 drop in 5–10 min. Secretion periods started 47, 47½, 57, 66 and 72 h, respectively, after the division of the nerves. The periods were not longer than 1–2 h and once the salivation had ceased in a gland it did not reappear, although the observation periods in 3 of the animals were as long as 36 h. Some 3–4 h before and after the appearance of the degeneration secretion, the responses to injected methacholine or acetylcholine were found to be prolonged. During this phase of 'provoked' degeneration secretion [4] salivation continued for 30 min or more after a single injection of methacholine, 5 μg/kg, whereas the normal gland only secretes for 2–3 min in response to such a dose. The degeneration secretion was often seen to terminate when supplementary doses of chloralose were given. It was, however, not caused by reflex activation because it occurred in the gland where the tympanic nerve had been divided and it was not affected by hexamethonium, 20 mg/kg i.v. Atropine, on the other hand, in a dose as low as 10 μg/kg i.v., totally abolished the salivation.

Discussion. Degeneration secretion thus appears in the parotid gland of the dog, but it seems to be much slower and of a shorter duration than in most other salivary glands, for example the canine submandibular gland [5]. The most probable explanation to this is that the former gland seems to have a more sparse cholinergic secretory innervation than the latter; the canine parotid contains fewer cholinesterase positive nerves (J. R. GARRETT, personal communication) and its postganglionic cholinergic nerves seem to release less acetylcholine on electrical stimulation than those of the submandibular gland [6]. The time of onset of the degeneration secretion in the present work correlates well with the finding in an earlier report that the secretion induced by injecting eserine into the parotid duct of dogs was increased above the normal on the second day after division of the postganglionic secretory nerves to the gland [2].

Zusammenfassung. In der Parotisdrüse von Hunden erscheint eine kurz anhaltende sogenannte Degenerationssekretion 2–4 Tage nach postganglionär-parasympatischer Denervierung.

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The Effect of Pancreozymin and Acetylcholine on the Membrane Potential of the Pancreatic Acinar Cells

In a number of secretory tissues, e.g. the adrenal medulla [1], the pancreatic islets [2] and the salivary glands [3], the mechanism of action of the physiological stimulant has been suggested to be an increase in membrane permeability leading to the observed change in membrane potential. It was recently reported that stimulation of the pancreatic nerve depolarized the pancreatic acinar cell membranes. It was also shown that the membrane potentials measured in the presence of a relatively high concentration of pancreozymin (CCK-Pz) were significantly lower than the membrane potentials measured in the absence of stimulation [4].

The aim of the present work was to compare more closely the membrane effects of acetylcholine (ACh) and CCK-Pz and examine the role of membrane depolarization in the secretory process.

Methods. The pancreas from young mice was quickly removed after killing the animals and part of the gland mounted in a perspex tissue bath (2 ml) through which a Krebs-Henseleit solution (103 m*M* NaCl, 25 m*M* $NaHCO_3$, 4.7 m*M* KCl, 2.56 m*M* $CaCl_2$, 1.13 m*M* $MgCl_2$, 1.15 m*M* NaH_2PO_4, 2.8 m*M* glucose, 4.9 m*M* Na-pyruvate, 2.7 m*M* Na-fumarate and 4.9 m*M* Na-glutamate) equilibrated with 5% CO_2 in O_2 was pumped at a constant rate of 1.2 ml/min. This solution was in some experiments modified by augmenting the K^+ concentration and reducing the Ca^{2+} concentration. In these cases corresponding changes in the Na^+ concentration ensured constant osmolarity. Membrane potentials were measured according to methods already described [5] by using high resistance (100–300 *MΩ*) K-citrate-filled microelectrodes.

Results. Figure 1 shows typical microelectrode recordings from the acinar cells of the pancreas. Both addition of ACh and CCK-Pz to the bath resulted in temporary depolarization of the acinar cell membrane. The time course and magnitude of the effect of CCK-Pz did not appear to be different from that of ACh. Figure 2 shows examples of dose-response curves for the depolarizing action of ACh and CCK-Pz. When the cells were depolarized by a maximal dose of ACh, stimulation with a maximal dose of CCK-Pz failed to produce any further depolarization. It is seen that in the presence of atropine, CCK-Pz, but not ACh still depolarized the cell membrane.

Adrenaline or noradrenaline in the same molar concentration routinely used for ACh ($5.5 \times 10^{-5} M$) were without effect on the membrane potential. Addition of cyclic AMP or dibutyrylcyclic AMP to achieve a concentration of 1 m*M* in the bathing fluid likewise had no effect on the membrane potential.

Exposing the pancreatic tissue to a high K^+ Krebs solution ($[K^+] = 50$ m*M*) depolarized the acinar cell membranes from the control value ($[K^+] = 4.7$ m*M*) of

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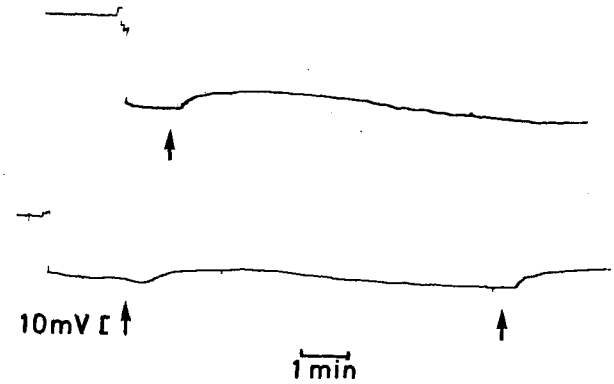

Fig. 1. Two tracings of membrane potential recordings from mouse pancreatic acinar cells. A deflection downwards represents an increased negativity of the microelectrode. The sudden jump in potential seen in the left part of both tracings corresponds in time to the insertion of the microelectrode into the acinus. The arrow in the upper tracing denotes addition of ACh to the bath to obtain a concentration of 10^{-5} g/ml ($5.5 \times 10^{-5} M$), whereas in the lower tracing the arrows denote additions of CCK-Pz to obtain concentrations in the bath of 150 mU/ml (Crick-Harper-Raper units).

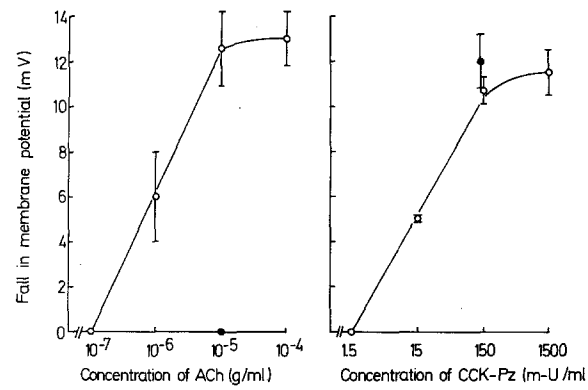

Fig. 2. Examples of dose-response curves for the depolarizing effect of ACh and CCK-Pz on the pancreatic acinar cells (open circles). The closed circles represent results of measurements carried out with the addition of atropine ($1.4 \times 10^{-6} M$) to the superfusion fluid. Each circle represents the mean value of 4–6 measurements from the same 2 glands. The length of the vertical bars is equal to 2 S.E.

-38.9 mV $\pm$ 1.8 (S.E., $n = 10$) to -18.3 mV $\pm$ 1.1 (9). This depolarization was not mediated by release of ACh from depolarized nerve endings, since in the presence of a concentration of atropine preventing ACh-induced depolarization ($1.4 \times 10^{-6} M$) exposure of the pancreas to the high K^+ medium still depolarized the acinar cells from the control value of -36.9 mV $\pm$ 1.2 (12) to -18.4 mV $\pm$ 0.9 (9).

When measured between 30 and 60 min after exposure of the pancreas to a Ca^{2+}-free solution the resting membrane potential was -27.9 mV $\pm$ 1.7 (11). Stimulation with ACh reduced the membrane potential to -14.2 mV $\pm$ 1.1 (11). In the control periods of the same experiments the resting membrane potential was -37.1 mV $\pm$ 1.0 (18) and ACh reduced the membrane potential to -22.0 mV $\pm$ 0.7 (18).

Discussion. The physiological stimulants acting on the pancreatic acinar cells are ACh and CCK-Pz. CCK-Pz mimicks the stimulatory effect of ACh on protein secretion from the pancreas, except in the presence of atropine where the effect of ACh but not that of pancreozymin is blocked[6]. The results presented in Figure 2, that both drugs cause depolarization of the acinar cell membrane, that the dose-response curves show a striking parallelism and that atropin blocks the ACh-induced, but not the CCK-Pz-induced membrane effect, strongly suggest that membrane depolarization, or perhaps rather the underlying permeability change, is the first necessary step in the number of events leading to extrusion of proteins into the acinar lumen.

The result that depolarization of the acinar cell membrane by a high external K^+ concentration was independent of the presence of atropine is of interest since the ability of a high external K^+ concentration to release amylase from the exocrine pancreas is abolished in the presence of atropine[7,8]. Therefore depolarization per se is not an adequate stimulus for secretion from the pancreatic acinar cells in contrast to the adrenal medulla where potassium depolarization directly releases adrenaline[1].

It is generally agreed that Ca^{2+} plays a crucial role in stimulus-secretion coupling[1,8,9]. The importance of Ca^{2+} for the secretion of proteins from the exocrine pancreas has also been established[8,10]. It is, perhaps, not so surprising that the ACh-induced depolarization of the acinar cells was of the same magnitude during exposure of the glands to a Ca^{2+}-free solution as during control conditions since in some other secretory tissues it has also been found that reduction of the extracellular Ca^{2+} concentration does not seriously affect the stimulation-induced change in membrane potential in spite of a severely reduced secretory ability[1,3]. The ACh- or CCK-Pz-induced change in membrane permeability, giving rise to the depolarization of the acinar cells, is probably a necessary but not a sufficient requirement for protein secretion; the presence of extracellular Ca^{2+} is also obligatory[10].

Zusammenfassung. Acetylcholin und Pancreozymin induzieren beide eine Depolarisation der Azinuszellmembran in der Bauchspeicheldrüse. Nur die Wirkung von Acetylcholin wird von Atropin blockiert. Das Ruhepotential kann auch durch eine erhöhte Kaliumkonzentration im Extrazellularraum reduziert werden, diese Wirkung wird nicht von Atropin beeinflusst. Die Acetylcholin-induzierte Depolarisation ist nicht von extrazellularer Kalziumkonzentration abhängig, sondern ist wahrscheinlich eine notwendige Bedingung für den Sekretionsprozess.

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