accomplished with a Northern Scientific Digital Memory Oscilloscope (NS-550) with 1024 bit memory and 25 μsec bit^{-1} resolution. The temperature was controlled to within ± 0.5 °C by placing the experimental chamber on an aluminum plate containing 4 Peltier elements. A small thermistor, positioned approximately 5 mm from the recording site, continuously monitored bath temperature. The bathing solution contained NaCl, 195 m*M*; KCl, 5.5 m*M*; $CaCl_2$, 13.5 m*M*; $MgCl_2$, 3.0 m*M* and *Tris*-maleate buffer, 10 m*M*; pH 7.5.

Results. The Figure (A, B) shows the average response obtained with an extracellular microelectrode from an excitatory neuromuscular synapse at 9 °C and stimulus frequencies of 1 and 10 sec^{-1} in normal crayfish Ringer. The earliest response is a small, < 40 μV, monophasic positive (upward) potential which is all-or-none in individual records. This response results from the extracellular potential field produced by current flow across the nerve terminal membrane. Its shape indicates the site of recording to be in close proximity to the ultimate terminal of an excitatory axon branch[15–18].

Following the nerve terminal potential is the large, negative postsynaptic potential resulting from inward current through the postsynaptic membrane in response to transmitter action. However, comparison of the averaged high gain voltage traces (Figure B) reveals that at a stimulus frequency of 10 sec^{-1} (lower trace) the rising phase of a small positive potential slightly preceeds the EJP while at 1 sec^{-1} (upper trace) no significant voltage fluctuation occurs. The amplitude of this small positive potential is proportional to the negative synaptic potential recorded at various stimulus frequencies and probably results from outward synaptic current of neighboring junctions[12].

The Figure shows that with an increase in stimulus frequency from 1 to 10 sec^{-1} there is an ~ 2.5-fold increase in the extracellular EJP and essentially no change in the amplitude or duration (Figure, B) of the potential recorded from the presynaptic nerve terminal.

Discussion. The present experiments, performed at the excitatory neuromuscular junction of the crayfish *Orconectes virilis*, have utilized reduced temperature and signal averaging to differentiate the small presynaptic nerve potential from the large postsynaptic response resulting from released transmitter. It is concluded that at this synapse, facilitation in response to repetitive stimulation is not accompanied by any change in amplitude or duration of the extracellularly recorded nerve terminal potential. If such changes occur they are below the resolution of present recording techniques.

Zusammenfassung. Nachweis, dass die Verbindung in neuromuskulären Synapsen des Flusskrebses *Orconectes virilis* ohne Änderungen der Amplitude oder Dauer des extrazellulär registrierten Nervenendpotentials abläuft. Durch Abkühlen des Präparates auf 9 °C gelang eine zeitliche Trennung der prä- und postsynaptischen elektrischen Aktivitäten.

C. L. ORTIZ[19]

Department of Zoology, University of California at Los Angeles, Los Angeles (California 90024, USA),
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Degeneration Secretion from the Parotid Gland of the Dog

During degeneration of efferent nerve fibres there is a stage when the nerves seem to be unable to retain in a normal way the transmitter still produced. At this stage the amount of transmitter leaking from the degenerating nerves exceeds the normal leakage from intact nerves and as a consequence the denervated effector organ is activated temporarily. This phenomenon was first described in the parotid of the cat and has later been observed in many other organs (EMMELIN[1]). Recently, secretion induced by different means was studied in parotid glands of dogs before and at various intervals after division of the postganglionic cholinergic nerves to the gland[2]. The secretory responses to eserine, locally administered, were found to be elevated above the preoperative values on the second postoperative day, indicating an increased leakage of acetylcholine at that time, but no degeneration secretion could be observed in the absence of eserine. Degeneration secretion has so far mainly been demonstrated in chloralose anaesthesia and it might be that the phenomenon did not appear in the experiments described above, due to the atropine-like effect of the barbiturate used as an anesthetic. Therefore, the present study was undertaken, where the parotid secretion was studied in dogs under chloralose anaesthesia after division of the postganglionic cholinergic nerves to the gland.

Methods. Five mongrel dogs were used. The auriculotemporal nerve and the secretory fibres on the internal maxillary artery[3] were divided bilaterally in 4 of the dogs, unilaterally in the 5th. In order to cause denervation supersensitivity, 1 parotid was preganglionically denervated by section of the tympanic nerve 7 days before the postganglionic denervation. Ether anaesthesia was used in these operations. When 42 to 50 h had elapsed after section of the postganglionic nerves, the animals were anaesthetized with chloralose (100 mg/kg i.v.) after induction with ether and a tracheal cannula was introduced. Supplementary doses of the anaesthetic were given when necessary. Both parotid ducts were cannulated with glass cannulae. The drops of saliva, falling from these cannulae, were recorded with manually operated electromagnetic pens on a smoked drum. Methacholine, 1 or 5 μg/kg; acetylcholine, 5 μg/kg; hexamethonium bromide, 20

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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.

J. HOLMBERG

Institute of Physiology, University of Lund, S-223 62 Lund (Sweden), 28 February 1972.

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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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