Contraction of Myoepithelial Cells in Secreting Submandibular Glands of Dogs

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Summary. In slowly secreting submandibular glands of dogs, a sympathetic effect on myoepithelial cells was demonstrated as an acceleration of the flow, followed by retardation, on short-lasting sympathetic stimulation, particularly after β-adrenoceptor-blocking drugs.

In order to study contraction of the salivary myoepithelium, its effect on a continuous salivation can be recorded. Thus, in the parotid gland of the sheep sympathetic stimulation was found to accelerate the spontaneous secretion present in this gland, and afterwards there was a period of reduced flow[2]. Application of such a method to other salivary glands is rendered difficult by the fact that sympathetic stimulation may activate not only motor (and vasoconstrictor) fibres, as in the sheep parotid, but also secretory fibres, which will complicate the picture[3]; in addition, most salivary glands do not secrete spontaneously. Nevertheless, it has proved possible to use such a method in the submandibular gland of the dog.

Material and methods. In 16 dogs (weights 4–11 kg), under chloralose-urethane anaesthesia, the submandibular duct was cannulated in the neck and connected to a bottle where saliva secreted displaced water. Drops of water of a size of 20 µl falling from an outlet of the bottle were recorded by an ordinate writer on a smoked drum. Continuous salivation was evoked from an electrode on the chorda-lingual nerve; shocks of a strength of 8–12 V and a frequency of 0.2–0.5 Hz produced a flow at a steady rate of 2–6 drops/min. To activate sympathetic fibres, the vagosympathetic trunk was excited using 15–25 V and frequencies of 5–20 Hz.

Results and discussion. Sympathetic stimulation for 1 min was found to change the parasympathetically induced secretion in a complicated and variable manner. Complications arose from two sources.

First, sympathetically evoked vasoconstriction interfered with the flow[4]. This could be largely avoided by reducing the period of sympathetic stimulation to a few seconds. The Figure shows an experiment where sympathetic stimulation for 2 sec caused a quickening of the continuous, parasympathetically induced flow, followed, when stimulation ceased, by a compensatory retardation. Stimulation for 1 sec had a similar but smaller effect. It seems reasonable to attribute the acceleration to myoepithelial contraction, caused by stimulation of sympathetic motor nerves, and the retardation to subsequent relaxation of the myoepithelium. However, clearcut results of this type were obtained only in 6 of the 16 dogs. In all the dogs, sympathetic stimulation for 1–10 sec accelerated the salivary flow, but in 5 dogs the compensatory retardation was small and in 5 it was lacking. This was found to be due to a second complication, derived from the fact that sympathetic secretory fibres were activated. Sympathetic stimulation alone causes a slow secretion after a long latency, but when superimposed on parasympathetic stimulation its secretory effect is greatly augmented, appears with a very short latency and continues for some time after stimulation[3]. Interference from such a sympathetic secretion is therefore possible even when the nerve is stimulated for a very brief period, as in the present experiments; the quick secretory response will accelerate the parasympathetic secretion, and the persisting sympathetic aftersecretion may conceal an effect of the myoepithelial relaxation on the flow. Fortunately, in this particular gland sympathetic secretion can be abolished by β-adrenoceptor-blocking drugs[5], whereas the motor effect remains, being α-receptor mediated[6]. The second half of the Figure shows an experiment where sympathetic stimulation for 2 sec caused acceleration but no retardation of the parasympathetically induced flow. When,

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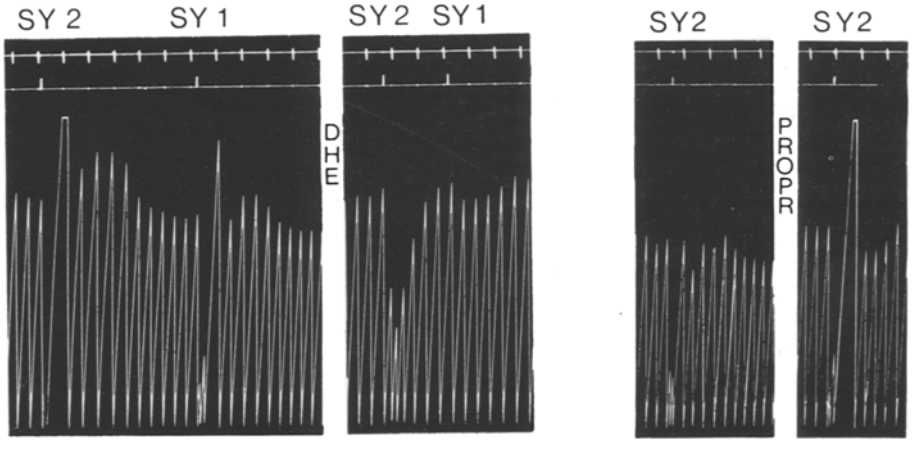

The 2 left sections are from one experiment, the 2 right ones from another. Records from above: minute marks; signal; salivary secretion recorded with an ordinate writer (each ordinate gives the time interval between 2 drops). Throughout both experiments the chorda-lingual nerve was stimulated, at 0.5 Hz in the left, and at 0.3 Hz in the right experiment. Left: SY2 and SY1, sympathetic stimulation at 10 Hz for 2 and 1 sec, respectively. DHE, dihydroergotamine, 0.5 mg/kg i.v. Right: SY2, sympathetic stimulation at 5 Hz for 2 sec. PROPR, Propranolol, 1 mg/kg i.v.

however, sympathetic secretion had been prevented by injection of the β-blocking propranolol, a temporary retardation, attributable to myoepithelial relaxation, appeared when stimulation ceased; before propranolol, this phase obviously did not come to light because the secretory rate was augmented for a short period after stimulation. In the first experiment of the Figure, it can be seen that, after the α-receptor-blocking drug dihydroergotamine, some acceleration of the flow persisted after sympathetic stimulation for 2 sec, but scarcely for 1 sec. Such a persisting acceleration, abolishable by propranolol, can be ascribed to augmented secretion, suggesting that part of the acceleration at stimulation for 2 sec before dihydroergotamine was in fact due to secretion, adding its accelerating effect to that of the myoepithelial contraction and possibly reducing the retarding effect of subsequent myoepithelial relaxation even in this experiment.

The investigation shows that sympathetically evoked myoepithelial contraction can be studied in the submandibular gland of the dog if allowed to act on a slow, parasympathetically induced secretion, provided the period of sympathetic stimulation is short and a β-adrenoceptor-blocking drug has been given.

Physiological Studies on the Effects of Nutritional Imbalance on the Central Nervous System. II. Effects of Thiamine Deficiency on Oxidative Enzymes in the Brain of Chicken, *Gallus domesticus*

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Summary. The activity levels of succinate dehydrogenase, glutamate dehydrogenase and pyruvate dehydrogenase in the fore, mid and hind brain regions of the thiamine deficient chicken, *Gallus domesticus* were determined. The activity levels of succinate dehydrogenase and glutamate dehydrogenase in all the 3 regions of brain showed augmentation on inducing thiamine deficiency. In contrast the activity levels of pyruvate dehydrogenase decreased in the brain of thiamine deficient animals. It is suggested that these changes in the oxidative enzymes indicate disturbance caused in the operation of the tricarboxylic acid cycle in thiamine deficiency.

Vitamin deficiency is known to cause metabolic defects in man and other vertebrates[1]. Many clinical signs and biochemical changes induced by vitamin deficiencies in chick have been described[2,3]. It has been reported that absence of thiamine causes cellular anorexia[2] and affects the distribution of thiamine-dependent enzymes that operate in the carbohydrate metabolism in birds and mammals[2,4,5]. Thiamine deficiency is also known to produce a rise in pyruvate content of the tissues in vertebrates, and this is believed to be the key to many deficiency-induced lesions[6]. However, information about the regional distribution of dehydrogenases in the CNS, and changes occurring in their activity during thiamine deficiency, is lacking. Hence it was felt desirable to study the dehydrogenases which play a significant role in the carbohydrate metabolism on inducing thaimine deficiency. The paper presents information about the pyruvate, succinate and glutamate dehydrogenases in the fore, mid and hind brain regions of the thiamine deficient chick, *Gallus domesticus*.

Materials and methods. 3-day-old white Leg-horn chicken, *Gallus domesticus*, ranging in weight from 15–20 g, were purchased from a local dealer and reared in the laboratory in electrically heated cages at 37 $\pm$ 1 °C. The controls were fed on standard chicken feed purchased from 'Mysore Feeds', Bangalore, India. The experimental birds were fed on polished rice for more than 3 weeks to induce thiamine deficiency[3,7]; water was given ad libitum.

The normal and thiamine deficient chicken were sacrificed by decapitation after 30 days. The brain was dissected with sterilized instruments and kept in normal saline on ice at 0 °C, and adhering blood vessels were removed. The fore, mid and hind brain regions were separated with sterilized bent forceps and scalpel; they were weighed quickly in Ringer[8] in an electric balance and were used for analysis.

Assay of dehydrogenase. A 10% (wt/vol) homogenate of brain cortical matter was prepared in 0.25 *M* cold sucrose solution using glass homogenizer and centrifuged at 3000 rpm for 15 min. The supernatant was used for the assay of enzyme activities. The levels of dehydrogenase activities were estimated by modified triphenyl tetrazolium chloride reduction method[9]. The incubation mixture contained 0.5 ml each of 0.09 *M* sodium succinate, and 0.27 *M* sodium glutamate and 0.1 *M* sodium pyruvate as substrates for the estimation of succinate, glutamate and pyruvate dehydrogenases respectively (these concentrations of substrates were found to give optimal activity[10]), 0.5 ml of sodium phosphate buffer of 0.1 *M*, 0.5 ml triphenyl tetrazolium chloride (0.2% solution at neutral pH) and 0.1 ml of the 10% homogenate. The incubation was carried out at 37 °C for 45 min after which 6 ml of glacial acetic acid (BDH) and 6 ml of toluene were added and kept in the refrigerator overnight. The toluene layer was extracted and the optical density was read at 505 nm in Du[2] Beckman's spectrophotometer.

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