Ca\(^{2+}\) dependence of the release of noradrenaline during nerve degeneration in the parotid gland

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Summary. Amylase secretion "in vitro" was used as an indication of the degeneration activity of sympathetically denervated parotid glands. Seventeen and a half hours after sympathetomy, slices of parotid gland released amylase into the incubation medium at a rate higher than that observed for non-denervated glands. The time course of amylase release from denervated glands followed a bell-shaped pattern similar to that observed in other denervated structures. Lowering extracellular Ca\(^{2+}\) to 0.25 mmol/l diminished significantly the release of amylase. Low Ca\(^{2+}\) however, did not decrease the amylase release in response to added noradrenaline.

These results indicate that Ca\(^{2+}\) is required for the release of noradrenaline from degenerating nerve endings.

Key words: Sympathectomy — Degeneration activity — Ca\(^{2+}\) requirement — Amylase secretion — Noradrenaline release

Introduction

The mechanism by which neurotransmitter is released from degenerating terminals of sympathetic nerves is still not well understood. The fact that adrenergic neuron blocking agents inhibit degeneration activity (Pluchino et al. 1970; Lundberg 1970; Arbilla et al. 1980) suggests that the release of noradrenaline is not solely due to leakage from damaged terminals. Moreover, "in vitro" studies have shown that the metabolic pattern of the released neurotransmitter resembles that seen during nerve stimulation (Stefano et al. 1974). Because of these similarities between nerve stimulation and nerve degeneration-induced release, it was of interest to analyze the Ca\(^{2+}\)-dependence of the latter process. For this purpose we studied amylase release from denervated parotid gland in vitro, since Asking et al. (1982) have shown this preparation to be an adequate model to analyze degeneration activity "in vitro".

The data presented here indicate that the release of noradrenaline from degenerating nerve terminals is a Ca\(^{2+}\)-dependent process.

Methods

Female rats of the Wistar strain (150 — 200 g) were used. The right superior cervical ganglion was removed under ether anesthesia. The contralateral innervated gland was used as control.

The animals were fasted for 18 h before the experiment but water was administered ad libitum.

Seventeen and a half hours after the surgical procedure, rats were anesthesitized with sodium pentobarbital (66 mg/kg) and both parotid glands were carefully dissected in the incubation medium equilibrated with O\(_2\). Each gland was divided in two portions and incubated in 10 ml of a continuously gassed medium which was changed every 30 min. The total incubation time was 3 h.

When the effect of low Ca\(^{2+}\) was assayed, the concentration of Ca\(^{2+}\) was 0.25 mmol/l during the first 2 h of incubation and 2.5 mol/l thereafter.

When the effect of exogenous noradrenaline was assayed, fresh noradrenaline was added at the beginning of each 30 min incubation period.

The composition of the incubation medium was the following (millimolar concentrations): NaCl, 140; KCl, 5; CaCl\(_2\), 2.5; Tris HCl, pH 7.8, 10; Na\(_2\)EDTA, 0.04; ascorbic acid, 0.11 and β-hydroxybutyrate, 5. The final pH of the solution at 37°C was 7.5.

Amylase release was determined as described by Bernfeld (1955) with some minor modifications (see Peusner et al. 1979). One unit of amylase was defined as the enzyme activity liberating reducing groups corresponding to 1 gmol/l of maltose per min at 37°C.

The amylase released into the successive washings was added to that remaining in the tissue at the end of the experiment; in this way the initial content of amylase was known. The release of amylase for any given 30 min incubation period was expressed as percentage of the calculated amylase content in the tissue at the end of each incubation period. The innervated glands had an initial content of amylase of 79 ± 8 units/mg tissue and the denervated had 80 ± 11 units/mg tissue (n = 11 each).

Results

In agreement with the report of Asking et a. (1982), the slices of the parotid gland that had been denervated 17.5 h earlier released more amylase into the incubation medium than their corresponding controls. During the first incubation period, the release of amylase from the denervated gland was almost three times higher than that from the innervated one. There-
after this difference was further increased. While the innervated gland had a steady rate of secretion, in the denervated side the rate rose sharply and remained at a relatively high level for the next 90 min. During the last hour of the experiment, there was a decline in the release of amylase although it still remained higher than that observed in the innervated gland (Fig. 1 A).

The release of amylase was affected by lowering the Ca$^{2+}$ concentration in the incubation medium. Figure 1 B shows that, except in the first period of observation, the release of amylase from slices exposed to low Ca$^{2+}$ was significantly lower than that observed in the presence of normal Ca$^{2+}$. Also, the sharp increase in secretion at the 18th hour after denervation in denervated controls was not observed in the glands incubated with low Ca$^{2+}$. Reintroduction of Ca$^{2+}$ to the medium did not increase the rate of secretion of amylase from denervated glands. Low Ca$^{2+}$ also reduced the basal release from innervated glands (Fig. 1). To test whether the decremental effect of low Ca$^{2+}$ on degeneration secretion was due to a decrease in the responsiveness of the effector cells, innervated glands were exposed to a submaximally effective concentration of noradrenaline. This concentration was chosen because it elicited a secretion of amylase comparable to that observed in denervated glands. One group was incubated with 2.5 mmol/l Ca$^{2+}$ and the other with 0.25 mmol/l Ca$^{2+}$ (Fig. 2). Noradrenaline elicited a similar rate of secretion in both groups indicating that the low concentration of Ca$^{2+}$ does not alter the amylase secretion elicited by noradrenaline.

**Discussion**

Slices of parotid gland which had been adrenergically denervated 17.5 h earlier showed a sustained release of amylase into the incubation medium. This output of amylase was significantly greater than the spontaneous leakage of the enzyme from control glands. Asking et al. (1982) showed that the enzyme secretion observed after denervation is related to the release of noradrenaline from degenerating nerve terminals.

The bell-shaped pattern of amylase release from denervated glands seems to indicate that the process of nerve degeneration was taking place during the period of incubation. A bell-shaped time course of the response is characteristic of the process of degeneration (Langer 1966; Geffen and Hughes 1972; Asking et al. 1982).

On the other hand, the response to a fixed concentration of exogenous noradrenaline showed an almost constant rate of release throughout a similar period of observation. This indicates that the bell-shaped patterns observed in denervated glands is related to changes in the release of the neurotransmitter and not to changes in the sensitivity of the effector cells.

Since the first description of the phenomenon of degeneration activity, several reports have been concerned with the nature of the process by which the neurotransmitter is released from the degenerating nerve endings. Evidence has accumulated which excludes a simple leakage of the transmitter as an explanation of the release observed. For instance, adrenergic nerve blocking agents can interrupt degeneration activity of smooth muscle in the cat (Pluchino et al. 1970) or in the rat (Lundberg 1970) or in salivary glands (Arbilla et al. 1980; Sanz et al. 1982). These findings would indicate similarities between the mechanism of noradrenaline release by nerve impulses and that by the degeneration process.

In this respect Geffen and Hughes (1972) showed that degeneration contraction of the isolated "expansor seismaturium" muscle of the chicken was blocked by withdrawal of Ca$^{2+}$ from the incubation medium. However since calcium is necessary for both, neurotransmitter release and muscle contraction, their data did not fully prove a presynaptic requirement of Ca$^{2+}$ for degeneration activity.

Amylase release mediated via β-receptors, on the other hand, is a mechanism independent of external Ca$^{2+}$ (Dormer and Ashcroft 1974). Hence, the degeneration activity of the parotid gland offers a useful model for the evaluation of the role of Ca$^{2+}$ in the release of neurotransmitter during nerve degeneration.