A SPECIFIC FORM OF ACETYLCHOLINESTERASE IS SECRETED BY RAT SYMPATHETIC GANGLIA

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1. Introduction

The function of acetylcholinesterase (AChE) in the superior cervical ganglion (SCG) remains unclear.

(1) In contrast to the neuromuscular junction, AChE of the SCG does not seem to be directly involved in the process of transmission [1], since anti-AChE drugs do not prolong the time course of the excitatory postsynaptic potential and the ganglion remains able to sustain repetitive activity [2,3].

(2) A large part of the AChE activity revealed in the rat SCG by ultrastructural cytochemistry is concentrated in the cisternae of the rough endoplasmic reticulum of the neurones and little activity is found at the synaptic and plasmic membranes [4–7].

We have recently reported that the rat SCG contains 4 molecular forms of AChE – two major forms sedimenting at 4 S and 10 S, and two minor forms at 6.5 S and 16 S – which differ in their solubility properties [5]. And we have concluded from our biochemical and cytochemical studies [4,5] that the reticulum-bound enzyme mainly corresponds to the pool of the 10 S form, which requires a detergent for its solubilization. It seems difficult to assign a clear physiological role to the reticulum-linked AChE since it has no direct access to the acetylcholine released by the nerve endings. Several authors have reported a release of AChE from cholinesterase-rich structures (myoblasts [8], nerves [9], adrenal gland [10]) into the culture medium and from brain into the cerebrospinal fluid [11]. Arguments have been presented suggesting that the release may result from a secretion [12], but no physiological significance has been proposed for this phenomenon.

We wish to report here that the rat SCG in vitro releases 10 S AChE by a secretory process. We discuss the physiological role of such a secretion in SCG and suggest that such a process might play an important role in the peripheral nervous system.

Abbreviations: ACh, Acetylcholine; AChE, Acetylcholinesterase, EC 3.1.1.7; DFP, Diisopropylfluorophosphate; SCG, Superior cervical ganglion

2. Materials and methods

The preparation and incubation procedures of the SCG have been described in detail [4,5]. The desheathed ganglia obtained from anaesthetized rats were incubated in 0.2–1 ml modified Eagle-Dulbecco medium and kept in an O_2–CO_2, water -saturated incubator at 37°C. For experimental point, two ganglia were used.

AChE was inhibited by DFP in the following manner: the desheathed ganglia were incubated in the presence of 8×10^{-4} M DFP (Sigma) for 30 min, rinsed rapidly and placed in 5 ml Eagle-Dulbecco medium for

30 min. After washing, the ganglia were incubated in 0.2–1 ml medium.

AChE activity was extracted by homogenization of the ganglia in a Triton–NaCl buffer (0.01 M Tris/HCl, pH 7.0, 1 M NaCl, 1% Triton X-100). The AChE activity was measured with a modified Ellman's method [5]. One unit of activity was defined as that producing a one unit A_{412} increase/min at 20°C (1 ml assay medium, 1 cm path-length). Activity of the culture media was referred to their volume and not to the protein concentration.

Sedimentation analyses were performed as described [5]. Aliquots, 100 μl, of the supernatants and of the incubation medium were layered on 5–20% w/v sucrose gradients made up in the homogenization buffers. Sedimentation coefficients were determined by comparison with horse liver alcohol dehydrogenase (ADH : 4.8 S) and *E. coli* β-galactosidase (Z : 16 S) used as markers.

3. Results

When ganglia were incubated in vitro, a cholinesterasic activity appeared in the incubation medium, and increased with time during 24 h incubation (fig.1). At the end of this period, the incubation medium contained an amount of esterasic activity as high as 10% of that found in the ganglia. It was due exclusively to AChE since no reduction of the acetylthiocholine hydrolysis was observed in the presence of 10^{-5} M ethopropazine. Sedimentation analysis of the incubation medium showed that the released enzyme distinctly differed in its composition from that found in the incubated ganglia (fig.2): about 90% of the AChE

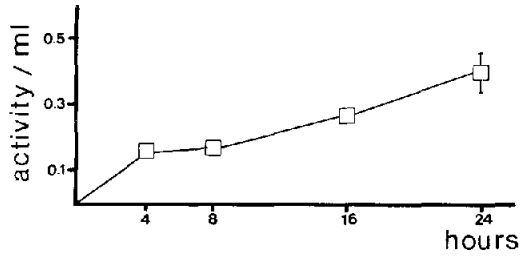

Fig.1. Time course of the appearance of AChE activity in the medium. For each point, two desheathed ganglia, obtained from urethane-anaesthetized rats were incubated in 0.2–1 ml modified Eagle-Dulbecco medium [1] and kept in an O_2–CO_2, water-saturated incubator at 37°C. The AChE activity was measured with a modified Ellman's method [5]. One unit of activity was defined as that producing a one unit A_{412} increase / min at 20°C. The activity of the media was referred to the volume and not to the proteins. Each value represents the mean ± SD of 2–3 independent values.

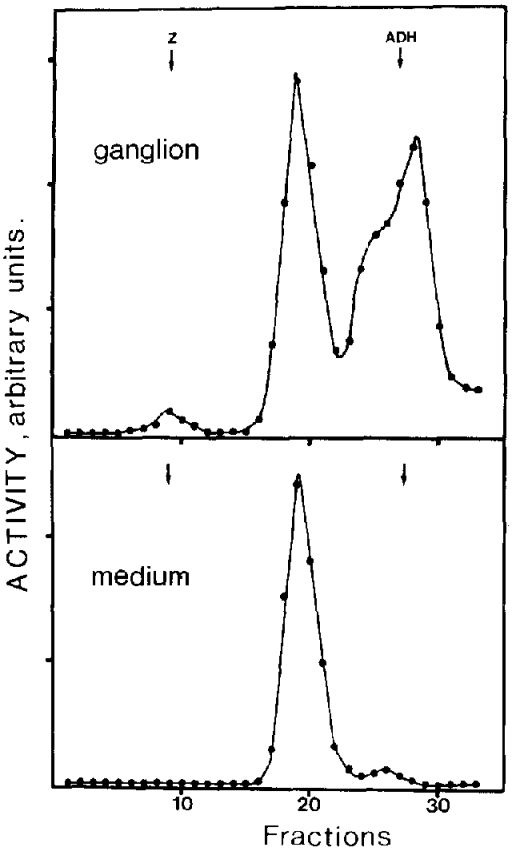

Fig.2. Molecular forms of AChE extracted from the ganglia, and found in the medium at the end of a 24 h incubation period. AChE activity was extracted by homogenization of the ganglia in a Triton–NaCl buffer (0.01 M Tris/HCl, pH 7.0, 1 M NaCl, 1% Triton X-100). Aliquots, 100 μl, of the supernatants and of the incubation medium were layered on a 5–20% w/v sucrose gradient made up in the homogenization buffer. Centrifugation was performed in a Beckman SW-60 rotor, at 38 000 rev/min, for 15 h at 2°C. Sedimentation coefficients were determined by comparison with horse liver alcohol dehydrogenase (ADH : 4.8 S) and *E. coli* β-galactosidase (Z : 16 S) used as markers. Relative cholinesterasic activities were determined with a modified Ellman's method [5]. Otherwise as in fig.1.

activity sedimented at 10 S and the remaining at 6.5 S. The sedimentation coefficients observed were strictly identical to those of the corresponding forms of the ganglia.

The rate of appearance of the enzymatic activity in the medium was not reduced by ligature of the ganglionic nerves, indicating that AChE is released by the ganglion body and not through the extremities of the nerves. To exclude the possibility that this activity originated from the presynaptic axons, we incubated ganglia from which more than 99% of the AChE activity had previously been inhibited by diisopropyl-fluorophosphate (DFP). After DFP treatment, ganglia incubated in vitro recovered about 30% of their initial activity within 24 h (fig.3), in a way very similar to

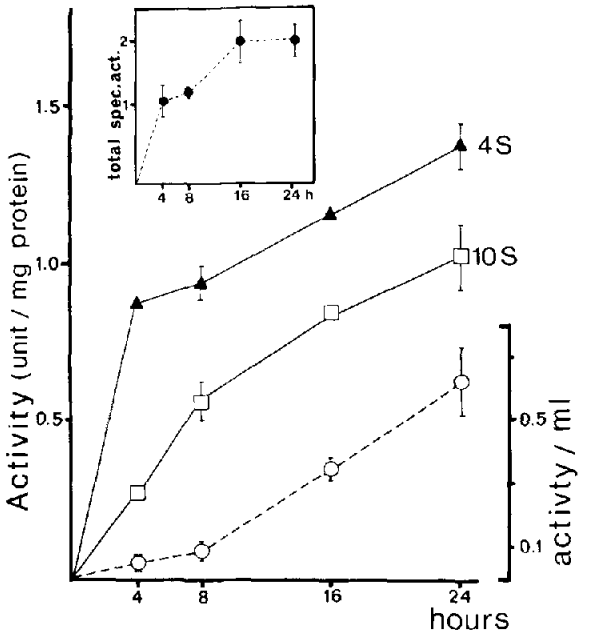

Fig.3. Recovery of the total activity and of the molecular forms by DFP-treated ganglia incubated in vitro and the concomitant appearance of AChE in the incubation medium. (● (insert)) Total activity extracted from the ganglia at different times after the DFP treatment. (▲) 4 S form and (□) 10 S form activities extracted from the ganglia. (○) Activity of the medium. The desheathed ganglia were incubated in the presence of 8×10^{-4} M DFP for 30 min, rapidly rinsed and placed in 5 ml Eagle-Dulbecco medium for 30 min. After washing, the ganglia were incubated in 0.2–1 ml medium. The specific activities of the forms were computed using the total specific activity and the proportions of forms measured in each extract. Otherwise as in figs 1 and 2, values represent the mean ± SD of 2–3 independent values.

that observed in vivo [7], (Gisiger and Vigny, unpublished results). The recovery was inhibited by 20 μM cycloheximide, implying that it resulted from a neosynthesis of the enzyme. Analysis of ganglia incubated after DFP treatment showed that the 4 S form reappeared first, the 10 S form only being found in substantial proportions more than 8 h after DFP treatment (fig.3). The 4 S and 10 S forms reestablished their original proportions within less than 24 h. In this situation also, an AChE activity, sedimenting almost exclusively at 10 S, appeared in the medium containing the DFP-treated ganglia. However, in this case, the rate at which the activity increased in the medium was low until 8 h after DFP treatment and thereafter rose markedly so that the activity measured in the medium after 24 h incubation was about 150% of that found in the medium containing the untreated ganglia (fig.3). At this time, 40% of the recovered 10 S activity was found in the incubation medium. The reason for this increased rate of release remains unclear at the moment.

4. Discussion

In the two situations described here, the incubation media were found to contain 10 S AChE and a little 6.5 S, both forms requiring the presence of Triton X-100 for quantitative extraction [5,13]; the 4 S form, present in large quantities in the ganglia (fig.2), is absent, although it is readily solubilized by aqueous homogenization. It seems very unlikely that such a selective outflow of enzyme results from a passive diffusion consecutive to either modification of the membrane permeability or cell death. This selective release of the 10 S form, considered together with the localization in the endoplasmic reticulum of most of this form [4,5,14,15], clearly suggests an active secretion of the enzyme. It probably represents a physiological event occurring in vivo, although it is likely that the rate of enzymatic release is different from that observed in vitro.

The evidence for this secretion by sympathetic neurones enables us to propose a functional role for the reticulum linked AChE. Electrophysiological data [1–3], together with our own biochemical and cytochemical results [4,5], indicate a low AChE activity at the synapses of the SCG. According to

[16], the main action of ACh in the SCG is terminated by diffusion of the transmitter out of the synaptic zone. The hydrolysis of ACh would then take place, as a subsequent step, far away from the synapses, catalyzed by the secreted AChE, which would thereby assume two functions: providing the precursors necessary for the resynthesis of ACh [17,18], and preventing an overwhelming of the ganglion by ACh by setting up a cholinesterasic barrier, as proposed by Eccles [19]. This hypothesis is presently under investigation.

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