Some Characteristics of Sulphate Uptake into Synaptosomes

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Z. Naturforsch. **34 c**, 487–489 (1979);
received December 22, 1978/February 19, 1979

Synaptosomes, Sulphate Uptake, Sulphated Glycoproteins

Incorporation of $Na_2{}^{35}SO_4$ by ovine brain Ficoll-prepared synaptosomes were studied as a function of time, temperature and substrate concentration. The rate of uptake obeyed Michaelis-Menten kinetics and apparent K_m value of 4.4 mM Na_2SO_4 was determined. In the presence of *p*-hydroxymercuribenzoate a reduction in sulphate uptake was noted which suggests that thiol groups may possibly be implicated in the uptake mechanism.

Introduction

An important component required by nervous tissue is inorganic sulphate. Unfortunately owing to limited yields and other practical problems little is known of the uptake process associated with this anion into brain. Studies using dried material have shown that sulphate groups are associated with brain lipids (cerebrosides), glycosaminoglycans (chondroitin sulphate and heparan sulphate) and glycoproteins [1, 2]. The enzyme catalysing the transfer of sulphate groups via the intermediate 3-phosphoadenosine 5-phosphosulphate to suitable endogenous accepted has been located in nerve terminals [3]. This raises the possibility of using isolated nerve endings as a model in studying the incorporation and metabolism of sulphate in brain.

The isolation of nerve ending particles from guinea-pig and rat cortex was first demonstrated by Whittaker [4] and De Robertis [5]. The nerve terminal becomes "pinched off" during homogenisation in sucrose, forming sealed supramolecular structures (synaptosomes). Synaptosomes contain specific enzyme markers, for example, choline acetyltransferase which is associated with cholinergic terminals. They also contain synaptic vesicles and mitochondria. Studies on nerve terminal preparations have demonstrated that these structures respire, synthesise and release neural transmitters [6]. Recently a lot of interest has been focused on carrier-mediated transport of a number of metabolic precursors across the synaptosomal membrane. The uptake and metabolism of glucose has been closely studied [7, 8] whereas the catabolism of glucosamine is still yet undefined [9]. This present communication describes some characteristics of the uptake of sulphate ions into nerve endings and the possible metabolic fate of this group.

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0341-0382 / 79 / 0500-0487 $ 01.00/0

Materials and Methods

Fresh adult ovine brain was freed of blood vessels and other adhering tissue. Cortex slices were homogenised in 0.32 M sucrose [8] and the crude mitochondria fraction collected after centrifugation at $14{,}000 \times g$. Synaptosomes were isolated using a Ficoll (Pharmacia) discontinuous density gradient procedure [10]. After removal of the 7.5 – 13% (w/v) Ficoll interface layer, the synaptosomal preparation was washed twice with 0.32 M sucrose by centrifugation ($40{,}000 \times g$) and the pellet stored on ice. Further experiments were confined to material which had been prepared less than 2 h previously.

The uptake of $Na_2{}^{35}SO_4$ (specific activity 103 m Ci/mol; The Radiochemical Centre, Amersham, UK) by synaptosomes (0.3 – 0.5 mg synaptosomal protein) was performed in a medium contain 10 mM Tris/HCl (pH 7.4), 15 mM $MgCl_2$, 146 mM NaCl and 4 mM KCl. The total volume of the medium was 1 ml and all incubations were carried out at 25 °C unless otherwise stated. The uptake of $Na_2{}^{35}SO_4$ was terminated by the addition of 8 ml of medium (4 °C) and synaptosomal particles collected by filtration on a 0.8 μm Millipore filter. Filters were now washed with 2×20 volumes of cold medium and ^{35}S remaining on the filter was detected in an Isocap 300 (Nuclear Chicago) liquid scintillation counter using Unisolve (Koch Light) as a scintillant. All samples were measured in triplicate. Protein concentrations were determined colormetrically using bovine serum albumin as a standard [11].

Results and Discussion

The uptake of sulphate ions into adult ovine brain synaptosomes increased linearly with time; up to about 3 minutes at an initial substrate concentration of 10 mM Na_2SO_4 (Fig. 1). All subsequent experiments were done over a 2 minute period. Incorporation of sulphate into nerve endings appeared to be temperature dependent. At 4 °C little ^{35}S was

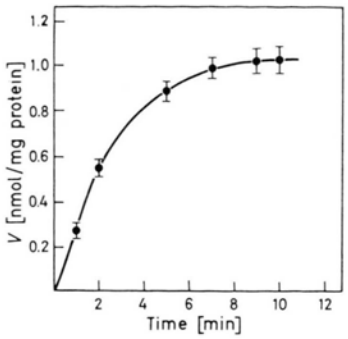

Fig. 1. Time course of synaptosomal uptake of sulphate. Synaptosomes were incubated with 10 mM Na_2SO_4 (see text) at 25 °C. Means and standard deviations are given ($n=10$).

detected on the Millipore filter. However, twice as much radioactivity was noted after incubating at 37 °C compared to 25 °C. Owing to the increased probability of lysis of the sealed synaptosomal particle at higher temperatures [8] further experiments were performed at 25 °C. The incorporation of sulphate seemed to follow saturation kinetics and the apparent affinity constant (K_m) for the uptake of 4.4 mM Na_2SO_4 was determined from the Lineweaver-Burk plot (Fig. 2). It should be noted that the above experiments do not discriminate between sulphate incorporation into outer synaptosomal membrane and uptake into the cytoplasm or cytoplasmic organelles present in nerve terminals. Unspecific binding of sulphate ions was taken as ^{35}S present after incubation at 4 °C, and all values reported here have been corrected accordingly. A parallel experiment in which synaptosomes were first dialysed prior to determining radioactivity showed little variation in the amount of ^{35}S present

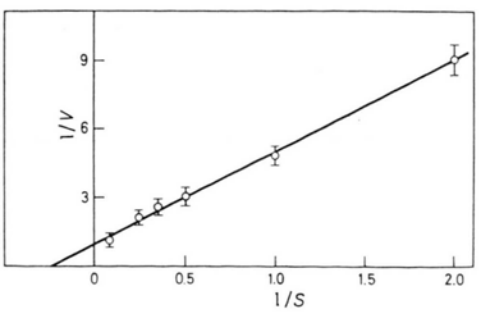

Fig. 2. Double reciprocal plot of synaptosomal sulphate uptake. See text for experimental details. V and S correspond to nmol/mg protein/min and mM respectively. Means and standard deviations are given ($n=10$).

in the dialysate when compared with material collected on Millipore filters.

Na_2SO_4 incorporation was further examined in the presence of a number of chemicals which are known to influence the transport of glucosamine into synaptosomes [9]. In a medium containing 10 mM oubain no observable inhibition of ^{35}S was found which suggests that this process does not rely on an active transport system involving a Na^+-K^+ dependent ATP'ase. In the presence of 10 mm *p*-hydroxymercuribenzoate a 50% reduction in ^{35}S incorporation was noted. Although conclusive evidence at this time is not possible this result could imply that sulphydryl groups may be involved in the uptake mechanism.

Using "pinched off" synaptosomes as a model system for the study of nerve terminal function has in the past given rise to criticism due to the occurrence of contaminating material in the preparation. It is generally accepted that reduction in foreign membrane components derived from microsomal and glial sources occurs using Ficoll in preference to sucrose-prepared gradients [10]. This finding has been clearly demonstrated using electron microscopy [12]. The purity of the synaptosome preparation in this study was assessed by using specific enzymic markers and similar specific activities of alkaline phosphatase (E. C. 3.1.3.1) and (Na^+-K^+)-APT'ase (E. C. 3.6.1.4) were found to those previously reported [10]. Therefore, the incorporation of sulphate into sealed membrane structures other than those derived from nerve endings seems unlikely.

The total amount of sulphate in synaptosomes is still unclear, values between 0.1 – 2 μmol sulphate per mg protein have been reported [13, 14]. In this present study about 1 nmol of sulphate per mg protein per 10 min was found to be incorporated (Fig. 1). This apparent low value could be due to either a slower turnover of sulphate-containing material recognised in mature nervous tissue [15], since maximum ^{35}S uptake into brain occurs during premyelination stages of development [1, 16]. Alternatively an over estimation of total sulphate could have been made possible due to the presence of glial and microsomal membranes present in sucrose-prepared synaptosomes which are enriched with sulphated glycosaminoglycans [17]. Preliminary work in my laboratory has shown the presence of ^{35}S in

a number of protein-containing fractions derived from both the synaptosomal soluble and particulate components. Characterisation studies has also demonstrated that some of these fractions are rich in fucose and hexosamine. In a previous study using pronase-treated whole rat brain tissue the occurrence of sulphated glycopeptides has also been demonstrated. Furthermore, after partial acid hydrolysis galactose-6-sulphate and N-acetylglucosamine-6-sulphate were characterised [2].

Recently much interest has been focused on the metabolism of glycoproteins in nerve terminals and their possible function in a number of neuronal processes [18]. The results presented in this communication demonstrate that synaptosomes are capable of incorporation sulphate obeying the Michaelis-Menten kinetics. This phenomenon could influence the structure/function relationship of a variety of sulphate-containing components in the region of the nerve terminal.

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