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EFFECT OF CELLULAR DESIALYLATION ON CHOLINE HIGH AFFINITY UPTAKE AND ECTO-ACETYLCHOLINESTERASE ACTIVITY OF CHOLINERGIC NEUROBLASTS

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It is not definitely established whether choline can be synthesized by nerve tissue (1,2). Its uptake by the neuronal membrane may be an important regulator of neuronal phosphatidylcholine and acetylcholine metabolism. An energy-dependent component of the choline uptake system has recently been found in neuroblastoma cell cultures (3). Choline uptake in cholinergic neuroblasts is markedly reduced by inhibition of cholinesterase activity (4). Further information about the system for choline transport still is lacking.

To gain insight into the biochemical mechanism(s) at the cell surface which are involved in choline uptake by nerve cells, cultured cholinergic neuroblasts in the present study were subjected to gentle sialidase treatment, and choline uptake and acetylcholinesterase activities (AChE, EC 3.1.1.7) were determined in parallel. Removal of a portion of the cell surface sialic acid markedly reduced choline uptake and concurrently enhanced AChE activity. These findings suggest an interrelatedness of choline uptake and acetylcholinesterase activity mediated by sialic acid components in the outer surface of the cell.

METHODS

Cell cultures

Neuroblastoma C1300 cells, clonal line S_{21} (a gift of Dr. M. Nirenberg), were defined as cholinergic (5) by the presence of choline acetyltransferase

(ChAC, EC 2.3.1.5). The cells were seeded at a rate of approximately 5×10^4 cells per Petri dish (Falcon, 25 cm^2 growth surface area). The growth medium was Dulbecco's modification of Eagle's medium supplemented with 10% fetal calf serum (Gibco, Grand Island, N.Y.) and containing 50 units of sodium penicillin G and 25 ug of streptomycin sulfate/ml. The cells were grown at 37° under an atmosphere of humidified 5% CO_2 - 95% air (v/v). The medium was changed once after 3 days. The cells were taken for experimental treatment with Clostridium perfringens sialidase, generally 1 day after the change of medium.

Treatment with sialidase

Sialidase (neuraminidase, EC 3.2.1.18) free of protease, phospholipase C, and hemolysin was obtained from Worthington. The cells were treated with 5×10^{-3} units sialidase/ml in a 1.5 ml volume of buffered glucose-salt solution containing 130 mM NaCl, 5.5 mM glucose, 0.2 mM $MgCl_2$, and 20 mM Tris-HCl, pH 7.0. At the end of the incubation period, the incubation solution was removed, to be analyzed for sialic acid by the method of Warren (6). The cells were immediately rinsed with sialidase-free glucose-salt solution, and the growth medium was replaced for 4 min in order to remove sialidase adhering to the cells (7). For the subsequent incubations, this medium was removed and the fully attached confluent monolayer cultures were rinsed with the respective incubation solutions for choline uptake and for acetylcholinesterase activity determinations.

Choline uptake

Experiments were performed with 2 uM ^{14}C-methyl choline chloride (60 mCi/mmole, Amersham, England). Details are given elsewhere (4).

Acetylcholinesterase (AChE)

Enzyme activity was assayed by the photometric method of Ellman et al. (8) which was modified so that the enzyme activity of intact, attached neuroblasts could be determined reliably. The incubation medium contained 130 mM NaCl, 5.5 mM glucose, 20 mM Tris-HCl buffer (pH 7.8), 10^{-5} M iso-OMPA, a selective

inhibitor of non-specific cholinesterase, and 0.5 mM acetylthiocholine. Incubation was started upon adding substrate, and was conducted for 15-30 min at 37°. The reaction was stopped and color development initiated by the addition of (final concentration): 1.5 mM physostigmine sulfate and 10^{-4} M dithiobisnitrobenzoic acid. Corrections were made for the effect of sulfhydryl groups on the color reaction and for non-enzymatic hydrolysis of substrate. Incubation media were examined for the presence of cytoplasmic lactic dehydrogenase activity (9) to ensure that no cell breakage had occurred. Protein was measured by the method of Lowry et al. (10), modified by first adding 2 ml concentrated formic acid to the samples to dissolve the protein so that an aliquot could be taken for assay of radioactivity and then permitting the formic acid to evaporate completely before performing the analyses for protein as described (10).

RESULTS

Results are summarized in Fig. 1. The achievement of a rapid progressive removal of a portion of the cellular sialic acid by the action of C. perfringens sialidase is evident from the progress curve. Upon enzymatic removal of cell surface sialic acid, a concomitant increase in ecto-AChE activity appeared. There is overall parallelism between removal of cell surface sialic acid and appearance of additional ecto-AChE activity. A substantial activation of ecto-AChE activity occurred upon removal of a fraction of the total cell surface sialic acid. At the same time, uptake of radioactive choline by treated cells was markedly reduced. A 53% inhibition of choline uptake occurred after a 2 min treatment with sialidase. Prolonged treatment reduced choline uptake to 39% of the control value.

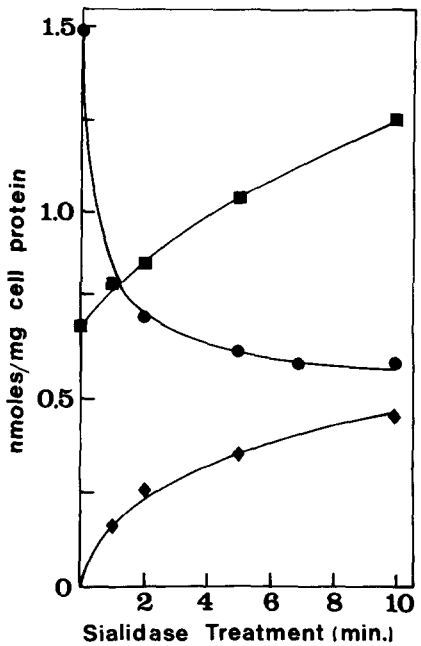

Fig. 1. Removal of cell surface sialic acid from S_{21} neuroblasts by Clostridium perfringens sialidase, and concomitant activation of acetylcholinesterase and decrease in high affinity choline uptake. Key: (◆—◆) enzymatically released cell surface sialic acid X 10^{-1}; (■—■) acetylcholinesterase activity per hr X 10^{-2}; and (●—●) high affinity choline uptake/hr. Experimental details are given in the text.

All cells in the monolayer culture remained attached and viable during the entire course of the experiments. No cell breakage was detectable under the conditions of the assay, as judged by the lack of any measurable extrusion of cytoplasmic lactic dehydrogenase into the medium.

DISCUSSION

The data presented here indicate the possibility of a relationship in cholinergic neuroblasts between cell surface sialic acid on the one hand and AChE activity and high affinity choline uptake on the other hand. Neurons appear to take up choline by two major systems which differ in their affinity for choline. The high affinity system for choline uptake seems to be directed primarily toward the synthesis of acetylcholine (11), whereas the general metabolism of choline may depend upon the low affinity system. Considering the possible influence of high affinity choline uptake upon choline metabolism,

decreased choline uptake and activation of ecto-AChE activity of S_{21} cells upon partial removal of cell surface sialic acid suggest a role for cell surface sialic acid in controlling acetylcholine metabolism. It has been shown previously that careful treatment of a number of different mammalian cells with sialidase removed about 20% of their sialic acid, mostly from sialoglycoproteins, which may be among the extreme outer structures on the surface of the cell (12). Substantial activation of ecto-AChE activity and inhibition of high affinity choline uptake have been obtained in the present study after only a 2 min treatment with sialidase when a small part of the enzymatically available sialic acid has been released from the surface of the cells. Several kinds of mammalian cholinesterases have been shown to contain sialic acid (13,14). Heilbronn and Cedergren (15) observed that treatment of brain slices with sialidase reduced but did not abolish uptake of acetylcholine. Recently, Brodbeck et al. (16) have shown an activation of plaice muscle cholinesterase by treatment with sialidase. In this regard, treatment with sialidase might be a useful tool in further unraveling the nature of choline transport and acetylcholine metabolism. Bound sialic acid has been implicated as part of the 5-hydroxytryptamine receptor complex in smooth muscle (17,18). Thus, there is increasing evidence that membrane sialic acid may be involved in the action of certain neurotransmitters.

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