transferase activity from a rat brain high-speed supernatant was
Fig. 1. Titrations of the C-2 and C-4 protons of the
Chemical shifts were measured relative to sodium 2,2-dimethyl-
titratable histidine residue of native phospholipase C
2-silapentane-5-sulphonate as internal standard.

Lysis of cholinergic synaptosomes by an antiserum to choline acetyltransferase

MAUREEN DOCHERTY,* H. F. BRADFORD* and B. H.
ANDE RTON†

*Department of Biochemistry, Imperial College of Science and
Technology, London SW7 2AZ, and † Department of
Immunology, St. George's Hospital Medical School, Cranmer
Terrace, London SW17 ORE, U.K.

A major limitation in the use of synaptosomes from mammalian
brain is the heterogeneity of the preparations with respect to
transmitter type. An ability to discriminate between sub-
populations of these nerve terminals would allow the sub-
populations to be counted and even separated. Elimination of a
sub-population would allow greater certainty in attributing
specific properties to a nerve-ending type. As a result, the
mammalian synaptosome preparation would be more versatile
in application to studies of transmitter systems.

In the present experiments an antiserum to choline acetyl-
transferase was found to lyse cholinergic synaptosomes. It was
prepared from the partially purified bovine enzyme (Sigma
Chemical Co.) by injection of an oil emulsion into rabbits using
standard procedures. The serum was prepared by allowing the
blood to clot overnight at 4°C, and was tested for antibodies to
the choline acetyltransferase preparation by double immuno-
diffusion. Direct immunoprecipitation of choline acetyl-
transferase activity from a rat brain high-speed supernatant was
achieved using dilutions of antiserum and Pansorbin (Gibco
Laboratories). The enzyme was assayed using 0.4 mM-[14C]-
acetyl-CoA (4 Ci/mol; Amersham International) by the method
(sp. radioactivity 15 Ci/mmol; Amersham International) into rat
cerebrocortical synaptosomes (Bradford et al., 1973) was
measured at choline concentrations of 0.0625–2 μM as described
by Simon et al. (1976), using smaller volumes (500 μl) in
Eppendorf tubes. Na+ dependent uptake of 4-amino-n-[2,3-
H]butyric acid, at concentrations of 0.125–5 μM, into synap-
tosomes was measured by the same method. Eadie–Hofstee plots
were constructed to calculate Vmax and Km values. Lactate
dehydrogenase activity was measured by the method of Johnson
(1960).

The actions of antisera were tested by incubating cortical
synaptosomes (400 μg of protein/ml) in 10 ml of Na+-free
Krebs–Ringer phosphate which contained either antiserum (0.2
or 2.0 ml) plus 1.0 ml of guinea-pig complement (Gibco
Laboratories), or the antiserum alone, or complement alone, for
30 min at 37°C. The synaptosomes were deposited by centri-
fugation in a Beckman 50 rotor. The supernatant was retained
for lactate dehydrogenase assay, and the pellets were resuspended
in 0.32 M sucrose (2 mg/ml) for assay of choline or
γ-aminobutyric acid uptake. Lactate dehydrogenase was measured
after lysis caused by the addition of 8 mM Tris/ClHCl, pH 8.1.
A dilution of 1:16 of the antiserum with phosphate-buffered
saline produced a precipitate in the double immunodiffusion test
against the partially purified choline acetyltransferase pre-
paration. The more sensitive method of direct immuno-
precipitation showed 100% precipitation of the choline acetyl-
transferase activity without loss of this activity at a dilution of
antiserum of 1:5.

The results of Table 1 show that the antiserum caused total
suppression of the Na+-dependent uptake of choline at a dilution
of 1:5 in the incubation medium, provided complement was
added. A dilution of antiserum of 1:50 caused a halving of the
Vmax, without altering the Km, showing a dose-dependent

This work was supported by a research grant from NATO.

326–333
Biochem. 27, 238–243

1982
Table 1. Effects of partially purified choline acetyltransferase antiserum on cerebrocortical synaptosomes prepared from rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Na⁺-dependent choline uptake</th>
<th>Na⁺-dependent γ-aminobutyrate uptake</th>
<th>Lactate dehydrogenase activity (% release)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$V_{\text{max}}$</td>
<td>$K_m$</td>
<td>$V_{\text{max}}$</td>
</tr>
<tr>
<td>Pre-immune antiserum (2ml)</td>
<td>55 ± 3</td>
<td>0.52 ± 0.04</td>
<td>739 ± 83</td>
</tr>
<tr>
<td>Antiserum (200μl) + complement</td>
<td>25 ± 4</td>
<td>0.53 ± 0.03</td>
<td>Not estimated</td>
</tr>
<tr>
<td>Antiserum (2ml) + complement</td>
<td>Not measurable</td>
<td>Not measurable</td>
<td>698 ± 72</td>
</tr>
<tr>
<td>Antiserum (200μl)</td>
<td>54 ± 5</td>
<td>0.60 ± 0.05</td>
<td>Not estimated</td>
</tr>
<tr>
<td>Antiserum (2ml)</td>
<td>53 ± 4</td>
<td>1.1 ± 0.09</td>
<td>Not estimated</td>
</tr>
<tr>
<td>Complement</td>
<td>52 ± 5</td>
<td>0.53 ± 0.05</td>
<td>Not estimated</td>
</tr>
</tbody>
</table>

Relationship. Antiserum alone at these dilutions caused an increase in $K_m$, the 1:5 dilution producing a 2-fold change. Complement alone, however, was without effect. The uptake of γ-aminobutyrate was not changed by the antiserum at these dilutions, with respect to either $V_{\text{max}}$ or $K_m$, though 2,4-diaminobutyric acid (0.1 mM) did block its uptake.

Measurement of lactate dehydrogenase showed that this soluble enzyme was released into the incubation medium to the extent of 30-34% by the antiserum at the 1:5 dilution, and by 15-20% at the 1:50 dilution, provided complement was added. Neither complement alone, nor antiserum alone, caused any release of lactate dehydrogenase above control levels.

The results indicate that the antiserum contains antibodies able to lyse about one-third of the cortical synaptosomes, whilst totally preventing Na⁺-dependent choline uptake. The lack of action of γ-aminobutyrate uptake indicates a degree of specificity in the lysis, and suggests it may be confined to cholinergic terminals. An action on choline uptake due to inhibition of choline acetyltransferase activity and hence acetylcholine synthesis seems to be ruled out since the enzyme activity was not changed by the antiserum. The immuno-precipitation tests are consistent with the presence of antibodies to choline acetyltransferase. If this is the effective agent in the antiserum, the data suggest that it is interacting with choline acetyltransferase, which, unexpected, may be present and accessible in the outer membrane of cholinergic synaptosomes, addition of complement leading to lysis of this synaptosome sub-population. Alternatively, antibodies and complement may gain access to the inner face of the membrane by entry to the synaptosomes via endocytosis and thus cause lysis, but this seems a less tenable explanation.

Richardson (1981) and Jones et al. (1981) have reported similar complement-mediated lysis of guinea-pig synaptosomes in the presence of an antiserum to the nerve terminal (T' sacs) of Torpedo electric organ.


Brain glutaminase activity in relation to transmitter glutamate biosynthesis

H. K. WARD, C. M. THANKI, D. W. PETERSON and H. F. BRADFORD
Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ, U.K.

Glutamate generated from glutamine may be more directly related to the pool from which transmitter glutamate is released than is that generated from glucose by transamination of 2-oxoglutarate in the Krebs cycle. Evidence for this view comes principally from studies of the compartmentation of glutamate in neural tissue slices or synaptosomes in relation to the fraction of this transmitter released to incubation media as Ca²⁺-dependant responses to treatments with depolarizing stimuli (Reubi et al., 1978; Hamberger et al., 1978; Bradford et al., 1978).

In addition, glutamine addition to incubated brain slices or synaptosomes substantially increases the glutamate content and the amounts that are released during depolarization (Bradford & Ward 1976; Hamberger et al., 1978, 1979b).

One hazard of conclusions from experiments in vitro is the possibility that glutamine conversion into glutamate could occur extracellularly followed by uptake and release of the glutamate formed. Various reasons why this would be minimal have been considered (Bradford et al., 1978; Hamberger et al., 1979b), but evidence of conversion in vivo of glutamine into releasable glutamate under conditions of minimal tissue damage and other artefacts would add substantially to the case proposed.

In the present study 62.5 nmol of [U-14C]glutamine (sp. radioactivity 40 Ci/mmol) or 40 nmol of [U-14C]glucose (sp. radioactivity 268 Ci/mol) were slowly infused in saline or 2% ethanol into the lateral ventricle of Sprague-Dawley rats (female; 200-250 g) previously implanted with cannulae. After 1.5 h, the rats were culled and slices of cerebral cortex were prepared and incubated for 10 min at 37°C in Krebs/Tris medium gassed with O₂. Slices were then transferred to fresh medium containing veratrine alone (75 μM) or veratrine plus tetrodotoxin (1 μM). Amino acids in the medium or the tissue were extracted with methanol/formic acid/water (1:5:4, by vol.) containing standard norleucine. Amino acids were separated on an autoanalyser as described by Norris et al. (1980) with a fluorescence detector, and samples were collected and counted for radioactivity by liquid scintillation.

Calculation of the ratio of specific radioactivities of amino acids in incubation medium to that remaining in the tissue showed that when [U-14C]glutamine was employed as precursor, the medium tissue ratio was about 1.7 for glutamate and 2.0 for γ-aminobutyrate. When [U-14C]glucose was substrate the ratio was about 0.8 for glutamate and 1.2 for γ-aminobutyrate. Thus, glutamine appeared to be about twice (2.2) as efficient at