Specific lysis of noradrenergic synaptosomes by an antiserum to dopamine-β-hydroxylase

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An antiserum to dopamine-β-hydroxylase purified from bovine adrenal medulla, acting in the presence of complement, caused the release of 12% of lactate dehydrogenase, 20% of tyrosine hydroxylase activity, and 40% of noradrenaline (NA) content from synaptosomes prepared from rat brain cerebral cortex. Uptake of [^{3}H]NA was reduced by 54%. Anti-serum alone or complement alone were without action. The antiserum plus complement had no effect on choline uptake and did not release choline acetyltransferase, glutamate decarboxylase, dopamine or 5-hydroxytryptamine. These results suggest selective lysis of noradrenergic terminals had occurred. An enhancement of lysis was not observed when synaptosomes were stimulated with 75 mequiv./l K^+ and exposed to a sub-maximal dose of antiserum, plus complement.

Synaptosome *Noradrenergic nerve terminal* *Lysis* *Antiserum* *Dopamine-β-hydroxylase*

1. INTRODUCTION

Dopamine-β-hydroxylase (DBH), the enzyme responsible for the synthesis of noradrenaline (NA) in vesicles and granules of the mammalian nervous system, is thought to exist both in a membrane-bound form and as a granular complex in the vesicle matrix [1,2]. Antibodies against the enzyme purified from bovine adrenal medulla have been shown to be capable of selectively destroying in vivo, in the presence of complement, noradrenergic nerve terminals in guinea pig and rat cerebral cortex [3] and guinea pig iris [4]. These observations have been used to support the case for exocytosis and subsequent recycling and incorporation of vesicular membranes into those of the axon terminal. Such a process would permit the antibody to bind to the DBH present in the membrane of NA-containing vesicles when these vesicles fuse with the axonal membrane during neurotransmitter release.

We now report that this selective immunolytic action of DBH can be shown to occur in vitro using synaptosomes prepared from rat brain cerebral cortex.

2. MATERIALS AND METHODS

Antiserum to DBH, purified from bovine adrenal medulla, was prepared as described previously and has been shown to be monospecific for DBH by a variety of immunochemical tests including immunodouble-diffusion, immunoelectrophoresis and immunotitration [5].

The action of the antiserum was tested essentially as described in [6] by incubating cortical synaptosomes (500–1000 μg protein/ml) prepared from rat brain with (a) anti-DBH (10–40 μl) plus complement (50 μl) (b) anti-DBH (10–40 μl) alone (c) complement alone (50 μl) and (d) Krebs-phosphate buffer alone in a total volume of 1 ml for 30 min at 37°C. After incubation the synaptosomes were deposited by centrifugation at 10000 × *g* for 30 s. The supernatant was retained for measurement of tyrosine hydroxylase (TH), choline acetyltransferase (ChAT), glutamate decarboxylase (GAD)

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and lactate dehydrogenase (LDH) activities and for measurement of NA, dopamine (DA) and 5-hydroxytryptamine (5-HT) content. After surface washing the pellet was resuspended in Krebs-phosphate medium. An aliquot of this synaptosomal suspension was used to estimate [^{3}H]NA and [^{3}H]choline uptake, to assay for TH, ChAT, GAD and LDH activities, and to measure NA, DA and 5-HT content.

Release of enzymes, catecholamines and 5-HT from synaptosomes due to the combined action of antiserum plus complement could thus be calculated. This was expressed as a percentage of the total, after subtraction of both the amount present at the start of the incubation and that due to non-specific release (the sum of which never amounted to more than 6% of the total). Neither anti-DBH nor complement acting alone had any significant effect on either NA or choline uptake.

The effect of potassium stimulation on the lytic action of anti-DBH was also investigated. Synaptosomes (mg/ml) were preincubated in Krebs-phosphate medium containing 75 mequiv./l K^+ for 2 min, before addition of anti-DBH and/or complement.

TH activity was assayed by the production of [^{3}H]dopa from 10 μM L-[2,6-^{3}H]tyrosine (40 Ci/mmol, Amersham) as described [7].

ChAT activity was measured using 0.4 nM [1-^{14}C]acetyl-CoA (4 mCi/mmol, Amersham) as in [8].

GAD activity was assayed by the production of $^{14}CO_2$ from L-[1-^{14}C]glutamic acid (59 mCi/mmol, Amersham) at a final concentration of 2.5 mM according to [6,9].

LDH activity was measured as in [10]. Synaptosomal catecholamine and indoleamine levels were determined by electrochemical detection using an LCA 15 electrochemical detector (EDT Research) after HPLC separation on a Spherosorb column (S5 ODS 2) under reverse phase. Prior to measurement, synaptosome samples were extracted by homogenization in ice-cold column mobile phase (0.1 M KH_2PO_4, 186 μM disodium EDTA, 378 μM sodium acetyl sulphonate, 14% methanol; pH 3.5) which contained appropriate concentrations of 3,4-dihydroxybenzylamine and 5-hydroxyindole as internal standards. After homogenization, the samples were centrifuged at 10 000 × g for 10 min in the cold and the supernatants were then filtered using regenerated cellulose microfilters (0.2 μM, Anachem).

The sodium-dependent uptake of DL-[7-^{3}H]noradrenaline hydrochloride (13 Ci/mmol, New England Nuclear) at 0.0625–4 μM and [^{3}H]choline (15 Ci/mmol, Amersham) at 0.125 μM was measured as described [6]. In some experiments the effect of varying concentrations of Triton X-100 on the uptake of NA into synaptosomes was investigated. Synaptosomes were preincubated in the presence of Triton X-100 for 5 min at 37°C prior to the addition of [^{3}H]NA.

3. RESULTS AND DISCUSSION

When cortical synaptosomes were incubated with varying dilutions of anti-DBH serum in the presence of complement, lysis occurred as reflected by the release of LDH into the supernatant to a maximum of 12% (fig.1). The response was proportional to the amount of antibody used, 0.08 μl antiserum/μg synaptosomal protein antiserum producing the maximal effect. TH activity was

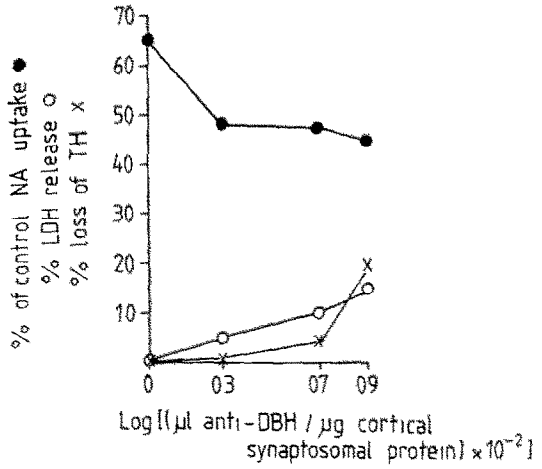

Fig.1. Action of anti-DBH serum plus complement on synaptosomes prepared from rat cerebral cortex. Values represent the mean of triplicate observations on single synaptosome preparations, a total of 4 separate preparations having been used. Control noradrenaline uptake was 15 ± 1.1 pmol/2 min per mg (mean ± SD, n = 4). Untreated synaptosomes had a total LDH activity of 1.3 ± 0.09 μmol/min per mg protein (mean ± SD, n = 4) and a total TH activity of 12 ± 2 pmol/min per mg protein (mean ± SD, n = 4).

detected in the supernatant obtained after centrifuging treated synaptosomes. When optimal concentrations of antiserum were used, this release amounted to 20% of the total TH present at the start of the incubation (fig.1). NA was differentially lost from the synaptosomes when they were treated with anti-DBH and complement, resulting in a maximal loss of 40% of total content, with no detectable loss occurring in 5-HT or DA (fig.2). [^{3}H]NA uptake into these treated synaptosomes was reduced to 46% of control values (fig.1). A loss of uptake also occurred after lysis of synaptosomes using the detergent Triton X-100. When synaptosomes were treated with 0.01% Triton X-100, NA uptake was reduced to 3.4–7.5% of control values when substrate concentrations of 0.0625, 0.5 and 4 μM were employed.

These results suggest that after exposure to both antiserum and complement, cortical noradrenergic synaptosomes are lysed with a subsequent loss of NA uptake and release of both TH and NA.

That such immunolysis was selective for noradrenergic synaptosomes was demonstrated by the absence of ChAT and GAD release (total control values 490 ± 32 and 932 ± 43 pmol/min per mg,

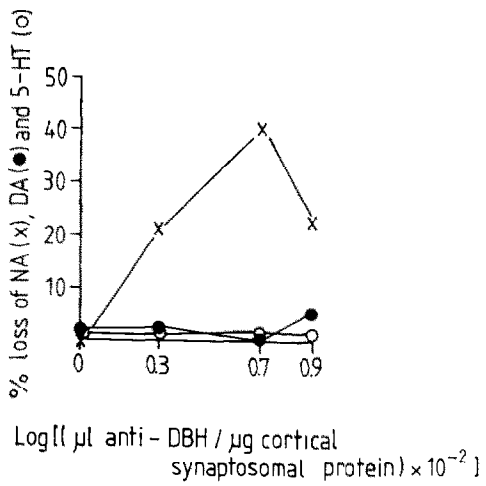

Fig.2. Action of anti-DBH serum plus complement on catecholamine and indoleamine content from synaptosomes prepared from rat cerebral cortex. Values represent the mean of duplicate observations on single synaptosome preparations, a total of 4 separate preparations having been used. Untreated synaptosomes had a total NA, DA and 5-HT content of 432 ± 20, 10.6 ± 2.3 and 2.5 ± 0.4 pmol/mg, respectively (mean ± SD, $n = 4$).

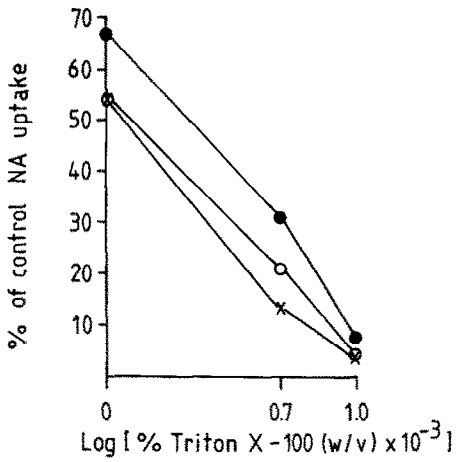

Fig.3. Effect of increasing Triton X-100 concentrations (0.001, 0.005 and 0.01%) on noradrenaline uptake into cerebral cortical synaptosomes prepared from rat brain; using 0.0625 μM NA (×), 0.5 μM (○), 4 μM (●). Each point represents the mean of triplicate observations using a single synaptosome preparation. Control noradrenaline uptake was 11 ± 3 using 0.0625 μM, 61 ± 5 using 0.5 μM and 120 ± 13 pmol/2 min per mg using 4 μM NA (mean ± SD, $n = 3$).

respectively, mean ± SD, $n = 4$ and total values after exposure to antibody and complement 473 ± 40 and 899 ± 52 pmol/min per mg, respectively, mean ± SD, $n = 4$) and the lack of action on choline uptake (control uptake 6 ± 0.5 pmol/2 min per mg, mean ± SD, $n = 4$ and uptake after exposure to antibody and complement 7 ± 1.5 pmol/2 min per mg, mean ± SD, $n = 4$). Furthermore, neither DA or 5-HT was lost from the treated synaptosomes (total control values 10.6 ± 2.3 and 2.5 ± 0.4 pmole/mg respectively, mean ± SD, $n = 4$ and total values after exposure to antibody and complement 12.5 ± 3 and 2.9 ± 0.7 pmole/mg respectively, mean ± SD, $n = 4$), thus neither dopaminergic nor serotonergic terminals appeared to have lysed during treatment.

The present data suggest that an antiserum against DBH, acting in the presence of complement, will cause specific lysis of noradrenergic synaptosomes. It is probable that lysis occurs because the antibody recognizes DBH in the synaptosomal membrane. This DBH could well originate from the vesicular membrane. However, when cortical synaptosomes were stimulated by incubation in Krebs medium containing elevated potassium and then treated with a sub-maximal dose of anti-

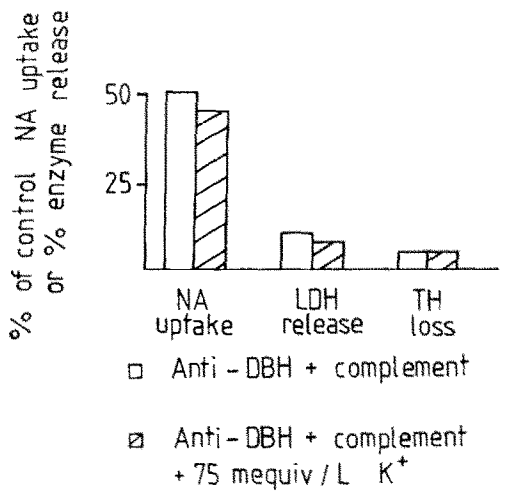

Fig.4. Effect of potassium stimulation on immunolysis produced by 0.03 μl anti-DBH/μg synaptosomal protein. Uptake values are given as a percentage of those obtained in untreated synaptosomes (uptake measured at 0.0625 μM NA, 14 ± 2 pmol/2 min per mg, mean $\pm$ SD, $n = 3$) and LDH and TH values are given as a percentage of total activities released into supernatant after treatment. Total LDH values were 1.0 ± 0.08 μmol/min per mg protein (mean $\pm$ SD, $n = 3$) and total ChAT values were 720 ± 90 pmol/min per mg protein (mean $\pm$ SD, $n = 3$), in untreated synaptosomes. Values given are the means from two separate synaptosome preparations.

DBH, in the presence of complement, lysis was not enhanced (fig.4). This lack of enhancement after such treatment suggests that an increase in the rate of membrane recycling via exocytosis, which should occur during potassium stimulation [11], does not lead to an increase in the steady-state concentration of DBH molecules in the synaptosomal membrane. However, it is also possible that the DBH present in this membrane does not occur there as a consequence of vesicular exocytosis.

We have previously reported that antisera to ChAT, GAD, TH [6] and tryptophan hydroxylase [7] also evoke complement-mediated lysis of specific subpopulations of synaptosomes. This suggests that these observations might be explained by the existence of either the enzyme itself or a peptide chain common to both the enzyme and another synaptosomal membrane protein. These components could serve as phenotypic surface markers for the nerve terminals concerned.

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REFERENCES

[1] Kirksey, D.F., Klein, R.L., Baggett, J.McC. and Gasparis, M.S. (1978) Neuroscience 3, 71–81.
[2] Fischer-Colbrie, R., Schaching, M., Zangerle, R. and Winkler, H. (1982) J. Neurochem. 38, 725–732.
[3] Costa, M., Geffen, L.B., Rush, A., Bridges, D., Blessing, W.W. and Heath, J.W. (1979) Brain Res. 173, 65–78.
[4] Furness, J.B., Lewis, S.Y., Rush, R., Costa, M. and Geffen, L.B. (1977) Brain Res. 136, 67–75.
[5] Joh, T.H. and Ross, M.E. (1983) in: Immunohistochemistry, IBRO Handbook Series: Methods in the Neurosciences, vol. 3 (Cuello, A.C. ed.) pp. 121–138, Wiley, Chichester.
[6] Dochtery, M., Bradford, H., Wu, J.-Y., Joh, T.H. and Reis, D.S. (1985) Brain Res. 339, 105–113.
[7] Dochtery, M., Bradford, H., Cash, C.D. and Maitre, M. (1985) FEBS Lett. 182, 489–492.
[8] Fonnum, F. (1975) J. Neurochem., 24, 407–409.
[9] Dochtery, M., Bradford, H., Anderton, B. and Wu, J.-Y. (1983) FEBS Lett. 152, 57–61.
[10] Bergmeyer, H.U., Bernt, E. and Hess, B. (1963) in: Methods of Enzymatic Analysis (Bergmeyer, H.U. ed.) pp. 736–741, Academic Press, New York.
[11] Fried, R.C. and Blaustein, M.P. (1976) Nature 261, 255–256.