Distribution of acetylcholinesterase, proteolipids and cholinergic receptor in cat brain

SIR,—Acetylcholinesterase-rich nerve-ending membranes isolated from the cerebral cortex have also the highest binding capacity for radioactive cholinergic blocking agents (De Robertis, Alberici & others, 1966). This binding capacity is not due to the lipids or glycolipids but probably to a proteolipid protein (De Robertis, Fiszer & Soto, 1967). Since myelin has the highest proteolipid content in the central nervous system but only a small binding capacity for dimethyl-[^{14}C]-(+)-tubocurarine, it was postulated that the cholinergic receptor may be a special type of proteolipid.

These results warranted the study of the content of proteolipids, acetylcholinesterase and of the binding capacity for dimethyl-[^{14}C]tubocurarine in the proteolipids extracted from different neuroanatomical regions of the brain of the cat including the retina (Table 1). Such regions were dissected in the cold room and homogenized in distilled water pH 7·4 to 10%. A total particulate was obtained by sedimenting at 100,000*g* for 30 min. Aliquots of this particulate were used for assay of total protein, and of the proteolipid protein extracted with chloroform–methanol (2:1) according to Folch & Lees (1951). Protein (Lowry, Rosebrough & others, 1951) acetylcholinesterase, (Ellman, Courtney & others, 1961) and the binding capacity for dimethyl-[^{14}C]tubocurarine (De Robertis, Azcurra & Fiszer, 1967) were estimated.

The results obtained are summarized in Table 1. To better correlate acetylcholinesterase activity with the binding capacity, ratios in which white matter was considered as 1·0 were used. It may be observed that there is a correlation of enzyme and the amount of dimethyl-[^{14}C]tubocurarine bound to the proteolipid protein. The parallelism is good in white matter, cerebral cortex, cerebellum and nucleus lentiformis but not as exact in corpora quadrigemina anterior and in nucleus caudatus, in which acetylcholinesterase is higher than the receptor capacity, and in retina in which this situation is reversed.

The content of proteolipid protein is 20–25 mg/g wet tissue in bovine white matter and in grey matter is 1/5 of that concentration (Folch & Lees, 1951). Amaducci (1960) studied the localization of proteolipids in numerous anatomical regions of the human brain. Our results in the cat are in general agreement although our figures for nucleus lentiformis and specially for corpora quadrigemina anterior are much higher. This may be due to species difference or to a wider inclusion of white areas in the sample.

The acetylcholinesterase activity has been examined by Nachmansohn (1939) and Burgen & Chipman (1951) in various regions of the CNS. Our results in

TABLE 1. CONTENT OF PROTEOLIPID PROTEIN, ACETYLCHOLINESTERASE (AChE) AND UPTAKE OF DIMETHYL-[^{14}C]TUBOCURARINE ([^{14}C]DMTC) IN THE CHLOROFORM–METHANOL (2:1) EXTRACT OF VARIOUS ANATOMICAL REGIONS OF THE CAT CNS

	Proteolipid protein mg/g tissue	AChE μM/hr ACh used/mg protein	[^{14}C]DMTC counts/min/mg proteolipid protein	Ratio AChE in X / AChE in *a*	[^{14}C]DMTC in X / [^{14}C]DMTC in *a*
White matter[a] ..	20·4	0·57	5,410	1·0	1·0
Cerebral cortex ..	7·9	2·30	36,160	4·0	6·7
Cerebellum ..	7·3	4·70	36,146	8·2	6·7
N. lentiformis ..	21·2	5·67	36,810	9·9	6·8
Corpora quadrigemina anterior	23·6	7·40	23,320	13·0	4·3
N. caudatus	12·3	23·40	43,540	41·0	8·0
Retina	7·5	3·90	51,177	6·8	9·5

the cat agree in general terms with those found in the rabbit, dog, ox and man by Nachmansohn (1959). In these species the concentration is highest in the caudatus and lentiformis nuclei. In the cat the highest acetylcholinesterase activity is the nucleus caudatus and this is followed by the corpora quadrigemina anterior and nucleus lentiformis.

The general relation between the enzyme and binding capacity indicates that both reside in the same cholinergic pathways in the CNS. Cell fractionation studies have demonstrated that the enzyme and the cholinergic receptor are concentrated in the same type of nerve-ending membrane (De Robertis & others, 1966). Such membranes comprise also the junctional complex formed by the synaptic membranes and associated structures. Recently, using a mild detergent, most of the limiting membrane of the ending was dissolved, together with the main proportion of acetylcholinesterase and other bound enzymes. With this technique the junctional complexes were isolated and shown to contain intact the binding capacity for the cholinergic blocking agents (De Robertis, Azcurra & Fiszer, 1967). This result suggested that the receptor protein has a postsynaptic localization while acetylcholinesterase has a wider distribution being mainly presynaptic. The enzyme and the receptor can also be dissociated by the chloroform-methanol treatment, which inhibits the enzyme without affecting the binding capacity. Some of these findings may explain the apparent lack of parallelism between the enzyme and the cholinergic receptor in certain neuroanatomical regions. Thus the predominance of acetylcholinesterase in the nucleus caudatus could be due to the presence of large amounts of the enzyme in presynaptic segments of the axons while in the retina the postsynaptic receptor protein may be proportionally higher. This is also confirmed by histochemical studies which show that the enzyme reaction is very strong and homogeneously distributed in the nucleus caudatus (see Gerebtzoff, 1959) while in the retina it is confined to the inner plexiform layer (Francis, 1953).

The fact that the retina has a very high choline-acetylase content (Hebb, 1957) is also in line with the high content of cholinergic receptor found in the present study.

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Interactions between monoamine oxidase inhibitors and sympathomimetic amines in the rat isolated vas deferens

SIR,—It has been assumed (Iversen, 1967) that monoamine oxidase inhibitors do not potentiate the effects of catecholamines administered exogenously or released by nerve stimulation. However, it has been shown (Bhargava, Kar & Parmar, 1963) that amphetamine, pheniprazine and tranylcypromine potentiate the responses of hypogastric nerve-vas deferens preparation elicited by electrical stimulation, and that monoamine oxidase inhibitors produce a large potentiation of the pharmacological actions of tyramine (Griesemer, Barsky & others, 1953; Goldberg & Sjoerdsma, 1959; Spano, 1966). Tranylcypromine and pheniprazine potentiate also the effects of catecholamines on the heart of reserpinized cat but not in the normal cat (Lee, Shin & Shideman, 1961).

In view of these conflicting reports, we have thought it worth while to study the interactions between some monoamine oxidase inhibitors and sympathomimetic amines in the rat isolated vas deferens preparation, using the technique of Laporte, Jané & Valdecasas (1966), since this is one of the most suitable preparations to assess *in vitro* noradrenaline supersensitivity (Cuenca & Valdecasas, 1965; Ursillo & Jacobson, 1965; Benvenutti, Bonaccorsi & Garattini, 1967). The monoamine oxidase inhibitors assayed were: pheniprazine, a hydrazine derivative, and tranylcypromine, amphetamine and pargyline, all non-hydrazine derivatives. Sympathomimetic agents used were a direct-acting amine, noradrenaline, and an indirect-acting amine, tyramine.

Tranylcypromine and pheniprazine have no action on the rat isolated vas deferens in concentrations up to 1×10^{-6} g/ml. Stronger concentrations elicit small and irregular contractions, and an increase of the residual tonus. To see the same effects with amphetamine, higher concentrations must be used (1×10^{-4} g/ml).

After these apparently ineffective stimulations with tranylcypromine the sensitivity of the vas deferens to catecholamines increases markedly. This

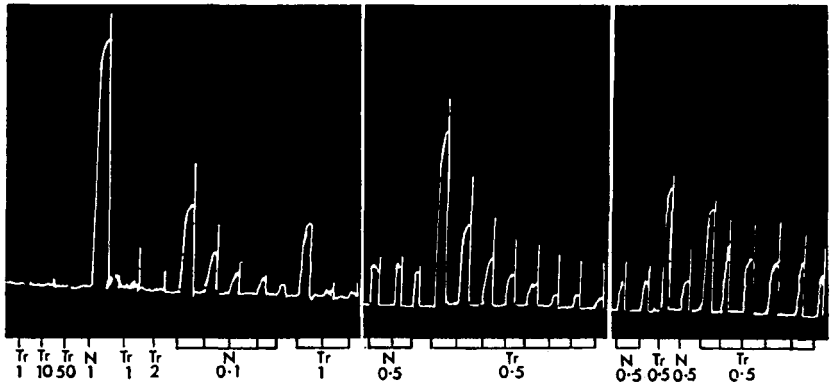

FIG. 1. The responses of isolated rat vas deferens to succesive doses of tranylcypromine (Tr) and noradrenaline (N), added to the bath at the points indicated. Drug concentrations μg/ml.