An adrenoceptor proteolipid isolated from the spleen capsule

Our laboratory has been engaged in the isolation of proteolipids (i.e. hydrophobic lipoproteins) from the central nervous system having high affinity binding for acetylcholine-like drugs (De Robertis, Fiszer & Soto, 1967), 5-hydroxytryptamine (Fiszer & De Robertis, 1969) and adrenergic blocking agents (Fiszer & De Robertis, 1968, De Robertis & Fiszer de Plazas, 1969). All these proteolipids with receptor properties are eluted in chloroform–methanol (4 : 1) from a Sephadex LH20 column. Furthermore a special proteolipid peak eluted in chloroform was isolated from electric organ of *Electrophorus* and *Torpedo* (La Torre, Lunt & De Robertis, 1970) and from skeletal muscle (Lunt, Stefani & De Robertis, 1971) and shown to bind [^{14}C]acetylcholine and other acetylcholine-like ligands. These findings led us to use a similar approach for the isolation of a peripheral adrenoceptor using ($\pm$)-[^{3}H]noradrenaline and a tissue having a rich sympathetic innervation, such as the spleen capsule of bovines.

After mechanical isolation, the tissue was freeze-dried and extracted with chloroform–methanol (2 : 1, by volume). The ($\pm$)-[^{3}H]noradrenaline (6·6 Ci/mM, New England Nuclear) was added to the extract in final concentrations of $1{\cdot}6 \times 10^{-8}$ to 5×10^{-5}M. For the isolation of the different proteolipids, the extract was then submitted to column chromatography on Sephadex LH20 and elution with chloroform followed by mixtures of chloroform–methanol of increasing polarity according to De Robertis, Fiszer & others (1969).

Fig. 1 shows the chromatographic pattern which was constantly obtained, in the presence as well as in the absence of noradrenaline. There are four peaks of protein eluted in the chloroform and one in the chloroform–methanol (4 : 1 by volume) at the end of the chromatogram. The [^{3}H]noradrenaline, at concentrations up to 5×10^{-5}M, appeared in a single peak coinciding with peak 1 of proteolipid, which hence will be called the adrenoceptor proteolipid. The shape of the saturation curve of the binding of noradrenaline suggested the presence of multiple sites of

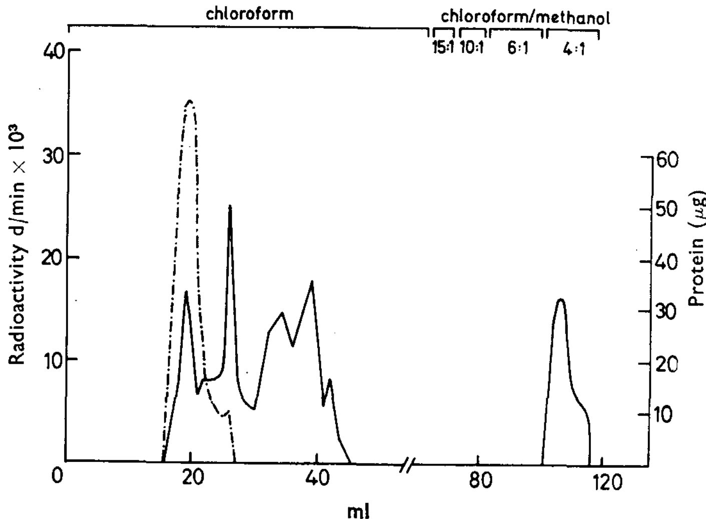

FIG. 1. Chromatographic pattern of the proteolipids extracted from freeze-dried spleen capsule The chloroform–methanol (2:1, v/v) extract was submitted to binding with $1{\cdot}6 \times 10^{8}$ ($\pm$)-[^{3}H]-noradrenaline and then eluted through a column of Sephadex LH20. The radioactivity appears with peak 1 of proteolipid protein determined by the Lowry's method. — · — [^{3}H]noradrenaline. —Protein.

binding having different dissociation constants. The high affinity binding has an apparent dissociation constant $K_1 = 3{\cdot}3 \times 10^{-7}$ while for the sites of lower affinity the K_2 was $1{\cdot}8 \times 10^{-5}$. The saturation of binding sites of high affinity was apparently obtained when 1 mol of noradrenaline was bound to 200 000 g of proteolipid.

The amount of receptor proteolipid that may be extracted from 2 grams of freeze-dried tissue is extremely small. In fact there is only 80–100 μg of protein in this peak and, assuming a molecular weight of 200 000 for this proteolipid, it may be calculated that in the spleen capsule there are $1{\cdot}5 \times 10^{11}$ receptor molecules per mg dry tissue. Such figure agrees with data of binding sites for [^{3}H]phenoxybenzamine to the rat seminal vesicle (Lewis & Miller, 1966) and of ^{3}H-Sy28 [(2-bromoethyl)-ethyl-1-naphthalene-methylamine], bound to rabbit aortic strips (Moran & Triggle, 1970) in which 2×10^{11} and $1{\cdot}5 \times 10^{12}$ receptor sites per mg dry tissue were respectively estimated.

This work was supported by Grants from the Consejo Nacional de Investigaciones Cientficas y Técnicas, Argentina (CONICET) and the National Institutes of Health (5 RO1 NS 06953–05 NEUA).

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September 1, 1971

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The effect of (−)-noradrenaline on artificial lipidic membranes containing a proteolipid with adrenoceptor properties

Previous work from this laboratory has demonstrated that proteolipids extracted from several tissues show pharmacological receptor properties (for a review see De Robertis, 1971). It was thus possible to obtain information relevant to the first step of the drug-receptor interaction, i.e., that of the high affinity binding. The second step, that of producing a response, was recently explored by Parisi, Rivas & De Robertis (1971). They incorporated the proteolipid, with cholinoceptor properties extracted from the electric tissue of *Electrophorus electricus* into artificial membranes separating two water compartments containing ions. These membranes responded to the local application of acetylcholine with a sudden increase in electrical conductance which was transient and reversible with time.

As shown in the previous communication, Fiszer de Plazas & De Robertis (1971) isolated a proteolipid with adrenoceptor properties from the bovine spleen capsule. It seemed thus pertinent to incorporate this proteolipid into artificial membranes and to study the conductance changes after the local application of (−)-noradrenaline.

Artificial membranes were made with a brush across a 1 mm hole in a Teflon septum separating two chambers containing 100 mM NaCl and 50 mM tris buffer