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.

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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

Table 1. *Composition of artificial membranes, their resistance at 75 mV, and mean conductance changes in nanoamperes (± s.e.) produced by the injection of various concentrations of (−)-noradrenaline.* n: number of experiments.

Composition		n	ohm/cm²	Conductance change 5×10^{-4}M	5×10^{-3}M	5×10^{-2}M
Cholesterol ..	.. 10 mg/ml	15	$9{\cdot}4 \times 10^6$	—	—	—
Cholesterol ..	.. 10 mg/ml					
Proteolipid with adrenoceptor properties ..	.. 3·5 μg/ml	7	$7{\cdot}6 \times 10^6$	0·04 ± 0·002	0·08 ± 0·001	0·12 ± 0·03
Cholesterol ..	.. 10 mg/ml					
Proteolipid without adrenoceptor properties ..	.. 3·5 μg/ml	6	$3{\cdot}9 \times 10^6$	—	—	—

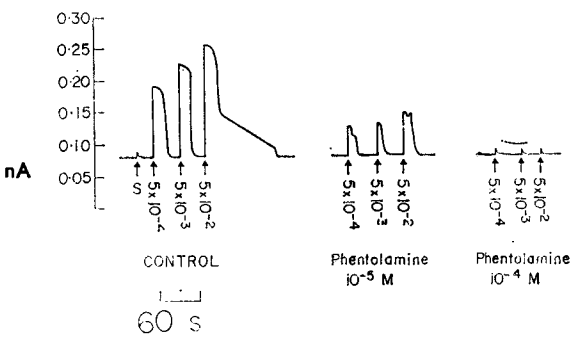

FIG. 1. Left, conductance changes produced in artificial membranes, containing the proteolipid with adrenoceptor properties by the injection of 50 μl aliquots of (—)-noradrenaline at different concentrations. S, injection of bathing solution. The arrows indicate the time of injection. Middle and right, the effect of phentolamine on the (—)-noradrenaline response. The antagonist was incorporated into the bathing solution before membrane formation.

(pH 7·2) according to Vásquez, Parisi & De Robertis (1971). Since a definite constant voltage is applied across the membrane, the recorded DC change reflects the variation in membrane conductance. The drugs, dissolved in water, were applied in 50 μl aliquots by means of a fine capillary tube ending at 2 mm from the positively charged side of the membrane. The concentration of the drug within the pipette refers to the base and is expressed in mol/litre. The composition of some of the membranes used is indicated in Table 1. These components were dissolved in redistilled chloroform, methanol (Merck) and tetradecane (BDH) in the following proportions 1·0 : 0·8 : 0·4 (v/v), while the proteolipids were added in the corresponding solvent in which were eluted from the chromatographic column.

The intensity : voltage curves for the several membranes showed an ohmic relation between 0 and 175 mV. For the study of drug action the membranes were kept at 75 mV. The electrical resistance at this voltage and the effect of noradrenaline on the membrane conductance is indicated in Table 1. Fig. 1 (Control) shows the effect produced by (—)-noradrenaline (as the bitartrate monohydrate, Sterling Winthrop) injected upon membranes containing the proteolipid with adrenoceptor properties. The addition of the drug resulted in a sudden increase in conductance which is transient and returned to the original level in 20-40 s. The amplitude and the

length of the response was dose-dependent, reaching a maximum with 50 μl of 5×10^{-2}M noradrenaline. The fact that the electrical resistance of these membranes is higher than those of biological origin (see Henn & Thompson, 1969) may explain why we could not obtain a response with the injection of aliquots of 10^{-4} (—)-noradrenaline. The membranes gave no response to the application, in similar doses, of the (+)-isomer and also when acetylcholine chloride, (+)-tubocurarine (Calbiochem) or hexamethonium bromide (Squibb) were injected. Other control experiments, giving negative results, included the injection of the bathing saline solution, distilled water and 10^{-2} sodium bitartrate. Similar experiments with proteolipid not associated with the adrenoceptor and which does not bind [^{3}H]noradrenaline, also gave negative results with (—)-noradrenaline (Table 1).

Since the proteolipid associated with the [^{3}H]noradrenaline binding peak contains relatively large amounts of phospholipids, and in view of the controversy on the role of phospholipids in adrenoceptor mechanisms (Belleau, 1967), we studied the phospholipid composition of the proteolipid with adrenoceptor properties by thin-layer chromatography on silica gel (Skipski, Peterson & Barclay, 1964). The peak contained phosphatidylinositol (10 μg/ml) and phosphatidylethanolamine (50 μg/ml) but lacked phosphatidylcholine.

Membranes of cholesterol (Sigma, 99%, standard for chromatography) alone, or of cholesterol plus phosphatidylinositol (100 μg/ml), or phosphatidylethanolamine (100 μg/ml) (both from Mann Research), did not react to (−)-noradrenaline. Membranes containing 10 μg/ml of phosphatidylcholine [(±)-α-phosphatidylcholine, Sigma] also gave negative results, but a response was obtained in membranes with higher concentrations of this phospholipid.

To study drug competition, the adrenoceptor antagonist phentolamine mesylate (Ciba) was incorporated into the bathing solution before the formation of the artificial membrane containing the 'adrenoceptor containing' proteolipid. Fig. 1 shows that this α-adrenoceptor antagonist produces a blockade of the response to (−)-noradrenaline. The results reported here provide further evidence that the proteolipid of peak 1 isolated from the spleen capsule (Fiszer & De Robertis, 1971) possess adrenoceptor properties which depends on the protein and not on the phospholipid moiety.

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