THE INTERACTION BETWEEN ATROPINE SULPHATE AND A PROTEOLIPID FROM CEREBRAL CORTEX STUDIED BY LIGHT SCATTERING*

Eduardo De ROBERTIS, José GONZALEZ-RODRIGUEZ** and David N.TELLER***
Instituto de Anatomía General y Embriología, Facultad de Medicina, Universidad de Buenos Aires, Argentina

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1. Introduction

A special proteolipid, having high affinity binding for dimethyl ^{14}C-*d*-tubocurarine [1], ^{14}C-serotonin [2] and some adrenergic blocking agents [3] has been isolated from gray matter and shown to be concentrated in the nerve-ending membranes [1]. While studying the polarization of fluorescence of such a proteolipid, dissolved in chloroform-methanol (4:1), a notable change in Rayleigh scatter was observed on the addition of atropine sulphate (AS). This finding led us to study the drug-proteolipid interaction under various conditions and using different drugs that may have interfered with such a phenomenon. Although our findings are still preliminary the dose-response curves obtained show a sigmoid shape characteristic of a cooperative interaction [4], which is reminiscent of similar phenomena observed in excitable membranes [5].

2. Methods

The gray matter of the cerebral cortex of cat and ox was dissected, homogenised in water and the total particulate submitted to chloroform-methanol (CM) extraction and partition with water [6]. The proteolipid was purified further by precipitation at 0° with ether and redissolving in CM (4:1). Such a procedure eliminated cholesterol, 80% of phospholipids and about 50% of cerebrosides [7]. The ether precipitate was used as such for some titrations, but in other cases it was passed through a column of Sephadex LH_{20} and eluted with organic solvents of increasing polarity [7]. Four proteolipid peaks were obtained (fig. 1) of which the last two showed the high affinity binding for dimethyl C^{14}-*d*-tubocurarine [8] and ^{14}C-serotonin [2]. These peaks also had a low content of phospholipids [7].

The light scattering was determined with an Aminco-Bowman Spectro-fluorometer. The incident light was set at 430 mμ and the scattered light was measured at 90°, both radiations being vertically polarized by means of Glann prism polarizers. All the titrations were made in 2 ml of CM (4:1) containing 8–30 μg of proteolipid protein. The intensity of the scattered light of these solutions before the beginning of the titration is called T_0. To the cuvette 1 μl samples of a 10^{-3} M AS solution in CM (4:1) was added and the increase in light scattering (T-T_0) was registered at each point, waiting for a maximal response (fig. 1). In other experiments various amounts of AS were added and the response obtained was recorded for 100 seconds. The initial velocity $V_0 = d(T-T_0)/dt$(d in min) was calculated.

T_{max}-T_0 indicates the maximal light scattering change obtained at the end of the titration. Standard and Hill plots of the titrations were made and the Hill coefficients (n_H) were determined:

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** Fellow of Juan March Foundation.
*** On leave from the Research Division, Biochemistry Laboratory, Manhattan State Hospital, Wards Island, New York 10035.

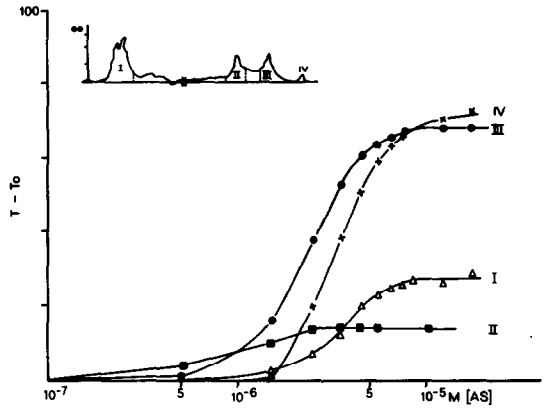

Fig. 1. Light scattering changes (T-T_O) produced by different concentrations of AS on the various protein peaks separated by column chromatography (see inset). Peaks I-IV are proteolipids of total particulate from bovine cerebral cortex precipitated with ether and eluted from Sephadex LH_{20} by organic solvents [7].

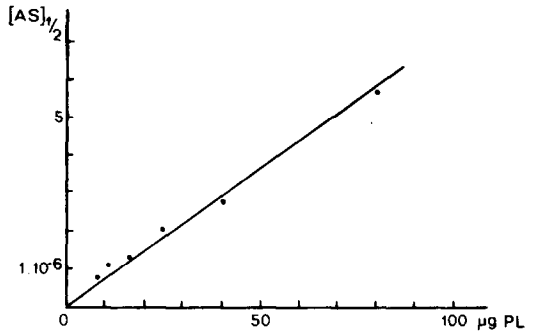

Fig. 2. Plotting of (AS) $\frac{1}{2}$ and proteolipid concentration in µg using an ether precipitate of bovine cerebral cortex.

$$n_H = (\log \frac{T\text{-}T_o}{T_{max}\text{-}T})/\log\ [AS]\ .$$

3. Results

Fig. 1 shows dose-response curves obtained with the different proteolipid peaks eluted from the Sephadex LH_{20} column using bovine cerebral cortex. Peaks III and IV give the typical rapid increase in light scattering with AS, while peaks I and II give much lower responses. The scatter does not change with the first addition of AS but, with further additions it increases, following a characteristic sigmoid shape which reaches saturation level.

The light scattering is proportional to the amount of protein introduced into the cuvette. In the experiment of fig. 2 it is observed that there is a linear relationship between the proteolipid concentration and the concentration of AS needed to obtain half saturation of the optical change. This concentration of AS is referred to as [AS] $\frac{1}{2}$.

The light scattering effect of AS was tested in the presence of increasing concentrations of acetylcholine (ACh) (fig. 3). This treatment reduced the amplitude of the response (T_{max}-T_o), which at 10^{-3} M ACh was only of the order of 40% of the control value but without altering the [AS] $\frac{1}{2}$ with respect to the control (table 1). Dimethyl-*d*-tubocurarine (DMTC) had a very striking effect. At low concentrations it produced no change or even increased the intensity of the scattering; however with higher concentrations (5×10^{-4} M) there was a definite blocking effect which was surmounted only when 5.5×10^{-6} M of AS were added.

The differences between the effects produced by ACh and DMTC are even of greater interest when Hill plots are made (fig. 3). While in the control the n_H is about 3, indicating a certain degree of cooperativity, with ACh it remains the same or tends to decrease (table 1). On the contrary with DMTC the n_H increases considerably reaching 8.6 at 5×10^{-4} M (fig. 3).

In table 1 it may be observed that succinylcholine (Sch) and hexamethonium (Hex) produce an inhibition of the light scattering which is in some way intermediary between those produced by ACh and DMTC. In effect, at high doses, there is a reduction of T_{max}-T_o but at the same time the n_H increases indicating greater cooperativity. This effect is without marked changes in the [AS] $\frac{1}{2}$.

Fig. 4 shows three kinetic experiments done with the same amount of proteolipid and different concentrations of AS added in a single dose. The light scattering increase was registered continuously for the first 100 sec. The initial velocity (V_o) increased with increasing AS concentration up to 4×10^{-6} M. V_o did not increase with higher concentrations of AS but there was a slight increase in T_{max}-T_o. When log V_o is plotted against log AS a straight line is obtained which has a slope of about 3. So the apparent

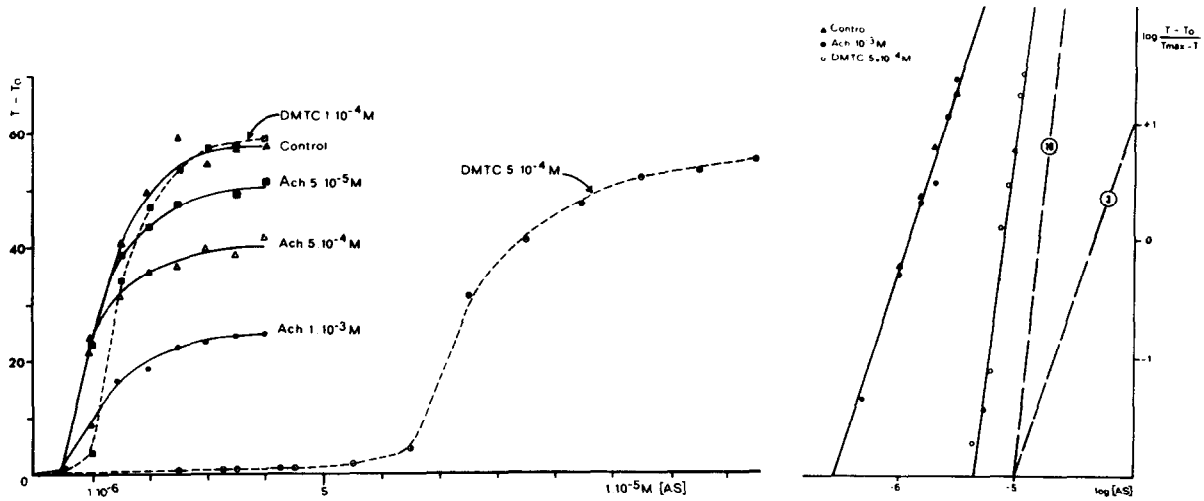

Fig. 3. Left. Standard plotting of the light scatering changes (T-T_0) by AS in proteolipid peak III from the cat cerebral cortex treated as in fig. 1. Observe the effect of AS in the presence of various concentrations of ACh and DMTC. Right. Hill plotting of the same experiments showing the strong change of the n_H by DMTC.

Table 1
Characteristics of the effect of some ligands on the light scattering changes by atropine sulphate.

	T_0	T_{max}-T_0	$[AS]\frac{1}{2}$ M	n_H
CM (4:1)	30	–		
PL (1) 10.6 μg	33	57	1.1×10^{-6}	3.15
PL (2) 21.2 μg	35	100	1.25×10^{-6}	3.59
ACh 5×10^{-5} M	34	51	1.1×10^{-6}	2.75
ACh 5×10^{-4} M	41	41	1.1×10^{-6}	1.98
ACh 5×10^{-3} M	45	24	1.2×10^{-6}	3.38
DMTC 10^{-4} M	35	59	1.4×10^{-6}	4.34
DMTC 5×10^{-4} M	35	55	7.4×10^{-6}	8.63
Sch 10^{-4} M	36	52	1.2×10^{-6}	3.27
Sch 5×10^{-4} M	38	43	1.15×10^{-6}	3.98
Sch 10^{-3} M	36	36	1.9×10^{-6}	7.50
Hex 5×10^{-4} M	37	48	1.8×10^{-6}	3.73
Hex 10^{-3} M	40	34	1.85×10^{-6}	6.36

For all titrations the same amount of proteolipid (PL) as in control (1) was used. The PL was from fraction III (fig. 1) of cat cerebral cortex.

order of reaction with respect to AS concentration coincides with the n_H for the same reaction. V_0 was strongly affected by DMTC and ACh (table 2).

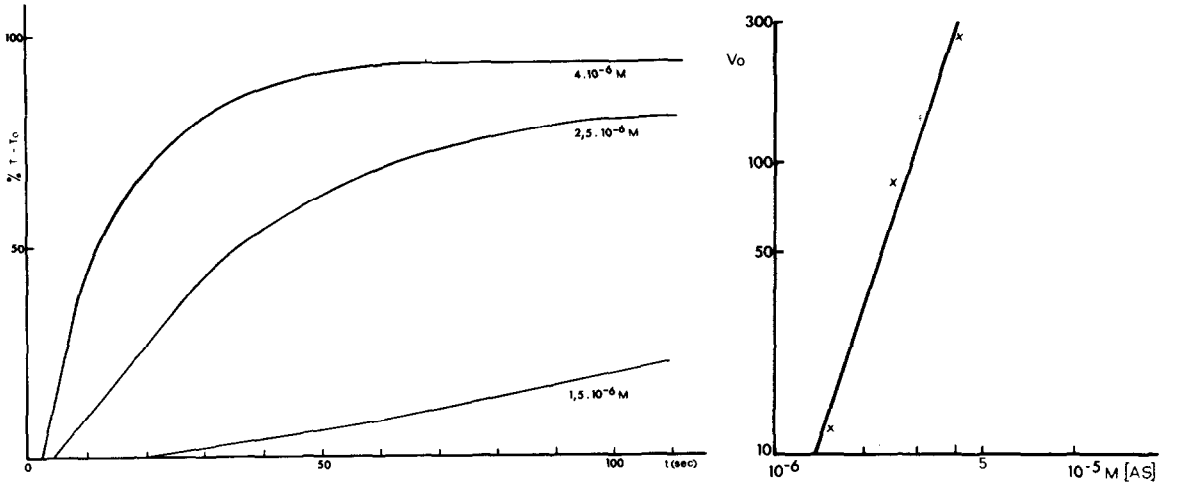

Fig. 4. Left. Light scattering changes (T-T_0), as a function of time, using the same amount of proteolipid (27 μg of ether precipitate of bovine cerebral cortex) and increasing concentrations of AS administered in a single dose. Right. The same experiment but plotting the initial velocity (V_0) against AS concentration.

Table 2
Changes in V_0 of the light scattering by AS with DMTC and ACh.

[DMTC] M	V_0	[ACh] M	V_0
–	83.3	–	83.3
5×10^{-6}	37.1	1.5×10^{-5}	73.8
1×10^{-6}	30.1	5×10^{-5}	55.4
2.5×10^{-5}	11.7	1×10^{-4}	42.8
5×10^{-5}	3.6	5×10^{-4}	16.6

The origin and amount of proteolipid used was the same as in fig. 4. The amount of AS added at $t = 0$ was 2.5×10^{-6} M.

4. Discussion

Light scattering may be used as a method for measuring molecular weight and size of proteins (see [9]). Although at present we do not have information about the degree of homogeneity and the size of the proteolipid isolated from the cerebral cortex it seems possible that the increase in light scattering with AS could be due to the association of molecular or micellar units into larger assemblies. This phenomenon is not observed using atropine base, suggesting that, due to the low polarity of the medium, the AS is only a little ionized and able to bind to two units of proteolipid. This type of conformational change, resulting in an increase in particle size, could easily explain the changes in light scattering. Furthermore with atropine base and with homatropine bromide it has been possible to block to a certain extent the effect of AS.

Another point of interest is the sigmoid shape of the dose-response curve and the n_H of 3 which indicates a certain degree of cooperativity in the AS-proteolipid interaction. In a study of the changes in membrane potential of the electroplax with cholinergic activators such as carbamylcholine an n_H of about 2 was found by Changeux and Podlesski [5], who on this basis suggested an analogy between excitable membranes and regulatory enzymes. In our study the degree of cooperativity changed little with ACh but had a dramatic change with DMTC and also with hexamethonium and succinylcholine with which, at high concentrations, the n_H was more than doubled. This light scattering change probably does not correspond to a stereospecific binding since it can be obtained with other amine sulphates such as: eserine, amphetamine, dibenzylamine and strychnine. However it is evident that there is a certain group-specificity between the various ligands and the proteolipids; further studies may clarify this point.

These light scattering changes are of great interest

in relation to our previous studies on the high affinity binding of labelled serotonin, DMTC and other drugs to the proteolipid [1–3]. In fact in the proteolipids eluted from the Sephadex LH_{20} column there is a coincidence between the property of binding and that of reacting with AS and other amine salts linked with bivalent anions. As a working hypothesis it may be postulated that in the subsynaptic membrane of central synapsis there is a special receptor proteolipid [1] which has group specificity rather than stereo specificity for the various transmitters. However the possibility should also be considered that once separated from the membrane the receptor could have undergone conformational changes by which the stereospecificity became lost.

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