BINDING OF ACETYLCHOLINE AND CHOLINERGIC DRUGS TO PROTEOLIPID FRACTIONS FROM RAT CEREBRAL CORTEX AND TO PHOSPHOLIPIDS FROM BOVINE BRAIN

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1. Introduction

An earlier report [1] described preparations of proteolipid fractions at -60° C from quenched rat cerebral cortex using column chromatography. When the proteolipids, after precipitation from the extract by diethyl ether, were incubated with a particular cholinergic substance, this substance was eluted in coincident peaks with specific proteolipid fractions. In elution with chloroform, the first peak of acetylcholine appeared in coincidence only with the second proteolipid peak and that of dimethyltubocurarine (or decamethonium) with the first.

We shall now discuss results obtained when these proteolipid fractions dissolved separately or together in chloromorm–methanol (2:1 v/v) at room remperature were introduced in the biphasic system resulting when the above organic solutions were mixed with aqueous solutions containing the individual cholinergic substances. In the absence of proteolipids these substances remain almost entirely in the aqueous phase but in their presence, depending on the proteolipid fraction, they are shifted into the organic phase in response to binding. Such a distribution method had been used by Weber et al. [2] with a fluorescent cholinergic drug together with proteolipid fractions prepared at room temperature when no resolution had been observed between fractions binding acetylcholine and dimethyltubocurarine (or decamethonium). Changes in fluorescence intensities had reflected the shift in concentrations of the drug. In the present experiments the concentration changes in the two phases were followed by scintillation counting of the radioactively tagged cholinergic substances. This analytical procedure was applied also to three phospholipids in place of the proteolipids.

2. Materials and methods

Total proteolipids of the extract in the form of the dried ether precipitate were warmed to 0°C, dissolved in chloroform–methanol (2:1 v/v) and diluted by the same solvent to the appropriate sequential concentrations of fig. 1, expressed as μg protein (absorption at 280 nm) per 3 ml of solvent mixture. To the 3 ml was added at 0° C 1 ml aqueous solution (2×10^{-5} M) acetyl-1-^{14}C choline iodide, decamethonium 1, 10-^{14}C diiodide or dimethyl ^{14}C-d-tubocurarine dichloride (all from New England Nuclear, Boston, Massachusetts). The tubes were shaken vigorously using Vortex mixer for 2 min at room temperature and centrifuged to get a clear separation of upper aqueous (2 ml) and lower organic phases (2 ml). The radioactivities of the phases were estimated by a standard liquid scintillation method.

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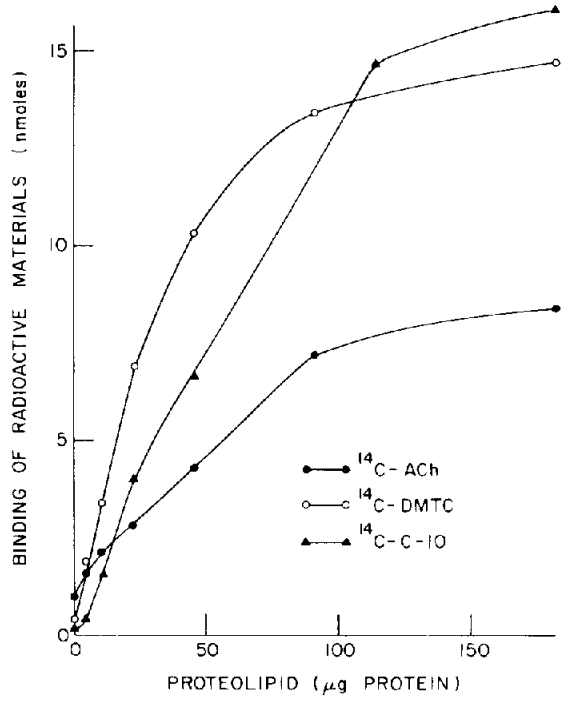

Fig. 1. The bindings of radioactive acetylcholine (ACh), dimethyltubocurarine (DMTC), decamethonium (C-10) to proteolipids from rat cerebral cortex.

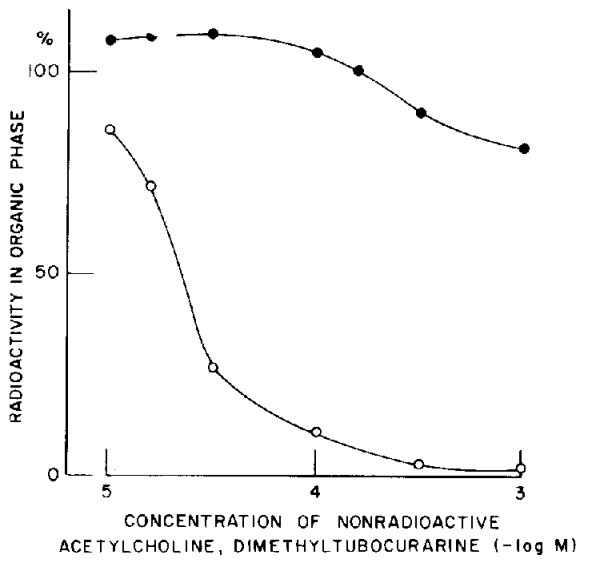

Fig. 2. Reciprocal inhibition to the binding by radioactive cholinergic substances to unchromatographed proteolipids in the organic phase. (○) Dimethyl-^{14}C tubocurarine + nonradioactive acetylcholine. (●) ^{14}C-acetylcholine + non-radioactive dimethyltubocurarine. 100%: ^{14}C-acetylcholine, 0.71×10^{-8} moles; Dimethyl-^{14}C tubocurarine, 1.34×10^{-8} moles.

The proteolipid fractions were each similarly dissolved in chloroform–methanol (2:1 v/v), brought to the appropriate concentrations and treated in the same way as the total lipids from which they had been fractionated.

For experiments on reciprocal inhibition to binding (fig. 2), ether precipitates of proteolipids dried completely by evaporation at −60°C under high vacuum were warmed to ice temperature and dissolved in the chloroform–methanol mixture to form a solution containing 45 μg proteolipid per ml. Two ml of this solution were mixed at ice temperature with 1 ml of the chloroform–methanol containing non-radioactive acetylcholine or dimethyltubocurarine and kept at this temperature 5 min before using. The concentrations of non-radioactive materials were expressed in the figure as the concentration in the 3 ml of chloroform–methanol mixture. One ml aqueous solution of ^{14}C-acetylcholine (2×10^{-5} M) was added to the tubes containing non-radioactive dimethyltubocurarine and similarly dimethyl-^{14}C-tubocurarine (2×10^{-5} M) was added to that containing non-radioactive acetylcholine. Subsequent procedures were the same as given for fig. 1.

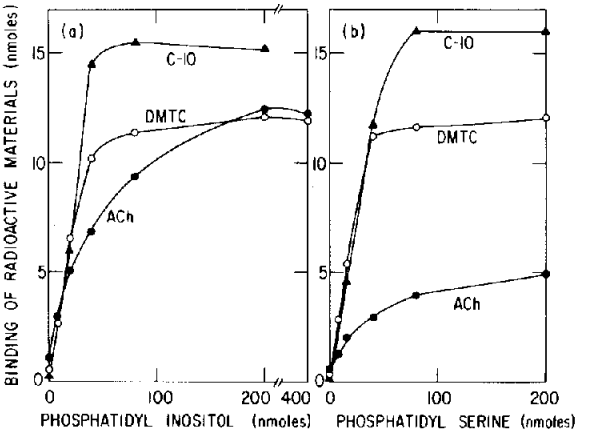

Fig. 3. The bindings of radioactive acetylcholine (ACh), dimethyltubocurarine (DMTC) and decamethonium (C-10) to phospholipids from bovine brain.

For fig. 3 phosphatidyl serine (and also phosphatidyl ethanolamine) of high purity had been freshly prepared by Applied Science Laboratories (State College, Pennsylvania) and similarly monophosphatidyl inositol by General Biochemicals (Chagrin Falls, Ohio). These phospholipids were dissolved in the chloroform–methanol mixture and diluted to the desired concentrations. The subsequent operations were the same as given above for the proteolipids.

3. Results

Fig. 1 represents the concentrations in the biphasic system of three cholinergic substances in the organic phase (in which they are only slightly soluble in the control) as a function of the concentrations of unchromatographed proteolipids introduced into this phase. The saturation values of binding of dimethyltubocurarine and of decamethonium are higher than that of acetylcholine and their initial slopes are steeper. The proteolipid fractions were tested in the same way for binding in biphasic distributions. Bindings to different proteolipid fractions by acetylcholine and by the blocking agents made evident at -60°C were confirmed at room temperature with some loss, however, in specificity by acetylcholine in that it was bound in addition to the second proteolipid peak also to the proteolipid fraction of the first peak but to a lesser extent.

Fig. 2 makes evident the disparity in the reciprocal inhibitions of acetylcholine and dimethyltubocurarine in their bindings to proteolipids. Clearly the inhibition by dimethyltubocurarine against binding by acetylene is much stronger than the reverse inhibition.

Fig. 3 represents the affinities of cholinergic substances for phosphatidyl serine (PS) and for monophosphatidyl inositol (PI). The saturation concentration of acetylcholine induced by the presence of phosphatidyl inositol in the organic phase is about twice that induced by phosphatidyl serine. The binding of acetylcholine has actually not reached saturation at the highest concentration of the phosphatidyl serine of the figure. The initial slope of the curve for acetylcholine in the presence of phosphatidyl inositol is higher than in the presence of phosphatidyl serine. The affinities of dimethyltubocurarine and of decamethonium for both these phospholipids seem about equal and greater than the affinities for acetylcholine. Similar biphasic analyses proved that dimethyltubocurarine had but slight affinity for phosphatidyl ethanolamine and acetylcholine had virtually none.

4. Discussion

A proper analysis of strengths of binding of the unchromatized proteolipids of fig. 1 requires substantially more measurements than we have made (see Weber et al. [2]). From the steeper slope at the origin comes the suggestion that the blocking agents bind more tightly at their most tightly bound site, (if the site next in tightness is considerably weaker) than does acetylcholine to a corresponding site. Further suggestion of this is shown (fig. 2) in the greater inhibition by dimethyltubocurarine against the binding by acetylcholine than the reciprocal inhibition, acetylcholine against dimethyltubocurarine.

We may consider two factors that would induce loss in the specificity of binding by acetylcholine at room temperature as compared with that at -60°C. (1) The organic solvents used at the higher temperature may have modified the conformation of the proteolipids of the first fraction and dissociated off some of their lipid components or exchanged some with others in the extract [3]. On the other hand, the specificity observed at -60°C was of proteolipids whose highest temperature of contact with chloroform–methanol was -60°C. It had originated in rat cerebral cortex quenched to -195°C and warmed to -60°C for extraction and fractionation. (2) The conformation of the first fraction of proteolipids had been modified by its contact with water in the biphasic distribution and the water may have brought new attracting groups to the surface of the proteolipid.

Another factor differentiates the preparation of -60°C and of room temperature, namely their different populations of proteolipids. In the extraction at -60°C a fraction of the proteolipids in the tissue dissociated off upon extraction, that on the average had weaker bonds with the residue than that requiring higher temperatures for extraction. Extraction at successively higher temperatures suggests itself for future work.

Phospholipids are known to be components of the

proteolipids. Phosphatidyl inositol [4] and probably phosphatidyl serine are among them. Although without specificity for binding when free, they would be expected if within the active binding sites of the proteolipids to contribute to the specificity as well as to the strength of binding with cholinergic substances.

Acknowledgement

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References

[1] Izumi, F. and Freed, S. (1974) FEBS Letters (in press).
[2] Weber,G., Borris, D.P., De Robertis, E., Barrantes, F.I., La Torre, I.L. and Llorente de Carlin, M.G., (1971) Mol. Pharmacol. 7, 530.
[3] Lees, M.B., Carr, S. and Folch, J. (1964) Biochim. Biophys. Acta 87, 489.
[4] Lunt, G.G., Canessa, O.M. and De Robertis, E.(1971) Nature New Biology 230, 187.