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LOCALIZATION AND CHARACTERIZATION OF CONCANAVALIN A RECEPTORS IN THE SYNAPTIC CLEFT

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INTRODUCTION

At the synapse the specialized pre- and postsynaptic membranes are connected via a structure called the synaptic junction. In order to understand the nature of the synaptic junction or connection, it is necessary to determine which molecules are associated with the surfaces of the specialized pre- and postsynaptic membranes and which are present in the cleft itself. Cytochemical evidence indicates the possible presence of carbohydrates in the cleft. The cleft area stains with ruthenium red and periodic acid-Schiff stain (Rambourg and Leblond, 1969; Bondareff, 1967). Recently Matus et al. (1973) suggested that concanavalin A (Con A) binding sites may be present in the synaptic cleft. Since Con A binds to mannopyranosyl or glucopyranosyl receptors, the cleft would appear to contain these sugars or closely related molecules. At present it is not known whether Con A sites are soluble or membrane bound and nothing is known about the type of molecule to which the lectin binds.

In this paper we describe the organization of Con A binding sites in the synaptic cleft of isolated synaptosomes and synaptic junctional complexes (SJC) by the use of ferritin-Con A conjugates and quantitate the binding and nature of the receptors by the use of radioactive Con A. We confirm the observations of Matus et al. (1973) and demonstrate that within the cleft a portion of the Con A binding sites are present on the external surface of the postsynaptic membrane overlying the postsynaptic density (PSD). These sites appear to be mainly glycoproteins. In contrast to the surface membrane the underlying PSD binds very little, if any, Con A conjugate.

MATERIALS AND METHODS

Con A was purified from Jack Bean meal (Sigma Chemical Co., St. Louis, Mo.) by affinity chromatography (Agrawal and Goldstein, 1967) and made radioactive by iodination with ^{125}I ($[^{125}I]$ Na, New England Nuclear, Boston, Mass.) by the procedure of Greenwood et al. (1963) in the presence of 0.1 M α-methyl glucoside (α-MG). Excess free iodide was removed by repeated di-

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alysis. Specific activities of Con A varied from 0.1 to 15×10^6 cpm/mg protein. Conjugates between Con A and ferritin (Polysciences, Inc., Warrington, Pa.) were prepared based on earlier methods (Nicholson and Singer, 1971; Stobo and Rosenthal, 1972). Con A was coupled to purified ferritin with 0.05% glutaraldehyde (Polysciences, Inc.) in the presence of 0.1 M α-MG. The conjugate was separated from free Con A and most free ferritin on an Agarose column (Bio-Rad Laboratories, Richmond, Calif.) or by affinity chromatography on Sephadex G-50 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), followed by separation on Agarose. The latter procedure gave lower backgrounds. In some experiments iodinated Con A was mixed with nonlabeled Con A in order to prepare an [^{125}I]Con A-ferritin conjugate. Subcellular fractions were prepared from the forebrain of adult rats by standard procedures. Synaptosomal and synaptic plasma membrane fractions were prepared as described by Cotman and Matthews (1971) and SJC, previously referred to as synaptic complexes, were prepared by the method of Cotman and Taylor (1972). In the isolation of SJC, a synaptic membrane (SM) fraction was treated with 0.5% Triton X-100 before density gradient centrifugation. All fractions were washed with 0.1 M NaCl 0.05 M Bicine, pH 7.0 before incubation. The amount of Con A bound to various fractions was determined by incubating fractions (30–180 μg protein) with 30 μg ^{125}I-labeled Con A in buffer (1.0 M NaCl 0.05 Bicine, pH 6.5) for 1 h at room temperature. Under these conditions Con A was saturating and binding was proportional to the amount of tissue protein present. Free Con A was removed by pelleting the fraction (50,000 *g* for 10 min) and washing the pellet three times with buffer. The specific binding was determined by the difference in Con A bound in the presence and absence of 0.1 M α-MG. Con A specifically binds α-MG and related sugars so that the difference in binding in the absence and presence of excess α-MG provides a measure of specific interactions. Binding in the absence of α-MG was five- to tenfold above that in the presence of α-MG. Incubation of samples with Con A-ferritin conjugates was carried out with saturating concentrations of Con A-ferritin. Experiments on the localization of conjugate binding in the SJC, SM, and synaptosomal fractions were carried out at least twice for each fraction, with similar results.

RESULTS

All subcellular fractions bound substantial quantities of ^{125}I-labeled Con A (Table I). SM fractions bound slightly more than the SJC fraction. Myelin and synaptic membranes bound the highest amounts and a mitochondrial fraction the lowest. These data suggest that the average number of receptors per milligram protein in SJC is slightly less than in the SM fractions.

Con A-ferritin conjugates provide a means to localize the Con A sites more precisely. In isolated SJC, ferritin-Con A selectively interacted with the external surface of the plasma membrane overlying the PSD (Fig. 1 *a*). The ferritin molecules were seen lined up along the cleft in close correspondence to the surface specializations ("bristles") of the postsynaptic membrane previously reported (Cotman and Taylor, 1972). α-MG inhibited the labeling of the cleft area (Fig. 1 *b*). In each case, conjugate was present only in areas where the cleft was dissociated, indicating that the cleft area was impermeable to the large ferritin molecules. Little conjugate was bound to the PSD or on either membrane segments outside the confines of the synaptic region or other membrane fragments present in the fraction. The majority of Con A binding sites in the SJC fraction were present on the postsynaptic membrane overlying the PSD. These data were generally reproduced in SM or synaptosomal fractions. In addition, the labeling of membrane areas outside the confines of the cleft can be examined in these fractions which have not been treated with detergent. In these fractions the conjugates bound to open (Fig. 2 *f*) or partially opened clefts (Fig. 2 *a–f*). Ferritin molecules were aligned over the PSD and diminished in number in outlying membrane areas away from the synaptic region. Membrane areas outside the cleft displayed only scattered ferritin molecules under our conditions. Ferritin on the presynaptic plasma membrane at the

TABLE I

The Quantity of ^{125}I-Labeled Con A Bound to Various Subcellular Fractions

Fraction	Relative binding*
SM	1.34, 1.10, 1.53
SC	0.96, 0.65
Myelin	1.41, 1.38
Mitochondria	0.12, 0.05
Synaptosomes	0.40, 0.38

Data are given as the ratio of the specific activity of Con A in the subcellular fraction to that in the total particulate fraction of brain.

* Each value represents the result of an individual experiment. The specific activity of the homogenate total particulate fraction in a typical experiment was 5×10^5 cpm/mg protein which amounted to binding in the range of 30 μg of Con A/mg protein. Binding was determined by the difference in Con A bound in the presence and absence of α-MG as described in Materials and Methods. The homogenate total particulate fraction was prepared by pelleting the homogenate at 100,000 *g* for 0.5 h.

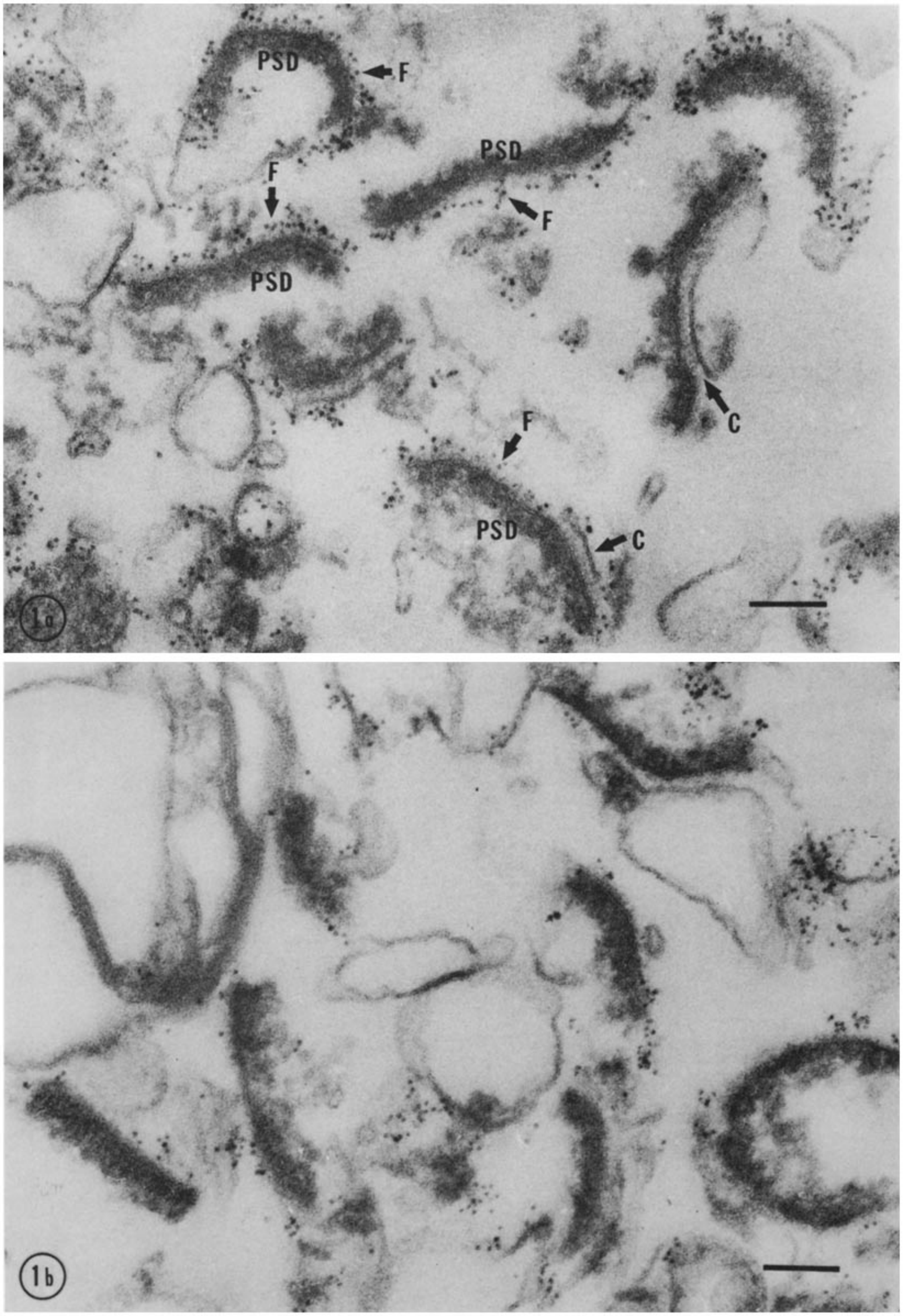

FIGURE 1 Electron micrograph of a SJC fraction which has been incubated with Con A-ferritin conjugates in the absence (*a*) and presence (*b*) of α-MG. In those complexes where the cleft is open, exposing the outer surface of the postsynaptic membrane, the ferritin molecules are seen localized on the external surface of the postsynaptic membrane (*F*) overlying the postsynaptic density (*PSD*). Little ferritin is found on the PSD. Ferritin is excluded from the cleft where the presynaptic membrane remains attached to the postsynaptic (*C*). α-MG selectively inhibits the binding of conjugate to the postsynaptic membrane surface. Bar is 0.1 μm. $\times$ 120,000.

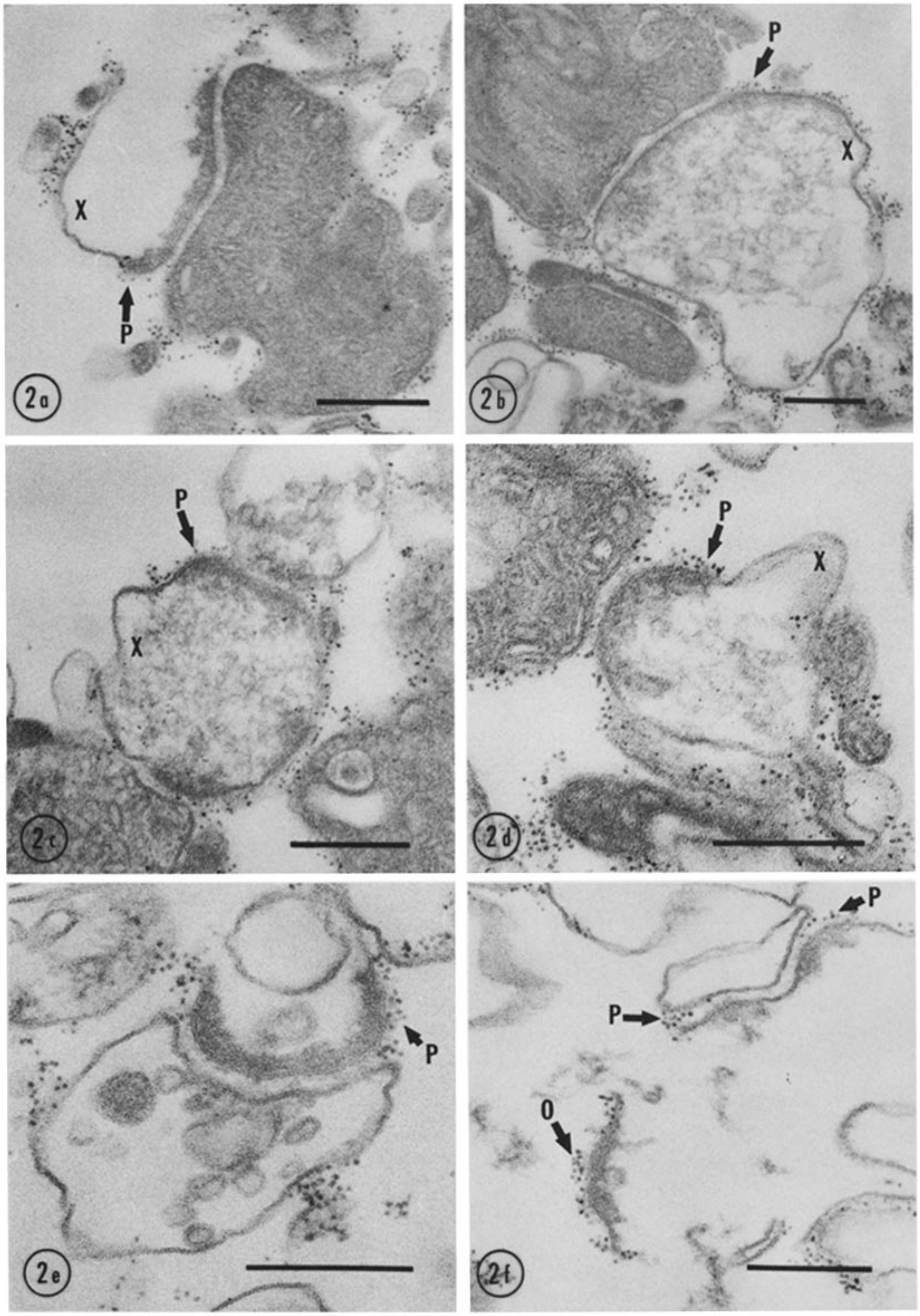

FIGURE 2 Electron micrograph of synaptosomal (*a–d*) or synaptic membrane fractions (*e, f*) which have been incubated with Con A-ferritin conjugate. Ferritin is found at open (*O* in Fig. 2 *f*) or partially opened clefts (*P* in Fig. 2 *a–f*). Outside the cleft region ferritin is often absent or is unevenly distributed (*X* in Fig. 2 *a–d*). The inner surface of the postsynaptic membrane or the PSD bind very little conjugate (Fig. 2 *a* and *f*). Bar is 0.2 μm. (*a*) × 85,000, (*b*) × 60,000, (*c*) × 90,000, (*d*) × 115,000, (*e*) × 124,000, (*f*) × 95,000.

surface which is complementary to the postsynaptic membrane surface overlying the PSD was scattered and sometimes not apparent.

A possible difficulty with the localization determined by conjugate arises from its inaccessibility to some of the binding sites which is introduced by the large ferritin molecules. In order to determine if the distribution of ferritin failed to reveal some Con A binding sites, the amount of free Con A bound by an SJC fraction was compared with that of conjugate. ^{125}I-labeled Con A and ^{125}I-labeled Con A-ferritin were prepared to identical specific activity and binding was tested at saturating concentrations. SJC bound 25–50% less ^{125}I-labeled Con A-ferritin than ^{125}I-labeled Con A. The SJC fraction contains SJC with intact clefts as well as those in which the presynaptic membrane is removed (see Fig. 1). The reduced binding probably results in part from the presence of a number of intact clefts which exclude conjugate. Thus, although differences exist, conjugated and unconjugated Con A bound in similar quantities so that the cytochemical analysis revealed the majority of binding sites in SJC fractions.

The interaction of Con A-ferritin conjugates with the external surface of the postsynaptic plasma membrane provides strong evidence for the presence of specific carbohydrate residues within the cleft. The receptors could be glycoproteins, glycolipids, or tightly bound carbohydrates or carbohydrate polymers. The receptors appeared to be in part protein bound since chloroform/methanol (2:1) extraction of glycolipids and lipids from the SJC fraction removed only 30% of the Con A binding. Whether the lost binding was due to a loss of glycolipids or an inactivation of binding sites cannot be definitely determined at present. Nonetheless, part of the Con A binding appeared to be to proteins which is consistent with the finding that the majority of carbohydrates in SJC fractions appeared protein bound.[1]

DISCUSSION

Our findings demonstrate that at least a part of the Con A binding in the synaptic cleft is membrane bound and localized on the surface of the postsynaptic membrane exactly overlying the PSD. These Con A sites closely correspond in location to the postsynaptic membrane projections (bristles) which are also localized on the surface of the postsynaptic membrane overlying the PSD (Van der Loos, 1963; Cotman and Taylor, 1972). By comparison, in agreement with data reported by Matus et al. (1973) both the inner surface of the synaptic membrane and the PSD bind very little Con A conjugate. Essentially all postsynaptic membranes with a prominent PSD display Con A binding sites which indicates that the presence of Con A receptors may be a property of all asymmetric type synapses (type I) (Gray, 1959; Colonnier, 1968) in rat brain. The postsynaptic Con A receptors are in part glycoprotein(s) and, on the basis of the selective binding of Con A to mannopyranosyl or α-*O*-glucopyranosyl residues, the glycoprotein(s) appear to contain these residues. Thus the postsynaptic membrane of asymmetric synapses contains a glycoprotein(s) which probably has terminal mannopyranosyl or glycopyranosyl residues.

The Con A receptors which form part of the postsynaptic membrane surface appear to be a stable part of the membrane in two ways: (*a*) they appear to be unusually tightly structured into the postsynaptic membrane complex and (*b*) they are probably immobile. First, as part of the isolation procedure for SJC the PM fraction is treated with Triton X-100. Yet the Con A receptors are present and appear similar in the cleft in both SM fractions and SJC fractions. Unlike the cleft Con A sites, those outside the area delineated by the PSD are reduced in SJC fractions and rarely evident. The fact that Con A sites persist selectively in the cleft after Triton treatment is striking evidence that they are tightly bound in the postsynaptic membrane complex. In the erythrocyte membrane, on the other hand, Triton X-100 removes the majority of the glycoproteins and in doing so removes proteins known to span the membranes (Yu et al., 1973). Second, in nerve membrane areas outside the cleft Matus et al. (1973) obtained indications that Con A receptors are mobile. In aldehyde-fixed preparations Con A conjugates distributed diffusely and uniformly over the surface whereas in the absence of prior fixation Con A sites can be induced to reorganize into small clusters. In our study, samples are fixed after incubation with conjugate, so it would be expected that Con A receptors capable of mobility could reorganize into a scattered array. As shown by

[1] Churchill, L., G. Banker, and C. W. Cotman. 1974. The protein and carbohydrate composition of a synaptic complex and postsynaptic density fractions. Manuscript in preparation.

Matus et al. (1973), and as described in this study, extrajunctional Con A receptors are not organized in a reproducible pattern, and thus may be mobile. By contrast, Con A receptors in the cleft are quite consistently found overlying the PSD under conditions which favor mobility. Hence the Con A receptors in the cleft appear to be immobile. It may be, as suggested previously (Cotman and Taylor, 1972), that the specialized postsynaptic binding sites are stabilized by the PSD, which serves to restrict the diffusion of these and perhaps other synaptic molecules away from the synaptic area. This possibility derives some support from studies on other membranes where the lateral diffusion of proteins on the surface is restricted by proteins located on the cytoplasmic side of the membrane (Nicolson, 1973; Nicolson and Painter, 1973).

Con A receptors in the synaptic cleft could play any number of roles in synaptic function. These molecules could be a part of the specific transmitter receptor or allied structures of the postsynaptic membrane. Because Con A sites are associated with most, if not all, synapses characterized by a PSD, a role in synaptic transmission which is general, rather than specific to individual transmitter systems, is implied.

The molecules which bind Con A within the cleft may be involved in the junction of pre- and postsynaptic membranes through either a covalent or noncovalent interaction. In one model, the carbohydrates on the pre- and postsynaptic membrane surfaces interact with either soluble or membrane lectin-like molecules organized on the complementary membrane surface and thus join the pre- and postsynaptic membranes. Such an interaction could provide a stable strong attachment. In addition, a lectin-like interaction could be quite specific and assist in selective formation of synaptic contacts. Recently, Churchill and Cotman[2] obtained evidence that certain sugars (glucose, sucrose, fucose, etc.) bind to SJC and PSD fractions. This finding suggests that lectin-like receptors for various sugars are present at synaptic areas.

In summary, our data suggest that presence of lectin receptors and lectin-like molecules at the synaptic cleft which may join the pre- and postsynaptic membranes or serve other functions on the postsynaptic side.

[2] Churchill, L., and C. W. Cotman. 1974. Interaction of carbohydrates with isolated synaptic junctional complexes and postsynaptic densities. Manuscript in preparation.

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