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SERUM ALBUMIN STIMULATION OF SYNAPTOSOMAL PROLINE UPTAKE: PARTIAL IDENTIFICATION OF THE ACTIVE SITE

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Summary

Synaptosomal uptake of proline is stimulated by serum albumin. Evidence is presented which indicates that peptide fragments isolated from the N-terminal region of serum albumin do not stimulate synaptosomal uptake of proline, while fragments derived from the C-terminal region have stimulatory capacities comparable to that of the parent albumin. The site responsible for the stimulatory effect has been tentatively located in the sequence region 377-504 of the albumin molecule.

Nerve terminals isolated from brain tissue (synaptosomes) possess a high affinity transport system for proline that is strictly Na+-dependent (1). The existence of this transport system and other evidence as well (2,3) raise the possibility that proline may have a neurotransmitter function in the central nervous system. Such a transport system would provide a reuptake mechanism for proline release at the nerve endings. Studies in our laboratory have revealed that the synaptosomal transport

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of proline is markedly stimulated by serum albumin. We have examined a number of characteristics of this stimulatory effect and these will be dealt with elsewhere (manuscript in preparation). In these studies, evidence was obtained that the stimulatory effect is not due to any contaminating molecules adsorbed onto the albumin but, rather, is an intrinsic property of the albumin molecule itself.

The primary structure of albumin is now fairly clearly established (4) and the availability of peptide fragments of known amino acid sequences isolated from albumin by a variety of procedures enables us to delineate, with some precision, the site responsible or needed for the stimulatory effect (5-7). In the present paper we show that peptide fragments isolated from the N-terminal region of the albumin do not possess proline uptake stimulatory capacity but fragments derived from the C-terminal third of the molecule demonstrates stimulatory activities comparable to the intact albumin molecule. The site responsible for the stimulatory effect has been identified as the sequence region 377-504 in the albumin molecule.

Materials and Methods

[^{14}C]-labeled proline was obtained from New England Nuclear Corp., Boston, MA. and had a specific radioactivity of 230mCi/mmole. Crystalline bovine serum albumin was obtained from Pentex Corporation. Glycylglycyl-L-histidine-N-methyl amide, and L-aspartyl-L-alanyl-L-histidine-N-methyl amide were gifts of Dr. B. Sarkar. The other peptide fragments used in this study were C_2, C_3, P_A, P_B, T_A and Phe (see table 1). C_2 and C_3 were obtained by cyanogen bromide cleavage of serum albumin essentially by the method of King and Spencer (8). P_A and P_B were isolated by peptic digestion of serum albumin by the method of Feldhoff and Peters (9). Fragment T_A was obtained by tryptic hydrolysis of serum albumin as described by Peters and Feldhoff (11). The peptide fragment, Phe, was

isolated by pepsin treatment of albumin and subsequent fractionation with TCA as described by Peters and Hawn (11).

Synaptosomal fractions were prepared from homogenates of adult Sprague-Dawley rat brain cortices by the method of Kurokawa et al. (12). The fractions were suspended in a medium containing 10mM Tris-HCl (pH 7.4), 15mM $MgCl_2$, 150mM NaCl and 1mM KCl. One ml portions of the suspension containing 0.1mg of synaptosomal protein were incubated for 30 min at 25° with 0.1μCi of labeled proline and each of the different peptide fragments or albumin at the concentrations indicated. At the end of the incubation period, the synaptosomal particles were collected on Millipore filters and assayed for radioactivity as described elsewhere (1).

Results and Discussion

The peptide fragments described in this paper all have established amino acid sequences and the location of these fragments within the parent molecule is given in Table 1. Table 1 also summarizes the effects of these peptide fragments on the uptake of proline by isolated nerve endings. The results clearly indicate that fractions isolated from the N-terminal portion of the albumin molecule have no stimulatory effect on the uptake of proline. Thus C_3 (residues 1-183) and P_B (residues 1-306) have little effect on the synaptosomal transport of proline. On the other hand, peptide fragments derived from the C-terminal region of the molecule consistently stimulate synaptosomal uptake of proline. Thus fragments C_2 (residues 184-582), P_A (residues 307-582) and T_A (residues 377-582) stimulate proline uptake to the same extent as the parent albumin. These results strongly suggest that in the albumin molecule the site responsible for stimulation of proline transport occurs between residues 377 and 582. Furthermore, since the fragment Phe (residues 505-582) shows only minimal stimulation of proline transport, the principal site for stimulation may involve only residues 377-504. The fact that pure peptide fragments of established amino acid

TABLE 1

EFFECTS OF PEPTIDE FRAGMENTS FROM SERUM ALBUMIN ON SYNAPTOSOMAL PROLINE UPTAKE

Experimental conditions are as described in the text. Values represent mean ± S.D. of the numbers of experiments given in parentheses.

Fragment	Residue Numbers in Albumin Molecule	Concentration (mM)	[^{14}C]-Proline Uptake (cpm)
None	--	--	3823 ± 267 (6)
Serum Albumin	1 - 582	0.03	7169 ± 123 (6)
P_A	307 - 582	0.03	6921 ± 168 (4)
P_B	1 - 306	0.03	4190 ± 87 (4)*
C_3	1 - 183	0.04	4028 ± 110 (4)**
C_2	184 - 582	0.03	6878 ± 241 (4)
Phe	505 - 582	0.03	4728 ± 310 (4)**
T_A	377 - 582	0.04	6896 ± 292 (4)

* Differs significantly from control value ($P < 0.1$)

** Differs significantly from control value ($P < 0.05$)

sequences stimulate uptake of proline demonstrates that the stimulatory effect is an intrinsic property of the albumin molecule per se and not due to any contaminants adsorbed on to the albumin molecule.

Serum albumin has the ability to bind a wide variety of molecules. The list of ligands includes Cu^{++}, fatty acids, bilirubin, and steroids (see ref. 9). The precise site for the binding of some of these ligands has been elucidated in recent years. The Cu^{++}-binding capacity of albumin has now been identified to be present in the N-terminal region (13,14). The observations that C_3 (residues 1-183) and P_B (1-306) have no effect on synaptosomal proline uptake indicate that the site needed for stimulation of proline transport is distinct from the Cu^{++}-binding

site. Consistent with this conclusion is the observation that the peptides glycylglycyl-L-histidine-N-methylamide and L-aspartyl-L-alanyl-L-histidine-N-methylamide, both of which mimic albumin in their Cu^{++} binding properties (15) had no effect on proline uptake (data not shown). The observation that dog serum albumin, which lacks the specific first binding site for Cu^{++}, stimulates the uptake of proline to the same extent as bovine or human albumin is again in line with the conclusion that the Cu^{++}-binding site in serum albumin is not the site needed for stimulation of synaptosomal proline uptake.

King (5) reported that the primary organic ligand binding site of albumin resides in the carboxy terminal two-thirds of the molecule. Reed et al. (7) have extended this finding and showed that different binding sites are involved in the binding of different organic ligands. They have shown that amino acid residues 186-238 of the albumin molecule constitute the essential region for bilirubin binding. The observation that P_B (residues 1-306) has no effect on the uptake of proline suggests that the site responsible for stimulation of proline transport is distinct from the bilirubin binding site. The studies of Reed et al. (7) have also shown that the strongest fatty acid binding site involves residues between 377 and 582. Furthermore, since in their studies peptide fragment Phe (residues 505-582) showed little or no affinity for fatty acids, they concluded that the principal interaction of albumin with fatty acids involves only residues 377-504. The results of the present study indicate that the same site (residues 377-504) is also involved in the stimulation of proline uptake by isolated nerve endings.

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