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LOCALIZATION IN THE SYNAPTIC JUNCTION OF THE CYCLIC AMP STIMULATED INTRINSIC PROTEIN KINASE ACTIVITY OF SYNAPTOSOMAL PLASMA MEMBRANES

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Summary

Synaptosomal plasma membrane fragments contain a tightly bound protein kinase which can catalyse the phosphorylation of endogenous protein the reaction bein stimulated by cyclic AMP. A fraction enriched in synaptic junctions, which can be isolated from Triton X-100-treated synaptosomal plasma membranes, is also enriched in the cyclic AMP stimulated intrinsic protein kinase. The location of the enzyme in the synaptic junction suggests that cyclic AMP-stimulated phosphorylation may have some role in synaptic transmission.

It was first reported in 1970 that synaptic membrane fragments contain a protein kinase which phosphorylates endogenous membrane proteins, and which is stimulated by cyclic AMP [1]. This finding has since been confirmed by other workers [2—4]. The microsomal fraction also contains intrinsic protein kinase activity but the highest activity, and particularly the highest cyclic AMP-stimulated activity is present in synaptosomal plasma membrane fragments [5]. The protein kinase responsible for the phosphorylation of synaptosomal membrane proteins is tightly bound to the fragments and cannot be solubilised by hypotonic washes, or by using detergents which solubilise up to 40% of the membrane protein [5,6]. Synaptosomal plasma membrane fragments have been purified to a point at which they can hardly catalyse the phosphorylation of histones or phosvitin [5] and this, together with the observation that a specific inhibitor of histone kinase activity does not inhibit the intrinsic protein kinase activity of synaptic membrane fragments [3],

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strongly suggests that the phosphorylation reaction is due to a protein kinase which acts specifically on certain membrane proteins.

Techniques are now available for preparing fractions enriched in the synaptic junction from preparations of synaptosomal plasma membranes [7,8] and we have used a modification of these methods to show, that the cyclic AMP-stimulated intrinsic protein kinase activity is concentrated in the synaptic junction.

Synaptosomal plasma membrane fragments were prepared from adult rat brain as previously described [9], except that 50 μM $CaCl_2$ was substituted for 0.1 mM EDTA in some solutions. The synaptosomal plasma membranes were homogenized in 0.2% Triton X-100, 10 mM Tris·HCl (pH 7.0) at a concentration of about 2 mg of protein/ml and layered directly onto discontinuous sucrose gradients consisting of 10 ml portions of 1.4, 1.2, 1.0, 0.85 and 0.6 M sucrose in 1 mM potassium phosphate (pH 7.6) following the method of Davis and Bloom [7]. The gradients were spun at 53 500 $\times$ g for 90 min in an SW 25.2 rotor. All material above the 1.0—1.2 M sucrose interface was pooled and diluted with 0.2% Triton X-100 in 10 mM Tris·HCl (pH 7.0). Material at the 1.0—1.2 and 1.2—1.4 M sucrose interfaces was pooled and diluted with 1 mM potassium phosphate (pH 7.6). The two lots of pooled material were then centrifuged at 78 000 $\times$ g for 2 h, giving rise to pellets of residual membrane (Fraction I) and synaptic junctions (Fraction II), respectively. The supernatant from the first material was taken as the Triton-soluble fraction.

The intrinsic protein kinase activity of the various fractions obtained after detergent treatment of the synaptic membrane fragments was determined, as previously described, by incubating the material with [γ-^{32}P]ATP and measuring the transfer of [^{32}P]phosphate to the endogenous proteins of the fractions [5].

Cyclic AMP stimulated activity is defined as the difference between the basal activity and the activity measured in the presence of cyclic AMP (10 μM). Incubations were carried out for 30 s to allow an estimate of the initial rate of phosphorylation. Due to the presence of intrinsic protein phosphatase activity the time course of phosphorylation is markedly curvilinear [10] complete linearity being not even strictly observed over 30 s. The amount of phosphate incorporated after 30 s incubation is, however, a reasonably close approximation to the initial rate of phosphorylation [6]. (It should be noted that the time-course of protein phosphorylation of synaptic plasma membrane fragments is considerably longer [5] than reported by Ueda et al. [3]).

The distribution of intrinsic protein kinase activity following fractionation of the detergent-treated synaptosomal plasma membranes is shown in Table I. The synaptosomal plasma membranes suspended in 0.2% Triton X-100 had only 50 percent of the activity of synaptosomal plasma membranes previously studied [5], probably due to the use of Ca^{2+} during the preparation which is necessary to preserve synaptic junction morphology, but which tends to inactivate the enzyme [11]. Although over 30 percent of the protein was soluble in Triton X-100, the intrinsic protein kinase activity of this fraction (both with and without cyclic AMP) was negligible. Fraction 1, which does

TABLE I

DISTRIBUTION OF INTRINSIC PROTEIN KINASE ACTIVITY IN FRACTIONS OBTAINED FROM SYNAPTOSOMAL PLASMA MEMBRANE FRAGMENTS

Synaptosomal plasma membranes were homogenized in 0.2% Triton X-100 (0.5 ml/mg protein), and fractionated as described in the text. The intrinsic protein kinase activity was determined as described and specific activity of the intrinsic protein kinase is expressed as nmol of phosphate incorporated/mg protein/30 s. Results are shown as mean ± S.D., with the number of observations in brackets.

Fraction	Intrinsic protein kinase activity				Protein distribution (%)
	Without cyclic AMP		Cyclic AMP stimulated activity		
	Specific activity	% distribution	Specific activity	% distribution	
Synaptosomal plasma membranes	0.72 ± 0.07 (9)	100	0.2 ± 0.02 (9)	100	100
Residual membranes (Fraction I)	1.34 ± 0.24 (8)	80 ± 10 (4)	0.06 ± 0.03 (8)	15 ± 7 (8)	49.5 ± 7 (4)
Synaptic junctions (Fraction II)	0.98 ± 0.2 (12)	10.5 ± 2.5 (12)	1.3 ± 0.3 (12)	60 ± 10 (11)	7.9 ± 2.0 (11)
Triton-soluble fraction	0.04 ± 0.05 (8)	1.8 ± 2 (5)	0	0	34 ± 2.5 (5)

not contain morphologically identifiable synaptic junctions, has some intrinsic protein kinase activity, but is not stimulated by cyclic AMP. By contrast, Fraction II, which consists predominantly, although not entirely, of synaptic junctions [7], has appreciable intrinsic protein kinase activity which is more than doubled by the addition of 10 μM cyclic AMP, 60 percent of the original cyclic AMP-stimulated intrinsic protein kinase activity of the synaptosomal plasma membranes (over 80 percent of the recovered activity), was found in the synaptic junction fraction. It thus appears that the cyclic AMP stimulated, intrinsic protein kinase activity of synaptic membranes is concentrated in the region of the synaptic junction.

It has been shown that cyclic AMP stimulates the phosphorylation of at least two of the several synaptosomal plasma membrane proteins which are phosphorylated by the intrinsic protein kinase [3,5]. We therefore incubated samples of the original synaptic membrane fragments, and the fractions obtained from them, with [^{32}P]ATP for 30 s as described above, and separated the phosphorylated proteins by polyacrylamide gel electrophoresis. The pattern obtained (Fig. 1) shows that two bands (I and II) are concentrated in the synaptic junctions whereas band III is more concentrated in the residual membrane fraction. Our bands II and III correspond approximately in molecular weight to bands I and II of Ueda et al. [3] who reported that cyclic AMP stimulated the phosphorylation of their band I (our band II) more than that of band II (our band III). These results, while not as clear-cut as our observations on the localization of the cyclic AMP-stimulated intrinsic protein kinase, are in agreement with our conclusion.

The function of the phosphorylation of synaptic plasma membrane proteins is not yet known. It is possible that it may regulate passive ion permeability [12] and it has been shown that there is a correlation between the

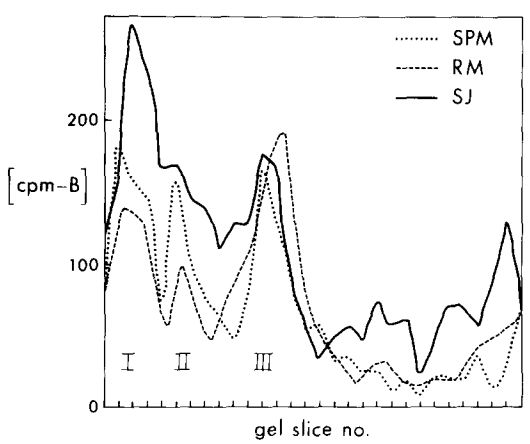

Fig. 1. Distribution of phosphorylated proteins on 12% polyacrylamide gels after electrophoresis in sodium dodecyl sulfate. Samples of the original synaptosomal plasma membrane fragments (. . . . . SPM), residual membranes (- - - - - RM) and synaptic junctions (———SJ) were incubated for 30 s with [γ-^{32}P]ATP and 10 μM cyclic AMP as described in the text. The phosphorylated fractions were processed for gel electrophoresis as previously described [5], 2 mm lengths of gel were treated overnight with 1 ml of hydrogen peroxide, mixed with 10 ml of aquasol (Intertechnique, Paris) and counted in an Intertechnique scintillation counter using the preset ^{32}P channel.

time course of changes in the state of phosphorylation of a specific membrane protein and changes in Na^+ permeability in toad bladder epithelial cell [13] and avian erythrocyte [14] membranes. More directly it has been found that increased phosphorylation of retinal rod outer segment membrane proteins lowers the permeability of the membranes to Ca^{2+} [15] and similar results have been obtained using intact synaptosomes (Weller, M. and Morgan, I., unpublished). It has been shown that electrical stimulation of respiring slices of brain tissue or treatment with agents such as noradrenaline, histamine or dopamine causes increased phosphorylation of membrane proteins, presumably by causing an increase in the concentration of cyclic AMP which stimulates the membrane bound protein kinase [12,16,17]. The location of the cyclic AMP stimulated, intrinsic protein kinase activity in the synaptic junction supports the possibility that it may have a role in the control of synaptic transmission. It is not yet, however, possible to say if the phosphorylation reaction is pre- or post-synaptic.

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