Responses of Synaptosomes to Electrical Stimulation

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When mammalian cerebral-cortical tissue is ruptured in iso-osmotic media by mild dispersion techniques, nerve-endings tear away from their axon and the post-synaptic cell to which they are attached, and survive as discrete cytoplasmic bodies (Gray & Whittaker, 1962; De Robertis, de Iraldi, de Lores Arnaiz & Salganicoff, 1962). The boundary membrane of these particles appears to be largely intact, as judged by such properties as their ability to concentrate K^+ , extrude Na^+ and carry out active glycolysis and oxidation of added glucose linked to ATP and creatine phosphate production (Bradford, 1969; Ling & Abdel-Latif, 1968). Moreover, considerable pools of amino acids are generated from glucose and are concentrated within the synaptosome (Bradford & Thomas, 1969).

The capacity to maintain ion gradients suggests the presence of a synaptosomal transmembrane potential, but, because of the small size of the synaptosomes ($0.5-1.0\mu$), direct measurement of such potentials with intracellular electrodes would be difficult, and indirect methods of detection must be sought. In the present study, one such approach was to expose synaptosomes incubated in Krebs phosphate medium containing 10mM-glucose to oscillating electrical fields and examine changes in their composition and metabolic performance for evidence of membrane depolarization. Such electrical stimulation caused a loss of K^+ and an increase in Na^+ of the synaptosomes, which occurred in parallel with a large (60–80%) increase in glycolytic and respiratory rates. These changes are also seen to accompany electrical stimulation of slices of cerebral cortex and have been interpreted as responses to the measurable membrane depolarization that occurs in the cellular elements of the tissue (McIlwain, 1963; Hillman, Campbell & McIlwain, 1963).

In addition, electrical stimulation caused the loss from synaptosomes to the medium of certain amino acids, those showing loss in by far the greatest proportion being the putative transmitter agents, glutamate, aspartate and α -aminobutyrate. Prisms of cortical tissue incubated under the same conditions showed a similar complex of responses to such stimulation, but loss of amino acids was greatly diminished.

Increasing the K^+ concentration of the incubation medium from 5 to 55mM evoked a similar pattern of responses from synaptosomes, including the release of physiologically active amino acids, thus supporting

the hypothesis that membrane depolarization is the triggering event.

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Peptidase Activity in the Hypothalamus

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Hooper (1962, 1963 & 1966) showed that vasopressinase is present in the hypothalamus of the dog in greater concentration than elsewhere in the brain and presented evidence suggesting that oxytocin was inactivated by the same enzyme. The enzyme was unaffected by mM-EDTA (15min preincubation) but was inhibited by mM- Cu^{2+} .

Hypothalamic oxytocinase was shown to be associated with two subcellular fractions: the supernatant and a mitochondrial fraction. The activities of those fractions changed independently of one another in response to endocrine stimuli.

To assess the importance of these changes the activities of hypothalamic peptidases were studied with the synthetic substrates cystine β -naphthylamide and leucine β -naphthylamide. The enzyme(s) splitting the cystine substrate (cystine aminopeptidase) and those splitting the leucine substrate (various aminopeptidases) were found to have a similar subcellular distribution to oxytocinase. However, both sets of enzymes were found to be evenly distributed throughout the brain except for white matter, which had a low activity towards cystine β -naphthylamide.

Cystine aminopeptidase was almost completely inhibited by Zn^{2+} , Cu^{2+} , Hg^{2+} , Pb^{2+} and Ag^+ (each at 1mM). EDTA preincubated for 15min caused 30–50% inhibition, and diethyl *p*-nitrophenyl phosphate had no effect. CN^- was a weak activator and cysteine a strong one. The velocity of reaction decreased rapidly with time, owing mainly to enzyme instability, the reaction products being only slightly inhibitory and excess of substrate being present throughout. The pH-activity curve was broad, with optimum pH 7.0.

Leucine β -naphthylamide was split by brain homogenate ten times as rapidly as cystine β -

naphthylamide. The pH-activity curve was similar to that of cystine aminopeptidase. Zero-order kinetics were observed, suggesting that most of the enzymes splitting this substrate are different from those splitting cystine β -naphthylamide.

The enzymes splitting both cystine β -naphthylamide and leucine β -naphthylamide were unaffected by the injection of oestrogen either alone or with progesterone. Ovariectomy also had no effect.

It is suggested that changes in hypothalamic oxytocinase activity in response to endocrine stimuli are specific effects and not a reflection of changes in general activities of brain peptidases. Enzymes splitting the synthetic substrates do not behave in the same way as oxytocinase. These results support suggestions that hypothalamic oxytocinase and vasopressinase have a role in the functioning of the hypothalamus as a neuroendocrine organ.

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Dissociation and Reassociation of Rat Liver Mitochondrial Monoamine Oxidase

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The multiplicity of forms of mitochondrial monoamine oxidase (EC 1.4.3.4) of several tissues and species has been established (Youdim & Sandler, 1967; Collins, Youdim & Sandler, 1968; Hartman, Kloepper & Yasunobu, 1969; Youdim, Collins & Sandler, 1969). These multiple forms differ in their physicochemical properties and it appears unlikely that this variation is the result of the preparative procedure (Youdim, Collins & Sandler, 1968, 1969). Genetically related multiple forms of proteins may occur as a result of covalent or non-covalent differences or, in certain cases, as mixed multimers (Epstein & Schechter, 1968). Gomes, Igaue, Kloepper & Yasunobu (1969) concluded that the multiplicity of ox liver mitochondrial monoamine oxidase is due to aggregation of a subunit to produce a polymeric series. Collins & Youdim (1969), on the other hand, found that the molecular weights of the electrophoretically separated bands of the rat liver enzyme were similar. The present study is an attempt to determine whether the isoenzymes of rat liver monoamine oxidase are generated solely by conformational differences or whether other factors are involved.

Whole native and multiple forms of monoamine oxidase were individually dissociated by using 8M-urea in the presence of 0.1M-2-mercaptoethanol; electrophoresis on 5% polyacrylamide gel containing 8M-urea and 0.1M-2-mercaptoethanol showed a single band migrating from cathode to anode. The molecular weight of this subunit was 76740 ± 880 when measured by the method described by Andrews (1964). Exposure of the native enzyme to 8M-urea and 0.1M-2-mercaptoethanol for 10min resulted in a complete loss of activity. Reassociation was initiated by dialysis of the mixture against 0.1M-phosphate buffer, pH 7.4, containing 0.1M-KCl for at least 24h. Electrophoresis of the dialysis residue (Youdim *et al.* 1968) showed that the original five isoenzymes had been re-formed. Similarly, each individual isoenzyme, when treated as described above, re-formed all five forms of the enzyme. Any possibility that the multiple band formation may also be due to oxidation of the thiol groups of monoamine oxidase (Erwin & Hellerman, 1967) is excluded since electrophoresis of whole native enzyme after incubation with 0.1M-2-mercaptoethanol gave the same pattern of multiple bands as that of the control. From these results we conclude that the multiplicity of rat liver monoamine oxidase is due to conformational differences.

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