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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