The binding of neurohypophysial hormones in van Dyke protein

Since the isolation of the carrier protein (van Dyke protein) containing oxytocin and vasopressin from the ox posterior pituitary gland (van Dyke, Chow & others, 1942) the nature of the binding of these hormones to the protein has been thought to be a loose one involving the amino-group of the hormones (van Dyke, 1968). In addition, oxytocin and lysine vasopressin can be inactivated by exposure to acetone which is thought to act on this amino-group (Yamashiro, Havran & others, 1965; 1967). The following experiments add to the evidence presented on the nature of the binding of these hormones to their carrier protein.

van Dyke protein was subjected to dialysis, boiling before dialysis, and to gel filtration through Sephadex G-25 (fine bead) (Sawyer, Freer & others, 1967) in a column 2·5 cm diameter and 100 cm long, at a flow rate of 1·33 ml/min in attempts to separate the hormones from the protein. Acetic acid eluates were collected in 6 ml samples in a fraction collector. van Dyke protein was also subjected to exposure to acetone for 16 or 65 h, without boiling, then after boiling for 2½ h, with vasopressor activity monitored. For comparison, synthetic arginine vasopressin (Sandoz) was similarly subjected to acetone treatment, and the time sequence for the "regeneration" of activity by boiling was also noted. Protein was measured spectrophotometrically by the Folin-Lowry method, oxytocic activity by the rat uterus method of Munsick (1960) in magnesium free van Dyke and Hastings solution, and vasopressor activity by changes in the blood pressure of the dibenzylene pretreated rat (Dekanski, 1952).

The neurohypophysial hormones were easier to separate from their carrier protein than was thought. Simple dialysis for 16 h reduced the vasopressor activity of the protein by 40%. Boiling before dialysis increased the reduction to 52%. Boiling alone reduced the potency by 29%.

Gel filtration separated the hormones completely from the carrier protein. 90% of the weight of the protein was recovered, but this contained only 0·06% of its original vasopressor and oxytocic activities. The hormones could also be separated from each other, and 90% of both oxytocic and vasopressor activities were recovered.

Acetone treatment did not affect the vasopressor activity in van Dyke protein, but boiling to liberate the hormones before acetone treatment reduced the vasopressor activity by 40%. The activity was "regenerated" by boiling in acetic acid. In contrast, synthetic arginine vasopressin was almost completely inactivated by exposure to acetone, when 98% of the potency was lost (Table 1), but regained 86% of its vasopressor activity after boiling in 0·002M acetic acid for about 15 min.

Table 1. *Effect of acetone on the vasopressor activity of van Dyke protein and arginine vasopressin*

Treatment	Vp activity van Dyke protein U/mg	%	Vp activity arginine vasopressin	%
Control	16·7 ± 1·7*	100	0·48 U/ml	96
Acetone (65 h)	14·2 ± 1·4	85	0·01 U/ml	2
Acetone (16 h)	17·3 ± 1·8	102		
Boiled before acetone treatment (65 h)	10·2 ± 1·4	61		
Boiled before acetone (16 h)	10·3 ± 1·5	61		
Regenerated acetone powder (16 h)	13·3 ± 1·2	80		
Regenerated acetone powder (65 h)	14·7 ± 1·6	88	0·33 U/ml	66

* Limits of error of estimations.

The binding of the neurohypophysial hormones to their carrier protein seems to be loose as they can be separated not only by electrophoresis and counter-current distribution (van Dyke, 1968) but also by gel filtration, dialysis and boiling. Acetone did not inactivate the vasopressor activity of van Dyke protein as the hormone was still bound to the carrier protein. But a simple procedure like boiling which liberated about 30% of the hormone, exposed this amount to the action of acetone, and reduced potency by 40%; the extra 10% was probably inactivated before it could re-establish binding with protein. The inference is that acetone acts on the same amino-group with which the hormone attaches itself to the carrier protein.

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REFERENCES

DEKANSKI, J. (1952). *Br. J. Pharmac. Chemother.*, **7**, 567–572.

VAN DYKE, H. B. (1968). *Proc. R. Soc. B.*, **170**, 3–5.

VAN DYKE, H. B., CHOW, B. F., GREEP, R. O. & ROTHEN, A. (1942). *J. Pharmac. exp. Ther.*, **74**, 190–209.

MUNSICK, R. A. (1960). *Endocrinology*, **66**, 451–457.

SAWYER, W. H., FREER, R. J. & TSENG, TSUI-CHIN (1967). *Gen. comp. Endocr.*, **9**, 31–37.

YAMASHIRO, D., AANNING, H. L. & DU VIGNEAUD, V. (1965). *Proc. natn. Acad. Sci. U.S.A.*, **54**, 166–171.

YAMASHIRO, D., HAVRAN, R. T., AANNING, H. L. & DU VIGNEAUD, V. (1967). *Ibid.*, **57**, 1058–1061.

Inhibition by *p*-chloroamphetamine of the conversion of 5-hydroxytryptamine to 5-hydroxyindoleacetic acid in rat brain

p-Chloroamphetamine (PCA) causes a lowering of 5-hydroxytryptamine (5-HT) in whole brain of rats (Pletscher, Bartholini & others, 1964; Fuller, Hines & Mills, 1964, 1965). Two possible mechanisms are, the inhibition of 5-HT synthesis, and the release of 5-HT from binding sites in brain. The fact that PCA also lowers 5-hydroxyindoleacetic acid (5-HIAA) concentrations in brain pointed to the first possibility, since releasing agents make 5-HT susceptible to attack by monoamine oxidase and thus raise the 5-HIAA concentration (Roos & Werdinius, 1962). An alternative explanation for the lowered 5-HIAA concentration was proposed (Fuller, 1966) on the basis of the ability of PCA to inhibit the oxidation of 5-HT by brain mitochondria from rats. Based on the *in vitro* potency of PCA as an inhibitor and the concentrations found to be present in the brains of rats given PCA, we suggested that the conversion of 5-HT to 5-HIAA in rat brain might be inhibited. To provide direct evidence for this possibility, we have now examined the metabolism of [^{3}H]5-HT formed from [^{3}H]5-hydroxytryptophan (5-HTP) in rats treated with PCA.

In these experiments, male albino rats, about 150 g, were injected intraperitoneally with saline or with PCA at a dose of 20·6 mg/kg (0·1 mmol/kg). 16 h later the rats were given an intraperitoneal injection of [^{3}H]DL-5-HTP (generally labelled, from Volk Radiochemical Company). A tracer amount of the 5-HTP was injected