An improved method of estimation of small amounts of antidiuretic activity

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Jeffers, Liverzey & Austin (1942), and Dicker (1953) developed a sensitive method for the estimation of small amounts of antidiuretic hormone by measuring antidiuresis in ethanol-anaesthetized hydrated rats. Two factors which limit the suitability of this preparation are the degree of diuresis produced and the development of tachyphylaxis to ADH observed when doses are administered at intervals shorter than 45–60 min (Dicker, 1953). This communication describes the effects of propranolol which increases the rate of urine formation and allows ADH to be administered at shorter time intervals.

Male Wistar rats (200–280 g) fasted for 24 h, but with free access to water were anaesthetized with 12% ethanol, 5 ml/100 g administered orally through a stomach tube. Following anaesthesia, the total volume was made up to 8·0 ml/100 g wt by the oral administration of 3·0 ml of 0·2% ethanol/100 g. The degree of hydration was then maintained with 0·2% ethanol and not water using the method described by Boura & Dicker (1953). The trachea and bladder were cannulated, and drugs were administered through a cannula in the femoral vein in 0·1 ml saline and washed through the cannula with 0·1 ml saline. The urine produced was collected into an airtight jar and displaced an equal volume of glycerol and the drops of glycerol were recorded by means of a photo electric cell and SRI drop counter.

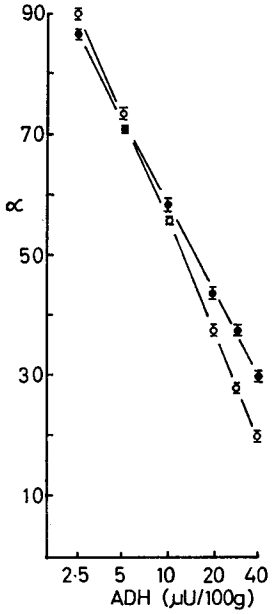

FIG. 1. Log dose regression lines giving values for antidiuretic effect (α) of ADH in ○ — ○ untreated and ● — ● propranolol-treated rats. Each point is the mean of 6 determinations, vertical lines show the standard error of the mean. Ordinate: values for α (see text), abscissa: log dose of ADH (μU/100g).

The diuresis produced was regular for up to 5 h. A typical example was in a rat (238 g) whose rate of urine formation remained unchanged at 0·14 ml min^{-1} (within a range of ± 5%) for 5 h. The degree of diuresis was increased by the administration of 0·1 mg kg^{-1} propranolol which produced a slowly developing increased urine flow reaching a maximum after 9 min. In 24 experiments, the mean rate of urine formation (± s.e.) for 10 min periods before the administration of propranolol was 1·32 ml (± 0·02) and following propranolol, the mean maximal rate of urine formation (after 9 min) was 2·60 ml (±0·03). The increased diuresis produced by propranolol was a maximal effect, a second dose of 0·1 mg kg^{-1} having no further affect on urine formation and the diuresis was well maintained for more than 2 h and in some experiments in excess of 3 h. Where the effects of propranolol decreased with time, the diuresis could be re-established to former values by intravenous administration of 0·05 mg kg^{-1} propranolol.

The dose range of ADH which was employed was 2·5–30·0 μU/100 g, and antidiuretic activity was measured by the method of Boura & Dicker (1953). Between the time of administration of ADH and the onset of the antidiuretic effect there is a latent period of 2 min and the antidiuretic activity (α) was calculated over an empirical period of 10 min as: $\alpha = (a/b) \times 100$ where b is the volume of urine collected for the period from 8 min before to 2 min after the injection of ADH

Table 1. *The results from one rat at each dose level showing the effects of repeated administration of a standard amount of ADH every 30 min to untreated and propranolol-treated rats.*

Preparation	ADH (μU/100 g)	Antidiuretic activity (α) at time (min)			
		0	30	60	90
Untreated	2·5	90	92	98	100
Propranolol	2·5	86	88	85	88
Untreated	5·0	74	90	94	100
Propranolol	5·0	72	70	73	70
Untreated	10·0	55	86	100	100
Propranolol	10·0	54	58	58	62
Untreated	20·0	38	86	100	100
Propranolol	20·0	46	60	80	90
Untreated	30·0	29	85	100	100
Propranolol	30·0	36	60	80	100

and a is the volume collected from the 3rd–13th min after the ADH injection.

The antidiuretic potency of ADH is shown in Fig. 1. Values of antidiuretic effect obtained from 10 untreated and 10 propranolol-treated rats are expressed in terms of dose of ADH. Each point is the mean of 6 determinations at each dose level of ADH administered at 1 h intervals, and shows that ADH produces a similar degree of antidiuresis in different animals and that the antidiuretic response increased linearly with the logarithm of the dose.

The untreated and propranolol-treated rats exhibited a similar sensitivity to ADH, but the log dose response regression line of the propranolol-treated animals was more shallow than that for the untreated rats, and is due to the quicker recovery of diuretic activity from the effects of ADH.

Dicker (1953) found that tachyphylaxis developed to low concentrations of ADH if the time interval between doses was less than 45–60 min. Table 1 summarises results which confirm this finding, tachyphylaxis being observed with doses of ADH as low as 2·5 μU/100 g when given at 30 min intervals. Propranolol (0·1 mg kg^{-1}) treatment allowed the estimation of low concentrations of ADH (2·5–10·0 μU/100 g) at 30 min intervals, but tachyphylaxis was seen with higher doses.

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Some observations on the attempted separation of isoenzymes of monoamine oxidase

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Studies of the varying activity of monoamine oxidase (MAO) [monoamine: O_2 oxidoreductase (deaminating) EC 1.4.3.4.] isolated from different species and tissues towards various substrates (Alles & Heegaard, 1943) inhibitors (Hardegg & Heilbron, 1961; Johnston, 1968; Hall, Logan & Parsons, 1969) and heat sensitivity (Youdim & Sourkes, 1965) have suggested that MAO exists in several forms or may be represented as a family of related enzymes. These suggestions have been strengthened by the frequent demonstration of multiple bands of enzymatic activity on gel electrophoresis of solubilized enzyme preparations (Youdim & Sandler, 1967; Kim & D'Iorio, 1968). The number of such bands detected however, varies with the tissue and species employed in the isolation (for review see Sandler & Youdim, 1972).

The present work was initiated as a study of the potential substrate specificity or selectivity of the various separable isoenzymic forms of MAO. Since that time however, more recent studies have raised doubts regarding the specificity of the detection methods employed for the colourimetric detection of the enzyme in polyacrylamide discs (Diaz Borges & D'Iorio, 1973) and the methods employed in isolating the enzyme (Houslay & Tipton, 1973) suggesting that some of these bands of activity may be artifacts. Our own results, reported here, are in agreement with the more recent studies, demonstrating a lack of specificity in the detection procedure. In addition, it is suggested that the presence of non-ionic surfactants employed in the solubilization of the enzyme e.g. Triton X-100 may influence the behaviour of the enzyme preparation on gel filtration.

The enzyme MAO was isolated from the liver and brain of male Sprague-Dawley rats (250–300 g) by previously reported methods (Youdim & Collins, 1971) with only slight modification. Thus, the enzyme preparation, after solubilization in Triton X-100 (1·5% w/v) in phosphate buffer (0·05 M; pH 7·4), was precipitated between 25 and 55% ammonium sulphate saturation and the product dialysed, after redissolving in phosphate buffer (0·05 M; pH 7·4), against a total of 500 volumes of buffer for up to 60 h. The effect of dialysis time upon the subsequent elution of the enzyme

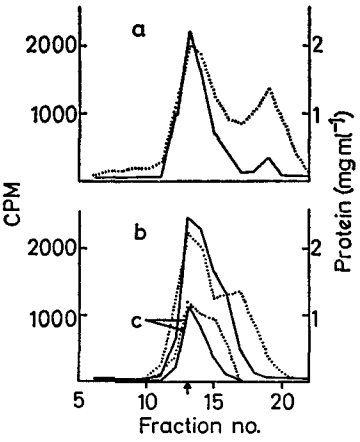

FIG. 1. Effect of dialysis time upon gel filtration behaviour of rat MAO. (a) Liver enzyme preparation dialysed 24–48 h. (b) Liver enzyme preparation dialysed 60 h. (c) Brain enzyme preparation dialysed 60 h. Solid line—enzyme activity, substrate tyramine. Dotted line—protein concentration. Arrow indicates void volume (105 ml).

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