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Some effects of dihydroergocristine and of phentolamine mesylate on renal function in rats

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Single injections of dihydroergocristine (DHE) (25, 50 and 100 μg, i.p.) did not depress the systemic arterial pressure but antagonized the pressor effects of (−)-noradrenaline (100 μg, s.c.) in unanaesthetized rats. The effects of these doses of DHE on water diuresis were minimal: slight K retention, a rise in urinary Na : K and a slight but significant fall in the clearance of *p*-aminohippuric acid were noted. Single injections of phentolamine mesylate (10, 20 and 40 μg, i.p.) greatly depressed systemic arterial pressure and reduced the glomerular filtration rate and *p*-aminohippuric acid clearance and the excretion of water and Na proportionately, but markedly. The Na : K in the urine rose. Chemical denervation of the kidneys either with DHE, 32 μg, or phentolamine, 2 mg, thrice daily for 5–7 days decreased, but for 14 days increased, the juxtaglomerular index of the kidneys. Parallel changes were found in the extractable renin. Continued treatment with DHE (32 μg thrice daily for 2 weeks) raised, and hyperduric adrenaline (250 μg twice daily for 1 week) lowered the stores of growth hormone in the adenohypophysis. Continued treatment with DHE, 32 μg thrice daily produced antidiuresis and retention by the third day lasting to the fifth. This condition had reversed by the eighth day.

Recent observations have indicated that the sympathetic nervous system plays some part in the control of the secretion of renin from the juxtaglomerular apparatus (Bunag, Page & McCubbin, 1966; Bozovic & Castenfors, 1967). A rich innervation of the epithelioid cells which lie within the wall of the afferent glomerular arteriole and which constitute the "polkissen" of the juxtaglomerular apparatus has been demonstrated by electronmicroscopy (Barajas, 1964). More recently these fibres have been identified as adrenergic by histochemical fluorescence (Wägermark, Ungerstedt & Ljungquist, 1968). The smooth muscle cells of the afferent arterioles also receive sympathetic innervation.

The object of the present work has been to discover whether the influence of the sympathetic nervous system on the secretion of renin is mediated through α-receptors within the "polkissen" or through changes in tension within the arteriolar wall. Hence, two α-blocking drugs have been selected for use: the first, dihydroergocristine is capable of blocking the vascular action of exogenous noradrenaline without affecting systemic arterial pressure; the second, phentolamine, is not. The urinary changes induced by these drugs have also been examined since renin is secreted into the blood stream and into the interstitial fluid within the kidney (Peart, 1965) and the interstitial fluid contains renin substrate (Thurau, 1964), It is therefore theoretically possible that part of the renal actions of these blocking agents may be due to change in the rate of the intrarenal secretion of renin and hence to change in the rate of the intrarenal formation of angiotensin.

EXPERIMENTAL

Methods

Female Wistar rats, 160–205 g, were housed in a single room at 20–22°, drank water freely and ate a pellet diet supplied by Wesfarmers Ltd. Since the sodium concentration in this diet varied from batch to batch during the period of these experiments it was necessary to stabilize groups of control and experimental animals on each new batch of diet before the start of treatments.

Renin measurements. Rats were anaesthetized with sodium pentobarbitone (6 mg/100 g/weight, i.p.) before the kidneys were removed, weighed separately, and assigned R and L alternately for determination of renin content or of juxtaglomerular index.

Measurement of the renin content of kidneys. Weighed kidneys were chilled and separately homogenized in NaH_2PO_4, 0·05M, pH 7·0 3·5 ml/g tissue, at 8° as described by Katz, Cockett & Moore (1966). Renin was prepared from each kidney homogenate by Skinner's method (1967) modified only in so far that pH 3·0 was substituted for pH 3·3 in the first dialysis at 8° and the subsequent step at 32°. The final renin preparations obtained, free of angiotensinase and endogenous substrate, were diluted 1 in 10 to 1 in 100 with 0·1M NaH_2PO_4 buffer, pH 7·0 for incubation with an excess of ox substrate (Lever, Robertson & Tree, 1964) at 37° and pH 7·0. The incubation mixtures were sampled at 1, 4 and 8 h for immediate assay of the angiotensin formed on the blood pressure of the ganglion blocked rat (Peart, 1955). Aspartyl[1], valyl[5], angiotensin II β amide (Hypertensin, CIBA), similarly incubated, was used as reference standard. All assays were of 2 × 2 Latin Square design and afforded data which were subjected to variance analysis. The renin content of each kidney was expressed as ng angiotensin formed per g kidney per hour.

Measurement of juxtaglomerular index (JG index). Transverse slices of cortex 4 mm thick, were fixed for 18 h in Smith's fluid: $K_2Cr_2O_7$, 5g: 38–40% formaldehyde, 10 ml; glacial acetic acid, 2·5 ml; distilled water 88 ml, washed for 2 h in running tap water and embedded in Paraplast wax using a Shandon Elliot automatic tissue processor. Sections, 7 μm, were stained by Smith's method (1966). The juxtaglomerular granules stained deep purple, the nuclei lavender to pink, the cytoplasm blue, collagen pink and erythrocytes red. The JG index was determined as described by Hartroft & Hartroft (1953): estimates of JG index were invariably found reproducable amongst those experienced in the technique.

Measurement of the concentrations of adenohypophyseal hormones. The pituitary glands were removed from rats decapitated under pentobarbitone anaesthesia; all adenohypophyseal tissue from groups of 4 rats were separately homogenized with 1 ml sample gel, 0·1 ml of which was applied to each column for electrophoresis. Disc electrophoresis was carried out on standard 7·5% acrylamide gel at pH 9·0 as described by Ornstein (1964). Polymerization of the large pore spacer and sample gels was assisted by the addition of tetramethylene diamine (Lewis, 1963). A constant current of 5 mA was applied to each tube for 40 min. All gels were stained for 2 h with Amido Schwartz reagent and were destained electrophoretically. The protein bands were examined by means of a double beam recording and integrating densitometer (Joyce, Loeble & Co. Ltd.) speed 2 mm/s, gain 5.

Measurement of the effects of drugs on mean arterial pressure. Pentobarbitone anaesthesia was used for the insertion of indwelling polythene cannulae, heparin (Evans Medical Ltd.) filled, into carotid arteries 36 h before their use. The cannulae

were exteriorized at the back of the neck. The animals were enclosed in a small dark, well ventilated, fabric space during measurement of mean arterial pressure. The polythene cannulae projected from this space to connect with an E & M transducer coupled to a Heathkit pen recorder.

Experiments made during water diuresis. Every experiment was designed as a series of cross-over tests in which each animal received each treatment and served as its own control. Equal numbers of each treatment were allocated to each day. Tests were made every third day and began with a 2 h period during which rats were deprived of solid food. The oral water load, equivalent to 2·5% body weight, was given at the end of this period immediately before each animal was put into a separate cage for the collection of all urine entering the bladder in the next hour. Gentle suprapubic pressure was applied to empty the animals' bladders at the termination of each collection of urine. All tests constituting a single experiment were performed at a time of day fixed for each experiment. Phentolamine and dihydroergocristine were injected intraperitoneally in 0·1 to 0·2 ml of 0·9% NaCl at the time of water-loading. Creatinine 4·0% and *p*-amino-hippuric acid (PAH) 1% in 0·9% NaCl, neutralized by addition of solid $NaHCO_3$, were injected subcutaneously (0·6 ml per 100g 30 min) before administration of the water load. Concentrations of sodium (Na) and potassium (K) in urine samples were determined by flame photometry. Two channels of an autoanalyzer (Technicon Ltd.) were used to estimate urinary concentrations of creatinine and PAH. Predetermined mean plasma values for these substances, found 60 min after their subcutaneous injection were applied to obtain individual clearance values for each animal: mg substance excreted in 1 h divided by mg substance per ml plasma $\times$ 60. The data from each experiment was subjected to variance analysis: *t*-tests were applied within groups to determine the significance of drug actions and interactions.

Drugs. Dihydroergocristine (DHE) was received as a gift from Sandoz Basle. Phentolamine mesylate (CIBA), (−)-noradrenaline (Winthrop) and hyperduric (−)-adrenaline chloride (Parke Davis) were obtained commercially. Bovine growth hormone (NIH, GH B9) and thyrotropic hormone (Thyrotropar) were received as gifts.

RESULTS

The influence of dihydroergocristine (DHE) and phentolamine on water diuresis in rats. Nine 4 day cross-over tests (Lees, Lockett & Roberts, 1964) were made on groups of 10 to 12 rats to determine the effect of DHE on water diuresis. The combined results of these experiments are shown on the left of Fig. 1. DHE in doses of 25, 50 and 100 μg/rat was without significant effect on the elimination of a water load and on the excretion of Na. The rate of excretion of K tended to decrease as the dose of DHE increased hence both 50 and 100 μg/rat caused a small but significant increase in the ratio Na : K of the urine. The glomerular filtration rate (GFR) remained unchanged but the clearance of PAH(C_{PAH}) was slightly depressed: this depression reached significance ($P < 0{\cdot}05$) overall, but not at the individual dose levels in individual experiments. Data obtained from six similarly designed cross-over tests made to determine the effects of phentolamine on water diuresis in rats is summarized on the right of Fig. 1. Phentolamine in doses of 10, 20 and 40 μg/rat caused a small reduction in the urine flow in these water-loaded rats accompanied by retention of Na and K and significant reduction in GFR and C_{PAH}. Since the urinary excretion of K was

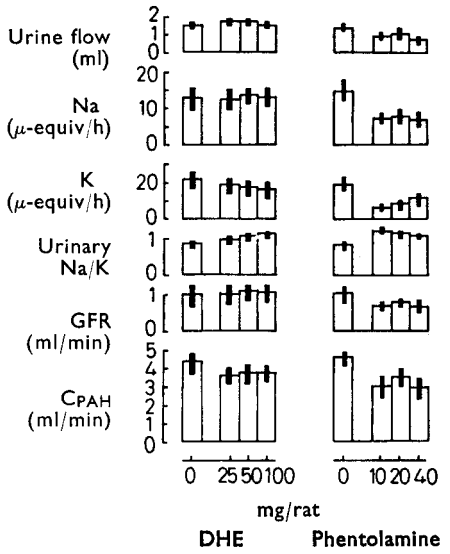

FIG. 1. Contrasting effects of single i.p. doses of dihydroergocristine (DHE) and of phentolamine on water diuresis in rats. All values are expressed per 100g weight. The heights of the columns indicate mean values and the inset columns the standard errors of these means. The columns on the left of each series indicate control values, the other columns represent the effects of treatment with DHE 25, 50 and 100 μg per rat (left) and phentolamine, 10, 20 and 40 μg per rat.

even more markedly reduced than that of Na, the ratio Na:K in the urine rose. The higher the dose of phentolamine the less the effect on this ratio: by contrast, only the higher doses of DHE (25 and 50 μg) produced a rise in the Na:K of the urine.

The influence of DHE *and phentolamine on the systemic arterial pressure of unanaesthetized rats.* The injection of DHE (50 μg, i.p.) into rats equipped with indwelling

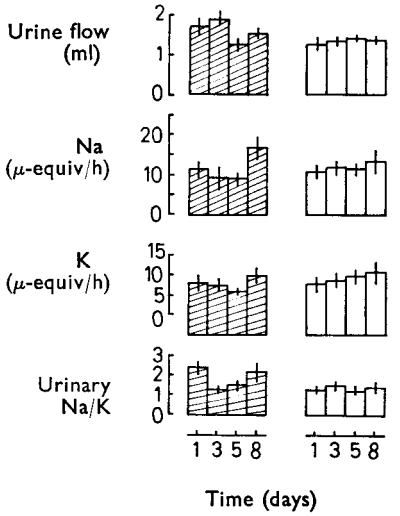

FIG. 2. The effects of continuous treatment with DHE, 32 μg thrice daily per rat, for 8 days, on the excretion of a water load and on urinary electrolytes. Shaded columns are treated and open columns control results. Values per 100g rat per h: on the first, third, fifth and eighth day of the experiment.

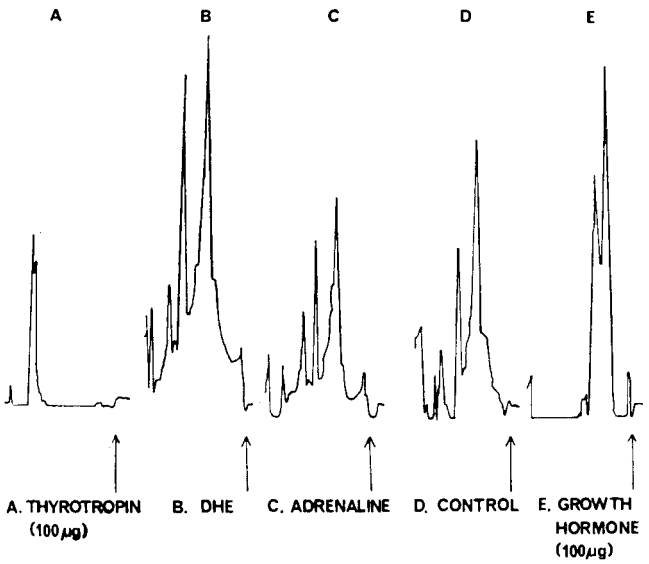

FIG. 3. Typical densitometer tracings of disc electrophoretic patterns obtained by mixing A, 100 μg thyrotropin, E, 100 μg bovine growth hormone, B, C and D each one tenth the homogenate from the adenohypophyses of four rats treated with DHE, 32 μg thrice daily, 2 weeks, hyperduric adrenaline 250 μg, s.c. thrice daily, 1 week and 0·9% NACl thrice daily, 2 weeks, respectively. The arrows indicate the starting line of each tracing.

carotid cannulae caused no significant fall in mean arterial pressure during subsequent periods of observation lasting 1½ to 3 h. By contrast, phentolamine (20 μg) decreased mean arterial pressure by as much as 40 mm Hg within 30 min. Noradrenaline (100 μg, s.c.) produced no sustained elevation of mean arterial pressure in the presence of either 50 μg of DHE or 20 μg of phentolamine.

The effect of continued treatment with DHE 50 μg *thrice daily on water diuresis in rats.* The effect of continuous treatment with DHE (32 μg, i.p.) thrice daily for 8 days is shown in Fig. 3. During this period there were no significant variations in the urinary responses of a group of 24 rats weighing 287 ± 4·3 g to water loading. By contrast, 14 rats (191 ± 4·1 g) receiving DHE significantly reduced their urinary Na by the 5th day of treatment. The K output also tended to fall during this period but to a lesser extent so that the ratio Na : K in the urine was very significantly decreased. This initial effect of continuous treatment with DHE was not maintained: normal ability to excrete Na was restored by the 8th day of treatment.

The influence of renal denervation, adrenal demedullation and of continuous treatment with DHE *or with phentolamine on the renin content and juxtaglomerular index of rat kidneys.* Both 5 and 7 days of treatment with DHE (32 μg, i.p., thrice daily) caused significant decrease in the JG index but phentolamine (2 mg, i.p., thrice daily) did not influence the JG index. Two weeks of continuous treatment with either DHE or phentolamine at the above doses significantly increased both the JG index and the renin content of the kidneys of normal rats (Table 1).

The influence of continuous treatment with DHE (32 μg, *thrice daily*) *on the stored hormones in the adenohypophyses of rats.* Fig. 3 shows typical patterns of the adenohypophyses of control (D), DHE-treated (B) and adrenaline-treated (C) rats. Moving right to left from the starting arrow, the growth hormone peak (E) precedes the albumen

Table 1. *The influence of various treatments on the juxtaglomerular index and the renin content of rat kidneys*

Treatment	Body weight g	Weight of a single kidney mg	Juxtaglomerular index	Renin content ng angiotensin/g kidney h^{-1}
Normal rats (controls)	183 ± 5·2 (10)	646 ± 14·7	15·5 ± 0·37	371 ± 39·8
Dihydroergocristine 32 μg thrice daily				
for 5 days	141 ± 0·4 (6)	612 ± 8·4	11·6 ± 1·27*	
for 7 days	174 ± 1·6 (4)	642 ± 12·6	12·8 ± 0·63*	
for 14 days	191 ± 3·0 (10)	668 ± 15·2	18·6 ± 0·74	449 ± 41·3*
Phentolamine 2 mg thrice daily				
for 7 days	179 ± 2·1 (6)	632 ± 11·7	16·6 ± 1·08	394 ± 42·4
for 14 days	175 ± 4·8 (10)	660 ± 14·7	19·6 ± 1·82*	456 ± 47·4*

The values shown are means ± their standard errors. The numbers of animals used are shown in parentheses. The significance of differences caused by treatments has been examined by *t*-tests and is indicated by an asterisk if P is $< 0{\cdot}05$.

peak in the normal adenohypophysis (D); thyrotropin (A) and lactogenic hormone (not shown) migrate together more rapidly. Adrenocorticotrophin does not migrate under the conditions of these experiments. Continuous treatment with DHE for two weeks 32 μg, thrice daily) has increased the stores of growth hormone markedly. The albumen content of the glands has also increased. Stores of thyrotropic and lactogenic hormones remain unaffected. Conversely, hyperduric adrenaline (250 μg, twice daily) for 1 week has caused some reduction in the stores of somatotrophin.

DISCUSSION

It is generally agreed that sympathetic vasoconstrictor tone is small or absent in the normal kidney (Smith, 1939; Thurau, 1964). Therefore, the very marked reduction in GFR and in C_{PAH} caused by single full α-adrenergic blocking doses of phentolamine are attributed to the large decrease in systemic arterial pressure which these doses caused. This decrease in arterial pressure does not appear to have provoked the release of renin since GFR and C_{PAH} were proportionately reduced and so the filtration fraction remained unchanged. Any increase in the plasma concentration of renin would have increased the concentration of circulating angiotensin (Peart, 1965) and a rise in the filtration fraction is characteristic of the renal actions of this peptide (Finnerty, Massaro & others, 1961; Lockett, 1967). Our observations are only superficially in conflict with previous work, for Vander (1965) has demonstrated that electrical stimulation of the renal nerves or infusions of either adrenaline or noradrenaline increase the secretion of renin. Hence the raised rate of renin secretion which results from a fall in systemic mean arterial pressure (Skinner, McCubbin & Page, 1963a, b) is probably a consequence of reflexly increased sympathetic activity. However, the α-receptor blockade induced by phentolamine would be expected to abolish the effects of enhanced sympathetic nervous activity on the release of renin. The reduction in GFR caused by phentolamine suffices to explain both the antidiuresis and reduction in Na excretion caused by single injections of this drug. The doses of DHE used in these single injection experiments were adequate to prevent the pressor effects of (−)-noradrenaline (100 μg, s.c.) per rat, but did not depress mean arterial pressure. These doses of DHE reduced C_{PAH} but not the GFR and therefore caused a rise in the filtration

fraction. This rise in the filtration fraction, observed to result from single doses of DHE is probably attributable to the release of noradrenaline from the terminals of the postganglionic sympathetic nerves supplying the juxtaglomerular apparatus since DHE has been shown to release catecholamine from the adrenal medulla (Lockett & Wadley, 1969). An effect of DHE on the postglomerular renal vasculature cannot however be excluded since DHE is known to possess weak direct constrictor properties (Rothlin & Cerletti, 1949).

The changes induced by the continued administration of these same doses of phentolamine and DHE are more complex. Denervation of a kidney decreases its renin content (Tacquini, Blaquier & Tacquini, 1964) and lowers the JG index, (Tobian, Branden & Maney, 1965). Since 7 days of treatment with full α-adrenergic blocking doses of phentolamine failed to influence the JG index, and the same period of treatment with DHE, in doses sufficient to block the effects of exogenous noradrenaline but insufficient to inhibit vasomotor tone, reduced the JG index, our observations cannot be explained in terms of the blocking action of these drugs on a pure α-receptor mechanism for the release of renin by catecholamine. Moreover, 2 weeks of continuous treatment with these same doses of either drug caused a secondary rise both in the JG index and in the renin content of the kidneys. Possibly a prolonged α-blockade brings about a change in the sensitivity of the juxtaglomerular apparatus either to a feed back system or to an intermediary substance within the apparatus itself or to both.

Rise and fall in the JG index or the renin content of the kidneys, or both, is widely accepted as evidence of a rise or a fall, respectively, in the rate of secretion of renin (Tobian, 1960). The effects of continuous treatment with DHE on the excretion of water and electrolytes by rats do not reflect the changes in renin secretion indicated by the changes in the JG index. A small but significant retention of water and Na was evident from the third to the fifth day of treatment but reversed by the 8th day. Stores of electrophoretically identifiable growth hormone had however accumulated in the adenohypophysis during 2 weeks of treatment with DHE. No conclusion can be drawn until it be determined whether these increased stores of growth hormone reflect change in the rate of synthesis or release or both of these. This hormone has however been shown powerfully Na-retaining in the rat (Lockett & Nail, 1965) and necessary for the normal sensitivity of the nephron to aldosterone (Lees & others, 1964; Lockett & Roberts, 1963).

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