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A STUDY OF THE β-ADRENERGIC RECEPTORS IN RAT KIDNEYS

BY

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Dichloroisoprenaline (1 mg), injected intramuscularly into the rat, retarded the elimination of water and retained sodium, depressing both glomerular filtration rate and renal blood flow. A dose of 4 mg had a similar but more intense action. These actions of dichloroisoprenaline resembled those of 2 or 4 μg of (±)-isoprenaline given subcutaneously. Dichloroisoprenaline and (±)-isoprenaline summed in salt retaining effect but competed in renal vascular action. 2-Isopropylamino-1-naphth-2′-ylethanol hydrochloride (nethalide, 1 mg intramuscularly) was without action during water diuresis. A dose of 4 mg inhibited solely the excretion of water. Nethalide (1 mg) completely antagonized the antidiuretic and vascular actions of 2 μg of (±)-isoprenaline; antagonism of the salt retention caused by (±)-isoprenaline was less complete. A dose of 4 mg of nethalide antagonized both the salt retaining and the vascular actions of 4 μg of (±)-isoprenaline.

Ahlquist (1948) demonstrated the existence of two types of receptor for sympathomimetic amines. Those he classified as α-receptors had especial sensitivity to noradrenaline; β-receptors, by contrast, were preferentially activated by very low concentrations of compounds like isoprenaline. The receptors which mediate vasoconstriction in the kidney were shown to belong to the α-group. It does, however, seem probable that there are also β-receptors in the kidney, since (±)-isoprenaline is five to ten times as active as (−)-adrenaline in provoking antidiuresis with retention of sodium and potassium, when these compounds are given subcutaneously in very low doses to unanaesthestized rats (Botting & Lockett, 1961). Very small amounts of (±)-isoprenaline (0.5 to 1.0 μg per 100 g of body weight) induce this antidiuresis without changing measurably either glomerular filtration rate or effective renal plasma flow. Larger doses of (±)-isoprenaline (2 to 3 μg per 100 g) reduce both the renal blood flow and the filtration rate (Botting, Farmer & Lockett, 1961).

The object of the present work was to confirm the existence of β-adrenergic receptors in the kidney by means of selective β-adrenergic blocking drugs. Those used were dichloroisoprenaline and 2-isopropylamino-1-naphth-2′-ylethanol hydrochloride (nethalide). Dichloroisoprenaline, introduced as a β-adrenergic blocking drug by Powell & Slater (1958), exhibits some isoprenaline-like actions (Furchgott, 1959; Dresel, 1960) which appear absent from nethalide (Black & Stephensen, 1962; Dornhorst & Robinson, 1962). Rats have been used for this work.

METHODS

Male Wistar rats, fed the 41b diet of Stein and given water *ad libitum*, were accustomed to stomach tubes and handling before use. All subsequent experiments were designed as a series of cross-over tests in which every animal received each treatment in sequence according to Latin Square or random block designs. Tests were made at intervals of 2 or 3 days. The data provided by each complete experiment were submitted to analysis of variance. Throughout, the mean squares of variance attributable to the use of different treatments were of high significance, and those arising from variation in the animals from one test to the next were of little or no significance. Significant differences between individual animals appeared in five experiments; hence each animal was made to serve as its own control in the *t* tests by which differences between means were examined to assess their significance.

The test procedure. Animals were deprived of solid food at a zero time which was kept constant throughout each complete experiment. Subcutaneous injections of 3.0 ml. of a 5% solution of inulin in 0.9% saline were made at 3 hr; subcutaneous injections of 0.4 or 0.5 ml. of a 35% solution of diodone and intramuscular injections of a β-adrenergic blocking agent in 0.1 ml. of propylene glycol, or intramuscular injections of propylene glycol alone, were made at 3.5 hr. Subcutaneous injections of (±)-isoprenaline hydrochloride in 0.1 ml. of 0.9% saline were made at 4 hr, immediately after administration of an oral water load equivalent to 5% of body weight. The urine made by each animal in the next hour was then separately collected as described by Botting & Lockett (1961). The clearances of inulin and diodone and the concentrations of sodium and potassium in the urine were estimated as described by Botting *et al.* (1961).

TABLE 1

ACTIONS OF DICHLOROISOPRENALINE ON THE EXCRETION OF WATER AND SODIUM BY RATS

The values are means±the standard errors. The significance of differences in means, attributable to the effect of a treatment, was examined by the *t* test and is indicated by asterisks. *, $P<0.05$; **, $P<0.01$. Values in parentheses are the numbers of rats. Isoprenaline was given subcutaneously, dichloroisoprenaline intramuscularly

Expt.	Body weight (g)	Observation	Treatment: None	Isoprenaline 2 μg	Dichloroisoprenaline 2 mg	Dichloroisoprenaline and isoprenaline 2 mg+2 μg
1	157±5·0 (9)	ml. urine/rat/hr	2·1±0·40	0·5±0·11**	0·6±0·21**	0·8±0·23*
		μ-equiv. of Na/ 100 g of rat/hr	4·5±1·12	1·2±0·54**	1·0±0·26**	0·6±0·12**
		mg of inulin/rat/hr	35·6±1·81	29·5±2·04*	25·4±2·45**	28·5±1·57*
2	151±3·1 (10)	ml. urine/rat/hr	2·4±0·27	1·0±0·23**	2·0±0·27	1·2±0·14**
		μ-equiv. of Na/ 100 g of rat/hr	4·1±0·93	2·0±0·50*	2·2±0·43*	1·3±0·28**
		mg of inulin/rat/hr	30·0±1·59	27·2±1·55	24·9±1·79*	29·1±2·18
3	174±4·4 (6)	ml. urine/rat/hr	1·9±0·33	0·4±0·15**	0·6±0·24**	0·4±0·18**
		μ-equiv. of Na/ 100 g of rat/hr	6·6±1·10	2·9±0·92**	3·4±0·98*	3·3±1·06*
		mg of I_2 in diadone/ rat/hr	25·7±1·13	15·3±3·07**	14·1±2·04**	13·4±1·80**
				4 μg	4 mg	4 mg+4 μg
4	217±8·6 (6)	ml. urine/rat/hr	2·4±0·67	1·7±0·45	0·9±0·13*	0·8±0·23*
		μ-equiv. of Na/ 100 g of rat/hr	6·8±0·62	3·0±0·23**	3·8±0·66*	3·5±0·86**
		mg of I_2 in diadone/ rat/hr	26·7±2·04	20·5±1·91**	22·3±2·19*	20·8±2·45*

RESULTS

Dichloroisoprenaline, 1 to 4 mg injected intramuscularly into rats 30 min before the administration of standard water loads, had an effect on the subsequent water diuresis similar to that produced by the subcutaneous injection of 2 to 4 μg of (±)-isoprenaline (Table 1). Either drug given separately caused antidiuresis and retention of sodium, and each reduced the rate of elimination of inulin and diodone. Given together the two drugs summed in one action only: in producing sodium retention. By contrast, dichloroisoprenaline in the dose range used appeared unable to influence the elimination of water, inulin or diodone in the presence of isoprenaline (Table 1).

Nethalide (1 mg intramuscularly into the rat) was without measurable effect on the excretion of water, sodium, inulin or diodone. This same dose partially antagonized the effect of 2 μg of (±)-isoprenaline on sodium reabsorption, and completely antagonized the antidiuresis and reduction in effective renal plasma flow caused by the sympathomimetic amine (Table 2). A higher dose of nethalide (4 mg) reduced the rate of elimination of a water load but failed to influence the rates of excretion of sodium, inulin and diodone. This dose also completely antagonized the effects of 4 μg of (±)-isoprenaline on the excretion of sodium and diodone, but did not influence the antidiuresis (Table 2).

TABLE 2

ACTIONS OF NETHALIDE ON THE EXCRETION OF WATER AND SODIUM BY RATS

The values are means±the standard errors. Significance of differences between means was examined and is indicated as in Table 1. Values in parentheses are numbers of rats. Isoprenaline was given subcutaneously, nethalide intramuscularly

Expt.	Body weight (g)	Observation	Treatment: None	Isoprenaline 2 μg	Nethalide 1 mg	Nethalide and isoprenaline 1 mg+2 μg
1	157±5·0 (8)	ml. urine/rat/hr	2·2±0·41	1·1±0·23*	2·9±0·50	2·1±0·60
		μ-equiv. of Na/100 g of rat/hr	4·4±1·06	3·7±0·78**	6·7±1·22	4·0±0·28*
		mg of inulin/rat/hr	35·6±1·81	27·6±1·62*	29·7±1·61	31·4±2·91
2	150±3·5 (11)	ml. urine/rat/hr	2·4±0·29	1·1±0·24**	2·4±0·38	2·1±0·60
		μ-equiv. of Na/100 g of rat/hr	3·4±0·72	2·2±0·51*	3·3±0·39	2·3±0·27*
		mg of inulin/rat/hr	29·8±1·57	27·4±1·61	30·5±1·99	31·5±1·94
3	164±3·3 (7)	ml. urine/rat/hr	2·8±0·22	0·9±0·19**	1·5±0·34*	1·2±0·31*
		μ-equiv. of Na/100 g of rat/hr	6·0±0·42	3·6±0·48**	5·7±0·32	4·5±0·77*
		mg of I_2 in diadone/rat/hr	26·4±1·41	19·0±1·59**	22·4±1·52	23·3±1·34
				4 μg	4 mg	4 mg+4 μg
4	156±4·2 (6)	ml. urine/rat/hr	2·3±0·45	0·6±0·27**	1·3±0·56	1·0±0·34*
		μ-equiv. of Na/100 g of rat/hr	5·8±0·69	1·4±0·51**	5·4±1·09	5·5±1·13
5	206±3·9 (6)	ml. urine/rat/hr	2·4±0·28	1·2±0·27**	0·9±0·29**	0·8±0·18**
		μ-equiv. of Na/100 g of rat/hr	5·1±0·51	1·6±0·33**	6·0±0·44	5·4±0·90
		mg of I_2 in diadone/rat/hr	26·7±1·87	19·9±2·12*	26·5±1·33	21·9±2·19

DISCUSSION

Experimental results (Tables 1 and 2) confirm the presence of renal receptors which may properly be typified as β-adrenergic, since they are activated preferentially by very low concentrations of isoprenaline (Botting *et al.*, 1961). Threshold concentrations of isoprenaline cause retention of salt and water in the absence of demonstrable change either in glomerular filtration rate or in renal plasma flow (Botting *et al.*, 1961 ; Tables 1 and 2). It is therefore possible that the β-adrenergic receptors involved in a threshold response are those associated with the postganglionic sympathetic innervation of the proximal tubules (Smith, 1956). Higher concentrations of isoprenaline reduce the renal plasma flow (Tables 1 and 2) ; this action is probably mediated through a second group of β-adrenergic receptors lying in close association with sympathetic vasodilator fibres. Some support for an hypothesis involving two separate groups of β-adrenergic receptors is given by the actions of the β-adrenergic blocking drugs dichloroisoprenaline and nethalide. Nethalide blocks those actions of isoprenaline which have been attributed to activation of the β-receptors of the proximal tubules less readily than it inhibits the vascular actions of the drug (Table 2). Dichloroisoprenaline sums with isoprenaline in tubular actions and competes with isoprenaline in vascular effects (Table 1).

There are two further points of considerable interest. The newly discovered isoprenaline-like actions of dichloroisoprenaline on the kidney (Table 1) are not without precedent ; both Furchgott (1959) and Dresel (1960) have drawn attention to the tachycardia produced by dichloroisoprenaline both *in vivo* and *in vitro*. Nethalide, by contrast, has a pure β-blocking action in the kidney which is complicated, but only with high doses, by an antidiuresis. This antidiuresis resembles that caused by vasopressin, for there are no accompanying changes either in the glomerular filtration rate, in the renal plasma flow or in the reabsorption of salt.

(±)-Isoprenaline hydrochloride (Winthrop Sterling) and nethalide (I.C.I.) were kindly supplied by the makers. Dichloroisoprenaline was obtained from L. Light & Co.

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