A NOTE ON THE ANTIDIURETIC EFFECT OF SMALL AMOUNTS OF ISOPRENALINE IN RATS

By J. B. Farmer and Mary F. Lockett

From The Department of Physiology and Pharmacology, Chelsea College of Science and Technology, London, S.W.3

Received March 20, 1961

Small subcutaneous amounts of isoprenaline, 0·5–1·0 μg./100 g. weight, caused reduction in the rates of excretion of sodium, potassium and water, both in sham operated and in adrenalectomised rats, and also in unoperated rats in the presence and absence of mannitol diuresis. These effects of isoprenaline were attributed to enhanced reabsorption of sodium and water in the proximal tubules.

Botting and Lockett (1961) demonstrated the antidiuretic action of as little as 0·25 μg. (±)-isoprenaline/100 g. weight injected subcutaneously into unanaesthetised rats. Later experiments (Botting, Farmer and Lockett, 1961) showed that this antidiuresis was accompanied by retention of sodium and chloride, a decreased excretion of ammonia and potassium and a rise in urinary pH; there was no demonstrable change either in glomerular filtration rate or effective renal plasma flow.

The extremely small quantities of isoprenaline required to elicit the urinary changes, suggested that they were brought about by change in the rate of secretion of neurohypophyseal or adrenal cortical hormones rather than by direct action of the amine on the kidneys. It is this hypothesis that we have sought to confirm or exclude by making examination of the effects of very small subcutaneous doses of isoprenaline during osmotic diuresis and in adrenalectomised animals.

Methods

Male rats of a single Wistar strain were fed diet 41 b. of Stein and were given water *ad libitum* unless otherwise stated. All were accustomed to stomach tubes and handling before use in experiments.

Water diuresis was induced, after absence of solid diet for 12–14 hr., by the oral administration of a standard water load equivalent to 5 per cent weight preceeded by an hydrating dose (Botting and Lockett, 1961). Combined mannitol and water diuresis was produced by the subcutaneous injection of 3 ml. 10 per cent mannitol per rat weighing 200–230 g. 30 min. before administration of the standard water load. Subcutaneous injections of 0·9 per cent NaCl, vasopressin and isoprenaline in 0·9 per cent NaCl were made immediately after the standard water load had been given. Urine was collected from individual animals for the succeeding period of 50 min.

Bilateral adrenalectomy and sham operations were performed through a middorsal incision on rats weighing 190–230 g. under pentobarbitone anaesthesia. Half the adrenalectomised animals were maintained by the addition of 0·4 per cent NaCl to their drinking water. The rest each received a daily injection of 0·1 ml. adrenal cortical extract and were

given the choice of tap or salt water to drink. Sham operated animals drank tap water.

All experiments were designed as cross-over tests in which treatments were assigned, day by day, to individual rats in an order predetermined by deliberate randomisation. Numerical values quoted are means ± the standard error of the mean followed by the number of animals within brackets. The significance of differences between means has been determined by *t* tests.

The concentrations of sodium and potassium in the urine were measured by means of an EEL flame photometer. Creatinine and diodone were estimated and their clearances were compared in cross over tests as described elsewhere (Botting, Farmer and Lockett, 1961).

Drugs. Isoprenaline sulphate (British Drug Houses Ltd.) Eucortone (Allen & Hanbury Ltd.) and pitressin (Parke Davis & Co. Ltd.) were obtained commercially.

Results

A comparison of the effect of a small subcutaneous injection of isoprenaline on water diuresis in adrenalectomised and sham operated rats. Comparison was made of the effect of subcutaneous injections of 0·5 μg. (±)-isoprenaline/100 g. weight and of saline on the rates of excretion of water, sodium and potassium by adrenalectomised and sham operated animals in the 50 min. after the administration of a water load and the making of these injections. This purpose was effected by means of a two day cross-over test made on the 4th and 6th postoperative days on one group of sham operated and two groups of adrenalectomised rats. One group of adrenalectomised animals had been treated with salt, the other with injections of adrenal cortical extract. In either case the treatment had been inadequate, for the rates of elimination of the water load were significantly lower ($P = <0{\cdot}01$) and those of excretion of $Na^+ + K^+$ higher ($P = <0{\cdot}01$) for the adrenalectomised than for the sham operated animals after control injections of saline (Table I). The Na^+/K^+ ratio in the urine was also higher in the adrenalectomised rats, 2·97 ± 0·21 (8), than in those which had had sham operations, 1·97 ± 0·52 (4). Despite the presence of adrenal insufficiency, isoprenaline, 0·5 μg./100 g. body weight, reduced the percentage of the water load and the μ-equivalents of Na^+ and K^+ excreted in the 50 min. after administration of the water load. significantly. Similar changes were induced by isoprenaline in the mock operated animals (Table I).

A comparison of the effect of subcutaneous injections of 1·0 μg. (±)-*isoprenaline*/100 *g. body weight on the excretion of sodium, potassium and water by rats in the presence and absence of mannitol.* Simultaneous investigation was made in 12 rats of the effect of subcutaneous injections of 0·9 per cent NaCl, 1·0 μg. (±)-isoprenaline/100 g. body weight and 1·5 mU. vasopressin/100 g. body weight both during water diuresis and during a combined water and mannitol diuresis. A six day cross over test was used for this purpose in which the subcutaneous injections were made at the time of administration of the water load, and the rates of

TABLE I

A COMPARISON OF THE EFFECTS OF SUBCUTANEOUS ISOPRENALINE, 0·5 μg./100 g. RAT, ON THE EXCRETION OF SODIUM POTASSIUM AND WATER BY SHAM OPERATED AND ADRENALECTOMISED RATS

Urine, per rat, excreted in 50 min.	Sham operated rats		Adrenalectomised rats			
			Salt maintained		Treated with adrenal cortical extract	
	Without amine	With amine	Without amine	With amine	Without amine	With amine
Per cent water load.. ..	50·3 ± 6·77 (4)	24·1 ± 3·66 (4)*	17·9 ± 1·41 (4)	8·7 ± 1·91 (4)*	18·6 ± 2·78 (4)	5·3 ± 4·15 (4)*
μ-equiv. Na^+	59·8 ± 13·62 (4)	18·0 ± 1·67 (4)*	165·8 ± 20·49 (4)	62·0 ± 19·11 (4)*	160·4 ± 16·14 (4)	79·3 ± 13·38 (4)*
μ-equiv. K^+	32·4 ± 5·27 (4)	14·4 ± 2·26 (4)*	57·8 ± 6·67 (4)	22·4 ± 6·90 (4)*	54·5 ± 3·07 (4)	16·3 ± 1·76 (4)*
Na^+/K^+	1·97 ± 0·52 (4)	1·59 ± 0·20 (4)	2·97 ± 0·27 (4)	4·3 ± 1·21 (4)	2·97 ± 0·25 (4)	3·7 ± 1·00 (4)

Significance of differences caused by isoprenaline was determined by *t* test and is indicated by an asterisk where P = <0·95.

TABLE II

A COMPARISON OF THE EFFECTS OF 1·0 μg. ISOPRENALINE AND OF 1·5 mU. VASOPRESSIN INJECTED SUBCUTANEOUSLY, EACH PER 100 g. RAT, ON THE EXCRETION OF WATER, SODIUM AND POTASSIUM IN THE PRESENCE AND ABSENCE OF MANNITOL

Urine per rat, excreted in 50 min.		Mannitol absent		Mannitol present		
	Saline subcut	Isoprenaline subcut	Vasopressin subcut	Saline subcut	Isoprenaline subcut	Vasopressin subcut
Per cent water load..	60·7 ± 1·9 (12)	27·8 ± 3·2 (12)*	34·3 ± 3·4 (12)*	57·9 ± 2·9 (12)	33·7 ± 1·7 (12)*	50·3 ± 2·4 (12)*
μ-equiv. Na^+ ..	84·6 ± 8·0 (12)	32·4 ± 2·7 (12)*	107·8 ± 10·5 (12)*	78·1 ± 11·4 (12)	40·2 ± 9·5 (12)*	111·7 ± 11·0 (12)*
μ-equiv. K^+ ..	81·8 ± 4·6 (12)	58·3 ± 1·5 (12)*	84·8 ± 1·9 (12)	79·5 ± 2·1 (12)	60·6 ± 2·7 (12)*	84·8 ± 3·6 (11)
Na^+/K^+	1·1 ± 0·12 (12)	0·6 ± 0·05 (12)*	1·2 ± 0·11 (12)*	1·2 ± 0·14 (12)	0·6 ± 0·13 (12)*	1·35 ± 00·04 (11)
Creatinine mg. ..	17·5 ± 0·4 (12)	16·5 ± 0·6 (12)	19·2 ± 1·0 (12)	18·9 ± 0·5 (12)	15·3 ± 0·4 (12)*	16·9 ± 0·6 (11)*
Diodone mg. ..	2·9 ± 0·08 (12)	2·8 ± 0·07 (12)	3·0 ± 0·13 (12)	3·0 ± 0·07 (12)	2·7 ± 0·08 (12)*	2·9 ± 0·08 (11)

Significance of difference caused by drugs has been determined by *t* test, and is indicated by an asterisk where P = <0·05.

excretion of Na^+, K^+ and water were measured over the succeeding period of 50 min. The combined results of these experiments are shown in Table II. The concentration of mannitol did not alter the rates of excretion of water, Na^+ and K^+ found during water diuresis but did, in very large part, antagonise the antidiuretic action of 1·5 mU. vasopressin/100 g. without influencing the augmented Na^+ output which resulted from the natural vasopressin used. Isoprenaline however, caused antidiuresis and reduction in the rates of excretion of Na^+ and K^+ almost equally effectively in the presence and the absence of the mannitol. The slight tendency to reduction in filtration rate and effective renal plasma flow caused by this dose of the amine during water diuresis became significant only during mannitol diuresis (Table II).

Discussion

The fact that subcutaneous injections of 0·5 μg. ($\pm$)-isoprenaline/100 g. body weight caused antidiuresis and reduced the rates of excretion of sodium and potassium similarly in sham operated and in partially deficient adrenalectomised rats (Table I) proves that these actions of isoprenaline are not caused by changes in the rate of secretion of adrenal hormones. Neither are they in part the consequence of change in the rate of secretion of vasopressin, for, whereas mannitol effectively antagonised the antidiuretic effect of 1·5 mU. vasopressin/100 g. rat, it did not antagonise that of isoprenaline, (Table II). Whereas both vasopressin and isoprenaline reduced the glomerular filtration rate, only the isoprenaline reduced the excretion of sodium and water (see Table II, mannitol diuresis).

References

Botting, R., and Lockett, Mary, F. (1961). *Arch. Internat. de Physiol.* **69**, 36–45.
Botting, R., Farmer, J. B., and Lockett, M. F. (1961), *Ibid.*, **69**, June.