A NOTE ON THE INFLUENCE OF A METABOLITE OF ADRENALINE ON WATER DIURESIS IN RATS

By D. Roberts and Mary F. Lockett

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

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Solutions of an isoprenaline-like metabolite of adrenaline, and of isoprenaline itself which had been equated in potency both by depressor action on the mean arterial pressure of rats and by their inhibitory action on the rat uterus, equated also in effect on water diuresis in rats. The metabolite, like isoprenaline, reduced the rates of excretion of water, sodium and potassium and raised the pH of the urine.

A METABOLITE of (—)-adrenaline, indistinguishable from isoprenaline both in R_F value and in pharmacological action (Lockett, 1954, 1957, 1959) has been found in the plasma of blood withdrawn from the lower aorta (Eakins and Lockett, 1961). It is therefore expected to be present also in the plasma of renal arterial blood. Earlier work from this laboratory has shown that in unanaesthetised rats the subcutaneous injection of very small quantities of isoprenaline (0·75 μg. per 100 g.) diminishes the rates of excretion of sodium, potassium, ammonium and chloride and raises the pH of the urine (Botting, Farmer and Lockett, 1961; Farmer and Lockett, 1961, Lockett, 1959). We were interested, for these reasons, to compare the actions of isoprenaline and this metabolite of adrenaline on renal function in rats during water diuresis.

Methods

Preparation of the metabolite for injection. Rabbits, 1·5–2·7 kg., were anaesthetised by slow injection of 5 ml. of 20 per cent urethane per kg. into a marginal ear vein. A tracheal cannula was inserted and 5 mg./kg. harmeline in 10 ml. of 0·9 per cent aqueous NaCl was similarly injected; then a polythene cannula was introduced through a femoral artery so that its tip lay just above the bifurcation of the aorta. Next heparin, 1,000 u/kg., was injected into an ear vein and the needle fixed in position for an infusion of (—)-adrenaline bitartrate 10 μg./ml. in 0·9 per cent NaCl, begun 30 min. after the injection of harmeline and 5 min. before bleeding, and maintained at a rate of 4 μg./min. Blood was collected from the femoral arterial cannula into heparinised ice-cold tubes; plasma was separated without delay. The metabolite was isolated from protein free extracts of plasma by ascending paper chromatography, using phenol-hydrochloric acid as solvent in an atmosphere of carbon dioxide, and eluates were prepared for biological use as previously described (Lockett, 1954, 1957). These eluates were assayed in terms of (±)-isoprenaline activity by their depressor action on the mean arterial pressure of rats anaesthetised with pentobarbitone sodium and by inhibition of the response of a quiescent dioestrus rat uterus to a fixed dose of acetylcholine, submaximal in effect (Eakins and Lockett, 1961).

Renal function tests. Male wistar rats, weighing 225–275 g., accustomed to stomach-tubes and handling, were fasted for 12 hr. before experiments. Each received an hydrating dose of tap water, 2 ml./100 g. weight, by stomach-tube, at zero time. At 1 hr., 5 ml. of tap water/100 g. weight was similarly administered and the bladder was emptied by gentle suprapubic pressure. A subcutaneous injection of isoprenaline, adrenaline metabolite or of normal saline was now given and the rat was placed in an individual cage for the collection of all urine excreted in the subsequent 90 min., when the bladder was again emptied. Two groups of 12

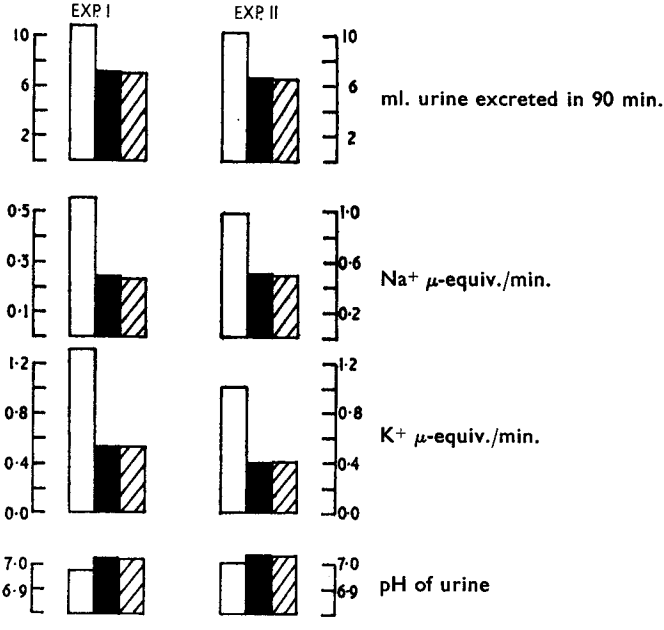

FIG. 1. A comparison of the effects of isoprenaline and a metabolite of adrenaline (assayed as isoprenaline) on the excretion of water, sodium and potassium in the 90 min. after administration of a water-load equivalent to 5 per cent weight. The mean results of two cross-over tests, each on 12 rats, are shown. Open rectangles, control values. Black rectangles, subcutaneous (±)-isoprenaline 0·75 μg./100 g. (Exp. I) and 1·0 μg./100 g. (Exp. II) weight. Hatched rectangles, Exp. I, 0·7 μg. and Exp. II, 1·0 μg. (±)-isoprenaline-like activity per 100 g. in eluate of metabolite injected subcutaneously (see also Table I).

rats were subjected to a 3 part cross-over test, made every third day, in which each animal received the three different subcutaneous injections in an order determined by randomisation within and between groups. Concentrations of Na^+ and K^+ in urine were measured in a flame photometer, and the pH of urines was determined by a glass electrode.

Drugs. (±)-Isoprenaline hydrochloride (Winthrop Sterling Inc.), Harmeline (L. Light & Co. Ltd.), (−)-adrenaline bitartrate (Burroughs Wellcome Ltd.), and heparin, Liquemin (Roche Products Ltd.) were obtained commercially.

RESULTS

Two three part cross-over experiments were used to compare the submaximal effects of subcutaneous injections of (±)-isoprenaline and of the isoprenaline-like metabolite of adrenaline on the elimination of a water-load by rats. The concentrations of metabolite in the eluates were assayed biologically for isoprenaline-like activity; these answers enabled a calculated dose to be used in the cross-over tests. The mean results of these two experiments are shown in Fig. 1, and the significance of differences caused by the administration of either isoprenaline or the metabolite are examined in Table I. In the first experiment the effects of 0·75 μg.

TABLE I

COMPARISON OF THE EFFECTS OF (±)-ISOPRENALINE AND A METABOLITE OF (−)-ADRENALINE ON THE EXCRETION OF WATER, SODIUM AND POTASSIUM BY RATS, IN THE FIRST 90 MIN. AFTER ADMINISTRATION OF A STANDARD WATER LOAD. THE VALUES SHOWN ARE MEANS ± STANDARD ERRORS OF THE MEAN. SIGNIFICANCE OF DIFFERENCES WAS DETERMINED BY *t* TEST, ONE ASTERISK, P = <0·05; TWO, P = <0·001.

Expt. I	Control	Isoprenaline 0·75 μg./100 g.	Metabolite as Isoprenaline 0·7 μg./100 g.
Water load excreted in 90 min., per cent ..	81·5 ± 3·55	52·6 ± 2·76**	52·0 ± 3·42**
Urine. m-equiv. Na^+/litre	8·5 ± 0·93	6·8 ± 0·63*	7·3 ± 0·88
m-equiv. K^+/litre..	9·1 ± 0·75	5·4 ± 0·71**	6·0 ± 0·53**
μ-equiv. Na^+/min.	0·9 ± 0·14	0·5 ± 0·06**	0·5 ± 0·04**
μ-equiv. K^+/min...	1·0 ± 0·09	0·4 ± 0·05**	0·4 ± 0·04**
pH	7·02 ± 0·03	7·28 ± 0·05*	7·24 ± 0·03**
Expt. II		1·0 μg./100 g.	1·0 μg./100 g.
Water load excreted in 90 min., per cent. ..	83·8 ± 2·78	51·8 ± 5·80**	51·6 ± 5·3**
Urine. m-equiv. Na^+/litre	3·9 ± 0·50	2·4 ± 0·32**	2·4 ± 0·31**
m-equiv. K^+/litre..	10·9 ± 1·05	6·7 ± 0·95**	6·3 ± 0·73**
μ-equiv. Na^+/min.	0·46 ± 0·06	0·20 ± 0·02**	0·19 ± 0·02**
μ-equiv. K^+/min...	1·31 ± 0·14	0·52 ± 0·06**	0·52 ± 0·07**
pH	6·94 ± 0·04	7·13 ± 0·01**	7·10 ± 0·03**

isoprenaline per 100 g. weight are compared with those of 0·7 ± 0·05 μg. isoprenaline-like activity of the metabolite per 100 g. weight in 12 rats weighing 250 ± 1·0 g. The second experiment differed in that the dose of metabolite was increased to 1·0 ± 0·1 μg./100 g. and compared with 1·0 μg. isoprenaline in rats weighing 254 ± 5·3 g. Fig. 1 demonstrates that isoprenaline and the metabolite caused equivalent reduction in the standard water load per cent excreted in 90 min. and similarly reduced not only the rates of excretion of Na^+ and K^+ (Fig. 1), but also their concentration in the urine (Table I). Both substances raised the pH of the urine, similarly. All these induced changes were of high significance (Table I). No significant differences were demonstrable by *t* tests between the effects of isoprenaline and the metabolite of adrenaline.

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