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The influence of β-adrenoceptive receptor blocking agents on urinary function in the rat

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1. Intramuscular or subcutaneous injections of isoprenaline and dichloroisoprenaline and subcutaneous injection of pronethalol reduced the rates of excretion of water, sodium, potassium and chloride in the urine of conscious, hydrated rats. Inulin excretion usually fell at high, but not at low, dose levels. These changes were attributed to direct stimulant actions on β-adrenoceptors in the kidney.

2. A reduction in perfusion pressure to the kidney may also have contributed to these urinary changes, because isoprenaline produced a transient fall in mean arterial blood pressure when given subcutaneously to anaesthetized rats.

3. Intramuscular injection of pronethalol and subcutaneous injection of antidiuretic hormone both reduced the rate of urine flow without modifying other parameters of urinary function (excretion of inulin and electrolytes were not diminished).

4. This latter action of pronethalol could not be ascribed to an increased secretion of antidiuretic hormone, for it also occurred in hypophysectomized rats.

5. Propranolol increased the renal excretion of sodium and chloride. A small rise in urinary potassium levels also occurred but urine volume and inulin excretion were not modified. Some possible modes of action of propranolol are discussed.

Previous investigations of Lockett and her co-workers have shown that small doses of isoprenaline and adrenaline reduce the rates of urine flow and electrolyte excretion, when given by subcutaneous injection to conscious, hydrated rats (Botting, Farmer & Lockett, 1961 ; Botting & Lockett, 1961 ; Farmer & Lockett, 1961a, b ; Lockett & Mrozowska, 1958 ; Roberts & Lockett, 1961). These responses were attributable to an action on β-adrenoceptive receptors, because they were antagonized by pronethalol (Lees & Lockett, 1963). The latter authors also found that the two β-receptor blocking agents, dichloroisoprenaline and pronethalol, reduced the rate of excretion of a standard water load in conscious rats. In addition, dichloroisoprenaline enhanced the reabsorption of sodium ions by the kidney. The action of this drug, being similar in nature to that of isoprenaline, was there-

fore ascribed to a stimulant action on β-receptors. Pronethalol, on the other hand, did not alter the excretion of sodium ions significantly, so it was tentatively suggested that this compound might act by increasing the secretion of antidiuretic hormone from the posterior pituitary.

The present paper records the results of investigations in which the latter hypothesis was subjected to experimental test. In addition, changes in volume and composition of the urine in the rat, resulting from the administration of isoprenaline and the β-receptor antagonists, dichloroisoprenaline, pronethalol and propranolol, have been examined in two experimental conditions. The compounds were administered subcutaneously immediately before or intramuscularly 30 min before the period of urine collection.

Methods

Male Wistar rats, fed diet 86 and allowed free access to water, were accustomed to stomach tubes, handling and the metabolism cages before use. The room temperature was maintained at 21° ± 1° C. All experiments were designed as cross-over tests, in which each animal received each treatment in an order pre-determined by deliberate randomization. The cross-over tests were performed twice weekly at intervals of 3 or 4 days. The differences in parameters of urinary function between treated and untreated animals were subsequently examined for statistical significance by *t* tests, in which the results from each animal were made to serve as their own controls.

Cross-over tests

The times of the various procedures in the cross-over tests were kept constant in each experiment, so that the effects of circadian rhythms of water and electrolyte excretion would be nullified.

Intact animals were deprived of solid food for 14–16 hr before the start of the test on experimental days but water was allowed *ad libitum* until the morning of the test. For the hypophysectomized rats, the starvation period was reduced to 3 hr, in order to avoid undue stress on these animals. At zero time drinking water was removed and a distilled water load, comprising 2.5% of the body weight, given orally by stomach tube. A second 2.5% water load was administered 1 hr later and the rats were then placed in individual metabolism cages for a period of 1 hr, having received either agonist or antagonist drugs for β-receptors. (In one experiment in hypophysectomized animals the duration of the urine collection period was extended to 2 hr.) The bladders were emptied by gentle suprapubic pressure immediately before urine collection commenced and again at the end of the collection period.

In one series of experiments subcutaneous injections of (±)-isoprenaline hydrochloride, (±)-dichloroisoprenaline hydrochloride, (±)-pronethalol hydrochloride and (±)-propranolol hydrochloride, each in 0.1 ml. of normal saline, were given immediately before the rats were placed in metabolism cages for the collection of urine. In other experiments the same drugs, dissolved in 0.1 ml. of propylene glycol, were injected into the gastrocnemius muscle 30 min before the commencement of urine collection. In all experiments the control rats received 0.1 ml. of the appropriate solvent. The rate of excretion of a standard inulin load, comprising

3 ml. of a 5% inulin solution per rat by subcutaneous injection, was employed as an approximate measure of the glomerular filtration rate ; inulin was administered 30 min before the second hydration. The previous experiments of Botting *et al.* (1961) have shown that plateau levels of inulin in the blood are maintained throughout the period of urine collection when the inulin load is given 30 min before the second water load.

Blood pressure recordings

Recordings of mean arterial blood pressure were made from a cannulated carotid artery in eight anaesthetized rats weighing from 180 to 285 g ; four animals were anaesthetized with pentobarbitone sodium and four with urethane, each given intraperitoneally.

Hypophysectomy

Twenty rats were hypophysectomized under ether anaesthesia by the transpharyngeal route, as described by Burn, Finney & Goodwin (1950). These animals were then maintained by making the solid food pellets into a stiff mash with water and received in addition 2% glucose in the drinking water for 3 days postoperatively. Thereafter, the animals ate solid food and drank tap water, like the intact, unoperated rats. Hypophysectomized animals were used in the cross-over tests from 13 to 28 days postoperatively. At post mortem, carried out 26 to 36 days postoperatively, no hypophysial tissue was visible in the pituitary fossa of any of the rats, the results from which are presented in this paper. Confirmation of the completeness of hypophysectomy was obtained during life by comparing the ability to excrete a standard water load, comprising 2.5% of the body weight during a period of 1 hr, and by measurement of adrenal and testicular weights after the animals were killed in normal and hypophysectomized animals. These data are summarized below, the mean values being expressed per 100 g body weight.

	Adrenals (mg)	Testes (g)	Water excretion (ml.)
Twelve normal animals	20.7	1.10	2.14
Twenty hypophysectomized rats	8.4	0.26	0.40

Analytical procedures

Concentrations of sodium and potassium in the urine were estimated photometrically using an Eel flame photometer, chloride was determined by potentiometric titration with silver nitrate and inulin by the diphenylamine method of Smith (1956).

Drugs

(±)-Isoprenaline hydrochloride (Winthrop Sterling), (±)-pronethalol hydrochloride (Imperial Chemical Industries) and (±)-propranolol hydrochloride (Imperial Chemical Industries) were kindly supplied by the makers. (±)-Dichloroisoprenaline hydrochloride (L. Light and Co.) and antidiuretic hormone (Pitressin, Parke Davis Co.) were obtained commercially. Doses of all drugs, excepting Pitressin, are expressed as the salts.

Results

Influence of isoprenaline on urinary function

Doses of 1.0–4.0 μg (±)-isoprenaline hydrochloride reduced the rates of urine flow and excretion of sodium, potassium and chloride ions to significant degrees during the 1 hr period of urine collection. These responses were obtained when the drug was given either by intramuscular injection 30 min before urine collection or subcutaneously immediately preceding the collection period. In neither instance were the changes in urinary Na/K ratio or the rate of excretion of the standard

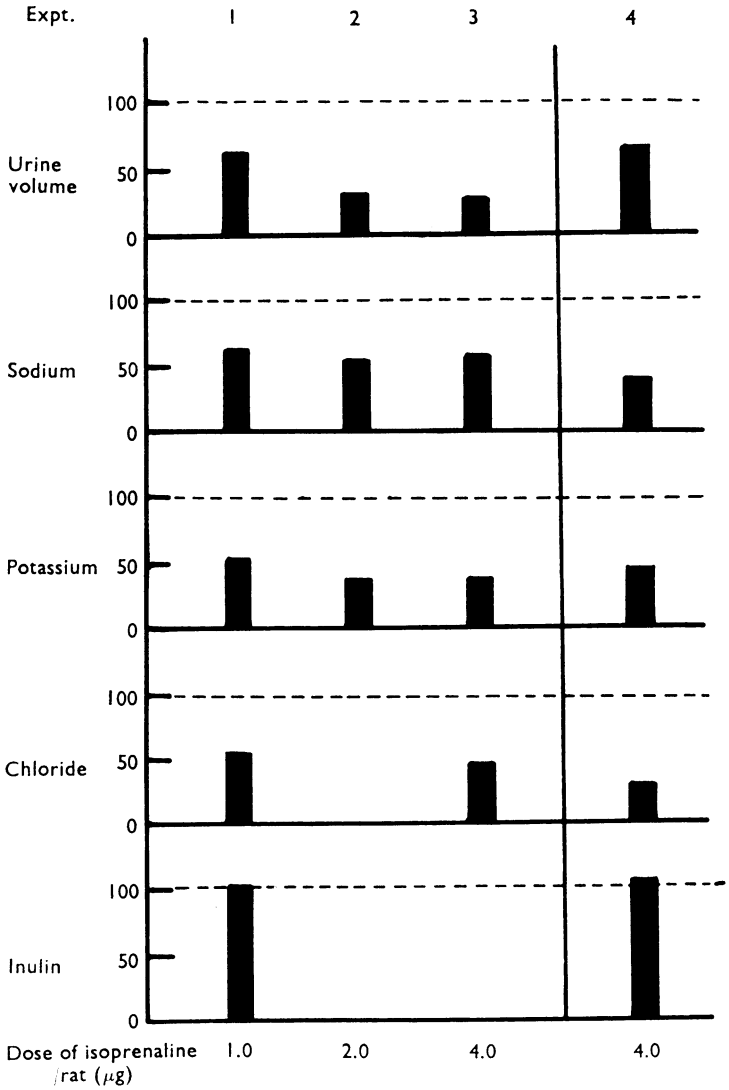

FIG. 1. Influence of (±)-isoprenaline hydrochloride on urinary function in normal rats. Ordinates: the heights of the rectangles represent the rates of excretion of water, sodium, potassium, chloride and inulin expressed as percentages of the control values (dotted lines). Isoprenaline was administered subcutaneously (Expts. 1, 2 and 3) in 0.1 ml. normal saline immediately before or intramuscularly (Expt. 4) in 0.1 ml. propylene glycol 30 min before the 1 hr urine collection period.

TABLE 1. *Influence of isoprenaline hydrochloride on urinary function in normal rats*

Expt.	Body weight (g)	Water (ml.)	Excretion rates/100 g body weight/hr: Na	K (μ-equiv)	Cl	Na/K	Inulin (mg)	Dose per rat (μg)
1	284±8·0 (12)	1·84±0·09	11·3±2·6	29·7±3·3	20·7±3·9	0·34±0·06	13·4±1·2	—
		1·15±0·10*	7·1±3·8*	15·4±2·1**	11·3±2·8*	0·38±0·08	13·5±0·8	1·0
2	177±4·9 (10)	2·14±0·44	4·8±0·19	13·8±2·8		0·34±0·16		—
		0·66±0·27**	2·6±0·26*	5·1±1·0**		0·41±0·13		2·0
3	268±6·0 (12)	1·90±0·08	7·7±2·2	19·0±2·5	10·1±2·1	0·38±0·07		—
		0·51±0·13**	4·4±1·2*	6·9±1·5**	4·5±1·5**	0·62±0·09		4·0
4	221±3·0 (12)	1·76±0·13	10·8±2·5	19·7±3·3	20·4±4·3	0·53±0·08	15·5±0·8	—
		1·17±0·16**	4·0±1·2**	8·4±1·3**	6·1±1·4**	0·44±0·11	16·3±1·2	4·0

Isoprenaline was administered subcutaneously (experiments 1, 2 and 3) immediately before or intramuscularly (experiment 4) 30 min before the urine collection period. The values are means±the standard errors. The significance of differences between means, attributable to the effect of isoprenaline, has been examined by *t* tests and is indicated by asterisks; one, $P<0·05$; two, $P<0·01$. Values in parentheses are the numbers of rats.

TABLE 2. *Influence of dichloroisoprenaline hydrochloride on urinary function in normal rats*

Expt.	Body weight (g)	Excretion rates/100 g body weight/hr: Water (ml.)	Na	K (μ-equiv)	Cl	Na/K	Inulin (mg)	Dose per rat (mg)
5	233±4·0 (12)	2·17±0·12	14·4±4·4	25·2±3·8	21·0±6·3	0·50±0·10	15·8±0·5	—
		0·43±0·09**	2·4±0·5**	6·2±1·0**	2·9±0·8**	0·53±0·16	9·4±1·3*	4·0
6	277±6·0 (10)	1·88±0·16	5·1±1·6	15·6±2·4	10·6±2·8	0·30±0·05	12·7±0·8	—
		0·67±0·12**	4·2±1·8	8·5±1·9*	3·1±1·5**	0·41±0·08	14·7±1·6	0·4
7	162±4·7 (9)	2·21±0·37	4·8±0·87	13·9±2·8		0·41±0·09	17·8±1·0	—
		0·68±0·19**	1·7±0·30**	3·0±0·4**		0·67±0·13	12·4±1·7*	1·0
8	283±7·0 (12)	1·67±0·15	4·6±1·0	13·0±0·5	6·0±1·0	0·34±0·06		—
		0·17±0·05**	1·1±0·2**	2·6±0·9**	1·0±0·2**	0·88±0·29**		4·0

Dichloroisoprenaline was administered subcutaneously (experiment 5) immediately before or intramuscularly (experiments 6, 7 and 8) 30 min before the urine collection period. The values are means±the standard errors. The significance of differences between means, attributable to the effect of dichloroisoprenaline, has been examined by *t* tests and is indicated by asterisks; one, $P<0·05$; two, $P<0·01$. Values in parentheses are the numbers of rats.

inulin load found to be statistically significant, although the reduction in potassium excretion usually exceeded the fall in urinary sodium. (Fig. 1 ; Table 1.)

Influence of isoprenaline on mean arterial blood pressure

The changes in mean arterial blood pressure, following the administration of 4.0 μg (±)-isoprenaline hydrochloride subcutaneously to anaesthetized rats, were recorded in eight experiments (Fig. 2). In general, when the initial pressure was low (less than 80 mm Hg) isoprenaline produced little or no reduction. At somewhat higher initial pressures (85 mm Hg or greater), isoprenaline caused a fall for periods of 10–18 min ; the maximal reduction in blood pressure was of the order of 10 to 15 mm Hg. The weights of rats used in these experiments were similar to those of the conscious animals employed in the urinary function tests. Alterations in volume and composition of the urine, which followed the administration of isoprenaline to conscious rats, may thus have resulted, in part, from a fall in renal perfusion pressure.

Influence of dichloroisoprenaline on urinary function

Doses of 0.4 4.0 mg (±)-dischloroisoprenaline hydrochloride, injected subcutaneously immediately before or intramuscularly 30 min before urine collection, exerted actions similar to isoprenaline (Fig. 3 ; Table 2). Thus the excretory rates of sodium, potassium and chloride ions, as well as the rate of urine flow, were significantly reduced in most experiments. In addition, the Na/K ratio of the urine tended to rise, while the renal elimination of inulin fell at high but not at low dose levels.

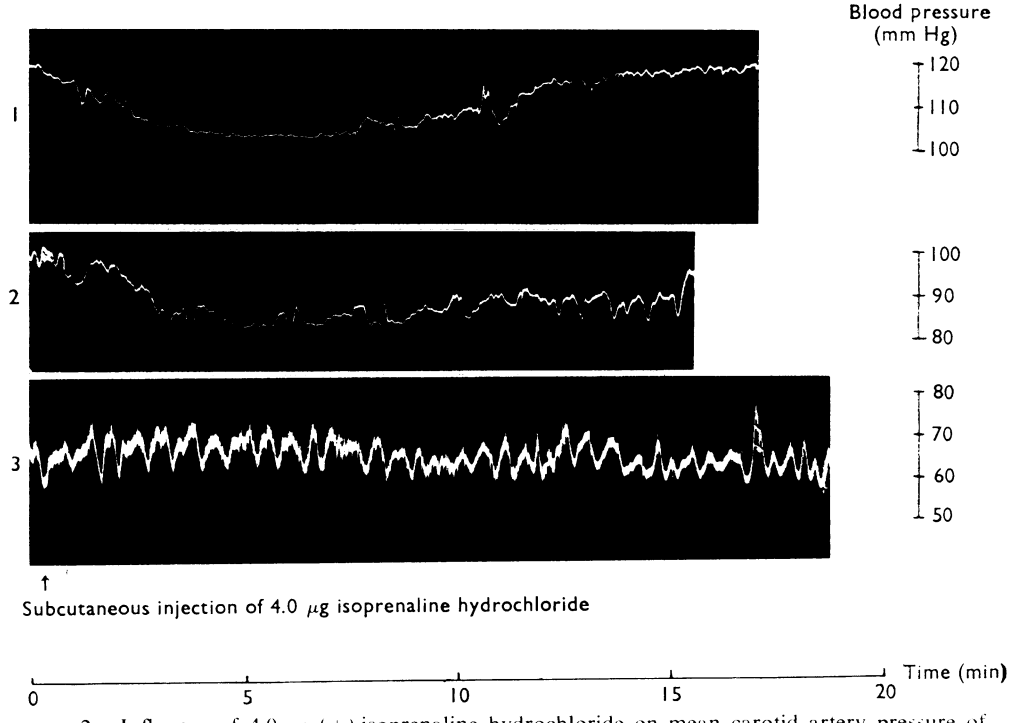

FIG. 2. Influence of 4.0 μg (±)-isoprenaline hydrochloride on mean carotid artery pressure of anaesthetized rats ; isoprenaline injected subcutaneously in 0.1 ml. saline at arrow. Anaesthetic : 1 and 2, pentobarbitone sodium ; 3 urethane.

Influence of pronethalol on urinary function

The actions of (±)-pronethalol hydrochloride in normal rats were found to be dependent on the route of injection and time of administration before the final water load (Fig. 4; Table 3). For example, at the dose level of 4.0 mg/rat, the drug, when given subcutaneously with the second water load, produced changes in urinary composition similar to isoprenaline and dichloroisoprenaline. The urine volume and rates of excretion of sodium, potassium, chloride and inulin were reduced. A dose of 2.0 mg pronethalol was sub-threshold in this respect. By contrast, the intramuscular injection of 2.0 and 4.0 mg pronethalol 30 min before urine collection

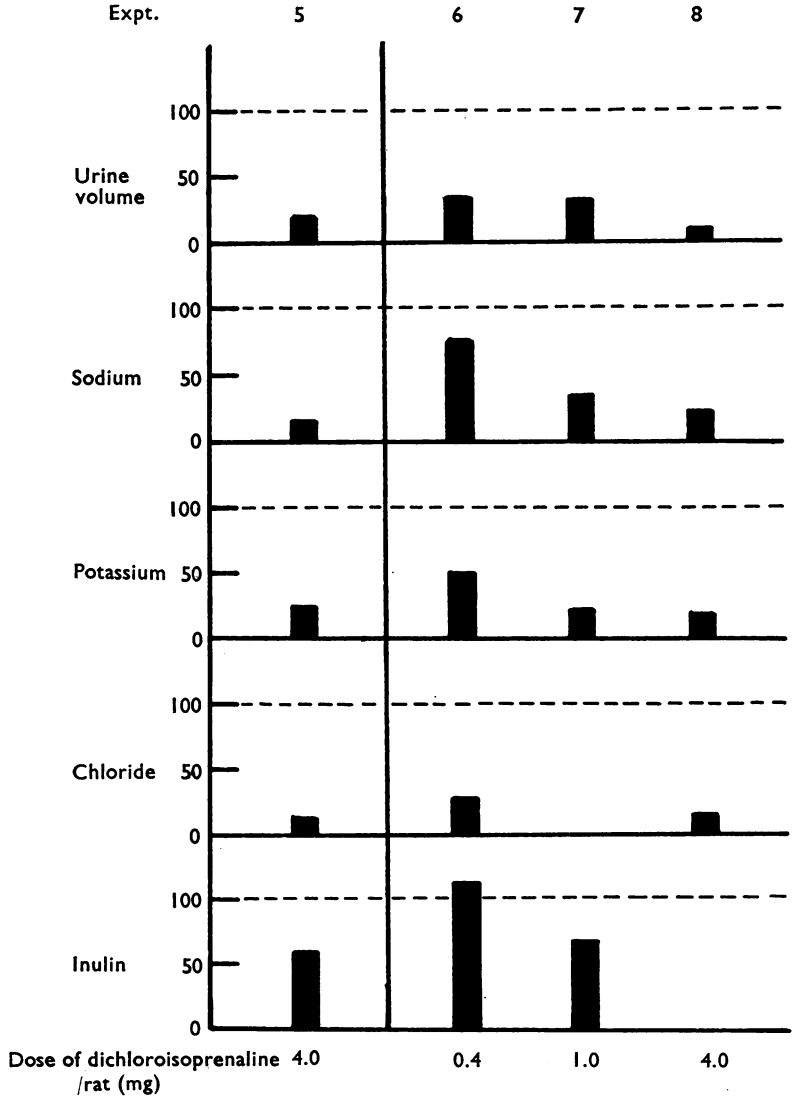

FIG. 3. Influence of (±)-dichloroisoprenaline hydrochloride on urinary function in normal rats. Ordinates: as in Fig. 1. Dichloroisoprenaline was administered subcutaneously (Expt. 5) in 0.1 ml. normal saline immediately before or intramuscularly (Expts. 6, 7 and 8) in 0.1 ml. propylene glycol 30 min before the 1 hr urine collection period.

failed to influence the excretory rates of sodium, potassium, chloride and inulin to statistically significant degrees. In most experiments, however, the renal excretion of sodium rose slightly, while urinary potassium levels tended to fall. Although these changes were not of themselves normally statistically significant, the resulting rise in urinary Na/K ratio did differ significantly from the control values in two of four experiments. The most consistent finding in the experiments involving intramuscular administration of pronethalol was an antidiuresis. This reduction in urine volume occurred in all four experiments at both 2.0 and 4.0 mg dose levels.

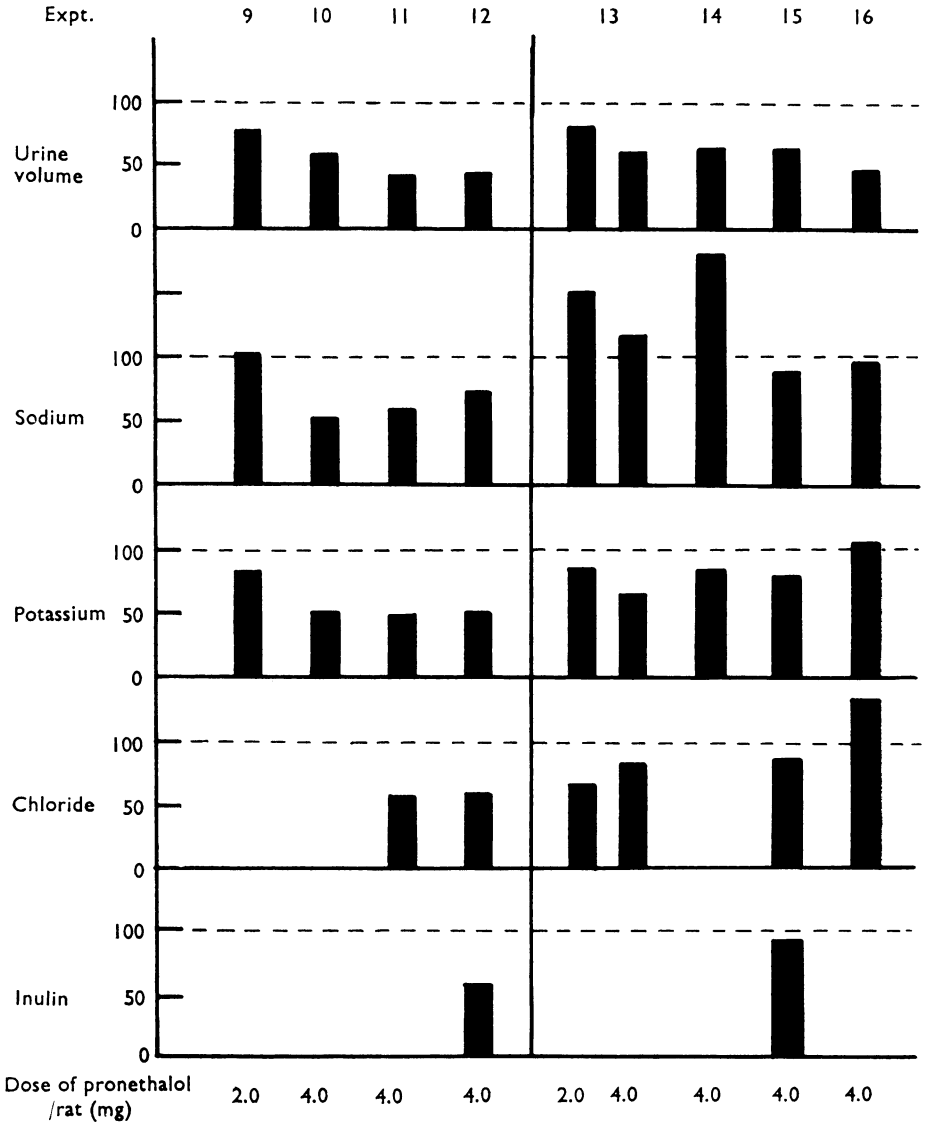

FIG. 4. Influence of (±)-pronethalol hydrochloride on urinary function in normal rats. Ordinates: as in Fig. 1. Pronethalol was administered subcutaneously (Expts. 9, 10, 11 and 12) in 0.1 ml. normal saline immediately before or intramuscularly (Expts. 13, 14, 15 and 16) in 0.1 ml. propylene glycol 30 min before the 1 hr collection period.

TABLE 3. *Influence of pronethalol hydrochloride on urinary function in normal rats*

Expt.	Body weight (g)	Excretion rates/100 g body weight/hr: Water (ml.)	Na	K (μ-equiv)	Cl	Na/K	Inulin (mg)	Dose per rat (mg)
9	177±3·0 (11)	2·19±0·16	20·0±4·6	31·8±4·7		0·66±0·10		—
		1·71±0·24	21·3±9·9	26·4±7·8		0·65±0·10		2·0
10	187±3·0 (12)	1·97±0·15	31·8±10·7	38·5±6·5		0·70±0·12		—
		1·18±0·17*	17·2±6·4*	19·3±4·2**		0·95±0·25		4·0
11	176±3·0 (12)	2·19±0·15	30·0±6·8	34·4±6·1	47·3±12·4	0·90±0·14		—
		0·91±0·15**	18·0±4·2*	17·1±3·1*	27·2±6·4**	1·03±0·17		4·0
12	185±4·0 (11)	2·07±0·17	16·1±4·2	21·4±3·6	26·1±6·3	0·82±0·24	13·8±1·0	—
		0·93±0·07**	12·0±3·3	10·9±1·8**	16·0±4·4*	0·97±0·17	8·1±0·9*	4·0
13	234±7·0 (11)	2·30±0·11	5·2±1·1	14·2±1·2	7·3±2·0	0·35±0·07		—
		1·87±0·14*	7·8±2·0	12·3±1·3	5·9±1·1	0·65±0·13*		2·0
		1·41±0·23**	6·1±1·0	9·4±1·0	6·1±1·9	0·64±0·08*		4·0
14	242±3·0 (12)	2·20±0·11	8·2±1·4	21·7±2·6		0·38±0·06		—
		1·41±0·22*	14·6±4·5*	18·0±1·6		0·82±0·24**		4·0
15	174±3·0 (12)	1·87±0·10	25·8±3·6	32·5±3·7	54·2±8·9	0·82±0·10	17·9±1·0	—
		1·17±0·24*	23·2±3·2	26·0±4·3	47·0±9·2	0·99±0·11	16·8±1·9	4·0
16	198±4·0 (12)	2·38±0·13	18·6±3·7	21·7±2·7	26·1±5·3	0·81±0·10		—
		1·12±0·33**	18·3±2·9	22·6±3·7	35·1±7·6	0·93±0·12		4·0

Pronethalol was administered subcutaneously (experiments 9, 10, 11 and 12) immediately before or intramuscularly (experiments 13, 14, 15 and 16) 30 min before the urine collection period. The values are means±the standard errors. The significance of differences between means, attributable to the effect of pronethalol, has been examined by *t* tests and is indicated by asterisks; one, $P<0·05$; two, $P<0·01$. Values in parentheses are the numbers of rats.

TABLE 4. *Influence of antidiuretic hormone on urinary function in normal rats*

Expt.	Body weight (g)	Excretion rates/100 g body weight/hr: Water (ml.)	Na	K (μ-equiv)	Cl	Na/K	Inulin (mg)	Dose per rat (m-u.)
17	155±3·7 (11)	2·47±0·17	52·3±5·9	23·2±4·3		2·64±0·33		—
		1·82±0·22*	52·2±8·7	21·3±6·0		3·31±0·54		0·25
		1·06±0·14**	46·2±5·1	18·3±2·4		3·02±0·37		0·50
18	234±3·0 (12)	1·69±0·96	20·7±4·6	31·3±3·7	30·1±6·8	0·62±0·10	14·6±1·0	—
		1·17±0·05*	14·5±3·5	25·4±2·8	27·3±5·3	0·54±0·10	13·3±1·0	0·50
19	236±5·0 (11)	1·98±0·07	13·2±3·0	26·2±3·2	20·0±4·3	0·48±0·08		—
		1·05±0·12**	14·0±4·5	22·3±2·3	22·0±4·4	0·55±0·12		0·50

Antidiuretic hormone was administered subcutaneously immediately before the period of urine collection. The values are means±the standard errors. The significance of differences between means, attributable to the effect of antidiuretic hormone, has been examined by *t* tests and is indicated by asterisks; one, $P<0·05$; two, $P<0·01$. Values in parentheses are the numbers of rats.

Influence of antidiuretic hormone on urinary function

Subcutaneous injections of doses of 0.25 and 0.50 m-u. antidiuretic hormone immediately before urine collection influenced neither the urinary electrolyte composition nor the excretion of the standard inulin load (Fig. 5 ; Table 4). Although potassium excretion tended to fall and the urinary Na/K ratio usually rose slightly, such changes were never significantly different from control values. On the other hand, the characteristic antidiuretic action of the hormone was recorded in all experiments. These effects of antidiuretic hormone were therefore both qualitatively and quantitatively similar to those resulting from the intramuscular injection of pronethalol.

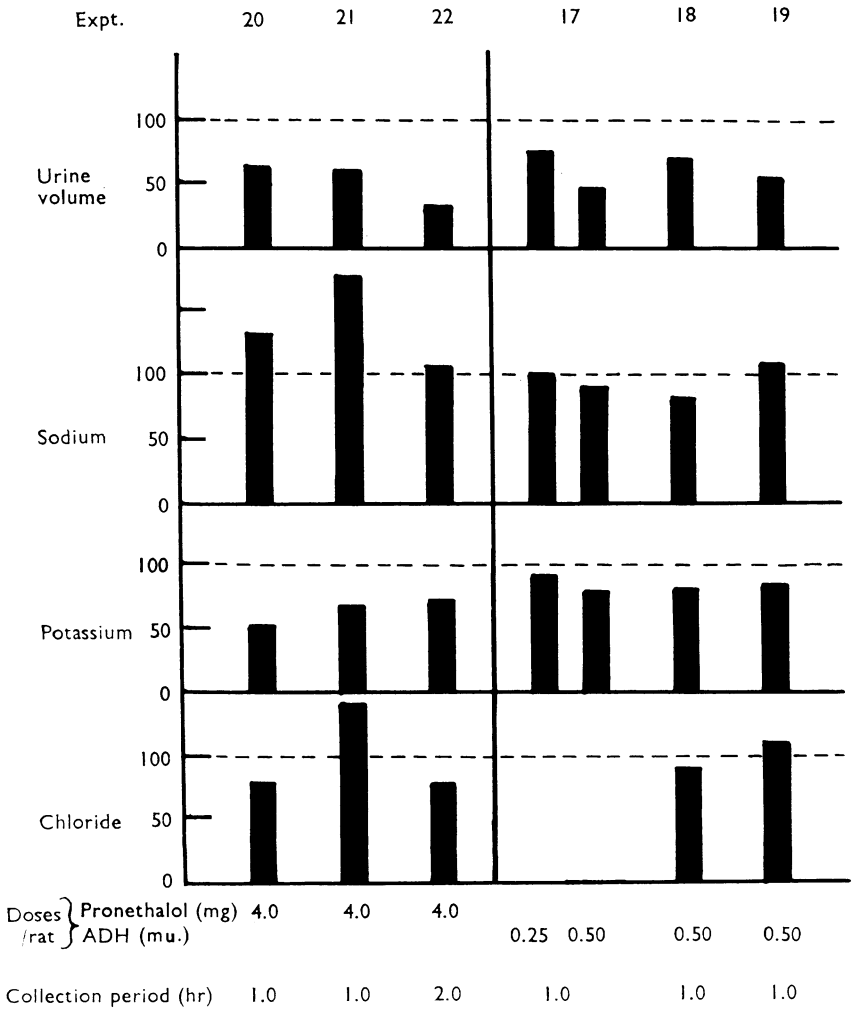

FIG. 5. Comparison of the effects of (±)-pronethalol hydrochloride on urinary function in hypophysectomized rats with the effects of antidiuretic hormone (ADH) in normal rats. Ordinates: as in Fig. 1. Pronethalol was administered intramuscularly (Expts. 20, 21 and 22) in 0.1 ml. propylene glycol 30 min before the collection period. ADH was given subcutaneously (Expts. 17, 18 and 19) in 0.1 ml. saline immediately before the 1 hr collection period.

Influence of pronethalol on urinary function in hypophysectomized rats

The similarity between the actions of pronethalol and antidiuretic hormone seemed to warrant an investigation of the hypothesis that the changes in volume and composition of the urine produced by the former drug were the indirect consequence of an increased secretion of antidiuretic hormone from the posterior pituitary (Fig. 5; Table 5). For this purpose, doses of 4.0 mg pronethalol were given intramuscularly to hypophysectomized rats 30 min before urine collection. In two experiments (17 and 18) a 1 hr urine collection period was employed and in a further test (experiment 19) the duration was extended to 2 hr, because the control rates of urine flow were very low in the former experiments. The characteristic actions of pronethalol were recorded in all three experiments, namely, an antidiuresis without significant alterations in urinary electrolyte excretion. As in the normal animals, however, the Na/K ratio and sodium excretion tended to rise, while the renal elimination of potassium was somewhat reduced. These findings indicate therefore that the actions of pronethalol do not result from increased secretion of antidiuretic hormone.

Influence of propranolol on urinary function

The actions of (±)-propranolol hydrochloride in normal rats were examined at the two separate dose levels of 0.4 and 0.8 mg, each being given either subcutaneously immediately before or intramuscularly 30 min before urine collection (Fig. 6; Table 6). The responses to propranolol were similar in both experimental conditions and comprised significant increases in the excretion of sodium and chloride ions, without altering the rate of urine flow. The rate of excretion of inulin fell slightly and that of potassium rose somewhat, but neither of these changes differed significantly from control values. Consequently, the urinary Na/K ratio was also elevated.

Discussion

The present experiments with isoprenaline and dichloroisoprenaline confirm and extend the previous findings of Lees & Lockett (1963). These authors showed that both drugs reduced the excretion of sodium and the rate of urine flow in conscious, hydrated rats. The results described in this paper further indicate that the excretory

TABLE 5. *Influence of pronethalol hydrochloride on urinary function in hypophysectomized rats*

Expt.	Body weight (g)	Water (ml.)	Excretion rates/100 g body weight/hr Na	K (μ-equiv)	Cl	Na/K	Dose per rat (mg)
20	224±7·0 (5)	0·52±0·11	4·8±2·1	12·0±4·4	7·3±3·2	0·35±0·04	—
		0·33±0·12	6·3±2·6	6·2±1·5	5·8±1·9	1·00±0·32**	4·0
21	225±8·0 (6)	0·25±0·04	3·8±0·7	5·8±1·9	4·5±2·3	1·72±1·16	—
		0·15±0·04	6·8±2·6	4·0±1·4	6·5±3·6	1·71±0·83	4·0
			Excretion rates/100 g body weight/2 hr				
22	213±4·7 (9)	0·84±0·18	7·0±2·1	15·9±3·1	10·6±4·1	0·40±0·06	—
		0·26±0·04**	7·3±2·1	11·4±1·8	8·2±2·0	0·64±0·16	4·0

Pronethalol was administered intramuscularly 30 min before the urine collection period. The values are means±the standard errors. The significance of differences between means, attributable to the effect of pronethalol, has been examined by *t* tests and is indicated by asterisks; one, $P<0·05$; two, $P<0·01$. Values in parentheses are the numbers of rats.

TABLE 6. *Influence of propranolol hydrochloride on urinary function in normal rats*

Expt.	Body weight (g)	Water (ml.)	Excretion rates/100 g body weight/hr				Inulin (mg)	Dose per rat (mg)
			Na	K (μ-equiv)	Cl	Na/K		
23	205±4·0 (12)	2·09±0·08	11·1±1·8	30·4±3·6	20·8±3·4	0·41±0·08		—
		2·07±0·19	17·6±2·4*	35·6±3·3	35·0±3·5**	0·51±0·06		0·4
24	220±4·0 (12)	2·02±0·15	6·4±1·7	23·5±3·5	14·7±2·6	0·27±0·03	17·3±0·8	—
		1·94±0·22	16·5±8·4**	25·7±5·1	27·9±9·1**	0·47±0·12*	15·9±1·4	0·8
25	197±5·0 (12)	2·15±0·14	10·0±2·5	28·5±5·3	18·8±5·4	0·36±0·04		—
		2·21±0·12	23·2±5·6**	36·6±3·4	40·5±8·2**	0·56±0·09**		0·4
26	218±5·0 (11)	1·88±0·07	4·3±0·8	18·9±3·4	12·0±2·8	0·29±0·08	16·3±2·5	—
		1·79±0·11	8·5±1·7**	21.4±2·5	22·5±3·7**	0·41±0·05*	14·3±0·9	0·8

Propranolol was administered subcutaneously (experiments 23 and 24) immediately before or intramuscularly (experiments 25 and 26) 30 min before the urine collection period. The values are means±the standard errors. The significance of differences between means, attributable to the effect of propranolol, has been examined by *t* tests and is indicated by asterisks; one, $P<0·05$; two, $P<0·01$. Values in parentheses are the numbers of rats.

rates of potassium and chloride ions are also diminished by these drugs. Moreover, these changes in urinary volume and composition were found to be similar when the agents were given by subcutaneous injection immediately before the 1 hr period of urine collection or when administered intramuscularly 30 min before collection. In view of the similarities between the actions of isoprenaline and dichloroisoprenaline it seems reasonable to attribute the effects of both drugs to β-adrenoceptor activation. This conclusion is substantiated by the fact that dichloroisoprenaline, in addition to its blocking action, is known to exert a stimulant action on β-receptors (Dresel, 1960 ; Furchgott, 1959 ; Vogin, Rice & Dhalla, 1965).

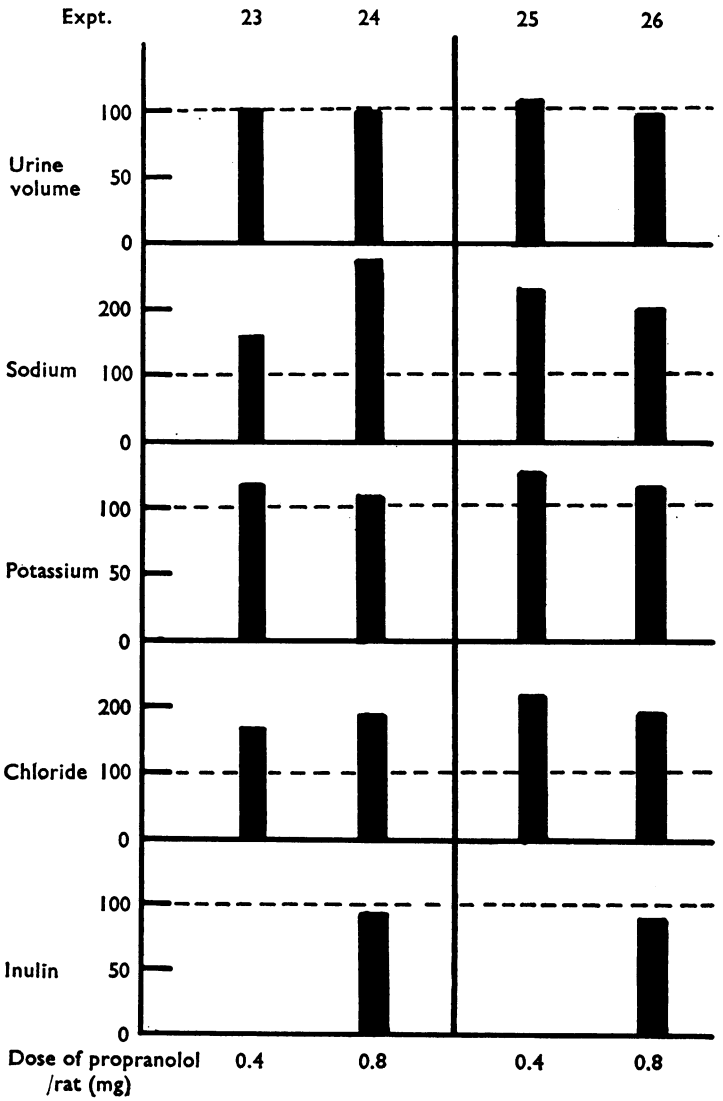

FIG. 6. Influence of (±)-propranolol hydrochloride on urinary function in normal rats. Ordinates: as in Fig. 1. Propranolol was administered subcutaneously (Expts. 23 and 24) in 0.1 ml. normal saline immediately before or intramuscularly (Expts. 25 and 26) in 0.1 ml. propylene glycol 30 min before the 1 hr collection period.

In the present experiments the dose levels of dichloroisoprenaline were 400 to 1,000 times greater than those of isoprenaline, so that an isoprenaline-like action of dichloroisoprenaline would, in fact, be expected.

In considering the possible site or sites of action of these two drugs, account must be taken of their effects, if any, on mean arterial blood pressure, because a reduction in perfusion pressure to the kidney would lead to corresponding falls in the rates of urine flow and electrolyte excretion, even over the autoregulatory range of arterial pressures (Davidson, Levinsky & Berliner, 1958; Selkurt, Hall & Spencer, 1949). In the case of isoprenaline the falls in blood pressure, produced by subcutaneous injection of 4.0 μg to anaesthetized rats were never very pronounced and in some instances were absent. In no experiment did this depressor effect last for more than 18 min. It is therefore unlikely that a similar action in conscious animals could explain wholly the urinary changes.

From the experiments of Lees & Lockett (1965) it seems likely that the main site of action of isoprenaline is directly on the kidney. These workers added small doses of isoprenaline and orciprenaline to the perfusion circuit of an isolated feline heart-lung-kidney preparation. With both drugs reductions in urine volume and in sodium, potassium and chloride excretion were recorded, despite the fact that perfusion pressures to the kidney remained constant. These responses were attributed to changes in renal haemodynamics, the glomerular filtration rate decreasing and the total renal blood flow increasing above control levels. Both renal vascular and urinary changes were antagonized by pronethalol. In their studies of the actions of isoprenaline and orciprenaline on renal function in anaesthetized dogs Heidenreich, Laaff, Fülgraff & Balshüsemann (1966) recorded similar changes to those reported by Lees & Lockett (1965) in the cat. In the dog intravenous or renal artery infusions of orciprenaline and isoprenaline reduced the excretion of sodium, potassium and water, but there was little or no accompanying reduction in glomerular filtration rate. Because the actions of isoprenaline and orciprenaline on the isolated, feline kidney were not essentially altered by mannitol diuresis, Lees & Lockett (1965) did not feel that it was necessary to invoke a direct tubular action of sympathomimetic amines in order to explain the observed responses. The present findings and the previous results of Lees & Lockett (1963) indicate that large doses of isoprenaline and dichloroisoprenaline reduce the rate of excretion of a standard inulin load in the rat, these values being taken as a measure of the glomerular filtration rate. The fact that lower doses of isoprenaline reduce the rates of excretion of water and electrolytes in this species without detectably altering the glomerular filtration rate (Botting *et al.*, 1961; Botting & Lockett, 1961) does not necessarily provide firm evidence for a tubular action, because the available methods for estimating the rate of glomerular filtration in the rat give, at best, only an approximate measure of the true values. Thus, although a direct action of isoprenaline on renal tubular function in the rat cannot be excluded, it is thought to be unlikely.

An antidiuretic action of pronethalol in the rat has been described previously by Lees & Lockett (1963), using conscious, hydrated animals, and by Poisner (1964) in ethanol-anaesthetized rats. According to the latter author, intravenous injections of 0.1 mg/kg frequently produced a prolonged antidiuresis, without changing mean arterial blood pressure. In the present investigation pronethalol always reduced the rate of urine flow, whereas changes in other parameters of urinary function were

found to vary with the experimental conditions. Thus subcutaneous injection of the drug in saline immediately before the rats were placed in metabolism cages for the collection of urine resulted in diminished excretion of sodium, potassium and chloride. On the other hand, the antidiuresis resulting from intramuscular administration of pronethalol 30 min before urine collection was not normally associated with significant changes in the excretion of electrolytes. In the former case, following subcutaneous injection, it appears that pronethalol, which in common with dichloroisoprenaline and isoprenaline possesses stimulant actions on β-receptors, is acting through the same pathway. By analogy, this action probably results from a direct effect of pronethalol on the kidney. By the intramuscular route, on the other hand, the similarities between the actions of pronethalol and antidiuretic hormone indicated that the former might act indirectly by increasing the secretion of antidiuretic hormone from the posterior pituitary. This hypothesis was not, however, borne out by experimental evidence, for 4.0 mg pronethalol still exerted an antidiuretic action when given intramuscularly to hypophysectomized animals. As in the normal unoperated rats, this dose of pronethalol also failed to modify the excretory rates of sodium, potassium and chloride ions significantly ; in both groups of animals pronethalol tended to promote retention of potassium, and loss of sodium in the urine but these effects were not statistically significant in most experiments. Hence these actions of pronethalol cannot be attributed solely to an isoprenaline-like action nor, from the evidence presented in this paper, to a rise in plasma levels of antidiuretic hormone.

A possible explanation for the latter action of pronethalol is provided by the results of the experiments with propranolol. The latter β-receptor blocker produced significant increases in the rates of excretion of sodium and chloride ions. The elimination of potassium, likewise, tended to rise but in no case was the kaliuresis statistically significant. In addition, the urine volume was not modified by propranolol. It therefore seems likely that the changes in urinary function produced by pronethalol represent the sum of two distinct actions in the body, an isoprenaline-like effect, tending to diminish the rates of excretion of water and electrolytes, and a propranolol-like action, whereby pronethalol tends to increase the excretion of sodium and chloride ions, and to a lesser extent potassium, without altering the rate of urine flow. The observed antidiuresis and the tendency for sodium excretion to rise and potassium excretion to fall, in response to intramuscularly administered pronethalol, are compatible with this hypothesis of two separate actions, because the effects are intermediate between those resulting from isoprenaline and dichloroisoprenaline on the one hand and propranolol on the other. This concept of the mode of action of pronethalol in the rat is also supported by the well-known, relative agonistic and antagonistic activities of β-receptor blocking agents ; pronethalol is intermediate in potency between dichloroisoprenaline and propranolol, both as a stimulant and as a blocking agent for β-receptors.

Finally, some consideration must be given to the mode of action of propranolol. This drug produced pronounced natriuretic and chloruretic responses and a smaller increase in potassium excretion. Urine flow was not, however, modified. These effects could, conceivably, have resulted from a direct dilator action of propranolol at some pre-glomerular site, for example, the afferent arteriole. This concept is not supported by the fact that the inulin excretion was not modified by propranolol. Clearance techniques in the rat, however, give only an approximate measure of the

rate of glomerular filtration. Moreover, Shanks (1967) noted that propranolol, when given directly into the left renal artery at a dose level of 1.0 mg/kg, increased renal blood flow in the dog. An alternative possibility, for which the present results again provide no direct evidence, is that under the experimental conditions employed, renal function is subject to some direct or indirect β-adrenergic influence. In such an event the β-receptor blocking actions of propranolol would be expected to lead to responses, which were the opposite of those produced by isoprenaline; the observed effects of propranolol on electrolyte excretion could be accounted for on this hypothesis. The inability of propranolol to evoke a diuretic response need not necessarily argue against this concept, for a number of drugs, which normally increase the rate of urine flow, do not do so when given to rats during a maximal water diuresis. Further discussion of the mode of action of propranolol must await additional experimental evidence.

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