Brit. J. Pharmacol. (1959), 14, 380.

THE ACTION OF ANTISYMPATHOMIMETIC DRUGS ON THE URINARY EXCRETION OF ADRENALINE AND NORADRENALINE

BY

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(RECEIVED MAY 11, 1959)

In cats anaesthetized with pentobarbitone sodium, phenoxybenzamine, dibenamine, phentolamine, and piperoxane reduced the blood pressure and increased the urinary excretion of noradrenaline. When the fall of blood pressure after phenoxybenzamine was prevented by repeated injections of vasopressin, the urinary excretion of noradrenaline did not rise. Hydergine and hydrallazine reduced the blood pressure without raising the urinary excretion of noradrenaline. In dogs, the infusion of small amounts of noradrenaline led to a significantly higher urinary recovery of the amine after phenoxybenzamine than before. It is concluded that antisympathomimetic drugs interfere with the destruction in the body of noradrenaline, whether released reflexly in hypotension or injected.

It had been found previously that phenoxybenzamine raised the urinary excretion of adrenaline and of noradrenaline in dogs (Benfey, Ledoux, and Melville, 1958). Hexamethonium, which markedly reduced the accompanying hypotension, prevented this effect. A similar hypotension produced by repeated injections of methacholine only elevated urinary adrenaline moderately (Benfey, Ledoux, and Melville, 1959). Similarly, phenoxybenzamine raised the plasma adrenaline and noradrenaline concentration in dogs (Millar, Keener, and Benfey, 1959).

In the earlier studies, phenoxybenzamine lowered the blood pressure for a prolonged period of time, and evidence was obtained that hypotension in the absence of anti-adrenaline agents differed from that in the presence of antisympathomimetic substances in the urinary excretion of adrenaline and noradrenaline in dogs. Whereas in the former there was only a moderate rise of adrenaline, in the latter there was a markedly increased adrenaline and noradrenaline excretion. It appeared to be of interest to investigate the effects of antisympathomimetic agents in the absence of hypotension. As experiments with dogs were unsuccessful we used cats. In addition, previous experiments to demonstrate the action of phenoxybenzamine on the urinary recovery of infused noradrenaline in dogs have been modified. At the autumn meeting of the American Society for Pharmacology and Experimental Therapeutics in 1957 we reported that antisympathomimetic drugs increased the urinary recovery of injected adrenaline and noradrenaline (Benfey, Mazurkiewicz, and Melville, 1958a).

METHODS

Cats were first anaesthetized with ether. The femoral vein was cannulated as quickly as possible, and the anaesthesia was continued with intravenous pentobarbitone sodium. After opening the abdomen, the bladder was cannulated with polyethylene tubing. Dogs were anaesthetized with pentobarbitone sodium and, after opening the abdomen, the ureters were cannulated with polyethylene tubing. The blood pressure was recorded from the femoral artery and an infusion of 0.9% NaCl solution was given into a femoral vein at a constant rate (0.2 ml./kg./min.) throughout the experiment. Total urine samples were collected every 30 min. The urine was passed through a column of alumina and adrenaline and noradrenaline were determined by the fluorimetric method described by Millar and Benfey (1958). The clearance of *p*-aminohippuric acid was determined according to Smith, Finkelstein, Aliminosa, Crawford, and Graber (1945) and that of creatinine according to Folin and Wu (1919).

Drugs were given after a control period of 60 min. which permitted two 30 min. control collections. The following agents were employed: phenoxybenzamine hydrochloride (Dibenzyline, Smith, Kline, and French) was dissolved in a small quantity of 95% ethanol,

brought to a volume of 20 ml. with physiological saline and injected intravenously over a 10 min. period; vasopressin (Benfey, 1953); phentolamine (Regitine hydrochloride), and hydrallazine (Apresoline hydrochloride, Ciba); piperoxane and hexamethonium bromide (Vegolysen, Poulenc). The drugs were kindly supplied by the companies named, and the Sterling Winthrop Research Institute kindly gave us (−)-noradrenaline bitartrate monohydrate. The amounts of adrenaline and noradrenaline given below refer to the base; they are not corrected for such errors in the method of determination as losses on purification of the urine samples.

Results

In cats, the application of 10 mg./kg. of phenoxybenzamine led to an increase in the urinary excretion of noradrenaline, but the output of adrenaline was not influenced (Fig. 1). This is in contrast to previous observations on dogs which showed an equal rise of noradrenaline and adrenaline; however, the rise in urinary noradrenaline in cats was of the same order as that in dogs (Benfey *et al.*, 1959). The blood pressure dropped under the influence of the drug and remained at about 90 mm. Hg for at least 3 hr.

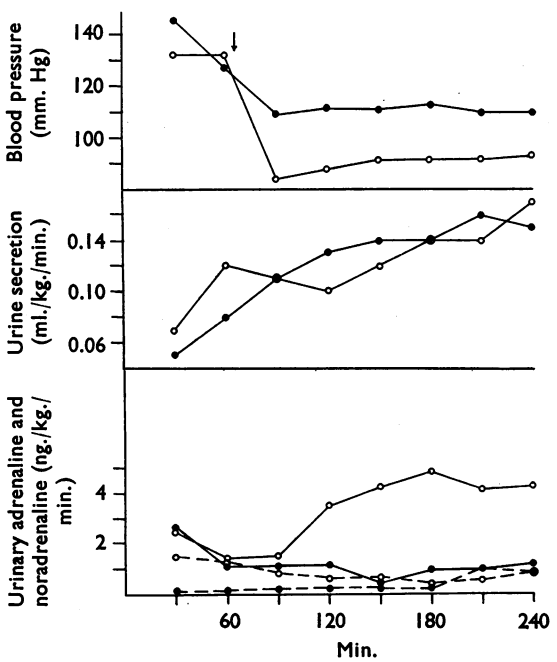

FIG. 1.—Cat anaesthetized with pentobarbitone. Blood pressure (top), urine flow (middle) and urinary excretion (bottom) of adrenaline (broken line) and noradrenaline (full line). At arrow, phenoxybenzamine (10 mg./kg.). O, without, and ●, with repeated injections of vasopressin (2 to 10 units). Mean of 5 experiments.

Intravenous injections of vasopressin (2 to 10 units at intervals of at least 10 min.) maintained a fairly stable blood pressure after phenoxybenzamine in five out of eight cats. In dogs this was achieved in only one out of six animals for a limited period of time. As seen in Fig. 1, maintenance of the blood pressure at approximately 110 mm. Hg prevented the increased urinary excretion of noradrenaline following phenoxybenzamine in cats. The total urinary excretion of noradrenaline in 3 hr. following injection of phenoxybenzamine was 667 ± 153 without, and 166 ± 83 ng./kg. ± S.E. with, vasopressin. The difference is statistically significant ($P < 0.05$). The rate of urine secretion was not affected by vasopressin.

Dibenamine similarly reduced the blood pressure to an average of approximately 80 mm. Hg and raised the urinary excretion of noradrenaline, but it also elevated moderately the urinary output of adrenaline (Table I).

TABLE I

URINARY EXCRETION OF ADRENALINE AND NORADRENALINE, RATE OF URINE SECRETION, AND BLOOD PRESSURE IN CATS BEFORE AND AFTER DIBENAMINE

Means of four experiments. 30 min. periods.

Period	Adrenaline (ng./kg./min.)	Noradrenaline (ng./kg./min.)	Urine (ml./kg./min.)	B.P. (mm. Hg)
Control 1 and 2	0·55	0·95	0·04	141→117
After dibenamine (20 mg./kg.) 3 to 10	2·5	4·7	0·07	70→85→67

Phentolamine (Table II) and piperoxane (Table III) increased both adrenaline and noradrenaline excretion as did dibenamine. However, the first two drugs reduced the blood pressure only moderately, to a mean of approximately 100 mm. Hg. In contrast, hydergine did not influence the urinary excretion of adrenaline and noradrenaline although it reduced the blood pressure (Table IV).

Finally, we tried to reduce the blood pressure by repeated intravenous injections of hydrallazine in amounts of 1 to 5 mg./kg. A mean of approximately 105 mm. Hg was obtained. Hydrallazine has little if any antisympathomimetic action. There was a moderate rise of both adrenaline and noradrenaline (Table V). The excretion of noradrenaline did not greatly exceed that of adrenaline.

The urine from the cat bladder obtained after opening the abdomen and before the bladder was cannulated was assayed. It contained a mean of 4.9 ng./ml. of adrenaline and 29.3 ng./ml. of noradrenaline in ten samples of a mean volume of 19 ml. In contrast, urine obtained from dogs

TABLE II

URINARY EXCRETION OF ADRENALINE AND NORADRENALINE, RATE OF URINE SECRETION, AND BLOOD PRESSURE IN CATS BEFORE, DURING, AND AFTER PHENTOLAMINE INFUSION

Means of four experiments. 30 min. periods. Phentolamine infusion, 2·5 mg./kg./30 min.

Period	Adrenaline (ng./kg./min.)	Noradrenaline (ng./kg./min.)	Urine (ml./kg./min.)	B.P. (mm. Hg)
1 and 2	1·4	1·9	0·09	140→131
Infusion started				
3 to 8	2·4	5·3	0·11	105→93→107
Infusion stopped				
9 and 10	3·0	4·6	0·15	98→95

TABLE III

URINARY EXCRETION OF ADRENALINE AND NORADRENALINE, RATE OF URINE SECRETION, AND BLOOD PRESSURE IN CATS BEFORE, DURING, AND AFTER PIPEROXANE TEST, AN INJECTION (5 MG./KG.) AND THEN AN INFUSION (2·5 MG./KG./30 MIN.) WERE MADE

Means of six experiments. 30 min. periods.

Period	Adrenaline (ng./kg./min.)	Noradrenaline (ng./kg./min.)	Urine (ml./kg./min.)	B.P. (mm. Hg)
1 and 2	0·85	2·9	0·07	130→118
Injection				
3	1·5	4·8	0·07	97
Infusion started				
4 to 8	2·6	5·3	0·12	109→102
Infusion stopped				
9 and 10	2·6	4·2	0·16	105→110

TABLE IV

URINARY EXCRETION OF ADRENALINE AND NORADRENALINE, RATE OF URINE SECRETION, AND BLOOD PRESSURE IN CATS BEFORE, DURING, AND AFTER HYDERGINE INJECTIONS

Means of four experiments. 30 min. periods.

Period	Adrenaline (ng./kg./min.)	Noradrenaline (ng./kg./min.)	Urine (ml./kg./min.)	B.P. (mm. Hg)
1 and 2	0·30	1·6	0·07	139→103
Hydergine (1 mg./kg.)				
3 and 4	1·9	1·1	0·09	94→108
Hydergine (1 mg./kg.)				
5 to 10	0·97	0·38	0·13	105→80

TABLE V

URINARY EXCRETION OF ADRENALINE AND NORADRENALINE, RATE OF URINE SECRETION, AND BLOOD PRESSURE IN CATS BEFORE, DURING, AND AFTER HYDRALLAZINE INJECTIONS

Means of five experiments. 30 min. periods. Injections of hydergine (1–5 mg./kg.) were given intravenously as needed.

Period	Adrenaline (ng./kg./min.)	Noradrenaline (ng./kg./min.)	Urine (ml./kg./min.)	B.P. (mm. Hg)
1 and 2	0·75	1·4	0·08	154→133
Injections started				
3 to 8	2·5	1·9	0·09	86→111
Injections stopped				
9 and 10	3·9	2·6	0·12	108→90

under similar conditions contained a mean of 4.2 ng./ml. of adrenaline and only 16.6 ng./ml. of noradrenaline (Benfey, Mazurkiewicz, and Melville, 1958b).

We have repeated, with certain modifications, experiments in which dogs were infused with noradrenaline before and after phenoxybenzamine (Benfey *et al.*, 1959). Hexamethonium was given at 0.1 mg./kg./min. throughout the experiment in order to eliminate an action of phenoxybenzamine on the excretion of endogenous noradrenaline. After a control period of 1 hr., 0.2 μg./kg./min. of noradrenaline was infused for 1 hr.; phenoxybenzamine was injected 1 hr. later. A second infusion of noradrenaline was started 1 hr. after phenoxybenzamine.

The clearances of *p*-aminohippuric acid and creatinine before and after phenoxybenzamine were also determined (Table VI and Fig. 2). The control values for noradrenaline (periods 1, 2, and 6 to 8) were low. Before phenoxybenzamine, the noradrenaline excretion in periods 3 to 5 was 290 ± 40 and after phenoxybenzamine in periods 7 to 9 it was 454 ± 49 ng./kg. $\pm$S.E. The difference was statistically significant ($P < 0.05$). Taking into account the control urinary noradrenaline output of 0.4 ng./kg./min. (Table VI), the urinary recovery of infused noradrenaline was 2.1% before and 3.5% after phenoxybenzamine. The urine output was 10.0 ml./kg. during periods 3 to 5 and 10.6 ml./kg. during periods 7 to 9. The mean *p*-aminohippuric acid clearance was 12.2 ml./kg./min. in periods 3 and 4 and 10.7 in periods 7 and 8, and the creatinine clearance was also somewhat reduced from 5.7 ml./kg./.min. during periods 3 and 4 to 4.7 during periods 7 and 8. A change in "kidney function" was therefore not responsible for the effect of phenoxybenzamine. There was no evidence that the infusion of noradrenaline raised the urinary excretion of adrenaline, so there was no indication that part of the infused noradrenaline might have been converted to adrenaline.

DISCUSSION

Our experiments have shown that phenoxybenzamine raised the urinary excretion of noradrenaline in cats only when there was hypotension. Its action was indirect and secondary to a reflex stimulation of sympathetic tone. Hypotension in the absence of antisympathomimetic agents had little influence on the excretion of noradrenaline. It has previously been shown in dogs that hypotension in the early stages of haemorrhage (Millar and Benfey, 1958) or after repeated injections of methacholine (Benfey *et al.*,

TABLE VI

URINARY EXCRETION OF ADRENALINE AND NORADRENALINE, BLOOD PRESSURE, URINE SECRETION, CLEARANCE OF *P*-AMINOHIPPURIC ACID, AND OF CREATININE, AND FILTRATION FRACTION IN DOGS DURING INFUSION WITH NORADRENALINE BEFORE AND AFTER PHENOXYBENZAMINE INJECTION

Intravenous infusion of sodium chloride (0.9%) and hexamethonium (0.05%) was given throughout the experiment (0.2 ml./kg./min.). A, indicates adrenaline (ng./kg./min.); N, noradrenaline (ng./kg./min.); B.P., blood pressure in mm. Hg; U, urine secretion (ml./kg./min.); C_{PAH}, clearance of *p*-aminohippuric acid (ml./kg./min.); C_{CR}, clearance of creatinine (ml./kg./min.); FF, filtration fraction. Noradrenaline infusion, 0.2 μg./kg./min. Phenoxybenzamine injection 10 mg./kg. Means of four experiments. 30 min. periods.

Period	A	N	B.P.	U	C_{PAH}	C_{CR}	FF
1 and 2	1·3	0·30	117→112	0·107	33·3→13·1	14·0→5·9	0·461
Noradrenaline infusion started							
3 and 4	1·2	4·1	119→120	0·121	12·2	5·7	0·478
Noradrenaline infusion stopped							
5 and 6	1·0	1·6→0·30	109	0·095			
Phenoxybenzamine injection							
7 and 8	1·0	0·45	109→97	0·113	8·2	4·1	0·523
Noradrenaline infusion started							
9 and 10	1·1	6·3	92	0·128	10·7	4·7	0·461
Noradrenaline infusion stopped							
11	0·50	2·7	88	0·098			

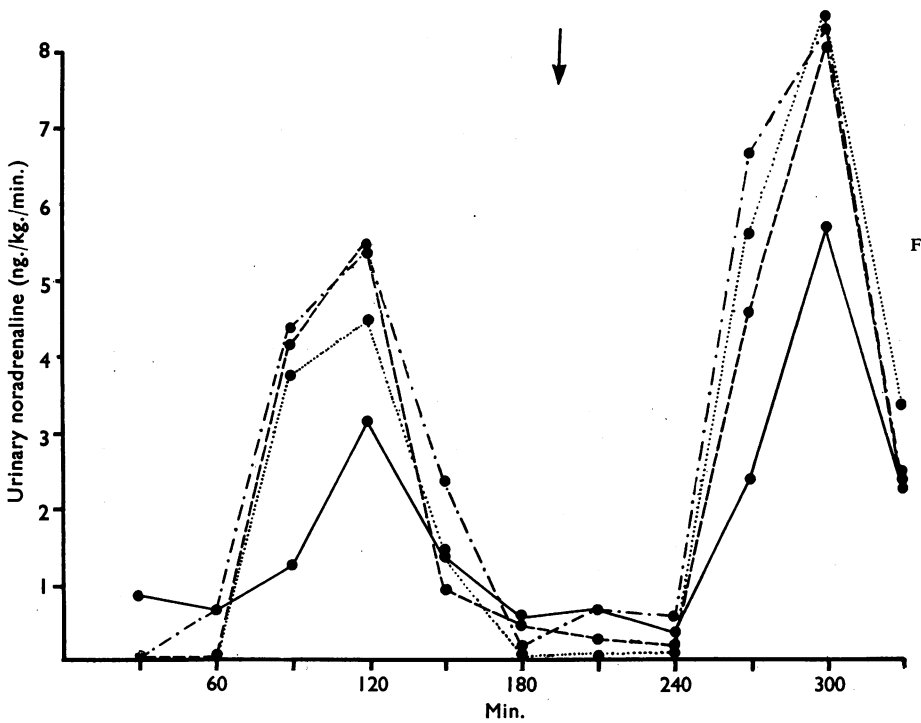

FIG. 2.—Urinary excretion of noradrenaline before and following administration (at the arrow) of phenoxybenzamine (10 mg./kg.). Each line represents a record from an individual dog. Noradrenaline (0.2 μg./kg./min.) was infused from 61 to 120 and 241 to 300 min.

1959) raises mainly the urinary excretion of adrenaline. In the present experiments, hydrallazine, of which the main action appears to be directly on the blood vessels (Moyer, Huggins, and Handley, 1953) and which possesses slight if any anti-sympathomimetic action, raised the noradrenaline excretion only a little and not greatly above that of adrenaline as did the other antagonists. It was difficult to find an agent which lowered the blood pressure for a prolonged period of time, was free from action on sympathetic ganglia or receptors and did not possess side actions which made it unfit for purposes of comparison.

The increase in urinary noradrenaline after phenoxybenzamine thus appeared to be accounted for satisfactorily by the contention of Brown and

Gillespie (1957) that "the mechanism for the destruction of noradrenaline at the nerve endings is linked to the receptors for this substance and can be inactivated with them." These workers found that, by giving phenoxybenzamine, they could raise the noradrenaline concentration in the venous outflow from the spleen when stimulating the splenic nerves in cats. It may be assumed that, in the present experiments, hypotension activated sympathetic fibres reflexly and liberated noradrenaline which, in the presence of antisympathomimetic drugs, appeared in the urine in higher concentrations because destruction was impaired. We have previously shown that phenoxybenzamine increased the urinary output of noradrenaline when large doses of acetylcholine were repeatedly given after atropine (Benfey *et al.*, 1959) and, further, phenoxybenzamine raised the plasma concentration of noradrenaline in dogs and increased the plasma noradrenaline concentration in adrenalectomized dogs subjected to haemorrhage (Millar *et al.*, 1959).

Phenoxybenzamine and dibenamine appeared to be the only antisympathomimetic drugs that were reasonably free from side actions. Piperoxane and phentolamine have been reported to stimulate the sympathetic nervous system by central action (Handovsky, 1935; Gross, Tripod, and Meier, 1951). Their effect on the urinary excretion of adrenaline and noradrenaline might therefore be due partly to direct sympathetic stimulation. In addition, their hypotensive action was not very strong. Hydergine, on the other hand, is known to depress the vasomotor centre, so reducing the blood pressure (Konzett and Rothlin, 1953). This might explain our divergent result with this drug, which moderately reduced blood pressure but did not elevate the urinary excretion of noradrenaline.

There were great variations in the urinary excretion of adrenaline and noradrenaline in the control as well as in the experimental periods. Generally, the control values in cats were higher than in dogs. This might have been due to the induction of anaesthesia with ether which is known to stimulate the sympathetic nervous system. This was followed by sympathetic depression during anaesthesia with pentobarbitone. The latter was generally deep but not controlled, and it is conceivable that the state of the animals (particularly the responsiveness of their sympathetic system) varied considerably. We have tried to relate the blood pressure to the adrenaline and noradrenaline output in the control period, but this proved impossible. A low blood pressure was often but not always associated with high urinary excretion of adrenaline and noradrenaline.

Hexamethonium appeared to exert a stabilizing influence; the blood pressure was well maintained after phenoxybenzamine and the urinary excretion of adrenaline and noradrenaline was uniformly low.

Studies of the action of antisympathomimetic drugs on the destruction of adrenaline and other catechol amines are in progress. In the present experiments the behaviour of adrenaline has been rather puzzling. The urinary excretion of adrenaline was reduced by phenoxybenzamine but increased by hydrallazine and the other anti-adrenaline substances.

The authors are indebted to Professor K. I. Melville for his interest in this work, which was supported by a grant from the National Research Council of Canada.

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