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DIURESIS IN RATS: EFFECTS OF SYMPATHOMIMETIC AND SYMPATHETIC BLOCKING AGENTS

BY

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Dopamine and tyramine possess diuretic properties resembling but weaker than those of noradrenaline in the rat. These three amines produce a relatively smaller loss of sodium than does adrenaline. Bretylium shows a diuretic action which is apparently associated with its sympathomimetic properties and which is antagonized by phenoxybenzamine. However, bretylium causes a relatively greater loss of potassium and chloride than do the sympathomimetic amines. A slight antidiuretic action is shown by bretylium after its diuretic effect has subsided and a similar effect is produced by BW 172C58, which has relatively weak sympathomimetic properties. In contrast to these adrenergic neurone blocking agents, phenoxybenzamine and a ganglion blocking agent (pentacynium) show powerful antidiuretic effects. These observations are consistent with the view that the adrenal medulla has an important role in facilitating water diuresis in rats.

Adrenaline and noradrenaline cause diuresis in the rat (Dexter & Stoner, 1952). It was therefore interesting to investigate the effects of dopamine, the noradrenaline precursor, and tyramine, which releases catechol amines (Burn & Rand, 1958; Lockett & Eakins, 1960; Schümann, 1961). Bretylium, a compound with early sympathomimetic action, and *N*-[2-(4-benzoyl-2,6-dimethylphenoxy)ethyl]-*N,N,N*-trimethylammonium methyl sulphate (BW 172C58), a compound with relatively little sympathomimetic effect (Boura & Green, 1959; Boura, Coker, Copp, Duncombe, Elphick, Green & McCoubrey, 1960), were compared with these amines.

However, the main and more persistent action of bretylium and BW 172C58 is to depress adrenergic nerve function without inhibiting the activity of the adrenal medulla (Boura & Green, 1959; Boura *et al.*, 1960), and it is therefore of further interest to compare their effects on kidney function in the rat with those of the ganglion blocking agent pentacynium (Green, 1956) and the adrenaline antagonist phenoxybenzamine.

METHODS

Male Wistar rats weighing 100 to 200 g from a closed colony were used. For 18 hr before testing they were allowed no solid food but water *ad lib.* They were divided at random into groups of 5, received water or saline by stomach tube and were placed in metabolism cages. Except where stated the drugs were administered subcutaneously immediately after the water or saline load. The drugs were given in saline, 0.5 ml./100 g rat, and a similar volume of saline was given to controls. Urine volumes were recorded at 30 min intervals usually for 3 hr. They have been expressed as percentages of the total volumes of fluid administered.

In diuretic tests, 0.9% sodium chloride solution at 37° C, 1 ml./100 g, was given by stomach tube. In antidiuretic tests water at 37° C, 5 ml./100 g, was given orally. At least three separate tests on groups of 5 rats at each dose level were combined to obtain the mean values shown in the tables.

Urine sodium and potassium concentrations were determined by flame photometry and chloride by Volhard's method.

The effect of phenoxybenzamine and pentacynium on the absorption of water from the alimentary tract and on the retention of urine was examined in groups of 6 fasted rats. Phenoxybenzamine was given 3 hr before and pentacynium immediately after giving a water load of 5 ml./100 g. Controls received saline 0.9% sodium chloride solution, 0.5 ml./100 g, at the same time as the water load. The rats were placed in individual metabolism cages. Each rat was killed 1 hr later and the volume of urine in the bladder was measured with a tuberculin syringe. The weight with contents of stomach, small intestine and caecum of each rat was also determined. The effect of bretylium on the urine content of the bladder was determined 1 hr after administering the drug at the same time as a 0.9% sodium chloride solution load of 1 ml./100 g orally in groups of 6 fasted rats.

Bretylium bromide and bretylium tosylate ("Darenthin") were used, but doses are expressed as bromide. BW 172C58 was given as bromide or methyl sulphate; amounts refer to the bromide equivalent. Doses of pentacynium *bis* (methyl sulphate), phenoxybenzamine hydrochloride, tyramine hydrochloride, dopamine hydrochloride are those of the salts used, but doses of (−)-adrenaline and (−)-noradrenaline are in terms of base. Chlorothiazide was dissolved in 0.9% sodium chloride solution by adding a minimum of sodium hydroxide.

Comparisons of differences between pairs of groups of animals were assessed by the exact method of Fisher, the 5% level being used as the criterion of significance.

RESULTS

Diuresis

The volumes of urine excreted by saline-treated rats at intervals after the administration of adrenaline, noradrenaline, dopamine, tyramine, bretylium and BW 172C58 are shown in Fig. 1. Each of the catechol amines tested showed powerful diuretic actions, noradrenaline being the most active and dopamine easily the least active. The effects of noradrenaline and dopamine were more rapid in onset than those of adrenaline. Tyramine was slightly less active than dopamine. A powerful diuretic action was also shown by bretylium but not by BW 172C58.

The effects of bretylium, adrenaline and noradrenaline on electrolyte excretion are compared in Fig. 2. Sodium concentration was slightly but significantly increased by diuretic doses of bretylium and adrenaline except where the latter was given at very high dosage (4 mg/kg). In contrast, all doses of noradrenaline (except 0.25 mg/kg) caused a significant fall in sodium concentration. The total output of sodium was increased by diuretic doses of all three drugs, but was least with noradrenaline. Potassium concentration was lowered by all three compounds to extents that increased with the dosage, the change being greatest with adrenaline and least with bretylium. Total potassium output was increased significantly only by bretylium (for all doses examined $P =$ or < 0.01). The mean chloride concentration, 155 mEq/l. (s.e. ± 7.6) in controls, was not appreciably changed by any of the doses of bretylium nor by 0.25 or 0.5 mg/kg adrenaline but was reduced to 110 ± 4.5 mEq/l. by 4 mg/kg adrenaline. All the doses of noradrenaline tested significantly lowered the chloride concentration, values of 120 ± 13 and 91 ± 12

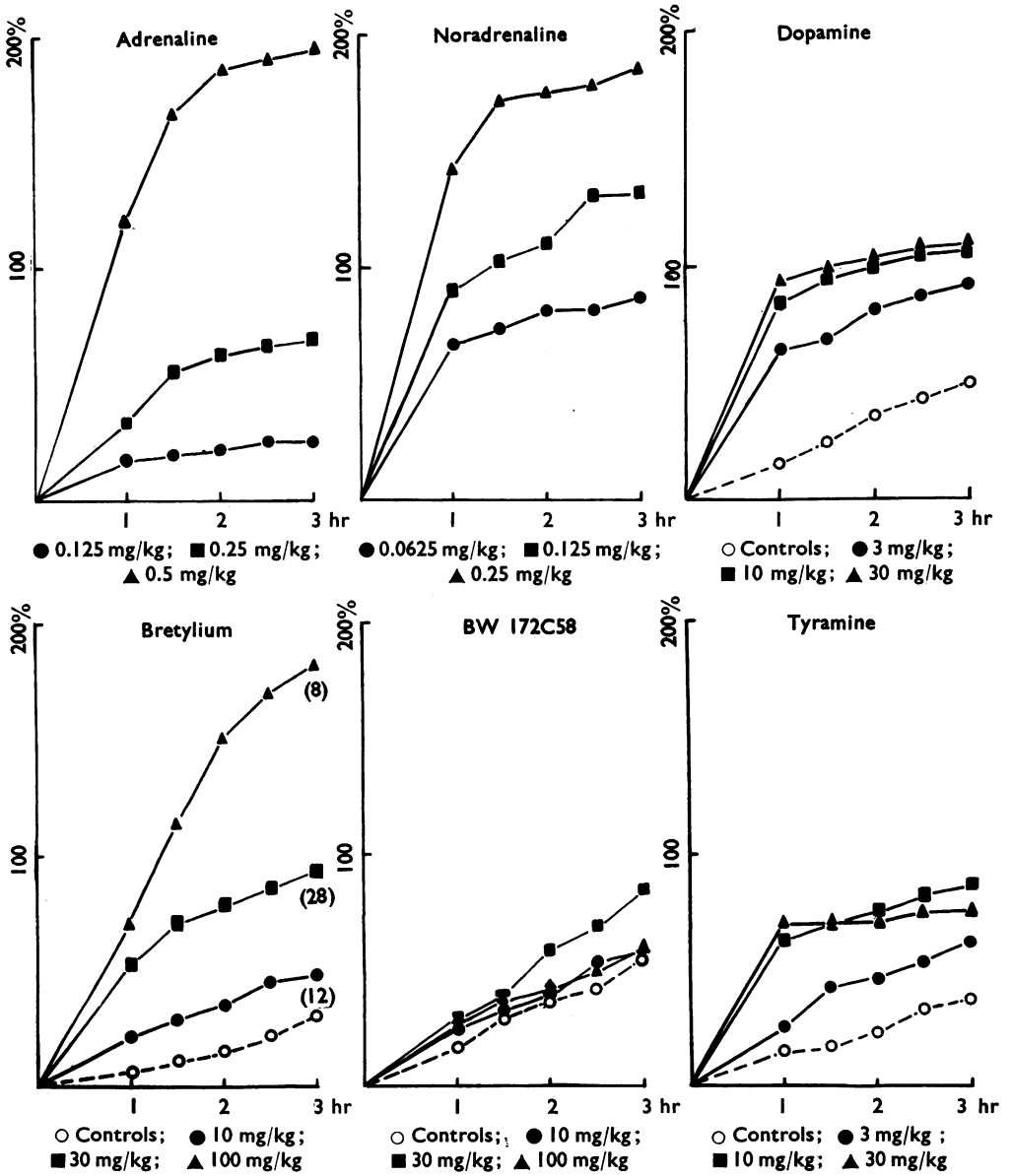

Fig. 1. Diuretic effects in rats. Ordinates: urine volume as a % of the total fluid load. Abscissa: hr after giving drug and saline load. The control shown under bretylium is from 26 experiments; those for dopamine, tyramine and BW 172C58 were concomitant. The numbers of groups of 5 rats given each dose level of bretylium are shown in brackets. All other treatments were given to 3 groups of 5 rats.

being found after the doses of 0.0625 and 2 mg/kg respectively. The mean ratio of the sum of the sodium and potassium concentration to the chloride concentration in mEq/l. was about 1.6 in controls. Similar ratios were found in bretylium-treated rats but significantly lower values occurred after adrenaline and noradren-

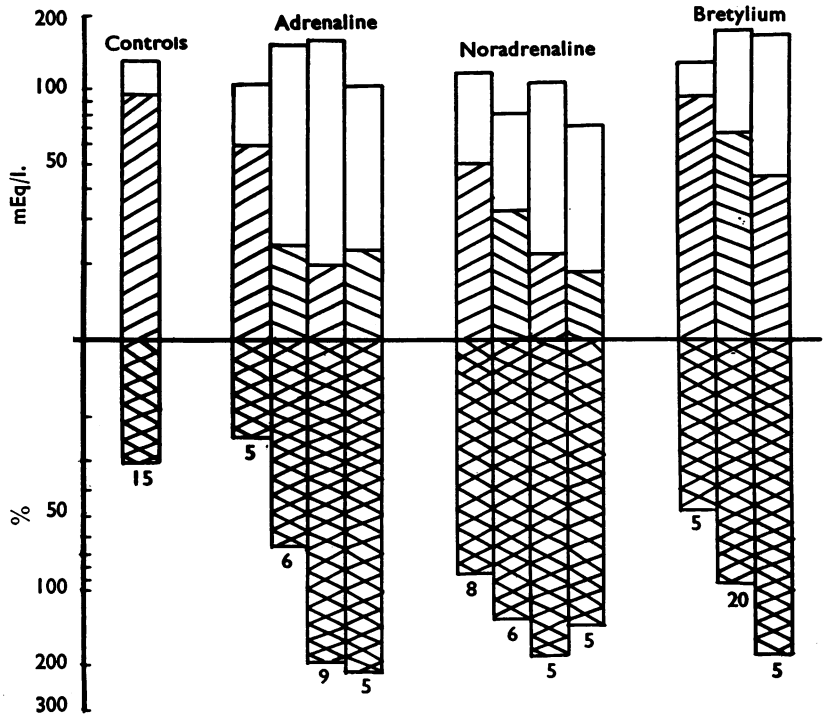

Fig. 2. Diuresis in rats. Electrolyte concentrations and total urine volumes excreted 3 hr following drug administration. The lower limits of the cross-hatched columns below the base line indicate urine volumes as a % of the total saline load. The heights above the base line of the clear and shaded portions of the columns represent sodium and potassium concentration respectively, in mEq/l. The logarithmic scales are arranged so that the total sodium and potassium excretions are proportional to the combined spans of the columns. The overall mean standard deviations of the % urine volumes, sodium concentrations and potassium concentrations were of the order of ±25, ±36 and ±16 respectively. The number of tests at each dose level is shown below the columns. Columns from left to right: controls; adrenaline, 0.125, 0.25, 0.5 and 4.0 mg/kg; noradrenaline, 0.0625, 0.125, 0.25 and 2 mg/kg; bretylium, 10, 30 and 100 mg/kg.

aline. Thus the mean ratios were 1.24, 1.35 and 1.16 after 0.25, 0.5 and 4 mg/kg adrenaline respectively, and 1.14 and 0.90 after 0.0625 and 2.0 mg/kg noradrenaline respectively.

A low dose of adrenaline (0.125 mg/kg) tended to reduce the urine output and significantly lowered sodium and potassium concentrations. These results in the rats loaded with 0.9% sodium chloride solution are in keeping with those in the water-loaded rat as reported by Botting & Lockett (1961) and Botting, Farmer & Lockett (1961).

A comparison between the effects on electrolyte excretion of bretylium, adrenaline, dopamine and tyramine in another series of similar experiments is shown in Fig. 3. The sodium:potassium balance was slightly different in the control rats of this series. Nevertheless the changes produced by bretylium and adrenaline were similar to those in the first series. Bretylium was again the only compound which signifi-

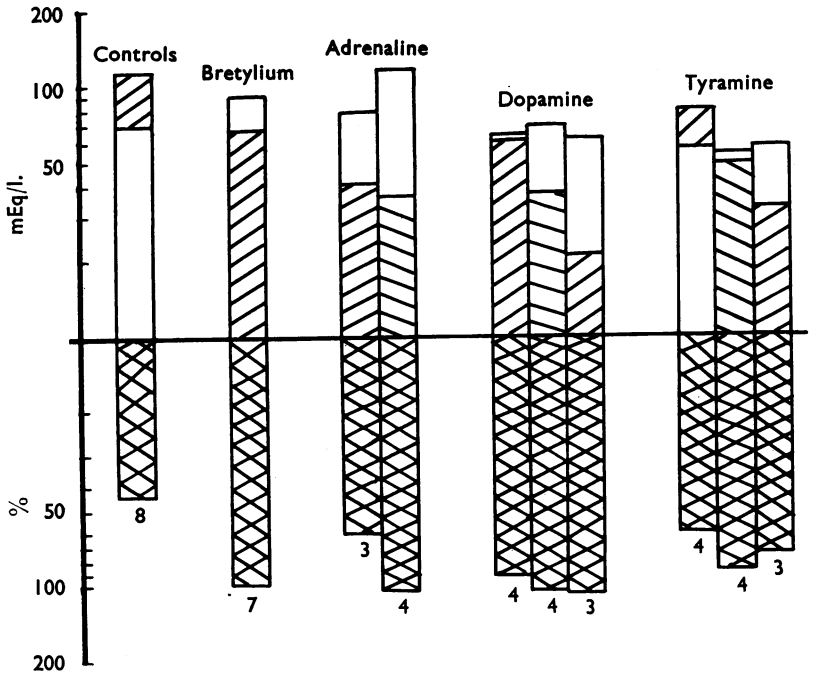

Fig. 3. As Fig. 2, but columns from left to right: controls; bretylium, 30 mg/kg; adrenaline, 0·25 and 0·5 mg/kg; dopamine, 3, 10 and 30 mg/kg; tyramine, 3, 10 and 30 mg/kg.

cantly increased potassium output. Dopamine and tyramine at submaximally effective doses (3 to 10 mg/kg) showed a relatively greater sodium-retaining effect and caused a relatively smaller change in the sodium:potassium ratio than did adrenaline. In these respects the effects of dopamine and tyramine resembled those of noradrenaline.

TABLE 1

ANTAGONISM OF THE DIURETIC EFFECTS OF ADRENALINE AND NORADRENALINE BY VARIOUS DOSES OF PHENOXYBENZAMINE GIVEN 3 HR PREVIOUSLY

	2 hr urine volume (% of fluid load)	
Phenoxybenzamine mg/kg	Adrenaline, 0·5 mg/kg	Noradrenaline, 0·25 mg/kg
0	113	173
0·0125	67	140
0·125	22	91
1·25	6	29

The diuretic effects of adrenaline and noradrenaline could be greatly reduced by treating the rats with phenoxybenzamine 3 hr earlier, the degree of antagonism being proportional to the dose of phenoxybenzamine (Table 1). Pretreatment with phenoxybenzamine 3 hr previously also reduced the diuretic response to bretylium but not to chlorothiazide (Table 2).

Just as bretylium potentiates many other effects of adrenaline and noradrenaline (Boura & Green, 1959), so also it increased the diuretic action of these substances. In these experiments sufficient time (4 hr) was allowed for the sympathomimetic

TABLE 2

EFFECT OF PHENOXYBENZAMINE ON THE DIURETIC EFFECT OF CHLOROTHIAZIDE AND BRETYLIUM

Phenoxybenzamine mg/kg	2 hr urine volume (% of fluid load)		
	Bretylium 30 mg/kg	Bretylium 100 mg/kg	Chlorothiazide 5 mg/kg
0	130	172	118
0·1	76	63	103

TABLE 3

DIURETIC EFFECTS IN RATS TREATED WITH BRETYLIUM

Bretylium was given 4 hr before the diuretic drugs

Bretylium mg/kg	2 hr urine volumes (% of fluid load)			
	Adrenaline 0·25 mg/kg	Adrenaline 0·5 mg/kg	Noradrenaline 0·0625 mg/kg	Chlorothiazide 5 mg/kg
0	30	161	104	107
30	105	227	127	96

action of bretylium to decline, before giving the catechol amines. Whereas the increase in the effect of adrenaline shown in Table 3 is large, that for noradrenaline is less prominent, but nevertheless significant at the 5% level. Under similar conditions bretylium tended to reduce the diuretic action of chlorothiazide.

Antidiuresis

When 30 mg/kg bretylium was given at the same time as a large water load, it increased the rate of diuresis. However, since an antidiuretic action might occur once the sympathomimetic diuretic action had subsided, water loads were given 2 or 4 hr after administering bretylium. An attempt was made to ensure that the bretylium-treated and control animals were in a similar state of hydration at the time of water loading, by giving orally, at the same time as the bretylium, a volume of 0.9% sodium chloride solution roughly equal to the urine excretion that would occur as a result of its diuretic action. These volumes of 0.9% sodium chloride solution given to the 30 mg/kg, 100 mg/kg and control groups were 1, 3 and 0.25 ml./100 g respectively. Table 4 shows that 30 mg/kg bretylium given 2 hr before the water load, or 30 to 100 mg/kg given 4 hr before the water, slightly retarded the onset of diuresis. No antidiuretic action was seen 2 hr after 100 mg/kg bretylium, presumably because the masking diuretic action of this dose was more persistent. BW 172C58, which has little sympathomimetic action, had a very weak antidiuretic effect when given at the same time as the oral water load. No antidiuretic action was seen in 3 other experiments where doses of 30 or 100 mg/kg BW 172C58 were given 2 hr before the water load. In contrast to the adrenergic neurone blocking agents, the ganglion blocking agent pentacynium and the adrenaline antagonist phenoxybenzamine showed powerful antidiuretic properties.

Effect on absorption of water and retention of urine. Large doses of phenoxybenzamine (12.8 mg/kg) had no effect and pentacynium (5 mg/kg) had only a small delaying effect on the absorption of water from stomach and small intestine, so far as

TABLE 4

ANTIDIURETIC EFFECTS OF BRETYLIUM, BW 172C58, PENTACYNIUM AND PHENOXYBENZAMINE GIVEN AT VARIOUS TIME INTERVALS BEFORE A WATER LOAD

Urine volumes at 1 and 2 hr after the water load are shown as % of total fluid load

Treatment		Interval	Urine volumes	
Drug	mg/kg	hr	1 hr	2 hr
Bretylium	30		16	56
Bretylium	100	2	41	67
Controls			33	75
Bretylium	30		16	80
Bretylium	100	4	14	62
Controls			28	73
BW 172C58	30		29	69
BW 172C58	100	0	23	43
Controls			22	71
Pentacynium	0·625		26	59
Pentacynium	1·25		11	34
Pentacynium	2·5	0	7	24
Pentacynium	5·0		3	13
Pentacynium	10·0		2	6
Controls			47	85
Phenoxybenzamine	0·05		30	74
Phenoxybenzamine	0·2		20	71
Phenoxybenzamine	0·8	3	15	66
Phenoxybenzamine	3·2		8	52
Phenoxybenzamine	12·8		3	22
Controls			44	79

could be ascertained by weighing these organs 1 hr after giving a water load. The alimentary tract of pentacynium-treated rats contained significantly more water than controls, but they had absorbed about 70% (5 ml.) of the water load. Thus this difference can only partly account for the antidiuretic effect of the compound. The volumes of urine found in the bladders of the rats given phenoxybenzamine and pentacynium tended to be less than those in the controls (mean 0.27 ml.). The antidiuretic action of these compounds cannot therefore be due to retention of urine. The volumes of urine found in the bladders of rats examined 1 hr after a saline load (1 ml./100 g) were similar in bretylium-treated rats and controls. Hence the diuretic effect of the drug cannot be attributed to any effect on the bladder.

DISCUSSION

Earlier reports that adrenaline and noradrenaline exhibit powerful diuretic effects in the rat are confirmed and it is further shown that in rats loaded with 0.9% sodium chloride solution noradrenaline causes a relatively smaller loss of sodium and chloride than does adrenaline. Low doses of adrenaline were found by Botting *et al.* (1961) to produce antidiuresis with retention of sodium and potassium in water-loaded rats as in our experiments on saline-loaded rats. Quantitative differences between the effects of adrenaline and noradrenaline in increasing water, sodium, and chloride output and decreasing urinary potassium concentration have been reported by Giere (1954), but these results in water-loaded rats are difficult to equate with our results in saline-loaded rats. Diuresis was also produced by dopamine and tyramine in the rat, and, in so far as they caused a relatively smaller

loss of sodium than did adrenaline, their action resembles that of noradrenaline. Just as dopamine is a weaker pressor agent than noradrenaline so also is its diuretic activity less. The diuretic action of tyramine is in keeping with its known releasing effect on noradrenaline stores (Burn & Rand, 1958; Lockett & Eakins, 1960; Schümann, 1961).

Bretylium also causes diuresis in rats. This may be another manifestation of its sympathomimetic action which is apparently due to catechol amine release in the rat (Gillis, 1960) as in other species (Boura & Green, 1959), since the diuresis like that of adrenaline and noradrenaline is readily inhibited by phenoxybenzamine. Moreover, BW 172C58, an adrenergic neurone blocking agent with relatively weak sympathomimetic properties (Boura *et al.*, 1960), showed very little diuretic action in rats. However, the relatively greater loss of potassium and chloride found in rats given bretylium, as compared with that in rats given adrenaline, noradrenaline or dopamine, indicates that the change in electrolyte excretion is not entirely due to bretylium releasing any one of these amines. Nevertheless, the possibility of the effect of bretylium being due to release of a combination of these amines and perhaps also isoprenaline is not excluded. Alternatively, the difference between the change in electrolyte composition seen with bretylium and that with the catechol amines studied, or with tyramine, could be related to the adrenergic neurone blocking effect of bretylium. This possibility deserves further investigation. Despite the increased potassium excretion seen with bretylium, no significant change was seen in the serum level of potassium when groups of 6 rats were examined 20 and 60 min after 100 mg/kg bretylium. Tissue levels have still to be examined.

Eversole, Giere & Rock (1952) found that noradrenaline caused an increase of glomerular filtration rate and also an apparent decrease in tubular reabsorption of water. Glomerular filtration rate is also likely to increase with diuretic amounts of adrenaline and bretylium. However, the changes in sodium: potassium ratios following the various compounds may indicate effects on the renal tubules. The rise of chloride, relative to sodium and potassium, found in the urine of rats treated with adrenaline or noradrenaline suggests that the amines may inhibit the tubular reabsorption of chloride, particularly as an analogous effect was not produced by bretylium. Botting *et al.* (1961) found that low doses of adrenaline and isoprenaline showed antidiuretic effects without reducing glomerular filtration rate, indicating an effect on renal tubular function.

Phenoxybenzamine and pentacynium showed powerful antidiuretic effects in the rat, and the action of phenoxybenzamine is similar to that described for Dibenamine (Horres, Eversole & Rock, 1950). The effective doses of phenoxybenzamine are similar to those that reduce the diuretic action of injected adrenaline and noradrenaline. The antidiuretic action of pentacynium occurs with doses comparable to those known to block autonomic ganglia and the mechanism of activation of the adrenal medulla in the rat (Green, 1956). Hence the antidiuretic effects of phenoxybenzamine and pentacynium can be related to their effects on sympathetic nerve function or on the adrenal medulla or both. In contrast, bretylium and BW 172C58 do not show powerful antidiuretic effects, despite ability to abolish adrenergic nerve function (at least in other species), which might be related to their lack of effect on

the adrenal medulla (Boura & Green, 1959 ; Boura *et al.*, 1960). Such an explanation would conform with other evidence indicating that the adrenal medulla plays a major role in bringing about water diuresis in rats (Dexter & Stoner, 1952). An alternative explanation of the lesser antidiuretic actions of bretylium and BW 172C58 would be that the fall of sympathetic tone produced by these compounds was less or was offset by their residual or inherent sympathomimetic actions. Though sympathetic blockade might cause antidiuresis by a reduction of glomerular filtration rate, this cannot at present be related to depression of systemic blood pressure. It has been reported that the blood pressure of unanaesthetized rats is increased by ganglion blocking agents and phentolamine (van Proosdij-Hartzema & de Jongh, 1955) and decreased by bretylium (van Proosdij-Hartzema, personal communication), even though the opposite effects are prominent in the anaesthetized rat. Further, the possibility is still open that sympathetic blockade may affect the activity of renal tubules, producing effects opposite from those of the sympathomimetic amines.

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