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PLASMA ADRENALINE AND NORADRENALINE AFTER PHENOXYBENZAMINE ADMINISTRATION, AND DURING HAEMORRHAGIC HYPOTENSION, IN NORMAL AND ADRENALECTOMIZED DOGS

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The intravenous administration of the antiadrenaline drug phenoxybenzamine (Dibenzyline) markedly raised the arterial adrenaline and noradrenaline concentration in dogs lightly anaesthetized with thiopentone. Graded haemorrhage led to a further rise in the amounts of amine. In adrenalectomized dogs, phenoxybenzamine moderately increased the plasma noradrenaline concentration. During haemorrhagic hypotension, previous treatment of adrenalectomized animals with phenoxybenzamine led to a significantly greater rise in plasma noradrenaline compared with that of adrenalectomized animals subjected to haemorrhage without treatment with phenoxybenzamine. Thus, phenoxybenzamine (1) raised plasma amine concentration largely due to adrenal medullary stimulation, and (2) led to increased plasma noradrenaline concentrations during sympathetic stimulation in adrenalectomized animals. The previous administration of phenoxybenzamine reduced the amount of blood which could be withdrawn before final circulatory collapse in both normal and adrenalectomized dogs.

Brown and Gillespie (1957) have recently reported that the administration of phenoxybenzamine increased the concentration of noradrenaline in splenic venous blood following splenic nerve stimulation in cats, and they have suggested that inactivation of sympathetic receptor sites by phenoxybenzamine allowed an accumulation and overflow of transmitter substance. In addition it has been shown by Benfey, Ledoux, and Melville (1958) that the administration of phenoxybenzamine to dogs greatly augmented the urinary excretion of noradrenaline and adrenaline.

This study reports arterial plasma adrenaline and noradrenaline concentrations before and after phenoxybenzamine administration in normal and adrenalectomized dogs. The effect of graded haemorrhagic hypotension induced after phenoxybenzamine injection has also been investigated. In addition, observations have been made during haemorrhagic hypotension induced in adrenalectomized dogs not previously treated with phenoxybenzamine.

METHODS

Eight dogs were very lightly anaesthetized with minimal amounts of thiopentone. A femoral artery was cannulated following infiltration of the groin with 5 to 10 cc. of 1% procaine hydrochloride. Heparin (5 mg./kg.) was then given intravenously. Arterial blood pressure was recorded continuously by means of a Statham strain gauge and Sanborn recorder. Arterial blood samples were withdrawn immediately before and after periods of 1 hr. and 2 hr. following the intravenous injection of phenoxybenzamine (20 mg./kg. given over 5 min.). Thereafter, a state of progressive haemorrhagic hypotension was induced by withdrawal of 12 ml./kg. of blood every 15 min. until circulatory collapse occurred.

Five healthy dogs were subjected to bilateral lumbar adrenalectomy, the operations being done in two stages at intervals of one week. Daily intramuscular injections of cortisone (0.5 mg./kg.) and desoxycorticosterone acetate (0.1 mg./kg.) were maintained for the five to seven days preceding each study using phenoxybenzamine which was performed in a similar manner to that described for the dogs with intact adrenal glands, except that more blood samples were taken for assay before induction of haemorrhagic hypotension.

Three adrenalectomized dogs were also subjected to graded haemorrhage as described previously (Millar and Benfey, 1958) by withdrawal of 12 ml./kg. of blood at intervals of 1 hr. until 36 ml./kg. had been lost, following which blood was withdrawn

at intervals of 15 min. until circulatory failure developed.

Samples withdrawn in the period before and after phenoxybenzamine administration (before haemorrhage) were limited to 30 to 40 ml., yielding the 15 to 20 ml. of plasma required for a single determination of adrenaline and noradrenaline concentration; the estimated values obtained from these samples therefore represent single assays. Subsequent samples (12 ml./kg.) from the normal and adrenalectomized dogs injected with phenoxybenzamine and the adrenalectomized dogs subjected to haemorrhage without phenoxybenzamine pretreatment were estimated in duplicate. Blood samples were withdrawn into glass tubes containing heparin and centrifuged for 15 to 20 min.

The fluorimetric method employed for estimation of adrenaline and noradrenaline has been outlined elsewhere (Millar and Benfey, 1958), but certain modifications were made for these studies.

Chromatographic Adsorption of Catechol Amines on Alumina

This was conveniently performed with a glass column consisting of a bulbous middle portion (capacity 30 to 50 ml.) with upper and lower limbs, the lower limb being narrower and constricted at its lower end. After placing a glass wool plug at the constriction in the lower limb, the column was partly filled with distilled water into which was poured 500 mg. of alumina (acid-washed, Merck). This was carefully stirred with a thin glass rod to eliminate air bubbles. The level of water was lowered to just above the alumina by applying air pressure to the upper limb of the column; 20 ml. of glass-distilled water was then run through to the same level. This was followed by the plasma sample (15 to 20 ml. from blood containing heparin) which was run through undiluted, or diluted with an equal volume of glass-distilled water; the plasma may be previously adjusted to *p*H 8.4 by gentle stirring and the addition of a few drops of 1% NaOH. It was run through the column at a rate of about 30 drops/min. If clogging occurred the upper surface of the alumina was stirred gently with a glass rod.

When the plasma level had almost reached the upper part of the alumina, 10 ml. of glass-distilled water was added and washed through, care being taken to avoid drying of the column. The adsorbed amines were eluted with 4 ml. of acetic acid (0.1N); recoveries 10 to 15% higher were obtainable by eluting with a second 4 ml. of acid.

The first and second 4 ml. eluates were collected in separate 15 ml. glass-stoppered tubes, and each eluate divided into 2 ml. portions for "sample" and "blank" fractions.

Recoveries, which should be above 70%, depended greatly on the technique of chromatographic adsorption, which must be studied carefully with the particular brand of alumina employed, and even with each individual batch. Although acid-washed alumina reduced the *p*H of the plasma percolate, so that satisfactory adsorption of catechol amines must occur to some extent at a *p*H lower than 8, so far this variety has given better results than the almost neutral type, which showed higher blank values unless specially prepared. The use of more concentrated acetic acid for elution from the column may be preferred, but the *p*H of the eluate/sodium acetate buffer mixture (see below) must then be brought to *p*H 6 to ensure complete oxidation of noradrenaline on addition of potassium ferricyanide.

Conversion of Adrenaline and Noradrenaline to Fluorescent Trihydroxyindole Compounds

The procedure resembled that of Lund (1950).

Treatment of "Sample" Fraction.—Reagents were added in the following sequence: (a) 1.0 ml. of 3 M-sodium acetate buffer (*p*H 8.0); (b) 0.1 ml. of 0.1% potassium ferricyanide and left for 2 min.; (c) 0.5 ml. of NaOH (20%), containing 1/10 of 2% ascorbic acid.

Treatment of "Blank" Fraction.—(a) 1.0 ml. of sodium acetate buffer; (b) 0.5 ml. of NaOH and ascorbic acid; (c) 0.1 ml. of 0.1% potassium ferricyanide.

Adrenaline and noradrenaline standard solutions, containing 0.05 μg. base/2 ml. acetic acid (0.1N), were run through the estimation procedure together with standard "blanks" as described. With the acid-washed alumina employed for these studies, it has not been found necessary to use acetic acid eluates from alumina columns for adrenaline and noradrenaline standard solutions.

Differential Estimation of Adrenaline and Noradrenaline

Fluorescence was measured 3 to 5 min. after addition of NaOH to the "sample" fraction. A Farrand or other photofluorimeter of comparably high sensitivity with the following filter arrangements allowed differential estimation of adrenaline and noradrenaline: (1) Primary filter at 400 mμ. with Wratten filter no. 35; secondary filter at 500 mμ. with Wratten no. 57. (2) Primary filter at 436 mμ. with Wratten no. 35; secondary filter at 500 mμ. with Wratten no. 57.

Two equations are constructed, where *a*, *b*, *c*, *d* represent the number of galvanometer divisions/0.01 μg. of adrenaline (A) and noradrenaline (N) for the two filter arrangements. "Net fluorescence" is the fluorescence of the sample minus that of the blank.

1. $a\mathrm{A}+b\mathrm{N}=$Net fluorescence at 400 mμ. primary wavelength.

2. $c\mathrm{A}+d\mathrm{N}=$Net fluorescence at 436 mμ. primary wavelength.

Solution of these equations gives the adrenaline and noradrenaline concentrations in μg./l. of plasma, when 20 ml. of plasma was used for assay and the eluates divided into two equal fractions (the "net fluorescence" values for each eluate were added). Smaller or larger plasma samples require the use

TABLE I

PLASMA ADRENALINE AND NORADRENALINE CONCENTRATIONS BEFORE AND AFTER PHENOXYBENZAMINE AND DURING HAEMORRHAGIC HYPOTENSION INDUCED AFTER THE 120 MIN. SAMPLE

The concentrations of adrenaline (A) and noradrenaline (N) are expressed in μg./l. Paired values in lowest line of the Table represent mean arterial blood pressure (B.P.) before and after withdrawal of each sample at each time.

Expt. No.	Before		Time (in Minutes) after Injection of 20 mg./ml. of Phenoxybenzamine											
			60		120		135		150		165		180	
	A	N	A	N	A	N	A	N	A	N	A	N	A	N
1	0·15	0·42	6·5	3·6	7·7	9·1	7·4	8·5	11	6·2				
2	0·18	0·04	1·7	1·6	1·2	4·5	2·9	4·3	3·3	3·9	4·9	4·8	19	6·4
3	0·00	0·00	6·6	2·7	4·9	5·2	9·8	7·3	9·2	7·4	20	9·9		
4	0·18	0·37	2·3	0·36	3·8	0·68	5·4	0·76	13	2·0	27	6·6		
5	0·10	0·20	0·71	0·27	1·9	0·47	3·2	0·65	6·2	1·1	14	2·7		
6	0·00	0·00	0·59	0·29	2·6	0·97	13	3·0	2·2	1·2				
7	0·12	0·09	1·8	1·5	2·6	1·7	3·1	1·3	4·0	1·3	9·4	2·7	27	11
8	0·05	0·29	10·0	2·0	9·3	2·1	12	5·5	9·2	6·2				
Mean ..	0·10	0·18	3·8	1·5	4·3	3·1	7·1	3·9	7·3	3·7	15	5·3	23	8·7
B.P. ..	143	146	95	80	70	65	63	35	42	22	26	14	17	

of a correction factor. Similar techniques have recently been described by Price and Price (1957) and Cohen and Goldenberg (1957).

This method allowed the estimation of less than 0.002 μg. adrenaline or noradrenaline, with an accuracy in mixtures of the order of ±25%. The physiological meaning of estimated plasma levels of adrenaline and noradrenaline which are close to zero is at present unknown, but serial quantitative changes, which are statistically significant, can be measured at levels below 0.5 μg./l.

RESULTS

In eight normal dogs the injection of phenoxybenzamine (20 mg./kg.) was followed by marked increases in arterial adrenaline and noradrenaline concentrations. Table I gives the results from individual experiments; the mean values are plotted in Fig. 1. From a mean adrenaline concentration of 0.10 μg./l. in the control samples there was an increase to 3.8 at 60 min. and 4.3 at 120 min. Noradrenaline increased from a mean value of 0.18 μg./l. before phenoxybenzamine injection to 1.5 and 3.1 after 60 and 120 min. respectively.

When the dogs were subjected to graded haemorrhagic hypotension by withdrawal of blood at 15 min. intervals starting 135 min. after phenoxybenzamine administration, the mean adrenaline concentration of the last samples obtained in each experiment increased to 16 μg./l. The increase in noradrenaline concentration was less pronounced, the final mean value being 6.2 μg./l.

Table II shows that, after phenoxybenzamine injection in five adrenalectomized dogs, there was no significant increase in plasma adrenaline concentration. The noradrenaline concentration, however, increased moderately in three out of five dogs after phenoxybenzamine had been given. More marked increases in plasma noradrenaline occurred when severe haemorrhagic hypotension was induced by withdrawal of blood at 15 min. intervals. The mean noradrenaline level in the final samples withdrawn before complete circulatory collapse was 2.4 μg./l., which differed significantly from the mean noradrenaline concentration of 0.19 μg./l. before injection of phenoxybenzamine (Fisher-Behrens test, $P<0.01$). By contrast the adrenaline plasma concentrations were not consistently altered in haemorrhagic hypotension, as is also shown in Table II.

In the three adrenalectomized dogs subjected to graded haemorrhage without prior injection

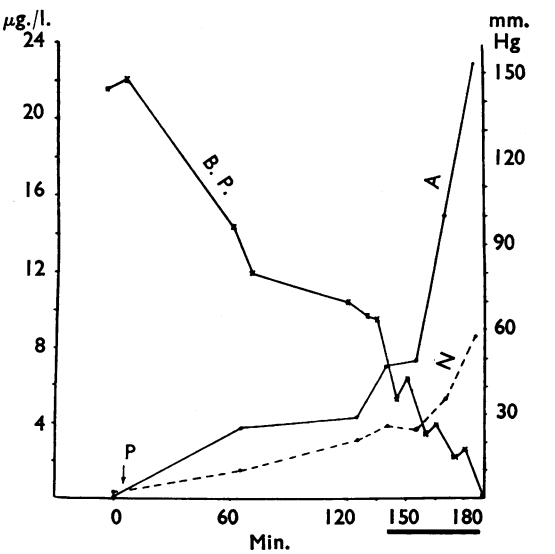

FIG. 1.—Concentrations of adrenaline (A), noradrenaline (N) in arterial blood plasma and mean arterial blood pressure (B.P.) (diastolic blood pressure +1/3 of pulse pressure) before and after phenoxybenzamine (20 mg./kg. being injected at P) and graded haemorrhage (horizontal bar).

TABLE II

PLASMA ADRENALINE AND NORADRENALINE CONCENTRATION IN ADRENALECTOMIZED DOGS BEFORE AND AFTER PHENOXYBENZAMINE, AND DURING HAEMORRHAGIC HYPOTENSION

The concentrations of adrenaline (A) and noradrenaline (N) are expressed in μg./l. Paired values in lowest line of each group represent mean arterial blood pressure (B.P.) before and after withdrawal of each blood sample. Asterisks indicate 12 mg./kg. of blood was withdrawn.

Expt. No.	Before		Time (in Minutes) after Phenoxybenzamine (10 mg./kg.)											
			60		120		180		195		210		225	
	A	N	A	N	A	N	A	N	A	N	A	N	A	N
1	0·13	0·10	0·03	0·43	0·07	0·55	0·17	0·55			0·00	1·3	0·14*	1·2*
2	0·12	0·06	0·07	0·24	0·07	0·32	0·11	0·47	0·09	0·59	0·10*	1·1*	0·12*	2·6*
3	0·27	0·12	0·38	0·61	0·41	0·94	0·12	0·77	0·11*	1·2*	0·27*	1·5*	0·22*	1·9*
Mean ..	0·17	0·09	0·16	0·43	0·18	0·60	0·13	0·60					0·16*	1·9*
B.P. ..	74	72	65	62	45	49	54	50					27	

Expt. o.	Before		Time (in Minutes) after Phenoxybenzamine (20 mg./kg.)									
			60		120		135		150		165	
	A	N	A	N	A	N	A	N	A	N	A	N
4	0·38	0·37	0·35	0·26	0·47	0·28	0·38*	0·71*	0·51*	2·1*		
5	0·04	0·32	0·04	0·32	0·20	0·29	0·11*	0·49*	0·30*	1·1*	0·81*	4·1*
Mean ..	0·21	0·35	0·20	0·29	0·34	0·29	0·25*	0·60*	0·42*	1·6*		
B.P. ..	119	116	104	77	74	51	71	27	41	13	32	

of phenoxybenzamine the plasma noradrenaline concentration increased progressively, as shown in Table III. The noradrenaline content of the final samples, withdrawn just before circulatory collapse, was in each case significantly greater than that of the first sample, as shown by analysis of variance using duplicate paired samples. The mean noradrenaline concentration in the final samples of this series (0.57 μg./l.) was significantly different ($P<0.05$) from the final mean noradrenaline concentration (2.4 μg./l.) in the five adrenalectomized dogs subjected to haemorrhagic hypotension after phenoxybenzamine treatment.

TABLE III

PLASMA ADRENALINE AND NORADRENALINE CONCENTRATION IN ADRENALECTOMIZED DOGS DURING HAEMORRHAGIC HYPOTENSION

The concentrations of adrenaline (A) and noradrenaline (N) are expressed in μg./l. Paired values in lowest line represent mean arterial blood pressure (B.P.) before and after withdrawal of each sample.

Expt. No.	0 Min.		60 Min.		120 Min.		135 Min.		150 Min.	
	A	N	A	N	A	N	A	N	A	N
1	0·00	0·25	0·00	0·31	0·00	0·43	0·01	0·60		
2	0·00	0·15	0·00	0·21	0·00	0·22	0·06	0·40	0·13	0·43
3	0·14	0·16	0·09	0·22	0·19	0·45	0·38	0·70		
Mean ..	0·05	0·19	0·03	0·25	0·06	0·37	0·15	0·57		
B.P. ..	98	92	100	68	78	59	52	27	23	

TABLE IV

TOTAL BLOOD VOLUME WITHDRAWN BEFORE CARDIAC ARREST IN VARIOUS STUDIES

	Mean (ml./kg.)	Range (ml./kg.)
8 normal dogs (Millar and Benfey, 1958) ..	58·9	46·8–69·6
8 normal dogs given phenoxybenzamine ..	38·7	9·3–51·9
3 adrenalectomized dogs	54·5	50·0–58·3
5 adrenalectomized dogs given phenoxybenzamine	44·1	31·1–58·3

Table IV illustrates the blood loss sustained in the experiments described; also included are results obtained previously by inducing graded haemorrhage in normal, untreated dogs (Millar and Benfey, 1958). The previous administration of phenoxybenzamine appeared to reduce the amount of blood which could be withdrawn before final circulatory collapse developed.

DISCUSSION

The results show that phenoxybenzamine administration in dogs led to marked increases in plasma adrenaline and noradrenaline concentration, which supports previous findings that the administration of phenoxybenzamine to dogs anaesthetized with pentobarbitone greatly augmented the urinary excretion of adrenaline and noradrenaline (Benfey, Ledoux, and Melville, 1958).

The absence of any significant rise in arterial adrenaline concentration in adrenalectomized dogs indicates that phenoxybenzamine induced liberation of adrenaline from the adrenal gland. Three adrenalectomized animals showed a gradual rise in noradrenaline concentration after administration of phenoxybenzamine, which suggests that the increased concentration of noradrenaline after phenoxybenzamine may be due partly to an extra-adrenal component.

The increased concentrations of adrenaline and noradrenaline when circulatory collapse due to

haemorrhage was imminent in the eight dogs treated with phenoxybenzamine were not significantly different from the terminal concentrations estimated previously in eight untreated dogs (Millar and Benfey, 1958). The prior administration of phenoxybenzamine did not appear to alter circulating plasma levels of adrenaline and noradrenaline at the stage of *severe* haemorrhagic shock in normal dogs.

However, in the adrenalectomized dogs treated with phenoxybenzamine, rises in arterial noradrenaline concentration could be measured when circulatory collapse was imminent as a result of haemorrhage which were significantly greater than those observed in adrenalectomized animals not treated with phenoxybenzamine. It is apparent that strong sympathetic stimulation induced by severe haemorrhage leads to higher plasma noradrenaline concentrations in adrenalectomized animals following the application of phenoxybenzamine. This could support the observations of Brown and Gillespie (1957) on the noradrenaline concentrations in splenic venous blood following splenic nerve stimulation in cats, when the amount of noradrenaline found was greater following treatment with phenoxybenzamine.

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