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# INCREASED URINARY EXCRETION OF ADRENALINE AND NORADRENALINE AFTER PHENOXYBENZAMINE

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In dogs anaesthetized with pentobarbitone sodium phenoxybenzamine greatly increased the urinary excretion of adrenaline and noradrenaline. This was associated with a transient increase in the volume of urine excreted and a fall in blood pressure. Hexamethonium prevented the effects on the urinary amines and on the blood pressure. Methacholine hypotension induced an increased adrenaline excretion, but no change in noradrenaline. The excretion of adrenaline released following ganglionic stimulation by acetylcholine did not appear to be affected by phenoxybenzamine, but that of noradrenaline was increased. During infusions of adrenaline or noradrenaline, phenoxybenzamine increased the excretion of both adrenaline and noradrenaline.

In earlier experiments (Benfey, Mazurkiewicz, and Melville, 1958a and b) it was shown that in dogs anaesthetized with phenobarbitone there was an increased urinary recovery of injected noradrenaline when various antisympathetico-mimetic agents were given (piperoxan, Hydergin, ergotoxine, chlorpromazine). As a continuation of these studies, the effects of phenoxybenzamine upon the urinary excretion of adrenaline and noradrenaline were investigated. It was observed that this agent led to a prolonged and increased excretion of both adrenaline and noradrenaline, and the experiments now described were undertaken to investigate this. Preliminary reports of this work have already appeared (Benfey, Ledoux, and Melville, 1958; Ledoux, Melville, and Benfey, 1958).

## METHODS

Dogs anaesthetized with pentobarbitone were used. After opening the abdomen, the ureters were cannulated with polyethylene tubing. The blood pressure was recorded from the femoral artery, and an infusion of physiological saline 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 determinations of adrenaline and noradrenaline were carried out by the fluorimetric method described by Millar and Benfey (1958).

Drugs were usually given after a control period of 60 min. (two 30 min. collections). The following agents were employed: phenoxybenzamine hydrochloride (Dibenzyline, kindly supplied by Messrs. Smith, Kline and French, Montreal), which was dissolved in a small quantity of propylene glycol, brought to a volume of 50 ml. with physiological saline and injected intravenously over a 10 min. period; (−)-adrenaline bitartrate and (−)-noradrenaline bitartrate monohydrate (kindly supplied by Sterling Winthrop Research Institute, Rensselaer, N.Y.); acetylcholine chloride (Hoffmann-LaRoche); methacholine chloride (Merck); and hexamethonium bromide (Vegolysen, kindly supplied by Messrs. Poulenc Ltd., Montreal). Amounts of adrenaline and noradrenaline given below refer to the base.

## RESULTS

Tables I and II contain observations from six experiments in which 10 and 20 mg./kg. of phenoxybenzamine respectively were injected. It is evident that after phenoxybenzamine output of both adrenaline and noradrenaline in the urine was increased, and that this increase was sustained for a long time. These changes were associated with an early moderately-increased urine output and a temporary fall of blood pressure. Fig. 1 shows the mean concomitant changes in urinary adrenaline and noradrenaline, urine flow, and blood pressure in these experiments. It is clear that towards the end of each experiment, although the blood pressure level was being restored to the control level, the rate of excretion of the amines was still progressively increasing.

When the effects of phenoxybenzamine were studied similarly during a continuous infusion of hexamethonium, as shown in Table III, the output of adrenaline and noradrenaline was not

TABLE I

URINARY EXCRETION OF ADRENALINE (A) AND OF NORADRENALINE (N) BEFORE AND AFTER PHENOXYBENZAMINE

Urinary catechol amine estimations are given in ng./kg./min. No urine secreted after 7th 30 min. period in Expt. No. 2; 9th period sample not analysed in Expt. No. 3.

| Expt. No.: | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | Mean (1 to 6) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 min. Period | A | N | A | N | A | N | A | N | A | N | A | N | A | N |
| 1 | 0 | 0 | 0·6 | 0·7 | 1·5 | 1·1 | 0 | 0 | 0 | 0 | 0·1 | 0·5 | 0·4 | 0·4 |
| 2 | 0 | 0 | 1·1 | 0 2 | 2·4 | 1·5 | 1·3 | 0 | 0 | 0 | 0·1 | 0·5 | 0·8 | 0·4 |
| | Phenoxybenzamine 10 mg./kg. | | | | | | Phenoxybenzamine 20 mg./kg. | | | | | | | |
| 3 | 0·5 | 2·5 | 3·1 | 0 | 3·1 | 0·8 | 0 | 0 | 0·5 | 1·2 | 0·1 | 3·4 | 1·2 | 1·3 |
| 4 | 2·0 | 5·0 | 3·1 | 0·4 | 2·9 | 0·6 | 2·6 | 7·1 | 2·5 | 1·7 | 0 | 9·4 | 2·2 | 4·0 |
| 5 | 2·5 | 4·1 | 4·7 | 0·3 | 4·3 | 1·9 | 3·0 | 3·3 | 6·0 | 2·2 | 0 | 2·6 | 3·4 | 2·4 |
| 6 | 2·5 | 5·4 | 2·9 | 0·6 | 5·8 | 2·9 | 3·0 | 5·4 | 3·6 | 1·1 | 0·6 | 4·0 | 3·1 | 3·2 |
| 7 | 1·1 | 1·6 | — | — | 1·9 | 3·0 | 2·0 | 2·6 | 13·6 | 5·1 | 0·6 | 4·0 | 3·8 | 3·3 |
| 8 | 1·2 | 2·7 | — | — | 1·1 | 2·1 | 1·6 | 2·3 | 17·0 | 9·5 | 11·0 | 5·6 | 6·4 | 4·4 |
| 9 | 1·2 | 3·1 | — | — | — | — | 1·0 | 1·0 | 13·6 | 12·5 | 11·0 | 5·6 | 6·7 | 5·5 |

augmented. The rate of urine secretion increased as usual, but there was no fall of blood pressure. Indeed, despite the continued infusion of hexamethonium, there was a small increase in blood pressure following the phenoxybenzamine injection. It was clear from the above that, during ganglionic block, phenoxybenzamine did not induce an increased output of adrenaline and noradrenaline, and the associated depressor response was also blocked.

In order to see if the increased excretion of the catechol amines after phenoxybenzamine resulted from a reflex response to the hypotension produced by the drug, experiments were performed in which subcutaneous injections of methacholine were given in small repeated doses in order to simulate the hypotension following phenoxybenzamine.

Table IV shows that, during methacholine injections, there was a decrease in mean blood pressure from the control readings of 147 and 139 mm. Hg (first and second period) to 108, 93, and 82 mm. Hg during the third, fourth, and fifth periods respectively. Whilst the blood pressure was depressed, the excretion of adrenaline was increased, but that of noradrenaline was not. Assuming that methacholine acts only at the end-organs innervated by the parasympathetic, these findings would suggest that the increased adrenaline output was caused by reflex release

TABLE II

MEAN BLOOD PRESSURE AND URINE SECRETION BEFORE AND AFTER PHENOXYBENZAMINE

Blood pressure (B.P.) in mm. Hg and urine secretion in ml./kg./min.

| Expt. No.: | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | Mean (1 to 6) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Period | B.P. | U | B.P. | U | B.P. | U | B.P. | U | B.P. | U | B.P. | U | B.P. | U |
| 1 | 166 | 0·01 | 104 | 0·14 | 160 | 0·08 | 138 | 0·02 | 130 | 0·03 | 160 | 0·01 | 143 | 0·05 |
| 2 | 154 | 0·01 | 90 | 0·06 | 150 | 0·10 | 140 | 0·02 | 138 | 0·04 | 158 | 0·02 | 138 | 0·04 |
| | Phenoxybenzamine 10 mg./kg. | | | | | | Phenoxybenzamine 20 mg./kg. | | | | | | | |
| 3 | 96 | 0·01 | 94 | 0·08 | 116 | 0·09 | 78 | 0·05 | 108 | 0·12 | 90 | 0·16 | 97 | 0·09 |
| 4 | 78 | 0·01 | 82 | 0·08 | 112 | 0·09 | 98 | 0·02 | 88 | 0·16 | 70 | 0·04 | 88 | 0·07 |
| 5 | 84 | 0·01 | 54 | 0·06 | 112 | 0·14 | 104 | 0·05 | 64 | 0·07 | 68 | 0·01 | 81 | 0·06 |
| 6 | 100 | 0·01 | 40 | 0·02 | 120 | 0·21 | 108 | 0·04 | 60 | 0·02 | 72 | 0·01 | 84 | 0·05 |
| 7 | 118 | 0·01 | 38 | 0 | 122 | 0·18 | 102 | 0·02 | 74 | 0·02 | 84 | 0·01 | 90 | 0·04 |
| 8 | 130 | 0·01 | 38 | 0 | 120 | 0·14 | 110 | 0·02 | 90 | 0·03 | 84 | 0·02 | 95 | 0·04 |
| 9 | 140 | 0·01 | — | — | — | — | 117 | 0·03 | 118 | 0·04 | 84 | 0·02 | 115 | 0·03 |

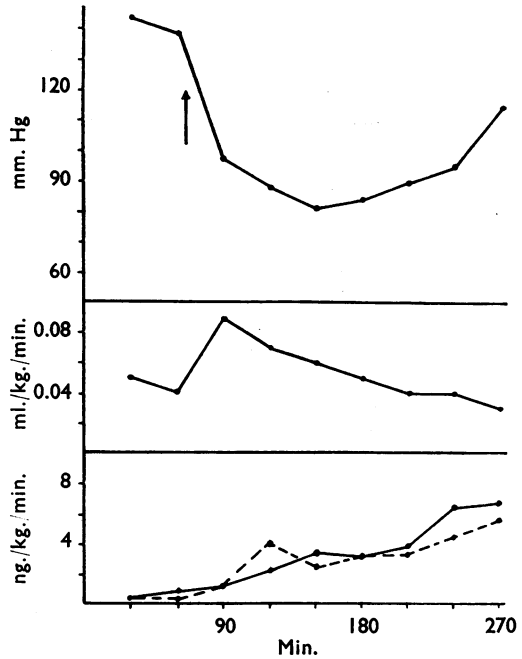


FIG. 1.—Blood pressure (upper curve), urine flow (middle curve) and urinary excretion (lowest two curves) of adrenaline (full line) and of noradrenaline (broken line) before and following administration (at arrow) of phenoxybenzamine (means of observations in Tables I and II).

of the amine from the adrenal medulla due to the fall of blood pressure. However, hypotension may not account for the equally high noradrenaline output following phenoxybenzamine.

TABLE III

MEAN URINARY EXCRETION OF ADRENALINE AND OF NORADRENALINE, RATE OF URINE SECRETION, AND BLOOD PRESSURE BEFORE AND DURING HEXAMETHONIUM INFUSION AND BEFORE AND AFTER PHENOXYBENZAMINE INJECTION

All values were based on 3 experiments. Means with range in parentheses.

| 30 min. Period | Adrenaline (ng./kg./min.) | Noradrenaline (ng./kg./min.) | Urine (ml./kg./min.) | B.P. (mm. Hg) |
|---|---|---|---|---|
| 1 | 0·3 (0·0–0·7) | 0·0 (0·0–0·1) | 0·03 (0·01–0·05) | 162 (146–178) |
| 2 | 0·2 (0·0–0·4) | 0·1 (0·0–0·1) | 0·05 (0·01–0·08) | 155 (132–179) |
| Hexamethonium (0·1 mg./kg./min.) infusion started | | | | |
| 3 | 0·4 (0·0–1·1) | 0·1 (0·0–0·4) | 0·05 (0·01–0·08) | 103 (48–130) |
| 4 | 0·0 (0·0–0·0) | 0·0 (0·0–0·0) | 0·03 (0·00–0·06) | 91 (62–120) |
| Phenoxybenzamine injection (20 mg. kg.) | | | | |
| 5 | 0·3 (0·0–0·6) | 0·1 (0·0–0·2) | 0·06 (0·01–0·09) | 105 (68–124) |
| 6 | 0·0 (0·0–0·1) | 0·1 (0·0–0·3) | 0·07 (0·01–0·11) | 97 (64–120) |
| 7 | 0·4 (0·0–1·1) | 0·1 (0·0–0·4) | 0·07 (0·02–0·12) | 106 (80–138) |
| Hexamethonium stopped | | | | |

TABLE IV

URINARY EXCRETION OF ADRENALINE AND OF NORADRENALINE, RATE OF URINE SECRETION AND BLOOD PRESSURE BEFORE, DURING, AND AFTER METHACHOLINE INJECTED SUBCUTANEOUSLY

All values are based on 3 experiments. Means with range in parentheses.

| 30 min. Period | Adrenaline (ng./kg./min.) | Noradrenaline (ng./kg./min.) | Urine (ml./kg./min.) | B.P. (mm. Hg) |
|---|---|---|---|---|
| 1 | 0·2 (0·0–0·6) | 0·3 (0·0–0·5) | 0·11 (0·03–0·14) | 147 (143–153) |
| 2 | 0·6 (0·0–1·2) | 0·3 (0·0–0·5) | 0·16 (0·13–0·18) | 139 (138–140) |
| Methacholine injections started | | | | |
| 3 | 0·4 (0·0–1·1) | 0·3 (0·0–0·6) | 0·08 (0·02–0·16) | 108 (90–120) |
| 4 | 1·4 (0·1–2·1) | 0·2 (0·0–0·6) | 0·05 (0·01–0·11) | 93 (83–104) |
| 5 | 1·3 (1·3) | 0·1 (0·1) | 0·03 (0·00–0·05) | 82 (80–84) |
| Methacholine stopped | | | | |
| 6 | 3·6 (0·0–7·2) | 0·5 (0·0–1·0) | 0·02 (0·00–0·06) | 105 (86–118) |
| 7 | 0·9 (0·1–1·3) | 0·2 (0·0–0·4) | 0·04 (0·01–0·10) | 122 (99–138) |
| 8 | 0·1 (0·0–0·1) | 0·3 (0·0–0·6) | 0·03 (0·03) | 113 (92–134) |
| 9 | 0·1 (0·0–0·1) | 0·3 (0·0–0·5) | 0·05 (0·03–0·06) | 119 (100–138) |

In order to test the effects of phenoxybenzamine on the recovery from the urine of amines released in greater amounts within the body, 5 mg./kg. of acetylcholine was injected intravenously every 3 min. after atropine had been given. Table V shows that during two successive periods (the third and fourth) during which acetylcholine was administered before phenoxybenzamine there

TABLE V

URINARY EXCRETION OF ADRENALINE AND OF NORADRENALINE, RATE OF URINE SECRETION, AND BLOOD PRESSURE BEFORE AND AFTER PHENOXYBENZAMINE INJECTION

During periods 3, 4, 8 and 9 (marked *) atropine (2 mg./kg.) followed by 10 injections of acetylcholine (5 mg./kg.) at 3 min. intervals were given. All values are based on 4 experiments. Means with range in parentheses.

| 30 min. Period | Adrenaline (ng./kg./min.) | Noradrenaline (ng./kg./min.) | Urine (ml./kg./min.) | B.P. (mm. Hg) |
|---|---|---|---|---|
| 1 | 0·2 (0·0–0·5) | 0·7 (0·0–1·6) | 0·03 (0·01–0·06) | 135 (70–197) |
| 2 | 0·6 (0·0–1·1) | 0·4 (0·0–1·6) | 0·03 (0·01–0·07) | 149 (102–186) |
| *3 | 2·6 (0·1–4·6) | 0·6 (0·0–1·2) | 0·02 (0·01–0·03) | 191 (100–270) |
| *4 | 4·8 (2·8–6·6) | 0·8 (0·0–2·1) | 0·02 (0·01–0·03) | 175 (110–230) |
| Phenoxybenzamine (10 mg./kg.) | | | | |
| 5 | 1·0 (0·7–1·5) | 0·4 (0·1–1·4) | 0·05 (0·01–0·11) | 87 (70–116) |
| 6 | 0·7 (0·2–0·9) | 1·1 (0·0–2·5) | 0·05 (0·03–0·07) | 72 (58–92) |
| 7 | 0·8 (0·0–1·5) | 0·9 (0·1–1·9) | 0·04 (0·03–0·05) | 73 (58–92) |
| *8 | 3·9 (1·4–8·3) | 2·4 (0·2–4·5) | 0·04 (0·01–0·08) | 55 (44–64) |
| *9 | 2·7 (0·4–5·7) | 2·4 (0·5–4·0) | 0·04 (0·01–0·12) | 51 (43–60) |
| 10 | 0·4 (0·0–0·7) | 0·4 (0·0–0·6) | 0·07 (0·01–0·17) | 94 (70–134) |

TABLE VI

CHANGES IN URINARY EXCRETION OF ADRENALINE (A) AND OF NORADRENALINE (N), URINE SECRETION (U), BLOOD PRESSURE (B.P.), DURING INFUSIONS OF ADRENALINE WITH AND WITHOUT PHENOXYBENZAMINE

The infusion of adrenaline was at the rate of 0·5 μg./kg./min. Estimates of A and N are in ng./kg./min.; urinary secretion in ml./kg./min. and blood pressure in mm. Hg. All values are means of four experiments with phenoxybenzamine and three without, with ranges in parentheses.

| 30 Min. Period | A | N | U | B.P. | A | N | U | B.P. |
|---|---|---|---|---|---|---|---|---|
| 1 | 4·8 (2·5–8·9) | 0·2 (0–0·4) | 0·07 (0·03–0·1) | 106 (98–114) | 1·1 (0·5–2·1) | 1·2 (0·7–1·9) | 0·22 (0·07–0·28) | 138 (90–170) |
| 2 | 6·5 (3·0–9·7) | 0·3 (0–0·8) | 0·08 (0·01–0·17) | 98 (68–146) | 1·4 (0·8–1·9) | 1·1 (0·3–1·9) | 0·27 (0·08–0·48) | 131 (98–162) |
| | Adrenaline infusion started | | | | Adrenaline infusion started | | | |
| 3 | 18·7 (16·5–21·5) | 0·3 (0–0·8) | 0·11 (0·01–0·27) | 111 (88–138) | 19·3 (12·6–24·1) | 0 | 0·21 (0·10–0·34) | 135 (100–166) |
| 4 | 23·5 (20·5–28·2) | 0 | 0·10 (0·02–0·21) | 111 (90–136) | 23·5 (17·0–27·8) | 0·9 (0–3·6) | 0·18 (0·12–0·29) | 144 (112–166) |
| | | | | | Phenoxybenzamine (10 mg./kg.) injected | | | |
| 5 | 20·8 (16·8–24·0) | 0 | 0·09 (0·02–0·16) | 114 (98–138) | 26·2 (21·1–30·0) | 0·7 (0–2·6) | 0·17 (0·11–0·27) | 101 (90–126) |
| 6 | 21·0 (17·6–25·4) | 0 | 0·09 (0·03–0·13) | 115 (100–138) | 27·7 (19·2–36·6) | 1·1 (0–4·2) | 0·13 (0·07–0·21) | 89 (70–116) |
| 7 | 18·1 (17·0–19·5) | 0·1 (0–0·3) | 0·08 (0·03–0·13) | 118 (100–146) | 27·2 (17·3–39·1) | 1·2 (0–4·9) | 0·09 (0 04–0·16) | 85 (62–106) |
| 8 | 20·9 (17·3–23·8) | 0 | 0·09 (0·04–0·13) | 124 (106–146) | 23·0 (15·0–33·2 | 1·3 (0–5·2) | 0·08 (0·03–0·14) | 88 (66–108) |
| 9 | 18·8 (18·6–19·2) | 0 | 0·11 (0·05–0·16) | 122 (94–146) | 27·7 (22·7–31·6) | 2·4 (0–9·4) | 0·09 (0·04–0·14) | 91 (70–108) |
| 10 | 21·5 (19·9–23·2) | 1·3 (0–3·8) | 0·12 (0·08–0·16) | 119 (90–146) | 23·5 (21·8–27·6) | 2·7 (0–9·4) | 0·10 (0·09–0·14) | 88 (66–104) |
| | Adrenaline stopped | | | | Adrenaline stopped | | | |
| 11 | 9·0 (4·1–13·6) | 0·5 (0–0·9) | 0·11 (0·07–0·15) | 109 (80–146) | 11·0 (6·6–16·4) | 3·5 (0·4–6·1) | 0·14 (0·06–0·18) | 99 (90–112) |
| 12 | 3·7 (0–8·9) | 0 | 0·10 (0·06–0·16) | 104 (70–146) | 3·3 (1·9–6·5) | 2·8 (0·2–4·7) | 0·18 (0·04–0·25) | 89 (78–104) |

was an increased excretion of adrenaline, but no significant change in noradrenaline. Variable pressor responses were observed after each injection of acetylcholine; the mean blood pressure during these periods reached 191 and 175 mm. Hg respectively, compared with the mean control values of 135 and 149 mm. Hg.

Following this, phenoxybenzamine induced only a slightly increased output of the amines, which was associated with an increased urine flow and a fall of blood pressure. Further acetylcholine injections after phenoxybenzamine (in the eighth and ninth periods) again increased the adrenaline excretion and, this time, increased the excretion of noradrenaline as well. These changes were associated with characteristic depressor (reversal) effects. Since identical "stimuli" were applied to release the amines before and after phenoxybenzamine, it would appear that phenoxybenzamine augmented not the excretion of endogenously-released adrenaline but that of noradrenaline.

It was of interest that, when a single injection of acetylcholine (5 mg./kg.) was given after atropine, there was always a good pressor response, but no significant change in the quantities of the amines excreted in the corresponding 30 min. sample was demonstrable. However, when adrenaline and noradrenaline were injected in amounts which had equivalent effects upon the blood pressure, excretion of the amines in the urine was greatly increased. It would appear, therefore, that either the amines released endogenously by acetylcholine are more active than those injected, or that, after release, they were so rapidly inactivated that none was detectable in the urine, except on repeated stimulation with acetylcholine. Our results permit no conclusion regarding the quantities of the amines released in this way.

With the hope of throwing some light upon the effects of phenoxybenzamine on adrenaline and noradrenaline "inactivation," we investigated the effects of the drug upon the excretion of infused adrenaline and noradrenaline. The results are shown in Tables VI and VII.

During infusion of small amounts of adrenaline (0.5 μg./kg./min.) without phenoxybenzamine, the output of adrenaline was increased, but that of noradrenaline slightly depressed. On the other hand, during a similar infusion of adrenaline with an injection of phenoxybenzamine added, the excretion of adrenaline was again increased, but this was associated with a progressively increasing excretion of noradrenaline. Indeed, even after the adrenaline infusion was stopped, the increased noradrenaline excretion continued. During infusion of noradrenaline, the output of noradrenaline was increased, but there was little change in that of adrenaline. On the other hand, during similar noradrenaline infusion with phenoxybenzamine added, the increased noradrenaline output was associated with some increase in adrenaline output (Table VII).

Assuming that the basal rate of excretion of the amines is constant and equal to that obtained in the control period, it was calculated that, during the first 3 hr. of infusion of adrenaline alone, 3% of the injected adrenaline was recovered in the urine, while in the 3 hr. period with phenoxybenzamine present the recovery was somewhat higher, 4.3%, although this difference appears unlikely to be significant. On the other hand, during noradrenaline infusion alone, 1.8% was recovered during the first 3 hr., while with phenoxybenzamine present the recovery was 1.9%. It must be concluded, therefore, that under the present experimental conditions the

TABLE VII

CHANGES IN URINARY EXCRETION OF ADRENALINE (A) AND NORADRENALINE (N), URINE SECRETION (U), BLOOD PRESSURE (B.P.), DURING INFUSIONS OF NORADRENALINE WITH AND WITHOUT PHENOXYBENZAMINE

Estimates of A and N are given in ng./kg./ml.; urinary secretion in ml./kg./min. and blood pressure in mm. Hg. The infusion of noradrenaline was at the rate of 0·5 μg./kg./min. All values are the means of four experiments with phenoxybenzamine and three without, with ranges in parentheses.

| 30 Min. Period | A | N | U | B.P. | A | N | U | B.P. |
|---|---|---|---|---|---|---|---|---|
| 1 | 1·9 (0–2·9) | 0·7 (0–2·2) | 0·14 (0·04–0·29) | 123 (102–148) | 0·4 (0–0·6) | 0·7 (0–1·9) | 0·07 (0·04–0·10) | 129 (121–140) |
| 2 | 3·5 (0–6·1) | 0·1 (0–0·4) | 0·16 (0·09–0·25) | 113 (98–135) | 1·4 (0–3·8) | 0·3 (0–1·2) | 0·10 (0·05–0·18) | 119 (88–140) |
| | Noradrenaline infusion started | | | | Noradrenaline infusion started | | | |
| 3 | 3·0 (0–5·7) | 7·5 (4·3–13·2) | 0·16 (0·10–0·22) | 115 (94–141) | 1·1 (0·3–1·9) | 7·0 (1·6–11·2) | 0·08 (0·04–0·14) | 126 (100–150) |
| 4 | 2·9 (0·1–6·4) | 10·0 (7·7–13·5) | 0·11 (0·07–0·15) | 118 (94–140) | 0·8 (0–1·5) | 13·1 (9·1–19·0) | 0·09 (0·04–0·12) | 124 (92–148) |
| | | | | | Phenoxybenzamine (10 mg. kg.) injected | | | |
| 5 | 2·2 (0–3·9) | 10·0 (6·3–17·5) | 0·11 (0·07–0·17) | 125 (98–148) | 0 7 (0–1·6) | 11·3 (4·1–18·7) | 0·08 (0·05–0·13) | 91 (68–128) |
| 6 | 1·3 (0·8–1·7) | 9·5 (7·0–10·8) | 0·13 (0·08–0·20) | 133 (106–154) | 3·0 (0–4·6) | 12·5 (10·7–15 8) | 0·04 (0–0·09) | 67 (45–97) |
| 7 | 1·8 (0·9–2·9) | 9·6 (7·1–10·9) | 0·15 (0·10–0·20) | 145 (118–164) | 6·1 (0–11·2) | 15·7 (13·9–18·6) | 0·04 (0–0·07) | 70 (50–104) |
| 8 | 1·6 (0·9–2·3) | 10·0 (5·6–13·2) | 0·14 (0·11–0·17) | 141 (120–164) | 3·3 (0–9·4) | 13·0 (6·2–17·1) | 0·06 (0·03–0·08) | 77 (56–121) |
| 9 | 1·7 (1·6–1·9) | 8·0 (6·1–10 0) | 0·13 (0·11–0·15) | 126 (118–134) | 2·0 (0–6·2) | 12·4 (5·6–15·9) | 0·05 (0·03–0·07) | 66 (60–73) |
| 10 | 1·5 (1·4–1·5) | 7·8 (5·2–10·5) | 0·14 (0·13–0·14) | 117 (104–130) | 2·4 (1·5–3·4) | 12·7 (8·6–16·9) | 0·08 (0·08) | 70 (64–76) |
| | Noradrenaline stopped | | | | Noradrenaline stopped | | | |
| 11 | 0·9 (0·2–1·6) | 2·1 (0·6–2·9) | 0·13 (0·10–0·14) | 113 (102–126) | 1·0 (0–2·2) | 9·2 (3·7–17·1) | 0·08 (0 04–0·13) | 106 (84–135) |
| 12 | 0·8 (0–1·6) | 1·2 (0–2·8) | 0·12 (0·08–0·17) | 110 (98–120) | 0·5 (0·1–0·6) | 2·1 (0–5·7) | 0·10 (0·05–0·13) | 104 (84–138) |

recovery in the urine of the small amounts of adrenaline or of noradrenaline infused was not significantly changed by phenoxybenzamine.

It was observed that, during 3 hr. of adrenaline infusion following phenoxybenzamine, the animals excreted a mean of 0.28 μg./kg. of noradrenaline, while following phenoxybenzamine alone the excretion of noradrenaline was 0.56 μg./kg. in the first 3 hr. Similarly, in the 3 hr. of noradrenaline infusion after phenoxybenzamine, the animals excreted a mean of 0.52 μg./kg. of adrenaline, whereas in the first 3 hr. after phenoxybenzamine alone the total excretion of adrenaline was 0.61 μg./kg. It may be concluded then that infusions of adrenaline or noradrenaline reduced the effect of phenoxybenzamine.

This may also account for the failure of the urine catechol amine content to increase markedly following 1 hr. of ganglionic stimulation by acetylcholine (Table V) which led to an increased output of amines in the urine.

In the above experiments, the infusions of adrenaline and noradrenaline in the dose employed (0.5 μg./kg./min.) did not appreciably influence the blood pressure or the rate of urine secretion. However, after phenoxybenzamine in both groups of experiments there was the usual fall of blood pressure and a slight decrease in urine flow.

## Discussion

Our results indicate that the increased urinary adrenaline excretion following the administration of phenoxybenzamine was due to nervous stimulation of the adrenal gland since this effect was blocked by hexamethonium. It may be a reflex elicited by the hypotension. Dontas and Nickerson (1957) reported an increase in the activity of the splanchnic nerve in cats following phenoxybenzamine which could be greatly reduced by tetraethylammonium. However, a reflex stimulation of the adrenal gland due to hypotension appears to account only partly for the elevated adrenaline in the urine. The methacholine hypotension caused only a moderate rise in adrenaline. Similarly, hypotension in the early stages of haemorrhage elevated the content of adrenaline only moderately (Millar and Benfey, 1958). We may, therefore, assume that part of the increased adrenaline in the urine arose from stimulation of sympathetic centres.

Our results do not afford any clue to the cause of the increased urinary noradrenaline. Brown and Gillespie (1957) and Brown, Davies, and Gillespie (1958) reported that, following phenoxybenzamine, greater amounts of noradrenaline could be recovered in the venous outflow of organs whose sympathetic supply was stimulated electrically. They suggested that inactivation of sympathetic transmitter substance occurred at receptor sites. Phenoxybenzamine interferes with the inactivation of noradrenaline by blocking sympathetic receptor sites. These authors do not appear to have investigated the effects of phenoxybenzamine on plasma noradrenaline in the absence of electrical stimulation. However, our results may be explained on their view that the increased urinary noradrenaline after phenoxybenzamine is derived from postganglionic sympathetic fibres, and is not adequately destroyed at receptor sites when these are blocked by phenoxybenzamine. The effect is not seen when hexamethonium paralyses the peripheral sympathetic system, because it depends on the state of activity of the sympathetic nervous system and is thus greatest in conditions of heightened sympathetic tone, as after ganglionic stimulation by acetylcholine or in hypotension. In the absence of phenoxybenzamine, hypotension induced by methacholine did not elevate urinary noradrenaline significantly, nor was there any significant rise in the content of noradrenaline in the early stages of haemorrhagic hypotension (Millar and Benfey, 1958). Recent studies on the plasma of normal and adrenalectomized dogs have shown that phenoxybenzamine elevated the concentration of amines, an effect which was greatly potentiated, in the case of noradrenaline, in adrenalectomized dogs subjected to acute and irreversible haemorrhage (Millar, Keener, and Benfey, 1958, 1959).

It is quite possible that the effect described by Brown and his colleagues is demonstrable only with endogenous noradrenaline. This is certainly handled differently by the body in comparison to exogenous noradrenaline (as indicated above in connexion with the effects of acetylcholine). This might explain our negative results with the infusion of small doses of adrenaline and noradrenaline before and after phenoxybenzamine. When large amounts of the amines were injected into dogs anaesthetized with phenobarbitone, a higher recovery of noradrenaline was obtained when antisympathomimetic substances were given (Benfey, Mazurkiewicz, and Melville, 1958a and b).

Our results also suggest that endogenous and exogenous adrenaline and noradrenaline

depressed ganglionic transmission as has previously been shown by Marrazzi (1939), Stehle and Melville (1943), and King and Marrazzi (1952). This effect may account for the reduction in urinary noradrenaline during adrenaline infusion, for the reduction in urinary adrenaline during noradrenaline infusion, and for the lessened effect of phenoxybenzamine when the amines were infused, as outlined above.

Whether or not a peripheral action of phenoxybenzamine on receptor sites accounts for the effect of noradrenaline cannot be stated definitely. Our findings could also suggest that phenoxybenzamine stimulates sympathetic centres and releases catechol amines, rather like reserpine. To what extent either or both of these actions is involved requires further study.

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