Br. J. Pharmac. (1968), **34**, 514–522.

The release of ^{3}H-dopamine from the isolated rabbit ileum

G. G. S. COLLINS* AND G. B. WEST†

Department of Pharmacology, School of Pharmacy, University of London, Brunswick Square, London W.C.1

1. Segments of rabbit ileum incubated with ^{3}H-DOPA or ^{3}H-dopamine and perfused with McEwen solution spontaneously release ^{3}H-dopamine ; this basal release is doubled when the sympathetic nerves are stimulated. Evidence is presented to show that the release is probably from the sympathetic nerve terminals.

2. Both cocaine and bretylium reduce the spontaneous release of ^{3}H-dopamine as well as the increased release resulting from sympathetic nerve stimulation. Guanethidine depresses the release during nerve stimulation but increases the spontaneous release of the amine. Phenoxybenzamine potentiates both the spontaneous release and that during nerve stimulation.

3. Tyramine releases ^{3}H-dopamine from the preparation but tachyphylaxis to the drug rapidly develops.

4. It is concluded that the release of ^{3}H-dopamine from the sympathetic nerves of the isolated perfused rabbit ileum possesses many of the characteristics of that of noradrenaline from other sympathetically innervated structures.

The drugs used to investigate the uptake, binding and release of noradrenaline from sympathetic postganglionic nerve endings include cocaine (a compound which blocks the uptake of catecholamines by sympathetic nerves), phenoxybenzamine (primarily an α-receptor blocking agent), guanethidine and bretylium (two adrenergic neurone blocking agents), and tyramine (an indirectly-acting sympathomimetic amine). A few ^{3}H-dopamine-containing sympathetic nerves have recently been found to release dopamine when stimulated (Musacchio, Fischer & Kopin, 1966 ; Austin, Livett & Chubb, 1967).

In this paper, the effects of drugs on the release of ^{3}H-dopamine from the isolated perfused rabbit ileum have been investigated. In addition, the uptake, binding and release of dopamine by sympathetic nerves in the ileum has been compared with that of noradrenaline.

* Present address: Department of Chemical Pathology, Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, Goldhawk Road, London W.6.

† Present address: British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey.

Methods

Drugs

(±)-3,4-Dihydroxyphenylethylamine-1-^{3}H-hydrobromide (45 mc/m-mole) and (±)-3,4-dihydroxyphenylethylamine-2-^{3}H-hydrochloride (1.8 c/m-mole) were obtained from the New England Nuclear Corporation, Boston, U.S.A., and dissolved in a 1% (w/v) solution of sodium metabisulphite in distilled water to provide stock solutions of 1 and 200 μc/ml. of radioactive dopamine. Different amounts of non-radioactive dopamine were then added to these solutions to produce the required concentrations of the amine, as stated below. L-3(3,4-dihydroxyphenyl)-alanine-ring-2,5,6^{3}H (DOPA, 28.7 c/m-mole) was obtained from the Radiochemical Centre, Amersham, and dissolved in the metabisulphite solution to provide stock solutions of 1 and 1,250 μc/ml. Other drugs used were cocaine hydrochloride (B.D.H.), phenoxybenzamine hydrochloride (Dibenyline ; Smith, Kline and French), guanethidine sulphate (Ismeline ; CIBA), bretylium tosylate (Burroughs Wellcome) and tyramine hydrochloride (B.D.H.).

Incubation

The incubation medium was McEwen (1956) solution to which ascorbic acid (20 mg/l.) and ethylenediamine tetraacetic acid (EDTA, 10 mg/l., disodium salt) were added to inhibit oxidation of the dopamine and DOPA. This concentration of EDTA was insufficient to reduce the calcium levels of the perfusate but it enhanced the antioxidant activity of ascorbic acid (Iversen, 1963). A segment of ileum, with its mesentery attached and weighing about 1 g, was removed from a freshly killed rabbit and placed in a small conical flask containing either 4.8 ml. of the incubation medium with 0.2 ml. of the ^{3}H-dopamine solution (200 μc/ml., to give a final concentration of 8 μc/ml. or 1 μg/ml.) or 4.9 ml. of the incubation medium with 0.1 ml. of the ^{3}H-DOPA solution (1,250 μc/ml., to give a final concentration of 25 μc/ml. or 1 mg/ml.). The mixture was then aerated with 5% carbon dioxide and 95% oxygen and the flask was agitated at 37° C for 1 hr. The segment was removed and perfused as described below.

Luminal perfusion

The perfusion apparatus consisted of a polythene trough-like organ bath, one end of which was open (Fig. 1). The segment of ileum was held in position by two pins protruding through each end at the base of the organ bath. The perfusion fluid was McEwen solution, aerated with 5% carbon dioxide and 95% oxygen and heated to 37° C by means of a heated water coil. This passed over the surface as well as through the lumen of the ileum and was collected at the open end of the organ bath, the flow rate being adjusted to about 4 ml./min. The whole organ bath assembly was surrounded by a constant-temperature water jacket at 37° C.

A piece of cotton was tied round the proximal end of the mesentery containing the sympathetic nerves and threaded through a bipolar platinum electrode connected to a square wave stimulator. After 1 hr of perfusion, the perfusate was collected for time-periods of 2.5 min. Sympathetic nerve stimulation was for time-periods of 5 min (pulse width, 1 msec ; pulse rate, 60/sec ; voltage, 10 V). All drugs were dissolved in McEwen solution. Cocaine hydrochloride, phenoxybenzamine hydrochloride, guanethidine sulphate and bretylium tosylate (10 mg/ml.)

were infused by means of a slow injection apparatus into the perfusion medium at such a rate as to give the required concentration of the drug. Tyramine hydrochloride (100 μg/ml.), on the other hand, was injected as a single dose of 10 or 20 μg directly on to the intestinal segment.

Extraction and purification of ^{3}H-dopamine and ^{3}H-noradrenaline

In most experiments it was necessary to separate unchanged ^{3}H-DOPA and ^{3}H-dopamine from newly formed ^{3}H-noradrenaline and radioactive metabolites present in the perfusion fluid. This was achieved by using alumina and Amberlite CG-120 (Type II), a strong acid ion-exchange resin. Briefly, each radioactive perfusate was collected in specimen pots containing 1 ml. 0.4 M perchloric acid, 1 ml. 1% (w/v) solution of ascorbic acid and 1 ml. 2% (w/v) solution of EDTA (disodium salt), and a mixture of 1 μg each of carrier DOPA, dopamine and noradrenaline was added; the pH value was adjusted to 8.4 with 0.5 and 0.1 N potassium hydroxide solutions, and each sample was passed through a column of alumina (made by pouring a slurry of 1 g. of alumina into a 150 mm × 6 mm glass column, the upper end of which contained a reservoir of about 50 ml. capacity). The flow rate was adjusted to 6 ml./min. The column was then washed with 5 ml. of sodium acetate solution (0.2 M, pH 8.4) and 5 ml. of distilled water, and finally the catechols were eluted with 10 ml. of 0.2 N-HCl. Ascorbic acid (1 ml. 1% (w/v) solution) and EDTA (1 ml. 2% (w/v) solution) were added to each eluate which was stored overnight at 4° C.

The pH of each sample was then adjusted to 2.0 by the dropwise addition of 0.5 and 0.1 N sodium hydroxide solutions and passed through a 50 mm × 6 mm column of Amberlite CG-120 (Type II) which had previously been treated according to the method of Häggendal (1962) thus ensuring that the resin was present in the

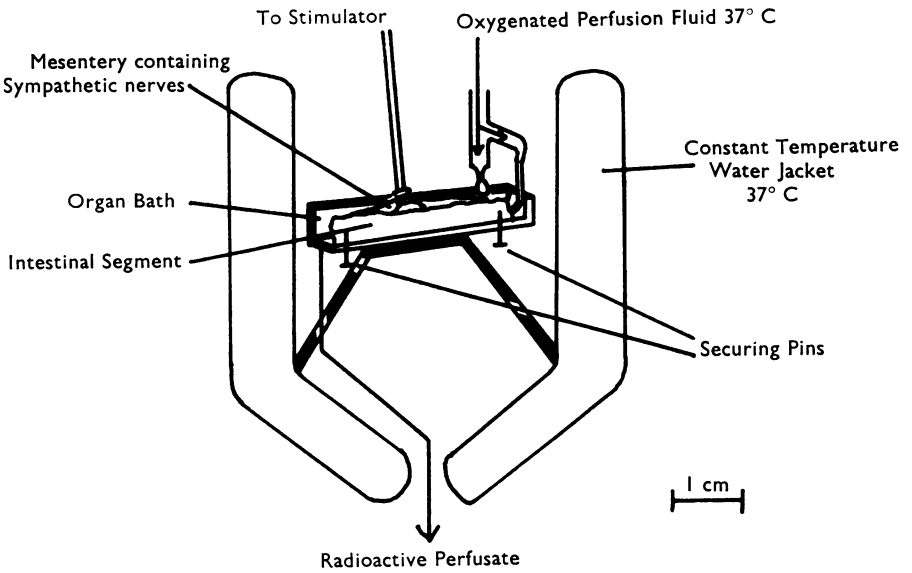

FIG. 1. Apparatus used for perfusing isolated segments of rabbit ileum.

sodium form. The flow rate was adjusted to about 6 ml./min. The column was washed with 10 ml. of distilled water to remove acidic and neutral substances, and DOPA was eluted with 10 ml. of phosphate buffer (0.1 M, pH 6.5). Excess buffer was washed off with a further 10 ml. of distilled water and the noradrenaline eluted with 10 ml. 2 N-HCl (this was called fraction I). Finally the dopamine was eluted with 10 ml. 6 N-HCl/ethanol mixture (1:1); this was called fraction II. In a series of fifty experiments with non-isotopic dopamine and noradrenaline added to perfusates using these extraction and separation procedures and the methods of estimation described by Collins & West (1968), the mean percentage recoveries ($\pm$S.E.) were 47.9 ± 2.1 and 79.8 ± 1.8, respectively. The recovery of ^{3}H-DOPA from the columns was about 30%. The amount of dopamine eluted in fraction I was less than 4% of the total dopamine and a corresponding amount of noradrenaline was eluted in fraction II. No correction has been made for these losses. The eluate obtained with a sample of perfusate from a segment of rabbit ileum incubated with ^{3}H-dopamine showed a peak of activity corresponding with that of authentic ^{3}H-dopamine and a much smaller peak corresponding with that of authentic noradrenaline.

Assay of ^{3}H-DOPA, ^{3}H-dopamine and ^{3}H-noradrenaline

Samples (1.5 ml.) of the eluates were placed in clean, dry low-potassium counting vials and evaporated to dryness *in vacuo*. The residue was taken up in 10 ml. of the counting solution (1,400 ml. toluene, 500 ml. absolute ethanol, 7.0 g 2,5-diphenyl oxazole and 0.2 g 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl)-benzene) and counted in a Packard Tri-Carb liquid scintillation spectrometer. The temperature was maintained at $-5°$ C. The counting efficiency was usually about 10% when calculated by the channels-ratio method of Baillie (1960).

Calculation of results

In each experiment, at least one blank (containing no radioactive material) was subjected to the extraction procedure and counted. The mean count of the blank samples was subtracted from the counts of the test samples to give the net counts, which have been expressed in the results as counts per min at 100% counting efficiency (c.p.m. at 100% c.e.).

Results

Effect of sympathetic nerve stimulation

When isolated segments of rabbit ileum are incubated with ^{3}H-dopamine and then perfused with McEwen solution, there is a basal release of ^{3}H-dopamine, and this increases at least two-fold when the sympathetic nerves to the ileum are stimulated (Fig. 2). During a 1-hr perfusion, the basal release as well as the release accompanying nerve stimulation are little reduced (maximum reduction 30%). Of the total radioactivity released into the perfusate (^{3}H-dopamine and metabolites), the ^{3}H-dopamine proportion increases from about 20% in basal conditions to over 50% during sympathetic nerve stimulation. When acetylcholine (2 μg) is injected into the perfusion fluid, a contraction of the intestinal segments results but the ^{3}H-dopamine content of the perfusate remains unchanged. When noradrenaline (2 μg) is injected, a marked relaxation of the tissue occurs, and again the

^{3}H-dopamine content of the perfusate remains unchanged. Thus the release of ^{3}H-dopamine which accompanies sympathetic nerve stimulation is probably derived from nervous tissue and not from the intracellular tissue spaces of the ileum.

Effect of cocaine

Cocaine (5 μg/ml. in the perfusion fluid) reduces both the basal release of ^{3}H-dopamine and its release during nerve stimulation (Fig. 3). The effect of cocaine is maximal after about 40 min of drug perfusion and does not alter the inhibitory response of the ileum to sympathetic nerve stimulation.

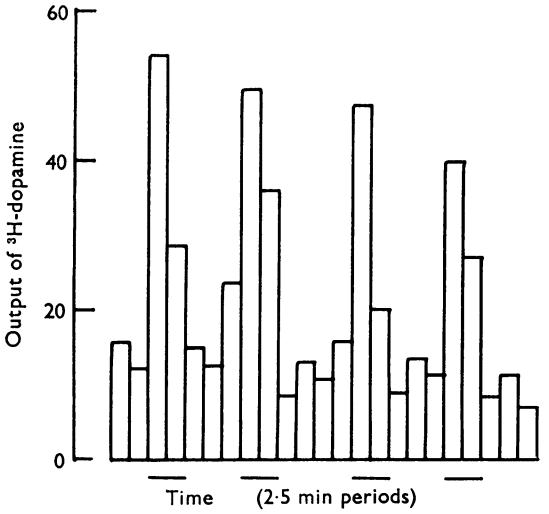

FIG. 2. Effect of four periods of sympathetic nerve stimulation (shown by the horizontal bars) on the output of ^{3}H-dopamine (c.p.m. at 100% c.e. $\times 10^3$) from an isolated, perfused segment of rabbit ileum. Each column represents the total ^{3}H-dopamine released into the perfusate over consecutive time periods of 2.5 min.

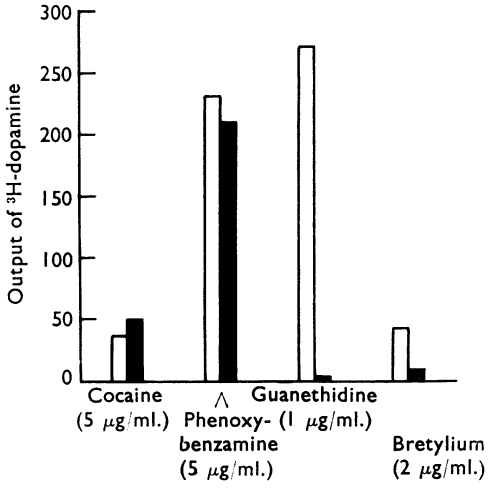

FIG. 3. Effects of different drugs on the basal release (open columns) and release during sympathetic nerve stimulation (filled columns) of ^{3}H-dopamine from an isolated, perfused segment of rabbit ileum. Results are expressed as percentages of the release from untreated segments. Each column is the mean of two separate experiments.

Effect of phenoxybenzamine

Phenoxybenzamine (5 μg/ml. in the perfusion fluid) more than doubles both the basal release of ^{3}H-dopamine and its release during nerve stimulation (Fig. 3). The effect of phenoxybenzamine is also maximal after about 40 min of drug perfusion and does not block the inhibitory response of the ileum to sympathetic nerve stimulation.

Effect of guanethidine and bretylium

Guanethidine (1 μg/ml. in the perfusion fluid) nearly doubles the basal release of ^{3}H-dopamine although its effect is short-lived and lasts for less than 30 min. Thirty minutes after drug perfusion commences, however, the release of ^{3}H-dopamine during nerve stimulation is reduced by 90% (Fig. 3). Bretylium, on the other hand, at concentrations of 2 μg/ml. in the perfusion fluid, reduces both the basal release of ^{3}H-dopamine (by 70%) and the increased release accompanying nerve stimulation (by 95%) (Fig. 3). Both guanethidine and bretylium, in the concentrations used, completely block the inhibitory response of the ileum to sympathetic nerve stimulation.

Effect of tyramine

A single injection of tyramine (10 μg) into the perfusion fluid releases ^{3}H-dopamine from the ileum for about 10 min and the amount is similar to that released during sympathetic nerve stimulation. A second dose of tyramine, however, releases much less (Fig. 4). When the dose is doubled, more ^{3}H-dopamine is released but this again is reduced when a second 20 μg of tyramine is injected into the perfusion fluid. Tyramine in these doses only partially inhibits the pendular activity of the ileum.

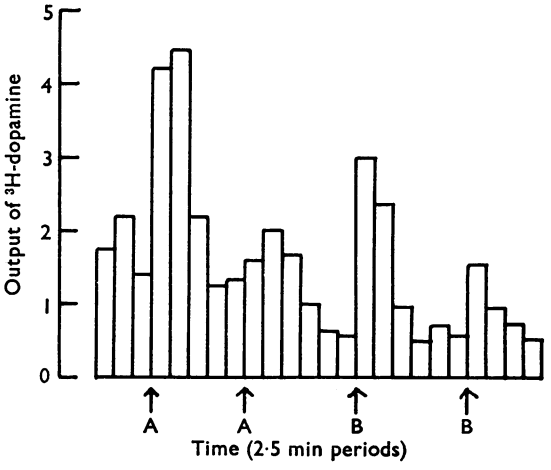

FIG. 4. Effect of tyramine (A, 10 μg ; B, 20 μg) on the release of ^{3}H-dopamine from the isolated, perfused rabbit ileum. For explanation of legend, see Fig. 2.

Effect of ^{3}H-DOPA

When segments of ileum are incubated with ^{3}H-DOPA (25 μc/ml.) and then perfused with McEwen solution, a basal release of ^{3}H-dopamine is detected. Sympathetic nerve stimulation nearly doubles this release and inhibits the pendular activity of the ileum.

Discussion

The release of ^{3}H-dopamine into the fluid perfusing segments of rabbit ileum previously incubated with ^{3}H-dopamine increases during periods in which the extrinsic sympathetic nerves innervating the ileum are stimulated, and it may be that this increased release is derived from nervous structures for the following reasons. First, a basal release of ^{3}H-dopamine occurs during perfusion and this may correspond with the spontaneous release of transmitter shown to produce excitatory junction potentials which interrupt the resting membrane potential of some sympathetically innervated organs (Burnstock & Holman, 1961, 1962a, 1962b). A high level of radioactivity in the perfusate immediately after perfusion commences probably represents washing out of the ^{3}H-dopamine from the extra-cellular tissue spaces as it declines and reaches a relatively constant basal level in about 30 min. Furthermore, contraction of the tissue by acetylcholine does not increase the radioactivity in the perfusate. Second, the drugs used in this investigation possess actions which are typical of interference with the uptake, binding and release of noradrenaline by the sympathetic nerves. Third, the increased ^{3}H-dopamine level in the perfusate is associated with sympathetic nerve stimulation and, in untreated preparations, inhibition of the pendular activity of the intestinal segments also occurs. It is also unlikely that the increased release of ^{3}H-dopamine resulting from sympathetic nerve stimulation is due to inhibition of intestinal motility, because noradrenaline added to the perfusion fluid does not release ^{3}H-dopamine into the perfusate. Finally, the fact that stimulation of the sympathetic nerves of segments of ileum which have been incubated with ^{3}H-DOPA releases ^{3}H-dopamine into the perfusate provides the strongest evidence that the amine is released from the sympathetic nerves.

No attempt has been made to estimate the non-radioactive compounds released during sympathetic nerve stimulation. The increased release of ^{3}H-dopamine during nerve stimulation is probably accompanied by a much greater release of non-radioactive noradrenaline. If the specific activity of the ^{3}H-dopamine released into the perfusate is the same as that of the ^{3}H-dopamine incubated with the preparation, however, then the basal release of the amine is equivalent to about 60 pg/min and the release during nerve stimulation to about 200 pg/min. Measurements of the total radioactivity in the perfusate, however, may be misleading because the radioactive metabolites present tend to mask changes in the level of ^{3}H-dopamine.

The similarity between the effects of cocaine, phenoxybenzamine, guanethidine, bretylium and tyramine on the release of ^{3}H-dopamine from the sympathetic nerves of rabbit ileum and those on the release of noradrenaline from other sympathetically innervated structures (see Costa, Boullin, Hammer, Vogel & Brodie, 1966) is of significance. By reducing both the basal release of ^{3}H-dopamine and that during nerve stimulation, cocaine is probably reducing the efflux of the amine

from the sympathetic nerves (Boullin, Costa & Brodie, unpublished observations quoted by Costa *et al.*, 1966) in addition to inhibiting its influx (Whitby, Hertting & Axelrod, 1960 ; Muscholl, 1961 ; Iversen, 1963). As with noradrenaline, cocaine does not increase the spontaneous release of ^{3}H-dopamine and so its effect cannot be explained on the basis of a simple inhibition of the membrane-pump mechanism which functions in sympathetic nerves. Phenoxybenzamine increases the ^{3}H-dopamine level in the perfusate both in the presence and absence of sympathetic nerve stimulation. Its effects on noradrenaline release from the sympathetic nerves of the spleen are similar (Gillespie & Kirpekar, 1965a, 1965b, 1966). Its mode of action may be by blocking the uptake of the amines by the sympathetic nerves (Iversen, 1965) as well as through its well known α-adrenergic receptor blocking activity. As cocaine reduces, whereas phenoxybenzamine increases, the efflux of ^{3}H-dopamine, the drugs probably have a different site of action. Guanethidine and bretylium both reduce the amount of ^{3}H-dopamine released during sympathetic nerve stimulation ; however, guanethidine, in contrast to bretylium, produces a transient increase in the basal release of the amine. This difference in action between the two drugs lends support to the suggestion of Chang, Costa & Brodie (1965) that the adrenergic neurone blocking activity of guanethidine depends on the persistent depolarization of the pre-synaptic nerve terminals (leading to transmitter loss) whereas bretylium prevents nerve impulses from invading the sympathetic nerve terminals (Boura & Green, 1959). It may be of significance that bretylium produces an initial blockade of the spontaneous excitatory junction potentials of the guinea-pig vas deferens (Burnstock & Holman, 1964) whereas guanethidine is inactive in this respect (Burnstock & Holman, 1966). Tyramine releases ^{3}H-dopamine, probably by increasing the permeability of the pre-synaptic nerve membrane to the amine, but tachyphylaxis of the release occurs. This corresponds with its tachyphylactic action in releasing noradrenaline, which is stated to be due to rapid depletion of the more easily releasable stores of the amine (Axelrod, Gordon, Hertting, Kopin & Potter, 1962). Thus it can be inferred that dopamine accumulating in the rabbit ileum occupies more than one binding site.

The finding that the sympathetic nerves of the rabbit ileum accumulate and release dopamine on sympathetic nerve stimulation may be analysed further. Both the catechol group and, to a lesser extent, the β-hydroxyl group of catecholamines are involved in their subcellular binding to the intraneuronal storage vesicles of sympathetic nerves (see Potter, 1966). As dopamine lacks the β-hydroxyl group, the particulate-bound fraction of this amine is probably rather less than that of noradrenaline in sympathetic nerves (Schümann, 1958a, b). Furthermore, this fraction in the case of dopamine is only weakly bound (Laverty, Michaelson, Sharman & Whittaker, 1963). Thus the presence of the β-hydroxyl group may not be so important and the particulate fraction need only be a small part of the total for the catecholamine to be released from sympathetic nerves on stimulation. von Orden, Bloom, Barrnett & Giarman (1966), using combined fluorescence and electron microscopic techniques, visualized a reduction in the intraneuronal storage vesicles of the vas deferens after treatment with α-methyl-meta-tyrosine (a drug that releases catecholamines from their storage sites) although the response to sympathetic nerve stimulation was unimpaired. Moreover, they were unable to find a recovery in the number of storage vesicles after the *in vitro* exposure of the preparation either to DOPA or to dopamine, whereas a recovery in their number occurred after exposure to noradrenaline.

REFERENCES

AUSTIN, L., LIVETT, B. G. & CHUBB, I. W. (1967). Increased synthesis and release of noradrenaline and dopamine during nerve stimulation. *Life Sci., Oxford.*, **6**, 97–104.

AXELROD, J., GORDON, E., HERTTING, G., KOPIN, I. J. & POTTER, L. T. (1962). On the mechanism of tachyphylaxis to tyramine in the isolated rat heart. *Br. J. Pharmac. Chemother.*, **19**, 56–63.

BAILLIE, L. A. (1960). Determination of liquid scintillation counting efficiency by pulse height shift. *Int. J. appl. Radiat. Isotopes*, **8**, 1–7.

BOURA, A. L. A. & GREEN, A. F. (1959). The actions of bretylium: adrenergic neurone blocking and other effects. *Br. J. Pharmac. Chemother.*, **14**, 536–548.

BURNSTOCK, G. & HOLMAN, M. E. (1961). The transmission of excitation from autonomic nerve to smooth muscle. *J. Physiol., Lond.*, **155**, 115–133.

BURNSTOCK, G. & HOLMAN, M. E. (1962a). Spontaneous potentials at sympathetic nerve endings in smooth muscle. *J. Physiol., Lond.*, **160**, 446–460.

BURNSTOCK, G. & HOLMAN, M. E. (1962b). Effect of denervation and of reserpine treatment on transmission at sympathetic nerve endings. *J. Physiol., Lond.*, **160**, 461–469.

BURNSTOCK, G. & HOLMAN, M. E. (1964). An electrophysiological investigation of the actions of some autonomic blocking drugs on transmission in the guinea pig vas deferens. *Br. J. Pharmac. Chemother.*, **23**, 600–612.

BURNSTOCK, G. & HOLMAN, M. E. (1966). Junction potentials at adrenergic synapses. *Pharmac. Rev.*, **18**, 481–493.

CHANG, G. C., COSTA, E. & BRODIE, B. B. (1965). Interaction of guanethidine with adrenergic neurones. *J. Pharmac. exp. Ther.*, **147**, 303–312.

COLLINS, G. G. S. & WEST, G. B. (1968). Some pharmacological actions of diethyldithiocarbamate on rabbit and rat ileum. *Br. J. Pharmac. Chemother.*, **32**, 402–409.

COSTA, E., BOULLIN, D. J., HAMMER, W., VOGEL, W. & BRODIE, B. B. (1966). Interaction of drugs with adrenergic neurones. *Pharmac. Rev.*, **18**, 577–597.

GILLESPIE, J. S. & KIRPEKAR, S. M. (1965a). The inactivation of infused noradrenaline by the cat spleen. *J. Physiol., Lond.*, **176**, 205–227.

GILLESPIE, J. S. & KIRPEKAR, S. M. (1965b). Uptake and release of ^{3}H-noradrenaline by the splenic nerves. *J. Physiol., Lond.*, **178**, 44–45*P*.

GILLESPIE, J. S. & KIRPEKAR, S. M. (1966). The uptake and release of radioactive noradrenaline by the splenic nerves of cats. *J. Physiol., Lond.*, **187**, 51–68.

HAGGENDAL, J. (1962). Fluorimetric determination of 3-0-methylated derivatives of adrenaline and noradrenaline in tissue and body fluids. *Acta physiol. scand.*, **56**, 258–266.

IVERSEN, L. L. (1963). The uptake of noradrenaline by the isolated perfused rat heart. *Br. J. Pharmac. Chemother.*, **21**, 523–537.

IVERSEN, L. L. (1965). Inhibition of noradrenaline uptake by drugs. *J. Pharm. Pharmac.*, **17**, 62–64.

LAVERTY, R., MICHAELSON, I. A., SHARMAN, D. F. & WHITTAKER, V. P. (1963). The subcellular localization of dopamine and acetylcholine in the dog caudate nucleus. *Br. J. Pharmac. Chemother.*, **21**, 482–490.

MCEWEN, L. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol., Lond.*, **131**, 678–689.

MUSACCHIO, J. M., FISCHER, J. E. & KOPIN, I. J. (1966). Subcellular distribution and release by sympathetic nerve stimulation of dopamine and methyl dopamine. *J. Pharmac. exp. Ther.*, **152**, 51–55.

MUSCHOLL, E. (1961). The effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. *Br. J. Pharmac. Chemother.*, **16**, 352–359.

VON ORDEN, L. S., BLOOM, F. E., BARRNETT, R. J. & GIARMAN, N. J. (1966). Histochemical and functional relationships of catecholamines in adrenergic nerve endings. I. Participation of granular vesicles. *J. Pharmac. exp. Ther.*, **154**, 185–199.

POTTER, L. T. (1966). Storage of norepinephrine in sympathetic nerves. *Pharmac. Rev.*, **18**, 439–451.

SCHUMANN, H. J. (1958a). Über den Noradrenalin- und ATP-Gehalt sympathischen Nerven. *Arch. exp. Path. Pharmak.*, **233**, 295–300.

SCHUMANN, H. J. (1958b). Über die verteilung von Noradrenalin und Hydroxytyramin in sympathischen Nerven (Milznerven). *Arch. exp. Path. Pharmak.*, **234**, 17–25.

WHITBY, L. G., HERTTING, G. & AXELROD, J. (1960). Effect of cocaine on the disposition of noradrenaline labelled with tritium. *Nature, Lond.*, **187**, 604–605.

(*Received April* 29, 1968)