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Mechanism of efflux of noradrenaline from adrenergic nerves in rabbit atria

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Summary

1. The mechanism of efflux of (−)-[^{3}H]-noradrenaline was examined in rabbit atria, which were pretreated with reserpine and pargyline.

2. Between 40 and 100 min, efflux occurred predominantly from a single intraneuronal compartment.

3. Efflux was rapidy increased by (−)- and (+)-noradrenaline, tyramine and (±)-metaraminol, but not by (±)-isopropylnoradrenaline or (±)-normetanephrine. The increase in efflux produced by (−)-noradrenaline was inhibited by cocaine and desipramine but not by lidocaine.

4. Spontaneous effluxes, and those accelerated by (−)-noradrenaline, were temperature-sensitive.

5. Efflux was increased by ouabain, omission of K^+, metabolic inhibition and lowering of the external Na^+ concentration. These effects were significantly reduced by cocaine and desipramine but not by lidocaine.

6. These findings provide evidence that the efflux of [^{3}H]-noradrenaline from adrenergic nerves occurs by a cocaine-sensitive, carrier-mediated process. The characteristics of the efflux process are compatible with, but not conclusive proof for, the Na^+-gradient hypothesis.

Introduction

Noradrenaline and certain structurally related amines are accumulated by adrenergic neurones in peripheral tissues and in the brain by a Na^+-dependent mechanism that has the characteristics of a carrier-mediated process (Iversen, 1967; 1971). Bogdanski & Brodie (1969) have proposed a model to account for noradrenaline transport, based on the 'Na^+-gradient hypothesis' of Crane, Miller & Bihler (1961). According to this model, Na^+ is considered to facilitate transport by increasing the binding affinity of a carrier for the amine, while the energy for influx of amine is imparted by the inward Na^+-gradient. However, changes in the external concentration of Na^+ altered the V_{max} for transport of metaraminol (Sugrue & Shore, 1969) and noradrenaline (White & Paton, 1972) but did not alter the K_m. In addition, the inwardly-directed Na^+ concentration gradients did not stimulate or restore uptake in metabolically-inhibited preparations (White & Keen, 1970; Paton, 1971). Since these findings were not predicted by the model of Bogdanski & Brodie, alternative models were considered by White & Paton (1972). In one, it was proposed that Na^+ increases the rate of movement of the carrier-noradrenaline complex across the membrane of adrenergic neurones, and in the other, that Na^+ increases the total number of active sites available for transport.

In order to evaluate such models further, it was necessary to examine the factors controlling the efflux of amine. However, such studies are difficult to interpret in normal animals because noradrenaline is either bound within intraneuronal vesicles or deaminated by intraneuronal monoamine oxidase after being transported into adrenergic neurones (Iversen, 1967). The only approach currently available for such studies was to impair intraneuronal storage (e.g., by pretreatment with reserpine) and simultaneously to inhibit monoamine oxidase before exposing tissues to exogenous noradrenaline. Under these circumstances, exogenous amine is accumulated predominantly within the axoplasm (Hamberger, Malmfors, Norberg & Sachs, 1964 ; Hamberger, 1967) and subsequent efflux of amine from the cytoplasm of the neurone can then be measured (Löffelholz, Lindmar & Muscholl, 1971 ; Lindmar & Löffelholz, 1972 ; Paton, 1973). This approach has been utilized in the present study. Tissues were exposed to (−)-[7-^{3}H]noradrenaline in order to avoid any possible differences in the disposition of the isomers (Iversen, Jarrott & Simmonds, 1971). A preliminary report of a portion of this work has been given (Paton, 1973).

Methods

Preparation of tissues

Adult male New Zealand rabbits, weighing 1·5–2·5 kg, were pretreated with reserpine (2·0 mg/kg s.c., 16–18 h ; 1·0 mg/kg i.v., 1 h) and were killed by a blow on the neck after which their hearts were rapidly excised. The atria from each animal were then dissected free of surrounding tissues and cut up into 6–8 pieces, weighing about 35 mg each. These pieces were then preincubated in medium at 37° C for 30 minutes. In each portion of the study, one piece of tissue from each animal served as a control while the rest were exposed to the experimental conditions.

Labelling of tissues with (−)-[7-^{3}H]noradrenaline

In order to inhibit catechol-*O*-methyl transferase, all media contained 1×10^{-4}M tropolone. After the preincubation, 5×10^{-4}M pargyline was added for 30 min to inhibit monoamine oxidase. Tissues were then placed in fresh media at 37° C for a further 30 min, at the end of which they were exposed to $5{\cdot}8\times10^{-7}$M (−)-[7-^{3}H] noradrenaline for 60 minutes.

Measurement of efflux of (−)-[7-^{3}H]noradrenaline

After labelling with (−)-[7-^{3}H]noradrenaline, each piece of tissue was removed, blotted, placed on a fine hook, and transferred (usually every 5 min), to a series of tubes containing 5 ml tracer-free medium. Media were at 37° C, unless otherwise indicated. Efflux was followed for 100 minutes.

At the end of this period, tissues were weighed, placed in Minivials (Nuclear Associates Inc.) and dissolved with NCS solubilizer (Amersham/Searle Corp.) (1 ml/100 mg tissue weight) at 37° C for 18 hours. When tissue dissolution was complete, scintillation fluor (5 ml per vial) was added and total ^{3}H content measured after the NCS had been neutralized with glacial acetic acid and the vials cooled and dark-adapted. The scintillation fluor used had the following composition: 5 g 2,5-diphenyloxazole ; 200 mg 1,4-bis-[2-4-methyl-5-phenyloxazolyl)] benzene ; 300 ml ethylene glycol monomethyl ether ; toluene to 1 litre.

One ml portions of all efflux media were added to Minivials and a scintillation fluor (5 ml/vial) added. The fluor used had the following composition: 8 g 2,5-diphenyloxazole; 200 mg 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)] benzene; 100 g naphthalene; 20 ml ethylene glycol; 100 ml ethylene glycol monomethyl ether; 1,4-dioxane to 1 litre.

In all cases, ^3H was counted with a Picker Nuclear Liquid Scintillation Spectrometer having an optimal counting efficiency for ^3H of at least 55%. Quenching was corrected for using the channels ratio technique.

The (−)-[7-^{3}H]noradrenaline content (At) of the tissue at various times during efflux was calculated by adding successively, in reverse order, the amount of (−)-[7-^{3}H]noradrenaline released into each tube during the efflux periods to that remaining in the tissue at the end of the experiment. Efflux was expressed as a rate coefficient (f) in min^{-1}

$$f = \frac{\Delta A}{\Delta t.\ At}$$

where, ΔA represents the disintegrations lost in the time interval Δt, and At is the amount of (−)-[7-^{3}H]noradrenaline in the tissue at the midpoint of the time interval Δt.

Determination of K^+ Content of Tissues

The K^+ content of tissues was determined by flame photometry as described by Munson & Paton (1972).

Unless otherwise indicated, the medium used had the following composition (mM): NaCl, 140; KCl, 5; $CaCl_2$, 1·5; $MgSO_4$, 1·2; Tris-HCl (pH 7·4), 10; D-glucose, 10. Disodium edetate (3×10^{-5}M Na_2EDTA) and sodium ascorbate (1×10^{-4}M) were added to all media to prevent oxidation of noradrenaline. The medium was equilibrated with O_2 and all incubations were at 37° C, unless otherwise indicated.

Chromatographically pure (−)-[7-^{3}H]noradrenaline with a specific activity of 6·41 C/mmol was obtained from New England Nuclear Corporation.

The variability of samples is expressed as the mean ± standard error of the mean.

Results

In Fig. 1 the efflux of (−)-[7-^{3}H]noradrenaline from rabbit atria has been plotted semilogarithmically as tissue desaturation against time. Efflux proceeded exponentially with an initially rapid rate followed by a slower rate which, after about 30 min, approached a straight line, suggesting that efflux was occurring predominantly from a single compartment at times greater than 30 minutes. This compartment accounted for about 85% of the amine present at time zero and had a half-time of about 170 minutes. It should be noted that the tissue content of (−)-[7-^{3}H]noradrenaline at time zero (i.e., at completion of loading) was 4·52 μmol/kg wet weight, representing a more than seven fold concentration compared to that present in the loading medium.

When tissues were labelled with amine in the presence of desipramine (a potent inhibitor of noradrenaline uptake (Iversen, 1967)), the amount of amine accumulated at time zero was reduced by more than 80%, to 0·88 μmol/kg wet weight. Under

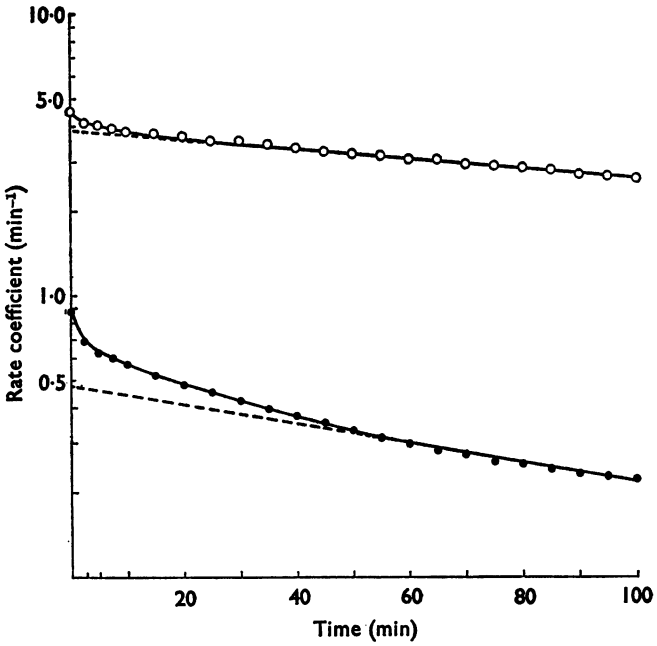

FIG. 1. Semilogarithmic plot of the [^{3}H]-noradrenaline content remaining in rabbit atrial tissues during efflux. ○——○, control tissues; ●——●, tissues exposed to 10^{-5}M desipramine during exposure to [^{3}H]-noradrenaline and subsequent efflux. (Each point is the mean of 5 observations.) The size of the slowest compartment was determined by extrapolating the line drawn through the terminal linear portion of the curve to $t=0$.

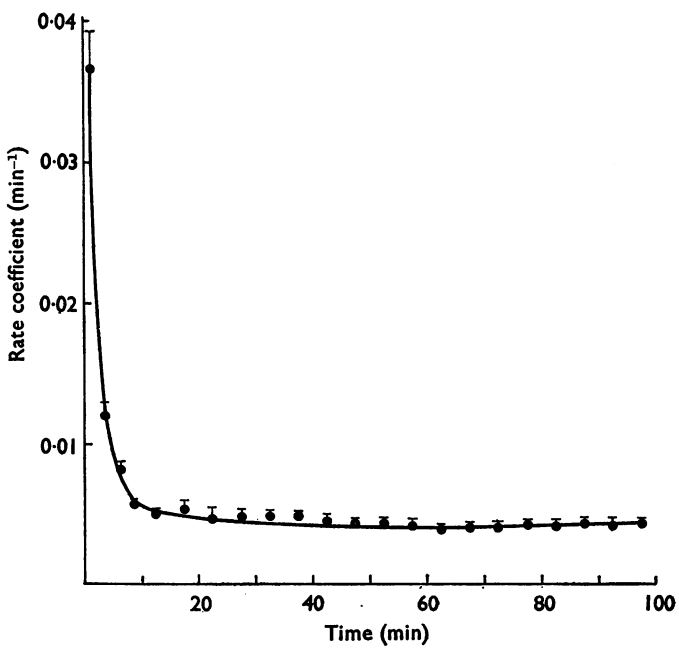

FIG. 2. Plot of [^{3}H]-noradrenaline efflux from rabbit atrial tissues expressed as rate coefficients. (Mean ±S.E. mean of 5 observations.)

these conditions (see Fig. 1), efflux of amine also proceeded exponentially, approaching a straight line after about 40–50 minutes. This slowest compartment accounted for about 55% of the amine present at time zero.

In Fig. 2, the efflux of (−)-[7-^{3}H]noradrenaline has been plotted as rate coefficient against time. The magnitude of the rate coefficients for efflux rapidly diminished over the first 10 min attaining an approximately constant value after about 30 minutes.

The above studies thus provided evidence that, at times greater than about 30 min, efflux was occurring predominantly from a single compartment. In addition, when tissues were loaded with amine in the presence of desipramine, the size of this compartment was reduced by about 90%, providing evidence that after 30 min the amine was predominantly intraneuronal in location. In subsequent studies, therefore, the effects of various procedures on efflux were generally examined between 60–100 min of efflux. After 60 and 100 min efflux, tissues contained 3·0 and 2·6 μmol (−)-[7-^{3}H]noradrenaline/kg wet weight respectively.

Effect on efflux of (−)-[7-^{3}H]noradrenaline of:

(a) Noradrenaline

Addition of (−)-noradrenaline caused a concentration-dependent increase in efflux (Figure 3). The threshold concentration appeared to be 1×10^{-6}M. The increase in efflux produced was immediately detectable and the maximal response to each concentration occurred within the first 5 minutes. Thereafter, the rate coefficient gradually decreased but remained considerably above control levels throughout the 40 min period of exposure to the amine. Efflux was also increased by the addition of (+)-noradrenaline ; the response to the (+)-isomer was slightly, but not significantly, smaller than that produced by the (−)-isomer.

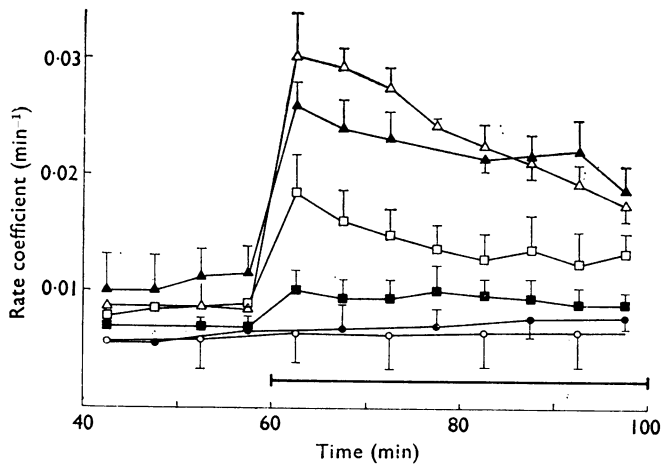

FIG. 3. Effect of (−)-noradrenaline on efflux of [^{3}H]-noradrenaline from rabbit atrial tissues. (−)-Noradrenaline (NA) was present from 60–100 minutes ●——●, control ; ○——○, $3\cdot3\times10^{-7}$M NA ; ■——■, 10^{-6}M NA ; □——□, $3\cdot3\times10^{-6}$M NA ; ▲——▲, 10^{-5}M NA ; and △——△, $3\cdot3\times10^{-5}$M NA. (Mean ± S.E. mean of 4 observations.)

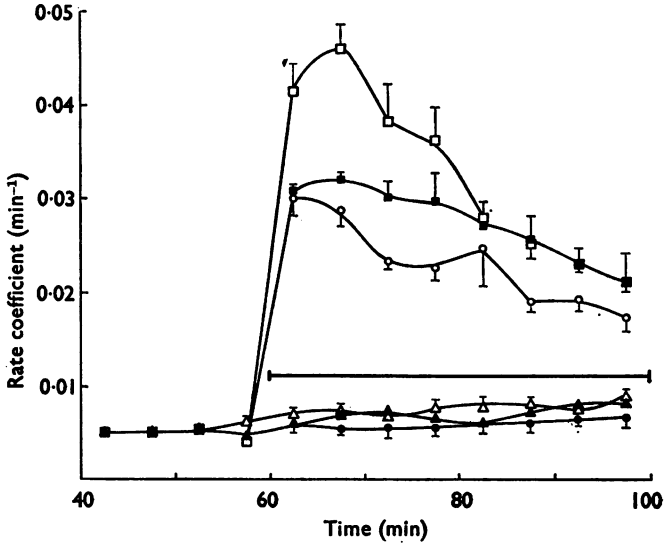

FIG. 4. Effect of amines on efflux of [^{3}H]-noradrenaline from rabbit atrial tissues. Amines were present from 60–100 min at $3{\cdot}3\times10^{-5}$M. ●——●, control; ○——○, (±)-noradrenaline; ■——■, (±)-metaraminol; □——□, tyramine; ▲——▲, (±)-isopropylnoradrenaline; and △——△, (±)-normetanephrine. (Mean ± S.E. mean of 5 observations.)

(b) Amines structurally related to noradrenaline

Addition of $3{\cdot}3\times10^{-6}$M and of $3{\cdot}3\times10^{-5}$M tyramine, (±)-metaraminol or (±)-noradrenaline all caused immediate increases in the efflux of (−)-[7-^{3}H]noradrenaline (Fig. 4), the maximal increase in efflux occurring after 5–10 min exposure to these amines. The maximal increase produced was not maintained but gradually declined. Addition of (±)-normetanephrine or (±)-isopropylnoradrenaline did not alter the rate coefficient significantly. Tyramine, metaraminol and noradrenaline are all transported into adrenergic neurones by a common membrane system while normetranephrine and isoproplynoradrenaline have an extremely low affinity for this system (Iversen, 1967; 1971).

(c) Cocaine and desipramine

Cocaine and desipramine are potent inhibitors of the process responsible for the accumulation of noradrenaline and related amines by adrenergic neurones (Iversen, 1967; 1971). Tissues were therefore exposed to these compounds within 60–100 min of efflux in order to determine whether they also altered efflux of amine. The rate coefficients for efflux were not, however, altered significantly by either 10^{-5}–10^{-4}M cocaine or 10^{-6}–10^{-5}M desipramine. However, both 10^{-5}M cocaine and 10^{-6}M desipramine very significantly reduced the increase in efflux produced by the addition of 3×10^{-6}M (−)-noradrenaline. Lidocaine (3×10^{-5}M) did not alter the effect on efflux of (−)-noradrenaline (3×10^{-6}M), suggesting that the inhibitory effects of cocaine and desipramine were not the result of a local anaesthetic action (Figure 5).

(d) Temperature

Since carrier-mediated processes are temperature-sensitive, the rate of efflux was observed at 37°, 27° and 2° C. A reduction in temperature to 27° C caused an

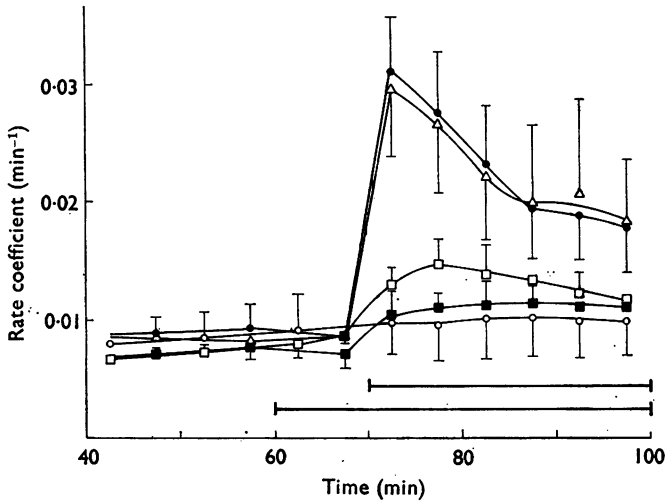

FIG. 5. Effect of drugs on the action of (−)-noradrenaline on efflux of [^{3}H]-noradrenaline from rabbit atrial tissues. (−)-Noradrenaline was present from 70–100 min, other drugs from 60–100 minutes. ○——○, control (no drugs added). In all other cases $3{\cdot}3\times10^{-6}$M (−)-noradrenaline added at 70 minutes. ●——●, control (noradrenaline only); △——△, 3×10^{-5}M lidocaine; □——□, 10^{-6}M desipramine; and ■——■, 10^{-5}M cocaine. (Mean± S.E. mean of 6 observations.)

immediate marked fall in rate coefficient (Fig. 6) with a Q_{10} of about 2·5—3·0. At 2° C, efflux was very markedly reduced. The increase in efflux produced by the addition of 3×10^{-6}M (−)-noradrenaline still occurred at 27° C but the magnitude of the response was reduced about three-fold. At 2° C, addition of (−)-noradrenaline did not alter efflux.

(e) Inhibition of Na pumping

Both ouabain (Dengler, Michaelson, Spiegel & Titus, 1962) and omission of K^+ from the external medium (Gillis & Paton, 1967) inhibit the net uptake of nor-

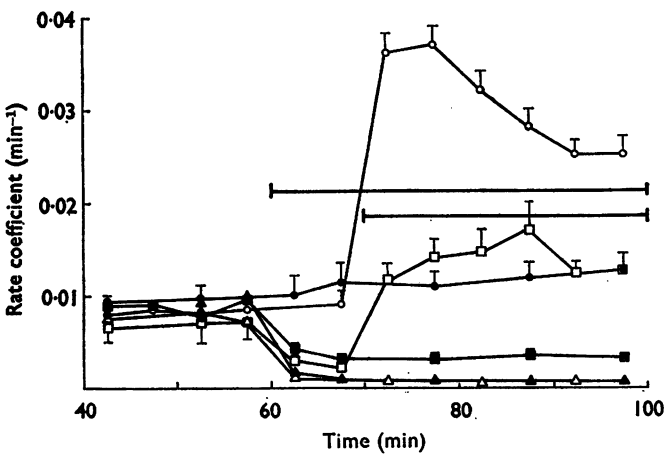

FIG. 6. Effect of temperature on efflux of [^{3}H]-noradrenaline from rabbit atrial tissues. Temperature reduced from 37° C to temperature indicated between 60–100 minutes. 3×10^{-5}M (−)-noradrenaline (NA) added between 70–100 min as indicated. ●——●, 37° C; ○——○, 37° C and NA; ■——■, 27° C; □——□, 27° C and NA; ▲——▲, 2° C; and △——△, 2° and NA. (Mean±S.E. mean of 7 observations.)

adrenaline and related amines by adrenergic nerves, possibly as a result of inhibition of the Na^+–K^+ activated ATPase (Bogdanski & Brodie, 1969; Paton, 1971). According to the Na^+-gradient hypothesis, inhibition of the net uptake of amine thus produced should result primarily from an increase in efflux (Schultz & Curran, 1970).

In the present study, the effect on efflux of the following procedures was determined between 60–100 min: omission of K^+ from the medium; 10^{-5} or 10^{-4}M ouabain; combination of omission of K^+ and addition of 10^{-5} ouabain. Omission of K^+ alone (Fig. 7) caused a gradual small increase in efflux, the maximal increase achieved being about twice the resting level. The addition of ouabain caused a much more rapid and marked increase in efflux. Ouabain (10^{-4}M) produced its maximal effect after about 20–25 min, the efflux rate decreasing thereafter. The maximal response to 10^{-5}M ouabain only occurred after about 35 minutes. The combination of 10^{-5}M ouabain and omission of K^+ caused a greater increase in efflux than either procedure alone.

The increase in efflux produced by the combination of 10^{-5}M ouabain and omission of K^+ was not altered by the addition of 3×10^{-5}M lidocaine but was very markedly reduced by 10^{-5}M cocaine or 10^{-6}M desipramine, suggesting that the increase in efflux was carrier-mediated (Figure 8). These agents did not, however, completely prevent the increase in efflux.

Exposure of tissues to 10^{-5}M ouabain and to omission of K^+ between 60–100 min of efflux resulted in a significant loss of tissue K^+, as would be anticipated from inhibition of coupled Na^+–K^+ pumping: the potassium content was $104{\cdot}2\pm17{\cdot}0$ ($n=6$) mmol/kg dry wt., compared with $201{\cdot}1\pm24{\cdot}8$ ($n=6$) mmol/kg dry wt. in normal atria. The loss of tissue K^+ so produced was not modified by the addition of lidocaine, cocaine or desipramine.

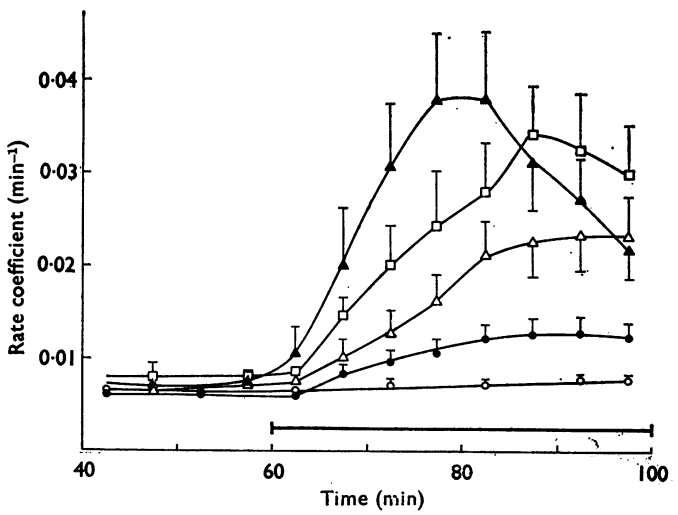

FIG. 7. Effect of ouabain and omission of K^+ on efflux of [^{3}H]-noradrenaline from rabbit atrial tissues. Ouabain added and/or K^+ omitted from 60–100 minutes. ○——○, control; ●——●, K^+ omission; △——△, 10^{-5}M ouabain; ▲——▲, 10^{-4}M ouabain; and □——□, K^+ omission and 10^{-5}M ouabain. (Mean ± S.E. mean of 7 observations.)

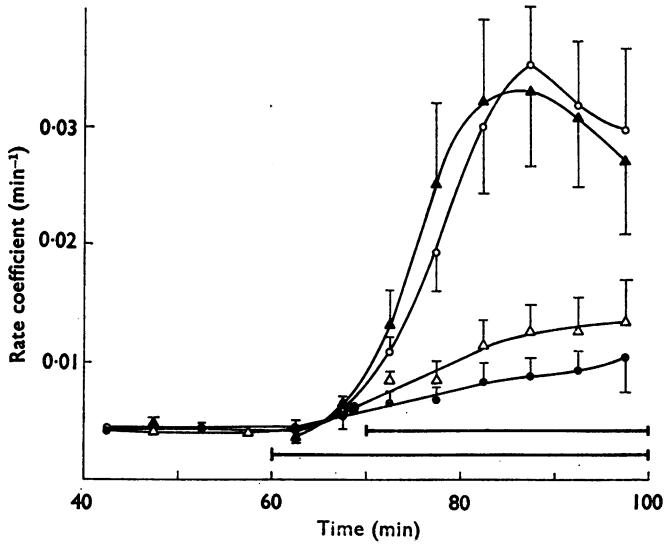

FIG. 8. Effect of drugs on action of ouabain and K^+ omission on efflux of [^{3}H]-noradrenaline from rabbit atrial tissues. All tissues were exposed to 10^{-5}M ouabain and K^+ omission from 60–100 minutes. Other drugs were present from 70–100 minutes. ○——○, control (addition of ouabain and K^+ omission only); ●——●, 10^{-5}M cocaine; △——△, 10^{-6}M desipramine; and ▲——▲, 3×10^{-5}M lidocaine. (Mean ± S.E. mean of 7 observations.)

(f) Metabolic inhibition

The net uptake of noradrenaline by rabbit atria was abolished by metabolic inhibition, possibly as a result of inhibition of coupled Na^+–K^+ pumping (Paton, 1972a). According to the Na^+-gradient hypothesis, metabolic inhibition should act primarily by increasing efflux (Schultz & Curran, 1970). In the present study, metabolic inhibition was produced by omission of D-glucose from the medium and by exposure of tissues to 100% nitrogen and 10 mM sodium azide. Previous studies (Paton, 1972a) have shown that the net uptake of noradrenaline by adrenergic nerves in rabbit atria is only markedly reduced by simultaneous inhibition of both glycolysis and oxidation via the Krebs cycle. Metabolic inhibition so produced was found to produce a rapid, very marked increase in efflux that was maximal

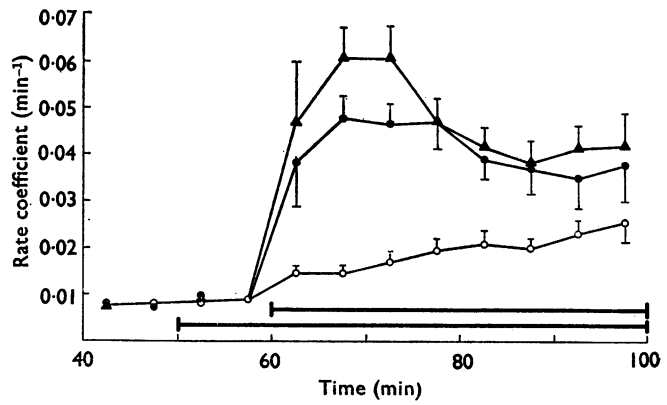

FIG. 9. Effect of metabolic inhibition on efflux of [^{3}H]-noradrenaline from rabbit atrial tissues. All tissues were exposed to 100% N_2, 10^{-2}M sodium azide and omission of D-glucose from 60–100 minutes. Other drugs were present from 50–100 minutes. ●——●, control; ○——○, 3×10^{-5}M cocaine; and, ▲——▲, 6×10^{-5}M lidocaine. (Mean ± S.E. mean of 6 observations.)

after about 10–15 min (Figure 9). Thereafter, the rate of efflux gradually declined. The effects of metabolic inhibition were not altered by 3×10^{-5}M lidocaine but were considerably reduced, but not abolished, by 3×10^{-5}M cocaine.

(g) Reduction of external Na^+

In order to determine the effect of Na^+ on efflux, NaCl in the medium was replaced by LiCl on an iso-osmolar basis. The modified solutions used had Na^+ concentrations of 20, 60 and 100 mM. Reduction of the external Na^+ to 20 mM caused a very rapid and very marked increase in efflux that was not maintained. Reduction of Na^+ to 100 mM resulted in a much more gradual and less marked increase in efflux. The effects of 60 mM Na^+ were intermediate between these extremes (Figure 10). The increase in efflux produced by lowering the external Na^+ to 20 mM was significantly reduced, but not abolished, by 3×10^{-5}M cocaine. Lidocaine (6×10^{-5}M) did not, however, modify the effect of 20 mM Na^+.

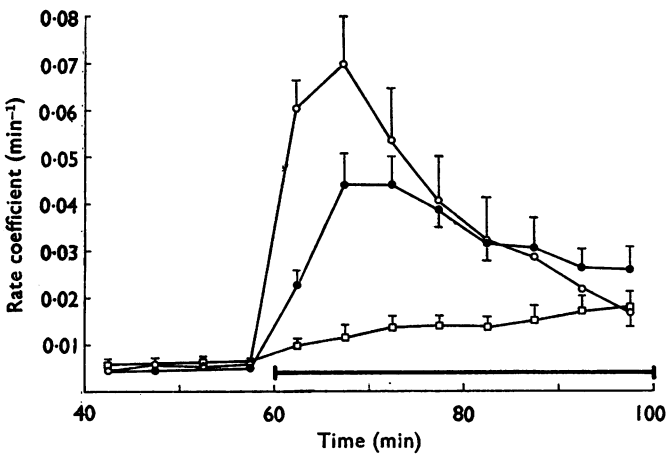

FIG. 10. Effect of Na^+ on efflux of [^{3}H]-noradrenaline from rabbit atrial tissues. Tissues were exposed to the Na^+ concentrations indicated from 60–100 minutes. NaCl was replaced by LiCl on an iso-osmolar basis. □—□, 100 mM Na^+; ●—●, 60 mM Na^+; ○—○, 20 mM Na^+. (Mean ± S.E. mean of 6 observations.)

Discussion

The following findings indicate that the alterations in rate coefficient observed in the present study reflect changes in efflux of [^{3}H]-noradrenaline from adrenergic nerves: (a) the size of the compartment from which efflux predominantly occurred was very greatly reduced when tissues were loaded with amine in the presence of desipramine; (b) the rate of efflux was increased by amines known to be transported into adrenergic nerves but not by amines which are not; and, (c) the increases in efflux produced by a number of procedures were reduced by cocaine and desipramine.

Considerable evidence was obtained that the efflux observed was (at least in part), carrier-mediated. The Q_{10} for efflux of 2·5–3·0 is compatible with such a mechanism. In perfused rabbit hearts, the Q_{10} of efflux was about 5·0, between 24° C and 34° C (Lindmar & Löffelholz, 1972). The difference in magnitude of the Q_{10} may be due to differences in experimental design, e.g., use of perfused hearts and determination of Q_{10} at an earlier time. The ability of noradrenaline, metaraminol and

tyramine to increase efflux is in keeping with an accelerative exchange diffusion process (Stein, 1967). However, the strongest evidence was provided by the finding that the increases in efflux produced by ouabain, omission of external K^+, reduction of external Na^+ and metabolic inhibition were all significantly inhibited by cocaine and desipramine, agents known to be potent inhibitors of the carried mechanism responsible for the accumulation of amines by adrenergic nerves (Iversen 1967 ; 1971).

[^{3}H]-noradrenaline, after efflux from adrenergic nerves, could possibly be transported back into the terminals on the membrane carrier. In order to assess the importance of such uptake, the effects of cocaine and desipramine on spontaneous efflux were examined. However, these agents did not alter the rate of efflux of amine significantly. Cocaine and desipramine may have inhibited efflux and uptake simultaneously so that no change in net flux occurred.

In the present study, ouabain, omission of K^+ and metabolic inhibition all produced marked increases in efflux of [^{3}H]-noradrenaline. It is possible that this effect is related to the inhibition of coupled Na^+/K^+ pumping produced by such procedures. Inhibition of the Na pump results in an increase in the intracellular Na^+ concentration and, according to the Na^+-gradient hypothesis, would be expected to increase efflux of solute by either reducing the K_m or increasing the V_{max} for efflux (Schultz & Curran, 1970). In addition, the effects on efflux of these procedures were inhibited by cocaine and desipramine. These findings thus provide evidence that the increases in efflux observed, may have resulted from an increase in carrier-mediated efflux. Inhibition of uptake of amine may have, however, contributed to the increases in efflux recorded, since the uptake of [^{3}H]-noradrenaline and [^{3}H]-metaraminol was inhibited by ouabain, omission of K^+ and metabolic inhibition (Paton, 1971 ; White & Paton, 1972). By contrast, efflux of amine was clearly not inhibited by inhibition of the Na^+/K^+ activated ATPase or by depletion of cellular ATP.

Previous studies (White & Paton, 1972) have shown that the uptake [^{3}H]-noradrenaline is Na^+-dependent. It is therefore possible that the increases in efflux produced by reduction in external Na^+ concentration may have resulted, in part, from impaired uptake. The results obtained are, however, also compatible with the Na^+-gradient hypothesis, since reducing the external Na^+ concentration would tend to reverse the direction of the Na^+ gradient.

It should be noted (Fig. 8) that cocaine and desipramine did not completely inhibit the increases in efflux of [^{3}H]-noradrenaline produced by either metabolic inhibition or the reduction of external Na^+ to 20 mM. Rather, efflux continued to increase in a time-dependent fashion until, at 100 min, efflux had increased three to four-fold. A generalized increase in cellular permeability resulting from these procedures could account for these findings. Certainly, in other tissues, metabolic inhibition is known to increase cellular permeability markedly as indicated by tissue swelling and increased permeability to such compounds as sucrose, inulin and dextran (Rangachari, Paton & Daniel, 1972 ; Osman, Munson & Paton, 1973).

The carrier-mediated uptake of noradrenaline is not sensitive to reserpine (Hamberger *et al.*, 1964 ; Iversen, Glowinski & Axelrod, 1965) but for the amine to be retained intraneuronally, monoamine oxidase must be inhibited. The amine accumulated under these conditions is, to a large extent, located in the cytoplasm

(Hamberger *et al.*, 1964 ; Hamberger, 1967) although a small amount of reserpine-resistant binding of noradrenaline occurs (Stitzel & Lundborg, 1967). It is possible that certain of the procedures used in the present study, particularly the addition of noradrenaline, metaraminol and tyramine, may have increased efflux, at least in part, by releasing [^{3}H]-noradrenaline from such binding sites or by initiating efflux from other compartments. The finding that the increases in efflux produced by such amines were not maintained but declined in a time-dependent manner, is also consistent with such a mechanism. It is, however, equally compatible with an accelerative exchange diffusion mechanism, since the intraneuronal accumulation of added amine (e.g., tyramine) would compete with [^{3}H]-noradrenaline for carrier sites for efflux thus producing a decline in efflux of [^{3}H]-noradrenaline. Further studies are being conducted to assess the role, if any, of reserpine-resistant binding in such effects.

Several workers have examined the efflux of [^{3}H]-noradrenaline in a number of adrenergically innervated tissues using a variety of compartmental analysis techniques (e.g., Bogdanski & Brodie, 1969 ; Löffelholz, Lindmar & Muscholl, 1971 ; Foster & O'Donnell, 1972 ; Starke, 1972). These workers reported that efflux could be resolved into two or three compartments. This approach was not followed in detail in the present study for the following reasons: it is not clear how one should correct for interactions between the extracellular, intraneuronal and extraneuronal compartments ; and, in the present study, not all the assumptions involved in such analyses had been met. (See also the discussions by Casteels & Droogmans, 1974 ; Cook, 1974 ; Jones, 1974).

Insufficient evidence is available to determine what was the rate-limiting step for efflux in the present study. After efflux for 60 min, however, the tissue content of [^{3}H]-noradrenaline was at least 3 μmol/kg wet weight. If, as seems likely, the great majority of this was located intraneuronally in adrenergic nerves, the concentration of amine within the axoplasm would be considerably in excess of the K_m for uptake of noradrenaline into adrenergic nerves in mammalian cardiac tissue (Jarrott, 1970). No information is available on the K_m or V_{max} for efflux of amine from adrenergic nerves. It is likely, however, that one or both parameters will differ from the values obtained for influx because intraneuronal Na^+ concentration is lower than that present in the bathing medium.

The ability of cocaine and desipramine to inhibit the increases in efflux produced by a number of different procedures, appeared to be due to an action at membrane carrier level rather than a non-specific local anaesthetic effect, since lidocaine, in higher concentration, did not have the same inhibitory properties. In previous studies (Paton, 1972b), lidocaine was more than a thousand times less potent than cocaine in inhibiting the net accumulation of [^{3}H]-metaraminol by rabbit atria. In addition, it was shown that cocaine and desipramine did not impair the downhill ion movements resulting from ouabain and omission of K^+.

The present study has thus demonstrated that [^{3}H]-noradrenaline efflux from adrenergic nerves occurs by a cocaine-sensitive, carrier-mediated process. However, the process is apparently not directly dependent upon either ATP or the activity of the Na^+/K^+ activated ATPase but may be markedly influenced by the prevailing Na^+ concentration gradient. The mechanism of efflux of amine is thus compatible with the model proposed by Bogdanski & Brodie (1969) in contrast to

the mechanism for uptake of amine (White & Keen, 1970; Paton, 1971; White & Paton, 1972).

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