Evidence for carrier-mediated efflux of noradrenaline from the axoplasm of adrenergic nerves in rabbit atria

When the intraneuronal inactivation of noradrenaline by deamination and by vesicular storage is impaired, exogenously administered amine accumulates in the axoplasm of adrenergic nerves (Graefe, Bönisch & Trendelenburg, 1971). Noradrenaline accumulated in the axoplasm in this manner, subsequently effluxes spontaneously with a half-time of about 30 min (Löffelholz, Lindmar & Muscholl, 1971). The aim of the present study was to examine the mechanism of efflux of amine from the axoplasm. Such information is essential in the evaluation of models that have been proposed to account for the transport of noradrenaline across the neuronal plasma membrane (Bogdanski & Brodie, 1969; White & Paton, 1972).

Rabbits were pretreated with reserpine (2·0–3·0 mg kg^{-1} 18 h, 1·0 mg kg^{-1} 1 h, s.c.). The animals were killed and pieces of atria were prepared and incubated at 37° in a physiological salt solution as described previously (Paton, 1972). The medium contained 10^{-4}M tropolone throughout and 5×10^{-4}M pargyline was added for 30 min. Tissues were then exposed to $5{\cdot}8 \times 10^{-7}$M [^{3}H](−)-noradrenaline for 60 min. At the end of this period, tissues were rapidly blotted, placed on fine metal hooks and transferred every 5 min thereafter to fresh media free of radioactivity. After 100 min of efflux, tissues were removed and blotted and the ^{3}H contents of all efflux and tissue samples determined by liquid scintillation spectrometry (Paton, 1972). The rate of efflux was expressed as a rate coefficient (f): where

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

ΔA represents the disintegrations lost in the time interval Δt, and At is the amount of radioactive compound in the tissue at the midpoint of the interval Δt.

(−)-Noradrenaline-7-[^{3}H] with specific activity of 6·41 Ci $mmol^{-1}$ was obtained from the New England Nuclear Corporation.

The effects of various procedures on the efflux of [^{3}H]noradrenaline were examined after 60–100 min of efflux because graphical analysis of efflux and the constancy of rate coefficients indicated that efflux during this time period was occurring predominantly from a single compartment. Amine in this compartment is apparently located within the axoplasm of adrenergic nerves (Löffelholz & others, 1971).

Addition of 10^{-5}M cocaine or 10^{-6}M desipramine or 3×10^{-5}M lignocaine for the final 40 min of efflux did not significantly alter the rate of efflux. Efflux was however markedly and rapidly increased by $1 \times 10^{-6} - 3{\cdot}3 \times 10^{-5}$M (−)-noradrenaline, $3{\cdot}3 - 6{\cdot}7 \times 10^{-6}$M (+)-noradrenaline, $3{\cdot}3 \times 10^{-6} - 3{\cdot}3 \times 10^{-5}$M (±)-metaraminol and $3{\cdot}3 \times 10^{-6} - 3{\cdot}3 \times 10^{-5}$M tyramine but not by $3{\cdot}3 \times 10^{-6} - 3{\cdot}3 \times 10^{-5}$M (±)-isoprenaline or (±)-normetanephrine. The ability of $3{\cdot}3 \times 10^{-6}$M (−)-noradrenaline to increase the rate of efflux was inhibited by 10^{-5} cocaine or 10^{-6}M desipramine but not by $3{\cdot}3 \times 10^{-5}$M lignocaine. These findings are summarised in Table 1.

The efflux of [^{3}H](−)-noradrenaline was also examined at 27 and 37°; the Q_{10} was found to be at least 2·5. In perfused rabbit hearts, the Q_{10} of efflux was about 5·0 between 24 and 34° (Lindmar & Löffelholz, 1972).

The finding that efflux was not increased by the addition of cocaine or desipramine in concentrations that markedly inhibit influx of amine (Iversen, 1967) might suggest that a unidirectional, and not a net, efflux was being measured. It is also possible, however, that cocaine and desipramine simultaneously inhibited both efflux and backflux of [^{3}H](−)-noradrenaline so that the net rate of efflux of amine was not altered.

Table 1. *Effect of amines on the efflux of* [^{3}H] (−)-*noradrenaline in rabbit atria.* Mean values of 5–7 observations ± s.e. $*P < 0{\cdot}05$: NA: (±)-noradrenaline. DMI: desipramine.

Experimental condition		Rate coefficient (min^{-1})					
Series I		52·2 min	57·5 min	62·5 min	67·5 min	72·5 min	77·5 min
$3{\cdot}3 \times 10^{-6}$ M NA added at 60 min	added at 70 min						
——	——	0·00425 ±0·00111	0·00447 ±0·00146	0·00452 ±0·00522	0·00450 ±0·00157	0·00483 ±0·00149	0·00472 ±0·00161
+	——	0·00406 ±0·00063	0·00442 ±0·00068	0·00459 ±0·00107	0·00423 ±0·00087	0·01546* ±0·00243	0·01375* ±0·00258
+	10^{-5}M cocaine	0·00355 ±0·00029	0·00352 ±0·00034	0·00395 ±0·00062	0·00434 ±0·00059	0·00636 ±0·00089	0·00723 ±0·00120
+	10^{-6}M DMI	0·00332 ±0·00026	0·00366 ±0·00043	0·00308 ±0·00045	0·00349 ±0·00052	0·00511 ±0·00104	0·00537 ±0·00072
+	3×10^{-5}M lignocaine	0·00434 ±0·00112	0·00419 ±0·00092	0·00406 ±0·00089	0·00423 ±0·00094	0·01470* ±0·00243	0·01322* ±0·00258
Series II (all $3{\cdot}3 \times 10^{-5}$M and all added at 60 min)							
——		0·00536 ±0·00075	0·00487 ±0·00079	0·00581 ±0·00106	0·00546 ±0·00075	0·00553 ±0·00130	0·00559 ±0·00093
(±)-noradrenaline		0·00477 ±0·00088	0·00459 ±0·00077	0·02996* ±0·00221	0·02860* ±0·00213	0·02325* ±0·00101	0·02263* ±0·00144
(±)-metaraminol		0·00445 ±0·00053	0·00443 ±0·00040	0·03071* ±0·00084	0·03182* ±0·00065	0·02990* ±0·00157	0·02963* ±0·00350
tyramine		0·00502 ±0·00086	0·00491 ±0·00069	0·04130* ±0·00292	0·04600* ±0·00247	0·03813* ±0·00396	0·03600* ±0·00350
(±)-isoprenaline		0·00418 ±0·00068	0·00461 ±0·00082	0·00604 ±0·00138	0·00680 ±0·00160	0·00704 ±0·00171	0·00645 ±0·00162
(±)-normetanephrine		0·00535 ±0·00097	0·00614 ±0·00060	0·00696 ±0·00085	0·00720 ±0·00080	0·00677 ±0·00071	0·00752 ±0·00087

Certain amines (i.e. noradrenaline, tyramine and metaraminol) that are transported into adrenergic nerves by a common mechanism (Iversen, 1967), increased the efflux of [^{3}H] (−)-noradrenaline. This effect was inhibited by cocaine and desipramine in concentrations known to inhibit the influx of noradrenaline and related amines into adrenergic nerves (Iversen, 1967). This effect of cocaine and desipramine was not apparently a local anaesthetic effect since higher concentrations of lignocaine did not prevent the action of noradrenaline. It should also be noted that isoprenaline and normetanephrine, amines with a high affinity for transport into extraneuronal sites but with a very low affinity for transport into adrenergic nerves (Iversen, 1967), did not increase the efflux of [^{3}H] (−)-noradrenaline. The ability of noradrenaline, metaraminol and tyramine, to increase efflux is in keeping with an accelerative exchange diffusion process (Stein, 1967). This, together with the Q_{10}, provides evidence that efflux of noradrenaline from the axoplasm of adrenergic nerves in rabbit atria is a carrier-mediated process.

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REFERENCES

BOGDANSKI, D. F. & BRODIE, B. B. (1969). *J. Pharmac. exp. Ther.*, **165**, 181–189.

GRAEFE, K. H., BÖNISCH, H. & TRENDELENBURG, U. (1971). *Arch. exp. Path. Pharmak.*, **271**, 1–28.

IVERSEN, L. L. (1967). *The uptake and storage of noradrenaline in sympathetic nerves*, p. 151. Cambridge: Cambridge Univ. Press.

LINDMAR, R. & LÖFFELHOLZ, K. (1972). *Arch. exp. Path. Pharmak.*, **274**, 410–414.

LÖFFELHOLZ, K., LINDMAR, R. & MUSCHOLL, E. (1971). *Ibid.*, **270**, R87.

PATON, D. M. (1972). *Pharmacology*, **7**, 78–88.

STEIN, W. D. (1967). *The movement of molecules across cell membranes.* p. 126. New York: Academic Press.

WHITE, T. D. & PATON, D. M. (1972). *Biochim. biophys. Acta*, **266**, 116–117.

Differential effects on mouse brain catecholamine turnover of chlorpromazine, trifluoperazine and closely-related non-tranquillizing analogues

There is convincing evidence that the major tranquillizing drugs accelerate the turnover of brain dopamine. Although they do not greatly change the brain level of dopamine itself, they cause a marked rise in the brain levels of dopamine metabolites (Carlsson & Lindqvist, 1963; Andén, Roos & Werdinius, 1964; Laverty & Sharman, 1965), they increase the rate at which dopamine disappears from the brain when synthesis is blocked by α-methyltyrosine (Sharman, 1966; Corrodi, Fuxe & Hökfelt, 1967), and they accelerate the turnover of [^{14}C] labelled dopamine formed in the brain from [^{14}C] tyrosine (Nybäck, Sedvall & Kopin, 1967; Gey & Pletscher, 1968). Their effects on brain noradrenaline turnover are less consistent; nevertheless, many tranquillizers raise the normetanephrine level in the brains of mice pretreated with monoamine oxidase inhibitors (Scheel-Krüger, 1972) and accelerate the disappearance of noradrenaline from the brains of rats when either tyrosine hydroxylase or dopamine-β-hydroxylase is inhibited (Andén, Corrodi & Fuxe, 1972).

The strongest reason for believing that these effects are causally related to the tranquillizing action is the correlation that exists between the effect of tranquillizers on catecholamine turnover in animals and their tranquillizing potency in man (Roos, 1965; Nybäck & Sedvall, 1970). However, other correlations have been observed between the tranquillizing potency of aminoalkylphenothiazines and some of their