Uptake kinetics and ion requirements for extraneuronal uptake of noradrenaline by arterial smooth muscle

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Quantitative investigations of extraneuronal uptake of noradrenaline (NA) have usually been carried out on whole organs or tissue slices. This method does not permit the localization of the particular tissue elements involved in the uptake process, and the values obtained may be some algebraic average of these elements. To overcome this disadvantage the microhistochemical technique of Avakian & Gillespie (1968) has been used to study extraneuronal uptake into arterial smooth muscle.

Isolated rabbit ear arteries were perfused with NA in Krebs or in ion-deficient solutions. When ion-deficient media were used, a 20 min equilibrium perfusion with the altered solution was allowed before the NA perfusion. Pieces of artery were removed at 1, 2, 5, 10, 20 and 40 min after the beginning of the NA perfusion, immediately quenched in liquid nitrogen-cooled isopentane, freeze-dried overnight, and subjected to the Falck technique. The fluorescence brightness of the NA taken up into smooth muscle cells was measured using a Leitz MPV microphotometer.

Perfusion with different concentrations of NA (10^{-4} M, 2×10^{-4} M, 5×10^{-4} M and 10^{-3} M) in unaltered Krebs showed that the uptake was concentration dependent. A Lineweaver-Burke plot of the reciprocal of the initial rate of uptake against the reciprocal of the concentration gave a straight line, showing that the uptake process obeys saturation kinetics. The K_m was found to be 5×10^{-4} M.

Perfusion at 2° C or in isotonic sucrose greatly reduced uptake, each giving 75% inhibition of control uptake. Sodium-free or 2·5 mM sodium solution produced about 50% inhibition and 25 mM sodium solution about 25% inhibition. Lithium was able to substitute for sodium. The omission of calcium, magnesium or potassium from the perfusion media had no effect on the uptake. Depolarization of the smooth muscle cells (by addition of solid K_2SO_4 to Krebs to give 100 mM potassium) gave 50% inhibition of uptake. In contrast to these results the binding of NA to collagen was unaffected by cold or alterations in the sodium ion concentration.

These results suggest first, that extraneuronal uptake in smooth muscle is a carrier-mediated process, and secondly that studies of uptake kinetics and the effects of ions on this in whole organs may yield complex results because of the contribution of different tissue components.

R. Towart is an M.R.C. scholar.

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Inhibitory nature of the adrenergic innervation in the guinea-pig vas deferens

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Twitches were elicited by one to eight pulses (10 Hz, 0·2–1 ms duration) and recorded isometrically. All other details of experimental procedures are given by

TABLE 1. *% Twitch-inhibition produced by noradrenaline, tyramine, cocaine and PGE_2 in desheathed vas deferens preparations from normal and reserpinized guinea-pigs*

		'Normal'		'Reserpinized'	
Noradrenaline	0·5 μg/ml	29·1 ±3·73	(6)	26·3±10·35	(3)
,,	2·5 μg/ml	39·05±4·65	(6)	38·3± 8·4	(3)
,,	10·0 μg/ml	48·5 ±5·5	(5)	55·1±13·3	(3)
Tyramine	0·5 μg/ml	20·9 ±5·5	(6)	0·6± 0·6	(3)
,,	2·5 μg/ml	38·4 ±9·15	(6)	0·7± 0·67	(3)
,,	10·0 μg/ml	58·0 ±7·56	(6)	4·9± 4·95	(3)
,,	50·0 μg/ml	64·1 ±8·85	(6)	9·0± 4·6	(3)
Cocaine	0·5 μg/ml	60·0	(2)	0·0	(2)
,,	5·0 μg/ml	78·5	(2)	−12*	(2)
,,	50·0 μg/ml	99·0	(2)	7·5	(2)
PGE_2	2·0 ng/ml	90·5 ±3·94	(4)	100·0	(3)

Values are means ± S.E. of mean. Number of experiments in parentheses. The negative value, marked with an asterisk, indicates % twitch potentiation by cocaine.

Ambache & Zar (1970, 1971), who presented evidence against motor transmission in the vas being adrenergic, and suggested that the adrenergic innervation might subserve an inhibitory function. This is corroborated by obtaining twitch-inhibition with the indirectly acting sympathomimetics, tyramine and cocaine.

Pretreatment with reserpine phosphate (0·5 mg/kg s.c. 2 days, and 1·5 mg/kg i.p. 1 day before use) abolished inhibition by tyramine and cocaine without altering noradrenaline or PGE_2 inhibitions (Table 1). This confirms that the mechanism of inhibition by tyramine or cocaine involves endogenous noradrenaline. If motor transmission were adrenergic, tyramine should have produced contractions in the normal vas deferens by releasing transmitter, but this was never seen in guinea-pig preparations.

The degree of inhibition produced by noradrenaline, tyramine or cocaine remained unaltered after exposure for 3 h to indomethacin or sodium meclofenamate ($1–2\times10^{-6}$ g/ml), both of which abolish prostaglandin-synthesis (Gryglewski & Vane, 1971). It is therefore unlikely that the noradrenaline inhibition is mediated by release of endogenous prostaglandin as proposed by Swedin (1971). Moreover, in guinea-pig (and rabbit) vas deferens, phentolamine (2×10^{-6} g/ml), antagonized noradrenaline inhibitions without affecting PGE_2 inhibitions. In addition, the motor transmission in other species (rat and *Meriones* vas deferens) was virtually unaffected by PGE_2 (2–500 ng/ml) but could be inhibited by tyramine or noradrenaline after the motor response to noradrenaline was abolished by phenoxybenzamine (10^{-6} g/ml).

In conclusion, the failure of tyramine to induce contractions, the ability of tyramine and cocaine to inhibit the motor transmission and the loss of this ability after reserpine, all substantiate the inhibitory adrenergic function postulated previously. The existence of a prostaglandin link in noradrenaline inhibition seems improbable.

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