

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 micro-photometer.

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.

REFERENCE

- AVAKIAN, O. V. & GILLESPIE, J. S. (1968). Uptake of noradrenaline by adrenergic nerves, smooth muscle and connective tissue in isolated perfused arteries and its correlation with the vaso-constrictor response, *Br. J. Pharmac. Chemother.*, **32**, 168-184.

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