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KINETICS AND SOME CHARACTERISTICS OF UPTAKE OF NORADRENALINE BY THE HUMAN UMBILICAL ARTERY

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The uptake of exogenously added noradrenaline (NA) (0.5-2.5 μg/ml) by the human umbilical artery was linear with time up to 10 minutes. The uptake was saturable and could be described by the Michaelis-Menten equation. The uptake was cocaine-resistant, normetanephrine-sensitive, was considerably inhibited in the cold and was partially inhibited by Na^+-deficiency. Of NA accumulated in the artery 31% could be washed out by NA-free medium. It is concluded that the mechanism of uptake of NA by the human umbilical artery is similar to the $uptake_2$ mechanism.

Introduction In innervated tissues, $uptake_1$ and the presence of metabolizing enzymes, catechol-*O*-methyltransferase (COMT) and monoamine oxidase (MAO) can interfere with the kinetic analysis of extraneouronal uptake of noradrenaline (NA). The human umbilical artery lacks adrenergic innervation and the MAO and COMT activity of this tissue is very low (Burnstock, McCulloch, Story & Wright, 1972). In the present study the kinetics and some characteristics of uptake of NA were determined using the human umbilical artery.

Methods Umbilical cords were obtained immediately after delivery from the Maternity Ward of Shree Sayaji General Hospital, Baroda and kept in the medium described below. After dissecting out both the arteries the lumen of each artery was cut open and 6 pieces each weighing about 100-200 mg were prepared from both arteries. Unless otherwise specified, all preincubations or incubations were carried out at 37°C in 30 ml of the medium (g/l: NaCl 9.0; KCl 0.42; $CaCl_2$ 0.24; $NaHCO_3$ 0.2; disodium edetate 0.01; and glucose 1.0) bubbled with 5% CO_2 in O_2 (pH 7.4). Preincubations and incubations were carried out in separate flasks. The Na^+-deficient medium contained sucrose (105.3 g/l) and $KHCO_3$ (0.238 g/l) instead of NaCl and $NaHCO_3$ respectively. NA was measured fluorometrically (Anton & Sayre, 1962). Fluorescence was read on a Turner fluorometer (Model 110). The mean recovery of NA added to alumina was 70 ± 2.5% (n = 10) and all values were corrected for 70% recovery.

For studying the kinetics of uptake four pieces of artery were preincubated for 10 min in individual flasks and then incubated with specified concentrations of NA for different times. The effects on uptake of the following drugs or procedures were studied: (i) preincubation of the artery for 10 min with normetanephrine (10 μg/ml) or cocaine (5 μg/ml) followed by incubation for 5 min with a specified concentration of NA and either normetanephrine (10 μg/ml) or cocaine (5 μg/ml); (ii) preincubation with Na^+-deficient medium with change of the medium every 10 min three times followed by incubation for 5 min in Na^+-deficient medium containing NA (1 μg/ml); (iii) preincubation with NA-free medium for 10 min, incubation for 5 min with medium containing NA (1 μg/ml) followed by incubation with NA-free medium with change of medium every 3 min; (iv) preincubation for 15 min at 4°C followed by incubation for 5 min at 4°C with medium containing NA (1 μg/ml).

For determination of the extracellular space, a piece of the artery was preincubated in the medium for 10 min and then incubated with alkali stable inulin (100 μg/ml) for specified times. The inulin content of the artery was estimated by the method of Schneyer & Schneyer (1960). The extracellular space (ml/g) of the artery was derived by the formula: inulin content per g of artery/inulin concentration per ml of incubation medium. The mean extracellular space values after incubation with inulin for 10, 20, 30 and 60 min were 0.23 ± 0.03, 0.3 ± 0.02, 0.37 ± 0.09 and 0.38 ± 0.02 ml/g respectively (n = 3 each). The values for 30 and 60 min are closely similar and, therefore, the 30 min value was used for correcting for NA present in the extracellular space. In washout experiments no correction was made for extracellular space.

The drugs used were: cocaine hydrochloride (May & Baker); (−)-noradrenaline (NA) (Rhone Poulenc); (±)-normetanephrine hydrochloride (Sterling-Winthrop). The concentrations of NA are in terms of base and those of other drugs are in terms of salt.

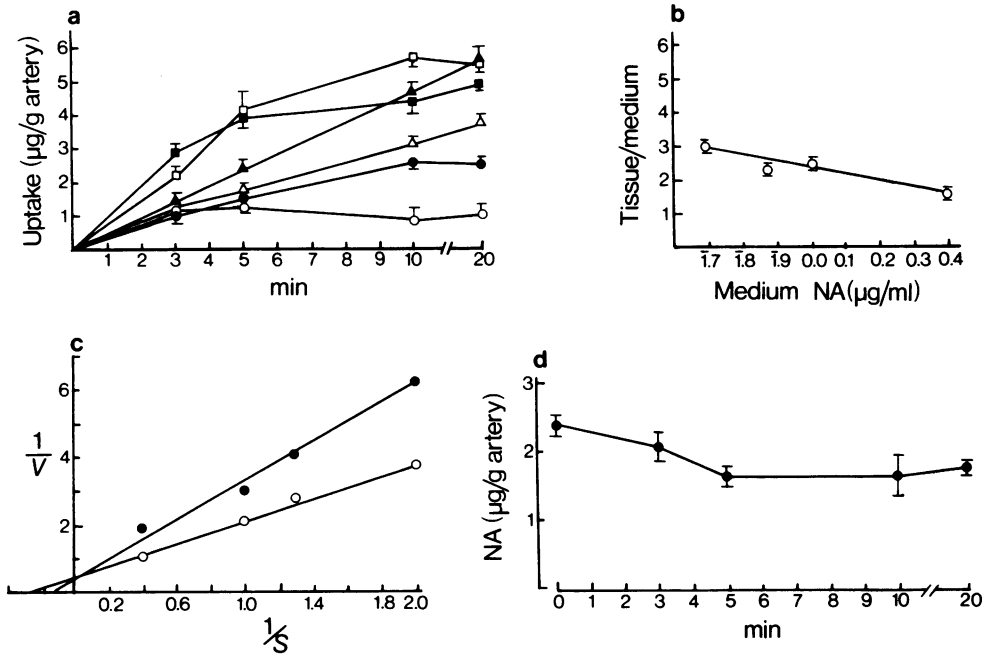

Figure 1 (a) The uptake of noradrenaline (NA) by the human umbilical artery incubated with 0.25 μg/ml (○), 0.5 μg/ml (●), 0.75 μg/ml (△), 1 μg/ml (▲), 2.5 μg/ml (□) and 5 μg/ml (■) of NA for different times. Each point is the mean of 3 values. (b) Five min data of (a) except for the lowest and the highest concentrations. The final medium concentration of NA is plotted against the ratio of tissue concentration vs. the final medium concentration of NA. (c) Kinetic analysis of NA uptake (data plotted according to regression analysis). S = concentration of NA in incubation medium (μg/ml). V = rate of uptake (μg g^{-1} min^{-1}). For control (○), the 5 min data as in (b) were used. (●) Represents the effect of normetanephrine (10 μg/ml; $n = 3$). (d) Effect of wash out with NA-free medium on the NA accumulated in the artery following incubation with NA (1 μg/ml) for 5 minutes. The time of incubation with NA-free medium is on the abscissa scale and the NA content of the artery is on the ordinate scale. Each point is the mean of three values. Vertical lines in (a), (b) and (d) indicate s.e. mean.

Results No endogenous NA could be detected in the human umbilical artery ($n = 10$) confirming the observation of Burnstock *et al.* (1972) that this tissue lacks adrenergic innervation.

The uptake of NA within the concentration range of 0.5-2.5 μg/ml of NA in the medium was linear with time up to 10 minutes. With lower and higher concentrations and beyond 10 min, the relationship was not linear (Figure 1a). The plot of tissue/medium concentration ratio vs. medium concentration indicates that the uptake was saturable (Figure 1b). The apparent K_m and V_{max} values obtained from a plot of $1/V$ against $1/S$ were 3.3 μg/ml (1.95×10^{-6} M) and 2.0 μg min^{-1} g^{-1} artery (0.0121 μmol min^{-1} g^{-1}) respectively (Figure 1c). Normetanephrine inhibited the uptake of NA and in its presence a linear relationship was observed between the plot of $1/V$ against $1/S$ (Figure 1c). The ordinate intercept for this line and the control line was common. The K_p value (i.e. the K_m of the substrate in the presence of the inhibitor) was 3.7×10^{-6} M. The K_i value of normetanephrine was 5.7×10^{-5} M.

The mean control uptake at the end of 5 min incubation with NA 1 μg/ml was 2.43 ± 0.15 μg/g ($n = 3$). The mean uptake in the presence of cocaine was 2.8 ± 0.16 μg/g ($n = 3$, $P > 0.5$). At 4°C, the mean uptake was 0.82 ± 0.02 μg/g ($n = 3$; $P < 0.01$). In Na^+-deficient medium, the mean uptake was 1.09 ± 0.33 μg/g ($n = 6$; $P < 0.01$).

Washing with NA-free solution was associated with reduction in the amount of NA accumulated, the maximum effect (31%; $P < 0.05$) being observed at 5 min (Figure 1d).

Discussion The uptake of exogenously added NA by the human umbilical artery was linear up to 10 min within the concentration range of

0.5-2.5 μg/ml, was saturable and was reduced by 66.3% of control in the cold, indicating that it was largely an active process. The uptake could be described by the Michaelis-Menten equation. The apparent K_m and the V_{max} values obtained by us are lower than the respective values (2.52×10^{-4} M and 0.102 μmol min^{-1} g^{-1}) obtained by Iversen (1965) for the isolated innervated rat heart; the lower values could be ascribed to the lack of adrenergic innervation and the very low activity of the enzymes MAO and COMT (Burnstock *et al.*, 1972) and are in accord with the observation that the uptake$_2$ of NA in innervated tissues occurs at low concentrations when the enzymes MAO and COMT are inhibited (Lightman & Iversen, 1969).

Cocaine, a potent inhibitor of uptake$_1$, was inactive while normetanephrine a potent inhibitor of uptake$_2$ (Iversen, 1967) inhibited the uptake competitively. The uptake was partially Na^+-dependent. Gillespie & Towart (1973) similarly demonstrated that the extraneuronal uptake of NA in the smooth muscle of the rabbit ear artery is partially Na^+-dependent. Finally 31% of NA accumulated in the umbilical artery could be washed out. The partial effect of washing out may be due to intracellular binding of NA (Burnstock, McLean & Wright, 1971).

In certain respects our results are at variance with those of Burnstock *et al.* (1971) e.g. they did not observe any inhibition of uptake of NA by the human umbilical artery with normetanephrine and in the cold. Gillespie, Hamilton & Hoise (1970) observed that the uptake of NA by the vascular smooth muscle of the spleen was abolished at 1°C and the NA accumulated in this tissue at 38° could be washed out. A possible reason for the discrepancy between the present results and those of Burnstock *et al.* (1971) may be that they used a much higher concentration of NA (100 μg/ml) and a longer incubation time (60 minutes).

note added in proof:

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