The substrate specificity of uptake2 in the rat heart*

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Summary. Experiments were carried out with hearts isolated from reserpine- and pargyline-pretreated rats; both noradrenaline-metabolizing enzymes and uptake1 were inhibited. Initial rates of extraneuronal uptake were measured after perfusion lasting for 2 min, either in the absence or in the presence of 100 μmol/l O-methyl-isoprenaline, a potent inhibitor of uptake2.

1. The ID50 (i.e., the concentration of unlabelled substance that halves the rate of uptake of a tracer concentration of 3H-(-)-isoprenaline) was determined for a variety of agents. Two types of stereoselective preference of (-)-isomers were observed: for isoprenaline and adrenaline (but not for noradrenaline) – and also for dobutamine.

2. The stereoselective preference for the (-)-isomers of isoprenaline and adrenaline is also evident from fluorimetric determination of initial rates of uptake of unlabelled isomers.

3. Experiments with various tritiated compounds indicate that uptake2 has a broad substrate spectrum: uptake2 is not restricted to 3H-catecholamines and 3H-phenethylamines, but extends to resorcinols (3H-orciprenaline), imidazoline derivatives (3H-clonidine), 3H-histamine and 3H-5-hydroxytryptamine (3H-5-HT).

4. Determinations of the Vmax of uptake2 revealed a correlation between the ID50 and the Vmax: the higher the ID50, the higher the Vmax.

5. These results indicate that uptake2 is a carrier-mediated process.

Key words: Extraneuronal uptake – Rat heart – Catecholamines – Stereoselectivity – Substrate specificity

Introduction

Trendelenburg et al. (1983) and Grohmann und Trendelenburg (1983) recently reported that 3H-catecholamines are subject to an isotope effect with regard to uptake2 (extraneuronal uptake). Since our earlier determinations of Km-values for uptake2 of various catecholamines in the rat heart (Bönisch et al. 1974; Bönisch 1978; Fiebig and Trendelenburg 1978) were obtained for labelled substrates, it was of interest to determine Km-values for unlabelled substrates. This can be done by determination of the initial rate of uptake of a tracer catecholamine, either in the absence or in the presence of various concentrations of the unlabelled catecholamine. That concentration of the unlabelled amine which halves the initial rate of uptake of the tracer amine (ID50) corresponds to the Km for the unlabelled catecholamine.

When Iversen (1965) compared (-) with (+)-noradrenaline and (-) with (+)-adrenaline, no evidence for stereoselectivity of uptake2 was obtained. However, Barone et al. (1983) recently reported stereoselectivity for the extraneuronal O-methylation of isoprenaline ((-)>(+)) in the rabbit aorta. Hence, it was of interest to determine, for the stereoisomers of noradrenaline, adrenaline and isoprenaline, whether uptake2 prefers the (-)-isomers to the (+)-isomers.

Furthermore, it was also of interest to extend the delineation of structure-action-relation for uptake2 of Burgen and Iversen (1965). For instance, Paiva et al. (1984) recently reported that 5-HT is a good substrate of the extraneuronal uptake and monoamine oxidase (MAO) of the dog’s saphenous vein.

Methods

Perfusion of isolated hearts. Male albino Wistar rats (160–250 g) were killed by cervical dislocation and bleeding. The hearts were perfused according to the Langendorff technique at a constant rate (of about 10 ml ⋅ g⁻¹ ⋅ min⁻¹) at 36°C. The perfusion pressure was monitored and was between 32 and 60 mm Hg. The perfusion fluid contained (in mmol/l): Na⁺ 149.2, K⁺ 2.7, Ca²⁺ 1.3, Mg²⁺ 1.05, Cl⁻ 144.3, H₂PO₄⁻ 0.4, HCO₃⁻ 11.9, glucose 5.0, L-(-)-ascorbic acid 0.3 and Na₂EDTA 0.04, and was bubbled with 95% O₂ and 5% CO₂. For further details see Bönisch and Trendelenburg (1974).

All hearts were initially perfused for 15 min with amine-free solution which contained 10 μmol/l U-0521 (to inhibit catechol-O-methyltransferase, COMT) and 30 μmol/l cocaine (to inhibit uptake1). The details of the subsequent perfusion with catecholamines (or other agents) for 2 min, are described under Results. At the end of the experiment, the heart was removed from the apparatus, blotted and weighed. The heart was then homoge-
nized in 10 ml ice-cold 0.4 mol/l perchloric acid and stored overnight.

Pretreatments. All rats were pretreated with reserpine, 20 h (5 mg/kg) and 2 h (2.5 mg/kg) prior to the experiment. 2 h prior to the experiment, 100 mg/kg pargyline was injected i.p. 20 min prior to the beginning of the isolation of the heart the rats received 5,000 IU/kg heparin i.p.

Measurement of initial rates of uptake. As both MAO and COMT were inhibited, only total activity was measured in the hearts perfused with \(^{3}H\)-compounds (scintillation counting). In some of the experiments (see Results) noradrenaline, adrenaline and isoprenaline were determined fluorimetrically as described by Laverty and Taylor (1968) for noradrenaline.

Determination of \(ID_{50}\)-values. Hearts were perfused for 2 min with 1–3 nmol/l \(^{3}H\)(±)-isoprenaline (in the presence of 10 nmol/l U-0521 and 30 nmol/l cocaine), either in the absence or in the presence of 3–4 different concentrations of unlabelled compounds that caused an inhibition of the uptake of the tracer amine by 5 to 95%. For each concentration of unlabelled compound 3–4 hearts were used. In separate experiments the initial rate of uptake of \(^{3}H\)(±)-isoprenaline was also determined in the presence of 100 nmol/l O-methyl-isoprenaline (OMI), i.e., when uptake1 is largely inhibited. After correction for the slightly incomplete inhibitory effect of OMI (see below), the OMI-resistant uptake of \(^{3}H\)(±)-isoprenaline amounted to 0.0725 ± 0.0019 ml.g⁻¹ (n = 5). To obtain “initial rates of uptake2”, this value was subtracted from the experimentally determined “total uptake”. The “percent inhibition” of the uptake of the tracer was transformed to probits (Do – c, where \(c\) is the concentration of the inhibitor, i.e., the \(ID_{50}\)-value) underestimates the true extent of “OMI-sensitive uptake”, this difference has to be multiplied by the factor 0.165 mCi/mmol) (Boehringer, Ingelheim, FRG). hystamine (spec.act. 5.8 Ci/mmol), \(^{3}H\)-1,2-serotonin creatinine sulphate (spec.act. 26.3 Ci/mmol), \(^{3}H\)-7-(±)-noradrenaline (spec.act. 14.8 Ci/mmol; 15% of the label in position 8), \(^{3}H\)-2,5,6-(±)-adrenaline (spec.act. 110.4 Ci/mmol), \(^{3}H\)-7-dopamine (spec.act. 30.4 Ci/mmol), \(^{14}C\)-L-tryamine (spec.act. 0.0588 Ci/mmol), \(^{3}H\)-L-2,6,7-corticosterone (spec.act. 105.0 Ci/mmol), \(^{14}C\)-D-sorbitol (0.346 Ci/mmol) (all from NEN, Dreieich, FRG); \(^{3}H\)-4-clonidine HCl (spec.act. 24 Ci/mmol; Amersham Buchler, Braunschweig, FRG); \(^{3}H\)-(±)-isoprenaline (spec.act. 0.504 mCi/mmol), \(^{3}H\)-(±)-fenoterol (spec.act. 0.165 mCi/mmol) (Boehringer, Ingelheim, FRG).

Correction for extracellular space and dead space. During the 2 min perfusion also 100 μmol/l \(^{14}C\)-sorbitol was present. As the dead space amounted to 0.37 ml, 0.06 rain were needed to clear the dead space. Hence, all rates were calculated as:

\[
v = \frac{\text{tissue content} - \text{\(^{14}C\)-sorbitol space} \cdot \text{perfusion concentration}}{\text{duration of total perfusion} - \text{time needed to clear dead space}}
\]

After 2 min of perfusion the \(^{14}C\)-sorbitol space amounted to 0.304 ± 0.002 ml/g (means ± SE; n = 201).

Correction for the incomplete inhibitory effect of a competitive inhibitor of uptake2. As the difference between the “initial rate of uptake in the absence of the inhibitor” and the “initial rate of uptake in the presence of 100 nmol/l OMI” underestimates the true extent of “OMI-sensitive uptake”, this difference has to be multiplied by the factor 0.165.

\[
F = \frac{S}{K_m + S} = \frac{K_m (1 + 1/K_i)}{K_m (1/K_i)}
\]

where \(S\) = concentration in the perfusion fluid, \(K_m = ID_{50}, I = 100 \mu\text{mol/l OMI, } K_i = 2.04 \mu\text{mol/l}\) (see Table 3). This value for “corrected uptake2” was then subtracted from the “initial rate of uptake determined in the absence of the inhibitor”, to yield the corrected value for OMI-resistant uptake.

Calculation of \(V_{max}\). After correction for the incomplete inhibition of uptake2 by the presence of 100 μmol/l OMI, \(V_{max}\) was calculated from the Michaelis-Menten equation:

\[
v_{max} = \frac{\text{corrected initial rate of uptake}_2 (K_m + S)}{S}
\]

where the \(ID_{50}\)-values of Table 1 or 3 were used as estimates of \(K_m\).