Accumulation of biogenic amines by rabbit erythrocytes

A.G.H. BLAKELEY & C.J.M. NICOL[1]

Department of Pharmacology, The University, Glasgow, G12 8QQ.

Adrenaline and noradrenaline (NA) enter cat and human erythrocytes during incubation at 37°C *in vitro* (Bain, Gaunt & Suffolk, 1937; Bain & Batty, 1956). Shanker, Nafpliotis & Johnson (1961) concluded that the entry of catecholamines and 5-hydroxytryptamine (5-HT) was by simple diffusion. Danon & Sapira (1972), disagreeing, proposed an active transport.

Blood was obtained from anaesthetized Dutch rabbits treated with heparin (1000 i.u./kg i.v.). An erythrocyte suspension in Krebs bicarbonate saline was prepared after removal of the white cells, platelets and plasma proteins by combined filtration through glass-wool and saline washes.

Samples of the erythrocyte suspension were incubated at 0°C or 37°C with [^{3}H]- or [^{14}C]-labelled NA, 5-HT or tyramine. The samples were then rapidly cooled in an ice-bath, spun (3100 *g* for 15 min) and the amount of radioactivity in the supernatant and packed erythrocyte layers determined by liquid scintillation counting. The activity in the red cells was calculated after correction for radioactivity trapped extracellularly.

Paper chromatography showed that only NA was broken down during incubation and that this was due to intracellular metabolism by catechol-O-methyltransferase. This breakdown was measured by column chromatography (Dubocovich & Langer, 1973).

At 37°C (−)-NA (1 μg/ml) entered the erythrocytes slowly and reached, after 4 h, a cell/medium concentration ratio of 2.13 ± 0.05 (s.e. of mean, $n = 12$). 5-HT entered faster, reaching a ratio of 2.75 ± 0.05 ($n = 19$) in 30 minutes. Tyramine achieved a ratio of 1.64 ± 0.03 ($n = 6$) within 5 minutes. Cooling to 0°C completely inhibited the entry of (−)-NA and 5-HT but had no effect on tyramine entry.

NA entry was stereoselective; the (−) isomer entering twice as fast as the (+) isomer.

(−)-NA entry obeyed Michaelis-Menten kinetics. The K_m and V_{max} were respectively 6.6×10^{-3}M and 3.5×10^{-7} mol min^{-1} ml RBC^{-1}. (+)-NA and 5-HT also entered by saturable processes. The K_m and V_{max} for (+)-NA were 11.3×10^{-3}M and 3.6×10^{-7} mol min^{-1} ml RBC^{-1}, respectively and for 5-HT were 5.6×10^{-3}M and 2.1×10^{-6} mol min^{-1} ml RBC^{-1}, respectively. Tyramine entry did not saturate.

5-HT and (−)-NA, when presented simultaneously to the erythrocytes, competed for entry. In the presence of 5-HT the K_m for (−)-NA entry was significantly ($P < 0.01$) increased to 10.5×10^{-3}M, while the V_{max}, 3.5×10^{-7} mol min^{-1} ml RBC^{-1} was unaffected.

The amines were apparently concentrated in the cells. This was a result of binding to haemoglobin and a Donnan equilibrium across the membrane.

NA and 5-HT were therefore crossing the erythrocyte membrane by a process of carrier-mediated diffusion. Tyramine entered these cells apparently by simple diffusion.

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[1] Present address: Dept. of Physiology, University of St. Andrews.

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