tic release accounted for 23.1% (±5.01%) of the total electrophoretic release of noradrenaline. The estimated 'real' transport number for noradrenaline was 0.220 (±0.025).

The relative contribution of electro-osmosis to total release seen in these experiments is considerably greater than the 11% estimated by Krnjević, Mitchell & Szerb (1963) for acetylcholine released from a 3.0 M solution. Apart from the different ionic species used, this could also reflect the weaker solutions used in our experiments. Electro-osmosis and iontophoresis may be thought of as the passage of current through two resistors in parallel; the use of weak solutions increases the resistance to iontophoretic current flow, thus causing more current to be carried by eclectro-osmosis.

This work was supported by the Wellcome Trust.

References

CURTIS, D.R. (1964). Microelectrophoresis. In: '*Physical Techniques in Biological Research*', Vol Va. ed. Nastuk, W.L. pp. 144–190. New York: Academic Press.

KRNJEVIĆ, K. MITCHELL, J.F. & SZERB, J.C. (1963). Determination of ionotophoretic release of acetylcholine from micropipettes. *J. Physiol. Lond.*, **165**, 421–436.

Homogeneity of β-adrenoceptors on rat erythrocytes

K. DICKINSON & S.R. NAHORSKI

Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, University Road, Leicester. LE1 7RH

Recent studies, using direct receptor labelling techniques, have provided evidence for the co-existence of β_1 and β_2 adrenoceptors in the same tissue, (Rugg, Barnett & Nahorski, 1978; Nahorski, 1978). We were particularly interested, therefore, to determine whether β-receptor sub-types could co-exist in the same population of cells. Rat erythrocytes represent a convenient source of single cell-types which contain β-receptors (Kaiser, Wiemer, Kremer, Dietz & Palm, 1978; Beckman & Hollenberg, 1979). The present study suggests that [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) binds to rat erythrocyte membranes in a manner indicating interaction with a physiological β-receptor, and that the receptors present are a homogenous population of β_2-adrenoceptors.

Erythrocyte membranes were prepared from male Wistar rats (150–200 g) essentially as described by Charness, Bylund, Beckman, Hollenberg & Snyder, 1976.

Specific [^{3}H]-DHA binding, that binding which was displaced by 200 μM (−)-isoprenaline (Nahorski, 1978), represented almost 100% of the total [^{3}H]-DHA bound to the membranes at 1 nM [^{3}H]-DHA.

Table 1 Affinities of adrenoceptor agonists and antagonists for β-adrenoceptors present on rat erythrocytes

	Ki (nM)	
Agonists		
(−)-Isoprenaline	33 (±3)	
(−)-Noradrenaline	3,000 (±370)	
(−)-Adrenaline	240 (±19)	
(±)-Salbutamol	360 (±26)	
Antagonists		*Hill coefficient*
(±)-Atenolol (β_1 selective)	2,500 (±220)	1.08 (±0.06)
(±)-Practolol (β_1 selective)	21,000 (±580)	1.03 (±0.04)
(−)-Alprenolol (Non-selective)	0.3*	0.98
(−)-Propranolol (Non-selective)	0.36 (±0.04)	1.11 (±0.03)
(+)-Propranolol (Non-selective)	25 (±3)	1.09 (±0.09)
(±)-OPC 2009 (β_2 selective)	23 (±2)	0.97 (±0.02)

Ki was determined from the equation $Ki = IC_{50}/1 + S/Kd$, where S is the concentration of [^{3}H]-DHA in the assay and Kd is the dissociation constant for [^{3}H]-DHA. The data are the mean (± s.e. mean) of 3–5 experiments conducted in duplicate.

* 2 determinations performed.

Specific binding was stereoselective, rapid, reversible and saturable. Scatchard analysis demonstrated a single, high affinity, binding site (Kd [^{3}H]-DHA = 0.2 ($\pm$0.02) nM, n = 9; B_{max} = 212 ($\pm$6) fmoles/mg protein, n = 9), while the Hill coefficient (n_H = 1.015 ($\pm$0.06), n = 7) revealed the absence of co-operative interactions.

The relative potencies of β-adrenoceptor agonists and selective adrenoceptor agents in displacing [^{3}H]-DHA from rat erythrocyte membranes is indicative of a β_2-adrenoceptor (Table 1). An indication of the homogeneity of these receptors is also suggested by an analysis of the displacement curves of these highly selective agents. In tissues which possess mixed β_1 and β_2 adrenoceptors, such agents produce non-law of mass action displacement curves with Hill coefficients <1. In rat erythrocytes, however, these agents produce displacement curves with Hill coefficients which are close to 1 (Table 1). Such results suggest that rat erythrocytes possess a homogenous β_2 adrenoceptor population.

References

BECKMAN, B.S. & HOLLENBERG, M.D. (1979). Beta-adrenergic receptors and adenylate cyclase activity in rat reticulocytes and mature erythrocytes. *Biochem. Pharmac.*, **28**, 239–248.

CHARNESS, M.E., BYLUND, D.B., BECKMAN, B.S., HOLLENBERG, M.D. & SNYDER, S.H. (1976). Independent variation of beta-adrenergic receptor binding and catecholamine stimulated adenylate cyclase activity in rat erythrocytes. *Life Sci.* **19**, 243–250.

KAISER, G., WIEMER, G., KREMER, G., DIETZ, J. & PALM, D. (1978). Identification and quantification of beta-adrenoceptor sites in red blood cells from rats. *Arch. Pharmac.*, **305**, 41–50.

NAHORSKI, S.R. (1978). Heterogeneity of cerebral beta-adrenoceptor binding sites in various vertebrate species. *Eur. J. Pharmac.*, **51**, 199–209.

RUGG, E.L., BARNETT, D.B. & NAHORSKI, S.R. (1978). Co-existence of beta$_1$ and beta$_2$ adrenoceptors in mammalian lung: Evidence from direct binding studies. *Mol. Pharmac.*, **14**, 996–1005.

A comparison of the effects of 2-2′-pyridylisatogen, 2-phenylisatogen and papaverine on calcium-stimulated respiration in mitochondria isolated from guinea-pig liver

G.S. LOVETT, A.J. SWEETMAN & D.F. WEETMAN

School of Health and Applied Sciences, Leeds Polytechnic, Leeds, LS1 3HE and School of Pharmacy, Sunderland Polytechnic, Sunderland, SR1 3SD

The ability of mitochondria to regulate the cytoplasmic level of Ca^{2+} by sequestering Ca^{2+} ions may be of importance in the control of the contractile process in smooth muscle. The demonstration that 2-2′-pyridylisatogen, 2-phenylisatogen and papaverine antagonised Ca^{2+}-induced contractions in isolated taenia of the guinea-pig caecum pointed to a possible involvement of Ca^{2+} in their mechanism of action (Spedding & Weetman, 1978). We have therefore continued our investigations on the mode of action of these smooth muscle relaxant drugs by studying their effects on calcium metabolism in isolated mitochondria.

The uptake of Ca^{2+} by tightly-coupled mitochondria, which is accompanied by a marked stimulation of respiration, can be supported by a variety of respir-

Table 1 Effect of relaxant drugs on Ca^{2+}-stimulated respiration in mitochondria in the presence of either phosphate or acetate

	$IC_{50} \pm$ s.e. mean (μM)	
	Phosphate-activated reaction	*Acetate-activated reaction*
2-2′-pyridylisatogen	12.2 ± 1.6	8.2 ± 0.4
2-phenylisatogen	10.2 ± 0.5	11.0 ± 0.8
Papaverine	5.5 ± 0.4	6.2 ± 0.7

Guinea-pig liver mitochondria (10 mg protein) were incubated at 30° in a medium containing 0.25 M sucrose, 10 mM tris-HCl buffer, pH 7.4, 3.3 mM sodium succinate (or 3.3 mM sodium glutamate plus 3.3 mM sodium malate) and 3.3 mM phosphate buffer, pH 7.4 (or 5 mM sodium acetate) for 2 minutes. The reaction was initiated by the addition of 1 µmol $CaCl_2$, to give a final volume of 3 ml; drugs were added 2 min before the $CaCl_2$. IC_{50} is the concentration of drug that produced a 50% reduction in the stimulation of respiration that followed the addition of $CaCl_2$ to the reaction chamber. The values were obtained using concentration-effect curves for each drug (n = 5). The IC_{50} values for each drug on the two reactions were not significantly different ($P > 0.05$).