CELL METABOLISM AFFECTS THE DENSITY OF β-ADRENERGIC RECEPTORS IN INTACT RAT RETICULOCYTES

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Received 3 January 1980

1. Introduction

Membrane receptors mediating the cellular effects of β-adrenergic compounds can be identified by studying stereoselective binding of radioactively labelled specific ligands. Such β-adrenoceptors have been identified in a large variety of different cells and tissues, among them nucleated avian red cells and mammalian reticulocytes [1–4]. Most authors have studied the properties of β-adrenoceptors in isolated membrane preparations rather than in intact cells in order to reduce possible interference from non-specific cellular uptake and binding processes. However, the few reports on β-adrenergic binding in intact cells [5–8] have tended to confirm the results obtained with membrane particles. We have used intact rat reticulocytes to study possible regulatory effects of cell metabolism on β-adrenoceptor binding. Stereospecific binding of the β-adrenergic antagonist (–) [^{3}H]dihydroalprenolol (DHAP) and its competitive displacement of unlabelled β-adrenergic agonists and antagonists was measured under conditions which cannot be mimicked in membrane fragments. We report that the number of β-adrenoceptors decreases considerably during metabolic inhibition. This finding challenges the assumption that regulatory changes in β-receptor densities of intact cells are faithfully reflected in the data derived from binding studies in fragmented membrane preparations.

2. Experimental

Reticulocytosis was induced by injecting rats with acetyl phenylhydrazide (30 mg/kg, i.m.) for 3 consecutive days [9]. The blood was obtained by decapitation on day 7 when the reticulocyte count was close to 50%. Leukocytes were removed by filtration through nylon wool (Leuko Pak, Fenwall Lab., USA). Reticulocytes and mature cells were completely separated by density gradient centrifugation in polyvinylpyrrolidone-coated silica gel (Percoll of Pharmacia AB, Sweden) adjusted to a tonicity of ~300 mOsm with KCl. Starved reticulocytes were prepared by incubating cells for 90 min in the presence of iodoacetamide and cyanide [10]. This procedure lowered the cellular ATP content measured by the firefly lantern assay [11] to ~1 μM. Binding equilibrium for DHAP was reached after 10 min at 37°C. No further change in specific binding occured during the following 60 min. All binding experiments were performed under equilibrium conditions. The density of β-adrenoceptors was determined from the difference between stereospecific and non-stereospecific (unspecific) binding of DHAP. Unspecific binding was defined as the amount of DHAP that could not be displaced by 0.2 or 1 μM unlabelled (–)timolol. Unlike (–)propranolol or (–)alprenolol [12] this β-adrenergic antagonist did not displace unspecific binding of DHAP in concentrations up to 3000-times its K_d value. Specific displacement of DHAP by (–)timolol was identical to the stereospecific effect of (–)propranolol, provided the specific displacement by propranolol was obtained from the differential effects of the (+) and (–) isomers.

3. Results and discussion

Table 1 gives values for total and for stereospecific binding of DHAP at ~80% saturation (6 nM) and lists also the maximal binding capacities extrapolated

Table 1
Total and specific binding of (−) [^{3}H]DHAP (fmol/μl cells) to intact rat reticulocytes (means ± SEM, no. independent experiments in brackets)

Cells	Total binding	Specific binding	Maximal specific binding
Native 37°C	29.7 ± 2.1 (11)	10.0 ± 0.8 (5)	12.6 ± 1.0 (6)
Starved 37°C	10.8 ± 0.6[a] (13)	6.7 ± 0.6[a] (6)	8.1 ± 0.6[a] (6)

[a] $p < 0.01$ compared to native cells by Student's t-test

Total binding of DHAP was determined in triplicate after a 40 min incubation in the presence of 6 nM DHAP in a medium containing (mM) 125 KCl, 20 Hepes buffer, 1 EGTA, 10 K_2HPO_4, 5.5 glucose, 1 ascorbic acid (pH 7.2) (native cells) or 140 KCl, 20 Hepes, 1 EGTA, 1 ascorbic acid (pH 7.2) (starved cells) Total incubation volume was 2.24 ml, haematocrit 8–15%. Unspecific binding was determined in triplicate in parallel incubations containing in addition 0 2 or 1 μM (−)timolol. Specific binding is the difference between total and unspecific binding. Maximal specific binding was obtained by extrapolating individual Scatchard plots for each binding curve to their intersection with the abscissa Cells were starved during a 90 min incubation period at 37°C in a solution containing (mM). 125. KCl; 10, NaCl; 20, Hepes, 5, inosin; 5, iodacetamide, 5, cyanide. Separation of cells and medium by filtration at 0°C across glass fibre filters (Whatman GF/F). The filters were incubated overnight in 0.6 ml 1:1 mixture of ethanol and 0.5 M quarternary ammonium hydroxide solution (Protosol of NEN Chemicals, Dreieich). ^{3}H activity was measured by liquid scintillation spectrophotometry after addition of 30 μl concentrated acetic acid and 10 ml scintillation fluid containing toluol and 4g/L BBOT scintillator (Ciba-Geigy, Basel) Specific activity in individual experiments varied from 25–38 cpm/fmol DHAP

graphically for 100% saturation of specific sites. Compared to control conditions, there was a significant decrease in β-receptor density, in starved reticulocytes. Unexpectedly, unspecific binding proved even more sensitive to the metabolic state of the cells than specific binding. The nature of unspecific binding sites is unknown. Intracellular uptake of the ligand does not explain the phenomenon. Total binding was the same whether or not the cells were haemolysed before analysis. The effects of metabolic inhibitors on apparent receptor numbers and on cellular ATP content were irreversible.

In fig.1 we have plotted binding curves and the corresponding Scatchard plots for specific DHAP binding under the same two conditions. The experimental points are fitted satisfactorily by straight lines (control $r^2 = 0.95$; starved $r^2 = 0.99$). The apparent dissociation constants (K_d) for DHAP calculated from the slopes of these plots were 0.8 and 1.06 μM. This finding suggests that a single class of antagonist binding sites existed under both conditions. The maximal binding capacity under control conditions corresponded to ~700 sites/cell. The no. sites was reduced to ~450 in starved cells.

In additional experiments (−)timolol and (−)isoprenaline were both used in native and in starved cells to displace DHAP (3 nM) from stereospecific binding sites. The concentration required to displace half the stereospecific DHAP binding (I_{50}) was used to calculate their K_d values according to the equation:

$$K_d = I_{50}/(1 + [\text{DHAP}]/K_{d,\text{DHAP}}) \qquad [13]$$

Under both conditions agonist and antagonist displaced identical amounts of DHAP. K_d for timolol remained unchanged (0.46 ± 0.1 nM in native cells versus 0.40 ± 0.06 nM in starved cells). However, the affinity for isoproterenol increased significantly in starved reticulocytes (K_d = 440 ± 68 nM in controls and 120 ± 24 nM in starved cells, $p < 0.01$). The K_d value for (+) isoprenaline (22.4 μM) was similar under both conditions.

The Hill coefficients of the DHAP displacement curves were similar in the presence of isoprenaline

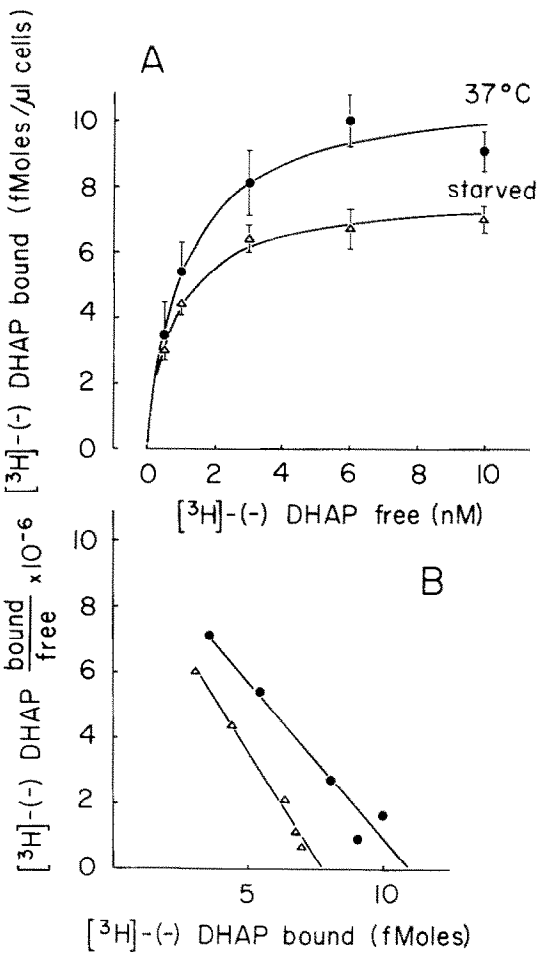

Fig.1. Binding of DHAP to native and to starved reticulocytes at 37°C. Method as in legend to table 1. (A) Binding curves. The points represent mean values of 4–6 independent expt ± SEM. (B) Scatchard plots of the data in (A). Least square regression lines were fitted to the experimental points. The curves in (A) are calculated according to the equation $r = n(x)K_{d,DHAP} + (x)$ (r = DHAP bound, n = no. binding sites, (x) = free concentration of DHAP). The parameters n and K_d were evaluated from the corresponding Scatchard plots. K_d (nmol) and n (fmol/µl cells) values for DHAP in non-starved cells, 1.06 and 10.9 at 37°C; in starved cells, 0.8 and 7.7.

and timolol. They varied between 0.8 and 1 and were independent from the metabolic state of the cell. This observation strongly suggests that agonist and antagonist both interacted with a single population of homogenous sites whether or not the hormone–receptor interaction was coupled to an activation of adenylate cyclase.

The decrease in receptor numbers during metabolic inhibition of intact cells indicates that metabolism is required to maintain the normal stationary state distribution of 'accessible' and 'cryptic' receptors. It is perhaps relevant in this context that we observed an even larger significant decrease in receptor numbers upon incubation of native cells at 1°C. A small decrease in specific binding during incubation at 4°C has been reported recently in intact cells of a cultured cell line [14]. However, we cannot decide whether incubation at low temperature and depletion of metabolic substrates lower receptor numbers by the same mechanism. It is interesting to note that stimulation of membrane phosphatidylcholine synthesis induced an increase in β-adrenergic binding sites in resealed rat reticulocyte ghosts but not in membrane fragments [15]. We conclude that cell metabolism is an important determinant of β-adrenoceptor properties. Metabolic regulation is not detectable in studies with isolated membranes.

Acknowledgements

We are grateful to Dr J. D. Fitzgerald of ICI, Macclesfield, who suggested the use of (−)timolol. (−)Timolol maleate was a gift of Merck, Sharp and Dohme, Rahway/NJ. The study was supported by the Swiss National Science Foundation.

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