Influence of Quinidine on the Binding of $[^3H]$-Ouabain and $[^3H]$-Digoxin by Human Lymphocytes

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Summary. To explore the molecular basis of the glycoside-quinidine interaction, the in vitro effect of quinidine on the binding of $[^3H]$-ouabain and $[^3H]$-digoxin to Na$^+$/K$^+$ATPase receptors on human mononuclear cells was investigated. The maximum $[^3H]$-ouabain binding capacity was $45.7 \pm 9.4 \times 10^3$ molecules/cell in pure lymphocyte preparations ($n=8$) and $75.5 \pm 7.3 \times 10^3$ molecules/cell in mixtures of mononuclear cells ($n=8$). These parameters were not influenced by $10^{-5}$M quinidine. In eight equilibrium experiments with pure lymphocytes, the dissociation constant of $[^3H]$-ouabain increased from $0.79 \pm 0.26 \times 10^{-8}$ M in the absence of $10^{-5}$ M quinidine to $1.56 \pm 0.74 \times 10^{-8}$ M in its presence ($p<0.01$), indicating that the affinity of the drug was decreased. Similar findings were observed using mixed mononuclear cells. In five uptake and release experiments, quinidine decreased the association rate constant of $[^3H]$-ouabain from $3.15 \pm 0.36 \times 10^4$ M$^{-1}$ s$^{-1}$ to $2.01 \pm 0.37 \times 10^4$ M$^{-1}$ s$^{-1}$ ($p<0.01$), whereas the dissociation rate constant was not affected. A therapeutic concentration of quinidine does not affect the number of glycoside receptors on lymphocytes, but it does appear to reduce fractional receptor occupancy by both $[^3H]$-ouabain and $[^3H]$-digoxin at lower tracer concentrations. This finding is compatible with the clinical observation that quinidine reduces the distribution volume of digoxin.

Key words: digoxin, quinidine; ouabain, cell binding constants, lymphocytes, mononuclear cells, drug interaction, glycoside receptor

Materials and Methods

Subjects

Twenty healthy hospital employees, aged 25–35 years, were studied. None was taking medicine and all had normal base-line biochemical tests, including plasma creatinine and electrolyte levels.
Materials

[3H]-ouabain and [3H]-digoxin (specific activity ~ 14–18 Ci/mmol), dissolved in ethanol-benzene (9:1 v/v), was purchased from New England Nuclear, West Germany. Dibutylphthalate (density ~ 1.044) and dionylphthalate (density ~ 0.98) were obtained from Merck, West Germany. Liquid scintillation fluid (Picoflour 30) came from the Packard Instrument Company, USA and Ficoll-Isopaque (density 1.077) from Pharmacia, Sweden. All chemicals were of analytical grade.

The cells were incubated in a potassium-free Krebs-Ringer bicarbonate buffer (KRBB) containing 5 mM glucose and 1% polyvinylpyrrolidine.

Cell Preparations

Fresh venous blood was defibrinated by slow agita-
tion with small glass beads for 10 min. After dilution with an equal volume of isotonic saline, the lymphocytes were isolated by centrifugation on a Ficoll-Isopaque gradient at 300 g for 20 min at room temperature. The cells were washed twice in potassium-free KRBB by alternate suspension and centrifugation at 500 g for 3 min, and incubated at a final concentration of 1.5–2.0 x 10^6 cells/ml.

This separation method was characterized by a comparatively low yield (35%, n=10) and a high purity (lymphocytes 94%, polymorph neutrophils 3%, and monocytes 2%; n=10). Cell viability was more than 97% as judged by trypan blue staining.

Mononuclear cells were also prepared with EDTA as the anticoagulant and using the same isolation procedure as described above. This method gave a higher yield (70%, n=10), but lower purity (lymphocytes 80%, monocytes 16% and polymorph neutrophils 4%).

Radioligand Binding

[3H]-Ouabain. Incubation was done at 37 °C in a shaking water bath, using potassium-free KRBB. Cells were preincubated for 30 min in the absence and presence of quinidine sulphate (10^-3–10^-5 M) before addition of the tracer. [3H]-ouabain was evaporated to dryness, redissolved in potassium-free buffer and added to incubation vials in final concentrations ranging from 10^-9 M to 3.5 x 10^-7 M. At each concentration level, parallel incubation vials containing tracer + 10^-3 M cold ouabain were run in order to measure the amount of [3H]-ouabain taken up by the cells, which was not displaced in the presence of excess unlabelled drug (non-specific binding). At specified times, duplicate 300 μl samples of the incubation medium were transferred to polystyrene microcentrifuge tubes containing dionylphthalate/dibutylphthalate 100 μl (3:1 v/v). Without delay these tubes were centrifugated at 10000 g for 1 min, which caused the cells to descend through the oil phase and to appear as a thin layer at the bottom of the tube. Repeated examinations revealed that the oil phase contained neither cells nor radioactivity in excess of the background, indicating complete partition of cells and incubation fluid. The bottom of the tubes containing the cell pellet and a small portion of the oil phase was then cut off and transferred to a scintillation vial, where the cells were lysed over-night in distilled water 1 ml. Finally, scintillation fluid 5 ml (Picoflour 30) was added, and the tritium activity measured in an Isocap 300 scintillation counter (Nuclear Chicago), with correction for quench (external channel ratio) and background activity. The counting error was less than 5%. In a few experiments, the cells were further digested by heating in 0.7 NaOH (70 °C, 1 h). This procedure had no effect on the radioactivity of the samples, indicating that cell lysis alone was sufficient to ensure complete release of cell-bound tracer.

In equilibrium experiments, the cells were incubated for 60–180 min, and the radioligand binding in each vial was repeatedly measured. Values were only accepted for Scatchard analysis, if the measurements clearly indicated the presence of a steady state. To ensure similar binding conditions, identical cell concentrations were used in paired vials with and without quinidine. The cells were gently resuspended every 30 min during incubation and immediately before sampling. In 10 experiments, the numbers of cells in samples obtained every 30 min during the 3 h incubation varied by less than 6%.

[3H]-Digoxin. Incubation was performed as described for [3H]-ouabain. The undisplaceable component of radioligand binding was measured by addition of 10^-3 M cold ouabain. Before addition of [3H]-digoxin, the cells were preincubated for 1 h without and with quinidine sulphate (5 x 10^-5 M – 1.6 x 10^-6 M), 0.7–1.0 ml of incubation medium was sampled and washed twice with 10 ml ice-cold buffer. Sampling of cells was also done by centrifugation through oil, as described for [3H]-ouabain. The isolated cells were lysed in distilled water overnight and further disintegrated by heating (70 °C) for 1 h in 0.7 N NaOH. After acidification with HCl, the cells were transferred to scintillation vials and counted.

Data Analysis

The number of glycoside molecules bound per cell was calculated from the number of cells in the sam-