INSULIN-SECRETING AND PROLIFERATIVE ACTIVITY OF TRANSPLANTABLE ISLET CELLS IN THE PRESENCE OF SULFONYLUREA


KEY WORDS: pancreatic cell culture; proliferation; insulin; secretion; sulfonylurea; regulation.

Hypoglycemia-inducing sulfonylureas (SU) are widely used for the treatment of patients with insulin-independent diabetes (IID). The SU bind with specific receptors on the surface of the β-cells and stimulate basal secretion of insulin and increase the sensitivity of the β-cells to secretogenic substances [1]. Receptors of SU are functionally linked with various regulatory systems of the β-cell, including with ATP-dependent potassium channels [2], voltage-dependent calcium channels [4], and the phosphoinositide system [10]. It can be tentatively suggested that SU affect not only the synthesis and secretion of insulin, but also other functions of the β-cell, and in particular, proliferation and differentiation of β-cells. In some cases the short-term (for 2-3 weeks) use of SU to treat patients with IID causes a prolonged (lasting several months) rise of the level of insulin secretion [5]. These findings are indirect evidence that SU increase the pool of functionally active β-cells in the islets of patients with IID. Experiments in vitro have shown that the 1st-generation SU tolbutamide stimulates incorporation of 3H-thymidine (3H-T) into the β-cells of rat islets [3].

The aim of this investigation was to study the effect of tolbutamide and the 2nd generation SU — gliclazide and glibenclamide — on the secretory and proliferatory activity of transplanted RIN and HIT islet cells [6, 9].

EXPERIMENTAL METHOD

Gliclazide was obtained from Servier (France), glibenclamide from Boehringer—Mannheim (Germany) and tolbutamide from Sigma (USA), mother solutions of SU (containing 1 mM gliclazide, 1 mM glibenclamide, or 1 mM tolbutamide) were made up in dimethyl sulfoxide (DMSO, from Sigma). Cells of rat insulinoma RINr and transformed hamster β-cells HIT T-15 were generously provided by Dr. Yoheved Berwald—Netter (Collège de France, Paris). The cells were grown in plastic dishes (Nunc, Denmark) in medium RPMI-1640 (Flow Laboratory, England) with 10% bovine fetal serum (BFS, from the N. F. Gamaleya Institute of Epidemiology and Microbiology), 2 mM glutamine, and 10 mM HEPES in a CO₂ incubator (5% CO₂ + 95% air) at 37°C. To study the effects of SU, the cells were seeded in 96-well Nunc planchets (20,000 cells per well) and cultured in medium with 10% BFS until a confluent layer of cells had formed. The medium with 10% BFS was then replaced by medium with 0.5% BFS. After 3 days the medium with 0.5% BFS was removed and the cultures were washed twice with Hanks’ solution containing 0.5% BSA, and medium with 5% BFS was introduced into the wells (to stimulate cell proliferation). Different doses of SU or 1% DMSO were added to the medium. Medium without SU and DMSO was added to the control wells. For each variant of the experiment eight wells were used. The planchets were incubated for 15 h at 37°C in an
Fig. 1. Insulin accumulation in RINr (a) and HIT T-15 (b) cell cultures during 15 h in presence of different sulfonylureas. Ordinate, insulin content in medium (PG/10^4 cells/100 μl). Results shown in the form $\bar{X} \pm S_X$ (n = 8). 1, 2, 3) Medium with tolbutamide (10, 100, and 1000 μM respectively); 4, 5, 6) medium with gliclazide (0.1, 1, and 10 μM); 7, 8, 9, 10) medium with glibenclamide (0.01, 0.1, 1, and 10 μM); 11) medium with 1% DMSO; 12) control medium. Significance of differences between control and experimental values indicated by asterisks: *p ≤ 0.05, **p ≤ 0.01.

Fig. 2. Changes in incorporation of 3H-T into DNA of RINr (a) and HIT T-15 (b) cells during 4 h in presence of different sulfonylureas. Ordinate, changes in incorporation of 3H-T (in % of control). Remainder of legend as in Fig. 1.

atmosphere with 5% CO₂, after which medium was taken from each well in order to determine the insulin concentration, and fresh medium with 5% BFS, 40 kBq/ml of 3H-T, and the corresponding supplements of SU or DMSO were introduced into the wells. The planchets were incubated for 4 h at 37°C, the medium was removed, and the cultures were washed 3 times with Hanks' solution with BSA and fixed with ethanol. The density of the cultures (the number of cells in the wells) was determined colorimetrically [7], using Unna's stain. After measurement of the density of the cultures the cells were lysed with alkali and the level of 3H-T incorporation into DNA was determined by a scintillation method. For radioimmunoassay of the insulin in the samples of medium, "RIA-INS-125I-PG" kits (Minsk) were used. The samples were diluted with bovine serum (1:3). The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

All the SU used affected insulin accumulation in cultures of RIN (Fig. 1a) and HIT (Fig. 1b) cells. Tolbutamide and gliclazide stimulated insulin secretion in RIN cell cultures; the secretory effect of these SU, moreover, was independent of their concentrations. During long-term continuous treatment of the RIN cells with tolbutamide or gliclazide, the insulin concentration in the medium evidently reached its limiting level (independent of the dose of SU) quickly, and thereafter it did not rise further as a result of blocking of insulin production on the negative feedback principle. Glibenclamide potentiated insulin secretion in RIN cell cultures only in doses of 1 and 10 μM.