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

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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 ^{3}H-thymidine (^{3}H-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 CO_2 incubator (5% CO_2 + 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

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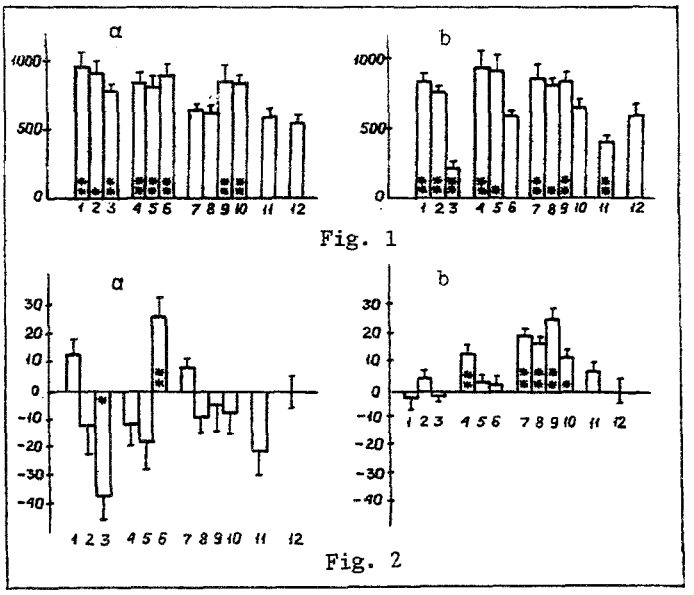

Fig. 1. Insulin accumulation in RINr (a) and HIT T-15 (b) cell cultures during 15 h in presence of different sulfonylureas. Ordinate, insulin content in medium (PG/10^4 cells/100 μl). Results shown in the form $\bar{X} \pm S_{\bar{x}}$ (n = 8). 1, 2, 3) Medium with tolbutamide (10, 100, and 1000 μM respectively); 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 $\leq$ 0.05, **p $\leq$ 0.01.

Fig. 2. Changes in incorporation of ^{3}H-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 ^{3}H-T (in % of control). Remainder of legend as in Fig. 1.

atmosphere with 5% CO_2, 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 ^{3}H-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 ^{3}H-T incorporation into DNA was determined by a scintillation method. For radioimmunoassay of the insulin in the samples of medium, "RIA-INS-^{125}I-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.

DMSD in a concentration of 1% did not affect insulin secretion in RIN cell cultures but significantly depressed the secretory activity of HIT cells. Tolbutamide, in doses of 10 and 100 μM, stimulated, whereas in a dose of 1000 μM it strongly inhibited insulin secretion in HIT cell cultures. The inhibitory effect of tolbutamide could be connected with the toxic action of a high dose of tolbutamide on HIT cells. Gliclazide in doses of 0.1 and 1 μM and glibenclamide in doses of 0.01-1 μM, stimulated insulin secretion in HIT cell cultures These same SU in a concentration of 10 μM did not change the secretory activity of the HIT cells, possibly due to desensitization of the SM receptors on HIT cells under the influence of maximal doses of gliclazide and glibenclamide and (or) the presence of 1% DMSO in the incubation medium. Just as in RIN cell cultures, so also in HIT cell cultures the secretogenic efficacy of glibenclamide was the same as that of gliclazide and tolbutamide. However, numerous investigations in vivo and in vitro have shown that glibenclamide is the most powerful stimulator of insulin secretion among all known SU and it potentiates insulin production in islet cell cultures in concentrations of 5-50 nM [2]. Glibenclamide is known to bind with albumin and other serum proteins [8]. In our experiments the incubation medium contained serum, and this could lead to a marked fall in the acting glibenclamide concentration.

The results of investigation of the action of SU on proliferation of RIN and HIT cells are given in Fig. 2. Incorporation of ^{3}H-T into RIN cells was intensified only by the maximal dose of gliclazide (10 μM). Smaller doses of gliclazide, and all doses of glibenclamide and tolbutamide used in concentrations of 10 and 100 μM did not affect proliferation of RIN cells. In the presence of 1000 μM tolbutamide, incorporation of ^{3}H-T into RIN cells was significantly inhibited. In HIT cell cultures, gliclazide, in the minimal concentration (1 μM), and glibenclamide in all concentrations used potentiated incorporation of ^{3}H-T, whereas in the remaining cases incorporation of ^{3}H-T into HIT cells was unchanged. DMSO (1%) did not affect proliferation of RIN and HIT cells.

Thus SU of the 1st and 2nd generations affect not only insulin-secreting activity, but also reproduction of RINr and HIT T-15 cells. Effects of SU on insulin secretion and on cell proliferation may be in opposite directions (depending on the doses and properties of the SU and on the particular features of the target cells). On the basis of these results it is impossible to formulate a hypothesis on the mechanisms of action of SU on islet-cell proliferation, but they do enable goal-directed investigations of these mechanisms to be planned. To begin with, it has to be noted that cultures of RIN and HIT cells are heterogeneous cell populations, containing different types of mature endocrine cells, and also precursor cells of endocrine cells [6, 9]. Evaluation of the proliferative activity of RIN and HIT cells on the basis of total incorporation of ^{3}H-T does not allow conclusions to be drawn on which types of cells are targets for SU. To shed light on this problem, a combination of autoradiographic and immunocytochemical methods is required. Effects of SU on proliferation of normal (untransformed) islet cells in vitro and in vivo must be studied. It is also essential to discover what causes changes in the proliferative status of the islet cells in the presence of SU – the primary effects of the SU themselves or secondary changes in the system of regulation of islet-cell reproduction.

The results of detailed investigations of the mechanisms of action of SU on islet-cell proliferation will have a definite role in planning of the tactics of hypoglycemia-inducing treatment of patients with IID and, in particular, for the correct choice of SU preparations, their doses, and their periods of administration.

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