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Gliclazide: Pharmacokinetic–Pharmacodynamic Relationships in Rats

V. Štětinová*, J. Květina, J. Pastera, A. Polášková and M. Pražáková
Institute of Experimental Biopharmaceutics, Joint Research Centre of the Czech Academy of Sciences and PRO.MED.CS Praha a.s., Hradec Králové, Czech Republic

ABSTRACT: The relationship between the pharmacokinetics of gliclazide and its antidiabetic efficacy were evaluated on the basis of experimental determination of changes with time in the plasma levels of this antidiabetic agent and those of glucose. The experiment included rats with both initial normal glycaemia and alloxan-induced hyperglycaemia (glycaemia increased by a minimum of 30%). Pharmacokinetic and pharmacodynamic parameters were examined in the interval of 30 to 180 min after p.o. administration of a single dose of 25 mg/kg of gliclazide. The drug was administered on day 4, following a single i.v. dose of either 50 mg/kg of alloxan (hyperglycaemic group) or the injection vehicle (control group). Even though the biological availability of gliclazide was similar in both normoglycaemic and hyperglycaemic animals, the gliclazide-induced hypoglycaemizing response was not uniform: until 60 min, the decrease of glycaemia was smaller in animals with alloxan hyperglycaemia (23% decrease at 60 min) in contrast to the normoglycaemic animals (36% decrease at 60 min), at later times, the intensity of this hypoglycaemizing effect of gliclazide persisted in the hyperglycaemic animals, while in the normoglycaemic ones, a reversal of the hypoglycaemizing effect occurred.

Key words: gliclazide; alloxan-induced diabetes mellitus; pharmacokinetics; rat

Introduction

With orally administered antidiabetic agents, the time relationships between their pharmacokinetics (t_{max}, C_{max}, bioelimination phase) and their hypoglycaemizing effects remain an as yet unresolved issue. Intervention in the homeostasis of glycaemia is determined by both glucose consumption and supply. Primarily, this depends on the coordination of the intensity of insulin secretion from the ß-cells of the islets of Langerhans in the pancreas, and that of glucagon, excreted by the α-cells. Glucagon, released in hypoglycaemia, increases liver glycogenolysis, thus increasing the level of blood sugar; on the other hand, insulin increases the utilization of glucose. Stimulation of glucose transport into tissue cells is a crucial component of the physiological response to insulin. The actions of insulin are initiated by binding to a cell-surface receptor (transmembrane glycoprotein composed of two α and two β subunits) [1,2].

Gliclazide, a second-generation sulfonylurea oral hypoglycaemic agent [3], has been shown to act directly on the pancreas, and to increase insulin secretion [4]. This effect appears to be initiated by the drug interaction with the cell-surface receptors on the pancreatic β-cells [5], resulting in reduced conductance of an

*Correspondence to: Institute of Experimental Biopharmaceutics, Joint Research Centre of the Academy of Sciences of the Czech Republic and PRO.MED.CS Praha a.s., Heyrovského 1207, Hradec Králové 500 03, Czech Republic. E-mail: stetinova@uebf.cas.cz

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ATP-sensitive K^+ channel. It seems probable that the receptor for the sulfonylureas is the ATP-sensitive K^+ channel itself [6,7]. Gliclazide is therefore prescribed in cases of diabetes with partially preserved insulin secretion. Recently, a number of *in vivo* and *in vitro* studies have shown that gliclazide functions effectively as an antioxidant as well [8–12].

The goal of this study was to evaluate the relationships between gliclazide plasma levels and gliclazide effect on the blood sugar levels in both normoglycaemic animals and those with induced hyperglycaemia. Normoglycaemic rats were used with the aim of analysing a potential difference in gliclazide effect in normoglycaemic and hyperglycaemic animals [13,14]. Hyperglycaemia was induced by the diabetogen alloxan, which is capable of destroying the pancreatic β-cells [15,16]. In the intact animal, alloxan is reduced to dialuric acid, and its autooxidation results in the formation of highly reactive oxygen radicals, which are responsible for the destruction of the β-cells [15,17]. Szkudelski [18] has shown that alloxan is not selectively toxic for the pancreatic β-cells, i.e. that its toxic effect is also the result of its interaction with other tissues. Most probably, the effect of alloxan on glycaemia can be divided into two stages. The first one, occurring immediately after i.v. alloxan administration to rats (half-life being about 1 min), gives rise to a sudden transient (short-lasting) insulin release as early as in min 2. The second stage, studied in this work, is characterized by the above described decrease of the plasma insulin level [19,20] as a consequence of the expected partial destruction of the pancreatic β-cells. In this period, the hypoglycaemic effect of gliclazide can be exerted in the remaining, functionally preserved cells, still capable of insulin production.

Thus, it was necessary to find a dose of alloxan, after the administration of which insulin production in the ß-cells in the experimental animals would be partially preserved, and would allow the decrease of the alloxan-induced hyperglycaemia by the administered gliclazide. In searching for the optimum dose of alloxan, an experiment on mice was tentatively started, in which alloxan in a dose of 120 mg/kg i.v. produced maximal hyperglycaemia after 3 days [21].

Material and Methods

Animals

Male Wistar Han II rats (BioTest, Konárovice breed, Czech Rep.) weighing 285 ± 27 g were housed under standard conditions (a temperature of $22 \pm 2^{\circ}$C, relative humidity 30–70%, 12-h light/dark cycle). The standard pellet diet (Biopo, Pozořice, Czech Rep.) and tap water were accessible *ad libitum*.

Chemicals

Gliclazide subst. (PRO. MEDIC. LIE, Liechtenstein) was dissolved in the vehicle (4.6% glycerine, 87.6% polyethylene glycol 400, 7.8% distilled water), alloxan tetrahydrate (Sigma-Aldrich s.r.o. Praha, Czech Republic) was dissolved in isotonic saline. Other reagents were purchased from Sigma-Aldrich s.r.o. (Praha, Czech Republic).

Induction of hyperglycaemia in rats

The estimation of the plasma glucose level on days 3 and 4 after alloxan administration served as the criterion of induction of the required level of hyperglycaemia. On the basis of preliminary experiments, a 30% increase in glycaemia compared with the values obtained prior to alloxan administration was selected as the minimum limit. In the above-mentioned pilot experiments, this criterion was met by about 30% of animals after a single i.v. (the lateral caudal vein) administration of 50 mg/kg of alloxan. Smaller doses of alloxan (20 mg/kg, or 30 mg/kg) did not increase glycaemia, and, after a larger dose (60 mg/kg), the induced hyperglycaemia was not susceptible to gliclazide. The validity of the use of this empirical criterion of selection was supported by tentative estimations of plasma insulin concentrations on day 4 after alloxan administration, which showed that even though a certain production of insulin was preserved, plasma concentrations were lower by an order of magnitude in hyperglycaemic rats (after 50 mg/kg of alloxan) in comparison with the normoglycaemic rats. In order to eliminate interindividual sensitivities against alloxan, the following selection was made when including experimental animals into the study:

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On day 3 after alloxan administration, the level of glycaemia was determined in all animals. The animals with a lower than a 30% increase of the glycaemia level were excluded from further experiments. In animals with at least 30% increase of glycaemia, the blood plasma sugar levels were determined again on day 4 after alloxan administration, and if hyperglycaemia persisted, they were included in the experiment.

Schedule of the treatment of animals (6–10 rats per group)

Group 1 (hyperglycaemic animals): day 0, alloxan; day 4, gliclazide.

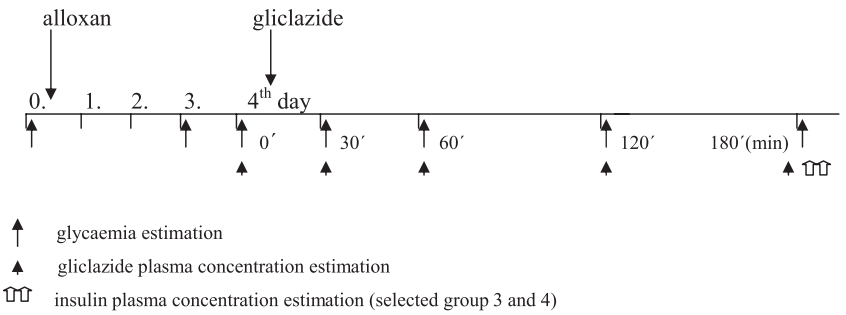
Group 2 (normoglycaemic animals): day 0, vehicle; day 4, gliclazide.

Group 3 (hyperglycemic control animals): day 0, alloxan; day 4, vehicle.

Group 4 (normoglycaemic control animals): day 0, vehicle; day 4, vehicle.

Time schedule of the experiment

The schedule is shown below.



Gliclazide was administered p.o. via a gastric tube at a dose of 25 mg/kg immediately after blood sampling for the determination of the glucose plasma concentration on day 4 (0 min).

Glucose concentration measurement

Blood for the glucose plasma concentration determination was sampled at 9 a.m. on days 0, 3 and 4. Glucose levels were determined from a blood drop using a glucometer-strip system (Senzori Test, Lachema, Brno, Czech Rep.).

Blood samples for the estimation of glycaemia and for the estimation of gliclazide were withdrawn on day 4 from the jugular vein under pentobarbital narcosis (60 mg/kg i.p.). The volume of the sample withdrawn (0.3 ml) was always replaced with the same volume of saline.

Estimation of plasma insulin levels

Plasma insulin levels were determined by radioimmunoassay (Set Insulin-CT [Cis Bio International]) in the last interval under study (180 min) in hyperglycaemic and normoglycaemic rats (groups 3 and 4) on day 4.

The data used in this work were obtained by simultaneous sampling and measurement of the drug plasma concentration and its effect within each individual, repeated at reasonable intervals, identically for all animals.

HPLC determination of gliclazide

The content of gliclazide in 100 µl plasma samples was determined by HPLC using the modified method of Zhang [22]. The HPLC system (TSP, USA) was equipped with a P1500 binary HPLC pump, a UV 3000 detector, and an autosampler AS 3000 with a 100 µl loop. A ChromQuest software communication network (TSP, USA) was used for data acquisition and reprocessing. LiChrospher 100 C8 (5 µm) 250 × 4 mm with a precolumn LiChroCart 4-4 packed with a LiChrospher C8 (5 µm) sorbent (both Merck, Germany) were used as analytical columns. The estimation was performed at ambient temperature using isocratic elution and UV detection at $\lambda = 229$ nm. The mobile phase, consisting of methanol/0.2%

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acetic acid (55:45) was pumped at a flow rate of 1 ml/min. Calibrations were carried out by the internal standard method. The quantification range was between 0.0037–0.074 μmol/ml. The accuracy and precision (RSD) of the method was below 15% over the whole concentration range.

Parameters under study

Direct. These included:

- Pharmacokinetics: the time course of the plasma gliclazide concentration in alloxan-treated and alloxan-untreated animals.
- Pharmacodynamics: the time course of the plasma glucose concentration (the time course and intensity of the gliclazide effect) in alloxan-treated and in alloxan-untreated animals.

Indirect (calculated). These included:

- The time dependent concentration–effect relationship for gliclazide (the data points are connected in chronological order).

The mathematical expression of the gliclazide effect employed the principal linear pharmacokinetic–pharmacodynamic model [23] expressed as $E = S \cdot C + E_0$, in which S represents (in our case S_{AUC}): the extent of the effect (mmol of glucose), induced by 1 unit of AUC of gliclazide (1 mmol.min/l) i.e. the area under the curve of gliclazide concentration/ in the time intervals 0 to x.

C = the relevant value of AUC

E_0 = values of the glucose level before gliclazide administration.

Statistical analysis

The results are represented as mean ± SD. The data obtained were analysed using the Jarque-Berra normality test for combined sample skewness and kurtosis [24]. Parametric data were analysed using an appropriate Student's *t*-test, nonparametric data were analysed with the Wilcoxon test. The results were considered significant if $p < 0.05$.

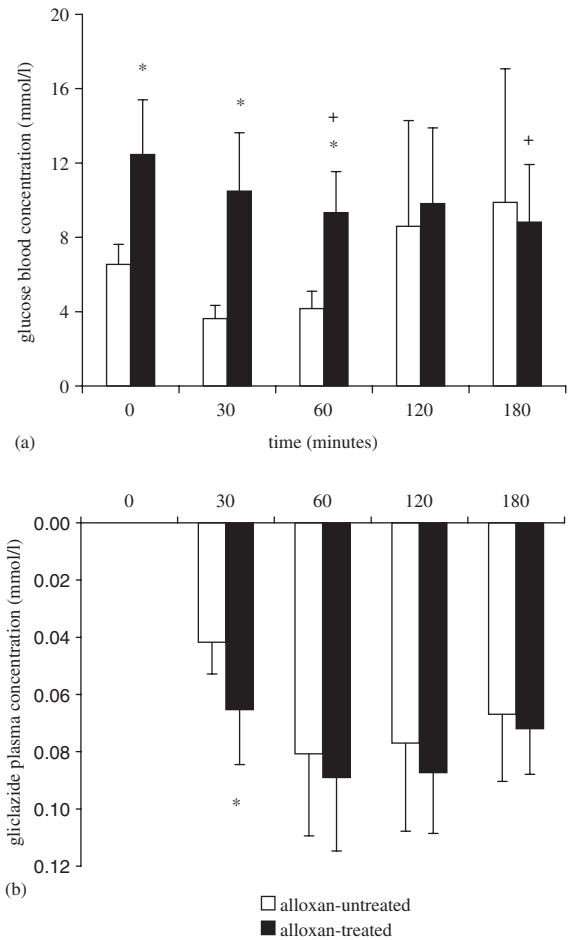

Figure 1. Correlation of the plasma gliclazide concentration (gliclazide 25 mg/kg p.o.) (b) with the blood glucose level (a) in the rats after alloxan administration (50 mg/kg i.v.) and those untreated with alloxan. Values represent the mean ± standard deviation ($n = 6-10$). Significant differences versus alloxan-untreated group are labeled with*, versus the corresponding initial value (in case of blood glucose concentration) are labeled with +, $p < 0.05$

Results

Estimation of plasma insulin levels: the mean (± SD) insulin concentration on day 4 (in min 180 after vehicle administration) in the hyperglycaemic animals was 19.4 ± 12 μIU/ml (lower by an order of magnitude in comparison with the normoglycaemic rats, in which the insulin level reached 188.2 ± 97 μIU/ml).

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Table 1. Effect of gliclazide (25 mg/kg) on the plasma glucose level in rats after alloxan administration and in rats untreated with alloxan

Group	0 min[a]	30 min	60 min	120 min	180 min
Alloxan untreated					
Control	5.2 ± 0.60 (100%)	5.4 ± 1.8 (107.0 ± 45.3%)	6.1 ± 2.0 (116.0 ± 35.4%)	5.3 ± 0.9 (106.3 ± 27.3%)	5.8 ± 1.70 (116.1 ± 39.2%)
Gliclazide	6.5 ± 1.1 (100%)	3.6 ± 0.7* (57.1 ± 16.7%)*	4.2 ± 0.9* (64.1 ± 14.1%)*	8.6 ± 5.7 (127.3 ± 73.8%)	9.9 ± 7.2 (157.8 ± 143.2%)
Alloxan treated					
Control	15.4 ± 3.9 (100%)	14.5 ± 4.7 (93.9 ± 14.1%)	14.9 ± 5.6 (94.6 ± 18.4%)	16.0 ± 6.2 (101.7 ± 23.91%)	15.1 ± 6.8 (96.0 ± 29.5%)
Gliclazide	12.5 ± 2.9 (100%)	10.5 ± 3.1 (85.5 ± 23.1%)	9.3 ± 2.2 (77.3 ± 18.1%)	9.8 ± 4.1 (79.9 ± 26.8%)	8.8 ± 3.1 (75.6 ± 18.5%)

[a]Initial values of glucose blood concentration (mmol/l) estimated on day 4 before gliclazide administration correspond to 100%. Values represent the mean ± standard deviation ($n = 6-10$).
*Statistical significance $p < 0.05$ (compared with the control group).

Gliclazide pharmacokinetics (Figure 1(b)): apart from the 30 min value where glicazide concentration was significantly lower in normoglycaemic rats, the plasma concentrations of gliclazide in both alloxan-treated and untreated animals were not affected by pretreatment with alloxan.

Gliclazide pharmacodynamics (Figure 1(a), Table 1): in alloxan-untreated animals (i.e. the normoglycaemic rats), gliclazide produced the most pronounced decrease in the glucose level as early as in min 30, with the drug level not having reached C_{max} at that time. In min 60, the decreased glucose level was maintained at an approximately identical value. In the following intervals after gliclazide administration (in min 120 and 180), the level of glycaemia increased, on average, above the mean of those determined prior to gliclazide administration, but a significant variability of glycaemic values was observed (SD increased to 73%).

In animals with alloxan hyperglycaemia, the decrease in glycaemia was, on average, smaller in comparison with the normoglycaemic individuals (e.g. by 23% in contrast to 36% in normoglycaemic animals in min 60), with the hypoglycaemizing effect being maintained at 120 and 180 min (in contrast to the normoglycaemic group, in which the above-mentioned variability of glycaemia and the indicated reversal of the hypoglycaemizing was observed at these times).

The above-mentioned relationships were also expressed in the time dependence between the concentration of gliclazide and its effect at chronologically arranged time intervals (Figure 2). In the alloxan-treated rats, a direct relationship between the plasma concentration of gliclazide and the hypoglycaemizing effect seems to exist. In the alloxan-untreated animals, this dependence is apparent only until min 60 following the administration of gliclazide. At later times, despite the persisting gliclazide plasma concentration, an increase in glycaemia

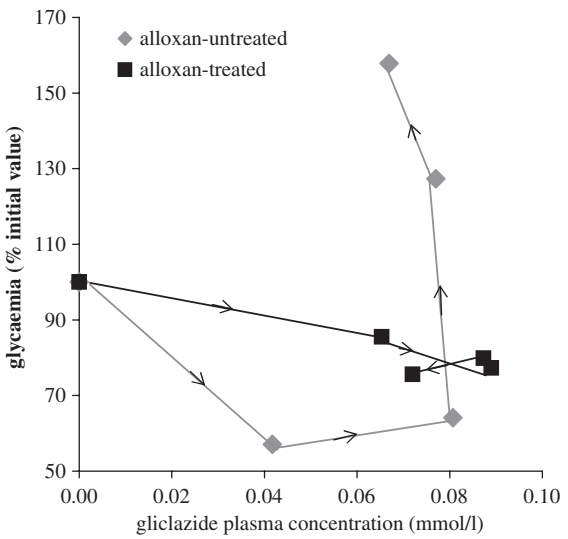

Figure 2. Concentration-effect profile of gliclazide in the rats after alloxan administration and those untreated with alloxan (the data points are connected in chronological order and the arrows indicate the direction of running time). Values represent the mean

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Table 2. Concentration-effect relationship of gliclazide (25 mg/kg) in rats after alloxan administration and in rats untreated with alloxan

	30 min	60 min	120 min	180 min
Alloxan-untreated rats				
S_{AUC}	4.93 ± 3.02	1.03 ± 0.63	−0.67 ± 1.47	−0.55 ± 1.19
Alloxan-treated rats				
S_{AUC}	1.85 ± 3.00*	0.96 ± 0.98	0.53 ± 0.34	0.28 ± 0.18

$S_{AUC} = (Ex - E_0)/AUC_{0\text{-}x}$ (effect induced by 1 unit of AUC). Values represent the mean ± standard deviation ($n = 6-10$).
*Statistical significance $p < 0.05$ (compared with alloxan-untreated group).

with large interindividual variability (Figure 1(a), Table 1) was observed.

These relationships are illustrated by a mathematical expression (the effect produced 1 mmol-gliclazide min/l [S_{AUC}]) (Table 2).

- As regards the evaluation of the effect related to the pertinent AUC of gliclazide (S_{AUC}), the maximum was achieved in both the normoglycaemic and hyperglycaemic group of animals in the interval up to 30 min;
- within the intervals of 120–180 min after gliclazide administration, the hypoglycaemizing effect lasted in the alloxan-treated group, whereas negative values were obtained (reversal of the effect) in the normoglycaemic one.

Discussion

As far as the relationships between the concentration of gliclazide and its hypoglycaemizing effect in the normoglycaemic group are concerned, a decrease in the plasma glucose level observed 60 min after gliclazide administration can be interpreted by the well described mechanisms of the effects of sulfonylurea derivatives, i.e. gliclazide-induced oversecretion of insulin, and extrapancreatic effects. Several studies suggest that there are extrapancreatic sites of action of gliclazide, assumed not to involve receptors, as well [25]. Target tissues may become more sensitive to insulin, [26] and it was demonstrated in cell cultures that sulfonylureas enhanced insulin action, and stimulated the synthesis of glucose transporters [27].

The interpretation of the pharmacokinetic–pharmacodynamic relationship is more difficult at later times (i.e. min 120 and 180 following gliclazide administration) in normoglycaemic animals, where a discrepancy is apparent between the persisting (relatively high) plasma level of gliclazide and pronounced variability of the level of glycaemia (from the occurrence of animals with a persisting hypoglycaemizing effect to those with marked hyperglycaemic values). Those cases where a reversal to hyperglycaemia occurred might possibly be explained by gliclazide-induced exhaustion of the capacity of the pancreatic ß-cells, or a change in the reactivity of insulin receptors.

The so-called down-regulation of insulin receptors under the influence of an increased insulin level, where the number of receptors decreases with an excess of the ligand as a result of the internalization of the insulin–receptor complex, is one possible explanation. Desensitization of the insulin receptors, resulting from an intense secretion of insulin, can serve as another possible explanation. This interpretation is supported by the character of the time dependence between gliclazide concentration and its effect (Figure 2), referred to as the clockwise hysteresis loop (proteresis) [23,28]. The brevity of the time interval between gliclazide administration, and a trend to a reversal of its hypoglycaemizing effect, observed in our experiments, would tend to support the idea of desensitization of the insulin receptors. This interpretation is further supported by an additional experiment, examining the effect of smaller doses of gliclazide (5 and 10 mg/kg) on the development of glycaemia in alloxan-untreated animals (Figure 3): with a small dose of gliclazide (5 mg/kg), no time reversal of the effect from the hypoglycaemizing to the hyperglycaemizing one occurred. Clearly, additional studies

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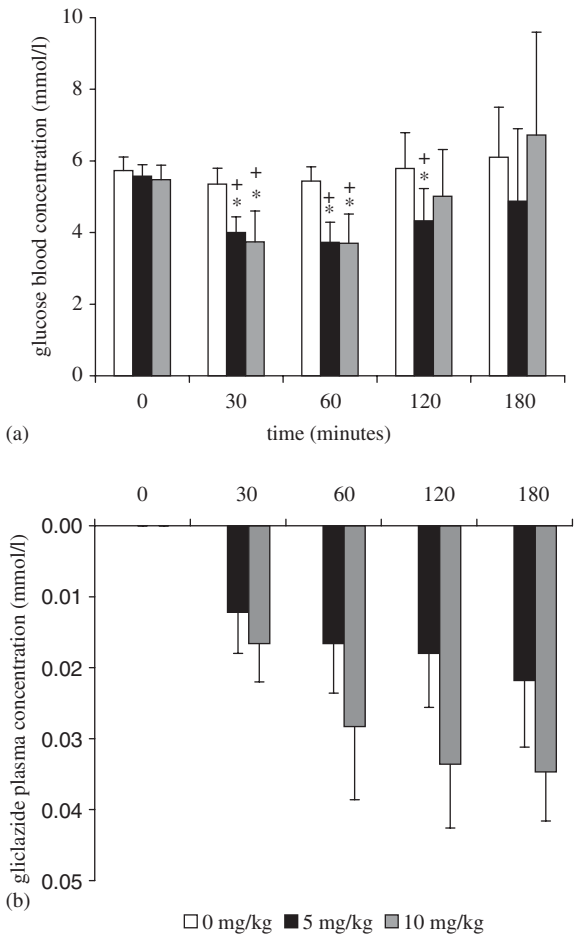

Figure 3. Correlation of the plasma gliclazide concentration (gliclazide 5 and 10 mg/kg p.o.) (b) with the blood glucose level (a) in normoglycaemic-control rats. Values represent the mean ± standard deviation ($n = 6-10$). Significant differences (in case of blood glucose concentration) versus the control group are labeled with*, versus the corresponding initial value are labeled with +, $p < 0.05$

will be needed in order to understand fully the exact mechanism of this gliclazide effect.

As far as the alloxan-treated group is concerned, the documented smaller hypoglycaemizing effect of gliclazide can be explained either by the decreased capability of the damaged ß-cells to produce insulin, or a reduction in the number of functionally preserved ß-cells. This assumption is supported by a decrease in glycaemia in the interval of 30–60 min after gliclazide administration (Table 1), which averaged 43% in alloxan-untreated animals, compared with 23% in the alloxan-treated ones. Thus, the employed dose of alloxan seems to have functionally changed about 50% of the ß-cells. The persistent hypoglycaemizing effect of gliclazide (after min 60) in the alloxan-treated animals (in contrast to the alloxan-untreated rats), can be interpreted by there being a quantitatively balanced interactive state between a relatively lower level of gliclazide-released insulin and those insulin receptors that retained their capability to react.

Conclusions

In summary, initially the pharmacodynamic efficacy of gliclazide, i.e. gliclazide-influenced hypoglycaemia, was significantly less in the animals with alloxan-induced hyperglycaemia in comparison with alloxan-untreated (normoglycaemic) rats, whereas the difference in gliclazide plasma concentrations between alloxan-treated and alloxan-untreated groups was relatively small (the levels in the alloxan-treated ones tended to be slightly higher). A reversal of the effect was observed in alloxan-untreated animals from min 120 onwards after administration of gliclazide.

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