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Pharmacodynamic Aspects of Two Sulphonylurea Derivatives Glipizide and Glibenclamide

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Summary. The results of experiments in dogs with and without two glucose loads and in the isolated pancreas to compare the pharmacodynamics of two low-dosage arylsulphonylureas, glipizide and glibenclamide, are described and discussed. — The results agree with those obtained by other authors confirming that glibenclamide shows delayed but prolonged activity on both plasma insulin and glucose levels. Moreover glibenclamide counteracts the hyperglycaemia induced by the second glucose load less efficently. Glipizide acts faster on insulin and glucose levels, which return quickly to normal. When a second glucose load was given it was still more active in reducing plasma glucose levels. The dynamics of insulin secretion following glipizide more closely resembles tolbutamide than glibenclamide.

Key words: Glipizide, glibenclamide; *in vitro*, *in vivo*, dynamics, of insulin secretion, dynamics of hypoglycaemic activity, effect on repeated glucose loads.

During pharmacological trials on a series of pyrazinoyl-amino-ethyl-benzene-sulphonylureas with oral hypoglycaemic activity [1, 2, 3, 4] the reference substance used was glibenclamide, a low dosage sulphonylurea.

Although the new molecules showed approximately the same degree of hypoglycaemic activity as glibenclamide, the blood insulin and glucose time courses differed, and it did not seem adequate to interpret the differences merely in the light of pharmacokinetics and bioavailability [5, 6].

This paper illustrates some pharmacodynamic aspects of one compound in this series, glipizide or K 4024 (N-{4-[beta-(5-methyl-pyrazine-2-carboxy-amido)ethyl]-benzene-sulphonyl}-N'cyclohexyl-urea), compared with glibenclamide.

Material and Methods

The first experiment was planned to check the plasma glucose and insulin pattern following oral administration of glipizide or glibenclamide in the dog. Twelve Beagle dogs from our stock were assigned at random to the two treatments. The dogs were fasted for 16 h. The products, micronised and suspended in 0.5% Methocel, were administered by gavage at the dose of 120 γ/kg. Blood samples were taken at time 0 (basal), and 20, 30, 40, 60, 90, 120 and 180 min following administration of the test products.

Glucose and insulin assay was carried out on plasma obtained by centrifuging immediately after taking the sample. The direct o-toluidine method of Frings *et al.* [7] was used for glucose assay; insulin was determined by the radio-immunological double antibody method of Hales and Randle [8], using the insulin immunoassay kit from the Amersham Radiochemical Centre.

Statistical analysis was carried out on the results of this trial to show up the following four aspects:

1. comparison of time courses: analysis of variance was carried out using three classification criteria (treatments, dogs, times). The least significant difference for $p = 0.05$ and $p = 0.01$ was also calculated to compare the means for the two drugs;

2. comparison of maximum peaks: for each animal the maximum peak was noted and the means for the two drugs were compared by analysis of variance;

3. comparison of maximum peak times: the time required to reach maximum peak was noted for each animal, and these times were transformed into reciprocals, then compared by analysis of variance. The harmonic mean of the times was also calculated;

4. comparison of the areas underneath the plasma glucose and insulin time course curves: the area was calculated for each dog, and the means of the areas were compared by analysis of variance.

The second experiment was set up to compare the effects of glipizide and glibenclamide on hyperglycaemia and hyperinsulinaemia induced by two glucose loads in the dog. Twelve Beagles were used, acting first as controls; they were then randomly divided in two groups of 6; one was given glipizide and the other glibenclamide. A blood sample was taken, and basal values (time 0) determined. The animals then received either the vehicle alone by gavage (controls) or else the glipizide or glibenclamide suspension in 0.5% Methocel at the dose of 120 γ/kg; immediately after taking the 40 and 420 min samples, the animals were given 4 ml/kg of a 33% glucose solution, by gavage. Plasma glucose and insulin levels were followed every 20 min for 100 min.

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Glucose and insulin assay methods were as described in the first experiment.

In order to evaluate the significance of the differences between the plasma glucose and insulin means at the different times for the three treatments, Kramer's test [9] was used, since the three samples involved different numbers. Further statistical analysis was made as described earlier.

Another series of experiments was carried out on isolated, perfused rat pancreas using the method described by Loubatières [10].

Outbred SPF CFE male rats from our stock, weighing 350—450 g, were used; access to food and water was *ad lib.* Anaesthesia was induced by intraperitoneal injection of ethyl urethane (1 g/kg), and respiration maintained throughout the experiment with a mixture of O_2-CO_2 (95—5%). The pancreas was separated from all surrounding tissue and perfused at 37° C, using an open circuit system. The basic perfusion medium was Krebs-Ringer solution containing 2‰ Grad/V bovine serum albumin (Sigma Ch. Co.) and 0.8 g/l glucose. The perfusion rate was approximately 2.5 ml/min, pressure 20—30 mmHg. Insulin secretion stabilized in all cases in about 30 min; from then on the test substance was added to the perfusion medium using an infusion pump, dissolved 2.5 γ in 5 ml of the perfusion solution. Infusion time was 15 min; while flowing through the pancreas the products reached a concentration of 50 γ/l. When stimulus with the drugs was stopped, perfusion was carried on with the basic solution.

Samples for radio-immunological insulin assay [8] were drawn from the solution as it came out of the pancreas at the times indicated in Fig. 5.

The two means at the various times were compared by a non-parametric statistical method, Wilcoxon's non-paired data test [11].

Results

Plasma Glucose and Insulin in the Dog Following Oral Administration of Glipizide or Glibenclamide

Fig. 1 gives the plasma glucose levels recorded in this test, and Fig. 2 the plasma insulin levels.

The effect of glipizide on plasma glucose starts to become evident 30 min after administration, reaching its maximum at 94 min (harmonic mean). Glibenclamide takes longer to act; its effect becomes evident only after 90 min, reaching maximum at a time which may be estimated at over 160 min (to obtain an exact figure the experiment would have had to be prolonged beyond 180 min).

Comparison of the plasma glucose means shows a significant difference between glipizide and glibenclamide at the 90th min.

Analysis of the plasma glucose time courses shows a highly significant interaction, times x drugs, confirming that glipizide acts faster. Comparison of the areas beneath the plasma glucose concentration curves also shows a significant difference between the two drugs.

The results in Fig. 2, regarding plasma insulin levels, confirm that glipizide acts faster than glibenclamide. Analysis of the time to peak shows a highly

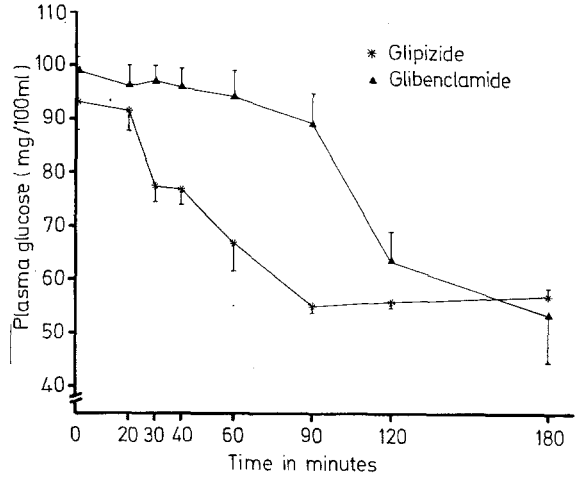

Fig. 1. Plasma glucose levels in dogs at different times after oral administration of glipizide and glibenclamide, 120 γ/kg

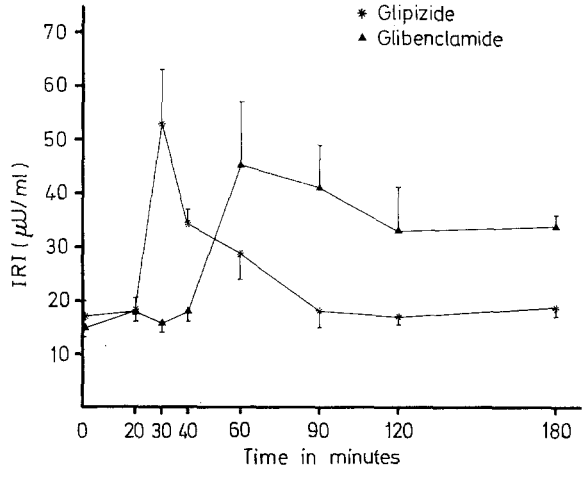

Fig. 2. Plasma insulin (IRI) levels in dogs at different times after oral administration of glipizide and glibenclamide, 120 γ/kg

significant difference between the two drugs; the harmonic mean was 31 min for glipizide and 72 min for glibenclamide.

The main difference between the two drugs, however, is the fact that plasma insulin remains high longer with glibenclamide.

With glipizide, following the peak the insulin level decreases rapidly to reach basal values at the 90th min, whereas with glibenclamide the decrease in

plasma insulin is much less marked and the plasma levels become normal only after the 180th min.

From the 60th min onwards, there was a highly significant difference between the mean plasma insulin level for the two drugs. Statistical analysis confirms the difference in the plasma insulin levels induced by the two drugs; there is a highly significant interaction times x drugs.

In view of the variations shown by the results, the means of the areas underneath the plasma insulin curves for the two drugs were not significantly different, although the mean for glibenclamide was 50% higher than for glipizide.

Effect of Glipizide and Glibenclamide on Plasma Glucose and Insulin (IRI) Levels Increase Induced in the Dog by Two Glucose Loads

Fig. 3 and Table 1 give the results of this experiment with reference to the plasma glucose levels, and Fig. 4 and Table 2 refer to plasma insulin levels.

In animals treated with glipizide the mean plasma glucose level at the 40th min was already significantly lower than both the controls and the glibenclamide group; this indicates that glipizide acts faster.

Throughout the first phase of the experiment (1 st glucose load) the glipizide-induced plasma glucose pattern remained lower than with the other two treatments; the means were significantly lower up to the 120th min. At the 140th min the means for the two hypoglycaemic compounds were not significantly different, although they differed significantly from the controls. The plasma glucose levels induced by glibenclamide start to differ from the controls only after the 60th min.

Seven hours after treatment, the mean plasma glucose level induced by glipizide was not significantly different from the controls, whereas the glibenclamide-treated animals showed a significantly lower mean than both the controls and those treated with glipizide.

During the second phase of the experiment, following the second glucose load, plasma glucose levels rose more in the animals treated with glibenclamide and the controls than in the group treated with glipizide. The effect of glibenclamide became evident only at the 480th min, and appeared more marked; in fact at the 500th min the glibenclamide-induced plasma glucose levels were significantly lower than in the other two groups.

Glipizide thus appears to act faster and less drastically, considering that following the stimulus represented by the hyperglycaemic load, the product lowered plasma glucose to within physiological limits, fairly close to control levels.

The figure giving the plasma insulin level time courses (Fig. 4) and the results in Table 2, show that at the 40th min the two groups treated with hypoglycaemic compounds showed higher plasma insulin levels than controls. However, in actual fact only the group receiving glipizide showed a real increase, since by chance the mean basal plasma insulin level in the glibenclamide group was higher than in the other two groups.

At 60th min glipizide also induced higher plasma insulin levels, with significantly higher means than

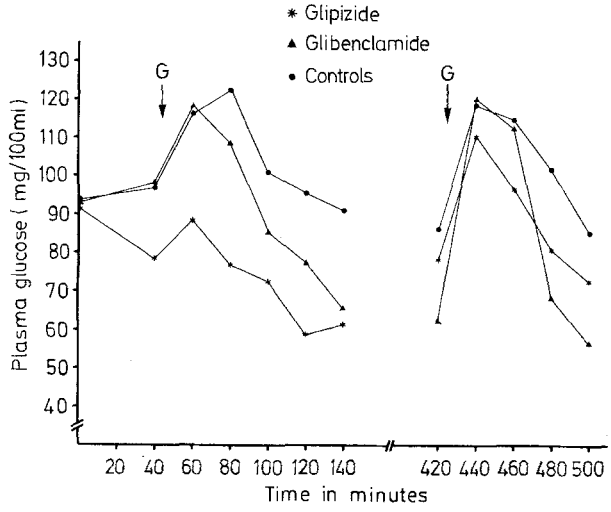

Fig. 3. Plasma glucose variations in control dogs and in dogs treated orally with glipizide or glibenclamide, 120 γ/kg, and given glucose loads (G) 40 and 420 min after administration (see Table 2)

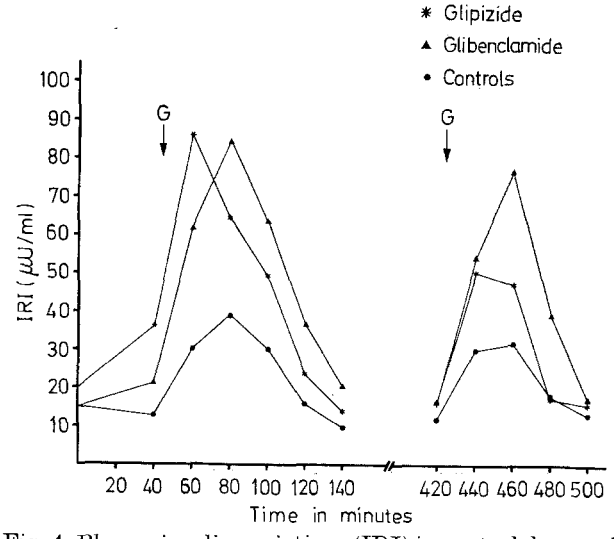

Fig. 4. Plasma insulin variations (IRI) in control dogs and in dogs treated orally with glipizide or glibenclamide, 120 γ/kg, and given glucose loads (G) 40 and 420 min after administration (see Table 2)

glibenclamide. At the 80th min plasma insulin was already decreasing in the group treated with glipizide, but in the glibenclamide and control groups a further rise was noted; after this the decrease in plasma insulin was almost parallel in all groups.

Statistical analysis of the results following the first glucose load further confirmed the difference between the two treatments. Both as regards the insulin peaks and the areas beneath the plasma insulin level curves, the means for the two hypoglycaemic agents are not significantly different, but they do differ significantly from the controls. A significant difference was found between the two drugs as regards the times required to reach peak. The harmonic means for these times were 40 min for glipizide and 63 min for glibenclamide (51 min for controls).

In the second experimental phase, i.e., after 7 h, insulin secretion was greater in the group treated with glibenclamide; this is evident comparing the levels from the 460th min onwards, the peaks and the areas, which were all highly significantly greater than controls and the glipizide group.

The three harmonic means for the peak times show no significant differences although the glibenclamide mean was delayed 7 min as compared to glipizide.

Insulin Secretion by the Isolated, Perfused Rat Pancreas Induced by Glipizide and Glibenclamide

Fig. 5 shows that at the start of perfusion with the two products, the pancreas responded rapidly to the stimulus, and a marked increase was noted in insulin secretion. At 5 min glipizide-induced secretion was significantly greater than that provoked by glibenclamide.

Insulin secretion remained high throughout the stimulus for both products; during this period no significant differences were noted.

When the drugs were withdrawn from the perfusing

Table 1. *Means, standard errors and results of comparisons of treatments for plasma glucose-levels obtained at different times in control dogs and dogs treated orally with glipizide or glibenclamide, 120 γ/kg, and given 2 oral glucose loads 40 and 420 min after drug administration*

Time in minutes	Plasma glucose (mg/100 ml). Means ± S.E.			[a] Comparisons by Kramer's test		
	Controls (C) no. = 12	Glipizide (GZ) no. = 6	Glibenclamide (GC) no. = 6	C vs GZ	C vs GC	GC vs GZ
0	93.75 ± 2.06	91.33 ± 3.50	93.50 ± 3.49	N.S.	N.S.	N.S.
40	96.75 ± 2.56	78.33 ± 2.72	98.17 ± 3.47	H.S.	N.S.	H.S
60	116.17 ± 2.45	88.33 ± 3.21	118.33 ± 4.98	H.S.	N.S.	H.S.
80	122.25 ± 2.88	76.50 ± 4.22	108.33 ± 3.89	H.S.	S	H.S.
100	100.58 ± 2.63	72.33 ± 3.79	85.17 ± 2.66	H.S.	H.S.	S
120	95.33 ± 2.44	58.50 ± 5.16	77.17 ± 2.69	H.S.	H.S.	H.S.
140	90.75 ± 2.26	61.33 ± 3.87	65.33 ± 2.19	H.S.	H.S.	N.S.
420	86.08 ± 2.09	78.00 ± 3.41	62.00 ± 3.78	N.S.	H.S.	H.S.
440	118.58 ± 2.03	110.33 ± 3.95	120.17 ± 3.47	N.S.	N.S.	S
460	114.50 ± 1.34	96.33 ± 3.98	112.33 ± 2.40	H.S.	N.S.	H.S.
480	101.33 ± 1.95	80.50 ± 2.75	68.17 ± 2.66	H.S.	H.S.	H.S.
500	85.00 ± 1.86	72.33 ± 2.50	56.33 ± 2.51	H.S.	H.S.	H.S.

[a] N.S. = Not Significant ($P > 0.05$)
S = Significant ($0.01 < P \leq 0.05$)
H.S. = Highly significant ($P \leq 0.01$)

Table 2. *Means, standard errors and results of comparisons of treatments for plasma insulin-levels obtained at different times in control dogs and dogs treated orally with glipizide or glibenclamide, 120 γ/kg, and given 2 oral glucose loads 40 and 420 min after drug administration*

Time in minutes	IRI (μU/ml). Means ± S.E.			[a] Comparisons by Kramer's test		
	Controls (C) no. = 12	Glipizide (GZ) no. = 6	Glibenclamide (GC) no. = 6	C vs GZ	C vs GC	GC vs GZ
0	14.42 ± 1.29	14.17 ± 0.98	20.00 ± 2.02	N.S.	S	S
40	12.58 ± 1.46	36.33 ± 2.35	21.17 ± 2.41	H.S.	H.S.	H.S.
60	30.00 ± 3.68	85.83 ± 6.52	61.67 ± 4.58	H.S.	H.S.	H.S.
80	38.83 ± 3.49	64.17 ± 5.28	84.17 ± 2.71	H.S.	H.S.	H.S.
100	29.83 ± 2.50	49.17 ± 3.20	63.33 ± 7.92	H.S.	H.S.	N.S.
120	15.67 ± 1.44	23.17 ± 3.91	36.33 ± 6.34	N.S.	H.S.	S
140	9.50 ± 0.63	13.83 ± 2.26	20.33 ± 3.15	N.S.	H.S.	S
420	11.75 ± 0.69	16.17 ± 1.81	16.17 ± 1.70	S	S	N.S.
440	28.92 ± 3.08	50.17 ± 3.63	54.33 ± 3.48	H.S.	H.S.	N.S.
460	31.83 ± 2.68	47.00 ± 3.87	76.83 ± 5.04	H.S.	H.S.	H.S.
480	17.92 ± 1.63	17.17 ± 2.14	39.17 ± 4.71	N.S.	H.S.	H.S.
500	13.00 ± 0.86	15.67 ± 1.14	17.00 ± 3.08	N.S.	N.S.	N.S.

[a] See note on Table 1

solution, a marked difference in behaviour was noted between the glipizide and glibenclamide treated pancreas. After glipizide perfusion insulin secretion returned rapidly to basal values, reaching them at the 35th min.

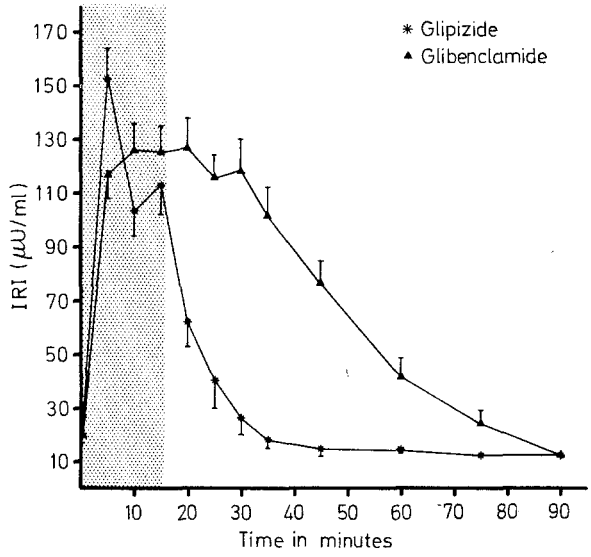

Fig. 5. Effect of glipizide and glibenclamide on insulin secretion by the isolated rat pancreas, perfused from time 0 to the 15th min. Except for times over 45 min, which were obtained with 9 replications, each point represents the mean of 11 replications

After glibenclamide perfusion insulin secretion remained at the levels reached while the product was being perfused until the 30th min, then started to decrease slowly, reaching basal values only after 90 min. From the 20th to the 75th min the differences between the means for the two treatments were highly significant.

Discussion

Comparison of glipizide and glibenclamide in the dog with no glucose load has shown that the two hypoglycaemic agents differ markedly. Glipizide acts rapidly, with a prompt hypoglycaemic effect, corresponding to an early insulin secretion peak which returns rapidly to basal levels. With glibenclamide the hypoglycaemic effect becomes evident only after a latent period, and the insulin response is of longer duration.

In the present experiment, the products were administered orally as they are used in therapy. Fuccella *et al.* [5] demonstrated that in man glipizide is absorbed faster than glibenclamide; this finding might suggest that the difference in the two products' pharmacodynamics was due only to different pharmacokinetics. The differences we observed, however, are similar to those reported by Loubatières *et al.* [12] Loubatières [13], Raptis *et al.* [14], and by Haupt *et al.* [15] between glibenclamide on the one hand, and tolbutamide, glibornuride and glisoxepide on the other, injecting the products intravenously in man. The results of this experiment therefore suggest that, pharmacokinetic characteristics apart, glibenclamide shows different pharmacodynamics from other sulphonylurea group drugs, while glipizide resembles them.

The experiment using two glucose loads in the dog, particularly the results referring to the first load, confirm the speed of action of glipizide, which completely inhibits the glucose-induced hyperglycaemia, whereas glibenclamide acts after a lag-time and in fact only partially antagonizes the hyperglycaemia.

The delay in glibenclamide's action is also seen in plasma insulin, although surprisingly, the insulin levels after glibenclamide treatment are not markedly different from those following glipizide, despite the former being only partially able to lower plasma glucose.

The plasma insulin pattern after the first glucose load also does not differ between glipizide and glibenclamide, contrary to the findings in the previous experiment on animals that had not received glucose. Similar results were obtained by Raptis *et al.* [14] in experiments comparing glibenclamide and tolbutamide.

Immediately before the second glucose load, the plasma glucose in glibenclamide-treated animals was significantly lower than in controls and the glipizide group. This might appear to suggest that glibenclamide shows more persistent action, even if not mediated by greater and longer-lasting insulin secretion; the results regarding the plasma glucose levels after the second glucose load, however, do not favour this hypothesis, since glibenclamide antagonizes the rise less than glipizide, even though plasma insulin levels were much higher and longer-lasting than in both control and glipizide groups.

That the insulin secretion dynamics following glibenclamide differ from those following glipizide, is confirmed by the results of our experiment on the perfused pancreas. Here the difference was easily seen, and agrees with the findings of Fussgänger *et al.* [16], Grodsky *et al.* [17] and Pfeiffer *et al.* [18] on comparison of glibenclamide with other known sulphonylureas. All these data show that the action of glibenclamide is less intense to start with, but is long-lasting.

These findings may provide at least a partial explanation of different pharmacodynamics of the two hypoglycaemic agents.

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