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# Effect of Glipentide on Galactose Absorption and Disaccharidase Activity in Rat Small Intestine *

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The effect of hypoglycemic sulfonylurea glipentide on galactose transport and disaccharidase activity has been studied in rat small intestine. When $2 \times 10^{-4}$ M glipentide is present in the mucosal bathing solution, galactose active transport is inhibited 30 % both *in vivo* and *in vitro*. Treatment or rats with 5 mg/kg glipentide p.o. for 10 days does not modify galactose absorption of disaccharidase activity. Incubation of the enzymes with glipentide shows no direct effect of the drug on its hydrolytic activity. The effects of glipentide on sugar transport are slight, or non significant in maintaining low blood sugar levels.

Glipentide, N-[4-$\beta$-(o-anisamidethyl)-benzenesulphonyl] - N' - cyclopentylcarbamide, is a hypoglycemic sulfonylurea whose pharmacological effects are attributed to stimulation on insulin release from the pancreas (2, 7). The drug also has extrapancreatic effects such as the inhibition of cAMP phosphodiesterase (6) and glycerol uptake and metabolism by adipose tissue (9). However, the glipentide dependent hypoglycemia is not strictly correlated with insulin secretion since the oral administration of a single dose of the drug is followed by a rapid increase in plasma insulin levels which lasts for 60 min, while the glycemia is decreased for 180 min (2).

The aim of the present work was to study whether glipentide has any effect on intestinal sugar absorption and disaccharidase activity because both processes may influence blood sugar levels. Since glucose is transported and metabolized, the transport of galactose was studied in order to examine a possible direct effect of the drug on intestinal transport mechanisms.

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## Materials and Methods

All experiments were carried out on Wistar rats (180-200 g). Animals were starved for 24 h with free access to water before the experiment.

Treated animals received a daily administration of glipentide at a dose of 5 mg/kg p.o., for 10 days. Since the drug had to be dissolved in 0.1 N KOH, control animals were administered with the solvent solution (5 ml/kg). Glipentide was a gift from Laboratorios Uriach (Barcelona).

In vitro *experiments.* The middle jejunum was removed under urethane anaesthesia (1.25 g/kg s.c.) and four everted gut sacs were prepared from each animal, according to the WILSON and WISEMAN technique (17). Mucosal and serosal bathing solution was Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 5 mM D-galactose and either $10^{-4}$M or $2\times10^{-4}$M glipentide. Flasks were gassed with carbogen (95% $O_2$:5% $CO_2$) during incubation (37°). Some experiments were done after preincubation of sacs with galactose free buffer solution containing $2\times10^{-4}$ M glipentide. After incubation mucosal and serosal fluids were analyzed for galactose with the SOMOGYI method (14).

In vivo *experiments.* Experiments were done in rats, anaesthetized with uretane, according to the SOLS and PONZ technique (13) with a subsequent modification (12). Intestinal perfusate was warm saline (37°) containing either 2 mM or 80 mM galactose and with or without $2\times10^{-4}$ M glipentide. When using 80 mM galactose the solution was corrected for osmolarity. Perfusion rate was 3.35 ml/min. Final galactose concentration in perfusate was determined as described before.

*Disaccharidase activity.* A piece of duodenum-proximal jejunum was excised both from intact and treated animals. After washing with saline it was longitudinally opened and the mucosa scraped and homogenized in an ice bath. The homogenate was then centrifuged at $1,500\times g$ (10 min) and the disaccharidase activity was analyzed in the supernatant by the method of DALQUIST (4). Glucose was determined by the glucose-deshydrogenase method (1). In some experiments glipentide was added to the reaction mixture (final concentration $2\times10^{-4}$ M) at the beginning of the test.

Protein content was assayed by the method of LOWRY *et al.* (10). Results are expressed as μmol disaccharide hydrolyzed/mg protein/h. All statistical analyses were made according to Student's t-test.

## Results

In vitro *experiments.* In previous experiments with $10^{-4}$ M glipentide in the serosal and mucosal bathing solutions no effect was observed (results not shown). At $2\times10^{-4}$ M concentration glipentide significantly inhibits the transport of galactose (table I). The observed inhibition was 28% and was not increased after subacute treatment of animals with the drug. Preincubation of sacs with glipentide for 15 min resulted in a slight increase in the inhibitory effect of glipentide on galactose transport (38% inhibition).

In vivo *experiments.* The absorption of galactose was not modified by drug treatment at both initial concentrations of sugar tested (table II). When glipentide $2\times10^{-4}$ M was added to the perfusion fluid an inhibition of sugar transport of 25% (2 mM) and 32% (80 mM) was observed.

*Disaccharidase activity.* Glipentide treatment did not affect any of the three disaccharidases tested: sucrase (EC 3.2.1.26), maltase (EC 3.2.1.20) and lactase (EC 3.2.1.23). Moreover, incubation of these enzymes with $2\times10^{-4}$ M glipen-

Table I. *Effect of glipentide on galactose transfer by intestinal everted sacs.* Sacs were incubated for 45 min in a Krebs-Henseleit bicarbonate buffer containing 5 mM galactose with or without glipentide. Some sacs were preincubated in a galactose free buffer with or without (controls) glipentide. Pretreated animals were administered p.o. with 5 mg/kg glipentide or 0.1 N KOH, 5 ml/kg (controls) for 10 days. n: number of experiments. Results are expressed as mean ± standard error. Statistical analysis: * $p < 0.05$, ** $p < 0.01$.

| | No preincubation | | | Preincubation | | |
|---|---|---|---|---|---|---|
| | Glipentide M | n | µmol galactose/ 100 mg wet tissue/ 45 min | Glipentide M | n | µmol galactose/ 100 mg wet tissue/ 45 min |
| *Untreated* | — | 11 | 2.62 ± 0.22 | — | 13 | 0.68 ± 0.07 |
| | $2 \times 10^{-4}$ | 11 | 1.89 ± 0.13 * | $2 \times 10^{-4}$ | 13 | 0.42 ± 0.09 * |
| *Treated* | | | | | | |
| Control | — | 10 | 2.43 ± 0.16 | | — | |
| | $2 \times 10^{-4}$ | 27 | 1.67 ± 0.08 ** | | — | |
| Glipentide | — | 12 | 2.51 ± 0.18 | | — | |
| | $2 \times 10^{-4}$ | 31 | 1.72 ± 0.12 ** | | — | |

Table II. *Effect of glipentide on galactose absorption* in vivo. Multiple pass perfusion for 10 min. The initial volume of perfusate was 10 ml and the rate 3.35 ml/min. Animals were treated as indicated in table I. n: number of experiments. Results are expressed as mean ± standard error. Statistical analysis: * $p < 0.05$.

| Galactose mM | Glipentide M | µmol galactose absorbed/cm Intest./10 min | | | |
|---|---|---|---|---|---|
| | | n | Control | n | Treated |
| 2 | — | 10 | 0.34 ± 0.02 | 16 | 0.36 ± 0.02 |
| 2 | $2 \times 10^{-4}$ | 9 | 0.28 ± 0.01 * | 6 | 0.27 ± 0.02 * |
| 80 | — | 14 | 7.35 ± 0.40 | 16 | 7.24 ± 0.57 |
| 80 | $2 \times 10^{-4}$ | 7 | 5.66 ± 0.67 * | 7 | 4.90 ± 0.47 * |

tide did not modify their hydrolytic activity.

## Discussion

Although the effects of hypoglycemic sulfonylureas like tolbutamide and glibenclamide on sugar absorption are known (15, 16), those of glipentide on intestinal tissue have not been described and the possible relevance of direct drug interaction with intestinal sugar transport and/or metabolism processes has not been studied.

Our results show that subacute treatment with glipentide does not affect the intestinal absorption of 2 mM or 80 mM galactose *in vivo*. The addition of glipentide $2 \times 10^{-4}$ M to the perfusion solution produces an inhibition of 25 % and 32 % at low or high initial galactose concentration, respectively. A similar degree of inhibition is obtained in everted sacs *in vitro* when the drug is present in both mucosal and serosal solutions and this effect is not enhanced by pretreatment of rats with glipentide for 10 days. Thus, this drug produces a partial inhibition on galactose transfer only when it is present in the lumen and this effect is increased when intestinal tissue is preincubated with glipentide in a Krebs galactose-free medium (table I). These effects are similar to those described by TEALE and LOVE for glibenclamide (15, 16). The lack of effect

after pretreatment suggests that the metabolic effects of this drug on intestinal tissue are low and easily reversible, which in part may be due to the fact that the drug is readily absorbed form the gut (3) and therefore is not accumulated in the intestine for long periods of time.

It has been suggested that the effects of glipentide and other sulfonylureas in some tissues may be explained through a reduction in ATP supply (5, 8, 9). Therefore a possible explanation for the effect of glipentide on galactose transport is that it may reduce the availability of ATP thus reducing the ionic gradient responsible for sugar active transport.

In conclusion, it seems that the observed effects of glipentide on sugar absorption together with the lack of effect on disaccharidase activity, are of low or no significance in the reduction of blood sugar levels *in vivo*. Considering the drug is administered in low doses in therapy (11), it is improbable that the drug maintains a sufficiently high intraluminal concentration long enough for a significant change in sugar absorption.

## Resumen

Se estudia el efecto de la glipentida, sulfonilurea hipoglucemiante, sobre el transporte de galactosa y actividad disacaridásica de intestino delgado de rata. La glipentida $2 \times 10^{-4}$ M, inhibe el transporte activo de galactosa en un 30 %, tanto *in vivo* como *in vitro*. Ratas tratadas durante 10 días con una dosis diaria de 5 mg/kg p.o. no presentan ninguna modificación de la absorción de galactosa ni de la actividad disacaridásica. Se concluye que los ligeros efectos que produce la glipentida sobre la absorción de azúcares tienen escasa influencia en el mantenimiento de la hipoglucemia.

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