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COMPARISON OF THE EFFECT OF TWO DIFFERENT HYPOGLYCEMIC AGENTS, GLIBENCLAMIDE AND HB 699, ON THE RAT SMALL INTESTINAL ABSORPTION OF SUGARS AND AMINO ACIDS

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Abstract—4-(2-[5-Chloro-2-methoxy-benzamido]-ethyl)-benzoic acid (HB 699) belongs to the group of hypoglycemic benzoic acid derivatives. Although lacking the sulfonylurea group, the structure of HB 699 partly resembles that of glibenclamide which is known to impair small-intestinal glucose absorption *in vitro* at high concentrations. Whereas this intestinal effect of glibenclamide is unlikely to contribute to its blood-glucose lowering properties, extrapancreatic and particularly intestinal effects may be important for the antidiabetic action of HB 699. Thus, HB 699 was compared with glibenclamide for the effect on the small-intestinal absorption of sugars and amino acids *in vitro* (everted-sac and tissue-accumulation technique) and *in vivo* (single-pass perfusion of the jejunum). *In vitro* both drugs inhibited the active transport of sugars and amino acids in a dose dependent manner. At equieffective doses (HB 699, 4.5 mmol/l and glibenclamide, 1 mmol/l) the mode of inhibition by the two drugs was similar. A 30-min incubation period reduced the uptake of methyl α-D-glucoside by about 75%. The degree of inhibition depended on the time of exposure of the tissue to the drugs. *In vitro* kinetic studies revealed a mixed type of inhibition. The *in vivo* effect of the drugs was in accordance with the *in vitro* findings. Inhibition, as *in vitro*, was not reversible and even increased further after reinfusion of a drug-free perfusate. *In vivo*, the drugs inhibited the absorption of methyl α-D-glucoside and leucine only at low (<20 mmol/l) but not at high (>30 mmol/l) solute concentrations. These results indicate that hypoglycemic benzoic acid derivatives may exert their blood-glucose lowering properties in part by impairing the small-intestinal active transport of glucose.

Hypoglycemic drugs used for the oral treatment of diabetes mellitus (type II) are almost exclusively represented by the class of sulfonylurea derivatives [1, 2]. These compounds generally stimulate the release of insulin from the β-cells of the pancreas [3, 4]. However, extrapancreatic effects of the sulfonylurea drugs may also contribute or even enhance their blood-glucose lowering properties [5, 6].

Recently, a new class of hypoglycemic agents was detected [7, 8]. Structurally they are derived from glibenclamide in which the sulfonylurea group is replaced by a carboxyl or an alkylcarboxyl group. These compounds also stimulate the release of insulin from the pancreas *in vitro* [7–9] and *in vivo* [8, 10]. Compared to the oral dose of glibenclamide that of HB 699 has to be larger (~100-fold), however, for a similar effect on the blood glucose levels after an oral glucose load since its potency resembles that of tolbutamide [8, 11, 12].

With regard to another class of antidiabetic drugs, the biguanides, and their inhibitory effect on the small intestinal absorption of glucose [13], previously, tolbutamide and glibenclamide were tested *in vitro* for their effect on rat small intestinal absorption of sugars [14, 15]. Both sulfonylureas inhibited glucose absorption, but, as concluded from these studies, the concentrations needed to produce a substantial effect were probably too high to be reached in the intestinal lumen after the oral application of a therapeutic dose of these drugs. For HB 699, however, luminal concentrations may be reached which might influence the absorption of glucose, thus contributing to extrapancreatic blood-glucose lowering mechanisms which were supposed recently.¶

We therefore studied the effect of HB 699 as a representative compound of the new class of hypoglycemic drugs on the small intestinal absorption of sugars and amino acids *in vitro* and *in vivo*. A comparison of the effect of HB 699 with that of glibenclamide was thought to provide an appropriate basis for the evaluation of the pharmacological properties of HB 699. We were also interested in establishing the extent to which the *in vitro* effects

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may reflect the situation in an *in vivo* perfusion experiment. A preliminary report was given at the spring meeting of the Deutsche Pharmakologische Gesellschaft, Mainz, F.R.G., 1985 [16].

MATERIALS AND METHODS

All studies were carried out using the small intestine from non-fasted female Wistar rats (Zentralinstitut für Versuchstiere, Hannover, F.R.G.) weighing 150–220 g. For the *in vitro* preparations, the animals were sacrificed by stunning and decapitation, and the proximal end of the mid-jejunum was identified 10–15 cm beyond the duodenojejunal flexure. A 30-cm segment was quickly exised, everted over a plastic rod, and chilled in oxygenated Krebs–Henseleit phosphate (KH) buffer (pH 7.4–7.6). The *in-vivo* perfusion of the jejunum was performed on animals anesthetized intraperitoneally with 70 mg sodium pentobarbital per kg body weight. The abdominal cavity was opened and a mid-jejunal segment was cannulated distally and proximally to provide a perfusion segment of approx. 20 cm in length.

Tissue accumulation method (everted rings). These *in-vitro* experiments were performed by incubating rings of everted rat jejunum according to previously described [17] and well established methods [18, 19]. The incubations were carried out in oxygenated (100% O_2) KH buffer at 37°. D-[^{3}H]mannitol together with unlabeled mannitol (1 mmol/l) was used to correct for the extracellular space. Tissue accumulation or uptake of the solute was expressed as a distribution ratio:

$$\text{Tissue uptake} = \frac{S_T}{S_M} = \frac{\text{Solute concentration in the intracellular fluid volume}}{\text{Solute concentration in the incubation medium}}$$

assuming an intracellular fluid volume of 80% of the tissue net weight [19]. The tissue uptake rates for the evaluation of the transport kinetics are expressed as the change in the intracellular solute concentration per minute. For the kinetic uptake experiments, the incubations were carried out for 8 min since within a 10-min period the tissue uptake was fairly linear both in the controls and solutions containing inhibitor. This short time period also minimizes the influence of intracellular solute accumulation on the results, thus reflecting mainly initial uptake rates which are required for the kinetic studies.

Everted sac technique. Everted sacs were prepared by a previously published method [20]. The experiments were performed as described recently [17]: one sac, 6–7 cm in length, was prepared from each animal and placed in chilled and oxygenated KH buffer. Five sacs were used for a single absorption measurement each prepared from a small intestinal segment approx. 20 cm distal from the duodenojejunal flexure. Sacs were filled with oxygenated KH buffer (0.75 ml) and incubated for 35 min at 37° in 25-ml Erlenmeyer flasks containing 10 ml KH buffer. The drugs were administered either at the mucosal or the serosal side of the preparation and the transport substrates only at the mucosal side. After incubation, the everted sacs were removed, blotted, opened and the sac fluid collected for analysis. The final serosal substrate concentration is given to characterize the mucosal-to-serosal transport in this preparation.

Single-pass perfusion. According to the methods employed in a recently performed perfusion study [21], the effect of the drugs on small intestinal absorption was also studied using a single-pass perfusion technique. The perfusion medium was KH buffer. The transport substrate, either methyl α-D-glucoside (α-Me-Glc) or leucine, was added at the expense of sodium chloride if its concentration exceeded 1 mmol/l. Since ^{14}C-labeled transport substrates were used, ^{3}H-PEG 4000 was employed as the impermeable volume marker. In all experiments the perfusion rate was 0.7 ml/min. In general, a pre-equilibration period of 30 min (in order to reach a steady state for water and solute absorption) was followed by a 30-min control period (perfusing the KH buffer without the drugs) during which three or four 10-min collections of the outflowing perfusate were obtained for analysis of radioactivity. The control period was followed by a second equilibration period of 30 min and subsequently by the test period employing in both these periods the drug to be tested. Again, three or four 10-min collections were made during the test period. When not otherwise expressed, absorption was calculated from these three or four samples to give a single value for each animal. For the reversibility experiments, the control conditions were re-established by reperfusing the drug-free KH buffer after the test period.

Materials. ^{14}C-labeled transport substrates, ^{3}H-mannitol and ^{3}H-PEG 4000 were purchased from NEN Chemicals GmbH (Dreieich, F.R.G.). Glibenclamide and HB 699 were provided by Hoechst AG (Frankfurt/M, F.R.G.). Other materials were obtained from standard commercial sources.

RESULTS

Both drugs, glibenclamide and HB 699, inhibited the uptake of α-Me-Glc into jejunal rings. The inhibition was strongly dose dependent, as depicted in Fig. 1. With a 10-min incubation period, 50% inhibition was achieved employing a HB 699 concentration of about 6 mmol/l. Longer incubation periods shifted the dose-dependency curve towards lower inhibitor concentrations so that a 30-min incubation period resulted in 50% inhibition with a HB 699 concentration of about 1.5 mmol/l. A similar pattern was observed for the inhibition of the methyl α-D-glucoside uptake by glibenclamide. This drug, however, was only soluble up to a concentration of about 0.7 mg/ml. Due to these findings, almost equieffective doses of glibenclamide (1 mmol/l or 0.5 mg/ml) and of HB 699 (4.5 mmol/l or 1.5 mg/ml) were used for the further experiments.

Experiments with everted rings were performed to study the effect of glibenclamide and HB 699 on the kinetics of the active transport of α-Me-Glc and leucine. Both drugs caused a mixed type of inhibition for both transport substrates, the sugar and the amino acid, as shown by the Lineweaver–Burk plots in Fig. 2.

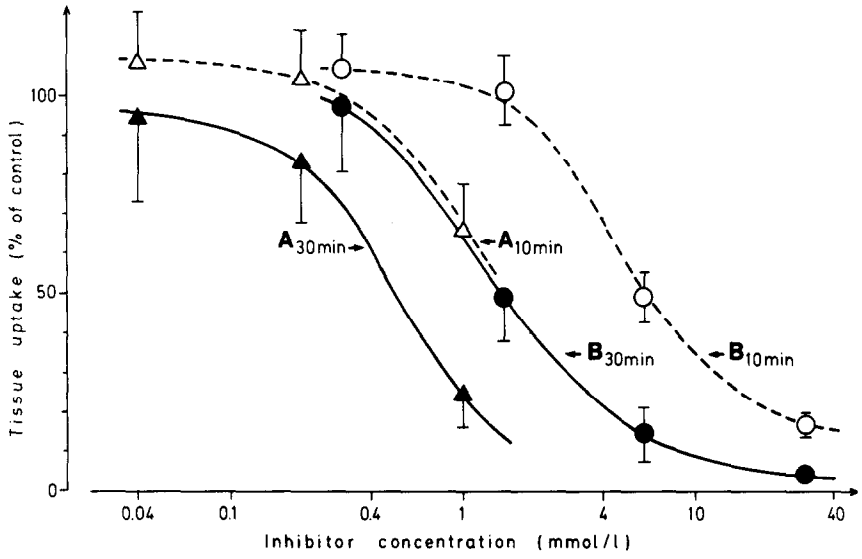

Fig. 1. Concentration-dependent inhibition of the small intestinal uptake of methyl α-D-glucoside (1 mmol/l) by glibenclamide (A) and HB 699 (B) in 10-min and 30-min incubations *in vitro*. The incubations were carried out in oxygenated Krebs–Henseleit buffer at 37° and 120 cycles/min using everted mid-jejunal rings. The values are means of six separate determinations (M ± SD).

Not only α-Me-Glc, a non-metabolizable glucose analog, and leucine uptake were inhibited by both drugs, but also other transport substrates tested in the present study (Table 1). Of the substrates transported by Na^+-dependent carriers, the amino acids were slightly more affected by the two drugs than the monosaccharides. The uptake of fructose, assumed to be Na^+-independent, was preferentially inhibited by glibenclamide to a slight extent. In general, HB 699 did cause slightly lower inhibition than glibenclamide. This, however, was probably due to the estimation of the equieffective doses according to the experiment shown in Fig. 1.

Additionally the effect of the drugs on the mucosal-to-serosal transport of α-Me-Glc was investigated using the everted sac technique (Table 2). Active transport was again inhibited when the drugs were administered at the mucosal side of the preparation. A slight increase in the passive permeability, as measured by an increased concentration of mannitol in the serosal sac fluid was observed for both drugs, but was not found to be statistically significant.

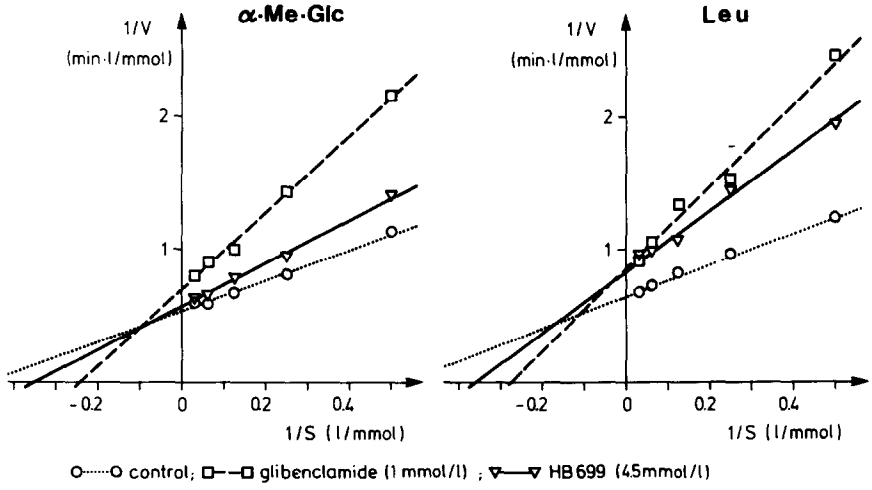

Fig. 2. Kinetic analysis of the *in-vitro* effect of glibenclamide and HB 699 on the intestinal uptake of methyl α-D-glucoside (α-Me-Glc) and leucine (Leu). The 8-min incubations were carried out in oxygenated Krebs–Henseleit buffer at 37° and 120 cycles/min using everted mid-jejunal rings. The results are means of four separate experiments and are plotted according to Lineweaver-Burk. Additional note: The V_{max} values from the four individual test experiments (HB 699, α-Me-Glc; 1.74 ± 0.39 mmol·l^{-1}·min^{-1}) are significantly different ($P < 0.05$, paired *t*-test) from the corresponding V_{max} values from the control experiments (α-Me-Glc; 2.03 ± 0.53 mmol·l^{-1}·min^{-1}).

Table 1. Effect of the two hypoglycemic agents on the *in vitro* uptake of various solutes in rat jejunum

Solute (1 mmol/l)	Tissue uptake (S_T/S_M) Control	Glibenclamide (1 mmol/l)	% of control	HB 699 (4.5 mmol/l)	% of control
α-Me-Glc	5.05 ± 1.46	3.03 ± 0.91*	60	3.74 ± 1.01	74
Gal	2.27 ± 0.57	1.61 ± 0.41*	71	1.82 ± 0.54	80
Fru	0.40 ± 0.07	0.12 ± 0.08†	30	0.26 ± 0.12*	65
Leu	4.66 ± 1.26	2.28 ± 0.61†	49	2.52 ± 0.61†	54
ACPC	2.66 ± 0.51	1.46 ± 0.16†	55	1.89 ± 0.48*	71

Tissue uptake was determined in 10-min incubations which were carried out in oxygenated Krebs–Henseleit phosphate buffer at 37° using everted rings of rat jejunum (tissue accumulation technique). Mean ± SD (N = 6) *$P < 0.05$; †$P < 0.005$; significance is related to the control incubation where no drug was added (unpaired Student's *t*-test). α-Me-Glc, methyl α-D-glucoside; Gal, galactose; Fru, fructose; Leu, leucine; ACPC, 1-amino-cyclopentane-1-carboxylic acid (cycloleucine).

Application of the inhibitors at the serosal side of the everted sac did not cause significant differences in the serosal substrate concentration compared to that of the controls. Furthermore, it should be pointed out that the serosal substrate concentration in the control was higher than the mucosal concentration, demonstrating the active transport of α-Me-Glc against its concentration gradient across the epithelium.

The *in vitro* inhibition was found to be irreversible as washing of the tissue with a drug-free buffer did not restore α-Me-Glc-uptake rates after pretreatment, (10-min incubation) of everted rings in the incubation medium containing the test drug (unpublished results).

Employing the single-pass perfusion technique with inhibitor concentrations of 1 and 4.5 mmol/l for glibenclamide and HB 699, respectively, the *in vivo* absorption of α-Me-Glc was impaired equally by both drugs. At a solute concentration of 1 mmol/l not only the extent but also the time course of inhibition by the two drugs were quite comparable (Fig. 3). As already suggested by the results of the *in vitro* experiments, inhibition *in vivo* was not reversible. In the present perfusion system the inhibition of the absorption of α-Me-Glc by glibenclamide and HB 699 persisted or even increased with time despite reperfusion of the drug-free control medium.

Although a steady state of the inhibition by the two drugs was not achieved after a 1-hr perfusion period with perfusate containing the test drug, this period was considered to be sufficient to evaluate the inhibitory effect of glibenclamide and HB 699 on the concentration-dependent absorption of some actively transported solutes. Therefore, the 40-, 50-, and 60-min sample of the test period (see Fig. 3), corresponding to a total perfusion period of 100, 110,

Table 2. Effect of glibenclamide and HB 699 on the mucosal-to-serosal transport of methyl α-D-glucoside and mannitol

Incubation medium mucosal concentration (mmol/l)	α-Me-Glc 1.00 Mannitol 1.00
Sac content serosal concentration (mmol/l)	
Control	α-Me-Glc 1.81 ± 0.48
	Mannitol 0.13 ± 0.05
Mucosal glibenclamide (1 mmol/l)	α-Me-Glc 0.41 ± 0.09*
	Mannitol 0.17 ± 0.03
Mucosal HB 699 (4.5 mmol/l)	α-Me-Glc 0.41 ± 0.14*
	Mannitol 0.17 ± 0.02
Serosal glibenclamide (1 mmol/l)	α-Me-Glc 1.74 ± 0.55
	Mannitol 0.13 ± 0.03
Serosal HB 699 (4.5 mmol/l)	α-Me-Glc 1.43 ± 0.49
	Mannitol 0.16 ± 0.03

Incubations were carried out in oxygenated Krebs–Henseleit buffer at 37° for 35 min using everted sacs of rat jejunum. The mucosal concentration of methyl α-D-glucoside and mannitol is given for the beginning of the experiment; serosal concentrations are those measured at the end of the incubations. Mean ± SD (N = 5) *$P < 0.01$ (related to the control); others are not significantly different from the control (unpaired Student's *t*-test).

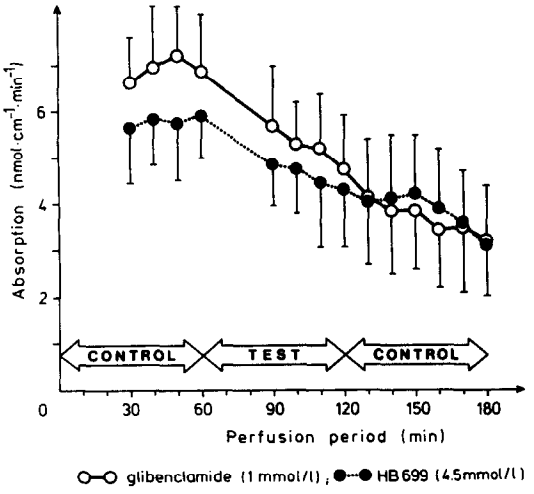

Fig. 3. Small-intestinal perfusion of glibenclamide and HB 699. The influence of the drugs on the absorption of methyl α-D-glucoside (1 mmol/l) was studied *in vivo* by a single-pass perfusion technique using the mid-jejunum of pentobarbital-anesthetized rats. The perfusion medium (Krebs–Henseleit buffer) contained the drug only in the 1-hr test period. During control periods a drug-free buffer was perfused. Perfusion rate: 0.7 ml/min; M ± SD (N = 9).

and 120 min, respectively, were chosen to determine the effect of the two drugs on the absorption of α-Me-Glc (Fig. 4). The absorption pattern of the solute was similarly changed in the presence of the two inhibitors. Despite a superimposed linear diffusion component—a typical feature of these *in vivo* perfusion experiments [21]—the control conditions revealed a saturation characteristic of the concentration-dependent absorption of α-Me-Glc. Addition of glibenclamide or HB 699 to the perfusate changed this pattern into a more linear relationship between solute concentration and rate of absorption. This indicates inhibition of the active, i.e. the saturable, transport system. At solute concentrations below 20 mmol/l, absorption rates were significantly lower in the presence of the drugs compared to those obtained under control conditions. Above solute concentrations of ~30 mmol/l absorption rates although statistically not significant were increased in the presence of glibenclamide or HB 699. Obviously the absorption of leucine was influenced by the two drugs in a similar manner (Table 3). Finally it should be mentioned that the water net absorption was not significantly altered by the presence of the drugs as judged by monitoring the concentration of the volume marker PEG 4000 during perfusion.

DISCUSSION

In the present investigation HB 699 and glibenclamide inhibited rat intestinal active transport of sugars and amino acids *in vitro* and *in vivo*. Employing equieffective doses, the mode and specificity of the inhibition by the two drugs was similar even under the various experimental conditions. This is remarkable since these doses were chosen only from the results of *in vitro* experiments using everted small-intestinal rings and α-Me-Glc as transport substrate. This behaviour of the drugs certainly suggests common mechanisms for their inhibitory effect.

The results of the present *in vitro* experiments with glibenclamide do not differ greatly from previous *in vitro* findings [14, 15]. In those studies, glucose uptake into the mucosal tissue of everted sacs was inhibited by about 40% by the drug (0.4 mmol/l) during a 1-hr incubation period. This value is comparable to that we obtained with everted rings after 30 min. In addition, previously reported [15] values on the inhibition of the mucosal-to-serosal transport of glucose in everted sacs of about 50% (glibenclamide concentration 0.15 mmol/l) after a 30-min incubation period are broadly similar to values

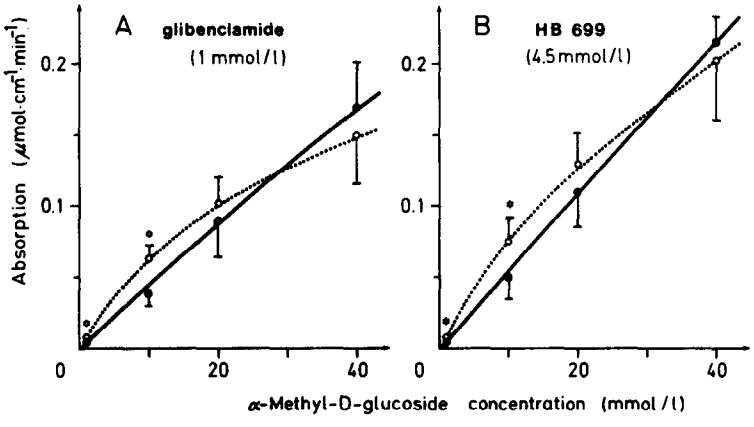

Fig. 4. Effect of small-intestinal perfusion of glibenclamide (A) and HB 699 (B) on the concentration-dependent absorption of methyl α-D-glucoside *in vivo*. A single-pass perfusion technique was used in the mid-jejunum. After perfusion of the drug-free control medium and determination of the control value for one α-Me-Glc concentration, the effect of the drug was determined at this concentration in a subsequent test period in the same animal. Perfusion rate: 0.7 ml/min; control (drug free): O····O; test (drug added): ●——●; M ± SD (N = 7) *P < 0.05 (unpaired Student's *t*-test).

Table 3. *In vivo* effect of glibenclamide and HB 699 on the absorption of leucine

Perfusion medium	Absorption ($nmol \cdot cm^{-1} \cdot min^{-1}$)	
	Control	+ Drug
(A) Glibenclamide (1 mmol/l)		
Leu 1 mmol/l	6.08 ± 1.02	4.02 ± 0.70*
Leu 40 mmol/l	173.6 ± 38.1	192.0 ± 30.5
(B) HB 699 (4.5 mmol/l)		
Leu 1 mmol/l	5.52 ± 0.99	4.12 ± 0.82*
Leu 40 mmol/l	185.9 ± 26.4	200.6 ± 20.4

A single-pass perfusion technique was used in the mid-jejunum. After perfusion of the drug-free control medium and determining of the control value, the effect of the drug was determined in a subsequent test period in the same animal (for further details see Methods). M ± SD (N = 7); *$P < 0.05$ (unpaired Student's *t*-test).

of about 80% inhibition of the transport of α-Me-Glc under the present conditions. A recent study employing the sulfonylurea glipentide [22]—a drug structurally resembling glibenclamide [23]—also showed comparable inhibition of galactose transport in rat small intestine *in vitro*. Differences between the results including the corresponding interpretations of the present and previous studies [14, 15, 22] are certainly due to the use of different experimental methods and procedures. In this connection it may be of some significance that we used mannitol as an extracellular space marker for our *in vitro* and PEG 4000 as an impermeable volume marker for our *in vivo* experiments.

As shown by our experiments, the longer the exposure of the tissue to the drugs the lower is the drug concentration required to produce the same degree of inhibition. Therefore it is plausible that the previous investigations [14, 15, 22] demonstrated inhibition of small intestinal sugar transport at lower drug concentrations than those required for the short-term experiments in the present study.

The inhibition of the sugar or amino acid transport or uptake was not able to be reversed either *in vitro* or *in vivo* under the conditions employed. These findings are in contrast to a certain extent with a previous interpretation of the results of everted-sac experiments [14] which suggested a slow restoration of the glucose transport after omitting glibenclamide from the incubation medium. However, in these studies no distinction was made between active and passive transport routes. Our results indicate rather that a certain drug accumulation might be required for the inhibition of active transport processes and for a simultaneous increase of the passive diffusion.

The short-term incubations with everted rings performed in our study were thought to provide some information on the effect of the drugs on the active, Na^+-dependent uptake step. The mixed type of inhibition caused by the two drugs means that the affinity of the transport substrates for the carrier (K_m effect) as well as the activity of the carrier itself (V_{max} effect) are affected. An altered intracellular metabolism of the transport substrate, as suggested previously [14, 15], may not be the reason because the present kinetic experiments were performed with methyl α-L-glucoside, a non-metabolisable D-glucose analog. Since a direct effect of the drugs on the sugar-binding moiety of the carrier, as seen for the inhibition of sugar uptake by phlorizin [24], appears unlikely due to the structural dissimilarity between sugar and drug, an interference with the sodium-binding site might be proposed. Recently, it was discussed whether the Na^+ concentration available for active, Na^+-dependent transport processes in the intestine in general may affect both transport parameters (K_m and V_{max}) making mixed-type kinetics unavoidable [25–27]. The interference proposed may not be merely a displacement of sodium ions which is thought to produce only a K_m effect, e.g. seen with harmaline [28, 29], or polycations [17], but could be of a more unspecific nature, e.g. alteration of the carrier or the membrane, or both, as suggested for the effect of certain arylalkylamines [29]. An unspecific interaction with membrane transport systems may also be indicated by the inhibition of fructose uptake, a mechanism of facilitated diffusion not mediated by a Na^+-dependent carrier mechanism.

Not only *in vitro*, but also *in vivo* transport of methyl α-D-glucoside was inhibited by both drugs. A tentative analysis of the perfusion experiments revealed an inhibition of the active transport component whereas passive diffusion was enhanced so that above a solute concentration of about 30 mmol/l a slight increase in the absorption of α-Me-Glc and leucine was observed. These findings are not necessarily in contrast to a previous study [22] which demonstrated inhibition of small-intestinal sugar transport at a galactose concentration of 80 mmol/l by glipentide. The conditions, i.e. 10-min multiple-pass perfusion experiments, were not comparable to the ones employed in our study; due to the short-term perfusion, the results may reflect the inhibition of sugar uptake into the tissue rather than the effect on the entire transport process.

In conclusion, HB 699, a non-sulfonylurea drug, inhibited the small-intestinal absorption of sugars and amino acids in the same way as the sulfonylurea glibenclamide. The effect was predominantly an inhibition of carrier-mediated and active transport; in addition, as seen particularly *in vivo*, passive diffusion was enhanced in the presence of the two drugs. These properties may not be of great significance for

glibenclamide, as pointed out previously [14], since the average single oral dose of 2–5 mg is unlikely to cause luminal concentrations high enough to produce effects as seen in our study. However, a single oral dose of HB 699 may be as high as 500 mg resulting in luminal concentrations sufficient to impair active transport of sugars, thereby contributing to the blood-glucose lowering properties of this drug.

In this connection it is worth mentioning recent findings about the action of HB 699 in humans.* In these studies it was shown that, in contrast to glibenclamide, HB 699 counteracted hyperglycemia induced by an oral glucose load without stimulating insulin release. Active transport in the small intestine may play an important role in triggering and regulating gastrointestinal hormone responses to ingested food. Thus, it was shown that the secretion of gastric inhibitory polypeptide (GIP), a hormone which potentiates the release of insulin, was inhibited by blocking the small-intestinal, active transport of glucose [30] by phlorizin or prenylamin. HB 699 might act in a similar fashion, at least partly explaining the findings mentioned above. Finally it is conceivable that the hypoglycemic mechanisms of oral antidiabetic drugs—particularly those of the class of benzoic acid derivatives such as HB 699—have small-intestinal components which might be as important as their effects on the pancreas.

REFERENCES

1. S. Raptis and E. F. Pfeiffer, *Therapiewoche* **21**, 578 (1971).
2. H. S. Seltzer, *Ann. Rev. Med.* **31**, 261 (1980).
3. R. S. Yalow, H. Black, M. Villazon and S. A. Berson, *Diabetes* **9**, 356 (1960).
4. A. Loubatières, M. M. Mariani and J. Chapal, *Diabetologia* **6**, 457 (1979).
5. J. M. Feldman and H. F. Lebovitz, *Arch. intern. Med.* **123**, 314 (1969).
6. T. G. Skillman and J. M. Feldman, *Am. J. Med.* **70**, 361 (1981).
7. R. D. Fussgänger and C. Wojcikowski, *Diabetologia* **13**, 394 (1977).
8. K. Geisen, V. Hitzel, V. E. Hrstka, W. Pfaff, G. Regitz and R. Weyer, *Arzneim.-Forsch./Drug Res.* **28**, 1081 (1978).
9. S. Efendić, F. Enzmann, M. Gutniak, A. Nylén and M. Zoltobrocki, *Acta Endocrin. (Kbh)* **98**, 573 (1981).
10. G. Ribes, E. R. Trimble, J. P. Blayac, C. B. Wollheim, R. Puech and M. M. Loubatierès-Mariani, *Diabetologia* **20**, 501 (1981).
11. C. Rufer and W. Losert, *J. med. Chem.* **22**, 750 (1979).
12. M.-G. Garrino, W. Schmeer, M. Nenquin, H. P. Meissner and J. C. Henquin, *Diabetologia* **28**, 697 (1985).
13. W. F. Caspary and W. Creutzfeldt, *Diabetologia* **7**, 379 (1971).
14. J. D. Teale and A. H. G. Love, *Biochem. Pharmac.* **21**, 1839 (1972).
15. J. D. Teale and A. H. G. Love, *Biochem. Pharmac.* **22**, 997 (1973).
16. B. Elsenhans, R. Blume and W. F. Caspary, *Naunyn-Schmiedeberg's Archs Pharmac.* **329**, Suppl., R67 (1985).
17. B. Elsenhans, R. Blume, B. Lembcke and W. F. Caspary, *Biochim. biophys. Acta.* **727**, 135 (1983).
18. W. F. Caspary, N. R. Stevenson and R. K. Crane, *Biochim. biophys. Acta* **193**, 169 (1969).
19. F. Alvarado and A. Mahmood, *Biochemistry* **13**, 2882 (1974).
20. T. H. Wilson and G. Wiseman, *J. Physiol.* **123**, 116 (1954).
21. B. Elsenhans, D. Zenker, W. F. Caspary and R. Blume, *Gastroenterology* **86**, 645 (1984).
22. P. L. Gómez, J. M. Planas, M. Moretó and J. Bolufer, *Rev. esp. Fisiol.* **36**, 445 (1980).
23. J. L. Chanal, M. T. Calmette, M. Khiat, V. Rimbau and A. Vera, *Arzneim.-Forsch./Drug Res.* **27**, 852 (1977).
24. F. Alvarado and R. K. Crane, *Biochim. biophys. Acta* **56**, 170 (1962).
25. F. Alvarado and M. Lherminier, *J. Physiol. (Paris)* **78**, 131 (1982).
26. J. W. L. Robinson and G. Van Melle, *J. Physiol.* **344**, 177 (1983).
27. A. B. R. Thomson, *Can. J. Physiol. Pharmac.* **61**, 1129 (1983).
28. F. V. Sepúlveda, M. Buclon and J. W. L. Robinson, *Naunyn-Schmiedeberg's Archs Pharmac.* **295**, 231 (1976).
29. B. Elsenhans, R. Blume, B. Lembcke and W. F. Caspary, *Biochim. biophys. Acta* **813**, 25 (1985).
30. R. Ebert and W. Creutzfeldt, in *Delaying Absorption as a Therapeutic Principle in Metabolic Diseases* (Eds. W. Creutzfeldt and U. R. Fölsch), p. 126. Georg Thieme Verlag, Stuttgart-New York (1983).

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