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Inhibition of Bile Salt Absorption by Blood-Sugar Lowering Biguanides

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Summary. The effect of blood sugar lowering biguanides (phenethyl-, butyl- and dimethylbiguanide) upon jejunal and ileal transport of bile salts (tauro- and glycocholate) was tested in rat small intestine by an *in vitro* technique. Biguanides inhibited active transport of bile salts in the ileum, but did not affect diffusional absorption of bile salts in the jejunum. The inhibitory effect was time-dependent and not reversible under *in vitro* incubation conditions, suggesting that biguanides must enter intestinal mucosal cells in order to exert their inhibitory action on active transport of glucose analogues, amino acids, calcium and bile salts. Since biguanides achieve high tissue concentrations in the small intestine even after parenteral administration, inhibition of ileal bile salt reabsorption by biguanides could possibly explain the lipid- and cholesterol-lowering effect of these oral antidiabetic drugs.

Key words: Bile salt absorption, biguanides, phenformin, buformin, metformin, active transport, bile salt malabsorption.

The mechanism of the blood sugar lowering action of bigunanides is not completely understood [3, 20, 22, 26]. An insulin-potentiating effect of biguanides upon glucose uptake by muscle [8, 9], inhibition of gluconeogenesis in the liver [1, 3, 16, 17, 20, 23, 36] and inhibition or delay of intestinal absorption [2, 3, 4, 6, 7, 11, 12, 13, 30, 21, 27, 29, 30, 41] have been considered as the possible mechanism. Biguanides inhibit intestinal absorption of sugar *in vitro* [11, 27, 29, 30, 41] and *in vivo* [2, 6, 7, 21], active transport of amino acids [12, 13] and calcium [12, 20] *in vitro* as well as vitamin B_{12}-absorption in patients on metformin [4, 5, 20, 37, 38] and phenformin [38]. The latter observation together with the findings of an increase of stool weight [4] and frequency [31], a slightly increased faecal fat excretion [4] and a lowering of serum cholesterol [3, 4, 19, 25, 26, 32, 34, 35] suggests that biguanides might affect transport processes confined to the distal small intestine. Since active transport of bile acids, in contrast to sugar and amino acid absorption, is confined to the ileum [24, 28], the effect of biguanides upon the active transport of bile acids in rat ileum has been examined by an *in vitro* technique.

Material and Methods

Materials

^{3}H-Na-taurocholate was obtained from New England Nuclear, ^{14}-C-glycyl-cholic acid from The Radiochemical Centre, Amersham. Taurocholate (Serva, Heidelberg) and glycocholate (Serva, Heidelberg) contained less than 0.1% impurities as previously reported [14, 15]. Phenethylbiguanide was supplied by Hoechst Pharmaceutical Company, Frankfurt-Hoechst, butylbiguanide was kindly supplied by Dr. R. Beckmann (Chemie Grünenthal, Stolberg, Rhld.) and dimethylbiguanide was provided by Dr. H. Haury, München.

Bile Acid Transport Technique

Female, Wistar rats (120 ± 10 g) were kept on a lab chow diet and fasted overnight with free access to water before the experiments were performed. Intestinal transport of bile salts was measured with the tissue accumulation technique of Crane and Mandelstam [18] and the everted sac technique of Wilson and Wiseman [40] with modifications as reported earlier [11, 12, 15]. Small intestinal tissue 15 cm proximal to the ileocecal valve was considered to represent ileum; intestinal segments 35–50 cm past the pylorus are referred to as 'mid-intestine' (= jejunum). 200–300 mg wet weight of tissue were incubated in Erlenmeyer flasks with 10 ml Krebs-Henseleit phosphate buffer under pure oxygen and the appropriate additions of substrate and biguanides. The serosal compartment of the everted sacs, measuring 7 cm in length, was filled with 0.5 ml of the same medium used on the mucosal side. The incubation medium contained tauro- or glycocholate at a concentration of 0.2×10^{-3}M and labelled ^{3}H-Na-taurocholate or ^{14}C-glycocholate to give 50000–100000 dpm/5 ml incubation medium.

Results are expressed as:

$$\text{per cent filling} = 100 \times \frac{\mu\text{moles/ml tissue water}}{\mu\text{moles/ml mucosal medium}}$$

assuming a tissue water content of 80% of the tissue wet weight [10, 15, 18]. Extracellular space corrections were performed by the use of ^{3}H- or ^{14}C-D-

mannitol [10]. In everted sac experiments results are expressed as serosal/mucosal (S/M) concentration ratio [40]. A value of $>100\%$ filling or a S/M-ratio >1 means accumulation of the substrate either in the tissue or in the serosal compartment against a concentration gradient.

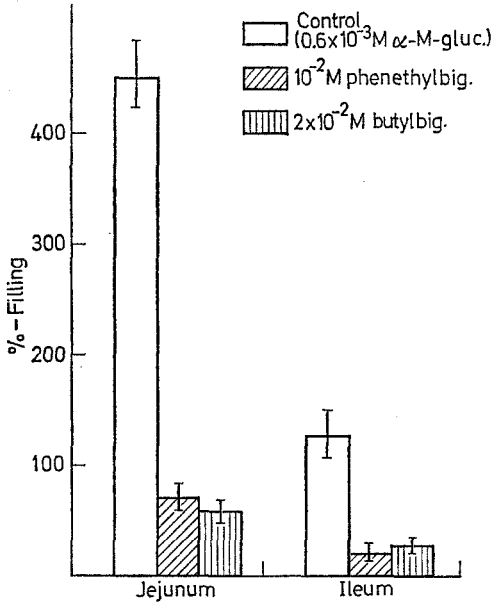

Fig. 1. Inhibitory effect of biguanides upon active transport of α-methyl-glucoside in rat jejunum and ileum. Segments of rat small intestinal tissue were incubated for 30 min with α-methyl-glucoside (0.6×10^{-3} M) in presence and absence of phenethyl- and butylbiguanide. Results are means ± SEM (n = 7)

Results are presented as means ± SEM. Statistical analyses were performed using the student t-test.

Results

In order to compare the effect of biguanides upon jejunal and ileal active transport processes tissue uptake of an actively transported glucose analogue, α-methyl-glucoside, was measured in the presence of biguanides by jejunal and ileal small intestine (Fig. 1). Phenethyl- and butylbiguanide inhibited active transport of the non-metabolizable α-methyl-glucoside in the ileum and the jejunum. α-methyl-glucoside was accumulated to a much higher degree in the jejunum than in the ileum (Fig. 1). Tissue uptake of conjugated bile salts (tauro-and glycocholate) occurred in the ileum against a concentration gradient, whereas minimal absorption took place in the jejunum by the mechanism of ionic and nonionic diffusion [24, 28] (Fig. 2). Biguanides (phenethyl-, butyl- and dimethylbiguanide) markedly inhibited active transport of bile salts in the rat ileum but did not affect the diffusional process in the jejunum (Fig. 2).

The onset of the inhibitory effect was observed 5 min after incubation by the simultaneous addition of substrate and biguanides (Fig. 3). The inhibitory effect increased with further incubation time. The inhibitory effect persisted even after preincubation with biguanides, subsequent washing of the tissue and reincubation with the substrate in a medium without

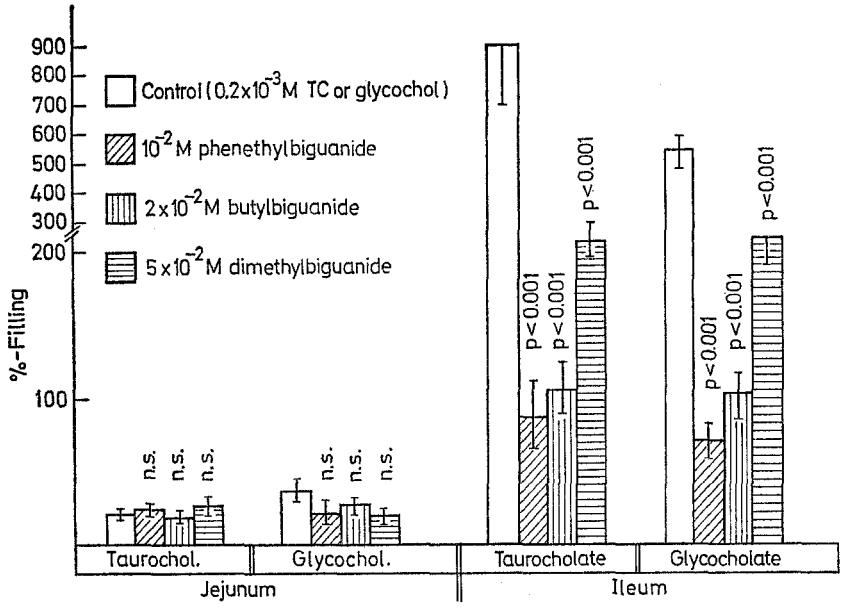

Fig. 2. Tissue uptake of bile salts in rat jejunum and ileum in the presence of biguanides. Segments of rat jejunum and ileum were incubated for 30 min in Krebs-Henseleit phosphate buffer with 0.2×10^{-3} M tauro- (TC) or glycocholate. Biguanides (phenethyl-, butyl- and dimethylbiguanide) were added simultaneously with the substrates. Results are means ± SEM (n = 6)

Table 1. *Effect of preincubation with biguanides upon subsequent tissue uptake of bile salts*

Preincubation conditions	Tissue-uptake after reincubation with 0.2×10^{-3} M taurocholate	%-inhibition
Buffer	911.0 ± 80	—
10^{-2} M Phenethylbiguanide	39.5 ± 6*	95.6
2×10^{-2} M Butylbiguanide	65.0 ± 8*	92.87
4×10^{-2} M Dimethylbiguanide	110.0 ± 12*	88.0

Segments of ileal rat intestine were incubated for 20 min under the conditions listed under 'preincubation conditions'. Tissue was then rinsed for 20 sec with buffer and reincubated in a Krebs-Henseleit phosphate buffer medium with 0.2 mM taurocholate in the absence of biguanides for further 20 min. Results are means ± SEM (n = 6)

* $p < 0.001$

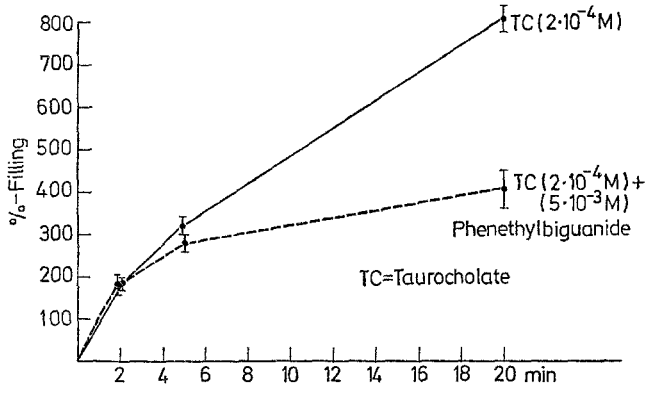

Fig. 3. Time-dependent inhibitory effect of phenethyl-biguanide upon tissue uptake of taurocholate. Tissue uptake of taurocholate by segments of rat ileum was measured in the presence of phenethyl-biguanide (5×10^{-3} M) after 2, 5 and 20 min incubation. Results are means ± SEM (n = 5)

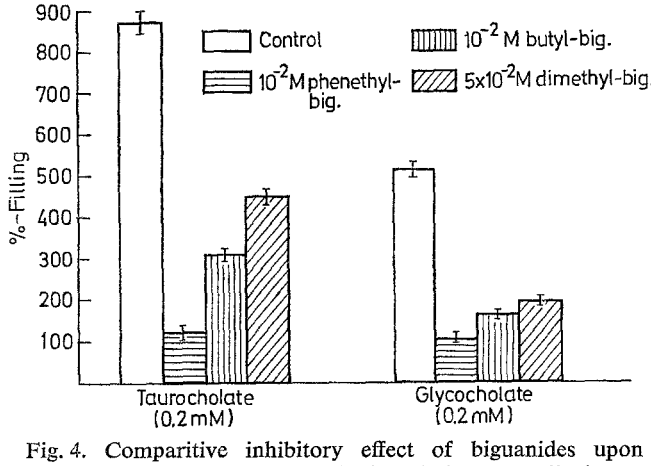

Fig. 4. Comparitive inhibitory effect of biguanides upon active transport of tauro- and glycocholate. Rat ileal segments were incubated with either 0.2×10^{-3} M taurocholate or 0.2×10^{-3} M glycocholate in the absence or presence of biguanides. Results are means ± SEM (n = 7)

the presence of biguanides (Table 1). The inhibitory effect of biguanides upon bile salt absorption was exerted by all biguanides tested (Fig. 4). Phenethylbiguanide was a more potent inhibitor of bile salt absorption than butylbiguanide. Five to ten times higher

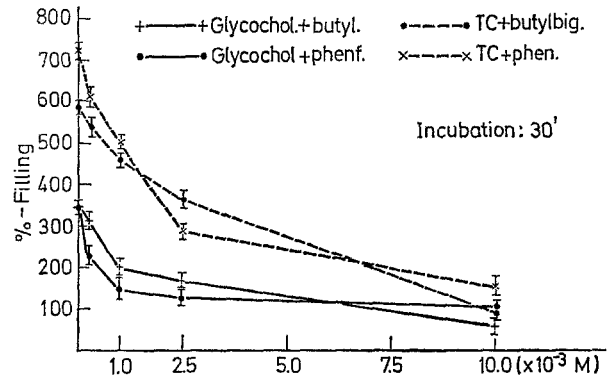

Fig. 5. Concentration-dependent inhibitory effect of phenethyl- and butylbiguanide upon transport of tauro- or glycocholate in rat ileum. Segments of rat ileal tissue were incubated with tauro- or glycocholate (0.2×10^{-3} M) in the presence of increasing concentrations of biguanides. Incubation time (30 min). Results are means ± SEM (n = 5)

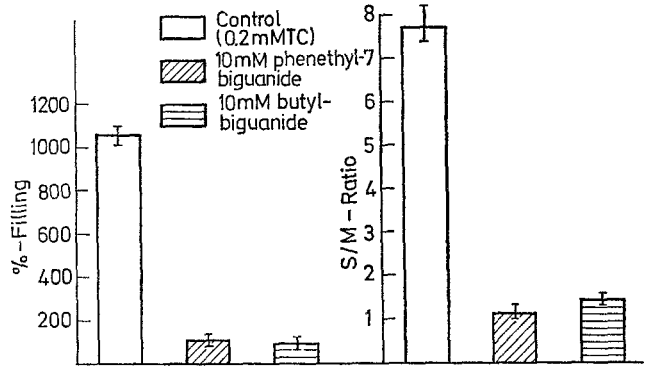

Fig. 6. Effect of biguanides upon tissue uptake and serosal accumulation of taurocholate in rat ileum. Sacs of everted rat ileum were incubated for 45 minutes in Krebs-Henseleit phosphate medium: Mucosal medium: 0.2×10^{-3} M taurocholate (TC) ± 10 mM phenethyl- or butylbiguanide. Serosal medium: 0.2×10^{-3} M taurocholate. Accumulation of taurocholate in the tissue compartment of the everted sac preparation is shown on the left side, accumulation of TC in the serosal compartment on the right side of the graph. Results are means ± SEM (n = 5)

concentrations of dimethylbiguanide were required to achieve an equal inhibitory effect to phenethyl- or butylbiguanide (Fig. 4). The minimal effective inhibitory dose of phenethyl- and butylbiguanide on tissue accumulation of tauro- and glycocholate was 2.5×10^{-4}M (Fig. 5). Since the inhibitory effect increased with incubation time (Fig. 3), minimal in-

hibitory concentrations might be even lower at longer incubation intervals.

Mucosal ⟶ serosal transport of taurocholate was as markedly inhibited by phenethyl- and butylbiguanide as tissue uptake (Fig. 6). A decrease of serosal accumulation of taurocholate was observed by mucosal addition of biguanides (Fig. 6).

Discussion

The assumption that biguanides inhibit active transport systems in general is confirmed by this and other studies: active transport of calcium in the duodenum [12, 20], jejunal and ileal transport of sugars [2, 6, 7, 11, 12, 21, 27, 29, 30, 41] (Fig. 1) and amino acids [7, 12, 20] as well as bile salt absorption in the ileum, whereas, diffusional transport is not affected (Fig. 2). The delayed onset of the inhibitory affect after the simultaneous introduction of substrate and inhibitor to the mucosal incubation medium and the persistence of the inhibitory effect after preincubation with biguanides and subsequent thorough washing suggests that biguanides must enter the intestinal tissue in order to exert their inhibitory effect upon active transport. The biguanide effect upon active transport systems is most likely mediated by the known inhibitory action of these antidiabetic drugs upon mitochrondrial respiration [3, 22]. Minimal inhibitory concentrations of phenethyl- and butylbiguanide were 2.5×10^{-4}M. Whether similar concentrations might be achieved in the ileum after therapeutic doses is not known. However, recent intestinal perfusion studies in man have shown that biguanides in therapeutic doses inhibited glucose absorption in man [2, 7]. Since metformin (dimethylbiguanide) and phenformin induce B_{12} malabsorption [4, 5, 37, 38] and an increase of faecal weight [4] and stool frequency [31], it might be assumed that biguanides can achieve tissue concentrations in the ileum after therapeutic doses sufficient to affect bile salt reabsorption. Biguanides achieve high tissue concentrations in the small intestine even after parenteral administration [3].

The confirmation that therapeutic doses of biguanides are able to inhibit ileal reabsorption of bile salts in man could explain possibly the cholesterol-lowering effect observed during treatment of diabetics with biguanides [3, 19, 25, 26, 32, 34, 35].

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