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Effects of two glucose absorption inhibitors: phenformin and 43-522 on hepatic gluconeogenesis

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The glucose absorption inhibitors, Phenformin and 3-phenylpropylaminoguanide HCl (SaH 43–522) have different effects on hepatic gluconeogenesis. Unlike phenformin, 43–522 in vivo does not inhibit hepatic gluconeogenesis; inhibition occurred only when 43–522 was added directly to a liver perfusion system. This suggests that when 43–522 is administered in vivo it is metabolized to an agent that does not inhibit hepatic gluconeogenesis whereas phenformin is not metabolized, and does inhibit gluconeogenesis. This is an advantage of 43–522 since it thereby specifically inhibits intestinal glucose absorption without a potential hypoglycaemic effect due to the suppression of gluconeogenesis.

3-Phenylpropylaminoguanidine HCl (43–522), like phenformin, is a potential oral anti-obesity agent (Ho et al 1977) and lowers the oral glucose tolerance curve in Rhesus monkeys. 43–522 causes no significant change in normal fasting blood sugar concentrations nor is it active when administered parenterally. Furthermore, it produces inhibition of glucose transport only when placed on the mucosal side of the intestine in in vitro studies. Phenformin, on the other hand, is active parenterally when placed on either side of the intestine in in vitro studies.

Inhibition of gluconeogenesis by phenformin has been considered to be one of the major causes of hypoglycaemia in normal animals. Since 43–522 did not show a hypoglycaemic effect in normal animals, we were prompted to investigate its actions compared with phenformin on hepatic gluconeogenesis.

METHODS

Animals. Male guinea-pigs, 200–300 g, of Hartley strain from Perfection Breeders, Douglasville, Pennsylvania, were fasted for 48 h before experiments. This species was used because: phenformin decreases hepatic gluconeogenesis both in vivo (Williams et al 1957) and in vitro (Altschuld & Kruger 1968), and it is sensitive to phenformin and 43–522 since marked inhibition of glucose absorption by both drugs has been demonstrated (Ho et al 1977).

Influence on DL-*alanine-induced gluconeogenesis.* Groups of five animals were treated with phenformin or 43–522 (20 mg kg^{-1} by mouth); two other groups received water. Thirty min later the treated groups and one of the control groups were injected with 10% DL-alanine in 0·9% NaCl (saline) (2 g kg^{-1} s.c.). The other control group was injected with saline. Four h later the animals were killed and blood sugar concentrations measured by Auto-Analyzer (Technicon Method N-2b). The liver glycogen concentrations were measured according to Seifter et al (1950).

Effect on nitrogen excretion of glucose primed guinea-pigs as an index of deamination in gluconeogenesis. Animals were kept in plastic metabolism cages without food or water during the experiment. The blood sugar concentrations were maintained by s.c. injections of 25% glucose solution at 7.30 and 17.30 h. Phenformin or 43–522 (20 mg kg^{-1} by mouth) was given each day at 12.30 h. At 14.30 h day 3 of the study, the animals were killed and blood sugar, plasma urea nitrogen (Technicon Method N-1C) and liver glycogen concentrations were determined. Total urine, collected in a flask containing 2 ml of M H_2SO_4, was used for the determination of total nitrogen according to Minari & Zilversmit (1963).

Effect on hepatic gluconeogenesis in vitro studies. Isolated livers were perfused via the portal vein by recycling 100 ml bicarbonate buffer (Krebs-Ringer; pH 7·4) that had been equilibrated with 95% O_2 and 5% CO_2. The buffer contained 100 mg glucose and 100 mg pyruvate as the gluconeogenic substrate. A symmetrical perfusion circuit containing two upper reservoirs with a three-way stopcock immediately above and below the liver platform permitted the instantaneous change to fresh medium without interruption of perfusion. Glucose in the perfusate was measured by Auto-Analyzer and pyruvate was determined enzymatically according to Bergmeyer (1963).

Effect on hepatic gluconeogenesis in vivo studies. Livers, removed from guinea-pigs one h after they had been treated with phenformin or 43–522 (20 mg kg^{-1} by mouth), were perfused and the perfusate

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analysed as described above. The viability of the perfused livers was judged by bile flow and perfusion rate.

Statistical analysis. Data were analysed using the Student's *t*-test, as described by Bernstein & Weatherall (1952).

RESULTS

The influence of 43–522 and phenformin on DL-*alanine-induced gluconeogenesis.* Alanine alone, or with phenformin or 43–522 had no significant effect on blood sugar concentrations. Liver glycogen was increased from 0·81 ± 0·29 mg g^{-1} mean ± s.e.m. to 2·74 ± 0·50 mg g^{-1} when DL-alanine was given. Pretreatment with phenformin (20 mg kg^{-1} by mouth) prevented (0·27 ± 0·17 mg g^{-1}, $P < 0·001$) the increase in liver glycogen produced by alanine; 43–522 did not affect this response.

Effect on nitrogen excretion of glucose-primed guinea-pigs. Neither drug had a significant effect on blood sugar concentrations in glucose-primed guinea-pigs (Table 1). Phenformin caused a 229% increase in plasma urea nitrogen; no such effects were found in 43–522-treated animals. Liver glycogen and urinary nitrogen excretion (0 to 52 h) were greatly decreased in animals receiving phenformin (Table 2) suggesting decreased gluconeogenesis.

Effect on hepatic gluconeogenesis when the drugs were added to perfusate. Fig. 1 (left panel) shows a typical experiment in which the liver was perfused with a medium containing pyruvate. The pyruvate uptake and the release of glucose are shown over 3 h during which fresh perfusate was introduced hourly. 43–522 (5 mg %) or phenformin (10 mg %) added to the perfusate after a control period reduced glucose release and pyruvate uptake indicating inhibition of gluconeogenesis.

Effect on hepatic gluconeogenesis by livers of pre-treated animals. Glucose release and pyruvate uptake

Table 1. Effect of DBI and SaH 43–522 on glucose primed guinea-pigs.

Treatment	Blood sugars mg % (mean ± s.e.m.)	Plasma urea nitrogen mg % (mean ± s.e.m.)	Liver glycogen mg g^{-1} (mean ± s.e.m.)	Urinary nitrogen excretion (0–52 h) mg (mean ± s.e.m.)
Control	93 ± 5 (4)†	35·7 ± 3·7 (7)	24·8 ± 5·0 (7)	329 ± 67 (5)
Phenformin (20 mg kg^{-1})	106 ± 20 (3)	81·9 ± 6·6*** (7)	3·9 ± 2·3** (7)	144 ± 13* (6)
SaH 43–522 (20 mg kg^{-1})	90 ± 8 (3)	40·0 ± 4·6 (5)	24·9 ± 4·9 (3)	367 ± 44 (4)

† Number of animals in parentheses.
* Statistically significant at $P < 0·05$. ** Statistically significant at $P < 0·01$. *** Statistically significant at $P < 0·001$.

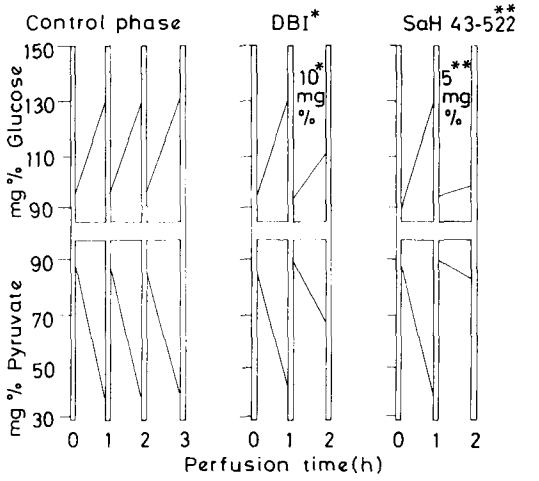

FIG. 1. In vitro effects of phenformin (DBI) (10 mg %) and 43–522 (5 mg %) on gluconeogenesis from pyruvate in perfused guinea-pig liver.

Table 2. Glucose release and pyruvate uptake in perfused liver of phenformin and 43–522 pretreated guinea-pigs.

Treatment	Glucose release: Initial glucose mg % (mean ± s.e.m.)	Glucose release: Final glucose mg % (mean ± s.e.m.)	Glucose release: % change in 60 min	Pyruvate uptake: Initial pyruvate mg % (mean ± s.e.m.)	Pyruvate uptake: Final pyruvate mg % (mean ± s.e.m.)	Pyruvate uptake: % change in 60 min
Control	94·5 ± 3·6	134·3 ± 9·3	↑ 42** (6)†	92·8 ± 3·9	34·1 ± 2·2	↓ 63*** (6)
Phenformin (20 mg kg^{-1})	97·5 ± 5·7	99·8 ± 9·7	↑ 2 (4)	95·0 ± 3·1	64·7 ± 17·2	↓ 32 (3)
SaH 43–522 (20 mg kg^{-1})	95·3 ± 1·9	132·3 ± 11·3	↑ 39** (7)	92·9 ± 4·6	38·2 ± 4·2	↓ 59*** (7)

† Number of determinations in parentheses.
** Statistically significant at $P < 0·01$. *** Statistically significant at $P < 0·001$.

in the livers of 43–522-pretreated animals did not differ from those of the controls (Table 2) whereas phenformin pretreatment produced marked inhibition of glucose release. Only about 30% of pyruvate in the perfusate was taken up by the livers pretreated with phenformin (20 mg kg^{-1}) compared with 63 and 59% for control and 43–522 pretreated groups, respectively (Table 2). Gluconeogenesis was indicated by the uptake of pyruvate, its conversion to and subsequent release as glucose by the liver. Phenformin caused a decrease in gluconeogenesis compared with controls, whereas 43–522 had no effect on gluconeogenesis in this test system.

DISCUSSION

Phenformin is widely recognized as an oral antidiabetic agent that inhibits hepatic gluconeogenesis in laboratory animals (Williams et al 1957; Altschuld & Kruger 1968). Its inhibitory effect on glucose absorption by the intestinal mucosa has also been extensively reported (Biro et al 1961; Czyzyk et al 1968; Hollobaugh et al 1970; Kruger et al 1970; Lorch 1971; Caspary & Creutzfeldt 1971; Ho et al 1977). 3-Phenylpropylaminoguanidine HCl (43–522), a potential antiobesity agent, was also found to markedly inhibit glucose absorption; it is two to three times more potent than phenformin (Ho et al 1977). The studies presented here were designed to determine the relative specificity of 43–522 compared with phenformin and show a direct comparison in regard to their hepatic gluconeogenic effect.

In the first study, hepatic glycogen was induced by an exogenous gluconeogenic substrate, DL-alanine. Surprisingly, the alanine injection did not affect the blood sugar concentrations although liver glycogen concentrations were increased markedly relative to the controls. In animals pretreated with phenformin, but not those pretreated with 43–522 (at twice the dose at which 50% of glucose active transport was inhibited as reported by Ho et al 1977), the liver glycogen concentrations were affected by alanine injection. Thus, 43–522, unlike phenformin, neither inhibits alanine-induced gluconeogenesis nor increases glycogenolysis in guinea-pigs.

To investigate whether 43–522 produced a change in gluconeogenesis, the nitrogen balance was studied. The amount of nitrogen excreted in the urine after phenformin treatment was much less than that of controls while there were no significant differences in urinary nitrogen excretion between control and 43–522. This suggests an effect of phenformin on the reduction of deamination as the result of decreased gluconeogenesis, but this effect is not seen with 43–522. The markedly elevated plasma urea nitrogen found in phenformin-treated animals supports this view and confirms the reports of Williams et al (1957) and Tyberghein & Williams (1957). The fact that 43–522 does not affect plasma urea nitrogen again highlights the differences between this drug and phenformin.

When hepatic gluconeogenesis was measured directly using liver perfusion, both 43–522 (5 mg %) and phenformin (10 mg %) added to the perfusate containing pyruvate as the gluconeogenic substrate, diminished glucose release and pyruvate uptake. This indicates both compounds inhibited hepatic gluconeogenesis when the agents were administered in vitro. However, glucose release and pyruvate uptake were unaffected when isolated livers of 43–522-pretreated guinea-pigs were used, while livers from phenformin-treated animals showed inhibited gluconeogenesis. This aspect of phenformin may also contribute to hypoglycaemia in normal animals, whereas the inhibition of glucose active transport by 42–522 in intestinal mucosa appears to be the dominant activity in all species so far tested.

The difference in the effect of 43–522 when added to the perfusate versus the result when it is administered to whole animals suggests the metabolism of 43–522 to a substance which does not affect hepatic gluconeogenesis at some site before the drug reaches the liver. Phenformin, on the other hand, reaches the liver with the ability to inhibit gluconeogenesis.

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