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3-Phenylpropylaminoguanidine·HCl (43-522): A Specific Carbohydrate Inhibitor in Rats (40747)

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It was previously reported that 3-phenylpropylaminoguanidine·HCl (43-522), a potential oral antiobesity agent, inhibited *in vitro* glucose active transport in rats, hamsters, guinea pigs, and dogs (1). This agent also flattened oral glucose tolerance curves in rats and monkeys, but did not affect the blood sugar levels in rats and monkeys when glucose was administered intracardiacly or intravenously. The 43-522 was found to be two or three times more potent than phenethylbiguanide (phenformin or DBI) in inhibiting glucose active transport *in vitro* of rat intestine. The present studies were conducted to define further the effects of 43-522 on galactose, fructose, maltose, lactose, sucrose, starch, methionine, and palmitic acid absorption in rats.

Materials and methods. Animals. Male Wistar rats (body weight 200 ± 10 g) supplied by Royal–Hart Laboratory Animals, New Hampton, New York, were used in the present studies. These animals were maintained on Purina Lab Chow *ad libitum*. They were, however, fasted for 16 to 18 hr before using, unless otherwise noted.

Influence of 43-522 on galactose absorption in rats. One hour after oral administration of H_2O (control), 43-522 (20 and 40 mg/kg) and DBI (40 mg/kg), all rats were given an oral galactose load (5 g/kg) and blood samples were taken for analysis of blood sugar levels (Technicon Method N-2b) by cardiac puncture at 0.5 or 1 hr later.

Effect of 43-522 on fructose-induced hypertriglycemic rats. Hypertriglycemia was induced in rats by incorporating 10% fructose into the drinking water according to Nikkila and Ojala (2). The 43-522 was administered orally to rats just before the animals were given 10% fructose in the drinking water for 24 hr. Twenty-four hours after the drug treatment, the animals were anesthetized with sodium hexobarbital ip (120 mg/kg), and blood was obtained from the carotid artery. Plasma triglyceride levels were determined as described by Timms *et al.* (3).

Effect of 43-522 on the elevated blood sugar levels after maltose, lactose, sucrose, and starch load. Maltose, lactose, sucrose or starch (corn) was intubated to groups of rats 1 hr following oral administration of 43-522 or DBI. Blood samples were taken by cardiac puncture at various time intervals (see Tables 3 and 4) after the disaccharide loads for blood sugar determination using the methods described previously.

Effect of 43-522 on methionine active transport in vitro. Rats were divided into four groups of six each, and were treated orally with 43-522 (20 and 40 mg/kg), DBI (40 mg/kg), or vehicle (H_2O) as control for 2 days. After an overnight fast, on the third day, the animals were given drug treatment. They were then sacrificed by a blow on the head 1 hr after the drug treatment. A piece of the upper jejunum from each animal was used to determine the methionine active transport *in vitro* as described by Ho *et al.* (1). However, in addition to 300 mg% of glucose, L-methionine was also added to the incubation buffer at the concentration of 100 mg%. One hour after incubation at 37°C, the concentrations of both glucose (determined by Auto Analyzer) and methionine (measured according to McCarthy and Sullivan (4)) in the fluids of both mucosal and serosal sides of the everted gut were determined.

[^{14}C]Palmitic acid absorption in vivo. The thoracic ducts of 10 male Wistar rats (275–300 g body wt) were cannulated according to Bollman *et al.* (5). A gastric fistula was also cannulated to each rat.

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Twenty-four hours was given for recuperation and then the animal was placed in the restraining cage, during which time food but not water was withdrawn. The rats were divided into two groups of five each. Group 1 received H_2O and served as control and the other group was given 43-522 (20 mg/kg) intragastrically. One hour later, D-[1-^{3}H]glucose (specific activity 1 μCi/135 mg glucose/ml H_2O) was given intragastrically to all rats at a dose of 1 ml/200 g body wt, immediately followed by [*carboxyl*-^{14}C] palmitic acid)[1] in corn oil (specific activity—2 μCi/4 mg/ml corn oil) at a dose of 0.5 ml/200 g body wt by the same route of administration. One-half hour after the D-[H^3]glucose administration, a blood sample was taken from the tail of the animal for an ^{3}H radioactivity count. Lymph was collected hourly after [*carboxyl*-^{14}C]palmitic acid administration for 24 hr in heparinized tubes. The ^{3}H radioactivity of blood and ^{14}C radioactivity of lymph were counted in a Packard Tri-Carb liquid scintillation spectrometer.[2] For blood, the sample from each rat was used for ^{3}H radioactivity counting by dissolving 0.05 ml blood in 1.5 ml Soluene,[2] after being decolorized by adding three drops of 30% H_2O_2; 15.0 ml of scintillation fluid consisting of 0.6% BBOT[3] in ethanolamine:methanol:toluene (6:44:50, v/v) was added.

To analyze lymph, 0.1 ml lymph was digested in 1.0 ml Soluene,[2] then 15 ml of the same scintillation fluid was added before ^{14}C radioactivity was counted; quenching corrections were made by using an automatic external standard.

Statistical methods. The significance of differences was calculated using Student's *t* test, as described by Bernstein and Weatherall (6).

Results. Effect of 43-522 on monosaccharide absorption. The fourfold elevation in blood sugar level of the rats after an oral galactose tolerance load was significantly lowered by both 43-522 (40 mg/kg) and DBI (40 mg/kg) (Table I). Using the plasma triglyceride rise after fructose as an indirect marker for fructose absorption, it was found that 20 mg/kg of 43-522 did not block fructose absorption but 40 mg/kg did (Table II). In contrast, DBI in a dose of 40 mg/kg had no effect on triglyceride levels.

Effect of 43-522 on disaccharides absorption. As shown in Table III, the blood sugar levels of maltose-, lactose-, and sucrose-loaded rats were increased by 59, 29, and 49 mg%, respectively. In animals pretreated with 43-522 (20 mg/kg), the increment of blood sugar due to maltose was blocked by 30%. Under the same conditions, 43-522 at 40 mg/kg markedly blocked this effect (reduced by 43%), whereas DBI (40 mg/kg) produced a response comparable with 20 mg/kg of 43-522. The inhibitory effect of 43-522 and DBI on the blood sugar after the loading of lactose and sucrose was much less than that found after the loading of maltose. The 43-522 inhibited the hyperglycemia induced by lactose and sucrose to the same degree, i.e., 15–16% at 20 mg/kg and 25–29% at 40 mg/kg. DBI (40 mg/kg) produced the same effect in inhibiting lactose as 43-522 at 20 mg/kg. However, under the same conditions, DBI did not significantly block the increase in blood sugar levels due to oral sucrose administration.

Effect of 43-522 on polysaccharides loaded rats. It can be seen in Table IV that the blood sugar level increased from the normal fasting level of 56 to 107 mg% in the starch-loaded group. Pretreatment with 43-522 at 40 mg/kg resulted in a 40% suppression in this response. The 43-522 was approximately two times more potent than DBI when using inhibition of hyperglycemia induced by corn starch load as the end point. These results are in agreement with those obtained using glucose active transport *in vitro* system (1).

Methionine active transport in vitro. In the control group, 53 mg% of the methionine was transported from the mucosal fluid into the fluid on the serosal side of the everted intestinal preparation. This was suppressed in rats treated with DBI (40 mg/kg). The 43-522 did not affect

[1] Both D-[1-^{3}H]glucose and [carboxyl-^{14}C]palmitic acid were purchased from Schwarz/Mann, Orangeburg, N.Y.

[2] Purchased from Packard Instrument Co., Downers Grove, Ill.

[3] BBOT, 2,5-bis-(5'-tert-benzoxazolyl[2']-thiophene, supplied by Ciba, Summit, N.J.

TABLE I. EFFECT OF 43-522 AND DBI ON GALACTOSE ABSORPTION IN RATS

Treatment (mg/kg)	Load	0.5 hr post galactose load		1 hr post galactose load	
		Blood sugars mg% (mean ± SEM)	Change as[a] % of control	Blood sugars mg% (mean ± SEM)	Change as[a] % of control
H_2O	H_2O	44 ± 2 (9)[b]	—	55 ± 4 (9)	—
H_2O	Galactose	150 ± 6 (11)	—	205 ± 21 (11)	—
43-522 (20)	Galactose	123 ± 13 (11)	↓18	190 ± 11 (9)	↓7
43-522 (40)	Galactose	84 ± 9 (9)	↓44**	116 ± 13 (8)	↓43**
DBI (40)	Galactose	118 ± 7 (10)	↓21*	129 ± 15 (9)	↓37*

[a] Compared with the group of rats which received no medication, but were galactose loaded.
[b] No. of animals in parentheses.
* Statistically significant at $P \approx 0.01$.
** Statistically significant at $P \approx 0.001$.

the methionine transport. Both 43-522 and DBI inhibited glucose transport significantly in this preparation (Table V).

Influence of 43-522 on palmitic acid absorption in vivo. The ^3H radioactivity in the blood of 43-522 (20 mg/kg) treated rats after intragastric administration of D-[1-^3H]glucose was lowered by 87% as shown in Table VI confirming previous results (1). The appearance of ^{14}C radioactivity in chyle after palmitic acid–[^{14}C]carboxyl in corn oil intragastric administration over a period of 24 hr was not different between the control and 43-522 treated group (48.38 and 49.90%, respectively). However, the ^{14}C radioactivity appearing in the lymph collected from the untreated rats was markedly higher than that of the 43-522 treated rats in the first 3 hr of collection (Fig. 1).

It would appear that absorption of this fatty acid was delayed somewhat as the peak of ^{14}C radioactivity in the lymph of 43-522 treated rats appeared at the fourth hour rather than at the third hour for the control group.

It may be concluded that 43-522 at 20 mg/kg, although inhibiting glucose absorption, did not significantly affect fatty acid absorption over a period of 24 hr; however, the data may suggest that a retardation of fatty acid absorption occurred in the 43-522 treated group.

Discussion. Our previous studies indi-

TABLE II. EFFECT OF 43-522 AND DBI ON HYPERTRIGLYCERIMIC RATS

Fructose administered	Treatment[a] (mg/kg)	Plasma triglyceride mg% (mean ± SEM)	Change as[b] % of control
–	None	98 ± 2	—
+	None	155 ± 19	—
+	43-522 (20)	179 ± 38	↑15
+	43-522 (40)	96 ± 11	↓38*
+	DBI (40)	190 ± 28	↑23

[a] Five animals per treatment.
[b] Compared with the rats which received 10% fructose in drinking water for 24 hours, but with no medication.
* Statistically significant at $P \approx 0.02$.

TABLE III. EFFECT OF 43-522 AND DBI ON BLOOD SUGAR LEVELS AFTER DISACCHARIDES LOAD

Disaccharides used (g/kg)	Time of sacrifice following disaccharide load (min)	Treatment[a] (mg/kg)	Blood sugars mg% (mean ± SEM)	Change as % of control
None	—	None	57 ± 2	—
Maltose (2.5)	15	None	116 ± 5	—
		43-522 (20)	81 ± 4	↓30**
		43-522 (40)	66 ± 4	↓43***
		DBI (40)	82 ± 3	↓29***
Lactose (2)	60	None	86 ± 2	—
		43-522 (20)	72 ± 3	↓16**
		43-522 (40)	61 ± 2	↓29***
		DBI (40)	70 ± 4	↓19**
Sucrose (10)	30	None	106 ± 5	—
		43-522 (20)	90 ± 2	↓15*
		43-522 (40)	79 ± 4	↓25**
		DBI (40)	96 ± 5	↓9

[a] Six rats per group.
* Statistically significant at $P \approx 0.01$.
** Statistically significant at $P \approx 0.002$.
*** Statistically significant at $P \approx 0.001$.

TABLE IV. EFFECT OF 43-522 AND DBI ON BLOOD SUGAR LEVELS OF STARCH (2.5 g/kg) LOADED HYPERGLYCEMIC RATS

Pretreatment[a] (mg/kg)	Load	Blood sugars mg% (mean ± SEM)	Change as % of control
None	None	56 ± 1	—
H_2O	Starch	107 ± 6	—
43-522 (20)	Starch	79 ± 3	↓26*
43-522 (40)	Starch	64 ± 2	↓40**
DBI (40)	Starch	81 ± 2	↓24*

[a] Six rats per treatment.
* Statistically significant at $P \approx 0.01$.
** Statistically significant at $P \approx 0.001$.

TABLE V. EFFECT OF 43-522 AND DBI ON GLUCOSE AND METHIONINE TRANSPORT *IN VITRO*

	Glucose transported from mucosa to serosa		Methionine transported from mucosa to serosa	
Treatment *(mg/kg)*	mg% *(mean* ± SEM)	% inhibition	mg% (mean ± SEM)	% inhibition
Control	330 ± 12	—	53 ± 4	—
43-522 (20)	75 ± 12	↓77**	47 ± 7	↓11
43-522 (40)	52 ± 14	↓84**	49 ± 9	↓ 8
DBI (40)	47 ± 16	↓86**	32 ± 5	↓40*

* Statistically significant at $P \approx 0.01$.
** Statistically significant at $P \approx 0.001$.

TABLE VI. EFFECT OF 43-522 ON RADIOACTIVITY IN BLOOD OF RATS AFTER D-1-^{3}H GLUCOSE INTRAGASTRIC ADMINISTRATION

Treatment[a] (mg/kg)	dpm/ml Blood (mean ± SEM)	Change as % of control
Control	4138 ± 577	—
43-522 (20)	552 ± 277	↓87**

[a] Five animals per treatment.
** Statistically significant at ≃ 0.001.

cated that 43-522 markedly inhibited D-glucose absorption in the intestinal wall of most of the laboratory animals used (1). The blood galactose level could be rapidly increased by oral galactose in rats; galactose is metabolized primarily by the liver, kidneys, and intestine but is otherwise nonutilizable to any extent. In the present study, both agents, 43-522, and DBI at 40 mg/kg significantly lowered oral galactose curve.

The method employed in present study to determine the D-fructose absorption was an indirect one. The lowering of plasma triglycerides due to 43-522 (40 mg/kg) in rats did suggest that there was an inhibitory or retarded effect of fructose absorption through the intestinal mucosa.

Studies conducted thus far showed that 43-522 inhibited transport of all hexoses (D-glucose, D-galactose, and D-fructose) tested. While DBI also exhibited the inhibitory effect on glucose and galactose absorption, it did not affect hypertriglycemia induced by incorporating fructose in the animals drinking water. Although this is an indirect measurement of fructose absorption, the data suggested DBI did not influence fructose uptake in rats. This is in agreement with the study by Caspary and Creutzfeldt (7).

The suppression of hyperglycemia produced by oral administration of disaccharides (maltose, lactose, and sucrose) can be explained by the inhibitory activity of 43-522 on the intestinal transport of glucose, galactose, and fructose. On the other hand, DBI at 40 mg/kg produced no significant effect on the hyperglycemia induced by sucrose load. This discrepancy may reflect the lack of effect of DBI on fructose absorption. As expected, both 43-522 and DBI significantly inhibited starch absorption in rats since glucose is the end product of starch hydrolysis in the intestinal tract, and both agents markedly depressed the glucose absorption in rats (1).

Caspary and Creutzfeldt (8) used the everted small intestine ring technique *in vitro* to test amino acid transport in the hamster and found that DBI inhibited active transport of α-aminoisobutyric acid, glycine, L-leucine, L-lysine, L-proline, and

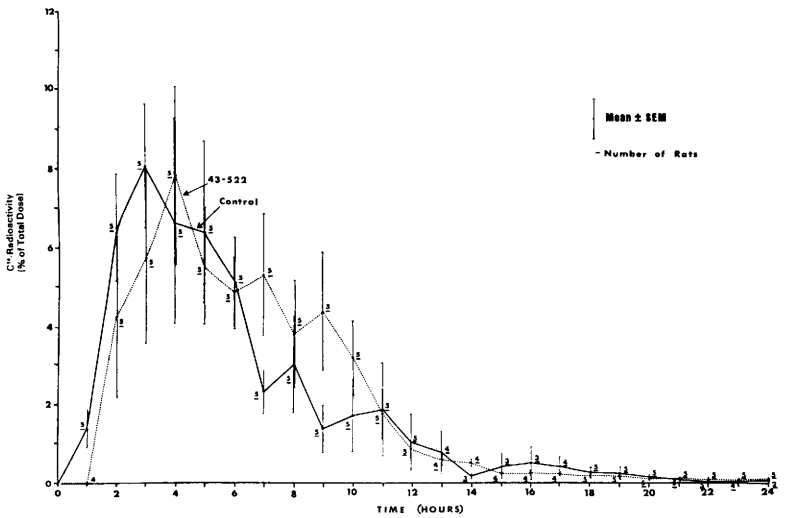

FIG. 1. The influence of 43-522 on ^{14}C radioactivity appearance in thoracic lymph after [carboxyl-^{14}C] palmitic acid intragastric administration in rats.

L-methionine. Amino acid transport in the small intestine exhibits similar features to sugar active transport; for example, they both are energy dependent, have saturation transport kinetic properties, and are also Na^+-dependent (9, 10). In the present study, the amino acid (L-methionine) transport was significantly inhibited in the everted rat intestine *in vitro* after three daily doses of DBI. In contrast, 43-522 showed no effect on methionine transport at doses capable of inhibiting glucose transport. Therefore, it appears that the inhibition of carbohydrate absorption in rats after 43-522 was not due to an effect of those features common to both sugar and amino acid transport, i.e., the dependency of energy (ATP) and so-called "Na^+ pump" mechanisms.

When the appearance of ^{14}C radioactivity in the chyle was used as the index of fatty acid absorption 43-522 had no significant effect. These data indicated that 43-522, unlike DBI, was rather specific in inhibiting carbohydrate absorption in rats.

In addition, experimental differences between these two drugs have been: First, 43-522 is active in *in vitro* glucose active transport when the drug is present in mucosal fluid only, whereas DBI must be present in both mucosal and serosal sides of the incubation fluids (1); and second, the hepatic gluconeogenesis is inhibited when 43-522 and DBI are added into the perfusates, but, unlike DBI, the hepatic gluconeogenesis is not altered when the liver is isolated from 43-522 pretreated animals (unpublished data).

Summary. Potential antiobesity oral agent 3-phenylpropylaminoguanidine·HCl (43-522) was further investigated for its effects on carbohydrates other than glucose, amino acids, and fatty acid absorption in rats. The present experiments were conducted to evaluate its action on absorption of galactose, maltose, lactose, sucrose, and starch. Fructose absorption, as measured by hypertriglycemia was prevented by 43-522 treatment. The 43-522 did not affect the active amino acid (methionine) transport *in vitro*. The fatty acid (palmitic acid) absorption was not significantly influenced by 43-522 treatment as the total ^{14}C radioactivity recovered over a period of 24 hr in thoracic duct lymph was unchanged. Therefore, 43-522, 3-phenylpropylaminoguanidine·HCl, is a useful antiobesity agent which inhibits carbohydrate absorption in the rat.

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