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EFFECT OF CHLORPROMAZINE ON RAT TISSUE UPTAKE OF ^{14}C-3-*O*-METHYL-D-GLUCOSE*

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Abstract—The influence of chlorpromazine (CPZ) pretreatment on tissue distribution of ^{14}C-3-*O*-methyl-D-glucose (3-MG), a nonutilizable glucose analog, has been investigated in rats. CPZ pretreatment reduced the uptake of label in diaphragm and elevated serum levels when ^{14}C-3-MG was administered intraperitoneally (i.p.). No significant effects were observed in brain and posterior tibialis muscle when tissue/serum ratios for each were evaluated. However, no CPZ-induced changes in serum or tissue levels were observed when ^{14}C-3-MG was intravenously administered. Furthermore, it was found that i.p. administration of CPZ promotes the absorption of both 3-MG and glucose from the peritoneal cavity. The data suggest that the reduction of glucose tolerance in rats after CPZ pretreatment is not due to a direct peripheral effect of CPZ on the permeability of tissue to glucose.

HYPERGLYCEMIA and glucose intolerance after the administration of chlorpromazine (CPZ) have been demonstrated in several species including man.[1–7] The mechanism of acute transient CPZ-induced hyperglycemia is, in part, indirect and mediated through the release of epinephrine from adrenal chromaffin tissue.[4,8,9] Acute CPZ-induced glucose intolerance, on the other hand, has been shown to be unaffected by bilateral adrenalectomy.[9,10] It has been suggested that the CPZ-induced alteration of carbohydrate metabolism may be due to changes in the permeability of membrane tissues to glucose.[9–11] The object of the present study was to investigate the effects of CPZ on sugar transport in rats. In order to circumvent possible effects of CPZ on sugar utilization, the results of which might appear to be an alteration of sugar transport, 3-*O*-methyl-D-glucose (3-MG) was employed in this study. This glucose analog has been shown to be nonmetabolizable,[12–14] insulin sensitive,[15,16] and to compete with glucose for transport into several tissues.[15–19]

METHODS

Male albino rats (Laboratory Supply Company, Indianapolis) weighing 110–175 g were used throughout the study. Prior to experimentation, rats were housed 10–12 animals per cage with free access to food and water. The animals were allowed a minimum of 3 days' acclimation to laboratory conditions, then were fasted 20–24 hr prior to experimentation.

Rats were pretreated with CPZ hydrochloride (15 mg/kg) intraperitoneally (i.p.) 60 min prior to the administration of either ^{14}C-3-MG or glucose. These sugars were administered in doses of 2·0 g/kg, i.p., or 0·5 g/kg, intravenously (i.v.). All solutions

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of 3-MG and glucose were prepared at a concentration of 250 mg/ml. Ambient temperature was 33–35° to maintain normal body temperatures, thus preventing the influence of CPZ-induced hypothermia on glucose metabolism.

At several intervals after the administration of the labeled compound, the animals were sacrificed by decapitation, free flowing blood was collected, and serum was obtained by centrifugation. Brains were immediately perfused with 5 ml normal saline via each carotid artery, removed from the cranium intact, washed with saline and blotted. Olfactory lobes were discarded and a frontal slice, approximately 100 mg, containing both grey and white matter was taken for analysis. Sections of diaphragm and right posterior tibialis muscle (p. tib.) were removed from the exsanguinated carcass, washed and blotted as above. All tissues were frozen and stored individually prior to analysis.

Aliquots of 0·1 ml serum and 50- to 150-mg sections of each tissue were placed in scintillation vials preparatory to digestion. Serum and tissue samples were digested essentially according to the method of Mahin and Lofberg.[20] Perchloric acid, 0·2 ml (70%), and then 0·4 ml hydrogen peroxide (30%) were added to each vial, thoroughly wetting the tissue. Vials were tightly capped and warmed for 1 hr at 80°.[20] After cooling, sample preparation was completed by adding 6·0 ml cellosolve and 10 ml 2,5-diphenyloxazole-toluene (6·0 g/l.). Samples were counted to 2 per cent efficiency in a Beckman LS 100 scintillation spectrometer. An internal standard was added to correct for quenching. Statistical comparisons between control and treatment groups were made using analysis of variance.

In experiments in which rats received glucose, the animals were sacrificed by decapitation and free flowing blood was collected in oxalated beakers. Blood glucose was determined by a glucose oxidase method.* Statistical comparisons between control and treated groups were made using the Student's *t*-test.

RESULTS

Since 3-MG is a nonmetabolizable analog of glucose, serum and tissue levels of ^{14}C found in this study are referred to as 3-MG.

The effects of chlorpromazine pretreatment on the serum levels of 3-MG after the sugar was administered i.p. are shown in Fig. 1. CPZ pretreatment resulted in a significant elevation of the serum levels of 3-MG. These results were not unexpected, since it has been shown that CPZ diminishes glucose tolerance in several species.[1–7]

Brain and p. tib. levels of 3-MG in CPZ-treated animals were also significantly elevated over control levels (data not shown). However, when tissue to serum ratios of 3-MG were evaluated, no differences between control and treated animals were observed for these tissues (Table 1). Thus, it would appear that the elevations of 3-MG in these tissues were merely a reflection of the increased serum levels.

Diaphragm levels of 3-MG were significantly reduced by CPZ pretreatment (Fig. 2). Concomitantly, a significant reduction of diaphragm/serum ratios of 3-MG was observed at each of the intervals studied (Table 1).

The results employing the i.v. route of administration of 3-MG are summarized in Table 2. When 3-MG was administered i.v., pretreatment with CPZ did not alter the tissue distribution of the glucose analog. No significant differences in tissue to serum ratios were detected at any of the time intervals studied. Also, it should be pointed out

* Glucostat (Worthington Biochemical Corp., Freehold, New Jersey).

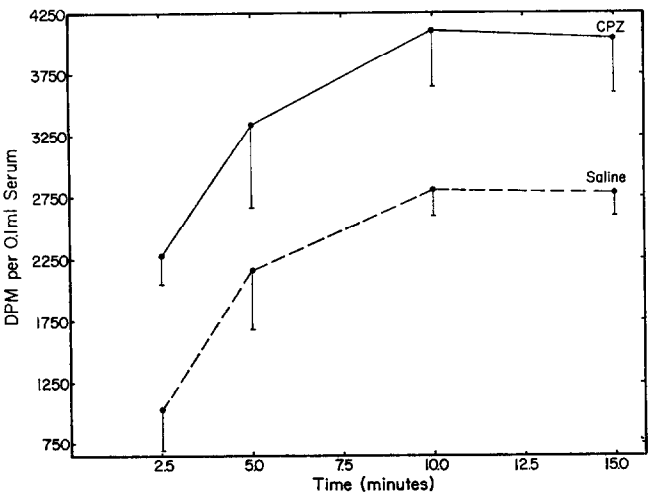

FIG. 1. Effect of chlorpromazine pretreatment on serum levels of ^{14}C-3-*O*-methyl-D-glucose after its intraperitoneal administration to rats. CPZ (15 mg/kg, i.p.) was injected 60 min before 3-MG (2·0 g/kg containing 10 μc/kg ^{14}C-3-MG). Each point represents average specific activity of serum samples from 3 rats with standard deviation. A significant treatment factor was found when analyzed by factorial analysis of variance ($P < 0{\cdot}001$, $F = 55{\cdot}5$, $df = 1/16$).

that CPZ pretreatment had no effect on serum levels of 3-MG at any of the time intervals tested (data not shown).

The results suggested that the effect of CPZ on the distribution of i.p. administered 3-MG is a reflection of an effect of CPZ on the absorption of the glucose analog from the peritoneal cavity. For this reason, the effect of CPZ on the absorption of i.p. administered glucose was evaluated. The data shown in Fig. 3 clearly demonstrate the effect of CPZ on glucose tolerance regardless of the route of administration of the glucose. At 45 min after either the i.p. or the i.v. injection of glucose, blood levels of the

TABLE 1. EFFECT OF CHLORPROMAZINE PRETREATMENT ON THE ^{14}C TISSUE/SERUM RATIOS AFTER INTRAPERITONEAL ADMINISTRATION OF ^{14}C-3-*O*-METHYL-D-GLUCOSE*

Tissue	Treatment	Mean tissue/serum ratio after ^{14}C-3-MG			
		2·5 min	5·0 min	10 min	15 min
Diaphragm	Saline	2·59 ± 0·69	1·35 ± 0·28	0·63 ± 0·14	0·81 ± 0·07
	CPZ	1·16 ± 0·26†	0·58 ± 0·29†	0·28 ± 0·03†	0·30 ± 0·11‡
Brain	Saline	0·13 ± 0·02	0·21 ± 0·01	0·28 ± 0·05	0·39 ± 0·05
	CPZ	0·12 ± 0·01	0·22 ± 0·03	0·27 ± 0·06	0·29 ± 0·04
Posterior tibialis	Saline	0·13 ± 0·05	0·12 ± 0·02	0·11 ± 0·02	0·12 ± 0·01
	CPZ	0·12 ± 0·05	0·10 ± 0·01	0·12 ± 0·03	0·12 ± 0·01

* CPZ (15 mg/kg, i.p.) was injected 60 min before 3-MG (2·0 g/kg containing 10 μc/kg of ^{14}C-3-MG). Each value ± S.D. represents the average of ratios: (dis./min per 100 mg wet tissue)/(dis./min per 0·1 ml serum) from 3 rats. Groups were compared using analysis of variance.

† $P < 0{\cdot}05$.

‡ $P < 0{\cdot}01$.

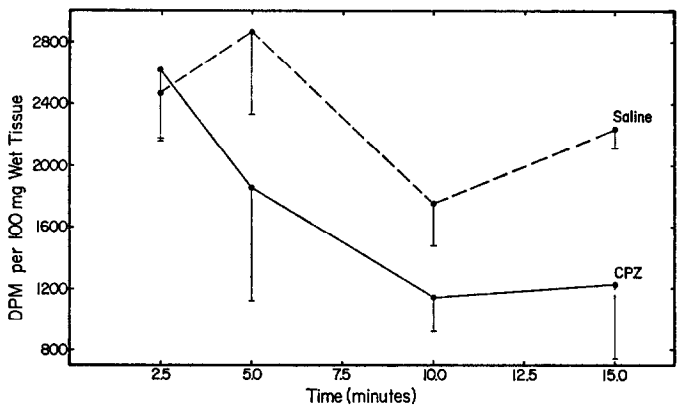

FIG. 2. Effect of chlorpromazine pretreatment on the uptake by diaphragm of intraperitoneally administered ^{14}C-3-*O*-methyl-D-glucose in the rat. CPZ (15 mg/kg, i.p.) was injected 60 min before 3-MG (2·0 g/kg containing 10 μc/kg ^{14}C-3-MG). Each point represents average specific activity of diaphragms of 3 rats with standard deviation. A significant treatment factor was found when analyzed by factorial analysis of variance ($P < 0{\cdot}01$, $F = 13{\cdot}10$, $df = 1/16$).

sugar were significantly higher in the CPZ-treated animals as compared to controls. It can also be seen that CPZ significantly enhances the absorption of glucose from the peritoneal cavity. At the 2·5-min interval after i.p. administration of the sugar, blood glucose levels in the CPZ group were nearly 2-fold higher than those of the control group. No difference was detected between control and CPZ-treated animals at the 2·5-min interval when glucose was administered intravenously.

TABLE 2. EFFECT OF CHLORPROMAZINE PRETREATMENT ON THE ^{14}C TISSUE/SERUM RATIOS AFTER INTRAVENOUS ADMINISTRATION OF ^{14}C-3-*O*-METHYL-D-GLUCOSE*

Tissue	Treatment	Mean tissue/serum ratios after ^{14}C-3-MG			
		2·5 min	5·0 min	10 min	15 min
Diaphragm	Saline	0·24 ± 0·02	0·22 ± 0·11	0·23 ± 0·01	0·23 ± 0·03
	CPZ	0·24 ± 0·06	0·23 ± 0·08	0·26 ± 0·04	0·24 ± 0·08
Brain	Saline	0·38 ± 0·04	0·48 ± 0·01	0·60 ± 0·06	0·59 ± 0·02
	CPZ	0·39 ± 0·04	0·50 ± 0·10	0·70 ± 0·02	0·62 ± 0·05
Posterior tibialis	Saline	0·18 ± 0·01	0·15 ± 0·03	0·16 ± 0·04	0·14 ± 0·01
	CPZ	0·17 ± 0·04	0·15 ± 0·03	0·18 ± 0·03	0·19 ± 0·04

* CPZ (15 mg/kg. i.p.) was injected 60 min before 3-MG (0·5 g/kg containing 8 μc/kg). The sugar was injected via lateral tail vein. Each value ± S.D. represents the average of ratios: (dis./min per 100 mg wet tissue)/dis./min per 0·1 ml serum) from 3 rats. Groups were compared using analysis of variance. No significant differences were observed.

DISCUSSION

The i.p. administration of chlorpromazine markedly influences the absorption of both 3-MG and glucose from the peritoneal cavity. Thus, CPZ-induced alterations in serum levels of 3-MG and glucose after their i.p. administration are probably the result of at least two factors: the effect of CPZ on the rate of absorption from the site

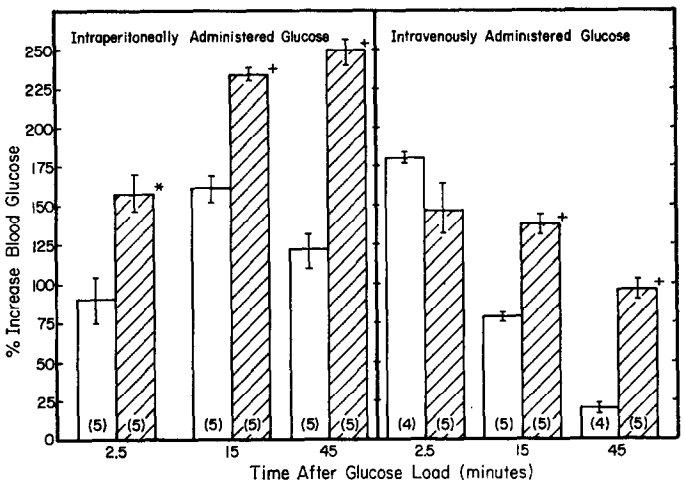

FIG. 3. Effect of CPZ on intraperitoneally and intravenously administered glucose load to rats. CPZ (15 mg/kg, i.p.) was administered 60 min prior to a glucose load, either 2·0 g/kg i.p. or 0·5 g/kg, i.v. ■, Indicates per cent increase of blood glucose of CPZ-glucose rats over CPZ-saline group; □, indicates per cent increase of blood glucose of saline-glucose rats over saline-saline group. Groups were compared by Student's *t*-test. Parentheses indicate number of animals per treatment (*P < 0·01; †P < 0·001).

of administration, and the effect of CPZ on glucose tolerance. Preliminary experiments indicate that the effect of CPZ on the absorption of glucose from the peritoneal cavity is a local effect of the drug. We have found that when CPZ is administered subcutaneously (s.c.) it does not influence the rate of absorption of i.p. administered glucose. Earlier experiments have demonstrated a local effect of CPZ on absorption. Gorbatova[21] claimed that CPZ given s.c., together with either strychnine or coramine, altered the onset of convulsions caused by the latter compounds. However, when CPZ was administered s.c. while the convulsants were administered i.v., no effects on the onset of convulsions were observed.

The ability of CPZ to reduce the incorporation of i.p. administered 3-MG into the diaphragm may also be related to the effect of the drug on the absorption of the sugar. The diaphragm is in direct contact with the peritoneal cavity, being the upper limiting structure. Intraperitoneally administered 3-MG has ready access to this muscle through direct contact, and transport to this tissue via the circulation may not be necessary. Interestingly, initial levels of 3-MG in the diaphragm were high and decreased with time. In the other tissues investigated, levels of 3-MG increased with time. The rapidly decreasing concentrations of the sugar from the peritoneal cavity as a result of the CPZ-increased rate of absorption into the circulation might be reflected as a decreased rate of incorporation into the muscle. Since CPZ failed to alter the accumulation of i.v. administered 3-MG into the diaphragm, we have concluded that in our initial experiment the levels of 3-MG in the diaphragm were probably influenced more by the concentration of the sugar in the peritoneal cavity than by an effect of CPZ on tissue permeability.

It has been suggested that CPZ-induced glucose intolerance is due to a reduction in

glucose permeability.[9–11] These earlier experiments failed to separate an effect of CPZ on utilization from an effect on tissue permeability to the sugar. The suggested decrease in permeability could be interpreted as a decrease in utilization. The present experiments have circumvented a possible effect of CPZ on glucose utilization, since 3-MG is a nonutilizable analog of glucose. Thus, any change in tissue concentration would be a reflection of decreased permeability. Since CPZ failed to alter the distribution of i.v. administered 3-MG, a direct effect of the drug on tissue permeability to glucose now seems unlikely.

An indirect effect of CPZ on tissue permeability to glucose cannot be ruled out at this time. The uptake of both 3-MG and glucose into skeletal muscle is increased by insulin. Glucose, but not 3-MG, stimulates the release of insulin from the pancreas.[22,23] It is possible that CPZ reduces or delays glucose-stimulated insulin release, thus indirectly reducing tissue permeability to the sugar and reducing glucose tolerance. Studies are currently in progress to assess the effects of CPZ on glucose-stimulated insulin release.

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