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Effect of Ganglionic Blocking Agents Chlorisondamine and Mecamylamine on Glucose Metabolism and Serum Insulin in the Rat (37741)

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In previous studies we found that the administration of chlorisondamine (1), a ganglionic blocking agent, prevented the fall of blood glucose concentration in 24-hr fasted rats, while it did not affect the rise in serum free fatty acid concentration (2). This unusual finding prompted us to study this phenomenon in greater detail, partly because very little is known about metabolic effects of ganglionic blocking agents, and partly because an understanding of the mechanism(s) causing this effect could contribute to our knowledge of metabolic control during fasting.

There are a number of possible explanations for the relatively high blood glucose concentration in chlorisondamine-treated fasting rats. The first possibility is that a ganglionic blocking agent prevents or slows down the development of fasting hypoglycemia by depressing glucose utilization. The second possibility is that the drug stimulates gluconeogenesis. Finally, the possibility cannot be excluded that relatively high blood glucose is the result of prolonged carbohydrate digestion and absorption.

We have attempted to evaluate these possibilities by examining the effects of chlorisondamine on glucose uptake by muscle and fat tissue, on liver glycogen, on serum free fatty acids, amino acids, and insulin, and on sensitivity of peripheral tissues to insulin added *in vitro*. Evidence will also be presented indicating that mecamylamine (3), another ganglionic blocking agent of markedly different chemical structure, has metabolic effects similar to those of chlorisondamine.

Methods. Adult male Sprague Dawley rats weighing approximately 200 g were fed a normal laboratory diet (Wayne Lab-Blox, Allied Mills) *ad libitum* until the beginning of fasting at 9:00 AM. This time, which coincided with the beginning of the light phase, is referred to as zero time of fasting. Food was removed at this time, but the animals were allowed tap water *ad libitum*. At zero time, the experimental rats were injected with 1 ml of chlorisondamine or mecamylamine solution/200 g of body weight intraperitoneally. Chlorisondamine chloride (Ecolid Ciba, Lot No. H-9279) was kindly supplied by Dr. A. J. Plummer, and mecamylamine HCl (Merck + Co., Lot No. L-551785-1-29) was kindly supplied by Dr. W. B. Gall. Solutions of ganglionic blocking agents were always freshly prepared just before each injection by dissolving the substance in 0.9% NaCl. Additional doses of the blocking agents were given every 4 hr. In order to overcome the developing tolerance to ganglionic blocking agents, the doses of the drugs were gradually increased during the fasting period. In the case of chlorisondamine chloride, the initial dose administered at zero time of fasting was 2.5 mg/kg of body weight, and each subsequent dose was one-third higher compared with the previous dose. When mecamylamine HCl was used, the initial dose was 5 mg/kg of body weight, and each subsequent dose was one-half higher than the previous one. Control rats, which were always run simultaneously with experimental animals, were injected with 1 ml of 0.9% NaCl solution/200 g of body weight intraperitoneally

every 4 hr starting at zero time of fasting. Animals were sacrificed by stunning and a rapid exsanguination from the cut chest at zero time of fasting and then at approximately 3, 6, 12, and 24 hr of fasting. Rats killed at zero time received neither saline nor ganglionic blocking agent. Other groups were sacrificed 2 to 4 hr after their last injection. Blood was collected for serum and plasma determinations, and tissues were immediately removed for analysis or incubation.

Study on glucose uptake and insulin sensitivity in vitro. The diaphragm and the epididymal fat tissue from control and chlorisondamine-treated animals fasted for 24 hr were used in this study. The diaphragms were removed and rinsed, cut in halves and cleaned in ice-cold 0.9% NaCl solution. The halves were blotted on filter paper, weighed, and then preincubated in an ice-cold medium for 30 min as recommended by Krahl (4). Two pieces of epididymal fat tissue (150–200 mg each) were taken from each rat, weighed, and preincubated in the same way as the diaphragms. The preincubation medium was Krebs–Ringer bicarbonate solution with 140 mg glucose/100 ml. At the end of the preincubation period, the tissues were transferred into a fresh medium (1 ml for each tissue sample) and incubated in a metabolic shaker bath at 37° for 3 hr. One portion of either tissue from each rat was incubated in Krebs–Ringer bicarbonate solution containing 140 mg glucose/100 ml, and the other portion in the same solution to which insulin had been added (0.1 unit/ml). Beef crystalline insulin (Eli Lilly & Co., Lot No. PJ-4609) with a potency of 23.6 units/mg and less than 0.0003% glucagon was kindly supplied by Dr. J. M. McGuire. All media were gassed with a 95% O_2–5% CO_2 mixture for 15 min before use, and preincubations as well as incubations were carried out under the atmosphere of 95% O_2–5% CO_2. Initial and final glucose concentrations in the incubation media were determined in duplicate.

Study on rats fasted 48 hr. This study differs in experimental protocol from experiments described above because the animals were first fasted for 24 hr in order to minimize or eliminate the possibility of carbohydrate absorption from the gastrointestinal tract and were then treated with repeated doses of saline or chlorisondamine as previously, while fasting continued for another 24 hr. A group of rats sacrificed at 24 hr of fasting was administered neither saline nor a ganglionic blocking agent and served as a reference point for saline-treated and chlorisondamine-treated rats sacrificed at 48 hr of fasting. Blood glucose, serum free fatty acids, and liver and muscle glycogen concentrations were determined in all groups.

Determinations. Glucose concentration in blood, serum, and incubation media was determined on a Technicon Autoanalyzer using a modification of the ferricyanide–ferrocyanide method (5). Glycogen in tissues was analyzed by the method of Good, Kramer and Somogyi (6) modified by heating 30% KOH before introduction of the tissue, precipitating glycogen with methanol, and hydrolyzing the sediment with 2 *N* H_2SO_4. Glucose in the neutralized hydrolysate was determined on a Technicon Autoanalyzer. The concentration of free fatty acids in serum was determined by the spectrophotometric method of Novak (7), and the concen-

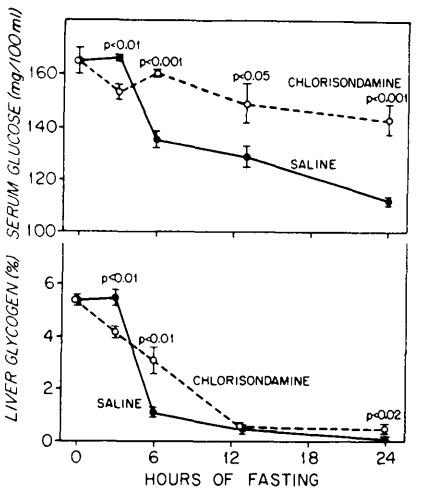

FIG. 1. Effect of chlorisondamine on changes in serum glucose and liver glycogen concentrations during fasting in rats. Each value is an average of six determinations. Vertical bars represent ± SE. *P* indicates the significance of the difference between saline-treated and chlorisondamine-treated rats fasted the same number of hours.

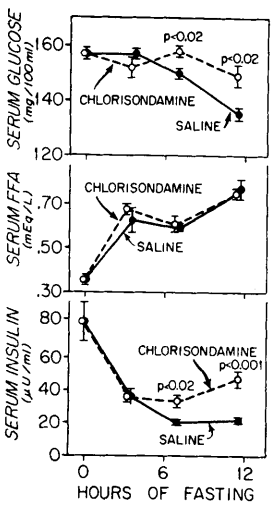

FIG. 2. Effect of chlorisondamine on changes in serum glucose, free fatty acids, and insulin concentrations during fasting in rats. Each value is an average of six determinations. Vertical bars represent ± SE. *P* indicates the significance of the difference between saline-treated and chlorisondamine-treated rats fasted the same number of hours.

trations of plasma amino acids and ammonia nitrogen were determined by the method described by Piez and Morris (8). Serum insulin concentration was measured by radioimmunoassay using a commercial insulin immunoassay kit (Amersham/Searle Corp.) with human insulin as standard.

Results. Effect of ganglionic blocking agents on serum or plasma concentrations of glucose, free fatty acids, amino acids, ammonia nitrogen, and insulin and on the concentration of glycogen in the liver during 24-hr fasting. As indicated in Fig. 1, during a 24-hr fast the serum glucose concentration in saline-treated rats fell 53 ± 1 (SE) mg/100 ml, while in chlorisondamine-treated rats there was a fall of only 21 ± 6 mg/100 ml ($P < 0.001$). Comparatively higher concentrations in chlorisondamine-treated rats were observed starting from 6 hr after deprivation of food. Similar findings in another experiment are depicted in Fig. 2. Treatment with mecamylamine, another ganglionic blocking agent, was found to have the same effect on serum glucose as chlorisondamine (Table I).

Liver glycogen stores were severely depleted in both control and experimental rats during the first 12 hr of fasting. However, at the end of the 24-hr fasting period, animals treated with either one of the ganglionic blocking agents did not show the complete exhaustion of liver glycogen observed in the controls ($P < 0.02$) (Fig. 1 and Table I).

Serum free fatty acid concentrations in the saline-treated rats doubled during the first 12 hr after deprivation of food and continued to rise. However, neither chlorisondamine nor mecamylamine had any significant effect on the development of these changes (Fig. 2 and Table I).[1] Plasma concentrations of individual amino acids and ammonia nitrogen determined in 24-hr fasted rats are shown in Table II. In chlorisondamine-treated animals, plasma glycine concentration was 30% low-

[1] In previous experiments, chlorisondamine delayed the rise of serum FFA in response to fasting (2). A different breed of rats and a different dosage regime was used at that time.

TABLE I. Comparison of the Effects of Chlorisondamine and Mecamylamine on Certain Metabolic Indexes in 24-hr Fasted Rats.[a]

Treatment	Serum glucose		Liver glycogen		Serum FFA	
	mg/100 ml ± SE	P^b	% ± SE	P^b	mEq/liter ± SE	P^b
Saline	99 ± 2	—	0.110 ± 0.020	—	1.24 ± 0.09	—
Chlorisondamine	135 ± 6	<0.001	0.410 ± 0.101	<0.02	1.11 ± 0.11	NS
Mecamylamine	130 ± 3	<0.001	0.560 ± 0.089	<0.001	1.25 ± 0.03	NS
	(P^c) NS		(P^c) NS		(P^c) NS	

[a] Each value is an average of 6 determinations.

[b] Significance of the difference between the given value and the saline-treated group.

[c] Significance of the difference between chlorisondamine-treated and mecamylamine-treated groups.

TABLE II. Effect of Chlorisondamine on Plasma Amino Acid Profile in 24-hr Fasted Rats.[a]

Amino acid	Saline-treated rats $\mu M \pm$ SE	Chlorisondamine-treated rats $\mu M \pm$ SE	Difference %
Methionine	54 ± 2	48 ± 4	−11
Tyrosine	73 ± 6	64 ± 6	−12
Phenylalanine	174 ± 8	198 ± 12	+14
Tryptophan	None	None	
Valine	266 ± 12	235 ± 17	−12
Isoleucine	122 ± 6	113 ± 7	−7
Leucine	190 ± 7	177 ± 12	−7
Histidine	38 ± 8	38 ± 6	0
Ornithine	73 ± 5	58 ± 6	−21
Lysine	675 ± 47	581 ± 49	−14
Arginine	198 ± 29	217 ± 25	+10
Glutamic acid	184 ± 13	168 ± 13	−9
Proline	289 ± 12	272 ± 23	−6
Glycine	617 ± 28	433 ± 30	−30[b]
Alanine	481 ± 48	374 ± 28	−22
Cystine/2	228 ± 35	235 ± 62	+3
Ammonia-N	179 ± 5	250 ± 17	+40[b]

[a] Each value is an average of 5 determinations. The differences were not statistically significant, except for glycine and ammonia-N.
[b] $P < 0.01$.

er and ammonia nitrogen 40% higher than corresponding values in saline-treated rats. The remaining differences were not statistically significant.

Serum insulin concentration fell considerably ($P < 0.01$) in both groups of rats during the first 3 hr after deprivation of food (Fig. 2), and as fasting progressed it continued to decrease in saline-treated animals, while chlorisondamine-treated rats showed an upward trend. Starting from 7 hr after food deprivation, chlorisondamine-treated animals had significantly higher insulin levels than comparably fasted controls. In a separate experiment a similar difference was observed ($P < 0.01$) after 24 hr of fasting.

Effect of chlorisondamine treatment in vivo *on glucose uptake* in vitro *and responsiveness to insulin* in vitro. Administration of chlorisondamine during a 24-hr fast had no effect on glucose uptake by diaphragm or adipose tissue (Table III). However, when tissues from chlorisondamine-treated rats were incubated in the presence of insulin, both the epididymal fat and the diaphragm showed significantly higher increases in glucose uptake than similarly treated tissues from saline-treated rats (Table III).

TABLE III. Effect of Chlorisondamine Treatment *in Vivo* on Glucose Uptake *in Vitro* and Responsiveness to Insulin *in Vitro* by Tissues from 24-hr Fasted Rats.[a]

	Glucose uptake (mg/g wet wt of tissue/3 hr ± SE)			
	Epididymal fat in medium containing		Diaphragm in medium containing	
Treatment *in vivo*	Glucose	Glucose + insulin	Glucose	Glucose + insulin
Saline	1.99 ± 0.19	3.42 ± 0.27	2.59 ± 0.12	2.56 ± 0.28
Chlorisondamine	2.08 ± 0.14	4.59 ± 0.44	2.64 ± 0.18	3.51 ± 0.19
	(P^b) NS	$P^b < 0.05$	(P^b) NS	$P^b < 0.02$

[a] Each value is an average of 6 determinations.
[b] Significance of the difference between saline-treated and chlorisondamine-treated groups in the same column.

Effect of chlorisondamine administered during the period between 24 and 48 hr of fasting. To investigate the possible role of prolonged carbohydrate absorption in the chlorisondamine-treated rats, we attempted to test the effects of chlorisondamine in experimental conditions which would minimize or eliminate the possibility of glucose absorption from the gastrointestinal tract during the treatment period. We assumed that if under such conditions there was a difference in blood glucose between the chlorisondamine and saline rats, the difference would have to be due to either a change in glucose uptake by the tissues or a change in gluconeogenesis.

In order to minimize the possibility of carbohydrate absorption from the gastrointestinal tract, animals were first fasted for 24 hr and then treated with repeated doses of saline or chlorisondamine, while fasting continued for another 24 hr. The findings are shown in Table IV. Treatment with saline during the second 24-hr period resulted in no changes in blood glucose, serum free fatty acids and muscle glycogen concentrations, but it did result in a significant increase in liver glycogen. By contrast, chlorisondamine treatment increased blood glucose by 11 mg/100 ml and increased liver glycogen to a significantly higher level than that in the saline-treated group. Serum free fatty acids were found to be significantly lower compared to saline-treated rats.

Discussion. The fact that chlorisondamine and mecamylamine, two ganglionic blocking agents with markedly different chemical structures, both diminished the fall in serum glucose during fasting suggests that their action on glucose metabolism is mediated primarily via their effect on the autonomic nervous system. Slower development of fasting hypoglycemia in chlorisondamine- or mecamylamine-treated rats does not seem to be caused by a decreased uptake of glucose by peripheral tissues, since the diaphragm and the epididymal fat from chlorisondamine-treated animals took up the same amount of glucose as the comparable tissues from fasted controls in the absence of added insulin. Actually, glucose uptake in chlorisondamine-treated rats *in vivo* may be even higher than that in comparable saline-treated animals, since the former had significantly higher plasma insulin levels and the diaphragm and the epididymal fat from chlorisondamine-treated animals showed higher sensitivity to insulin. We cannot completely exclude the possibility that the slower development of fasting hypoglycemia in chlorisondamine-treated rats is at least partly contributed to by prolonged absorption of carbohydrate. Indeed, findings of Drzhevetskaya and coworkers (9–12) suggest that ganglionic blockade inhibits glucose absorption. However, prolonged absorption of carbohydrate does not seem to be the sole explanation, since chlorisondamine treatment increased blood glucose in rats previously fasted for 24 hr.[2] We failed to find a higher gluconeogenic capacity of the renal cortex from 24-hr fasted chlorisondamine-treated rats compared to fasted controls (unpublished data), and it is therefore possible that the slower development of fasting hypoglycemia in chlorisondamine-treated rats is due to an increased hepatic gluconeogenesis. Our experiments do not shed light on the mechanism of the presumed stimulation of hepatic gluconeogenesis. A rise in the level of gluconeogenic amino acids in plasma can enhance hepatic gluconeogenesis (13), but there was no significant difference between the plasma amino acid concentrations in fasted chlorisondamine-treated rats and fasted controls, except for a decrease in plasma glycine in the former.

Fasting is normally associated with decreased sensitivity to insulin (14). It was, therefore, of interest that diaphragm and adipose tissue from 24-hr fasted chlorisondamine-treated rats showed higher sensitivity to insulin added *in vitro* than tissues from comparably fasted controls. Feldman and Lebo-

[2] In normal 24-hr fasted rats, the gastrointestinal tract is practically empty as evidenced by wet weights of the contents: 0.15 ± 0.01 (SE) g in the stomach, 0.35 ± 0.04 g in the small intestine, and 1.00 ± 0.14 g in the large intestine ($n = 6$). For comparison, the same parts of the gastrointestinal tract of rats sacrificed at zero time of fasting were found to contain 2.2 ± 0.5, 3.2 ± 0.4, and 4.6 ± 0.2 g, respectively ($n = 5$).

TABLE IV. Effects of Chlorisondamine Administered During the Period Between 24 and 48 hr of Fasting.[a]

Length of fasting (hr)	Treatment	Blood glucose		Serum FFA		Glycogen in liver		Glycogen in muscle	
		mg/100 ml ± SE	P[b]	mEq/liter ± SE	P[b]	% ± SE	P[b]	% ± SE	P[b]
24	None	65 ± 2	—	1.16 ± 0.08	—	<0.035 (unmeasurable)	—	0.227 ± 0.008	—
48	Saline	65 ± 3	NS	1.15 ± 0.05	NS	0.160 ± 0.043	<0.01	0.224 ± 0.010	NS
48	Chlorisondamine	76 ± 4	<0.05	1.00 ± 0.04	NS	0.310 ± 0.044	<0.001	0.265 ± 0.018	NS
		$P^c < 0.05$		$P^c < 0.05$		$P^c < 0.05$		(P^c) NS	

[a] Unlike all other experiments in this paper, the rats were first fasted for 24 hr to minimize or eliminate the possibility of carbohydrate absorption from the GI tract and were then treated with repeated doses of saline or chlorisondamine while fasting continued for another 24 hr. Each value is an average of 7 determinations.

[b] Significance of the difference between the given value and the value at 24 hr of fasting.

[c] Significance of the difference between chlorisondamine-treated and saline-treated rats sacrificed at 48 hr of fasting.

vitz (14) have reported that insulin resistance does not develop in fasting adrenalectomized mice given cortisol, leading them to suggest that the insulin insensitivity of fasting is most likely due to alterations in the secretion of adrenal medullary hormones. In our experiments, chlorisondamine treatment may have slowed the development of the insulin resistance of fasting in muscle and adipose tissue by inhibiting the sympathoadrenal system. However, if catecholamines were the only factor responsible for the development of insulin resistance in fasting, it would be expected that their secretion would change; but it has been found that fasting in the rat does not change urinary excretion of epinephrine and norepinephrine (15). Randle *et al.* (16) have proposed that the elevation of serum free fatty acids which occurs during fasting may be the cause of the decreased insulin sensitivity. However, in our chlorisondamine-treated rats the insulin sensitivity in fat and muscle was higher than in the controls, despite the fact that serum free fatty acid levels were the same in both groups. It is apparent that further studies of mechanisms controlling the sensitivity of peripheral tissues to insulin are necessary.

Summary. In rats treated with the ganglionic blocking agent, chlorisondamine, serum glucose fell only 21 ± 6 (SE) mg% during a 24-hr fast, compared to a fall of 53 ± 1 in saline-treated controls ($P < 0.001$), and another ganglionic blocker, mecamylamine, also lessened the fall in serum glucose during fasting. In 24-hr fasted rats, serum insulin was significantly higher in the chlorisondamine-treated group than in the controls; liver glycogen was increased and plasma glycine decreased in the chlorisondamine group, while the concentrations of 15 other plasma amino acids and serum FFA were the same in both groups; diaphragm and epididymal fat from chlorisondamine-treated rats and control rats took up the same amount of glucose *in vitro* in the absence of insulin, but both tissues from the chlorisondamine rats had an increased response to insulin added *in vitro*. Chlorisondamine given to rats previously fasted for 24 hr significantly increased blood glucose and hepatic glycogen. It is sug-

gested that the effect of ganglionic blocking agents on glucose metabolism may be mediated by stimulation of gluconeogenesis.

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