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CHLORPROPAMIDE–INDUCED LEUCINE HYPOGLYCEMIA *

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Previous reports from this laboratory have demonstrated that L-leucine-induced hypoglycemia can be experimentally produced in normal dogs during periods of either exogenous (1) or endogenous (2) hyperinsulinism. On the basis of these experiments, it was suggested that L-leucine acts to inhibit hepatic glucose output and that "leucine hypoglycemia" occurs in situations where increased hepatic glucose output is necessary for maintenance of euglycemia. To extend these observations of experimental leucine hypoglycemia, it seemed desirable to investigate the effect of L-leucine in other situations characterized by hypoglycemia, hyperinsulinism, or both. Consequently, we have measured the blood glucose response to L-leucine at varying intervals after both the acute and chronic administration of chlorpropamide.[1] In addition, we have attempted to ascertain in controlled experiments whether or not L-leucine by itself has any effect on blood glucose concentration. Finally, we have attempted to demonstrate that L-leucine inhibits hepatic glucose output by comparing hepatic-vein–femoral-vein (HV–FV) blood glucose concentration differences in dogs, with and without pretreatment with chlorpropamide.

METHODS

A. *Effect of* L-*leucine on peripheral blood glucose concentration with and without chlorpropamide pretreatment*

Nine mongrel dogs were used in these experiments, and the hypoglycemic response to L-leucine was tested in each dog under all five of the following experimental conditions: *1*) 2 hours, *2*) 12 hours, and *3*) 24 hours after a single oral dose of chlorpropamide, *4*) 24 hours after 3 consecutive days of the same oral dose of chlorpropamide, given once daily, and *5*) without chlorpropamide pretreatment.

Chlorpropamide was given in a dose of 250 or 375 mg. All dogs weighing less than 10 kg were arbitrarily given 250 mg, and 375 mg was used in dogs weighing over 10 kg. Each experiment was performed as follows. A dog was anesthetized with pentobarbital, and two blood samples were drawn 15 minutes apart for glucose determination, with the average of these two values used as a baseline. Then 30 mmoles of L-leucine in 200 ml of distilled water or an equal volume of 0.45 per cent NaCl was given by an intravenous infusion lasting approximately 15 minutes. Blood was then drawn for measurement of glucose concentration 30 and 60 minutes after L-leucine or saline had been administered. The results have been expressed as percentage of fall in glucose concentration (100 × maximal fall observed in blood glucose concentration divided by the baseline value). The percentage of fall in blood glucose due to L-leucine in any given dog under each experimental condition is then expressed as follows: maximal fall in blood glucose after leucine × 100/average baseline minus maximal fall in blood glucose after saline × 100/average baseline. Blood glucose was measured by the Somogyi method as modified by Nelson (3).

Serum chlorpropamide levels were measured in eight individual experiments by the method of Toolan and Wagner (4).

B. *Effect of* L-*leucine on hepatic metabolism*

1. *Effect of* L-*leucine on hepatic-vein and femoral-vein blood glucose concentration in dogs without prior pretreatment.* Five experiments were performed with the following protocol. After the induction of anesthesia with pentobarbital, a radio-opaque catheter was passed percutaneously into the vena cava from the femoral vein and inserted in a hepatic vein under fluoroscopic control. A second catheter was placed in the other femoral vein for collection of peripheral venous blood. After three blood samples were drawn from each catheter over a 15-minute period, each dog received a rapid intravenous infusion of 30 mM of L-leucine in 200 ml of distilled water. Paired blood samples were then drawn from each catheter every 10 minutes for the next 60 minutes. The location of the catheter in the hepatic vein was established before each sample was taken. Blood glucose was measured by the iodometric technique of Somogyi (5, 6).

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[1] 1-propyl-3-(*p*-chloro-benzenesulfonyl) urea.

TABLE I

Effect of L-*leucine on blood glucose concentration with or without chlorpropamide pretreatment**

Dog	Blood glucose concentration	2 Hours after chlorpropamide		12 Hours after chlorpropamide		24 Hours after chlorpropamide		24 Hours after 3 days of chlorpropamide		Without chlorpropamide	
		Leucine	Saline	Leucine	Saline	Leucine	Saline	Leucine	Saline	Leucine	Saline
	mg/100 ml										
145	Baseline	60	78	95	73	72	69	51	51	86	89
	After treatment	25	72	72	75	54	63	35	48	75	83
	% fall	58	8	24	−3	25	9	32	6	13	7
404	Baseline	66	36	60	59	69	69	57	72	65	83
	After treatment	33	41	41	59	46	66	33	60	51	79
	% fall	50	−14*	32	0	33	4	42	17	22	5
145	Baseline	78	63	71	70	75	82	71	63	91	91
	After treatment	54	63	48	69	69	82	63	63	73	86
	% fall	31	0	32	1	8	0	11	0	20	5
450	Baseline	63	66	46	51	69	57	59	38	75	81
	After treatment	46	59	35	51	47	57	38	30	70	83
	% fall	27	11	24	0	32	0	35	21	7	−2
M.A.	Baseline	41	60	57	72	75	66	48	36	79	76
	After treatment	25	48	38	75	58	63	43	43	73	76
	% fall	39	20	33	−4	23	5	10	−19	8	0
574	Baseline	51	42	65	61	60	68	75	81	82	80
	After treatment	40	56	52	72	56	71	84	79	75	78
	% fall	18	−33	20	−18	7	−4	−12	2	9	2
657	Baseline	51	48	68	70	74	78	54	58	70	85
	After treatment	47	47	58	71	64	77	44	55	62	79
	% fall	8	2	15	−1	14	1	19	5	11	7
42	Baseline	67	76	74	72	89	85	79	88	85	85
	After treatment	51	68	50	70	71	83	85	90	79	84
	% fall	24	11	32	3	20	2	−8	−2	7	1
21	Baseline	65	52	62	67	64	70	63	70	72	87
	After treatment	47	49	65	72	69	71	53	68	62	90
	% fall	28	6	−5	−7	−8	−1	16	3	14	−3
Average of 9 dogs	Baseline blood glucose	60	58	66	66	72	72	62	62	78	84
	Blood glucose after treatment	41	56	51	68	59	70	53	60	69	82
	% fall	32	3	23	−3	18	3	16	3	12	3

* Negative number signifies rise.

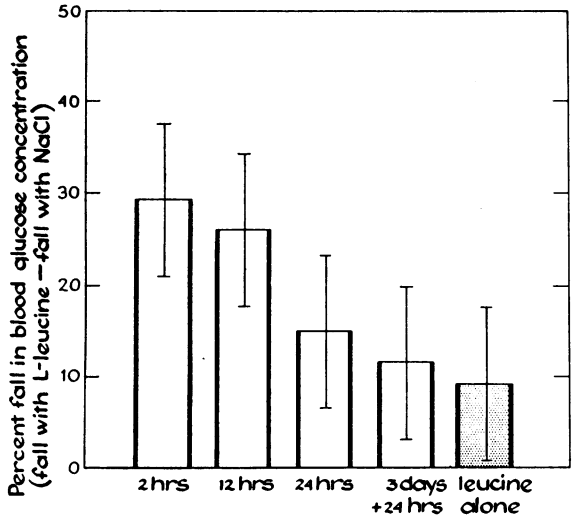

FIG. 1. MEAN HYPOGLYCEMIC RESPONSE TO L-LEUCINE OF ALL DOGS TESTED (9). I-bars indicate SE of ± 2.

2. *Effect of* L-*leucine or saline on hepatic-vein and femoral-vein blood glucose concentration in dogs pretreated with a single oral dose of chlorpropamide.* Fourteen experiments were performed, seven with L-leucine and seven with saline. In this series of experiments, each dog was pretreated with a single oral dose of chlorpropamide 4 hours before the beginning of the experiment. In every other respect, the experiments were like those described in section B1.

3. *Effect of* L-*leucine on hepatic-vein, femoral-vein, and femoral-artery blood glucose concentration in dogs with portacaval shunt.* Three experiments were performed on dogs in which end-to-side portacaval shunts had been performed several weeks previously. In each of these experiments the dogs were studied 4 hours after a single oral dose of chlorpropamide. In addition to the previously described catheters in the hepatic and femoral veins, a catheter was also passed percutaneously into the femoral artery, and blood was sampled from all three of these catheters for determination of blood glucose concentration. In this series of experiments, hepatic blood flow was also estimated by the bromsulphalein (BSP) extraction method of Bradley, Ingelfinger, Bradley, and Curry (7). Bromsulphalein was measured by a modification (8) of the method of Rosenthal and White (9). In other respects, the protocols for these experiments were as described previously.

RESULTS

A. *Effect of* L-*leucine on peripheral blood glucose concentration with and without chlorpropamide pretreatment*

The blood glucose response of each dog to L-leucine under the five experimental conditions is listed in Table I, and the mean hypoglycemic response of all dogs is illustrated in Figure 1. The data were pooled and a single standard error of 4.10 was calculated for each mean. Three conclusions may be drawn from these experiments. First, L-leucine by itself produces a modest but statistically significant fall in blood glucose concentration ($p < 0.03$). Second, the mean hypoglycemic response to L-leucine 2 and 12 hours after a single oral dose of chlorpropamide is significantly greater than that due to L-leucine alone. Finally, the mean hypoglycemic response to L-leucine in the same dogs receiving chlorpropamide on a chronic basis is significantly less than that 2 to 12 hours after one acute dose, and no greater than that produced by L-leucine alone. The comparison between means was made by the method of the 5 per cent least significant difference (10), in which means that differed by more than 11.7 were significant at the 0.05 level.

It appears unlikely that the diminished response to L-leucine 24 hours after 3 consecutive days of chlorpropamide pretreatment results from a decrease in the degree of hypoglycemia produced by chlorpropamide. First, baseline blood glucose concentration was as low after the chronic administration of chlorpropamide as it was after one acute dose. Second, the serum levels of chlorpropamide 24 hours after 3 days of chlorpropamide administration were as high as those measured 2 hours after chlorpropamide (Table II). Although there was no significant difference between the measured chlorpropamide levels, the leucine effect was more impressive in the 2-hour group.

TABLE II

Correlation between per cent fall in blood glucose concentration after L-*leucine administration and serum level of chlorpropamide*

	Time L-leucine given			
	2 Hours after chlorpropamide		24 Hours after 3 days of chlorpropamide	
Dog no.	Fall in blood glucose	Serum level chlorpropamide	Fall in blood glucose	Serum level chlorpropamide
	%	μg/ml	%	μg/ml
911	43	107	27	174
21*	31	123	15	91
574*	23	106	−20†	106
42*	30	75	7	120

* Experiments performed at different times from those in Table I.
† Negative number signifies rise.

TABLE III

Effect of L-*leucine alone on hepatic-vein (HV)–femoral-vein (FV) blood glucose concentration differences*

Dog		Baseline (time in minutes)			Minutes after L-leucine administration					
		−15	−7	0	10	20	30	40	50	60
		mg/100 ml			*mg/100 ml*					
404	HV	85	88	91	86	83	78	80	79	90
	FV	83	84	85	85	81	77	72	74	85
	HV-FV	+2	+4	+6	+1	+2	+1	+8	+5	+5
901	HV	86	88	84	84	78	78	73	73	70
	FV	72	75	81	84	78	70	72	66	63
	HV-FV	+14	+13	+3	0	0	+8	+1	+7	+7
657	HV	82	92	90	77	76	77	78	95	92
	FV	78	80	80	72	72	69	71	71	73
	HV-FV	+4	+12	+10	+5	+4	+8	+7	+24	+19
450	HV	90	76	92	82	81	82	81	82	87
	FV	74	81	81	79	78	77	74	74	80
	HV-FV	+16	−5	+11	+3	+3	+5	+7	+8	+7
609	HV	89	86	92	85	83	83	82	80	81
	FV	82	88	87	83	81	78	79	74	72
	HV-FV	+7	−2	+5	+2	+2	+5	+3	+6	+9

B. *The effect of* L-*leucine on hepatic metabolism*

1. L-*Leucine alone.* L-Leucine produced a small but consistent decrease in blood glucose concentration of every dog studied (Table III). Although there was considerable variation from time to time and from dog to dog, it appeared as if HV–FV glucose concentration differences tended to narrow during the first 20 minutes after administration of L-leucine. This observation is somewhat more apparent when the mean response of all dogs is examined (Figure 2). Mean HV–FV glucose concentration differences 10 and 20 minutes after L-leucine were compared with mean baseline values and found to be significantly decreased (t test, $p < 0.01$). Although the mean HV–FV glucose concentration difference was decreased at these times, it remained significantly positive.

2. L-*Leucine given 4 hours after chlorpropamide.* The effect of L-leucine or saline on blood glucose concentration of each dog studied is seen in Table IV. L-Leucine consistently lowered blood glucose concentration of both hepatic and femoral veins, whereas saline had no significant effect.

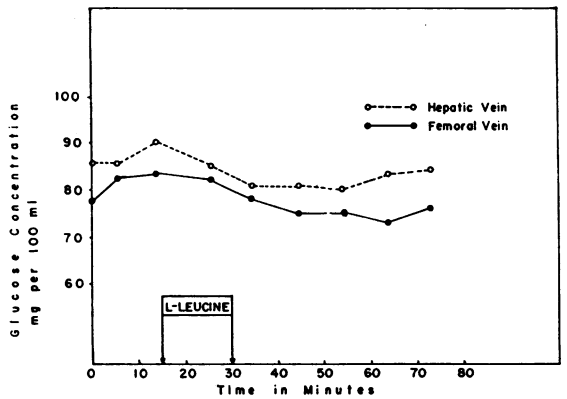

FIG. 2. EFFECT OF L-LEUCINE ON HEPATIC-VEIN–FEMORAL-VEIN GLUCOSE CONCENTRATION DIFFERENCES. Average response of 5 dogs not pretreated with chlorpropamide is shown.

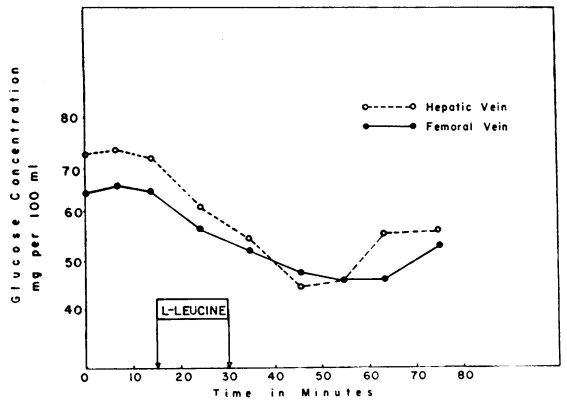

FIG. 3. EFFECT OF L-LEUCINE ON HEPATIC-VEIN–FEMORAL-VEIN GLUCOSE CONCENTRATION DIFFERENCES. Average response of 7 dogs pretreated with chlorpropamide is shown.

TABLE IV

Effect of L-*leucine on the hepatic-vein (HV)–femoral vein (FV) blood glucose concentration difference (HV–FV) in dogs pretreated with chlorpropamide**

Dog	Blood glucose concentration	Baseline (time in minutes) −15	−7	0	Minutes after L-leucine administration +10	+20	+30	+40	+50	+60
		mg/100 ml					*mg/100 ml*			
402	HV	63	73	60	59	34	37	41	34	36
	FV	58	59	59	44	52	45	29	25	29
	HV-FV	+5	+14	+1	+15	−18	−8	+12	+9	+7
901	HV	53	56	49	46	43	41	35	52	58
	FV	46	47	50	50	37	33	32	36	40
	HV-FV	+7	+9	−1	−4	+6	+8	+3	+16	+18
383	HV	74	75	74	63	58	42	54	58	67
	FV	67	65	66	56	54	52	49	52	60
	HV-FV	+7	+10	+8	+7	+4	−10	+5	+6	+7
609	HV	72	73	71	63	60	54	60	61	60
	FV	72	64	64	56	54	52	54	50	56
	HV-FV	0	+9	+7	+7	+6	+ 2	+6	+11	+4
657	HV	71	71	69	67	61	46	43	54	47
	FV	70	69	66	62	51	56	59	56	56
	HV-FV	+1	+2	+3	+5	+10	−10	−16	−2	−9
190	HV	88	83	96	79	72	54	57	73	91
	FV	81	83	81	78	69	62	70	60	84
	HV-FV	+7	0	+15	+1	+3	−8	−13	+13	+7
450	HV	82	81	81	53	49	47	41	67	50
	FV	52	62	72	54	50	43	37	50	60
	HV-FV	+30	+19	+9	−1	−1	+4	+4	+17	−10
					Minutes after saline administration					
574	HV	77	79	82	78	84	85	87	86	82
	FV	70	71	73	75	77	80	81	81	77
	HV-FV	+7	+8	+9	+3	+7	+5	+6	+5	+5
901	HV	74	74	71	78	81	80	81	82	75
	FV	69	75	72	74	74	73	68	68	64
	HV-FV	+5	−1	−1	+4	+7	+7	+13	+14	+11
760	HV	78	77	75	73	72	65	67	66	66
	FV	75	73	72	66	68	64	63	63	63
	HV-FV	+3	+4	+3	+7	+4	+1	+4	+3	+3
609	HV	63	70	65	69	66	69	68	69	67
	FV	59	59	60	61	62	60	63	61	60
	HV-FV	+4	+11	+5	+8	+4	+9	+5	+8	+7
657	HV	57	55	54	55	51	55	57	52	55
	FV	51	53	55	51	48	48	48	47	43
	HV-FV	+6	+2	−1	+4	+3	+7	+9	+5	+12
764	HV	83	86	88	92	89	87	88	87	87
	FV	78	77	78	79	80	79	83	79	80
	HV-FV	+5	+9	+10	+13	+9	+8	+5	+8	+17
450	HV	82	84	86	84	87	92	93	93	96
	FV	76	77	79	79	78	82	86	83	81
	HV-FV	+6	+7	+7	+5	+9	+10	+7	+10	+15

The mean response of all dogs receiving L-leucine after chlorpropamide pretreatment is illustrated in Figure 3. Although there was again considerable variation from time to time and from dog to dog, certain conclusions seem warranted. First, the hypoglycemic response to L-leucine 4 hours after chlorpropamide is much greater than that resulting from L-leucine alone. Second, although the effect of L-leucine on HV and FV glucose concentration appears similar during the

first 20 minutes, regardless of pretreatment, certain differences become apparent 30 and 40 minutes after the administration of L-leucine. In animals receiving L-leucine alone, the HV blood glucose concentration tended to increase from then on.

In contrast is the continued, accelerated fall in HV glucose concentration during the augmented hypoglycemic response after pretreatment with chlorpropamide. Under these conditions, the mean HV–FV glucose concentration difference continued to decrease until, 30 and 40 minutes after the administration of L-leucine, the normal positive hepatic balance was abolished, and then the mean HV–FV glucose concentration difference was not statistically significantly different from zero (t test). The mean response to saline 4 hours after chlorpropamide is illustrated in Figure 4, which shows that HV–FV blood glucose concentration differences did not vary appreciably, remained positive throughout, and were statistically different from zero ($p < 0.001$).

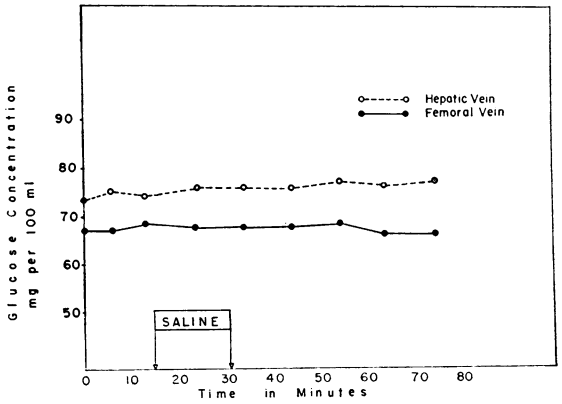

FIG. 4. EFFECT OF SALINE ON HEPATIC-VEIN–FEMORAL-VEIN GLUCOSE CONCENTRATION DIFFERENCES. Average response of 7 dogs pretreated with chlorpropamide is shown.

3. *L-Leucine 4 hours after chlorpropamide in dogs with portacaval shunt.* These experiments were performed in an effort to distinguish the effect of L-leucine on hepatic metabolism from any possible effect it might have on adipose tissue

TABLE V

Effect of L-leucine on hepatic-vein (HV), hepatic-artery (HA), and femoral-vein (FV) blood glucose concentration in dogs with end-to-side portacaval shunts and pretreated with chlorpropamide

Dog no.		Baseline (time in minutes) −20	−10	0	Minutes after L-leucine administration 10	20	40	60
379	Concentration, *mg/100 ml*							
	HV	72	75	70	48	44	28	18
	HA	60	62	58	47	39	24	14
	FV	55	57	54	42	38	22	12
	HV-HA	+12	+13	+12	+1	+5	+4	+4
	HV-FV	+17	+18	+16	+6	+6	+6	+6
	Estimated hepatic blood flow, *ml/min*	169	162	147	160	155	147	157
	Estimated net hepatic glucose output, *mg/min**	20.28	21.06	17.64	1.60	7.75	5.88	6.28
378	Concentration, *mg/100 ml*							
	HV	80	78	75		60	54	45
	HA	72	71	70		59	56	46
	FV	63	60	62		58	55	46
	HV-HA	+8	+7	+5		+1	−2	−1
	HV-FV	+17	+18	+13		+2	−1	−1
	Estimated hepatic blood flow, *ml/min*	182	170	196		200	186	160
	Estimated net hepatic glucose output, *mg/min**	14.56	11.90	9.80		2.0	−3.72	−1.60
377	Concentration, *mg/100 ml*							
	HV	69	68	67		57	36	36
	HA	63	62	62		60	38	40
	FV	57	57	56		51	34	35
	HV-HA	+6	+6	+5		−3	−2	−4
	HV-FV	+12	+11	+11		+6	+2	+1
	Estimated hepatic blood flow, *ml/min*	237	328	359		371	342	335
	Estimated net hepatic glucose output, *mg/min**	14.22	19.68	17.95		−11.13	−6.84	−13.40

* Negative value indicates absence of net hepatic glucose output.

drained by the splanchnic bed. In dogs with portacaval shunt, the blood glucose concentration difference between the hepatic vein and the peripheral arteries and veins is primarily a function of hepatic metabolism alone. Under these conditions, it is assumed that blood glucose concentration in the femoral artery is identical with that in the hepatic artery. The results of these experiments are seen in Table V and are similar to those observed under the same conditions in intact dogs, i.e., hypoglycemia produced by L-leucine was accompanied by a marked fall in HV–FV and HV–HA (hepatic-artery) blood glucose differences. In addition, estimated hepatic blood flow was shown not to change appreciably after L-leucine administration, and consequently net hepatic glucose output was markedly reduced during hypoglycemia. The observation that L-leucine does not affect hepatic blood flow was also confirmed in 3 other dogs by use of a noncannulating, square-wave, electromagnetic flowmeter (11).[2] Consequently it appears unlikely that the changes in HV–FV blood glucose concentrations in intact dogs could have resulted from increased splanchnic glucose utilization, and they indicate further that the effect of L-leucine is to inhibit mechanisms for the maintenance of hepatic glucose output.

DISCUSSION

Any theory attempting to explain the mechanism of leucine hypoglycemia must take into account two fundamental characteristics of this phenomenon. First, although the results we have described demonstrate that L-leucine can lower slightly the blood glucose concentration of normal subjects, significant hypoglycemia has only been produced in certain patients with idiopathic infantile hypoglycemia (12), or insulin-secreting tumors of the pancreas (13), or in normal men (14) and dogs pretreated with insulin (1) or chlorpropamide. Second, the response to L-leucine is not uniform in these situations and cannot be demonstrated in all patients with these clinical syndromes (15–19), nor, as we have shown, in all dogs pretreated with chlorpropamide. Since all the situations in which L-leucine had produced a significant hypoglycemic response seemed to share in common hyperinsulinism, or hypoglycemia, or both, we had suggested earlier that L-leucine "decreases the hyperglycemic response to hypoglycemia, specifically, hepatic glucose output" (1). The present demonstration that net hepatic glucose output is markedly reduced during hypoglycemia produced by L-leucine supports this thesis. On the other hand, even if it is assumed that L-leucine produces hypoglycemia by inhibiting hepatic glucose output during hypoglycemic stress, it is difficult to explain the variable response to L-leucine in what appear to be identical situations.

An attempt to solve this dilemma has been made by differentiating between patients who are "leucine-sensitive" and those who are "leucine-insensitive" (20). Implicit in this distinction is the assumption that these two categories are reflections of different disease processes, and that patients can be separated on the basis of their response to L-leucine. Since the etiology or etiologies of idiopathic infantile hypoglycemia are obscure, the merit of a distinction based on the patient's response to L-leucine is difficult to evaluate. On the other hand, the distinction between "leucine-sensitive" and "leucine-insensitive" seems to have little meaning when applied to patients with insulin-secreting tumors of the pancreas, and avoids the question of why L-leucine produces hypoglycemia only in some patients with organic hyperinsulinism (15–17). This problem is not confined to clinical situations, and it is as difficult to account for the variable hypoglycemic response to L-leucine that has been observed in experimental leucine hypoglycemia. The administration of L-leucine to normal dogs, as to normal men, does not elicit significant hypoglycemia. In contrast, if L-leucine is administered within 2 to 12 hours after a single oral dose of chlorpropamide, normal dogs, like "leucine-sensitive" patients, demonstrate a significant hypoglycemic response. Furthermore, when L-leucine was given to normal dogs who had received chlorpropamide chronically, there was only a modest fall in blood glucose concentration, similar to that described in "leucine-insensitive" patients. Although the analogies are obvious, it is clear that they are only analogies. On the other hand, the conditions under which experimental leucine hypoglycemia has been studied were varied purposefully, and it is possible that a more detailed analysis of these ex-

[2] These measurements were obtained with the aid of Dr. S. Kountz, Department of Surgery, Stanford University School of Medicine, Palo Alto, Calif.

periments may prove helpful in explaining the considerable variation in the hypoglycemic effect of L-leucine.

In the experiments described, two basic patterns of response to L-leucine were demonstrated. A normal dog receiving L-leucine exhibited a small fall in blood glucose concentration. The same dog, given the same amount of L-leucine 2 hours after a single oral dose of chlorpropamide, demonstrated a significantly greater hypoglycemic response. In the first instance, normal dogs are not reacting to any hypoglycemic stimulus, and it might be predicted that the ability of L-leucine to inhibit hepatic glucose output would result in only a minor fall in blood glucose concentration. The degree, however, of hypoglycemic stress in a normal dog 2 hours after 250 to 375 mg of chlorpropamide is certainly greater, and in this case, inhibition of hepatic glucose output could possibly result in a much greater fall in blood glucose concentration. On the other hand, it is clear that the degree of hypoglycemia produced by L-leucine is not directly related to the severity of the hypoglycemic stimulus alone. For example, dogs receiving chlorpropamide chronically were, if anything, reacting to a more profound hypoglycemic stimulus than were dogs 2 hours after a single oral dose of chlorpropamide. Yet the administration of L-leucine to dogs after chronic chlorpropamide pretreatment elicited a fall in blood glucose concentration no greater than that observed after L-leucine alone. Perhaps the explanation for the diminished effect of L-leucine in normal dogs after chronic pretreatment with chlorpropamide is that the counter-regulatory mechanisms for the maintenance of euglycemia had been so well stimulated that it was more difficult to produce hypoglycemia in this case. Obviously there are insufficient data to explain precisely why L-leucine acts differently under certain conditions. On the other hand, it is clear that different hypoglycemic responses to L-leucine can occur in the same subject under different conditions, and it is difficult in the light of these observations to view "leucine sensitivity" as an "inborn error of metabolism." It seems more reasonable to assume that the specific metabolic action of L-leucine is relatively constant under all conditions and that only the resultant fall in blood glucose concentration varies from situation to situation. Finally, it would appear that a more thorough understanding of the various mechanisms by which the liver maintains euglycemia is essential for ultimate understanding of the phenomenon of leucine hypoglycemia.

SUMMARY

The administration of L-leucine alone to normal dogs slightly lowered blood glucose concentration, which was greatly increased after suitable pretreatment with chlorpropamide. Experiments performed on both intact dogs and dogs with portacaval shunt have indicated that this augmented hypoglycemic response to L-leucine after chlorpropamide is accompanied by a marked decrease in positive hepatic glucose balance. It is suggested that L-leucine acts similarly in all situations to inhibit hepatic glucose output and that the resultant fall in blood glucose concentration will vary considerably, depending on the status of other homeostatic mechanisms for maintenance of euglycemia at the time L-leucine is administered.

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REFERENCES

1. Reaven, G., and Lucas, C. The use of insulin in the production of L-leucine-induced hypoglycemia in normal dogs. J. clin. Invest. 1962, **41**, 654.
2. Reaven, G., Lucas, C., and Winkelman, C. Hypoglycemic effect of L-leucine during periods of endogenous hyperinsulinism. Diabetes. In press.
3. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. J. biol. Chem. 1944, **153**, 375.
4. Toolan, T. J., and Wagner, R. L., Jr. The physical properties of chlorpropamide and its determination in human serum. Ann. N. Y. Acad. Sci. 1959, **74**, 449.
5. Somogyi, M. Determination of blood sugar. J. biol. Chem. 1954, **69**, 160.
6. Somogyi, M. Notes on sugar determination. J. biol. Chem. 1952, **19**, 195.
7. Bradley, S. E., Ingelfinger, F. J., Bradley, G. P., and Curry, J. J. The estimation of hepatic blood flow in man. J. clin. Invest. 1945, **24**, 890.
8. Henry, R. J., Chiamori, N., and Ware, A. G. Determination of bromsulfonphthalein in serum by means of acetone precipitation of proteins. Amer. J. clin. Path. 1959, **32**, 201.

9. Rosenthal, S. M., and White, E. C. Clinical application of the bromsulphalein test for hepatic function. J. Amer. med. Ass. 1925, **84**, 1112.
10. Fisher, R. A. Design of Experiments, 6th ed. New York, Hafner, 1951, pp. 56–58.
11. Denison, A. B., Jr., Spencer, M. P., and Green, H. D. A square wave electromagnetic flowmeter for application to intact blood vessels. Circulat. Res. 1955, **3**, 39.
12. Cochrane, W. A., Payne, W. W., Simpkiss, M. J., and Woolf, L. I. Familial hypoglycemia precipitated by amino acids. J. clin. Invest. 1956, **35**, 411.
13. Flanagan, G. C., Schwartz, T. B., and Ryan, W. G. Studies on patients with islet-cell tumor, including the phenomenon of leucine-induced accentuation of hypoglycemia. J. clin. Endocr. 1961, **21**, 401.
14. Fajans, S. S., Power, L., Gwinup, G. W., Knopf, R. F., and Conn, J. W. Studies on the mechanism of leucine hypoglycemia (abstract). J. Lab. clin. Med. 1960, **56**, 810.
15. Hartmann, A. F., Sr., Wohltmann, H. J., Holowach, J., and Caldwell, B. M. Studies in hypoglycemia. J. Pediat. 1960, **56**, 211.
16. Grumbach, M. M., and Kaplan, S. L. Amino acid and alpha-keto acid-induced hyperinsulinism in the leucine-sensitive type of infantile and childhood hypoglycemia. J. Pediat. 1960, **57**, 346.
17. Di George, A. M., and Auerbach, V. H. Leucine-induced hypoglycemia. A review and speculations. Amer. J. med. Sci. 1960, **240**, 792.
18. Cochrane, W. Idiopathic infantile hypoglycemia and leucine sensitivity. Metabolism 1960, **9**, 386.
19. Haworth, J. C., and Coodin, F. J. Idiopathic spontaneous hypoglycemia in children: report of seven cases and review of the literature. Pediatrics 1960, **25**, 748.
20. Cochrane, W. A. Studies in the relationship of amino acids to infantile hypoglycemia. Amer. J. Dis. Child. 1960, **99**, 476.