Leucine and Its Derivatives as Potential Elicitors of Insulin Secretion in Rats

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Abstract □ This report describes the testing of a number of leucine derivatives for their ability to increase serum levels of glucose and immunoreactive insulin. Insulin levels increased in rats when L-leucine, DL-leucine, and L-leucineamide were administered. Esters of L-leucine caused no significant effect on glucose or insulin levels.

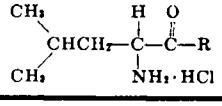
Keyphrases □ Leucine and derivatives—role in eliciting insulin secretion, rats, serum levels of glucose and immunoreactive insulin □ Insulin secretion, rats—effect of leucine and derivatives, glucose and immunoreactive insulin serum levels □ Immunoreactive insulin—role of leucine and derivatives in eliciting secretion, serum levels, rats

The amino acid, leucine, has been shown to cause hypoglycemia in normal subjects by increasing plasma levels of insulin (1, 2). Furthermore, an infusion of 10 essential amino acids, including leucine, results in a marked increase in insulin secretion, although the release of insulin by leucine appears to be *via* a different mechanism than that due to other amino acids (3, 4). In the present experiment, a number of derivatives of leucine were administered to rats, and glucose and immunoreactive insulin levels were obtained at subsequent intervals. It was found that only L-leucine (and DL-leucine to a lesser extent) had the ability to elicit insulin secretion in this preparation. Administration of L-leucineamide also caused an elevation in insulin levels, but it was secondary to a rise in blood glucose.

PROCEDURE

The compounds tested in this study are indicated in Table I. The methyl and ethyl esters of L-leucine were synthesized in the manner of Schneider and Schaeg (5), and the L-leucineamide hydrochloride and DL-leucineamide hydrochloride were synthesized from the corresponding methyl esters in the manner of Smith and Spackman (6). The drugs were all dissolved in distilled water to give a final concentration of 37.5 mg./ml. (w/v). The solutions were then coded according to Table I so that the persons injecting the rats, collecting and preparing the blood samples, and performing the biochemical determinations were unaware of the experimental conditions.

Table I—Compounds Studied



Name	R	Code on Figs. 1 and 2 and Tables II and III
—	—	A: Control solution (distilled water)
L-Leucineamide hydrochloride	NH_2	B
L-Leucine ethyl ester hydrochloride	OCH_2CH_3	C
L-Leucine methyl ester hydrochloride	OCH_3	D
DL-Leucine hydrochloride	OH	E
L-Leucine hydrochloride	OH	F
DL-Leucineamide hydrochloride	NH_2	G

A total of 118 mature albino rats of both sexes was used. All had had continuous access to both food[1] and water for at least a week prior to the experiment. For the experiment itself, all rats were deprived of food (but not water) for 24 hr. Each rat was then given an intraperitoneal injection of one of the seven solutions and returned to its individual home cage. The dose was always 150 mg./kg. of the appropriate drug or the equivalent volume of distilled water (Group A). The rats were removed from the home cage and decapitated by a guillotine at 2.5, 5, 10, or 20 min. after the injections. Uninjected rats (n = 5) were decapitated to provide baseline values for this population. Carotid blood was collected over ice and subsequently centrifuged. The serum portion was then frozen until the determinations were made.

Serum glucose determinations were done by the glucose oxidase method of O'Brien *et al.* (7), and serum insulin determinations were done by a modification (8) of the double antibody method of Morgan and Lazarow (9).

RESULTS

The results of the glucose and immunoreactive insulin determinations are summarized in Figs. 1 and 2 and Tables II and III, respectively. Baseline glucose and insulin values were 100.8 mg./100 ml. and 12.8 microunits/ml. serum, respectively. The placebo injection (Group A) caused a slight increase in glucose and a slight decrease in insulin, the magnitude and duration of which were previously observed in this laboratory (10, 11).

The administration of L-leucine hydrochloride (Group F) caused a large spike of insulin at 2.5 min., which then stabilized at a high level for the duration of the 20-min. test period. There was also a slight (nonsignificant) increase in glucose at that time. Although the small increase in glucose might have accounted for part of the initial rise in insulin in Group F, glucose levels in that group tended to

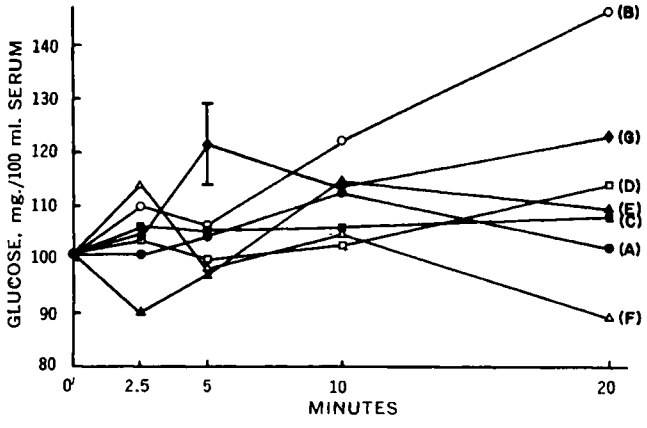


Figure 1—*Mean values of serum glucose for the different groups at the different intervals. Because there was homogeneity of variance, only the mean standard error of the mean was plotted.*

[1] Purina rat chow.

Table II—Serum Immunoreactive Insulin Values (microunits per milliliter) for the Different Groups[a]

Minutes after Injection	Group A	B	C	D	E	F	G
2.5	11.9 ± 2.8 (4)	23.2 ± 2.2 (4)	10.3 ± 4.0 (4)	11.6 ± 2.1 (4)	28.7 ± 5.2 (4)	38.3 ± 2.9 (4)	11.6 ± 3.5 (4)
5	9.8 ± 1.7 (4)	26.9 ± 5.7 (5)	10.8 ± 3.5 (4)	8.0 ± 2.3 (4)	22.5 ± 4.5 (4)	28.3 ± 3.7 (4)	9.4 ± 2.4 (4)
10	9.5 ± 2.9 (4)	28.1 ± 3.4 (4)	8.8 ± 2.2 (4)	15.4 ± 1.1 (4)	22.5 ± 2.1 (4)	32.0 ± 6.1 (4)	24.0 ± 1.9 (5)
20	9.4 ± 1.9 (4)	28.9 ± 6.1 (4)	10.3 ± 3.0 (4)	14.4 ± 2.4 (4)	23.8 ± 2.6 (4)	31.3 ± 8.1 (3)	17.2 ± 1.9 (4)

[a] Entries are expressed in the following form: mean ± standard error of the mean (number of animals).

Table III—Serum Glucose Values (mg./100 ml.) for the Different Groups[a]

Minutes after Injection	Group A	B	C	D	E	F	G
2.5	100.8 ± 1.9 (4)	109.8 ± 13.7 (4)	106.0 ± 8.7 (4)	103.4 ± 3.8 (4)	90.0 ± 5.4 (4)	114.0 ± 2.4 (4)	104.0 ± 10.0 (4)
5	104.3 ± 10.5 (4)	106.2 ± 12.4 (5)	105.5 ± 4.0 (4)	100.0 ± 10.7 (4)	96.8 ± 3.7 (4)	98.0 ± 10.4 (4)	121.8 ± 8.2 (4)
10	112.0 ± 6.4 (4)	122.0 ± 9.6 (4)	105.8 ± 6.8 (4)	103.0 ± 7.1 (4)	114.3 ± 3.5 (4)	104.0 ± 6.8 (4)	112.6 ± 15.3 (5)
20	102.0 ± 5.0 (4)	145.8 ± 6.2 (4)	108.0 ± 7.1 (4)	114.0 ± 9.2 (4)	109.0 ± 4.6 (4)	89.0 ± 5.0 (3)	123.0 ± 8.8 (4)

[a] Entries are expressed in the following form: mean ± standard error of the mean (number of animals).

decrease over the 20-min. test period while insulin values remained elevated. A similar but greatly attenuated insulin pattern was observed after the administration of DL-leucine hydrochloride (Group E); in this instance, the significant increase in insulin after 2.5 min. could not be attributed to an increase in glucose since that group showed a nonsignificant decrease in glucose at that interval.

The administration of L-leucineamide hydrochloride (Group B) also resulted in an elevation of insulin levels, although no early spike (2.5 min.) was observed. In this instance, the increased insulin levels can be attributed to a glucose level that continued to rise throughout the 20-min. period. Administration of DL-leucineamide hydrochloride (Group G) caused a similar pattern, which was again attenuated and which did not appear until 10 min. following the injection.

Administration of the methyl (Group D) and ethyl (Group C) esters had no reliable effect on either glucose or insulin values.

DISCUSSION

L-Leucine was previously shown to cause a biphasic insulin response in man (12). In that experiment, the initial peak occurred at 5 min. and the secondary peak occurred at 20 min. after the injection. The present results in rats are similar, although the initial peak appeared somewhat sooner (2.5 min.). The biphasic insulin response corresponds to the two-pool system of insulin postulated by Porte and Pupo (13) and Porte and Bagdade (14).

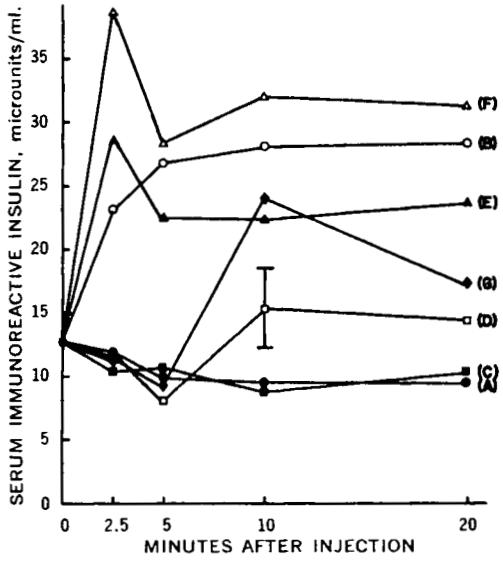


Figure 2—*Mean values of serum insulin for the different groups at the different intervals.*

The attenuated response to the DL-isomer of leucine was also reported previously (15). In that same paper, it was shown that the metabolic products of leucine have no effect in eliciting insulin secretion or hypoglycemia.

The delayed increase in insulin levels following the administration of L- and DL-leucineamide hydrochloride must be attributed to the elevated glucose levels in those rats. The mechanism for this increase in glucose is not known, although it probably is not secondary to a release of epinephrine because any epinephrine would be expected to prevent the observed increase in insulin output (16).

In summary, modification of the leucine molecule by addition of either an amide group or a methyl or ethyl ester eliminated the ability of the molecule to elicit insulin secretion. Furthermore, only the L-isomer of leucine appears to be effective because a DL-mixture responds in a manner predictable by simple dilution of the L-isomer.

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Selective Blockade of Muricidal Activity in the Rat by Anorectic Agents

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Abstract □ The ability of several anorectics to block selectively mouse killing in the rat was examined. A significant ratio between the muricide ED_{50} and the ED_{50} of a drug to produce debilitation in rotarod performance is a measure of selectivity. The mouse-killing behavior of a rat can be blocked with certain drugs at doses that do not render the animal debilitated. This selectivity, determined by the ratio of ED_{50}'s, was found in the four anorectics examined as follows: diethylpropion > dextroamphetamine > aminorex > fenfluramine.

Keyphrases □ Anorectic agents—ability to block mouse killing in rats, determination of selectivity, correlated with decreased rotarod performance □ Muricidal activity—selective blockade by anorectic agents, rat □ Mouse-killing behavior—killer rats, effect of four anorectic agents, determination of selective blockade

Horovitz *et al.* (1) demonstrated that several stimulants, antidepressants, and antihistamines block mouse-killing (muricide) behavior in rats. Barnes *et al.* (2) and Gogerty *et al.* (3) found that stimulants which possess anorectic activity abolish mouse-killing behavior. The stimulant-anorectic dextroamphetamine was shown by Horovitz's group and later by Sofia (4) to block selectively muricide behavior in the rat. Selectivity was observed when the dose of a drug causing muricidal blockade was significantly smaller than that resulting in debilitation of the animal. Selectivity was measured by the ratio between the ED_{50} (MKD_{50}) dose for mouse-killing blockade and the ED_{50} (NTD_{50}) for neurotoxicity (4), as found by the inability of the rat to perform on the rotarod *via* the method of Dunham and Miya (5).

Table I—Effects of Anorexigenic Compounds on Rat Rotarod Performance and Muricide Behavior

Drug	Muricide Blockade, MKD_{50}, mg./kg.[a]	Rotarod Debilitation, NTD_{50}, mg./kg.[a]	NTD_{50}/ MKD_{50}
Aminorex	1.67 (0.97–2.86)	12.1 (6.74–21.8)	7.25
Dextro-amphetamine	0.46 (0.30–0.72)	4.64 (1.82–11.8)	10.09
Diethylpropion	3.83 (2.13–6.89)	68.1 (no limits)	17.78
Fenfluramine	3.16 (1.69–5.92)	14.7 (8.5–25.3)	4.65

[a] MKD_{50} and NTD_{50} are given with fiducial limits in parentheses.

In the present experiment, the mouse-killing selectivity of other anorectic compounds was investigated. Rotarod performance was again used as a measure of neurotoxicity.

EXPERIMENTAL

The anorexigenics, aminorex, dextroamphetamine, diethylpropion, and fenfluramine, were tested in rats for their muricide-blocking ability and debilitation of performance on the rotarod as described in the following procedures.

Mouse Killing—Male Wistar rats[1], weighing 150–200 g. at the start of the experiment, were used. Food and water were freely available to each individually caged rat. To determine mouse-killing ability, a mouse was placed in each rat's cage for 10 min. This 10-min. period for the kill was gradually reduced to 5 sec. Eventually, only those rats that killed consistently and instantly upon confrontation with the mouse were saved for future drug studies. These animals, approximately 15% of the original group, were termed "killer rats."

Saline was administered intraperitoneally to condition the rats to the injection procedure and to eliminate any rats that did not kill after placebo administration. Mice were placed in the rats' cages 30, 60, and 120 min. after the saline injection. The same procedure was followed during the ensuing drug studies.

For a drug session, a single dose of one of the four drugs dissolved in saline was injected. A mouse was introduced to the rat at the three time intervals for a maximum of 30 sec. to test for muricide behavior. At the end of the 30 sec., all mice were removed from the cages. If a mouse was not killed at any of the three time intervals, muricidal behavior was considered blocked.

Killer rats were used for several drug injections. Therefore, at least 2 weeks elapsed between drug administrations. Between drugs, saline was given several times following the same procedure. Four rats were used for each dose of a drug, and the data were analyzed for an ED_{50} for mouse-killing behavior (MKD_{50}) by Horn's (6) method.

Rotarod Test—Wistar rats were also used for rotarod testing. Housing and food conditions were the same as in the mouse-killing experiments. Each rat was placed on a rotarod moving at a rate of approximately 4 r.p.m. and trained to maintain himself for 120 sec. Approximately 10% of the rats had difficulty in this conditioning and were discarded from the experiment. The rats were then intraperitoneally administered one dose of the four drugs to be examined. Thirty, 60, and 120 min. later, each animal was tested for its ability to remain on the rotarod for a 30-sec. period. If a rat fell off the rotating rod during any of the three testing sessions, he

[1] Harlan Industries, Cumberland, Ind.