

The Effect of Adrenergic and Ganglionic Blockers upon the L-Dopa-Stimulated Release of Glucagon in the Rat¹ (39978)

DAVID T. GEORGE AND PAUL T. BAILEY

United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701

The secretory function of the endocrine pancreas is altered by a number of neurotransmitters including epinephrine (1), norepinephrine (2), acetylcholine (3), serotonin (4), dopamine (5), and L-dopa (6, 7). The neural control of the endocrine pancreas has been reviewed in detail (8); there appear to be at least two mechanisms of action associated with these neurotransmitters. The most extensively studied mechanism involves the stimulation or suppression of α - and β -adrenergic receptor responses (8). Another mechanism seems to function, independent of the adrenergic nervous system, via the cholinergic (3) or dopaminergic (5) pathways. In fact, recent data from our laboratory have shown that L-dopa elicits the release of glucagon and promotes hyperglycemia in monkeys (6) and man (7).

Since the mechanism responsible for the L-dopa-induced release of glucagon is unknown, studies were designed to define more completely the role of the sympathetic nervous system in this phenomenon. Phentolamine and propranolol were used to block the α - and β -adrenergic receptors, respectively. In addition, dopamine was given to determine if it would mimic the L-dopa-induced release of glucagon.

Pentolinium tartrate, a ganglionic blocker, was given alone and in conjunction with L-dopa to ascertain the role of the autonomic ganglia in mediating the L-dopa-induced release of glucagon.

¹ In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Materials and methods. One hundred and twenty 14-hr-fasted, 180- to 220-g male Fisher-Dunning rats were randomly assigned to 12 groups of 10 animals each. Table I presents the experimental design used to assess the influence of L-dopa as well as selected adrenergic and ganglionic blockers upon the L-dopa-induced release of glucagon. The first study consisted of 30 rats which had no pretreatment, whereas in studies 2, 3, and 4 each rat received a prior intraperitoneal (ip) injection of phentolamine, propranolol, pentolinium tartrate 20 min before the intravenous (iv) injection of L-dopa or saline. Following sodium pentobarbitol (50 mg/kg, ip) anesthesia a midline laparotomy was performed exposing the hepatic portal and left femoral veins. A 21-gauge pediatric iv injection set was inserted into the hepatic portal vein, the basal (time = 0) 1.5-ml blood sample was taken, and the catheter was flushed with 1.5-ml of saline. Immediately after obtaining the basal sample a 25-gauge needle was inserted into the femoral vein for the administration of L-dopa, dopamine, or saline over a 30-sec time period. Two subsequent blood samples were obtained from the portal vein of each rat 5 and 15 min after the iv injection. Samples were immediately transferred to iced, capped, polypropylene test tubes containing 15 mg of disodium ethylenediaminetetraacetic acid and 1000 U (kallikrein inactivator units) of Trasylol/ml of whole blood. The samples were centrifuged at 4° for 15 min at 1000g; the plasma was separated and stored at -20° until assayed for glucose (9), glucagon (10), and insulin (11). Data were analyzed using Student's *t* test for paired or unpaired variates; *P* < 0.02 was the criterion of significance.

Results. The first study evaluated the effects of L-dopa, dopamine, and saline

upon the portal vein concentrations of glucagon, insulin, and plasma glucose in the rat. The iv administration of 10 mg/kg of L-dopa or dopamine induced a significant ($P < 0.01$) increase in portal plasma glucagon at 5 and 15 min which was accompanied by a significant ($P < 0.001$) elevation in portal plasma glucose (Fig. 1). The saline data demonstrate that the anesthesia and animal manipulation had no effect upon the subsequent portal vein concentrations of glucagon and glucose. Portal vein concentrations of insulin determined during the height of the glucagon response at 5 min were unaltered by L-dopa and dopamine treatments when compared to saline controls (Table I).

The second study evaluated the effect of an α -adrenergic receptor blocking agent on the L-dopa-induced release of glucagon in the rat. As shown in Table II, phentolamine blocked the L-dopa-stimulated

TABLE I. EXPERIMENTAL DESIGN, DRUG TREATMENTS, AND THE PORTAL PLASMA VEIN INSULIN^a CONCENTRATIONS 5 min AFTER INJECTION OF L-DOPA OR DOPAMINE.

Study No.	Treatments (dose)	Portal plasma insulin (μ U/ml)	P compared to controls ^b
1	Saline controls ^c	20 \pm 2	NS
	L-Dopa (10 mg/kg, iv)	31 \pm 7	
	Dopamine (10 mg/kg, iv)	16 \pm 5	
2	Phentolamine controls (4 mg/rat, ip)	243 \pm 82	<0.01
	Phentolamine + L-dopa	248 \pm 27	<0.001
	L-Dopa controls	48 \pm 10	
3	Propranolol controls (1 mg/rat, ip)	58 \pm 13	NS
	Propranolol + L-dopa	33 \pm 5	NS
	L-Dopa controls	28 \pm 4	
4	Phentolinium tartrate controls (5 mg/rat, ip)	53 \pm 12	NS
	Phentolinium tartrate + L-dopa	36 \pm 6	NS
	L-Dopa controls	31 \pm 6	

^a Ten rats per group; mean \pm SEM.

^b Saline, study 1; L-dopa, studies 2-4.

^c Saline was given iv in a volume equivalent to L-dopa.

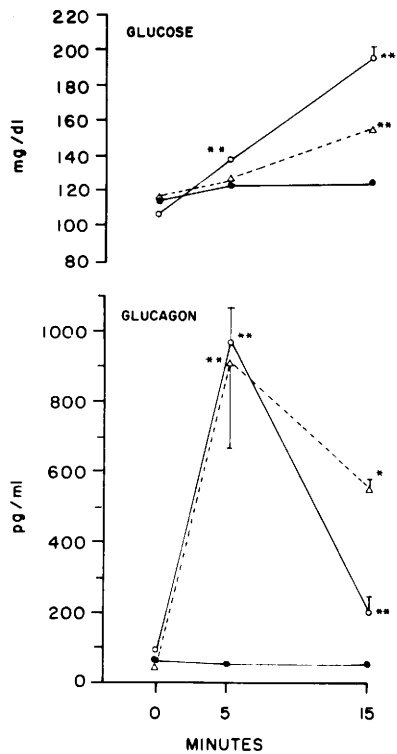


Fig. 1. Effect of dopamine (Δ) and L-dopa (\circ) on plasma glucose and glucagon levels in rats. * $P < 0.01$ and ** $P < 0.001$ by paired t test compared to time 0 values for each treatment. Standard errors are plotted when $P < 0.01$ by unpaired t test as compared to saline controls (\bullet).

release of glucagon and glucose. Pretreatment with phentolamine elicited a significant ($P < 0.001$) elevation of portal plasma insulin (Table I). L-dopa had no effect upon the phentolamine-induced insulin response.

The third study evaluated the influence of prior β -adrenergic receptor blockade established with propranolol (1 mg/rat); there was no effect upon the portal plasma glucagon. However, it did significantly elevate plasma glucose at 5 ($P < 0.01$) and 15 ($P < 0.001$) min when compared to basal levels (Table II). During β -receptor blockade, L-dopa was still an effective stimulator of glucagon release at 5 and 15 min ($P < 0.01$) but there was a significant muting of the response ($P < 0.02$) when compared to the L-dopa controls (Table II). Although propranolol induced an elevation of plasma glucose, the L-dopa controls

TABLE II. PORTAL PLASMA LEVELS OF GLUCAGON AND GLUCOSE IN RATS DURING ADRENERGIC AND GANGLIONIC BLOCKADE AT 0, 5, AND 15 min AFTER L-DOPA.

Group	0 Minutes		5 Minutes		15 Minutes	
	Glucagon (pg/ml)	Glucose (mg/dl)	Glucagon (pg/ml)	Glucose (mg/dl)	Glucagon (pg/ml)	Glucose (mg/dl)
Phentolamine + saline	73 ± 7	114 ± 2	76 ± 6 ^{*c}	118 ± 3	93 ± 12 ^b	120 ± 6 ^b
Phentolamine + L-dopa	66 ± 5	98 ± 6	82 ± 10 ^e	130 ± 9	90 ± 10 ^b	122 ± 7 ^a
L-Dopa controls	118 ± 14	107 ± 3	1169 ± 163 ^f	140 ± 3 ^f	255 ± 33 ^e	187 ± 6 ^f
Propranolol + saline	114 ± 21	122 ± 5	73 ± 5 ^c	144 ± 4	100 ± 16	161 ± 3 ^b
Propranolol + L-dopa	68 ± 5	120 ± 4	451 ± 80 ^b	150 ± 6	209 ± 68 ^a	188 ± 9
L-Dopa controls	76 ± 11	121 ± 10	773 ± 88 ^f	138 ± 14	155 ± 27 ^d	208 ± 17 ^f
Pentolinium tartrate + saline	75 ± 11	102 ± 4	46 ± 2 ^c	111 ± 3 ^b	51 ± 5 ^b	120 ± 3 ^b
Pentolinium tartrate + L-dopa	46 ± 3	111 ± 9	688 ± 99	118 ± 5 ^b	148 ± 20 ^c	176 ± 10 ^b
L-Dopa controls	92 ± 12	114 ± 5	807 ± 103 ^f	151 ± 11 ^e	246 ± 43 ^f	213 ± 8 ^f

* Statistically significant, as follows: (a) $P < 0.02$ vs L-dopa controls; (b) $P < 0.01$ vs L-dopa controls; (c) $P < 0.001$ vs L-dopa controls; (d) $P < 0.025$ vs 0-time values; (e) $P < 0.01$ vs 0-time values; (f) $P < 0.001$ vs 0-time values.

were significantly ($P < 0.01$) higher than the propranolol controls (Table II). Portal plasma insulin levels tended to be higher but were not significantly altered by pre-treatment with propranolol (Table I).

The ganglionic blocker pentolinium tartrate was examined in the fourth study. It is apparent in Table II that this agent had no effect upon the portal plasma concentrations of glucagon or the L-dopa-mediated glucagon response. Ganglionic blockade diminished the plasma glucose response to L-dopa at 5 and 15 min. Portal levels of insulin in pentolinium tartrate-treated rats were not significantly different from the L-dopa controls (Table I).

Discussion. The data presented demonstrate that L-dopa induces a significant release of glucagon which is detectable at high concentrations in portal plasma within 5 min. The magnitude and timing of this response in rats is in agreement with previous studies in monkeys (6). Since dopamine is unable to cross the blood-brain barrier (13) but mimics the L-dopa-induced release of glucagon, the effect of these compounds appears to be peripheral, and not central nervous system-mediated as postulated previously (6, 7). The inability of ganglionic blockade to modify the L-dopa-induced glucagon release indicates that the response probably is not mediated via the autonomic ganglia. Since it has been shown that L-dopa may be metabolized within the pancreas to norep-

inephrine and epinephrine (14) and that both the α - and β -adrenergic blockers are effective suppressors of the glucagon release, it appears likely that the response is mediated via the adrenergic receptors. These findings are consistent with the report of Harvey *et al.* (15), but in direct contrast to our previous reports in monkeys. The reasons for these differences appear to be, first, species differences, or second, a more likely explanation, differences in dosage of the α - and β -blockers. Although our previous studies in monkeys indicated effective blockade of some parameters, it was not sufficient to alter the insulin release prior to L-dopa treatment (6). In the present study the α -blocker engendered a striking increase in plasma insulin. Furthermore, Wilson *et al.* (5) have reported inhibition of the L-dopa-mediated insulin responses with similar high doses of α -blockers.

An alternate explanation for the L-dopa-mediated endocrine pancreas responses, as proposed by others (5, 7), is the presence of dopaminergic receptors within the endocrine pancreas. However, recently Lorenzi *et al.* (16) have reported that this is not a likely explanation since a known dopaminergic agonist, apomorphine, was unable to stimulate the release of glucagon and insulin in man.

Therefore it appears likely that the response is due to metabolism of L-dopa and dopamine to norepinephrine and epineph-

rine and is a result of stimulation of the α - and/or β -adrenergic receptors.

Summary. To help characterize the L-dopa-mediated release of glucagon, rats were given either L-dopa or dopamine (10 mg/kg) intravenously; portal plasma levels of insulin, glucagon, and glucose were measured in the presence and absence of adrenergic and ganglionic blockers.

The α -adrenergic blocker (phentolamine) suppressed the glucagon and glucose responses to L-dopa and increased plasma insulin levels. β -Adrenergic blockade with propranolol ameliorated the L-dopa-mediated glucagon and glucose responses but had no effect on plasma insulin levels.

Ganglionic blockade with pentolinium tartrate was ineffective and did not alter any of the L-dopa-mediated responses of glucagon, insulin, or glucose.

These data indicate that L-dopa and dopamine probably act via the sympathetic nervous system through known pathways enhancing the release of glucagon. Furthermore they substantiate the fact that the adrenergic nervous system is involved in the regulation of the endocrine pancreas.

The authors express their gratitude to George W. Saukel, Gary L. Dickson, Timothy B. Lantry, Jr., Saul Miller, and Karen Bostian for their expert technical assistance; Dr. Mary A. Root, Eli Lilly and Company, Research Laboratories, Indianapolis, Indiana, for a supply of human insulin standard (Lot 258-1064B-27); Dr. Robert J. Hosley, Eli Lilly and Company, for the supply of highly purified glucagon; and the Norwich

Pharmaceutical Company, Norwich, New York, for their donation of a supply of L-dopa.

1. Gerich, J. E., Karam, J. H., and Forsham, P. H., *J. Clin. Endocrinol. Metab.* **37**, 479 (1973).
2. Feldman, J. M., Boyd, III, A. E., and Lebovitz, H. E., *J. Pharmacol. Exp. Ther.* **176**, 611 (1971).
3. Kaneto, A., and Kosaka, K., *Endocrinology* **95**, 676 (1974).
4. Feldman, J. M., and Lebovitz, H. E., *Endocrinology* **86**, 66 (1970).
5. Wilson, J. P., Downs, R. W., Jr., Feldman, J. M., and Lebovitz, H. E., *Amer. J. Physiol.* **227**, 305 (1974).
6. George, D. T., and Rayfield, E. J., *J. Clin. Endocrinol. Metab.* **39**, 618 (1974).
7. Rayfield, E. J., George, D. T., Eichner, H. L., and Hsu, T. H., *N. Engl. J. Med.* **293**, 589 (1975).
8. Woods, S. C., and Porte, D., Jr., *Physiol. Rev.* **54**, 596 (1974).
9. Asrow, G., *Anal. Biochem.* **28**, 130 (1969).
10. Aguilar-Parada, E., Eisentraut, A. M., and Unger, R. H., *Amer. J. Med. Sci.* **257**, 415 (1969).
11. Juan, C., and AvRuskin, T. W., *J. Clin. Endocrinol. Metab.* **33**, 150 (1971).
12. Rocha, D. M., Faloona, G. R., and Unger, R. H., *J. Clin. Invest.* **51**, 2346 (1972).
13. Oldendorf, W. H., *Amer. J. Physiol.* **221**, 1629 (1971).
14. Ericson, L. E., Ekholm, R., and Lundquist, J., *Acta Endocrinol.* **67**, (Suppl. 155), 201 (1971).
15. Harvey, W. D., Faloona, G. R., and Unger, R. H., *Endocrinology* **94**, 1254 (1974).
16. Lorenzi, M., Tsalikian, E., Bohannon, N. V., Gerich, J. E., Karam, J. H., and Forsham, P. H., Program and Abstracts, Endocrine Society, 58th Annual Meeting, p. 229 (1976).

Received January 26, 1977. P.S.E.B.M. 1978, Vol. 157.