Cholinergic Stimulation of Norepinephrine Release in Man

EVIDENCE OF A SYMPATHETIC POSTGANGLIONIC AXONAL LESION IN DIABETIC ADRENERGIC NEUROPATHY

STEVEN A. LEVESTON, SURESH D. SHAH, and PHILIP E. CRYER, *Metabolism Division, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110*

ABSTRACT Amplification of endogenous cholinergic activity—produced by the intravenous injection of edrophonium, an acetylcholinesterase inhibitor which does not enter the central nervous system, into normal subjects—resulted in significant and briefly sustained increments in the plasma concentrations of norepinephrine (153±15–234±29 pg/ml, $P < 0.01$) and epinephrine (16±3–34±5 pg/ml, $P < 0.01$) measured with a single-isotope derivative method. These increments were not attributable to reflex responses to hemodynamic changes and similar increments in plasma norepinephrine occurred in adrenalectomized (epinephrine deficient) patients. Thus, cholinergic activation results in direct stimulation of sympathetic postganglionic neurons, with augmented norepinephrine release, and of the adrenal medullae, with augmented epinephrine release, in man. Four diabetic patients with hypoadrenergic postural hypotension exhibited blunted sympathetic postganglionic neural responses, and normal adrenomedullary responses, to cholinergic stimulation (and to standing) indicative of the presence of a sympathetic postganglionic axonal lesion in diabetic adrenergic neuropathy. Nondiabetic patients with hypoadrenergic postural hypotension due to documented or probable central nervous system lesions exhibited normal responses to cholinergic stimulation produced in this fashion demonstrating the presence of intact sympathetic postganglionic neurons and adrenal medullae in these patients and providing further support for the conceptual soundness of this approach to the study of human adrenergic physiology and pathophysiology.

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Dr. Leveston's present address is Albany Medical College, Albany, N. Y. 12208.

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INTRODUCTION

Normal compensation for the abrupt decrease in venous return to the heart caused by assumption of the upright posture involves activation of a sympathetic neural reflex which results in maintenance of the blood pressure and is reflected in a sharp increase in the plasma concentration of the sympathetic neurotransmitter norepinephrine and a smaller increase in the plasma concentration of the adrenomedullary hormone epinephrine (1, 2). Patients with postural hypotension due to a defect in this sympathetic neural reflex exhibit blunted plasma norepinephrine responses to standing —hypoadrenergic postural hypotension (3–5).

Our experience with supine and standing plasma norepinephrine and epinephrine measurements in two groups of patients with hypoadrenergic postural hypotension—diabetic adrenergic neuropathy and primary autonomic dysfunction (idiopathic orthstatic hypotension)—is summarized in Fig. 1. The seven patients with diabetic adrenergic neuropathy were identified in a survey of 100 nonketotic diabetic patients (6). The functional localization of the defect in the sympathetic neural reflex arc in such diabetic patients with documented hypoadrenergic postural hypotension has not been previously examined. However, in diabetic patients with other evidence of autonomic disease the findings of divergent cardiovascular responses to the Valsalva maneuver and to apneic face immerson favor an efferent lesion (7, 8) and those of enhanced local responses to subcutaneous epinephrine (9) and enhanced pressor responses to systemic phenylephrine (10) favor a sympathetic postganglionic lesion. Further,

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posturally hypotensive diabetic patients have been found to have diminished vasoconstrictive responses to the indirect (norepinephrine releasing) sympathomimetic tyramine but enhanced pressor responses to norepinephrine (11), findings consistent with the presence of a postganglionic defect. Other investigators, however, have found increased pressor sensitivity to tyramine in posturally hypotensive diabetic patients and concluded that the postganglionic neurons are intact (12). These apparently conflicting observations may well be attributable to the fact that postural hypotension in diabetic patients can be caused by mechanisms other than adrenergic neuropathy—and associated with normal or even exaggerated sympathetic responses (6)—and, therefore, that postural hypotension per se cannot be assumed to be indicative of the presence of diabetic adrenergic neuropathy in a given diabetic patient.

Primary autonomic dysfunction is undoubtedly a heterogeneous group of disorders of unknown etiologies. Evidence of sympathetic hypofunction—hypoadrenergic postural hypotension—is a cardinal feature and evidence of parasympathetic hypofunction is common (13). Associated central nervous system disease, especially parkinsonism, is often, but not invariably, present. As shown in Fig. 1, our experience in eight patients confirms that of Ziegler et al. (4) in that patients with primary autonomic dysfunction (PAD)[1] without asociated central nervous system disease (designated PAD type I for purposes of this report) have low mean supine plasma norepinephrine concentrations whereas patients with PAD with associated central nervous system disease (designated PAD type II) have normal mean supine plasma norepinephrine concentrations. These findings, coupled with other data from the literature, prompted those authors to suggest that patients with PAD type I have a peripheral sympathetic defect whereas those with PAD type II have a central sympathetic defect (4, 5). This suggestion requires independent confirmation, however, since it cannot be assumed that all patients with hypoadrenergic postural hypotension and low supine plasma norepinephrine concentrations have a peripheral sympathetic lesion. For example, patients with cervical spinal cord transections have been repeatedly shown to have low supine plasma norepinephrine levels (14, 15).

The plasma epinephrine response to standing in patients with diabetic adrenergic neuropathy and in patients with primary autonomic dysfunction has not been reported by other investigators. As shown in Fig. 1, we find the plasma epinephrine response to standing

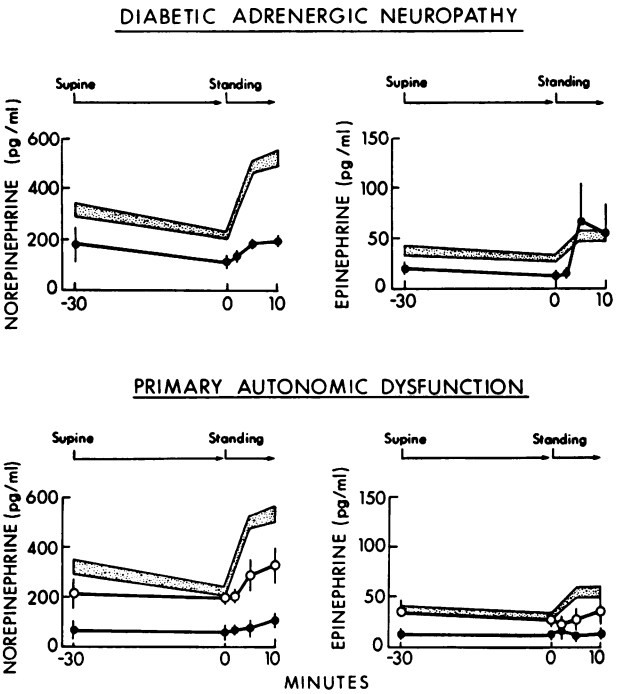

FIGURE 1 Mean (±SE) supine and standing plasma norepinephrine and epinephrine concentrations in patients with hypoadrenergic postural hypotension. The shaded areas represent 1 SE around the mean for 40 normal subjects. The upper panels show data from seven diabetic patients with hypoadrenergic postural hypotension—diabetic adrenergic neuropathy. The lower panels show data from eight patients with postural hypotension due to PAD including three patients with PAD type I (●) and five patients with PAD type II (○).

to be blunted in patients with PAD whereas the epinephrine response is not reduced in patients with diabetic adrenergic neuropathy. This finding, along with the view that the adrenal medullae can be conceptualized as sympathetic postganglionic neurons without axons, led to a hypothesis—that patients with diabetic adrenergic neuropathy have a postganglionic axonal lesion whereas those with PAD have a more central lesion.

To test this hypothesis, a new method to functionally dissect the sympathetic nervous system, specifically to directly stimulate the postganglionic sympathetic neurons, was needed. Since sympathetic pre- to postganglionic neurotransmission is cholinergic and since cholinergic stimulation has been reported to stimulate epinephrine release from the adrenal medullae of animals (16), we reasoned that cholinergic stimulation would also result in stimulation of sympathetic postganglionic neurons with release of norepinephrine. We elected to produce cholinergic stimulation in human subjects by the intravenous injection of edrophonium, an acetylcholinesterase inhibitor, because of its rather good safety record, as evidenced by its widespread

[1] *Abbreviations used in this paper*: PAD, primary autonomic dysfunction; PAD type I, PAD without associated central nervous system disease; PAD type II, PAD with associated central nervous system disease.

use in diagnostic testing for myasthenia gravis, because of its rapid onset and offset of action (minutes) and because, as a quarternary ammonium compound, it does not enter the central nervous system (17).

METHODS

Studies were performed in 10 normal, young adults (5 men and 5 women), 2 patients (1 man and 1 woman) who had previously undergone bilateral adrenalectomy for Cushing's disease, 3 patients undergoing diagnostic cardiac catheterization (without anticholinergic premedication), and 7 patients with hypoadrenergic postural hypotension. The characteristics of the latter patients are summarized in Table I. For purposes of our studies, we have defined postural hypotension as a supine to standing decrement in the mean blood pressure of 20 mm Hg or greater without bradycardia. Posturally hypotensive patients with a supine to standing increment in plasma norepinephrine of <140 pg/ml, the smallest response we have seen in a normal subject, are classified as having hypoadrenergic postural hypotension. With the exception of the cardiac catheterization studies, all procedures were performed on the Washington University Clinical Research Center. All subjects gave written consent before study.

Studies were performed in a quiet, darkened room with the overnight fasted subjects resting in the supine position throughout. Venous blood samples were drawn through an intravenous sampling needle inserted into an antecubital vein 30 min before the start of sampling. 10-ml blood samples were drawn at −10, −5, 0, 1, 2, 4, 6, 8, 10, 15, and 20 min with the injection of saline or edrophonium (Tensilon, Roche Laboratories, Div. of Hoffmann-La Roche Inc., Nutley, N. J.), 10 mg, intravenously after the zero time sample. All 10 normal subjects, both adrenalectomized patients and all 7 hypoadrenergic patients received both saline and edrophonium in varied sequence. Pulse rates were recorded at all sampling points. Blood samples were distributed at the bedside into four iced tubes containing heparin, heparin and GSH (5 mM, Sigma Chemical Co., St. Louis, Mo.), aprotinin (Trasylol, 500 U/ml, FBA Pharmaceuticals, New York), and perchloric acid (3 M). These were promptly centrifuged and the supernates were frozen until subsequent analysis.

Plasma norepinephrine and epinephrine were measured with a single-isotope derivative method (1, 6). Plasma insulin (18) and glucagon (19) were measured by radioimmunoassay, plasma glucose with a glucose oxidase method (20), and blood lactate (20), pyruvate (20), glycerol (21), and β-hydroxybutyrate (22) with microfluorometric techniques.

Statistical analyses included use of *t* tests for paired and nonpaired data and of linear regression analysis.

TABLE I
Patients with Hypoadrenergic Postural Hypotension

	Diabetic adrenergic neuropathy				PAD		
					I	II	Brain stem infarct
Age, *yr*	24	57	61	67	57	34	62
Sex	M	F	M	F	M	F	M
Mean blood pressure, *mm Hg*							
Supine	80	117	109	92	123	110	104
Standing	10	73	53	52	64	55	68
Plasma norepinephrine, *pg/ml*							
Supine	135	226	53	241	40	238	250
Standing	181	311	186	266	50	328	315
Associated central nervous system disorder	None	None	None	None	None	Parkinsonism	Hemiparesis Horner's syndrome
Duration of diabetes, *yr*	11	24	20	3			
Insulin therapy	Yes	Yes	Yes	Yes			
Symptoms of hyperglycemia	No	No	No	No			
Ketonuria	No	No	No	No			
Complications							
Retinopathy	Yes*	Yes‡	Yes‡	No			
Nephropathy	No	No	Yes	Yes			
Peripheral neuropathy	Yes	Yes	Yes	No			
Overt arteriosclerosis	No	Yes§	Yes§	No			
Fasting plasma glucose, *mg/dl*	112	248	144	81			
Fasting serum cholesterol, *mg/dl*	169	218	292	278			
Fasting serum triglycerides, *mg/dl*	140	66	106	—			
Creatinine clearance, *ml/min*	120	104	27	61			
Urine protein, *g/24 h*	0.01	0.27	1.32	0.01			
Urine glucose, *g/24 h*	5.2	39.5	0.3	0			

* Background retinopathy.
‡ Proliferative retinopathy.
§ Peripheral vascular disease.

RESULTS

Normal subjects. Mean (±SE) plasma norepinephrine and epinephrine concentrations before and after the intravenous injection of edrophonium and of saline into the 10 normal subjects are shown in Fig. 2. Plasma norepinephrine rose from 153±15 to 234±29 pg/ml ($P < 0.01$) 4 min after edrophonium injection and was significantly higher than zero time values from 1 through 8 min ($P < 0.05$, <0.001, <0.01, <0.05, and <0.01, respectively). Plasma norepinephrine did not change after saline injection; postedrophonium values were significantly higher than postsaline values from 2 through 8 min ($P < 0.001$, <0.01, <0.05, and <0.05, respectively). Plasma epinephrine rose from 16±3 to 34±5 pg/ml ($P < 0.01$) 2 min after edrophonium injection and was significantly higher than zero time values from 1 through 4 min ($P < 0.05$, <0.01, and <0.02, respectively). Plasma epinephrine did not change after saline injection; postedrophonium values were significantly higher than postsaline values at 2 and 4 min ($P < 0.01$ and <0.01, respectively).

There were small increments in blood β-hydroxybutyrate (123±26–153±36 μmol/liter, $P < 0.01$, at 15 min) and in plasma insulin (10±2–18±5 μU/ml, $P < 0.05$ at 8 min) after edrophonium injection. Plasma glucagon and glucose and blood lactate, pyruvate, and glycerol levels did not change.

Mean pulse rates did not change significantly after edrophonium injection and increments in plasma norepinephrine and apparent decrements in pulse rate after edrophonium injection in individual patients were not correlated ($r = 0.320$, $P > 0.1$).

Adrenalectomized patients. Epinephrine was not detectable (<5 pg/ml) in plasma from the adrenalectomized patients. Plasma norepinephrine rose from 449 to 583 pg/ml and from 292 to 392 pg/ml after edrophonium injection and did not change after saline injection in the two patients as shown in Fig. 3.

Cardiac catheterization patients. There were no significant changes in aortic systolic or diastolic blood pressure or in aortic pulse pressure after edrophonium injection into three patients studied during cardiac catheterization.

Patients with hypoadrenergic postural hypotension. The increments in plasma norepinephrine and epinephrine after edrophonium injection into 10 normal subjects, 2 adrenalectomized patients, and 7 patients with hypoadrenergic postural hypotension are shown in Fig. 4. The four patients with postural hypotension because of diabetic adrenergic neuropathy had blunted norepinephrine responses to edrophonium as did the patient with PAD type I. In contrast, the patient with postural hypotension dated from a brain stem infarct and the patient with PAD type II had normal norepinephrine responses to edrophonium. Epinephrine responses to edrophonium were indistinguishable from normal except in the patients with PAD—no epinephrine response in the type I patient and an exaggerated epinephrine response in the type II patient.

DISCUSSION

Transient amplification of endogenous cholinergic activity, produced by rapid inhibition of the acetylcholine-degrading enzyme acetylcholinesterase, resulted in augmented release of norepinephrine and epinephrine in resting, supine human subjects. Since the adrenal medullae represent the sole source of epinephrine in plasma in normal man (2), the plasma

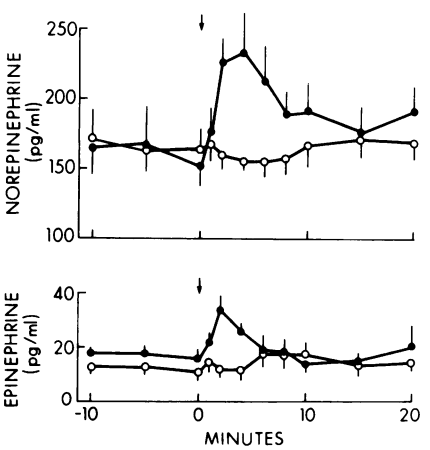

FIGURE 2 Mean (±SE) plasma norepinephrine and epinephrine concentrations before and after the intravenous injection (arrows) of edrophonium (●) or of saline (○) into 10 normal subjects.

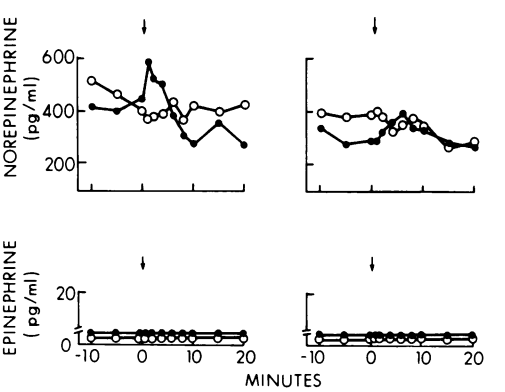

FIGURE 3 Plasma norepinephrine and epinephrine concentrations before and after the intravenous injection (arrows) of edrophonium (●) or saline (○) into two bilaterally adrenalectomized patients. Epinephrine was not detectable in any of the plasma samples from these patients.

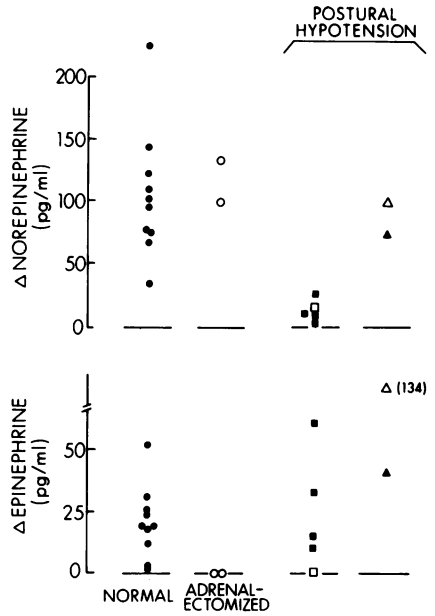

FIGURE 4 Increments in plasma norepinephrine and epinephrine concentrations after the intravenous injection of edrophonium into 10 normal subjects, 2 bilaterally adrenalectomized patients, and 7 patients with hypoadrenergic postural hypotension. The latter include four patients with diabetic adrenergic neuropathy (DAN, ■), one patient with PAD type I (□), one patient with PAD type II (△), and one patient with postural hypotension dated from a brain infarct (BSI, ▲).

epinephrine response to cholinergic stimulation must have been derived from the adrenal medullae. Since the adrenal medullae do release some norepinephrine (2), as well as epinephrine, the plasma norepinephrine response to cholinergic stimulation could have been derived from either the adrenal medullae or the sympathetic postganglionic neurons. However, the fact that the mean plasma norepinephrine response to cholinergic stimulation was more than fourfold greater than the mean plasma epinephrine response suggests that at least a proportion of the norepinephrine was of neural origin since predominant adrenomedullary activation typically results in greater release of epinephrine than of norepinephrine (2, 23). Furthermore, the finding that cholinergic stimulation resulted in comparably augmented norepinephrine release in adrenalectomized patients in whom the absence of functional adrenomedullary tissue was documented by the absence of detectable plasma epinephrine provides strong evidence that the plasma norepinephrine response to cholinergic stimulation is derived predominantly, if not exclusively, from sympathetic postganglionic neurons.

Although it was our premise that the increase in plasma norepinephrine reflected direct cholinergic stimulation of sympathetic postganglionic neurons (and the increase in plasma epinephrine reflected direct cholinergic stimulation of the adrenal medullae), the theoretical possibility that activation of these catecholamine releasing systems could have been indirect and due to reflex responses to hemodynamic changes produced by cholinergic stimulation must be considered. Decrements in pulse rate, blood pressure and pulse pressure are known to activate such a reflex (24). In the subjects studied, the mean pulse rate did not decrease significantly and increments in plasma norepinephrine were not correlated with apparent decrements in pulse rate in individual subjects after cholinergic stimulation. Katz and co-workers (25) found that the intravenous infusion of edrophonium did not alter the intraarterial blood pressure in human subjects. Similarly, we found that there was no significant decrement in aortic systolic or diastolic blood pressure after edrophonium injection into patients undergoing diagnostic cardiac catheterization. Thus, we conclude that amplification of endogenous cholinergic activity results in direct stimulation of sympathetic postganglionic neurons, with augmented norepinephrine release, as well as of the adrenal medullae, with augmented epinephrine release, in man.

It is likely that this effect of cholinergic stimulation on sympathetic postganglionic neurons is exerted at the pre- to postganglionic neuronal synapses in the sympathetic ganglia (26). Although there is evidence that cholinergic effects on presynaptic receptors on axon terminals of sympathetic postganglionic neurons can modulate norepinephrine release, the muscarinic cholinergic action results in decreased norepinephrine release and the nicotinic cholinergic action, which results in increased norepinephrine release, requires very high concentrations of acetylcholine (27).

To date this approach to the study of human adrenergic function has been applied to seven patients with hypoadrenergic postural hypotension. Four patients with postural hypotension due to diabetic adrenergic neuropathy exhibited blunted sympathetic postganglionic neural, but normal adrenomedullary, responses to cholinergic stimulation. This response pattern, which is similar to that produced by standing in such patients, is further consistent with the presence of a postganglionic axonal lesion in patients with diabetic adrenergic neuropathy. The data from the other patients represent important controls with respect to this conclusion. It could be reasoned that a central nervous system lesion might result in diminished basal acetylcholine release from preganglionic neurons and, therefore, a diminished response to acetylcholinesterase inhibition. However, there is no evidence for diminished basal sympathetic outflow in patients with a defective sympathetic response to standing due to a presumed central lesion (i.e., the patients with PAD

type II have normal mean basal plasma norepinephrine concentrations). More importantly, the patient whose hypoadrenergic postural hypotension was almost assuredly due to a central lesion due to his previous brain stem infarct exhibited a normal basal plasma norepinephrine level and a normal plasma norepinephrine (and epinephrine) response to cholinergic stimulation documenting both the presence of intact sympathetic postganglionic neurons (and adrenal medullae) and their capacity to respond to cholinergic stimulation produced by acetylcholinesterase inhibition.

Although only two patients with PAD have been studied with edrophonium, the finding of a normal sympathetic postganglionic neural response, and perhaps an exaggerated adrenomedullary response, to cholinergic stimulation in the patient with PAD type II is consistent with the presence of a lesion central to the sympathetic postganglionic neurons, whereas the finding of a blunted postganglionic neural and adrenomedullary response to cholinergic stimulation in the patient with PAD type I is consistent with a sympathetic postganglionic lesion. Thus, these studies provide additional support, employing a different approach, for the conclusions of Ziegler et al. (4) from their studies in such patients.

In summary, amplification of endogenous cholinergic activity results in direct stimulation of sympathetic postganglionic neurons, with augmented release of norepinephrine, as well as of the adrenal medullae, with release of epinephrine, in man. Although these responses are rather small, they can, with appropriate attention to detail, be used to functionally dissect the sympathetic nervous system in patients with hypoadrenergic postural hypotension. The plasma norepinephrine and epinephrine response patterns to cholinergic stimulation, as well as those to standing, indicate the presence of a sympathetic postganglionic axonal lesion in patients with diabetic adrenergic neuropathy. Thus, measurement of the plasma norepinephrine and epinephrine responses to cholinergic stimulation with edrophonium represents a new approach to the study of hypoadrenergic disorders in man.

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