Increased Growth Hormone Response to Dopamine Infusion in Insulin-dependent Diabetic Subjects

INDICATION OF POSSIBLE BLOOD-BRAIN BARRIER ABNORMALITY

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ABSTRACT To test the hypothesis that cerebral capillaries, which share the embryologic and morphologic characteristics of retinal capillaries, might have the same abnormal permeability in diabetic patients, we investigated the growth hormone response to a small amount of peripherally administered dopamine (1.5 μg/kg·min). Consistent with the known exclusion of systemic dopamine from brain parenchyma, no rise was observed in 12 normal subjects. In 10 of 12 juvenile-onset, insulin-dependent diabetic patients, however, a substantial growth hormone rise occurred (peak value, 19.2±3.0 ng/ml [mean±SE]). Comparison of metabolic and cardiovascular responses to the infusion in both groups did not suggest that higher circulating levels of dopamine had been achieved in the diabetics. Other growth hormone stimuli (apomorphine in decreasing amounts, glucagon, and graded physical exercise) failed to indicate that hypothalamic hypersensitivity could account for the consistent rise.

We postulate that an abnormal permeability of the blood-brain barrier in the diabetic patients permitted exposure of the hypothalamic structures regulating growth hormone secretion to a greater fraction of the infused dopamine.

INTRODUCTION

It is well established that the structure of retinal capillaries—a continuous inner layer of endothelial cells connected by tight junctions—is essentially the same as that of capillaries within the central nervous system and other tissues derived totally or partially from embryonic neuroectoderm (1). Hypothesizing that embryologic and morphologic similarities could form a basis for parallel susceptibility to certain pathologic processes, one might speculate that the brain capillaries of diabetic patients exhibit the same abnormalities as those observed in their retinal capillaries. The early, and probably initial, lesion of retinal capillaries in diabetic patients manifests itself as increased permeability, as detected by the leakage of intravascular fluorescein in the surrounding tissue (2, 3) and into the corpus vitreum (4). Because noninvasive technics for comparable, direct evaluation of the integrity of the blood-brain barrier are not now available, we investigated its possible abnormal permeability by an indirect method.

In juvenile-onset, insulin-dependent diabetic subjects, we studied growth hormone responses to intravenous dopamine. This drug, unlike the dopaminergic agents L-dopa (5), apomorphine (6), bromoergocryptine (7), and piribedil (8), rarely raises serum growth hormone levels in normal individuals (9–13)—an inability most likely explained by its failure to cross cerebral capillary walls (14, 15), in contrast with the other dopaminergic drugs (16, 17). If, however, peripherally administered dopamine at a dosage shown to be ineffective in normal subjects (18) should succeed in stimulating growth hormone secretion in diabetics, the concept of a permeable blood-brain barrier would become more than hypothetical. Because of the fluctuations and hyperresponsiveness of growth hormone secretion sometimes observed in these patients (19, 20), we also evaluated their growth hormone dynamics basally and upon stimulation by secretagogues other than dopamine.

METHODS

Informed consent was obtained from 12 juvenile-onset, insulin-dependent diabetic patients (all men, aged 21–32 yr)

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and 12 age-matched normal subjects (3 women and 9 men). The presence of three female subjects in the control group was considered acceptable insofar as their growth hormone behavior during dopamine infusion was known from previous studies to be indistinguishable from that of male controls. Body weight was 73.0±7.4 kg (mean±SD) in the diabetic group and 73±15 kg in the normal subjects. Pertinent clinical characteristics of the diabetic patients are shown in Table I; none was hypertensive or on medication other than insulin, nor was any acutely ill at the time of study.

All procedures were performed after an overnight fast between 8 and 11 a.m. in outpatient facilities of the Metabolic Research Unit or in the General Clinical Research Center, unless otherwise noted. Ambulatory subjects were requested to rest in the supine position for at least 30 min before initiation of any procedure. The diabetics' usual morning dosage of insulin was withheld until completion of the experiment.

Five protocols were used. All subjects underwent a study with dopamine, but only some from each group were tested with additional stimuli.

Dopamine. Two antecubital intravenous lines were established (one for sampling, the other for drug administration) and kept patent with an infusion of 0.9% saline solution. After a 30-min equilibration period, base-line blood samples were obtained at −30 and 0 min. Dopamine was prepared by diluting 20 mg of dopamine hydrochloride (Intropin; Arnar-Stone Laboratories, Inc., Mount Prospect, Ill.) in 20 ml of 0.9% saline solution and infused at a constant rate of 1.5 μg/kg·min for a 60-min period by means of a Harvard pump (Harvard Apparatus Co., Inc., Millis, Mass.). Blood samples were obtained every 10 min during the infusion period and at 15-min intervals for the 30 min after discontinuation for the determination of growth hormone, plasma glucose, free fatty acid, glucagon, and prolactin levels.

Pulse rate (by palpation) and blood pressure (by auscultation) were monitored throughout the experiment and recorded before each blood sampling.

Blood osmolality was only retrospectively felt to be of interest and the measurement was therefore performed on stored serum from time 0 instead of plasma, as recommended (21). Serum osmolality was determined by freezing-point depression.

TABLE I

Clinical Characteristics of Individual Diabetic Patients

Patient	Age	Duration of diabetes mellitus	Retinopathy*	Neuropathy‡	Proteinuria§
		yr			*mg/24 h*
1	27	12	+		212
2	32	29	++	+	213
3	29	23		++	461
4	30	6	+	+	290
5	24	8	+		200
6	25	7			307
7	29	18	++	++	208
8	31	4			210
9	29	2			200
10	26	10	+		220
11	27	8			
12	21	7			288

* Determined by ophthalmoscopic examination: + background; ++ proliferative.

‡ + subjective complaints; ++ objective neurologic deficits.

§ Determined by the Biuret method (upper limit of normal = 100 mg/24 h).

Apomorphine. We investigated the possibility of any specific hypersensitivity of the growth hormone secretory system to dopaminergic stimulation in our diabetic patients by studying their growth hormone responses to decreasing amounts of apomorphine. This dopaminergic agent effectively crosses the blood-brain barrier (16) and induces similar growth hormone elevations in normal and diabetic subjects (6).

Four subjects from each group were tested in random order on different days with both 300 and 100 μg of apomorphine (Eli Lilly & Co., Indianapolis, Ind.) administered subcutaneously at time 0. Procedures were as described above, except that blood sampling was performed every 15 min throughout the experiment.

Saline solution. Five diabetic subjects underwent a control study during which only normal saline solution was administered. The procedures were the same as those outlined above.

Exercise. It has been reported that mild physical activity induces a rise in circulating growth hormone levels, detectable within 10 min, in juvenile-onset, insulin-dependent diabetics but not in normal subjects (19). To investigate a possible hyperresponsiveness, four diabetic and four control subjects (all men) underwent a regimen of graded physical exercise.

The experiments were performed in outpatient facilities of the Cardiovascular Research Institute, University of California, San Francisco, under continuous ECG monitoring. The test consisted of upright exercise on a cycle ergometer with the subject breathing through an Otis-McKerrow high-velocity valve, the exhalation port of which was connected to a 120-liter Tissot spirometer (Barnor Products, Clifton Heights, Pa.) to permit collection of expired gasses during exercise. Gas samples were analyzed for oxygen and carbon dioxide by the micro-Scholander technic, and respiratory quotients were obtained by dividing the calculated $\dot{V}CO_2$ by $\dot{V}O_2$ (22).

In an attempt to induce a comparable degree of stress in all subjects, the work load was adjusted in each individual to increase the heart rate to 120 beats/min for the first 15 min of exercise and to 150 beats/min for two additional 15-min periods. The three bouts of exercise were separated by two intervals of 10-min duration. From an indwelling needle inserted into a forearm vein and kept open with normal saline solution, blood samples were obtained for the determination of glucose, free fatty acids, and growth hormone before exercise (time 0), at the end of the first bout (min 15), at the end of the second (min 40), at the end of the second rest period (min 50), at the end of the third bout of exercise (min 65), and at 15-min intervals in the recovery phase (min 90 and min 105).

Glucagon. Upon observing that circulating glucagon levels during dopamine infusion were consistently higher in the diabetics than in normal subjects (137±12 vs. 114±12 pg/ml; mean±SE), and because glucagon has been reported to stimulate growth hormone secretion (23, 24), we studied growth hormone dynamics in three of the diabetic patients during the infusion of exogenous glucagon. Glucagon (Eli Lilly & Co.) was infused via a Harvard pump at the rate of 3 ng/kg·min, which has been shown (25) to produce circulating levels exceeding those observed during the dopamine studies. The experimental protocol was identical to the one described for the dopamine study, but blood was collected for the determination of growth hormone, glucagon, and glucose only.

Previously described methods were used for the measurement of serum growth hormone (26), plasma glucose (27), free fatty acid (28), and glucagon (29) levels. Plasma prolactin

was determined by a homologous radioimmunoassay using antibodies obtained from rabbits immunized with purified human prolactin extracted from a prolactin-secreting tumor. This procedure is a modification of the one described by Aubert et al. (30). The intra-assay coefficient of variation is 9.6% in the very low range of prolactin levels (2–3 ng/ml) and 3.7% at concentrations between 10 and 12 ng/ml. (The human prolactin standard was purchased from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif. [catalogue No. 869058].)

Statistical significance was determined with a two-tailed Student's *t* test (paired and unpaired analysis). For detection of outlying observations, we used the statistical criterion of Grubbs (31) for simultaneous testing of the two largest values.

All data are presented as the mean±SE.

RESULTS

Dopamine (Table II and Fig. 1). Whereas intravenous infusion of dopamine did not induce growth hormone secretion in normal subjects, an obvious growth hormone rise was observed in 10 of the 12 diabetic patients studied (Table II). In the remaining two patients (11 and 12), growth hormone levels were elevated in the basal state and fell progressively thoughout the experimental period. Because this behavior was suggestive of a state of stimulated growth hormone secretion and we were testing responsiveness of basal growth hormone, the two patients were considered possible "outliers" and their isolation was supported by statistical analysis (31). The growth hormone data relative to patients 11 and 12 were therefore not included in the comparison between diabetic and normal subjects, although they would have affected only slightly the level of statistical significance.

Plasma glucose values, which had remained essentially unchanged in normal subjects, only minimally increased in the diabetics (from 241±20 to 253±20 mg/dl; $P < 0.02$). Free fatty acid levels gradually increased in both normal (from 0.54±0.06 to 0.63±0.07 meq/liter; $P < 0.02$) and diabetic subjects (from 0.70±0.04 to 0.90±0.05; $P < 0.005$). A significant rise in plasma immunoreactive glucagon was also observed in both normal (from 82±9 to 128±14 pg/ml; $P < 0.005$) and diabetic subjects (from 90±11 to 145±14 pg/ml; $P < 0.005$), but the peak was significantly higher in

TABLE II

Effect of Dopamine Infusion on Serum Human Growth Hormone Levels in Insulin-dependent Diabetic and Normal Subjects*

	Human growth hormone									
Min	−30	0	10	20	30	40	50	60	75	90
						ng/ml				
Patient										
1	2.9	4.9			12.2	14.3	12.0	10.0	7.0	5.8
2	5.7	6.1	6.4	6.9	18.8	27.0	29.6	27.8	22.0	14.4
3	3.9	5.2	8.3	11.1	14.5	11.9	10.5	6.5	3.1	5.1
4	6.2	4.3	6.0	12.0	14.8	22.5	13.5	12.2	7.8	7.0
5	7.5	7.5	8.5	8.3	8.8	8.9	10.1	12.5	12.6	9.2
6	7.8	6.4	8.3	11.3	11.7	13.8	12.0	12.5	11.1	10.4
7	6.7	6.7	6.4	4.6	4.0	3.6	6.3	20.4	17.4	12.0
8	4.5	4.6	5.9	4.2	5.4	10.3	17.4	37.8	41.3	24.3
9	6.3	6.7	7.6	9.9	10.0	10.4	13.0	12.3	12.5	12.4
10	3.1	3.7	9.1	11.3	10.4	9.1	8.6	7.2	7.8	6.8
11	20.8	15.6	8.4	6.5	6.2	5.9	5.0	3.7	5.8	3.6
12	17.6	13.2	11.7	10.0	7.2	6.2	5.7	5.3	3.5	3.5
Mean	7.8	7.0	7.8	8.7	10.3	12.0	12.0	14.0	12.6	9.5
SE	1.6	1.0	0.5	0.4	1.2	1.9	1.9	2.0	3.0	1.6
P vs. time 0					<0.02	<0.02	<0.02	<0.025		
Mean‡	5.4	5.6	7.3	8.8	11.0	13.1	13.3	16.0	14.0	10.7
SE‡	0.5	0.3	0.4	1.0	1.3	2.1	2.0	3.0	3.4	1.7
P vs. time 0‡			<0.02	<0.02	<0.005	<0.005	<0.005	<0.01	<0.05	<0.02
P vs. controls‡			<0.025	<0.001	<0.001	<0.001	<0.005	<0.01	<0.02	<0.02
Controls										
Mean	5.2	5.7	5.1	4.0	3.9	4.8	5.2	5.9	6.0	5.1
SE	0.8	0.7	0.9	0.5	0.6	0.7	0.9	1.5	1.3	1.1

* 1.5 μg/kg per min.

‡ Statistical analysis exclusive of patients 11 and 12.

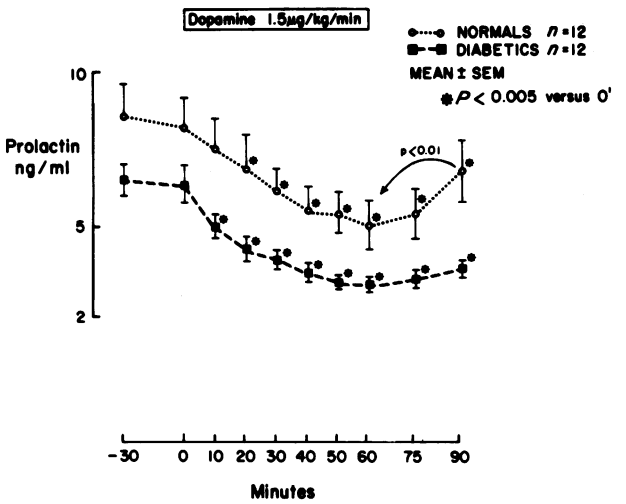

FIGURE 1 Effect of dopamine infusion on plasma prolactin levels in normal and insulin-dependent diabetic subjects.

the latter ($P < 0.05$). Upon discontinuation of the dopamine infusion, circulating levels of glucose, free fatty acids, and glucagon showed a gradual return toward base-line values in both groups.

Dopamine administration promptly and progressively suppressed prolactin levels (Fig. 1), from 8.2±1 to 5.2±0.8 ng/ml in normal subjects ($P < 0.005$) and from 6.2±0.6 to 3.2±0.3 ng/ml in the diabetic group ($P < 0.005$).

The pulse rate increased from 62±2 to 66±2 beats/min ($P < 0.05$) in normal subjects and from 70±4 to 78±4 beats/min ($P < 0.01$) in the diabetic patients. Pulse rates were not significantly different in the two groups at base line and only became so at 40 min (78±4 beats/min in the diabetics vs. 65±2 beats/min in the normals; $P < 0.02$). No changes in blood pressure or other side effects were observed in either group.

Serum osmolality at time 0 in the diabetic patients was 300±3 mosm/kg.

Apomorphine (Fig. 2). The subcutaneous injection of 300 μg of apomorphine induced a substantial growth hormone elevation in the normal subjects ($P < 0.025$) and in three of the four diabetic patients (NS). When the dosage was decreased to 100 μg, a rise occurred in only one of the normal subjects and in none of the diabetics.

It was of interest that 300 μg of apomorphine had no emetic or nauseating effect but consistently induced yawning in all subjects 10 min after administration.

Saline solution (Fig. 3). Infusion of normal saline solution in five of the diabetic patients failed to induce appreciable increases in growth hormone levels over a 2-h period. A minimal decline in prolactin levels (from 6.4±0.7 to 6.0±0.9 ng/ml; NS) was observed only during the first 60 min of the saline infusion.

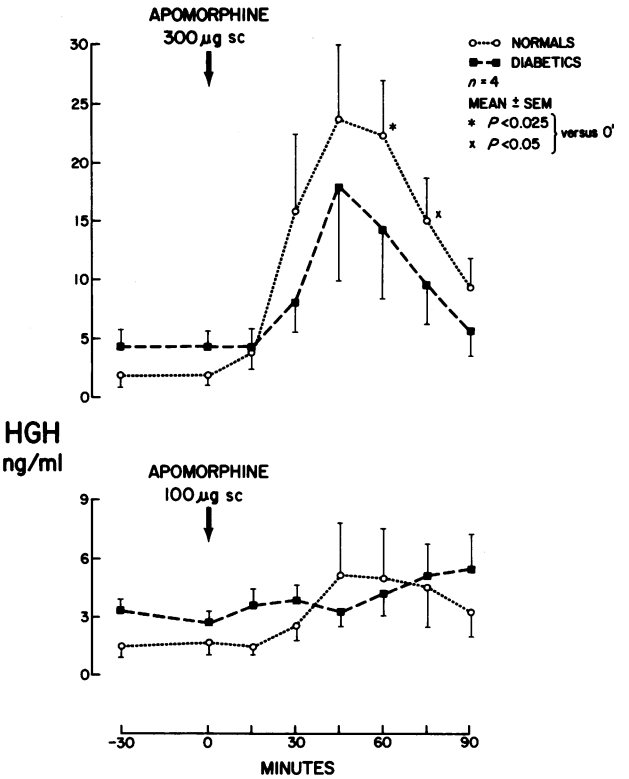

FIGURE 2 Effect of small doses of apomorphine on serum growth hormone (HGH) levels in normal and insulin-dependent diabetic subjects.

Exercise (Fig. 4). The heart rate was increased to 120 beats/min by a work load of 500±35 kg/min in the normal subjects and 375±52 kg/min in the diabetics; oxygen consumption was 1,531±99 and 1,394±118 ml/min, respectively. None of these differences were statistically significant, and no growth hormone rise was detected in either group.

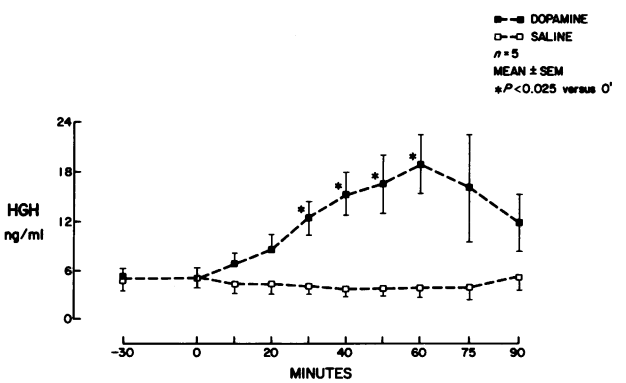

FIGURE 3 Serum growth hormone (HGH) behavior during infusion of dopamine or saline solution in insulin-dependent diabetic patients.

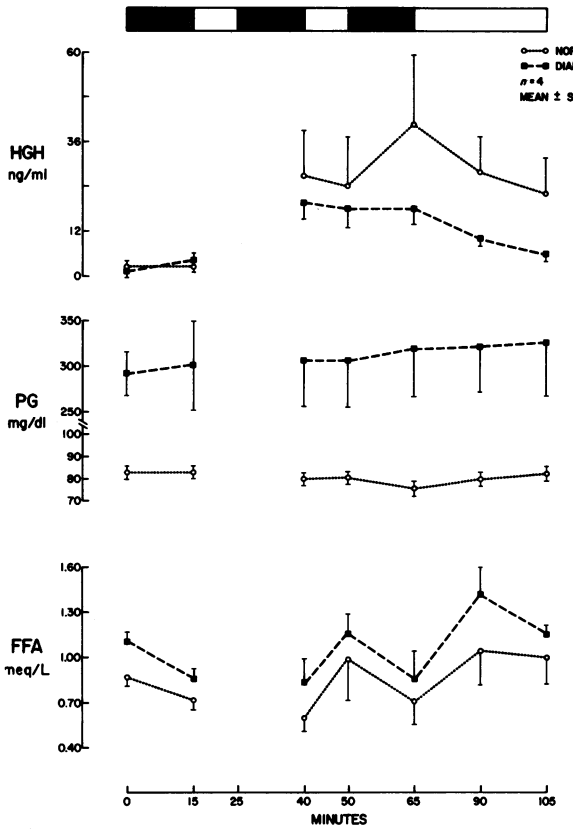

FIGURE 4 Serum growth hormone (HGH), plasma glucose (PG), and free fatty acid (FFA) responses to graded physical exercise in normal and insulin-dependent diabetic subjects.

During the second period of exercise, the heart rate was increased to 150 beats/min by a work load of 752±66 kg/min in the normals and 625±94 in the diabetics (NS); oxygen consumption was 2,157±199 and 1,963±169 ml/min (NS). Serum growth hormone had risen to 27±17 ng/ml in the control group and to 19±6 ng/ml in the patients. The third period of exercise required a work load of 700±70 kg/min in normals and 612±96 kg/min in the diabetics (NS), with a corresponding oxygen consumption of 2,047±146 and 1,897 ±175 ml/min (NS). Growth hormone levels rose to 38±19 ng/ml in the normal group and 17±5 ng/ml in the diabetics.

Plasma glucose did not change significantly throughout the exercise period in the control group, but it tended to increase in the patients (from 287±46 mg/dl at time 0 to 316±54 mg/dl at 65 min).

Free fatty acid levels were slightly but not significantly higher in the diabetics before the experiment (1.10±0.1 vs. 0.90±0.1 meq/liter) and behaved in a parallel fashion in both groups.

The respiratory quotient during the successive bouts of exercise was 0.78±0.09, 0.84±0.02, and 0.80±0.03 in the normal subjects and 0.86±0.01, 0.88±0.02, and 0.89±0.02 in the patients. These ratios were not statistically different.

Glucagon (Fig. 5). The infusion of glucagon produced mean circulating levels of 315±50 pg/ml, significantly higher than the mean values recorded in the same patients during the infusion of dopamine (180 ±32 pg/ml; $P < 0.01$). Despite the higher glucagon levels, no increase in growth hormone was observed during these experiments. The infusion was accompanied by a progressive rise in plasma glucose from 174±64 to 220±76 mg/dl, followed by a decline toward base line upon discontinuation.

DISCUSSION

Although growth hormone secretion in man is stimulated by a variety of dopaminergic agents (5–8), dopamine itself has not been found to be consistently effective. Two groups of investigators have reported a modest but statistically significant rise when 3–7 μg/kg·min were given to a total of 12 normal subjects (12, 13), but three other groups, using similar dosages, found no discernible effect in a total of 27 subjects (9–11). These discrepant results may be explained by the

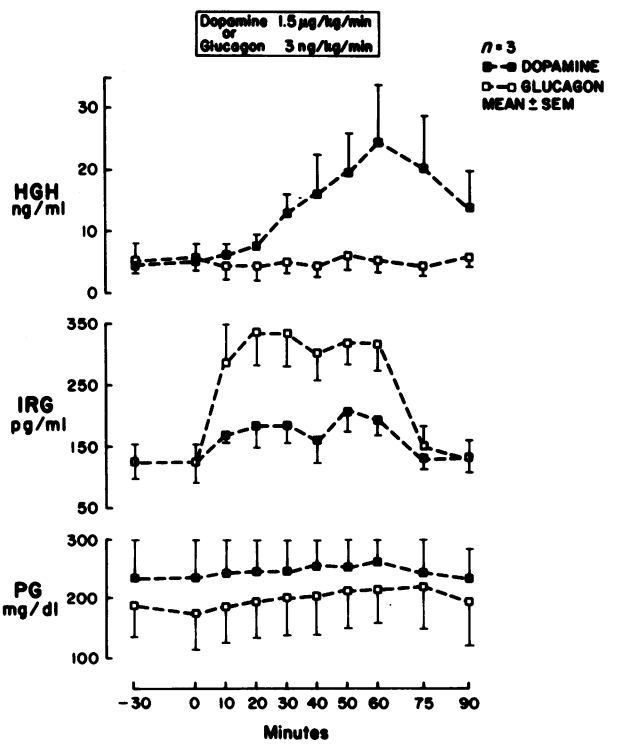

FIGURE 5 Effect of dopamine or glucagon infusion on serum growth hormone (HGH), plasma immunoreactive glucagon (IRG), and plasma glucose (PG) levels in insulin-dependent diabetic patients.

insensitivity of pituitary somatotrophs to the direct action of dopamine (32) and by the finding that the probable locus of the signal for growth hormone secretion is the ventral hypothalamus (33)—a region with transitional characteristics vis à vis the blood-brain barrier (34). Dopaminergic drugs that easily cross the barrier will consistently induce growth hormone secretion, but dopamine, which is normally excluded from the brain parenchyma (14), will succeed only when sufficient amounts escape the general circulation. The inconsistency of growth hormone responses to the dosages of dopamine used by others suggests that these yield barely effective concentrations at target structures situated beyond the blood-brain barrier. Consistent with this interpretation, the much smaller dosage in this study did not result in growth hormone stimulation in our normal subjects.

In contrast, in the 10 diabetic patients who exhibited a normal level of serum growth hormone in the pretest period, the infusion of dopamine was associated with a growth hormone rise that did not seem to stem from spontaneous fluctuations. We cannot exclude that higher circulating levels of dopamine might have been achieved in the diabetic group, but there is at the moment no reason to suspect different clearance of infused catecholamines in normal and insulin-treated diabetic subjects, as long as the latter are metabolically stable. Basal levels of plasma catecholamines do not differ in the two populations (35), blood volume is not decreased (36), and concentrations of plasma proteins, which might affect catecholamine binding (37) and hence distribution, were normal in the diabetic patients tested. Moreover, the degree of cardiovascular change and prolactin suppression—two peripheral effects of dopamine (32, 38)—was remarkably similar in the control and diabetic groups. The higher glucagon response to dopamine was probably related to increased α-cell sensitivity, observed in diabetic patients after other secretagogues (39, 40), and cannot be the main determinant of the differential growth hormone behavior because even higher circulating levels of glucagon, as achieved by exogenous administration, failed to induce a rise.

The possibility of hypersensitivity of the growth hormone stimulatory system in juvenile diabetics deserves serious consideration. Qualitatively and quantitatively abnormal responses have been reported in such patients to arginine (41), pharmacologic amounts of glucagon (41), and exercise (19). Although arginine was not used in our study, neither glucagon at physiologic concentrations nor exercise at increasing work loads disclosed growth hormone hyperresponsiveness. Moreover, the apomorphine studies failed to indicate a specific hypersensitivity to dopaminergic stimulation.

We would like to entertain the hypothesis that, in diabetic patients, an enhanced delivery of circulating dopamine to hypothalamic structures occurs and is responsible for the observed growth hormone stimulation. The increased escape of dopamine from the general circulation into cerebral parenchyma could be a consequence of augmented permeability of the blood-brain barrier. Increased brain uptake of norepinephrine after experimental damage of the barrier has been documented (42), and the suggestion that diabetic patients might have damaged cerebral capillaries stems from the consideration that cerebral capillaries have the same embryology, enzymatic properties and morphology as retinal capillaries (1), which suffer early and progressive insults in experimental (43) and human diabetes (2–4). At the time of this study, six of our patients had demonstrable clinical retinopathy confirmed by fluorescein angiography. Although the remaining six had not undergone fluorescein studies, it is likely that they too had abnormalities of the blood-retinal barrier because the prevalence of capillary leakage (as detected by the sensitive and specific [44] technique of vitreous fluorophotometry) is practically 100%, even very shortly after the diagnosis of diabetes and while fluorescein angiograms are still perfectly normal (4, 44, 45).

The effectiveness of dopamine as a growth hormone secretagogue in our diabetic patients shares the characteristics of the abnormal vitreous fluorophotometry in the general diabetic population: it is a consistent finding and its occurrence is independent of the duration of the disease. The two phenomena might well have a common etiology.

It has recently been reported that endothelial cells of brain capillaries have receptors for insulin (46) and that the transport of hexoses across the blood-brain barrier is altered in experimental diabetes (47). These preliminary studies support the contention that brain capillaries might be a target for diabetes-induced damage.

It is pertinent to ask if there are specific, clinically apparent counterparts to this hypothesized abnormality of the cerebral capillaries in diabetic patients. A negative answer must be qualified by the fact that early and even late stages of diabetic retinopathy are often totally asymptomatic and remain undetected until fluorescein angiography is performed or major events take place, such as a vitreous hemorrhage, macular involvement, or retinal detachment. In experimental disruption of the blood-brain barrier, no changes in EEG are detectable until the damaged system is challenged with acute administration of suitable substances (48).

It is therefore possible that we might not know if insulin-dependent diabetic patients have increased permeability of their blood-brain barrier until we search for the specific abnormality. At early stages this might be functional and reversible; however, if the analogy with retinal vessels is complete, morphologic altera-

tions would be present in long-standing diabetes. We hope that more direct experimental approaches will soon verify or disprove this hypothesis.

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