Effect of Diabetic Hyperglycemia and Other Sugars on Plasma Dopamine-β-Hydroxylase Activity

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SUMMARY

The plasma glycoprotein, dopamine-β-hydroxylase (DBH), is present in markedly increased amounts in experimental, streptozocin (STZ)-diabetic rats, reaching a maximum at about the first week and maintaining a plateau for several months afterward. High glycemia values are observed simultaneously. Insulin treatment is observed to keep the glycemia and plasma DBH activity values at levels seen in control rats. The heterologous half-life of DBH in STZ-diabetic rats is significantly increased compared with that of control animals. The glucose analogue, 2-deoxy-D-glucose, has a similar effect on plasma DBH activity levels, eliciting high glycemia values. In STZ-diabetic animals, this increase is more significant, as if it were the additive effect of the two sugars. Other sugars that can compete for glycoprotein catabolic receptors can also modulate the plasma DBH activity levels. The lack of effect of galactose on DBH levels, together with the induced increase of DBH by α-methyl-D-mannoside and, to a lesser extent, by inulin, suggest an important rate for the mannose/glucose/N-acetyl glucosamine/fructose receptor in the catabolic clearance of DBH from plasma and explain the abnormal values seen for DBH in diabetes mellitus. DIABETES 1984; 33:1127–32.

Dopamine-β-hydroxylase (DBH) (E.C. 1.14.17.1) is a Cu^{2+}-containing glycoprotein that catalyzes the conversion of dopamine to norepinephrine.[1,2] Dopamine-β-hydroxylase is localized in secretory vesicles[3] and is released by an exocytotic process,[4] which explains the finding of this enzyme in the blood of man and of other species.[5] Controversy exists concerning the significance of circulating DBH, which has frequently been measured as an index of sympathetic function.[6,7] Available evidence, however, suggests that circulating DBH levels are regulated by several factors besides sympathetic function.[8-10] Accordingly, high levels of circulating DBH activity were found in streptozocin (STZ)-diabetic rats,[10,11] with a markedly slowed half-life disappearance for this enzyme in plasma.[12]

Thus, the half-life for glycoprotein in serum depends on its carbohydrate moiety.[13] On the other hand, DBH is a glycoprotein with terminal mannose residues in the carbohydrate moiety in all enzymes studied, including human plasma and rat enzyme.[14,15] Sialic acid is also in a terminal position linked to a previous galactose residue.[2] This suggests that the circulating enzyme can be cleared from plasma by a galactose-lectin receptor when the sialic acid is lost.[13] Nevertheless, other lectin-recognizing receptors cannot be excluded.[16]

The purpose of this study was to discover the effect of hyperglycemia, induced by experimental diabetes, on serum DBH levels, as well as the effects of other sugars, to elucidate the receptor implicated in the catabolic recognition system for this plasma enzyme.

MATERIALS AND METHODS

Materials. Experimental animals were male Wistar rats, weighing 150 ± 15 g, approximately 7 wk old.

Bovine adrenal glands were obtained at a local slaughterhouse.

Bovine serum albumin, catalase from beef liver, concanavalin A-Sepharose 4B, 2-deoxy-D-glucose, fumaric acid, galactose, α-methyl-D-mannoside, octopamine, pargyline, PPO, dimethyl POPOP, streptozocin (STZ), and tyramine were purchased from Sigma Chemical Co., St. Louis, Missouri. Ascorbic acid, dextrane-T-70, and inulin were obtained from Merck, Darmstad, FRG. 2-Deoxy-D-(U-^{14}C) glucose (350 μCi/μmol) and ^{14}C-methyl-S-adenosyl-methionine (59 μCi/μmol) were obtained from Amersham International, United Kingdom.

Glucose kits for determinations were from Boehringer Mannheim, Barcelona, Spain. All other reagents were of the greatest purity available.

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TABLE 1
Plasma glucose concentrations and plasma DBH activity levels under experimental conditions

Groups	Pre		Post (48 h)			
	DBH Activity	Glucose	DBH Activity	P_1	Glucose	P_2
Streptozocin (8) (STZ)	15.81 ± 1.63	103.67 ± 5.53	48.22 ± 1.92	<0.001	305.71 ± 21.87	<0.001
Insulin (8) (I)	10.10 ± 0.52	94.03 ± 3.56	10.77 ± 0.51	NS	44.31 ± 6.51	<0.001
STZ + I (10)	12.41 ± 0.92	103.58 ± 3.56	10.90 ± 0.68	NS	44.06 ± 3.19	<0.001
2-Deoxy-D-glucose* (8) (2DG)	16.23 ± 1.01	109.83 ± 4.05	27.21 ± 1.14	<0.001	99.41 ± 5.68	NS
2-Deoxy-D-glucose (8) (2DG)	15.04 ± 1.03	90.37 ± 7.55	40.35 ± 4.79	<0.001	110.31 ± 4.86	NS
2DG + I (7)	13.84 ± 1.05	115.44 ± 9.53	28.05 ± 4.39	<0.001	51.18 ± 9.00	<0.001
STZ + 2DG* (5)	16.83 ± 1.49	96.92 ± 5.19	53.13 ± 5.81	<0.001	350.22 ± 17.32	<0.001
STZ + 2DG (10)	15.56 ± 1.57	89.74 ± 2.75	52.89 ± 3.40	<0.001	319.62 ± 28.34	<0.001
STZ + 2DG + I (9)	13.74 ± 0.60	111.68 ± 3.84	23.71 ± 2.64	<0.01	61.23 ± 3.21	<0.001

Wistar rats (150 ± 15 g) were administered: (1) STZ (65 mg/kg, intracardiac), (2) insulin (6 IU lente/day), and (3) 2DG (150 mg/kg* and 500 mg/kg, i.p.) every 6 h for 48 h before blood extraction. Blood was obtained by cardiac puncture, and plasma DBH and glycemia values were determined as described in the text. Enzyme activity is expressed in nmol octopamine/h/ml plasma, and glucose in mg/dl plasma. Each value represents the mean ± SEM with the number of experimental animals in parentheses. P was determined by Student's *t*-test, P_1 for DBH comparison between basal and 48-h-treated rats, and P_2 for glycemia values pre- and posttreatment.

Animal treatment. Male Wistar rats were housed in a room with controlled temperature (20°C), and received water and food ad libitum. The animals were anesthetized with ether before (basal determination) and after treatment (48 h), and 1 ml of heparinized blood was obtained by cardiac puncture.

STZ was dissolved in 0.5 ml of ice-cold 0.1 M citrate buffer (pH 4.5), and a dose of 65 mg/kg was injected by cardiac puncture.

Slow insulin (6 IU, lente; Novo, Copenhagen, Denmark) was administered s.c. daily.

2-Deoxy-D-glucose (2DG) was dissolved in 0.5 ml of 0.9% NaCl and was injected i.p. every 6 h for 48 h in doses of 150 and 500 mg/kg. In experiments assessing the half-life of disappearance of U-^{14}C-2DG, 5 μCi of this sugar was injected in a single dose by cardiac puncture.

α-Methyl-D-mannoside was dissolved in 0.5 ml of 0.9% NaCl and was injected i.p. every 8 h for 48 h in a dose of 500 mg/kg.

Inulin was dissolved in 0.5 ml of 0.9% NaCl and was injected i.p. every 8 h in a dose of 500 mg/kg.

Dextrane-T-70 was dissolved in 0.5 ml of 0.9% NaCl and was injected by cardiac puncture in a single dose of 1 g/kg.

Galactose was dissolved in 0.5 ml of 0.9% NaCl and was injected i.p. every 6 h for 48 h in a dose of 500 mg/kg.

Collection of blood samples. Blood samples were spun at 5000 × *g* for 10 min at 4°C. The plasma was stored at −20°C until assay. Blood was drawn (0.1 ml) for the determination of glucose, as described by Werner et al.[17]

Assay of DBH activity. DBH activity in plasma was assayed as described by Goldstein et al.[18] To obtain optimal enzymatic activity and to overcome the effect of endogenous inhibitors, 25 μl of plasma and 47.6 μmol of SO_4Cu/tube were used. The first step of the reaction was always run for 1 h and the second for 0.5 h.

The phenylethanolamine-N-methyl transferase (PNMT) necessary for the second step of the reaction was partially purified by the method of Axelrod,[19] as modified by Molinoff et al.[20] Its specific activity was 0.46 nmol synephrine/mg/h.

DBH was purified using the concanavalin-A method of Aunis et al.[21]

The optimal tyramine concentration in the assay was 0.645 mM. The concentration of octopamine standards was 0.2 nmol/tube. One unit of DBH activity was defined as the formation of 1 nmol octopamine, measured as (^{14}C)synephrine, per 1-h incubation at 37°C.

The variability between assays was 6% (N = 5) and between replicate assays, 4.1% (N = 5). Using DBH aliquots, the recoveries were always greater than 95%. All samples, blanks, and standards were assayed in duplicate at 4°C.

In the experiments carried out to determine the half-life of disappearance of exogenous bovine DBH, rats were anesthetized with ether, and DBH (225 U/ml) was injected in a single dose by cardiac puncture. A baseline and the required experimental time for the blood samples were obtained by

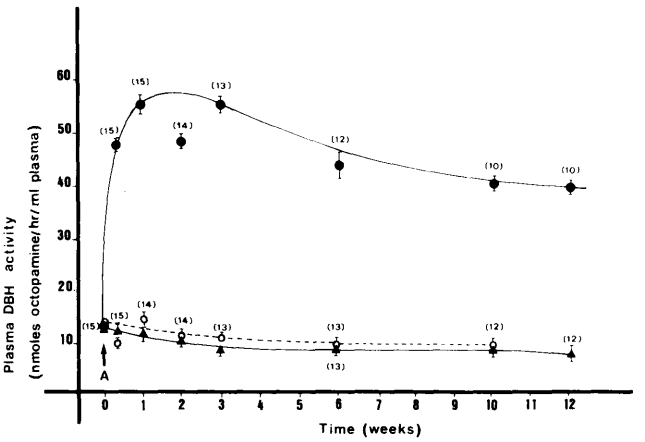

FIGURE 1. Changes in rat plasma dopamine-β-hydroxylase activity. Seven-week-old rats were injected with STZ (65 mg/kg, ●) or an equal volume of isotonic saline (control, ▲) at time A. The third group was STZ-diabetic rats that had received insulin (6 IU lente/day, ○). Values at each time interval represent the mean ± SEM, with the number of experimental animals in brackets.

the same procedure. Changes in DBH activity were calculated by subtracting the baseline rat plasma DBH activity from the total activity (endogenous rat DBH plus exogenous bovine DBH) at the required times.

All values in the text and tables are given as means ± SEM. To evaluate the significance of a difference between mean values, Student's *t*-test was used. Linear regression equations were calculated by the least-squares method using an Olivetti-P-6066 calculator with a linear regression program.

RESULTS

Plasma DBH activity in STZ-diabetic rats. The mean DBH activities in plasma from several groups are compared in Table 1. The effect of STZ (65 mg/kg, intracardiac) on rats is a significant increase in plasma enzyme activity with a maximum occurring at about the first week after treatment, reaching a plateau that is maintained for vast periods in experimental diabetes (Figure 1).

In separate experiments, a control was performed to test if rats had any change in plasma DBH activity with age. In Figure 1, we can observe that enzyme levels diminished slowly, but could be considered constant for the first 2 wk, when animals were 7 and 9 wk old.

Plasma glucose from the STZ group was maximally elevated within 2 days and remained so, without significant changes, during the next 3 mo of our experimental diabetes conditions. In Table 1, the glucose levels for all groups are summarized, before and after 48-h treatment. A significant increase was found for STZ-diabetic animals ($P < 0.001$).

When the STZ-diabetic animals (24 h after STZ treatment) were treated with insulin for a 48-h period (6 IU lente/day), DBH activity then decreased dramatically to a value near the initial one. The evolutionary pattern in time for this group was the same as that of the control animals, as shown in Figure 1.

Glycemia values were also significantly decreased in the first 48 h after insulin treatment ($P < 0.001$). Nevertheless, in this group the glycemia values fluctuated more than in other groups. Insulin administration in control rats had no effect on plasma DBH activity, but plasma glucose levels were significantly diminished in a way similar to that of the former group.

Plasma DBH turnover in normal and diabetic rats. Dopamine-β-hydroxylase purified from bovine adrenal medulla (sp act 10 μmol octopamine/min/mg protein) was administered as a single dose by cardiac injection. Usually, 225 U of enzyme activity was employed, which is enough to increase basal enzyme activity levels threefold.

The half-life disappearance for bovine DBH was prolonged almost twofold when animals were treated with STZ (Figure 2). A good correlation coefficient was obtained for both control ($r = 0.951$, $P = 0.0006$) and STZ-diabetic ($r = 0.95$, $P = 0.0001$) groups. The $t_{\frac{1}{2}}$ obtained from the slopes was 60 min and 96.6 min, respectively, and the difference in the regression coefficients between the two lines was statistically significant ($P < 0.02$). Maximal increase in plasma activities was reached between 0.5 and 1 h after an intracardiac pulse dose of DBH.

To determine the enzyme turnover, basal activity values were subtracted from total enzyme activities at all required times. It is necessary to point out that the volume obtained by cardiac puncture (in these experiments, 0.2–0.3 ml for each time) implied only a weak dilution in enzyme activity when vascular volume was restored, and was not considered significant.

Plasma DBH activity in STZ-diabetic and normal rats with 2DG administration. The high levels of DBH activity in STZ-diabetic animals can be explained by a direct insulin action at secretion or catabolic levels. On the other hand, the turnover experiments point to an effect at the catabolic level. This insulin action can be accomplished directly or indirectly as a result of high glycemia values or other altered parameters.

To determine whether glycemia values are the only factors responsible for increases in DBH activity, modulating the half-life of this enzyme, experiments were performed with the glucose analogue, 2DG. Two different concentrations of this sugar (150 and 500 mg/kg every 6 h, i.p.) were administered. 2DG induced a significant increase in plasma DBH activity ($P < 0.001$) for both groups. At the same time, no significant changes occurred in glycemia values, as can be seen in Table 1.

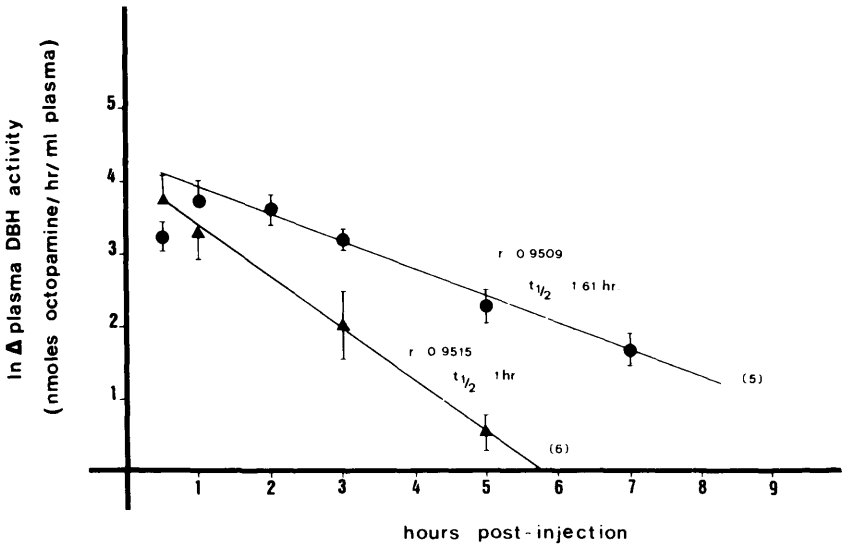

FIGURE 2. Serum disappearance curve of bovine DBH after pulse-dose injection into STZ-diabetic (●) (1 wk after treatment) and control (▲) rats. Animals were injected with purified bovine DBH (225 U) in a volume of 1 ml by intracardiac puncture. Serum bovine DBH activity was determined by subtracting the basal from the total level of DBH activity, and is expressed as ln of the remaining activity, at the indicated times, with the number of experimental animals in brackets.

When 2DG was administered to the STZ-diabetic group, the increase in plasma DBH activity was higher than that found either in the STZ-diabetic ($P < 0.02$) or in the 2DG ($P < 0.01$) groups individually. At the same time, glycemia values, which were not significantly modified in 2DG-treated rats, revealed a significant increase in the STZ-diabetic group treated with 2DG, similar to that found in the same group without 2DG.

Since the i.p. administration of 2DG was carried out every 6 h, it was necessary to ensure that its serum levels were high enough during this period. Thus, we determined the half-life for 2DG in plasma. Half-life for the control group was about 5.9 h with a 26% loss in urine for 24 h. The STZ-diabetic group showed a shorter half-life, 4.7 h, with an increased urinary excretion: 54% of total sugar was eliminated in 24 h and urinary volume was 9–10-fold increased.

Effect of several sugars on plasma DBH activity and glucose concentration. In previously reported experiments, it was found that STZ-diabetic animals that had high glycemia values, as well as a 2DG-treated group, had a significant increase in plasma DBH activity. The question arises whether other sugars exist with similar capabilities (Table 2).

Thus, α-methyl-D-mannoside (500 mg/kg every 8 h, i.p.) was administered. Forty-eight hours later, plasma DBH activity showed a significant increase, similar to that seen with 2DG. No changes in glycemia values were observed. After shorter time periods, such as 4 or 8 h, the DBH activity increase is not measurable; we have chosen the 48-h values because the cumulative effects can more easily be observed.

Inulin, a fructose polymer (about 50 residues/molecule), at doses of 500 mg/kg every 8 h, i.p., produced a weak increase in DBH levels at 48 h of treatment (P = NS). The glucose polymer, dextrane (about 300 residues/molecule), showed similar behavior.

On the other hand, galactose, which is the sugar recognized for catabolic receptors of many glycoproteins, did not increase plasma enzyme activity, but glycemia values were significantly decreased ($P < 0.001$).

DISCUSSION

Plasma DBH activity in STZ-diabetic rats. The large increase in plasma DBH activity for diabetic animals has been previously reported.[10,11] The threefold rise at 48 h with a maximum within the first week agrees with Schmidt et al.,[10] who demonstrated a similar result in the first 2 wk. A weak decrease in plasma activity was observed 3 wk after STZ treatment, and when the activity level of twelfth week posttreatment was compared with the maximum, a 28% decrease was observed. This effect can be related to the aging of the animals, because control rats have a similar evolutionary pattern of about a 40% decrease when the seventh and nineteenth weeks of life, which are the experimental times, are compared. A similar evolutionary behavior for DBH levels with aging was reported by Weinshilboum.[8]

Insulin administration returned the plasma DBH activity in STZ-treated animals to normal within a 48-h period, and the evolutionary pattern for plasma DBH was the same as in the control rats.

Insulin also reduced the hyperglycemia associated with diabetes, but glycemia values were more scattered than those shown by the control group. This could indicate that the standard insulin dose was not exactly adjusted for each animal; however, on the other hand, the glycemia values could have been the result of fast changes due to feeding and other factors. This was not the case for DBH due to its longer half-life in plasma. DBH may reflect changes in glycemia values before the glycemia determination.

The results obtained indicate that a lack of insulin was responsible for the plasma activity increase; however, it could have been the result of an indirect action of insulin through glycemia.

Thus, when purified bovine DBH enzyme was injected into STZ-diabetic animals, its half-life was longer than in controls as was found by Hurst et al.,[12] using homologous and the heterologous DBH injected into normal and STZ-diabetic rats.

If only glycemia values were responsible for the increase in DBH activity, as a consequence of its longer half-life in plasma, then other sugar analogues should have produced a similar effect.

Effects of 2DG. The glucose analogue, 2DG, produced a significant dose-dependent increase in plasma DBH activity in rats. No significant changes in glycemia values were observed by us; this is an important point, because other authors have reported an increase in glycemia during the 6 h after only one 2DG injection,[22,23] but glycemia studies with chronically administered 2DG have not been reported. In humans, plasma glucose levels reached a maximum be-

TABLE 2
Effects of administration of other sugars on plasma DBH activity and glucose concentration

Groups	Pre		Post (48 h)			
	DBH Activity	Glucose	DBH Activity	P_1	Glucose	P_2
α-Methyl-D-mannoside (6)	14.90 ± 1.38	81.14 ± 6.1	34.66 ± 1.96	<0.001	96.95 ± 4.6	NS
Inulin (8)	16.20 ± 1.86	80.00 ± 8.0	20.22 ± 2.30	NS	76.15 ± 3.8	NS
Dextrane-70 (6)	12.56 ± 1.68	101.10 ± 3.2	14.25 ± 2.60*	NS	105.16 ± 3.9	NS
Galactose (6)	17.19 ± 1.77	98.46 ± 3.17	15.62 ± 1.64	NS	73.04 ± 3.2	<0.001

Wistar rats (150 ± 15 g) were administered: (1) α-methyl-D-mannoside (500 mg/kg, i.p.), (2) inulin (500 mg/kg, i.p., in repeated doses every 8 h), (3) dextrane-T-70 (1 g/kg, only 1 dose, intracardiac), and (4) galactose (500 mg/kg, i.p., in repeated doses every 6 h). Blood for DBH activity and glucose measurement was obtained by cardiac puncture, before and after the first dose of 48-h treatment for all sugars, except for the dextrane-T-70 group before and after a single 24-h treatment*. Each value represents the mean ± SEM with number of experimental animals in parentheses. P was determined by Student's *t*-test between basal DBH and post-DBH (P_1) and between basal glucose and posttreatment (P_2).

tween 1 and 2 h after the injection of 2DG, and always returned to basal levels the following hour.[24]

2DG can be stored in cells as 2DG-6-P by the enzymatic action of hexokinase;[25] on the other hand, it can be eliminated in urine and its half-life in STZ-diabetic animals is shorter than that in controls, possibly due to major urinary excretion, but the levels are high enough in plasma to maintain the effect.

When 2DG and glucose effects are induced together, as in the case of STZ-diabetic animals with 2DG treatment, an accumulative effect is observed on plasma DBH activity. These experiments support the hypothesis that plasma DBH activity is closely connected to some sugar levels in plasma. Insulin produced a decrease in plasma DBH levels in the 2DG-treated rats (with and without STZ), perhaps due to the increase in transport of glucose and 2DG inside the cells.[28] This could explain why the 2DG plus insulin group had a significant decrease in plasma DBH in comparison with 2DG alone.

On the other hand, an increased release from sympathetic nerves in 2DG-treated rats is excluded, because 2DG diminishes nerve activity,[29] and most of the enzyme has a neural origin.[8] The stimulatory effect of 2DG on the adrenal medulla has very little effect on plasma DBH levels. Thus, insulin-induced hypoglycemia, which greatly stimulates the adrenal medulla, does not increase plasma DBH levels (Table 1).

As DBH has mannose and/or glucose and NANA-galactose as terminal residues[2,21,26] the question arises whether the clearance from plasma is due to the galactose-lectin receptor,[13] which needs the previous action of a neuraminidase, or if it can be recognized by the mannose/N-acetylglucosamine/lectin receptor, reported to be present in hepatic endothelial cells.[16]

Effect of other sugars. The interpretation of results of our in vivo galactose administration experiments is complicated, since plasma DBH levels did not increase and, at the same time, there was a significant decrease in glycemia values. Thus, the relative importance, if any, of hepatic galactose-lectin in the clearance of DBH from plasma was difficult to determine.

The significant increase in plasma DBH levels when α-methyl-D-mannoside, a nonmetabolizable sugar, was administered clearly indicates that the mannose/N-acetylglucosamine receptor must be important in the DBH clearance from plasma. This receptor also recognizes glucose, 2DG, fructose, and the corresponding glycoproteins that contain these sugars at nonreducing ends.[27] This could also explain our results in diabetic and 2DG-treated rats.

Inulin and dextrane, fructose and glucose polymers, respectively, weakly increased plasma DBH levels, perhaps due to their higher molecular weight, in addition to the corresponding problem of reaching a molar concentration sufficient to block the mannose/N-acetylglucosamine/glucose/fructose receptor.

The present results clearly indicate that plasma DBH activity can be increased by the presence of sugars that compete for the mannose-lectin receptor. The degree of this receptor blockade, or competition for its recognition site, can determine the enzyme activity level through its half-life modification.

Accordingly, plasma DBH levels can be seen not only as an effect of greater or lesser activity from the secretory events, but also as a parameter of the catabolic rate, which can be modulated at the mannose-lectin receptor level. The high levels for this enzyme in diabetes can be explained by glucose competition at the catabolic site.

The relevance of these findings for human diabetes is difficult to evaluate. Nevertheless, as human plasma DBH is a glycoprotein with mannose terminal residues and a half-life in plasma of about 1 day,[2,30] it is possible that the clearance rate can be modulated by the concentration of glucose in plasma, as occurs in experimental rats. In a study undertaken with diabetic patients suffering neuropathies, no increases in enzyme levels were observed.[31] However, this was not a study of recently diagnosed patients; the possibility of using plasma DBH values as a parameter for the diabetic state will require a long-term prospective study.

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