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Decreased *in vivo* Tyrosine Hydroxylase Activities with Normal Norepinephrine Levels in Streptozotocin-Induced Diabetic Rat Hypothalamus

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ABSTRACT

We studied changes in the hypothalamic norepinephrine(NE) metabolism in streptozotocin (STZ)-induced diabetic rats by measuring basal NE levels, turnover rate of NE, and *in vivo* tyrosine hydroxylase activities. Basal NE level did not change significantly upto 4 weeks after the establishment of diabetes with STZ(60 mg/kg, iv). But turnover rate of NE decreased to 62% of control rate($P<0.01$), and *in vivo* tyrosine hydroxylase activities decreased to 32% of control level($P<0.05$) at one week of diabetes. From these results, we concluded that, of the three parameters measured, *in vivo* tyrosine hydroxylase activity is the most sensitive index of altered hypothalamic NE metabloism in STZ-induced diabetic rats.

Key Words: *In vivo* tyrosine hydroxylase activity, Norepinephrine metabolism, Hypothalamus, Streptozotocin-diabetes

INTRODUCTION

Changes in various parameters of noradrenergic system have been reported in streptozotocin(STZ)-induced diabetic rat brains: decreased norepinephrine turnover rate(Trulson and Himmel, 1985), increased norepinephrine levels(Trulson and Himmel, 1985; Bitar *et al.*, 1986, 1987, 1990; Masiello *et al.*, 1987; Lackovic and Salkovic, 1990), decreased *in vivo*(Trulson and Himmel, 1983) and *in vitro* tyrosine hydroxylase activities(Bitar *et al.*, 1986) and increased densities of alpha 1(Bitar *et al.*, 1986) and beta 1 adrenoreceptor(Bitar and de Souza, 1990). But there has been no report that addressed what index of NE metabolism is most sensitively deranged in STZ-diabetes by measuring basal NE levels, NE turnover rate, and *in vivo* tyrosine hydroxylase activities in the same study. As the noradrenergic innervation to the hypothalamus is the densest in the brain, and hypothalamus functions key roles in the regulation of endocrine system, we studied hypothalamic NE metabolism in STZ-diabetes.

And we found that *in vivo* tyrosine hydroxylase activities and turnover rate of NE can be markedly decreased in the presence of normal basal NE levels, with *in vivo* tyrosine hydroxylase activities showing more marked changes than turnover rate of NE, suggesting that *in vivo* tyrosine hydroxylase activities is probably the most sensitive index in diabetes-induced changes in hypothalamic NE metabolism.

MATERIALS AND METHODS

Chemicals

Streptozotocin(STZ), norepinephrine HCl, do-

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pamine HCl, α-methyl-p-tyrosine, dihydroxyobenzylamine(DHBA), sodium octyl sulfate were obtained from Sigma Chemical Co.(St. Louis, MO, USA); NSD-1015(3-hydroxybenzylhydrazine HCl) from Aldrich Chemical Co.(Milwaukee, MI, USA) and all other reagents were of analytical grade.

Establishment of streptozotocin-induced diabetic rats

Streptozotocin-diabetes was induced as described in Kim *et al.* (1988) with slight modifications. Male Sprague-Dawley rats weighing 300~400 g were used. Diabetes was induced by an injection of streptozotocin(60 mg/kg b.wt.) prepared in 0.05 M citrate buffer, pH 4.5 via penile vein. One or 4 weeks after induction of diabetes, rats were sacrificed to study hypothalmic norepinephrine methabolism. Brains were removed immediately and stored at −80°C until assay. Blood was taken from retroorbital plexus and serum glucose levels were measured according to Hultman(1959).

Determination of basal levels of NE

NE levels were determined by high performance liquid chromatography with electrochemical dectection(HPLC-EC) according to Keller *et al.* (1976). Briefly, hypothalamus was homogenized in ten volumes of 0.1 N perchloric acid by sonication. After addition of Tris-HCl(pH 8.6) and activated alumina, homogenate was shaken for 15 min. After washing three times with Tris-HCl and distilled water, NE was eluted to perchloric acid(100 μl) and 5~10 μl was injected to C_{18}μ Bondapak column of 10 μm particle size, 10 mm inside diameter and 30 cm length. As a mobile phase, 25 mM phosphate buffer/5% acetonitrile(0.34 mM EDTA, 0.14 mM ocotane sulfonic acid) were used and the flow-rate was 0.8 ml/min(Waters model 600 pump) and the oxidation potential 0.5 V(Waters model 460 elctrochemical detector). Sample values were calculated relative to the peak height of the internal standard, DHBA.

***In vivo* TH activity assay**

One week after establishment of STZ-diabetes, *in vivo* TH activity was measured by using a modification of the method of Carlsson *et al.* (1971). Thirty minutes after the intraperitoneal injection of NSD-1015(100 mg/kg), an inhibitor of aromatic amino acid decarboxylase, dihydroxyphenylalanine(DOPA) accumulated in hypothalamus was measured by HPLC-EC.

Determination of NE turnover rate

One week after establishment of STZ-Diabetes, NE turnover rate was determined according to Brodie *et al.*(1996). Zero, 1, 2 or 3 hours after an intraperitoneal injection of α-methyl-p-tyrosine methyl ester(300 mg/kg), NE levels in hypothalamus were assayed with HPLC-EC.

RESULTS

In the STZ-diabetic rats, relative body weight decreased remarkably in a time-dependent manner. Serum glucose increased to 464%, and 409% of control values at 1 and 4 weeks, respectively, in the STZ-diabetic rats(Table 1). The basal

Table 1. Effect of STZ-diabetes on body weight and serum glucose in Sprague-Dawley rats

Duration of diabetes	Body weight		Serum glucose	
	Control	STZ	Control	STZ
weeks	g		ng/100 mg	
1	229±6	210± 7*	137±9	636±51*
4	353±2	194±10*	151±2	617±27*

STZ was injected i.v., 60 mg/kg in 0.05 M cirtate buffer, pH 4.5. Values are means ± SE for at least five animals. STZ, streptozotocin. *Significant at $P < 0.05$ compared to control values.

Table 2. Hypothalamic contents of norepinephrine and dopamine in control and STZ-diabetic rats

Duration of diabetes	Norepinephrine content		Dopamine content	
	Control	STZ	Control	STZ
weeks	ng/g tissue		ng/g tissue	
1	1908± 98	2116±128	346±28	407±43
4	1804±104	1902±115	308±51	302±27

Values are means ± SE for 4~8 animals.

concentration of NE in hypothalamus tended to increase without statistic significance. The slight increase in NE in 4 weeks of diabetes(5%) was less than the increase at 1 week(11%). There was no changes in dopamine(DA) contents (Table 2).

To study the dynamic aspect of NE metabolism, turnover rate of norepinephrine, and *in vivo* tyrosine hydroxylase activities were determined at 1 week after establishment of diabetes. In the STZ-diabetic rats, turnover rate of norepinephrine was decreased to 62% of control values(Table 3). *In vivo* TH activity in the hypothalamus was decreased to 32% of control value(Fig. 1), indicating that, of the three indexes measured, *in vivo* TH activity is the most sensitive index of altered NE metabolism in the SZ-diabetic rat hypothalamus.

Table 3. Effect of STZ-induced diabetes on hypothalamic norepinephrine turnover

Groups	n	Rate constant of norepinephrine decline(h^{-1})	Turnover time(h)	Turnover rate(ng/g/h)
Control	4	0.20±0.03	5.0	385±20
STZ	4	0.11±0.03*	8.9	239±16**

Subgroups of rats were killed for assay of norepinephrine 0, 1, 2, or 3 h after administration of α-mehtyl-p-tyrosine(300 mg/kg, i.p.). Rats were made diabetic 1 week prior to death. Values are means± SE. Weights of excised hypothalami were 36 ± 5 mg (mean ± SD). *P<0.05; **P<0.001; significantly different from control values.

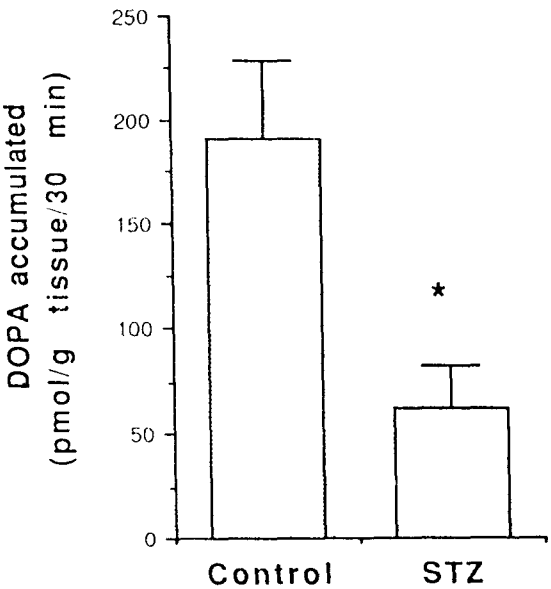

Fig. 1. Effect of STZ-induced diabetes on dihydroxyphenylalanine(DOPA) accumulation in the hypothalamus after an intraperitoneal injection of NSD-1015. Each rats were sacrificed at 30 min following NSD-1015 administration(30 mg/kg). Values are means ± SE for 5~8 animals. *Significant at P<0.05 compared to control values.

DISCUSSION

Several researchers reported increases in basal NE levels in STZ-diabetic rat brains, although the extent of the changes is more or less variable in the range of 10~60% depending on the location of brain tissue assayed and the duration of diabetes(Trulson and Himmel, 1985; Bitar *et al.*, 1986; Lackovic and Salkovic, 1990).

Our result that basal NE level did not increase significantly is rather unexpected. The blood glucose levels in our STZ-diabetic rats(> 600 mg/100 ml) were more or less higher than those of others. The only difference in experimental protocol between ours and others is that our rats were heavier(300~400 g) than others' (around 200 g). And we suggest that the response of hypothalamic basal NE to diabetes is more blunted in heavier or older rats than in lighter or younger rats.

But in the presence of normal NE basal levels, NE turnover rate remarkably decreased. Forty-one percent decrease in NE turnover rate in STZ-diabetic rat forebrain reported by Trulson and Himmel(1985) is good accordance with our reuslt(38% decrease). The extent of decrease in the hypothalamic *in vivo* TH activity(68% decrease) was very remarkable, compared to the extent of decrease in hypothalamic *in vitro* TH activities(around 10% decrease in the 10-day diabetic rats; Bitar *et al.*, 1986) and to the extent of decrease in the striatal and limbic *in vivo* TH activity(around 30~43% decrease; Trulson and Himmel., 1983), suggesting that *in vivo* TH activity is much more sensitive index of de-

creased NE synthetic rate than *in vitro* TH activity, and that hypothalamic *in vivo* TH activity is more severely deranged than that of striatum and limbic regions.

Hyperglycemia has been reported to inhibit hypothalamic NE neuronal activity(Smythe *et al.*, 1984). But the direct stimulus that results in depressed biosynthetic rate of dopa is unknown. Bitar *et al.*(1986) suggested two alternative hypotheses: i.e. hyperglycemia may directly inhibit the activiation or synthesis of TH. Alternatively, hyperglycemia induces enlargement of brain NE pool which may subsequently result in feedback-inhibition of the TH activity. Our finding of the profound decrease in *in vivo* TH activity in the presence of near-normal NE level support the former hypothesis. But the possiblity that small increase in NE levels may result in larger changes in the relative proportions of several different intraneuronal NE pools that may eventually suppress the TH activity can not be ignored.

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= 국문초록 =

Streptozotocin-유발 당뇨쥐의 시상하부에서 Norepinephrine 함량은 정상이나 *In vivo* Tyrosine Hydroxylase 활성은 감소함

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Streptozotocin(STZ)-유발 당뇨쥐에서 시상하부의 norepinephrine(NE) 대사를 기초 NE 함량, NE 교체율, *in vivo* tyrosine hydroxylase(TH) 활성을 그 지표로 하여 조사하였다. STZ (60 mg/kg, iv)로 당뇨를 유발한 후 4주까지 기초 NE 함량은 유의한 변화가 없었다. 그러나 당뇨 유발 후 1주째에 측정한 NE 교체율은 대조치의 62%($p<0.01$), *in vivo* TH 활성은 대조치의 34% ($p<0.05$)로 감소하였다. 이상의 결과로 본 실험에서 측정한 NE 대사의 세 지표 중에서 *in vivo* TH 활성이 STZ-유발 당뇨쥐의 시상하부 NE 대사의 변화를 가장 민감하게 반영하였다.