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Glucose Modulation of Release of Endogenous Catecholamines from Hypothalamic Fragments *in Vitro*

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ABSTRACT

Effects of glucose on the catecholamine release from the hypothalamic fragments in vitro were studied. Basal release of catecholamines was inversely related to the concentrations (5~30 mM) of glucose in the incubation medium. Glucose did not affect the 30 mM K^+-stimulated release of catecholamine. In the presence of tetrodotoxin (10 μM), the inhibitory effect of glucose on the basal release of catecholamines was largely persisted, but the inhibitory effect of 30 mM glucose on dopamine release was largerly blocked. In the presence of both tetrodotoxin (10 μM) and desipramine (3 μM), glucose failed to affect the basal catecholamine release. The results suggest that glucose modulates the catecholamine release through a direct action on the catecholaminergic nerve terminals, as well as through a trans-synaptical action. The glucose-modulation of the catecholamine release may explain, at least in part, the diabetes-induced changes in the hypothalamic catecholamine metabolism.

Key Words: Catecholamine release, Hypothalamus, D-Glucose, Tetrodotoxin, Desipramine

INTRODUCTION

Many studies have shown that rats and mice with streptozotocin-induced diabetes have altered metabolism of brain catecholamines, such as a decreased rate of catecholamine synthesis (Bitar *et al.*, 1986; Trulson and Himmel, 1983), a decrease in the metabolism (Kwok and Juorio, 1986) or turnover rate (Bellush and Reid, 1991; Steger *et al.*, 1989; Trulson and Himmel, 1985) of the catecholamines, and an increase in catecholamine level (Bitar *et al.*, 1986; Trulson and Himmel, 1985; Chen and Yang, 1991; Lackovic *et al.*, 1990) in several regions of brain.

The precise mechanism of the diabetes-induced changes in the brain catecholamine metabolism is still unclear. Streptozotocin-induced deficiency of insulin in diabetic animals produces many metabolis abnormalities, including hyperglycemia, altered plasma amino acid levels, or altered enzyme activities (Crandall and Fernstorm, 1983; Glanville and Anderson, 1985; Mayanil *et al.*, 1982a, 1982b). Such a complexity of diabetic state *in vivo* contributes to the uncertainty surrounding pathogenesis of brain neurochemical changes in diabetic animals. Therefore, the present investigation deals with the incubated fragments of hypothalamus, a region that shows a prominent change in the catecholamine metabolism in the diabetic state (Bitar *et al.*, 1986; Steger *et al.*, 1989; Shimizu, 1991), to test the direct effect of various concentrations of glucose on the *in vitro* release of catecholamines. We now report that

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glucose concentration-dependently decreases the basal release of norepinephrine (NE), and dopamine (DA).

MATERIALS AND METHODS

Hypothalamic incubation system

Female Sprague-Dawley rats weighing 250~300 g were used. The mediobasal hypothalamus, delimited by the hypothalamic fissures (laterally), the optic chiasm (anteriorly) and the rostral portion of the mamillary bodies (posteriorly) was removed by a horizontal cut of about 2 mm in depth and halved on ice. Each hypothalamus was incubated individually in a 5 ml polyethylene tube containing 0.5 ml of Krebs-Ringer-Henseleit solution (37℃) continuously bubbled from the bottom with 95% O_2/5% CO_2. The Krebs Ringer-Henseleit solution consisted of 117 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 24.8 mM $NaHCO_3$, 0.1 mg/ml ascorbic acid, 10 μM pargyline, and varying concentrations (5~30 mM) of glucose.

Experimental protocol

After equilibration for 60 min to stabilize the release of catecholamines, each hypothalamus was incubated for 2 consecutive 30-min periods (base-line period, and test period) and then stimulated for 15 min with 30 mM KCl (depolarization period). For experiment with tetrodotoxin (10 μM) and desipramine (3 μM), each drug was added to the incubation medium 15 min before the base-line period. From equilibration through the base line period, the concentration of glucose was 10 mM. From the test period through the 30 mM K^+ depolarization period, glucose was changed over a concentration range of 5~30 mM. In the depolarization period, hypothalami were challenged with 30 mM KCl with tonicity maintained by decreasing NaCl.

Data Analysis

The release of catecholamines during test period or depolarization period is expressed as a percentage of base-line release. Each incubation was terminated by careful aspiration of medium, which was subsequently acidified with 0.5 ml of 0.1 M perchloric acid containing 2 ng of dihydroxybenzylamine as an internal standard. The catecholamines were adsorbed on alumina, eluted with 0.1 M perchloric acid and measured by the high performance liquid chromatography procedure (Keller *et al.*, 1976). Data were presented as means±S.E.M. For the comparison of two or more means Student's t-test or Dunnett's test was used, respectively. The mean weight of hypothalami was 31.4±5.3 mg(mean±S.D., n=32), when measured after the depolarization period.

RESULTS

After 60-min equilibration period, the releases

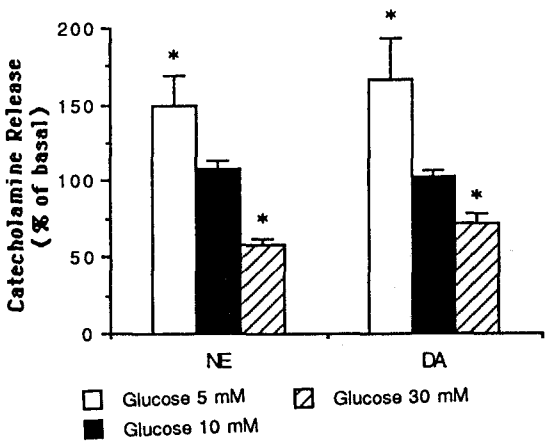

Fig. 1. Effect of glucose on the basal release of catecholamines from hypothalamic fragments. After equilibration for 60 min to stabilize the release of catecholamines, each hypothalamus was incubated for 2 consecutive 30-min periods (base-line period, and test period). From equilibration through the base-line period, the concentration of glucose was 10 mM. During the test period, glucose was changed over a concentration range of 5~30 mM. The release of catecholamines during the test period is expressed as a percentage of base-line release. Base-line releases of NE and DA were 3.37±0.40 and 1.77±0.29 pmol /30 min/hypothalamus (n=20), respectively. The mean±S.E.M. of 6 different experiments is shown. *$p<0.05$ vs. respective release of catecholamines in 10 mM glucose (Dunnett's test). Abbreviations: NE, norepinephrine; DA, dopamine.

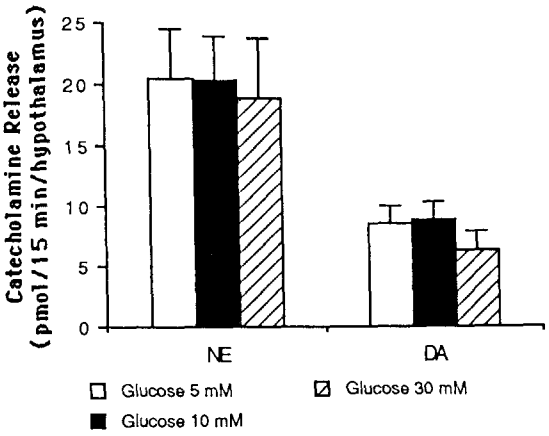

Fig. 2. Effect of glucose on the 30 mM K^+-stimulated release of catecholamines. After two consecutive 30-min incubation periods, hypothalami were challenged with 30 mM KCl for 15 min with tonicity maintained by decreasing NaCl. The mean±S.E.M. of 6 different experiments is shown. Abbreviations: NE, norepinephrine; DA, dopamine.

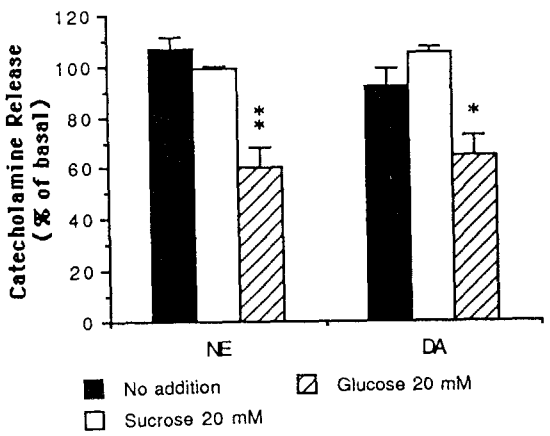

Fig. 3. Specificity for glucose in the modulation of the basal release of catecholamines. Same as the legend of Fig. 1. except that each hypothalamus was incubated in KRH containing 10 mM glucose or additional sugars (20 mM) indicated during the test period. The mean±S.E.M. of 3 different experiments is shown. $^*p<0.05$; $^{**}p<0.01$ vs. respective release of catecholamines in 10 mM glucose (Dunnett's test). Abbreviations; NE, norepinephrine; DA, dopamine.

of NE and DA from hypothalamic fragments *in vitro* during the base-line period (10 mM glucose) were 3.37±0.40 and 1.77±0.29 pmol/30 min/hypothalamus (n=20), respectively. Lowering the glucose concentration from 10 to 5 mM in the test period evoked an increase of releases of NE and DA 1.50-and 1.66-fold, respectively, expressed as a percentage of base-line value. When glucose concentration was increased from 10 to 30 mM, the releases of NE and DA were decreased by 42% and 28%, respectively (Fig. 1).

After two consecutive 30-min incubation periods, depolarization of the tissue by 30 mM K^+ increased the releases of NE and DA to 12.1-fold (20.4±3.6 pmol/15 min/hypothalamus, n=7) and 10.1-fold (8.9±1.4 pmol/15 min/hypothalamus, n=7), respectively. There was no significant difference in the K^+-stimulated catecholamine release among different concentrations of glucose, although 30 mM glucose tended to decrease catecholamine release, especially in the case of dopamine (Fig. 2).

To determine whether the inhibitory effect of high glucose (30 mM) on the basal release of catecholamines is due to a nonspecific hyperosmolar effects, equiosmolar concentrations of sucrose were substituted for the additional glucose. There was no effect on the basal release of catecholamines for an addition of surcrose, indicating the specific action of glucose (Fig. 3).

To determine the role of propagated action potentials in the glucose-modulation of release of catecholamines, tetrodotoxin (10 μM) was added into the incubation medium 15 min before the base-line period to block voltage-dependent Na^+ channels, and the glucose concentration was varied during the test period. The incubation with tetrodotoxin alone reduced the base-line release of NE and DA by 80% and 82%, respectively (0.68±0.15 pmol/30 min/hypothalamus n=9 for NE; 0.32±0.05 pmol/30 min/hypothalamus n=9 for DA). There was still dose-dependent decrease in the release of catecholamines in the presence of tetrodotoxin. Lowering the glucose concentration from 10 to 5 mM in the presence of tetrodotoxin (10 μM) increased NE and DA release 1.38-and

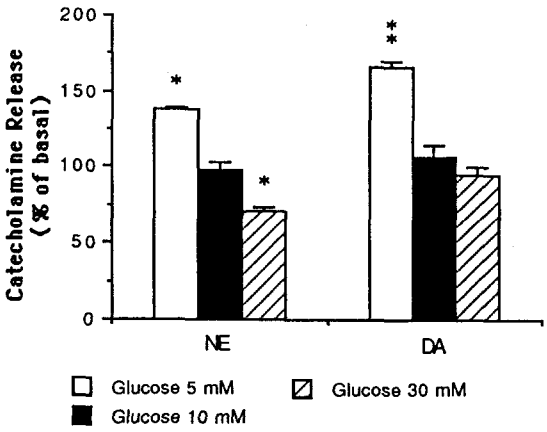

Fig. 4. Effect of glucose on the basal release of catecholamines in the presence of tetrodotoxin. Same as the legend of Fig. 1 except that tetrodotoxin (10 μM) was added into the incubation medium 15 min before the base-line period. Base-line releases of NE and DA in the presence of tetrodotoxin were 0.68±0.15 (n=9) and 0.32±0.05 (n=9) pmol/30 min/hypothalamus, respectively. The mean±S.E.M. of 3 different experiments is shown. *$p<0.05$; **$p<0.01$ vs. respective release of catecholamines in 10 mM glucose (Dunnett's test). Abbreviations: NE, norepinephrine; DA, dopamine.

Table 1. Glusoce fails to affect the basal release of catecholamines from incubated hypothalami in the presence of desipramine

Glucose mM	NE % of base-line release (Mean±S.E.M.(n))	DA
5	95.5±5.3(4)	95.5±6.4(4)
10	93.2±7.9(4)	94.2±4.2(4)
30	86.5±7.9(4)	96.0±3.9(4)

Same as the legend of Fig. 1 except that tetrodotoxin (10 μM) and desipramine (3 μM) were added into the incubation medium 15 min before the base-line period. Base-line releases of NE and DA in the presence of both tetrodotoxin and desipramine were 4.53±0.32 (n=12) and 3.21±0.22 (n=12) pmol/30 min/hypothalamus, respectively. The mean±S.E.M. of 3 different experiments is shown. Abbreviations: NE, norepinephrine; DA, dopamine.

1.67-fold, respectively. While the magnitude of 30 mM glucose-induced decrease in NE release was attenuated in the presence of tetrodotoxin, the 30 mM glucose-induced decrease in DA release was mostly abolished in the presence of tetrodotoxin (Fig. 4).

We determined the role of reuptake of catecholamines in the inhibitory effect of glucose in the presence of tetrodotoxin. Tetrodotoxin (10 μM) and desipramine (3 μM) were added into the incubation medium 15 min before the base-line period. Addition of desipramine increased the release of NE and DA by 7-and 10-fold, respectively, as compared with tetrodotoxin alone (4.53 ±0.32 (n=12) and 3.21±0.22 (n=12) pmol/30 min /hypothalamus, respectively. In the presence of both tetrodotoxin and desipramine, glucose did not affect the release of catecholamines (Table 1).

DISSCUSSION

The results of the present study demonstrated a reciprocal relationship between the glucose concentrations and the basal release of catecholamines from the hypothalamic fragments *in vitro*, with no significant effect on the K^+-stimulated release (Fig. 1, 2).

In the presence of tetrodotoxin, the inhibitory effect of glucose was, in large part, persisted (Fig. 4), implying a direct action of glucose on the catecholaminergic nerve terminals. This possibility is further supported by the blockade of glucose-induced response by desipramine in the presence of tetrodotoxin (Table 1), which suggests that glucose modulates reuptake of catecholamines to catecholaminergic terminals. The high affinity uptake of catecholamines is highly energy-dependent and is coupled with the electrical potential and Na^+ gradient, which parameters are dependent on the activity of Na^+, K^+-ATPase (for review see ref. Graefe *et al.*, 1988). Thus, it is tempting for us to speculate that high glucose may help to maintain catecholamine reuptake by maintaining Na^+, K^+-ATPase activity and Na^+ gradient in the catecholaminergic nerve terminals. In this regards, glucose has also been shown to facilitate the uptake of dopamine into striatal synaptosomal preparations (Dorris, 1978).

The inhibitory role of high concentrations of glucose on the basal release of catecholamines demonstrated in this study supports the previous *in vivo* findings that NE release from the ventromedial hypothalamus was reduced during push-pull perfusion with glucose (McCaleb *et al.*, 1979). The attenuation of the inhibitory effect of 30 mM glucose in the presence of tetrodotoxin (Fig. 4) suggests that glucose modulates the release of catecholamines not only through a direct action on the catecholaminergic nerve terminals, but also through trans-synaptic signals from some neurons which are sensitive to glucose. The exact nature of neurons which are sensitive to glucose and trans-synaptically related to the release of catecholamines remains to be clarified.

The slope of the catecholamine release change when glucose concentration was changed from 10 to 5 mM, was steeper compared with that of the change when glucose was changed from 10 to 30 mM (Fig. 1), implying a higher sensitivity of the hypothalamus to changes in glucose around physiologic levels. An inverse relationship between plasma glucose concentration and mediobasal hypothalamic noradrenergic activity was reported (Smythe *et al.*, 1984). Therefore, it is suggested that the release of catecholamines may be sensitively regulated by the local level of glucose within the hypothalamus in the rat.

Glucose decreases the release of somatostatin (Berelowitz *et al.*, 1982; Lengyel *et al.*, 1984), corticotropin releasing factor (Widmaier *et al.*, 1988), and growth hormone-releasing factor (Baes and Vale, 1990) from the hypothalamus *in vitro*. Although it is possible that glucose inhibits the release of these neuropeptides by acting directly on their respective neurons, another possibility is that it does so through an inhibition of the release of catecholamines which have been shown to stimulate the release of these hypothalamic neuropeptides (Miki *et al.*, 1984; Negro-Vilar *et al.*, 1978; Plotsky, 1987).

Although we examined only the acute effects of various concentrations of glucose on the release of catecholamines *in vitro*, the high glucose-induced decrease in the release of catecholamines may partially explain the diabetes-induced changes in the catecholamine metabolism.

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=국문초록=

시상하부 조각에서 내재성 카테콜아민의 분비에 대한 포도당의 조절작용

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송 동 근·김 용 식*·김 영 희

시상하부 조각에서 카테콜아민의 분비에 대한 포도당의 영향을 관찰하였다. 카테콜아민의 기초분비는 포도당의 농도(5~30 mM)에 반비례하였다. Tetrodotoxin (10 μM)의 존재하에서 카테콜아민의 기초분비에 대한 포도당의 억제 작용은 대부분 유지되었으나, 도파민에 대한 30 mM 포도당의 억제 작용은 거의 봉쇄되었다. Tetrodotoxin (10 μM)과 desipramine (3 μM)의 존재하에서는 카테콜아민의 기초분비에 대한 포도당의 영향이 없었다. 이상의 결과는 포도당이 transsynaptic action 뿐 아니라 카테콜아민 신경세포 말단에 대한 직접 작용을 통하여 카테콜아민의 분비를 조절할 것임을 시사한다. 카테콜아민의 분비에 대한 포도당의 조절작용은 당뇨상태에서의 시상하부 카테콜아민 대사의 변화를 적어도 부분적으로는 설명할 수 있으리라 사료된다.