ROLE OF THE SUPRACHIASMATIC NUCLEUS IN GLUCOSE HOMEOSTASIS

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ABSTRACT

Effect of the suprachiasmatic nucleus (SCN) on photoperiod-dependent hyperglycemia due to 2-deoxy-D-glucose (2DG) was investigated using SCN-injured rats. Lesions involving the SCN caused not only complete suppression of photoperiod-dependent hyperglycemia due to 2DG, but also hyperinsulinemia without hyperphagia, irrespective of the lighting conditions. We propose that besides acting as a circadian clock, the SCN is involved in the central glucoreceptor mechanism and also in the regulation of insulin secretion.

Deprivation of glucose by systemic (6) or central (8) administration of 2-deoxy-D-glucose (2DG) induces hyperglycemia in association with inhibition of insulin secretion through adrenomedullary function (3). We demonstrated that the hyperglycemia due to intracranial injection of 2DG showed photoperiodicity: it was more marked in the light period than in the dark period (12). Recent studies in rodents have established that the master clock of the circadian rhythm with a light-dark cycle as an environmental synchronizer is located in the suprachiasmatic nucleus (SCN) (7, 10, 11, 17, 21). It thus seemed likely that hyperglycemia due to 2DG injection would be affected by bilateral lesions of the SCN. In this experiment, we found that SCN lesions caused not only complete suppression of photoperiod-dependent hyperglycemia due to 2DG, but also hyperinsulinemia without hyperphagia, irrespective of the lighting conditions.

MATERIALS AND METHODS

Animals

Male Wistar rats, initially weighing 200 to 250 g, were used throughout. They were housed individually in plastic cages in a room maintained at $24 \pm 1^\circ\text{C}$ and at $60 \pm 10\%$ relative humidity, and illuminated for 12 h (0200-1400)

by an artificial fluorescent light bulb (80 lux). Food (MF, Oriental Yeast, Osaka) and water were given freely.

SCN Lesions

The SCN were injured as described previously (11). Locomotive activity was measured with an Animex (MK-Animex, Muromachi Kikai, Tokyo), since bilateral lesions of the SCN completely abolished the circadian rhythm of activity (11, 22). Only rats with complete loss of the circadian rhythm of locomotive activity were used. Rats treated in the same way but without the anodal current served as controls (sham-operated rats). After the operation, animals were killed with an overdose of pentobarbital and their brains were removed, fixed in 10% formalin, embedded in paraffin, and stained with Cresyl violet for histological examination.

Administration of 2DG

Under pentobarbital anesthesia, a polyethylene cannula (PE-10) was inserted into the right lateral cerebral ventricle by the method of Altaffer *et al.* (1). Another catheter made of silastic and polyethylene tubing (PE-50) was inserted into the right atrium of the heart by the

method of Steffens (20). Experiments were done 3 days after cannulation. 2DG (120 μ mol) and saline, each in a volume of 30 μ l, were injected through the brain catheter at 1000 (light period) or at 1700 (dark period). In the light period, 2DG was given to 5 rats with or without SCN lesions, and 3 of the 5 rats were used for administration of saline. In the dark period, 3 animals were used. Each injection was given at an interval of 7 days. Blood samples (0.2 ml each) were withdrawn from the right atrium at 0, 30, 60, 90, and 120 min after the injection. Food was removed from 2 h before the 2DG injection until the end of the blood sampling.

Measurement of Glucose and Insulin Concentration

The plasma glucose concentration was measured by the glucose oxidase method using a Blood Sugar-GOD-Perid Test Kit (Boehringer, Mannheim). The plasma insulin concentration was determined using a double antibody radioimmunoassay kit (Dainabot Radioisotope Institute, Tokyo) with porcine insulin as a standard.

Statistical Analysis

Data are expressed as means $\pm$ SEM. Statistical significance was examined by the analysis of variance and Student's *t*-test.

Fig. 1 Effect of lesions involving the SCN on hyperglycemia due to 2DG injected into the lateral cerebral ventricle. Values are expressed as means $\pm$ SEM. Statistical significance was examined by analysis of variance.

		Sham-operated	SCN-injured
2DG	light (A) vs. dark (B)	$P < 0.001$	NS
Saline	light (C) vs. dark (D)	NS	NS
		Light period	Dark period
2DG	sham vs. SCN-injured	$P < 0.001$	$P < 0.01$
Saline	sham vs. SCN-injured	NS	NS
SCN-injured	2DG vs. saline	NS	NS
Sham-operated	2DG vs. saline	$P < 0.001$	$P < 0.01$

RESULTS

Injection of 2DG into the lateral ventricle of sham-operated rats caused a significant increase in plasma glucose concentration. As previously reported (12), the increase was significantly ($P < 0.001$) higher in the light period than in the dark period (Fig. 1, A and B). Lesions involving the bilateral SCN did not affect the plasma glucose concentration, but completely suppressed the hyperglycemic response to 2DG in both the light and the dark period (Fig. 1, A and B). Intracranial injection of saline into sham-operated and SCN-injured rats did not elicit significant change in the plasma glucose level in either the light or dark period (Fig. 1, C and D). 2DG caused no change in the plasma insulin

level in sham-operated rats, although it induced hyperglycemia (>400 mg/dl; Fig. 2, A and B). Saline did not cause any change in the plasma level of insulin (Fig. 2, C and D).

However, even before 2DG or saline injection, lesions involving the SCN elicited hyperinsulinemia, irrespective of the lighting conditions (Fig. 2, A–D). 2DG injection caused further significant increase in plasma insulin concentration only in the light period (the plasma insulin levels at 30, 60 and 90 min were significantly ($P < 0.05$) higher than the level at zero time when examined by Student's t -test) (Fig. 2A), and the level remained high until the end of blood sampling in the dark period. The mean daily food intake during 5 days before the cannulation of SCN-injured animals did not significantly differ

Fig. 2 Effect of SCN lesions on the plasma insulin level after 2DG injection. Values are means $\pm$ SEM. Other conditions are given in the legend to Fig. 1. Statistical significance was examined by analysis of variance.

		Sham-operated	SCN-injured
2DG	light (A) vs. dark (B)	NS	$P < 0.01$
Saline	light (C) vs. dark (D)	NS	NS
2DG	sham vs. SCN-injured	Light period $P < 0.001$	Dark period $P < 0.001$
Saline	sham vs. SCN-injured	$P < 0.01$	$P < 0.01$
SCN-injured	2DG vs. saline	$P < 0.001$	NS
Sham-operated	2DG vs. saline	NS	NS

Fig. 3 The brain regions injured. The region injured in all 8 rats is indicated by black areas, and other regions affected in some rats by hatched area. GP: Globus pallidus. pom: Nucleus preopticus medialis. pol: Nucleus preopticus lateralis. sc: Nucleus suprachiasmaticus. CC: Crus cerebri. ha: Nucleus anterior (hypothalami). TO: Tractus opticus. ZI: Zona incerta. FH: Fimbria hippocampi. abl: Nucleus amygdaloideus basalis, pars lateralis. ac: Nucleus amygdaloideus centralis. hl: Nucleus lateralis (hypothalami). dm: Nucleus ventralis thalami, pars dorsomedialis. vm: Nucleus ventromedialis (hypothalami). rh: Nucleus rhombeideus. re: Nucleus reuniens

from that of sham-operated rats (26.3 ± 1.1 g/day for 8 control rats, and 23.0 ± 1.7 g/day in 8 SCN-injured rats). Thus, bilateral lesion of the SCN cause not only hyperinsulinemia without hyperphagia, but also complete suppression of the hyperglycemic action of 2DG administered intracranially.

Histological Examination

Fig. 3 shows the area of the lesions, which includes the SCN and parts of the periventricular and paraventricular nuclei, but not the ventromedial hypothalamus (VMH) or lateral hypothalamus (LH).

DISCUSSION

Glucoreceptor neurons are present in the VMH (16), and stimulation of the VMH results in hyperglucagonemia and hyperglycemia, but suppression of insulin secretion (2), whereas lesion of the VMH results in hyperinsulinemia (4). In contrast, glucose-sensitive neurons are present in the LH (16); stimulation of the LH lowers the blood glucose level (19). These observations suggest that the VMH and LH play important roles in control of insulin secretion and glucose metabolism. Bilateral lesions of the VMH or LH alone, or in combination do not suppress the hyperglycemia induced by intracranial injection of 2DG (9), suggesting that the SCN or its vicinity is responsible for the

hyperglycemia and suppression of insulin secretion observed after intracranial administration of 2DG. We reported that SCN lesions abolish the circadian feeding rhythm (10, 11) and presented evidence for neural connection between the SCN and VMH, and also between the SCN and LH (13, 14). We speculate that the circadian rhythm of feeding behavior is generated by time signals from the main circadian clock in the SCN through alternate excitations of the two relay stations, i.e. the VMH and the LH. In line with this speculation, it has been reported that the SCN exerts an excitatory influence on VMH activity and an inhibitory influence on LH activity (5, 15). Thus inhibition of insulin secretion mediated by excitation of the VMH or suppression of the LH can be explained on the basis of neuronal signals transmitted from the SCN. In this regard, the central control mechanism of insulin secretion seems to be in common with the generation mechanism of the circadian feeding rhythm.

Schwartz and Gainer (18) reported in the rat that uptake of [^{14}C]2DG into the SCN, which is greater than that into other brain areas, was more in the light than in the dark period. Our results are consistent with their findings in the sense that there is a glucoreceptor in the SCN and its reactivity with glucose or 2DG is enhanced during the light period. However, we do not know yet whether the SCN glucoreceptor exists in cells regulating the circadian clock.

This work was partly supported by a Grant-in-Aid for Cooperative Research on 'Biological Rhythm and Environmental Adaptation in Higher Animals' from the Ministry of Education, Science and Culture, Japan.

Received for publication 25 August 1983; and in revised form 14 November 1983

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