BILATERAL LESIONS OF THE SUPRACHIASMATIC NUCLEUS ENHANCE GLUCOSE TOLERANCE IN RATS

HIDEKI YAMAMOTO, KATSUYA NAGAI and HACHIRO NAKAGAWA

Division of Protein Metabolism, Institute for Protein Research, Osaka University, Suita, Osaka 565, Japan

ABSTRACT

Rats with or without lesions of the suprachiasmatic nucleus (SCN) were kept under a 12-h light (0800–2000) and 12-h dark (2000–0800) cycle, and oral and intravenous glucose tolerance tests were performed in the light period (at 1000) and dark period (at 2200). In control rats, glucose tolerance was higher in the dark period than in the light period in both tests, though increase in plasma insulin was significantly higher in the dark period than in the light period only in the intravenous test. Lesions involving the bilateral SCN markedly increased glucose tolerance and insulin secretion after oral and intravenous glucose loads in both the light and the dark period, and there was no difference between the results of either test in the light and the dark period. These findings suggest that in rats, glucose tolerance shows a daily rhythm and that the SCN may be involved in generation of this rhythm through photoperiodic control of insulin secretion.

Recently we observed photoperiodic hyperglycemia due to intracranial injections of sugars such as 2-deoxy-D-glucose (2DG), D-mannitol (MA), and D-glucose (GL) in rats (10); 2DG injection into the lateral ventricle elicited greater hyperglycemia in the light than in the dark period, but injections of MA and GL caused hyperglycemia only in the light and dark period, respectively. Furthermore, we observed that bilateral lesions of the suprachiasmatic nucleus (SCN) abolished not only the photoperiodic hyperglycemia due to 2DG but also hyperinsulinemia in rats (14). In this paper we report that SCN lesions increased glucose tolerance as a result of enhanced and more rapid insulin secretion.

MATERIALS AND METHODS

Animals

Male Wistar strain rats, weighing 250–300 g, were used. They were maintained in a room kept at $24 \pm 1^\circ\text{C}$ and illuminated for 12 h (0800–2000). Food (type MF, Oriental Yeast, Osaka) and water were given freely.

SCN Lesions

Electrical lesions of the SCN were made as described previously (9). Rats treated in the same way but without an anodal current served as controls (sham-operated rats). After the operation, animals were housed individually for about 3 weeks before beginning experiments. The completeness of SCN lesions was checked by monitoring the daily pattern of locomotor activity with an MK-Animex (Muromachi Kikai, Tokyo); bilateral lesions of the SCN cause loss of the circadian rhythm of locomotive activity (8). Only rats with complete loss of circadian locomotive activity were used as animals with SCN lesions. After the experiment, the brain was removed, fixed with formalin, embedded in paraffin, stained with Cresyl violet, and examined histologically.

Oral and Intravenous Glucose Tolerance Tests

A cardiac catheter made of silastic and polyethylene tubing was inserted into the right atrium

of the heart of the rats under pentobarbital anesthesia. For the intravenous glucose tolerance test, another catheter made of the same tubing was introduced into the superior vena cava by the method of Steffens (13). After catheterization, the animals were kept for 5 days. On the experimental day, food was withdrawn from 2 h before the glucose load until the end of blood sampling. For the oral glucose tolerance test, 1 ml of 50% glucose solution was given orally to the animals as described by Louis-Sylvestre (5). The animals took the solution within 1 min. For the intravenous test, 0.15 ml of 50% glucose solution was injected intravenously into the superior vena cava through the venous catheter. Blood samples (0.5 ml each) were removed from the heart through the cardiac catheter at 0, 3, 5, 15, 30, and 60 min, and at 0, 0.5, 1, 3, 5, 15, 30, and 60 min after glucose load in the oral and intravenous tests, respectively.

Measurement of Glucose and Insulin Concentrations

The plasma glucose concentration was measured by the glucose oxidase method using a Blood Sugar-GOD-Perid Test Kit (Boehringer, Mannheim). 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 analysis of variance and Student's *t*-test.

RESULTS

Oral Glucose Tolerance Test

In sham-operated rats, increase in the plasma glucose concentration after a glucose load was significantly higher ($P < 0.001$) in the light

Fig. 1 Effect of lesions involving the bilateral suprachiasmatic nucleus (SCN) on the plasma glucose level after an oral glucose load in the light and dark periods. A: Plasma glucose level of sham-operated rats. B: Plasma glucose level of SCN-injured rats. Three sham-operated and 3 SCN-injured rats were used. D-Glucose solution (0.5 g in 1 ml) was given orally to each rat at zero time. Statistical significance examined by analysis of variance. A: sham-operated; light vs. dark, $P < 0.001$. B: SCN-injured; light vs. dark, not significant. Light: SCN-injured vs. sham-operated, $P < 0.001$. Dark: SCN-injured vs. sham-operated, $P < 0.001$.

Fig. 2 Effect of bilateral SCN lesions on the plasma insulin level after an oral glucose load in the light and dark periods. A: Plasma insulin level of sham-operated rats. B: Plasma insulin level of SCN-lesioned rats. Three sham-operated and 3 SCN-injured rats were used. Statistical significance examined by analysis of variance. A: sham-operated; light vs. dark, not significant. B: SCN-injured; light vs. dark, not significant. Light: SCN-injured vs. sham-operated, $P < 0.001$. Dark: SCN-injured vs. sham-operated, not significant

period than in the dark period; the maximum value was reached within a shorter time (3 min) in the dark period than in the light period (15 min) (Fig. 1A). SCN lesions almost completely abolished the increase in glucose in both the light and dark period (Fig. 1B).

In sham-operated rats, change in the plasma insulin level immediately after a glucose load was similar in the two photoperiods, but the second phase of insulin secretion was significantly lower ($P < 0.05$) in the light period than in the dark period (Fig. 2A). SCN lesions caused significant increase in the plasma insulin level before a glucose load in both the light and dark period ($P < 0.01$ in the light period and $P < 0.05$ in the dark period by the *t*-test) (Fig. 2). Moreover, the insulin level after the oral glucose load in SCN-injured rats was significantly higher ($P < 0.01$) than in sham-operated rats in the light period, but similar to that in sham-operated rats in the dark period (Fig. 2). These findings suggest that insulin secretion from the pancreas after an oral glucose load also shows a daily rhythm, the response being low in the light period and high in the dark period, and that SCN lesions result in increased insulin secretion

and elimination of the rhythm. However, another possibility is that SCN lesions inhibited absorption of glucose from the gastro-intestinal tract and thus decreased the change in plasma glucose concentration after the glucose load. To exclude this possibility, we examined the effects of an intravenous glucose load on the level of plasma glucose and insulin.

Intravenous Glucose Tolerance Test

In sham-operated rats, the maximum increase in the plasma glucose concentration after a glucose load was significantly higher ($P < 0.001$) in the light period than in the dark period, though the maximum value was reached simultaneously (within 30 sec) in both groups (Fig. 3A). SCN lesions significantly lowered the increase in glucose in both groups ($P < 0.001$ in the light period, and $P < 0.01$ in the dark by the analysis of variance), resulting in disappearance of the difference in response between the two photoperiods (Fig. 3B).

In sham-operated rats, the maximum increase in the plasma insulin level after a glucose load was significantly lower ($P < 0.01$) in the light

Fig. 3 Effect of bilateral SCN lesions on the plasma glucose level after an intravenous glucose load in the light and dark periods. D-Glucose solution (75 mg in 150 μ l) was injected intravenously into each rat at zero time. A: Plasma glucose level of sham-operated rats. B: Plasma glucose level of SCN-injured rats. Three sham-operated and 3 SCN-injured rats were used. Statistical significance examined by analysis of variance. A: sham-operated; light vs. dark, $P < 0.001$. B: SCN-injured; light vs. dark, not significant. Light: SCN-injured vs. sham-operated, $P < 0.001$. Dark: SCN-injured vs. sham-operated, $P < 0.001$.

period than in the dark period (Fig. 4A). SCN lesions caused a significant increase in the plasma insulin level after the glucose load in both groups, which eliminated the difference in response between the two photoperiods. In the light period, the maximum value was reached in the sham-operated rats within 1 min, whereas in SCN-injured rats within 30 sec. In the dark period, the maximum increase was observed simultaneously in both the sham-operated and SCN-injured animals. These lesions also caused a considerable acceleration in insulin secretion, as can be seen from the difference between the maximum value in sham-operated rats and SCN-injured rats ($P < 0.01$ in the light period, and $P < 0.01$ in the dark period by the *t*-test) (Fig. 4). Also the lesions tended to increase the insulin level before the glucose load, though the difference between the rats with or without lesions was not statistically significant.

Histological Examination

Fig. 5 shows the area injured by the operation,

which included the bilateral SCN and part of the periventricular nucleus, but not the ventromedial hypothalamus (VMH) or lateral hypothalamus (LH).

DISCUSSION

In humans, glucose tolerance shows a daily rhythm in relation to daily changes in insulin secretion and the tissue sensitivity to insulin (2,3,7). On the other hand, Louis-Sylvestre (6) found in rats that glucose tolerance was higher in the dark period than in the light period, and that the increase in insulin secretion after an intravenous glucose load was also greater in the dark period. In the present experiment, we confirmed this finding in sham-operated rats. Furthermore, we demonstrated that lesions which involved the bilateral SCN caused a complete loss of the daily rhythm of glucose tolerance by causing complete suppression of increase in plasma glucose concentration in the oral test and partial suppression in the intravenous test. Moreover, we demonstrated that such

Fig. 4 Effect of bilateral SCN lesions on the plasma insulin level after an intravenous glucose load in the light and dark periods. A: Plasma insulin level of sham-operated rats. B: Plasma insulin level of SCN-injured rats. Three sham-operated and 3 SCN-injured rats were used. Statistical significance examined by analysis of variance. A: sham-operated; light vs. dark, $P < 0.01$. B: SCN-injured; light vs. dark, not significant. Light: SCN-injured vs. sham-operated, $P < 0.05$. Dark: SCN-injured vs. sham-operated, not significant

lesions caused increase in insulin secretion, which was more marked in the intravenous test than in the oral test. This eliminated the daily rhythm of the plasma insulin response after a glucose load, irrespective of the mode of administration. In accord with our previous findings, we observed that the plasma insulin level before a glucose load was higher in SCN-injured rats than in sham-operated rats (Fig. 2), suggesting that the SCN acts to suppress insulin secretion. Since the suppression is stronger in the light period than in the dark period, this may relate to generation of the daily rhythm of glucose tolerance.

Louis-Sylvestre (6) showed that the day-night differences in glucose tolerance and insulin secretion after a glucose load disappeared after vagotomy in parallel with loss of the feeding rhythm. These findings raise the question of whether the temporal signal from the SCN is transmitted via the vagus nerve to peripheral tissues for expression of the daily rhythms of insulin secretion and glucose tolerance. We (8, 9, 11, 12) presented evidence that in rats the SCN is a master clock of the circadian rhythms of feeding behavior and of metabolism, and that

the VMH and the LH are relay stations of the temporal signal from the SCN for these rhythms. According to Ban (1) the VMH is included in a 'B sympathetic zone,' and the LH in a 'C parasympathetic zone.' It thus seems quite likely that a temporal signal from the SCN is transmitted to the sympathetic nervous system via VMH, and to the parasympathetic nervous system (vagus nerve) via LH, to control insulin secretion and glucose tolerance.

Hara and Saito (4) recently reported that in rats subjected to a fixed feeding schedule, insulin secretion was higher in the feeding period than in other periods. We previously showed that bilateral lesions of the SCN in rats completely abolished the circadian feeding rhythm (8, 9). Together with the findings of Louis-Sylvestre (6), all these observations lead to a working hypothesis that the daily rhythms of glucose tolerance and insulin secretion in humans and rats are generated by the same mechanism as the circadian feeding rhythm. To substantiate this, questions of whether there is a daily rhythm of insulin sensitivity in various tissues, and if so whether this participates in generating the daily rhythm of glucose tolerance, and

Fig. 5 Brain regions injured in 7 rats. The region injured in all 7 rats is shown by black area, and other regions affected in some rats by hatched areas. 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 centrais. hl: Nucleus lateralis (hypothalami). dm: Nucleus ventralis thalami, pars dorsomedialis. Vm: Nucleus ventromedialis (hypothalami). rh: Nucleus rhombeideus. re: Nucleus reuniens

whether the same neurons responsible for the circadian feeding behavior are involved in suppression of insulin secretion and glucose tolerance should be examined.

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

REFERENCES

1. BAN T. (1966) The septo-preoptic-hypothalamic system and its autonomic function. *Prog. Brain Res.* **21A**, 1-43
2. CARROLL K. F. and NESTEL P. J. (1973) Diurnal variation in glucose tolerance and in insulin secretion in man. *Diabetes* **22**, 333-348
3. GIBSON T. and JARRETT R. J. (1972) Diurnal variation in insulin sensitivity. *Lancet* **II**, 947-948
4. HARA E. and SAITO M. (1980) Diurnal changes in plasma glucose and insulin responses to oral

- glucose load in rats. *Amer. J. Physiol.* **238**, E463-E466
5. LOUIS-SYLVESTRE J. (1978) Relationship between two stages of prandial insulin release in rats. *Amer. J. Physiol.* **235**, E103-E111
  6. LOUIS-SYLVESTRE J. (1978) Feeding and metabolic patterns in rats with truncular vagotomy or with transplanted β -cells. *Amer. J. Physiol.* **235**, E119-E125
  7. MALHERBE C., DE GASPARO M., DE HERTOOGH R. and HOET J. J. (1969) Circadian variations of blood sugar and plasma insulin levels in man. *Diabetologia* **5**, 397-404
  8. NAGAI K., MORI T. and NAKAGAWA H. (1982) Application of an immunological technique to behavioral studies: Anti-suprachiasmatic nucleus γ -globulin induced loss of the circadian rhythm. *Biomedical Res.* **3**, 294-302
  9. NAGAI K., NISHIO T., NAKAGAWA H., NAKAMURA S. and FUKUDA Y. (1978) Effect of bilateral lesions of the suprachiasmatic nuclei on the circadian rhythm of food-intake. *Brain Res.* **142**, 384-389
  10. NAGAI K., YAMAMOTO H. and NAKAGAWA H. (1982) Time-dependent hyperglycemic actions of centrally administered 2-deoxy-D-glucose, D-mannitol, and D-glucose. *Biomedical Res.* **3**, 288-293
  11. NAKAGAWA H., NAGAI K., KITA K. and NISHIO T. (1979) Control mechanism of circadian rhythm of feeding behavior and metabolism influenced by food intake. In *Biological Rhythms and Their Central Mechanism* (ed. SUDA M., HAYASHI O. and NAKAGAWA H.) Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 283-294
  12. NISHIO T., SHIOSAKA S., NAKAGAWA H., SAKUMOTO T. and SATOH K. (1979) Circadian feeding rhythm after hypothalamic knife-cut isolating suprachiasmatic nucleus. *Physiol. Behav.* **23**, 763-769
  13. STEFFENS A. B. (1969) A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiol. Behav.* **4**, 833-836
  14. YAMAMOTO H., NAGAI K. and NAKAGAWA H. (1984) Role of the suprachiasmatic nucleus in glucose homeostasis. *Biomedical Res.* **5**, (in press)