Effects of Ventromedial Hypothalamus Stimulation on Glycogenolysis in Rat Liver Using In Vivo Microdialysis

Akira Takahashi, Hirohisa Ishimaru, Yasushi Ikarashi, Eiko Kishi, and Yuji Maruyama

In vivo microdialysis was applied to study the effects of ventromedial hypothalamus (VMH) stimulation on liver glycogenolysis under anesthesia. We examined glucose output and norepinephrine (NE) outflow from the liver through analysis of glucose and NE in the liver dialyzate. Stimulation of the VMH increased glucose output and NE outflow from the liver and increased the plasma glucose level. Similar results were obtained on hepatic nerve stimulation. Bilateral adrenalectomy did not abolish the glycogenolysis induced by VMH stimulation. NE outflow increased to a much greater extent in adrenalectomized rats. These data show that VMH stimulation causes glycogenolysis and glucose output from the liver mainly via the hepatic nerves, and that microdialysis is a simple and useful method for the study of liver metabolism in vivo.

THE HYPOTHALAMUS is regarded as a critical locus for regulation and integration of peripheral intermediary metabolism, and the ventromedial hypothalamus (VMH) is believed to be one of the most effective regions in energy substrate metabolism.[1-4] Electrical stimulation of the VMH increased blood glucose,[3,4] and similar results have been obtained after stimulation of the peripheral sympathetic nerves innervating the liver.[5] The VMH–sympathetic nerve pathway can mediate efferent neuronal effects on peripheral glucose metabolism because the VMH is intimately involved in sympathetic facilitation.[6] The role of the sympathetic nervous system in VMH-induced hyperglycemia has been reported, including its partial dependence on the adrenal glands[7] and pancreas.[7,8] So far, the glycogenolytic effect of VMH stimulation has been indicated by the increase of liver glycogen phosphorylase activity, the increase of blood glucose, and the decrease of liver glycogen after a prolonged VMH stimulation for many hours.[3,4] Also, the increase of liver glyconeogenesis has been indicated in VMH stimulation[3,4] and in hyperglycemia induced by neostigmine injection into the third ventricle.[9]

None of the studies of hypothalamic stimulation directly and simultaneously analyzed glucose output and norepinephrine (NE) outflow from the liver in vivo to examine the effect of neural innervation of the liver. The isolated or in situ liver perfusion system is an important experimental model for the study of sympathetic nerve function in liver metabolism. Using the perfusion system, it has been clarified that hepatic nerve stimulation induces glycogenolysis from the analysis of glucose output into an artificial perfusion medium.[10,11] However, the perfusion system does not allow investigation of the metabolic effects induced by hypothalamic stimulation, although the system does allow investigation of the chronic effects such as changes in VMH lesions.[12]

On the basis of these facts and circumstances, we investigated the effects of VMH stimulation on liver glucose output and NE outflow using in vivo microdialysis in addition to the plasma glucose level. In this study, we analyzed glucose and NE concentrations in the liver microdialysis dialyzate. Microdialysis is a technique for sampling the extracellular space[13,14] that is being applied to many studies due to its potential for experimental analysis under physiological conditions and simplicity of application. This system allows monitoring of the whole time-concentration relation and allows direct in vivo analysis of glucose output and activity of the sympathetic nerves innervating the liver.

MATERIALS AND METHODS

Male Wistar rats weighing 260 to 320 g were used. They were kept at 24°C with a 12-hour light-dark cycle (lights on from 7 AM). The animals were fed ad libitum with laboratory food and water. One week before the experiment, excepting the hepatic nerve group, the rats were stereotaxically implanted with a bipolar electrode into the VMH under pentobarbital anesthesia as described previously.[15]

On the day of the experiment, a silicone heart catheter was inserted through the right jugular vein under pentobarbital anesthesia (55 mg/kg intraperitoneally) for blood sampling.[15] Bilateral adrenalectomy was performed in half of the VMH rat group directly after implantation of the heart catheter. Following implantation of the heart catheter, two microdialysis probes with a dialysis membrane 10 mm in length and 0.5 mm outer diameter (CMA 20; Carnegie Medicine, Stockholm, Sweden) were implanted into the left lateral lobe of the liver, and the wings of the probe were glued to the liver surface with a bonding agent. The probe was perfused with Ringer solution (in mmol/L: Na 147, K 4.0 and $CaCl_2$ 3.0) at 2 μL/min using a microinfusion pump. Perfusates from the two probes were collected together and used for analysis of glucose output and NE overflow from the liver. All experiments were made with fed rats and were started between 10 and 11 AM. After an equilibration period of 2 hours, the liver dialysate was collected every 10 minutes for 30 minutes, and then the VMH was stimulated electronically (monophasic square pulses, 50 Hz, 10 V amplitude, 0.3 millisecond duration, 20-second periods every minute for 10 minutes). In the hepatic nerve rat group, the hepatic nerve was stimulated (monophasic square pulses, 20 Hz, 20 V amplitude, 2 millisecond duration, 20-second periods every minute for 10 minutes) with a bipolar platinum wire electrode placed around the hepatic artery. Body temperature was kept stable at 37.5°C by a body temperature controller. To keep the anesthesia stable, pentobarbital (10 mg/kg) was administered intraperitoneally twice during the experiment. Measurement of in vitro recovery was performed at 37.5°C, and a Ringer solution containing glucose (2 mg/mL) and NE (40 pmol/mL) was used as the medium surrounding the dialysis probe. Recovery was determined as the ratio of the dialysate concentration to the concentration in the medium surrounding the probe. The recovery of glucose and NE at a flow rate of 2 μL/min was 42% and 51%, respectively. The recovery of glucose in the blood was similar to the results for the Ringer solution.

NE in the dialyzate (25 μL) was analyzed using high-performance

From the Department of Neuropsychopharmacology (Tsumura), Gunma University School of Medicine, Maebashi, Gunma, Japan.
Submitted August 29, 1996; accepted February 10, 1997.
Address reprint requests to Akira Takahashi, PhD, Department of Neuropsychopharmacology (Tsumura), Gunma University School of Medicine, 3-39-22 Showa-Machi, Maebashi, Gunma 371, Japan.

0026-0495/97/4608-0008$03.00/0

liquid chromatography with an electrochemical detector as reported previously.[16,17] Simultaneously, approximately 0.1-mL samples of blood were withdrawn from the heart catheter at 10-minute intervals for the purpose of measuring plasma glucose levels. After each sample was taken, 0.1 mL isotonic saline was injected through the catheter. Blood samples were transferred to microcentrifuge tubes containing trace amounts of heparin, and the plasma was separated by centrifugation. The glucose level in the dialyzate (10 μL) and plasma was determined by the glucose oxidase method. All values are presented as the mean ± SE. The data were evaluated by ANOVA with Tukey's post hoc analysis.

RESULTS

Effects of VMH Stimulation on Plasma Glucose Level and NE Outflow and Glucose Output From the Liver

After an equilibration period of 2 hours, basal glucose output and NE outflow were measured from an average of three samples collected over a period of 30 minutes. Basal levels were not changed significantly at least for the following 1 hour. Electrical stimulation of the VMH increased plasma glucose and NE and glucose concentrations in the liver dialyzate (Fig 1). The basal plasma glucose level was 1.68 ± 0.04 mg/mL. Plasma glucose increased to 0.86 mg/mL at the end of 10 minutes of intermittent stimulation, and the peak value for plasma glucose was equivalent to 1.51 times the basal level. Basal concentrations of NE and glucose in the dialyzate were 2.38 ± 0.45 pmol/25 μL perfusate and 0.307 ± 0.024 mg/mL, respectively, without correction for recovery across the probe membrane. NE outflow was increased rapidly by VMH stimulation and decreased after cessation of stimulation. The peak value for NE outflow was obtained in the dialyzate during (0 to 10 minutes) stimulation, and the peak for glucose output was obtained during the 10- to 20-min period after the beginning of stimulation. The peak value for liver dialyzate glucose reached 1.61 times the basal level. The increase in plasma glucose was in proportion to the increased rate of liver glucose output by measuring the dialyzate glucose concentration.

Effects of Adrenalectomy on VMH-Induced Glucose Output and NE Outflow

The increase of glucose output and NE outflow from the liver induced by VMH stimulation was not abolished in adrenalectomized rats (Fig 2), although the increase in plasma glucose was reduced. The increase in plasma glucose at 10 and 20 minutes was 0.51 and 0.52 mg/mL, respectively. The basal plasma glucose level was 1.44 ± 0.06 mg/mL, and basal concentrations of NE and glucose in the dialyzate were 3.41 ± 0.40 pmol/25 μL perfusate and 0.323 ± 0.016 mg/mL, respectively. The peak value for dialyzate glucose was 1.53 times the basal level. VMH stimulation–induced NE outflow in adrenalectomized rats was increased by about three times the NE outflow in intact rats. The rapid decrease in NE outflow immediately after cessation of the stimulation was not observed in this group of rats, and the peak value was obtained in the dialyzate during the 10- to 20-minute period.

Effects of Hepatic Nerve Stimulation on Glucose Output and NE Outflow From the Liver

Electrical stimulation of the hepatic nerve increased glucose output and NE outflow (Fig 3). The basal plasma glucose level was 1.81 ± 0.07 mg/mL. The increase of plasma glucose reached approximately 1.10 mg/mL at the end of a 10-minute intermittent stimulation. Basal concentrations of glucose and NE in the dialyzate were 0.386 ± 0.035 mg/mL and 2.36 ± 0.09 pmol/25 μL perfusate, respectively. NE outflow was increased rapidly by hepatic nerve stimulation and decreased rapidly after cessation of the stimulation. The peak value for NE outflow was obtained in the dialyzate during stimulation. The increase in NE outflow preceded the increase in glucose output. The peak for glucose output was obtained in the dialyzate during the 10- to 20-minute period after beginning the stimulation. Similarly in VMH stimulation, the increase of plasma glucose was in proportion to the increased rate of liver glucose output.

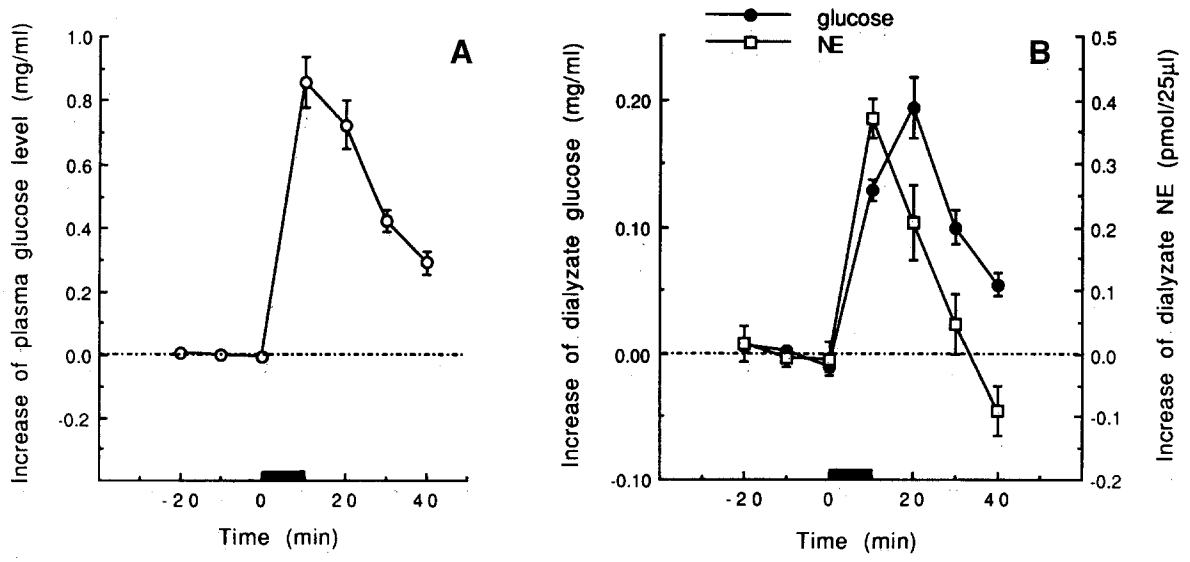

Fig 1. Effects of VMH stimulation on plasma glucose (A) and dialyzate NE and glucose concentrations (B) in intact rats. Values are the mean ± SE for 7 rats. Values for the increase of plasma glucose (A) and the increase of dialyzate glucose (B) during and after nerve stimulation (10 to 40 minutes) are significantly different from initial basal levels at $P < .05$. The increase of dialyzate NE during nerve stimulation (10 minutes) is significantly different from initial basal levels at $P < .05$.

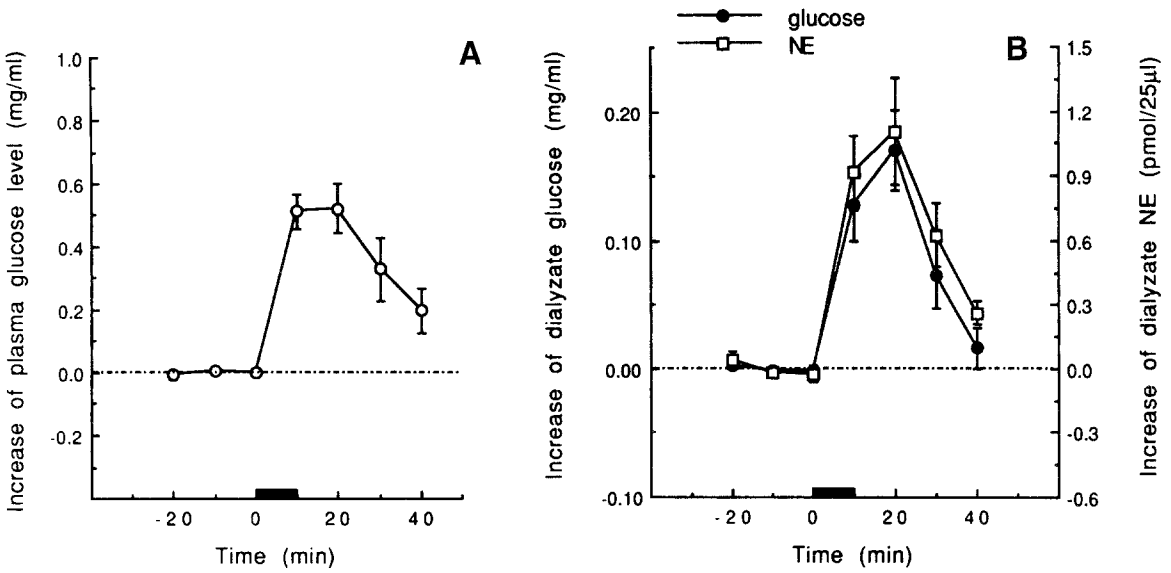

Fig 2. Effects of VMH stimulation on plasma glucose (A) and dialyzate glucose and NE concentrations (B) in adrenalectomized rats. Values are the mean ± SE for 6 rats. Values for the increase of plasma glucose at 10 to 30 minutes (A) and the increase of dialyzate glucose and NE (B) during and after nerve stimulation (10 to 20 minutes) are significantly different from initial basal levels at $P < .05$.

DISCUSSION

In this study, we confirm and extend the role of the VMH in the regulation of liver glycogenolysis through direct analysis of glucose output and NE outflow using in vivo liver microdialysis. The present approach provides new experimental data in the study of metabolic regulation in the liver. VMH stimulation rapidly increased NE and glucose concentrations in the liver dialyzate and simultaneously increased the plasma glucose level (Fig 1). Similar results were obtained with hepatic nerve stimulation (Fig 3), and adrenalectomy did not abolish the glycogenolytic effects induced by VMH stimulation (Fig 2). Our data clearly show that VMH stimulation caused glycogenolysis and glucose output mainly via activation of the sympathetic nerves innervating the liver. These nerve actions were assumed to be transmitted predominantly via α-adrenergic receptors, since an α-antagonist reduced the glycogenolytic effects induced by hepatic nerve stimulation in isolated rat liver perfused in situ.[10,18] Using this system, the role of eicosanoids, in addition to NE, in the action of the hepatic nerves has been suggested.[19,20] The hemodynamic changes are also induced by VMH or hepatic nerve stimulation. However, VMH stimulation did not produce a significant change in blood flow to the liver.[21]

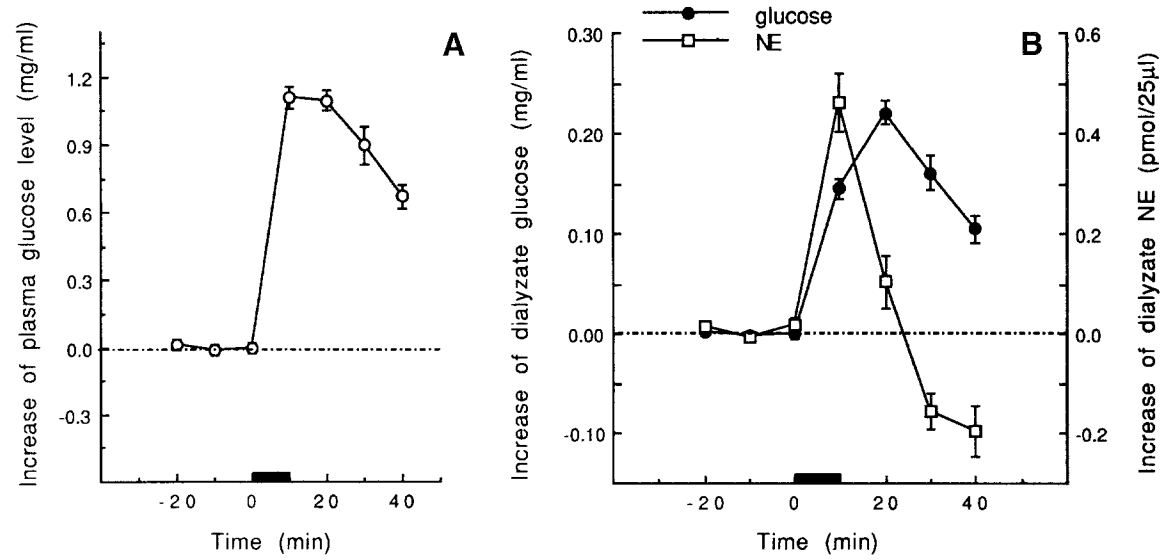

Fig 3. Effects of hepatic nerve stimulation on plasma glucose (A) and dialyzate glucose and NE concentrations (B) in intact rats. Values are the mean ± SE for 6 rats. Values for the increase of plasma and dialyzate glucose during and after nerve stimulation (10 to 40 minutes) are significantly different from initial basal levels at $P < .05$. The increase of dialyzate NE during nerve stimulation (10 minutes) is significantly different from initial basal levels at $P < .05$.

It is probable that the hemodynamic changes cannot be a primary cause of the glycogenolytic effects of hepatic nerve stimulation, because the stimulation still caused the effects in the presence of the smooth muscle relaxant.[10] Besides, it has been reported that VMH[7] or splanchnic nerve[22] stimulation increases glucagon output. In the hepatic nerve stimulation, epinephrine and pancreatic glucagon may account for the increased dialyzate glucose even after NE outflow appears to return to the basal level (Fig 3B).

This microdialysis system allows in vivo analysis of glucose output and activity of sympathetic nerves innervating the liver as outflow of NE; this cannot be easily accomplished with other methods. However, it is necessary to consider that microdialysis has a lag time with which to detect changes in concentration of the dialyzate. The time course study of dialyzate glucose after insertion of the dialysis probe into the liver shows that the changes in glucose concentration obtained in the dialyzate have a time lag of about five minutes or more (not shown). In our microdialysis data, it is assumed that an increase of NE outflow and glucose output occurred immediately after beginning the VMH or hepatic nerve stimulation. Several minutes may be required to realize the full effect of released NE on glucose outflow from the liver. Basal concentrations of glucose in the liver dialyzate, after corrections according to the recovery and hematocrit, were 75% to 90% of the basal plasma glucose levels. It seems that the rate of recovery of dialysis probe within the liver and perhaps other tissues is lower than the recovery in the blood and Ringer solution. Basal plasma glucose levels were high to some degree in this experimental condition, because our experiments were made with fed rats and the acute surgery affects the blood glucose level. The basal glucose level in the adrenalectomized rat was lower than in the intact rats. This indicates that an increase of adrenal epinephrine secretion increased the basal plasma glucose level in intact rats during and after the acute surgery.

In adrenalectomized rats, VMH stimulation–induced NE outflow was increased by about three times versus NE outflow in intact rats, and continued after cessation of the nerve stimulation (Fig 2). This increase may be one counterregulatory response to the lack of adrenal epinephrine to retain the supply of glucose to the blood. It is known that the physiological response to a variety of stresses is controlled in a redundant manner. We previously showed a compensation between sympathetic nerves and adrenal medullary activity.[23] Our system also makes it possible to analyze the relation of liver glycogenolysis and/or glyconeogenesis to blood glucose level. It becomes possible to investigate the interrelationships between the hepatic nerve and other organs such as the adrenals in the regulation of glycogenolysis using our microdialysis system. Practically, the liver is the only glucose-supplying organ; and except for the glucose absorption and supply from digested food, the increase of plasma glucose depends on glucose output originating from liver glycogenolysis, especially in fed rats.[9] The increase of plasma glucose was in proportion to the increased rate of liver glucose output in the intact rats (Figs 1 and 3). The hyperglycemia caused by VMH stimulation may include the influences of adrenal epinephrine, since the increase of plasma glucose in adrenalectomized rats was reduced to about two thirds of that in intact rats (Fig 2A). However, glucose output from the liver scarcely changed (Fig 2B). The ratio of the increase of plasma glucose to the increase of liver dialyzate glucose in the adrenalectomized rats was lower than in intact rats. Perhaps a lack of adrenal epinephrine increases insulin secretion from the pancreas, and this increase of insulin reduces the increment of glucose[24] even though liver glucose output scarcely changes. Tissue glucose uptake is another contributory factor to blood glucose level. Uptake and utilization of glucose in peripheral tissues can be estimated from glucose output from the liver and blood glucose level. Both efferent and afferent nerves may participate in the regulation of liver metabolism.[25] In liver metabolism and its regulation, our dialysis system allows investigation of the central nerves or hypothalamic function, as well as the peripheral aspects. Participation of the hypothalamic noradrenergic[3,4,26,27] and cholinergic[16,17,24] system has been indicated in the regulation of peripheral glucose metabolism.

ACKNOWLEDGMENT

The authors thank Yasutoshi Tsuda for valuable assistance.

REFERENCES

1. Shimizu N: Osaka Igakkai Zssh (in Japanese) 40:1029-1049, 1941

2. Bray GA, York DA: Hypothalamic and genetic obesity in experimental animals: An autonomic and endocrine hypothesis. Physiol Rev 59:719-809, 1979

3. Shimazu T: Neuronal control of intermediate metabolism, in Lightman SL, Everitt BJ (eds): Neuroendocrinology. Oxford, UK, Blackwell, 1986, pp 304-330

4. Shimazu T: Neuronal regulation of hepatic glucose metabolism in mammals. Diabetes Metab Rev 3:185-206, 1987

5. Edwards AV: The hyperglycaemic response to stimulation of the hepatic sympathetic innervation in adrenalectomized cats and dogs. J Physiol (Lond) 220:697-710, 1972

6. Saito M, Minikoshi Y, Shimazu T: Accelerated norepinephrine turnover in peripheral tissues after ventromedial hypothalamic stimulation in rats. Brain Res 481:298-303, 1989

7. Frohman LA, Bernardis LL: Effect of hypothalamic stimulation on plasma glucose, insulin, and glucagon levels. Am J Physiol 221:1596-1603, 1971

8. Frohman LA, Bernadis LL, Stachura ME: Factors modifying plasma insulin and glucose responses to ventromedial hypothalamic stimulation. Metabolism 23:1047-1056, 1974

9. Iguchi A, Kunoh Y, Miura H, et al: Central nervous system control of glycogenolysis and gluconeogenesis in fed and fasted rat liver. Metabolism 38:1216-1221, 1989

10. Hartmann H, Beckh K, Jungermann K: Direct control of glycogen metabolism in the perfused rat liver by the sympathetic innervation. Eur J Biochem 123:521-526, 1982

11. Jungermann K, Gardemann A, Beuers U, et al: Regulation of liver metabolism by the hepatic nerves. Adv Enzyme Regul 26:63-88, 1987

12. Karakash C, Hustvedt BE, Løvø A, et al: Consequences of ventromedial hypothalamic lesions on metabolism of perfused rat liver. Am J Physiol 232:E286-E293, 1977

13. Benveniste H: Brain microdialysis. J Neurochem 52:1667-1679, 1989

14. Ungerstedt U: Measurement of neurotransmitter release by

intracranial dialysis, in Marsden CA (ed): Measurement of Neurotransmitter Release In Vivo. New York, NY, Wiley, 1984, pp 81-105

15. Takahashi A, Shimazu T: Hypothalamic regulation of lipid metabolism in the rat: Effect of hypothalamic stimulation on lipolysis. J Auton Nerv Syst 4:195-205, 1981

16. Takahashi A, Ishimaru H, Ikarashi Y, et al: Intraventricular injection of neostigmine increases dopaminergic and noradrenergic nerve activities: Hyperglycemic effects and neurotransmitters in the hypothalamus. Neurosci Lett 156:54-56, 1993

17. Takahashi A, Ishimaru H, Ikarashi Y, et al: Hypothalamic cholinergic and noradrenergic neurons in hyperglycemia induced by 2-deoxyglucose. Brain Res 665:13-17, 1994

18. Gardemann A, Strulik H, Jungermann K: Nervous control of glycogenolysis and blood flow in arterially and portally perfused liver. Am J Physiol 253:E238-E245, 1987

19. Iwai M, Jungermann K: Possible involvement of eicosanoids in the actions of sympathetic hepatic nerves on carbohydrate metabolism and hemodynamics in perfused rat liver. FEBS Lett 221:155-160, 1987

20. Iwai M, Gardemann A, Puschel G, et al: Potential role for prostaglandin F2a, D2, E2 and thromboxane A2 in mediating the metabolic and hemodynamic actions of sympathetic nerves in perfused rat liver. Eur J Biochem 175:45-50, 1988

21. Iwai M, Shimazu T: Effects of ventromedial and lateral hypothalamic stimulation on chemically-induced liver injury in rats. Life Sci 42:1833-1840, 1988

22. Kaneto A, Kajinuma H, Kosaka K: Effect of splanchnic nerve stimulation on glucagon and insulin output in the dog. Endocrinology 96:143-150, 1975

23. Takahashi A, Ikarashi Y, Ishimaru H, et al: Compensation between sympathetic nerves and adrenal medullary activity: Effects of adrenodemedullation and chemical sympathectomy on catecholamine turnover. Life Sci 53:1567-1572, 1993

24. Iguchi A, Goto M, Matsunaga H, et al: Mechanism of central hyperglycemic effect of cholinergic agonists in fasted rats. Am J Physiol 251:E431-E437, 1986

25. Lautt WW: Afferent and efferent neural roles in liver function. Prog Neurobiol 21:323-348, 1983

26. Matushita H, Shimazu T: Chemical coding of the hypothalamic neurons in metabolic control. II. Norepinephrine-sensitive neurons and glycogen breakdown in liver. Brain Res 183:79-87, 1980

27. Smythe GA, Edwards SR: Suppression of central noradrenergic neuronal activity inhibits hyperglycemia. Am J Physiol 263:E823-E827, 1992