Glycogen Synthetase Activity in Liver: Regulation by the Autonomic Nerves

Abstract. *Electrical stimulation of the vagus nerve in intact or pancreatectomized rabbits resulted in a marked increase, within 5 minutes of the onset of stimulation, in the total activity of liver glycogen synthetase. The effect was completely counteracted by simultaneous stimulation of the splanchnic nerve, although stimulation of the splanchnic nerve alone had little effect on the enzyme. Injection of insulin caused an increase in both the total activity of glycogen synthetase and the activity that was independent of glucose-6-phosphate. The response of the enzyme to vagal stimulation was much faster than to administration of insulin.*

Liver glycogen is kept in metabolic equilibrium by catabolism for provision of energy or maintenance of proper amounts of glucose in the blood, by synthesis from dietary sources, and partly through gluconeogenesis from noncarbohydrate materials.

Dietary, hormonal, and nervous influences are thought to share in maintenance of the metabolic equilibrium of liver glycogen. Stimulation of the sympathetic nerves of rabbits causes rapid increase in the activities of glycogen phosphorylase and glucose-6-phosphatase in liver, followed by increase in the concentration of glucose in the blood and pronounced decrease in the content of glycogen in the liver; while stimulation of the parasympathetic nerves has, if any, a suppressive effect on these liver enzymes and causes decrease in blood glucose (*1, 2*).

I have studied a role of the autonomic nerves in the regulation of glycogen synthetase (uridine diphosphate glucose–glycogen glucosyltransferase) activity in rabbit liver. Of the enzymes that catalyze the steps from glucose-6-phosphate to glycogen, glycogen synthetase is known to be rate-limiting and so is likely to be important in control of the rate of glycogen synthesis. There are two types of activity of this enzyme in various mammalian sources (*3*): dependent on (G6P-d) and independent of (G6P-i) glucose-6-phosphate as a cofactor in the activity.

Male rabbits (about 2.5 kg) were lightly anesthetized with intravenous injections of pentobarbital sodium (10 mg/kg). Laparotomy was made under additional anesthesia with ether. The left vagus nerve or the left splanchnic nerve was exposed just under the diaphragm, and a bipolar platinum electrode was placed at the nerve. Fifteen to 20 minutes after placement of the electrode, one lobe of the liver was quickly removed by ligation and immediately immersed in liquid nitrogen. Stimuli delivered from an electronic stimulator were applied to the vagus nerve (frequency, 100 per second; duration, 0.3 msec; amplitude, 5 or 20 volts, with essentially similar results) or to the splanchnic nerve (frequency, 100 per second; duration, 0.3 msec; amplitude, 40 volts) through an isolation unit. After the indicated period of stimulation, the other lobe of the same liver was quickly removed and similarly frozen in liquid nitrogen while the stimulus was still being delivered.

For enzyme assay, a portion of each frozen liver was promptly pulverized and ground at −20°C with 1 volume of 40-percent glycerol solution containing 0.25*M* sucrose, 0.06*M* KF, and 0.001*M* EDTA (ethylenediaminetetraacetic acid) adjusted with KOH to *p*H 7.5 and already chilled close to freezing. After the material was ground, 3 volumes of cooled aqueous solution of the same salts were added. The suspension was centrifuged for 10 minutes at 600*g* and 0°C, and the supernatant was immediately analyzed for glycogen synthetase activity with (total activity) and without (G6P-i activity) added glucose-6-phosphate (see Table 1 legend).

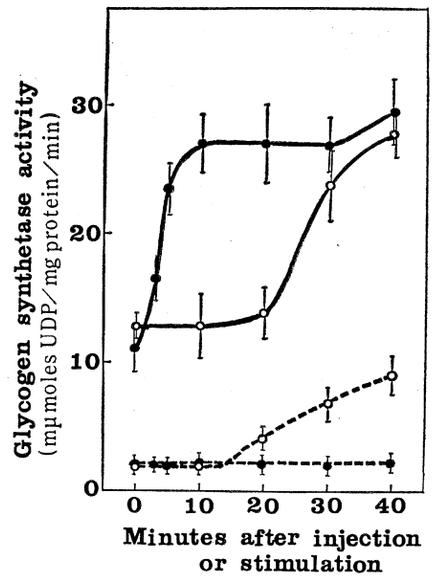

Fig. 1. The early time course of the increase in activity of liver glycogen synthetase after stimulation of the vagus nerve or administration of insulin. Solid lines: G6P-dependent activity of liver glycogen synthetase (total activity assayed with glucose-6-phosphate, minus G6P-independent activity). Dashed lines: G6P-independent activity assayed without glucose-6-phosphate. Solid circles: electrical stimuli were applied to the vagus nerves of pancreatectomized rabbits; pancreatectomy was carried out by laparotomy 15 to 20 minutes before the stimulation. Open circles: bovine insulin (2 units per kilogram), free of glucagon, was injected intravenously into intact rabbits. Each circle represents the average of four or more determinations; each vertical bar indicates the standard error of the mean. UDP, uridine diphosphate.

Table 1. Effect on the activity of glycogen synthetase in the liver of electrical stimulation of the vagus nerve, the splanchnic nerve, or both nerves simultaneously in intact and in pancreatectomized rabbits; activity was measured by the method of Leloir and Goldemberg (*6*), slightly modified. The reaction mixture contained (μmoles): 1.5 of uridine diphosphate–glucose, 3 of glucose-6-phosphate (when added), 15 of tris(hydroxymethyl)aminomethane (*p*H 8.0), and 0.75 of EDTA; plus 3 mg of glycogen and centrifuged liver extract in a total volume of 0.3 ml. After 10-minute incubation at 37°C, the reaction was stopped by heating, and uridine diphosphate was estimated by coupling with pyruvate kinase. Results are given as millimicromoles of uridine diphosphate per milligram of protein per minute. +G6P, activity assayed with glucose-6-phosphate added (total activity); −G6P, activity assayed without glucose-6-phosphate (G6P-i activity); int, rabbit intact; panc, rabbit pancreatectomized.

After stimulation for (min)	Nerve stimulated							
	Vagus (int)		Vagus (panc)		Splanchnic (int)		Vagus and splanchnic (int)	
	+G6P	−G6P	+G6P	−G6P	+G6P	−G6P	+G6P	−G6P
0	13.3 ± 2.5	2.3 ± 0.8	12.8 ± 1.8	2.0 ± 0.8	13.3 ± 0.5	1.8 ± 0.7	12.5 ± 1.2	2.0 ± 0.8
5	18.3 ± 3.7	2.5 ± 0.8	25.3 ± 4.2	1.8 ± 0.5	12.0 ± 1.2	1.8 ± 0.5	12.8 ± 1.2	1.8 ± 0.5
10	24.8 ± 4.5	2.5 ± 1.0	29.3 ± 2.3	2.2 ± 0.8	12.2 ± 1.0	2.0 ± 0.7	13.0 ± 2.0	2.0 ± 0.3
20	23.8 ±2.0	2.3 ± 0.8	29.0 ± 5.7	2.0 ± 0.8	11.0 ± 1.2	1.5 ± 0.8	13.5 ± 3.5	1.3 ± 0.5

The total activity of liver glycogen synthetase, assayed in the presence of glucose-6-phosphate, was greatly increased by electrical stimulation of the vagus nerve (Table 1), almost attaining maximum within 5 minutes of the onset of stimulation ($P<.01$). The G6P-i activity of liver glycogen synthetase, assayed without glucose-6-phosphate, changed insignificantly before and after stimulation of the vagus nerve ($P>.3$). Therefore the increase in total activity after vagal stimulation can be attributed to the increase in G6P-d activity of the enzyme, although the exact biochemical meanings of the two types of activity of glycogen synthetase in liver are still unclear.

Steiner *et al.* (*4*) reported that injection of insulin in rats, made diabetic with alloxan, markedly increased both G6P-d and G6P-i activities of liver glycogen synthetase. The result was confirmed in normal rabbits (Fig. 1): when anesthetized rabbits were injected intravenously with insulin (2 units per kilogram) free of glucagon, both types of activity of glycogen synthetase in the liver increased gradually during 20 to 40 minutes after the injection ($P<.01$).

The effect of vagal stimulation on glycogen synthetase activity of the liver is unlikely to be a secondary effect of insulin secreted from the pancreas, since pancreatectomized and intact rabbits gave essentially the same results (Table 1 and Fig. 1).

The response of glycogen synthetase to vagal stimulation is much faster than that to administration of insulin (Fig. 1): the maximum response was delayed only about 5 minutes with the former, versus about 40 minutes with the latter. My observations with liver phosphorylase have been similar: the response of liver phosphorylase to stimulation of the splanchnic nerve is faster than to injection of epinephrine (*1*, *5*). Again, vagal stimulation affected only the G6P-d activity of liver glycogen synthetase, whereas insulin influenced both its G6P-d and its G6P-i activity.

Neither G6P-d nor G6P-i activity of liver glycogen synthetase was affected significantly by electrical stimulation of the splanchnic nerve of rabbits ($P<.3$). Nevertheless, the effect of vagal stimulation on glycogen synthetase was counteracted almost completely by simultaneous stimulation of the splanchnic nerve (Table 1). A similar situation has already been observed with phosphorylase activation, the effect of splanchnic-nerve stimulation on liver phosphorylase being counteracted by simultaneous stimulation of the vagus nerve (*5*).

These results suggest that parasympathetic stimulation results in acceleration of glycogenesis in liver; this acceleration may affect the reported decrease in blood glucose after parasympathetic stimulation (*2*). It is noteworthy that the sympathetic and parasympathetic nerves regulate the activities of separate enzymes implicated in glycogen metabolism in the liver. Stimulation of the sympathetic nerve causes glycogenolysis in liver by stimulating the activation of phosphorylase and glucose-6-phosphatase (*1*), while stimulation of the parasympathetic nerve seems to cause glycogenesis in liver by increasing the activity of glycogen synthetase. These effects of the autonomic nerves on phosphorylase or glycogen synthetase are counteracted by simultaneous action of the antagonistic nerves. It seems that there is a dual regulatory system—hormonal and nervous—in glycogen metabolism in the liver, and that the nervous regulation is much faster than the hormonal.

TAKASHI SHIMAZU
Department of Anatomy, Osaka University Medical School, Osaka, Japan

References

1. T. Shimazu and A. Fukuda, *Science* **150**, 1607 (1965).
2. ———, T. Ban, *Nature* **210**, 1178 (1966).
3. M. Rosell-Perez, C. Villar-Palasi, J. Larner, *Biochemistry* **1**, 763 (1962); M. Rosell-Perez and J. Larner, *ibid.* **3**, 75 (1964); R. R. Traut and F. Lipmann, *J. Biol. Chem.* **238**, 1213 (1963); S. Hizukuri and J. Larner, *Biochemistry* **3**, 1783 (1964).
4. D. F. Steiner and J. King, *J. Biol. Chem.* **239**, 1292 (1964).
5. T. Shimazu and A. Fukuda, unpublished.
6. L. F. Leloir and S. H. Goldemberg, *J. Biol. Chem.* **235**, 919 (1960).

15 February 1967

Regulation of Intracellular Sodium Concentrations in Rat Diaphragm Muscle

Abstract. *The concept is suggested that the sodium pump mechanism is influenced by the transmembrane electrical potential, and that the pump acts to maintain a constant electrochemical gradient for sodium. Evidence leading to this suggestion was obtained in rat diaphragm muscle by altering systematically the transmembrane chemical gradient for sodium ions and the transmembrane voltage. The voltage changes were produced by varying the extracellular and intracellular potassium ion concentrations. In each case the intracellular sodium concentration changed, presumably by activity of the sodium pump, so that the total electrochemical gradient for sodium was restored.*

At the resting potential of the muscle cell the ratio of internal and external potassium concentrations is close to that predicted by the Nernst equation for a potassium electrode (*1*). Sodium, however, is far from its electrochemical equilibrium potential of about +40 mv. There is a large concentration gradient of sodium from the outside toward the inside, as well as a negative electrical potential in the cell attracting the positively charged sodium ions. The electrochemical potential of the cell is therefore 110 to 120 mv away from the sodium equilibrium, and a driving force of this magnitude is available to promote entry of sodium into the cell. The passive influx-efflux ratio can be calculated from this gradient (*2*) and can be shown to be greatly in favor of influx. Yet in the steady state, where internal sodium concentration is constant, the influx and efflux are necessarily equal. The sodium efflux that is in excess of the predicted passive efflux has been called active transport (*3*) and attributed to a "sodium pump." This part of the sodium efflux has been shown to be dependent on metabolic energy (*4*).

Since the action of the pump is to extrude sodium from the cell, it would be expected that the internal sodium concentration itself might be a factor in the regulation of pump activity. This relationship has been shown by several workers, and there also appears to be some dependence on external sodium concentration (*5*). In almost all studies of the ionic regulation of sodium efflux the assumption has been made that the transmembrane electrical potential gradient does not influence sodium "pumping." However, Conway and his co-workers (*6*, *7*) have suggested that the regulating factor is the total chemical and electrical gradient. According to their suggestion the cell maintains a constant electrochemical gradient for sodium (the "critical energy