MECHANISM OF SEROTONIN-INDUCED MUSCLE GLYCOGENESIS IN ADRENODEMEDULLATED RATS

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The effect of serotonin on glycogen content of liver, diaphragm and heart of adrenodemedullated rat has been demonstrated (Kobayashi *et al.*, 1960a; Leonard and Day, 1960). Intravenous injection of serotonin caused a significant increase of cardiac glycogen, as well as a depletion of hepatic and diaphragmatic glycogen 10 mins. after the injection. The mechanism of the serotonin action is still unclear. It was postulated that the reduction of hepatic blood flow and the resultant inhibition of glycogenesis might play some role in the depletion of hepatic glycogen, and that the increased blood lactate could participate in the effect of serotonin on cardiac glycogen.

This paper reports the experiments performed further to examine these and other possible mechanisms of the serotonin-induced changes in tissue glycogen levels by means of ^{14}C -labelled glucose.

MATERIALS AND METHODS

Male adult rats of Wistar strain weighing between 160 and 180 g were used. A litter mate control of the experiment was adopted in order to minimize individual variations. Bilateral adrenodemedullation was carried out by the usual procedure a week before use. They were fasted for 18 hrs. and fed by a stomach tube with 500 mg of glucose per 100 g body weight in 50 % solution 4 hrs. prior to the injection of serotonin. Serotonin (as a complex with creatinine sulfate) was injected through femoral vein at the dosage of 500 μg per rat in 0.25 % aqueous solution. The control rats were treated exactly similar to the test rats except receiving equal volume of redistilled water instead of the serotonin solution. Pairs of the rats were given 2 to 5 μC of uniformly ^{14}C -labelled glucose or carbon-1- ^{14}C -labelled glucose by an intraperitoneal or an intravenous injection through a femoral vein.

The rats were sacrificed by decapitation 7, 10 or 30 mins. after the administration of serotonin, and blood spouted out was collected and heparinized for analysis. The excised tissues were frozen immediately with solid carbon dioxide. The glycogen content of tissues was determined as described previously (Kobayashi *et al.*, 1960a).

For the estimation of the ^{14}C -activity, glycogen precipitated from tissue hydrolyzate with 1.5 vols. of ethanol was purified by repeating extraction with 10 % trichloroacetic acid solution and precipitation with 3 vols. of ethanol (Nadkarni *et al.*, 1960). Aliquots of aqueous glycogen solutions were plated directly on brass disks, the water evaporated under infrared lamp, and the samples were assayed for total ^{14}C -activity in a low background gas flow counter (Tracer Lab., Model CE-14). The specific activities of glycogen were presented as counts per min. per mg of glycogen.

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To examine the isotopic carbon pattern of the glucose molecule, the purified glycogen was hydrolyzed with 1N-HCl at 100°C for 30 mins., and then carrier glucose (400 mg) was added. The isolation of glucose carbons by chemical degradation was carried out according to Boothroyd (Boothroyd *et al.*, 1955), and each carbon was recovered and counted as BaCO₃.

Aliquots of blood samples were deproteinized, and the filtrates were added with carrier glucose and used for radioactivity assay. For the determination of the radioactivity of blood glucose, the glucose in the filtrate was precipitated with phenylhydrazine hydrochloride and counted as glucose phenylosazone. The radioactivity measurements were corrected for self-absorption and for carrier glucose.

Blood glucose and lactate was determined according to Somogyi-Nelson (Nelson, 1944; Somogyi, 1945) and Barker-Summerson (Barker and Summerson, 1945), respectively.

The ¹⁴C-labelled glucose (D-glucose-u-¹⁴C, Lot. No. 6259, Specific activity: 7.1 mc/m mole; more D-glucose-1-¹⁴C, Lot. No. 6202, Specific activity: 3.87 mc/m mole) was obtained from the Daiichi Kagaku Yakuhin Co., Tokyo. Serotonin creatinine sulfate was the product of Nutritional Biochemicals Corporation, U.S.A.

RESULTS AND DISCUSSION

Table 1 shows the effect of intravenously administered serotonin (500 µg/rat) on tissue glycogen content and specific activity in adrenodemedullated rats. These rats were injected intraperitoneally with 2 µc of glucose-u-¹⁴C 60 mins. prior to the serotonin treatment. They were sacrificed 10 mins. after the administration of serotonin. Both the glycogen specific activity as well as the glycogen content were significantly increased in heart, and lowered in liver of the serotonin-treated rats. No significant effect of serotonin on peripheral muscle glycogen was observed, even though the glycogen content of serotonin group was somewhat lower than the controls. It is likely that the present experimental condition was unfavourable for the demonstration of the serotonin effect as the change of specific activity in peripheral tissues, possibly because an isotopic steady state was partly established prior to the onset of the serotonin action. However the increased incorporation of ¹⁴C into cardiac glycogen suggests an enhanced glycogenesis in

Table 1. Effect of serotonin on tissue glycogen content and specific activity of intraperitoneally glucose-u-¹⁴C injected rats

	Heart		Diaphragm		Gastrocnemius		Liver	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Cont. ¹⁾	2.0±0.28	2.6±0.33**	5.7±0.66	4.2±0.67	4.3±0.44	3.2±0.58	12.6±2.58	6.1±1.24**
Sp. ac. ²⁾	17±3.6	56±1.26**	43±15.2	38±45.5	35±7.0	25±6.0	40±9.9	6±2.8**
	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)

1) Glycogen mg per g tissue

2) Counts per min. per mg glycogen

** Level of significance for the difference between the control value ($\alpha < 0.01$)

Figures in parentheses are the number of animals.

Serotonin dosage: 500 µg per rat intravenously

heart from endogenous sources rather than a depression of glycogenolysis by serotonin. On the contrary, the reduced incorporation into hepatic glycogen may ensue from a decreased glycogenesis, since it was shown separately that specific activity of hepatic glycogen in the control rat was still ascending at this phase under comparable conditions.

In an attempt to examine more closely the serotonin action on peripheral tissue glycogen, 2 μ c of glucose-u- 14 C was intravenously administered just in advance of the serotonin injection. The animals were sacrificed 7 (Experiment 1), 10 (Experiment 2) or 30 (Experiment 3) mins. after the injection (Table 2).

The distinct increase in the specific activity of cardiac glycogen was emphasized in Experiment 2, in which the time interval of glucose administration and sacrifice was reduced to one seventh that of the above described experiment. Table 2 further shows that shortness of the time interval favoured elucidation of serotonin effect on the diaphragmatic glycogen. The specific activities but not the contents of the diaphragmatic glycogen in the serotonin-treated rats were significantly higher than those of the controls. The serotonin effect on gastrocnemius muscle was also evident, though only in Experiment 3. This finding is compatible with the relatively low rate of turnover in skeletal muscle glycogen. On the other hand, the specific activity of hepatic glycogen failed to attain a significant value under the present experimental condition. Thus the detection of serotonin effect in this tissue was practicable only in the glycogen content.

Simultaneous determinations in the present experiment revealed that glucose level was elevated by serotonin in the 'central' blood samples collected from carotids at the time of decapitation (Table 3). This finding will be contrasted with the previous observation that intravenously administered serotonin into adrenodemodulated rats caused an instant hypoglycemia, in which the analyses were carried out on 'peripheral' blood samples obtained from tail veins (Kobayashi *et al.*, 1960b). In view of the present finding that the hepatic glycogen level was significantly depleted by serotonin, it will be reasonable to suppose that the elevated glucose level of the central blood was resulted from an increased glycogenolysis in liver. The observed failure of the blood glucose specific activity to reflect an increased dilution of labelled glucose in the serotonin-treated rats (Control; 7710 ± 346 , Treated; 7220 ± 480 cpm/mg glucose, 30 mins. after the administration) may best be accounted for by a counteracting effect of the decreased hepatic glycogenesis, as evidenced by the low specific activity of hepatic glycogen in Table 1.

It is an attractive hypothesis that the elevated 'central' blood glucose level in serotonin treated rats is the cause of the increased glucose incorporation into the cardiac as well as the diaphragmatic glycogen, because the rate of glucose transport in these tissues has been shown to be dependent of the external glucose concentration (Gemmell, 1941; Morgan *et al.*, 1961). However, a quantitative consideration on the available data suggests that the increment of 30 % in glycemic level of the present serotonin-treated rats can hardly explain sole of the group differences in the total activities of cardiac or diaphragmatic glycogen. It has been shown *in vitro* that doubling of external glucose concentration from 100 to 200 mg % failed to increase *in vitro* glucose uptake of cardiac and diaphragmatic tissue by

Table 2. Effect of serotonin on tissue glycogen content and specific activity of intravenously glucose- $u\text{-}^{14}\text{C}$ injected rats

Exp. no.	Time of sac. (mins.)	Heart		Diaphragm		Gastrocnemius		Liver	
		Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	7	Cont. ¹⁾ 3.5±0.87 16±9.1 (6)	3.3±0.53 30±7.4 (7)	5.0±0.55 11±3.5 (6)	5.1±0.97 23±5.8** (7)	4.4±0.65 7±3.7 (6)	5.0±0.59 4±1.7 (7)	15.9±3.53 4±1.1 (6)	16.1±4.03 3±1.1 (7)
2	10	Cont. 4.1±0.39 12±3.5 (4)	5.1±0.37 86±15.5** (4)	8.5±0.57 16±9.7 (4)	7.2±0.59 74±16.5** (4)	5.5±0.17 8±2.8 (4)	4.3±0.50 6±1.2 (4)	16.6±2.08 2±0.5 (4)	9.1±1.32* 2±0.4 (4)
3	30	Cont. 1.8±0.51 26±2.4 (3)	4.1±0.70 286±90.0**† (3)	4.7±1.05 4±2.0 (3)	4.9±1.33 145±24.9** (3)	5.9±1.26 25±11.3 (3)	5.4±0.71 83±7.3* (3)	10.6±1.57 2±0.4 (3)	3.1±0.87* 5±2.0 (3)

1) Glycogen mg per g tissue

2) Counts per min. per mg glycogen

*, ** Level of significance for the difference between the control value ($\alpha < 0.05$, 0.01)

† Heteroschedasticity

Figures in parentheses are the number of animals.

Table 3. Effect of serotonin on blood glucose and lactate level

Time of sac. (mins.)	Glucose (mg %)		Lactate (mg %)	
	Control	Treated	Control	Treated
7	111 ± 9.7 (6)	146 ± 13.1** (7)	59 ± 10.9 (6)	100 ± 13.3* (7)
10	97 ± 5.3 (4)	128 ± 9.8* (4)	51 ± 3.7 (4)	92 ± 4.6** (4)
30	92 ± 8.0 (5)	115 ± 7.9* (5)	25 ± 3.9 (5)	70 ± 2.5** (5)

*, ** Level of significance for the difference between the control value ($\alpha < 0.05, 0.01$)

Figures in parentheses are the number of animals.

more than 50 % of the initial value. There is no reason to believe that a similar situation does not exist *in vivo*. This view will be supported further by the fact that the increment of glucose uptake, accomplished by raising the glucose concentration, is poorly reflected in glycogen level of tissues (Larner *et al.*, 1959; Normn *et al.*, 1959).

In Table 3, it is also shown that lactate level of the serotonin-treated central blood was significantly higher than that of the controls. This finding is in accord with that obtained in the peripheral blood samples (Ui *et al.*, 1960). It was postulated previously that the increment of cardiac glycogen by serotonin might be caused by the hyperlactacidemia, since heart muscle was shown to remove lactate from blood and to utilize it as a source of glycogen (Kobayashi *et al.*, 1960a). Leonard and Day, on the other hand, in their study of serotonin effect on tissue glycogen contents, have speculated that increased mobilization and utilization of fatty acids might be the cause of the increment of cardiac glycogen level (Leonard and Day, 1960). These considerations urged us to study the metabolic route through which the labelled glucose carbon is incorporated into cardiac glycogen. This aim was accomplished by the use of the differentially ^{14}C -labelled glucose in place of the uniformly labelled one. The result in Table 4 shows the distribution of radioactivity in the glucose carbon of tissue glycogen, secured from rats after the intravenous injection of serotonin plus $5\mu\text{c}$ of glucose-1- ^{14}C . It will be seen that virtually all of radioactivity in the cardiac glycogen was recovered from position-1 of the glucose isolated, both in the control and in the serotonin treated rats. Under the condition of the present experiment, no significant radioactivity was found in carbons other than that in the position-1. A similar conclusion was reached in the diaphragmatic glycogen (Table 4). These findings are taken to show that direct glycogen formation from glucose was stimulated, and that contribution of the different route such as via lactate or fatty acids was minimum. Glycogenesis through the latter mechanism will inevitably lead to redistributions of radioactivity, especially a symmetrical distribution in C-1 and C-6.

The results detailed above show that incorporation of glucose into muscle glycogen is stimulated by serotonin, and that the stimulation is generic to muscle

tissues rather than specific to heart. The direct incorporation of glucose into glycogen, without passing through metabolic transformations, is the most likely site of serotonin action on muscle glycogen.

SUMMARY

When serotonin was intravenously injected into adreno-demedullated, glucose- $u\text{-}^{14}\text{C}$ administered rats, glycogen specific activity as well as content was increased in heart, and lowered in liver, 10 mins. after the injection. By shortening of the time interval of glucose- $u\text{-}^{14}\text{C}$ administration and serotonin injection, increased glycogen specific activity was observed not only in heart but in diaphragm, and the serotonin effect was also detected in gastrocnemius muscle 30 mins. after the injection. 'Central' blood glucose level was elevated in the serotonin treated rats, but the increment was not large enough to explain the increased incorporation of glucose- ^{14}C into muscle glycogen by serotonin. When glucose- $1\text{-}^{14}\text{C}$ was given to the control and the serotonin treated rats, all of radioactivity in cardiac and diaphragmatic glycogen was distributed in position-1 of the glucose. These data indicate that serotonin stimulates the incorporation of glucose into muscle glycogen rather than the resynthesis of glycogen from smaller fragments.

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REFERENCES

- Barker, S.B. and W.H. Summerson (1941). *J. Biol. Chem.* **138**, 535.
Boothroyd, B., S.A. Brown, J.A. Thorn and A.C. Neish (1955). *Can. J. Biochem. and Physiol.* **33**, 62.
Gemmill, C.L. and L. Hamman (1941). *Bull. Johns Hopkins Hosp.* **68**, 50.
Kobayashi, B., M. Ui and Y. Warashina (1960a). *Endocrinol. Japon.* **7**, 239.
Kobayashi, B., M. Ui and Y. Warashina (1960b). *Ibid.* **7**, 225.
Leonard, S.L. and T.H. Day (1960). *Proc. Soc. Exptl. Biol. Med.* **104**, 338.
Larner, J., C. Villar-Palasi and D.J. Richman (1959). *Ann. N.Y. Acad. Sci.* **82**, 345.
Morgan, H.E., M.J. Henderson, D.M. Regen and C.R. Park (1961). *J. Biol. Chem.* **236**, 253.
Nadkarni, G.B., B. Friedmann and S. Weinhouse (1960). *Ibid.* **235**, 420.
Nelson, N. (1944). *Ibid.* **153**, 375.
Norman, D., P. Menozzi, D. Reid, G. Lester and O. Hechter (1959). *J. Gen. Physiol.* **42**, 1277.
Somogyi, M. (1945). *J. Biol. Chem.* **160**, 61.
Ui, M., Y. Warashina and B. Kobayashi (1960). *Endocrinol. Japon.* **7**, 261.