THE ROLE OF SEROTONIN IN CARBOHYDRATE
METABOLISM IV.
MECHANISM OF SEROTONIN HYPERLACTACIDEMIA

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In the preceding publication (Ui *et al.*, 1960), it was found that administration of serotonin resulted in a marked increase in blood lactate of rats. An increase in blood lactate may be explained in terms of either increment of lactate production by extrahepatic tissues or depressed uptake of lactate by hepatic tissue, since rate of glycolysis in blood was demonstrated not to be modified. Though enhanced release of endogenous epinephrine by serotonin was ascertained, the possibility was excluded that the increase in blood lactate is solely due to the action of epinephrine released from adrenal medulla.

In the experiment described in this paper, some other endocrine substances involved in the regulation of carbohydrate metabolism were examined for their participation in serotonin-induced hyperlactacidemia. Eviscerated rat was treated with serotonin to examine the role of extrahepatic tissue. Liver perfusion was carried out as well to find any direct effect of serotonin on the uptake of lactate by hepatic tissue.

METHODS

The strain, breeding and maintenance of rats, technique of adrenodemedullation and determination of lactate were the same as reported elsewhere (Ui *et al.*, 1960). Serotonin creatinine sulfate was a commercial preparation (Nutritional Biochemical Co-op.) and dosage was expressed in terms of weight as creatinine sulfate per rat. Liver perfusion was carried out as described elsewhere (Warashina *et al.*, 1960). Functional evisceration was performed on adrenodemedullated rats by one-stage technique. Operation was completed in 5 mins. and glucose was constantly infused through femoral vein at the rate of 50 mg per hr. per rat. Adrenodemedullated rats were rendered diabetic by intravenous injection of alloxan monohydrate in a dose of 60 mg per kg body weight. Five ml of saline was simultaneously injected through intraperitoneal route. Blood sugar was determined in 0.02 ml blood specimen according to Somogyi-Nelson (Somogyi, 1945; Nelson, 1944). Bilateral adrenalectomy was carried out through lumbodorsal incision under pentobarbital anesthesia. Adrenalectomized rats were maintained on cortisone acetate (generously supplied by Teikoku Hormone Mfg. Co.) 1 mg per day and 1% NaCl as a drinking water for 2 days before experiment.

RESULTS

Effect of norepinephrine, insulin, glucagon and cortisone acetate on blood

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Fig. 1. Blood lactate level of adrenomedullated rat following injection of norepinephrine. Vertical lines indicate standard error of the mean, figures in parentheses are number of animals throughout the following figures.

Fig. 2. Blood lactate level of adrenomedullated rat following injection of insulin, glucagon and cortisone acetate
insulin, 2 I.U. per rat, intravenous; glucagon, 100 µg per rat, intravenous;
cortisone acetate, 2 mg per rat, subcutaneous

lactate of adrenomedullated rats was examined (Figs. 1 and 2). Subcutaneous injection of 200 µg norepinephrine into rats caused anoxemia and vasoconstriction in extremities but exerted little effect on blood lactate. Administration of 250 µg norepinephrine via intraperitoneal route also resulted in no alteration of blood lactate. Insulin (Glucagon-free insulin of 20 I.U. per mg was kindly given by Dr. O.K. Behrens and Dr. W.R. Kirtley, Lilly Research Laboratories), when large doses were intravenously injected, caused a rapid but transient increase which is consistent with the result cited by Tyberghein and Williams (1957). As is already known, adrenomedullated rats were highly sensitive to insulin and 2 received

Fig. 3. Blood lactate level of adrenal-ectomized rat following subcutaneous injection of serotonin

Fig. 4. Blood lactate level of alloxan-diabetic rat following intravenous injection of serotonin

— serotonin 100 μ g per rat

..... serotonin 50 μ g per rat

Figures on plotted lines indicate blood sugar level (mg %)

injection of glucose as indicated by arrows in Figure 2. Intravenous glucagon ($100\text{ }\mu\text{g}$ per rat) and subcutaneous cortisone acetate (2 mg per rat) had no effect on blood lactate level of adrenomedullated rats. For the purpose of examining the role of adrenal cortex, 1 mg of serotonin was subcutaneously injected into adrenalectomized rats. There observed a hyperlactacidemia as marked as seen in adrenomedullated rats (Fig. 3). The data appear to provide the evidence that adrenocortical hormones are not responsible for the action of serotonin.

Since a transient increase in lactate was produced by insulin treatment, alloxan-diabetic rats were treated with serotonin to observe the participation of insulin. If augment of lactate caused by serotonin were resulted from enhanced glycogenolysis, depletion of glycogen which appeared during development of diabetic state would reduce the action of serotonin. Therefore, rats were rendered diabetic only 2 days prior to experiments. The results were illustrated in Figure 4. Since diabetic rats were prone to differ to each other in blood sugar level, glycogen content in tissues and other state of metabolic defect, values of blood lactate were separately plotted for each rat in this figure, instead mean values provided with standard errors for all rats employed were drawn as before. Also in this case, increment in blood lactate was observed following intravenous injection of serotonin creatinine sulfate ($50\text{ }\mu\text{g}$ per rat).

Evisceration was superimposed on pre-existing adrenomedullation to examine the participation of hepatic tissue in serotonin action (Figs. 5 and 6). Removal of hepatic tissue from circulation system resulted in a rise of blood lactate, which may be accounted for by the cessation of hepatic utilization of lactate. Sometimes rapid rise of blood lactate was observed (Fig. 5), while very slow rise persisted in some cases (Fig. 6) following removal of liver. In both cases

Fig. 5. Blood lactate level of eviscerated rat following intravenous injection of serotonin. Representative data for the rat showing mild increase of blood lactate after evisceration. Serotonin, $50\text{ }\mu\text{g}$. Solid block indicates operation time; arrow, injection time. Blood lactate level is expressed in mg %.

Fig. 6. Blood lactate level of eviscerated rat following intravenous injection of serotonin. Representative data for the rat showing rapid increase of blood lactate after evisceration.

intravenous injection of serotonin caused a further rise of lactate. It may be reasonably deduced that production of lactate by extrahepatic tissue such as muscle is enhanced by serotonin.

Direct action of serotonin on liver was investigated *in vitro* in perfused rat liver (Fig. 7A and B). Effect of serotonin on perfused liver is presented in Figure 7B. Serotonin was constantly infused in a dose of 10 μ g per min. Constant infusion was adopted as means of administration into perfusate, because rapid injection itself brought about a instantaneous depression of blood flow rate and accordingly increase in lactate level (Kobayashi *et al.*, 1960b). Gradual decrease of lactate in perfusate was observed in the control experiment infused with saline; that is, hepatic utilization of lactate was demonstrated *in vitro* in perfused liver. As illustrated in Figure 7B a decrease of lactate was reduced by infusion of serotonin into perfusate. Since no significant alteration in blood flow rate was observed during the infusion, it may be concluded that serotonin directly inhibited hepatic utilization of lactate. As presented elsewhere (Warashina *et al.*, 1960) infusion of epinephrine was without effect on rate of lactate decrease in perfusate. Direct inhibitory action on lactate utilization is supposed, therefore, to be specific to serotonin and to differentiate the action of serotonin from that of epinephrine.

DISCUSSION

Interrelationship between serotonin and other endocrine factors has occasionally been suggested in the literature. Moussatche and Dereiro (1957) reported that serotonin stimulated the secretion of ACTH (adrenocorticotrophic hormone) from pituitary gland. Stimulation of serotonin secretion by growth hormone was postulated (Sirek, 1957) and indole derivatives were found to be insulinase inhibitors

Fig. 7. Effect of serotonin on lactate uptake by perfused liver
 7A Saline infusion; duration of infusion indicated by solid block
 7B Serotonin infusion at the rate of $10 \mu\text{g}$ per min.

(Mirsky, 1957). In the authors' laboratory some evidence for relation of serotonin to insulin as well as epinephrine was obtained (Kobayashi, 1960; Kobayashi *et al.*, 1960a; Ui *et al.*, 1960). Therefore, the possibility was examined that hyperlactacidemia caused by serotonin would not be resulted from direct action of serotonin but reflect an action of any endocrine factor, secretion of which was enhanced by serotonin.

As was evident in the preceding paper, though administration of serotonin into rats stimulated the release of endogenous epinephrine which was very active in increasing blood lactate, serotonin-induced hyperlactacidemia was not solely reflect of epinephrine from adrenal medulla because adrenalectomized rats were also responsive to serotonin.

Effect of norepinephrine on blood lactate level was of great interest since this compound gave rise to vasoconstriction and anoxemia to a extent seemingly comparable to those produced by serotonin. Administration of norepinephrine both subcutaneously and intraperitoneally as large as 200 μ g per rat resulted in no significant alteration of blood lactate. It may be suggested that lack of oxygen in circulation is not a direct stimulant to blood lactate level.

Participation of adrenocortical hormones was also excluded because serotonin produced an increment of blood lactate in adrenalectomized rats. Moreover, the authors lately investigated the effect of serotonin on hypophysectomized rats and obtained the evidence that pituitary hormones were not responsible for hyperlactacidemia (unpublished data).

Of pancreatic hormones, glucagon exerted no influence on blood lactate level, whereas insulin exhibited a transient but significant increase. The question then arose whether hyperlactacidemia was dependent on increased secretion, decrease of destruction, or activation of insulin in response to serotonin injection. In view of the fact that hyperlactacidemia was observed in alloxan diabetic rats, insulin appeared not to be solely responsible for serotonin action. Animals rendered diabetic by alloxan, however, would not be completely deprived of endogenous insulin. In this respect observed increment of blood lactate in eviscerated rats which was free of pancreatic activity might exclude at least participation of extra secretion of insulin. The fact that the degree of hyperlactacidemia which developed in the case of alloxan diabetes was considerably smaller than that in the animal with intact pancreas may be accounted for by depletion of muscle glycogen, which seemingly had occurred during diabetes.

In the light of the present knowledge, enhanced blood lactate level may be resulted from either depression of hepatic utilization or increased peripheral production. The results obtained in liver perfusion study showed that serotonin reduced the hepatic utilization of lactate, favoring the former of the above 2 alternatives. Non-responsiveness of perfused liver to epinephrine in rate of lactate utilization is of great importance; the action of serotonin was differentiated from that of epinephrine and now possibility may scarcely exist that serotonin-induced hyperlactacidemia is due to the action of endogenous epinephrine.

The possibility, however, that lactate production by extrahepatic tissues is augmented by serotonin can not be ruled out for the following reasons. The first, intraperitoneal injection of serotonin caused a hyperlactacidemia not so high

as that produced by subcutaneous injection; the second, eviscerated rats were also responsive to small doses of serotonin.

Viewed as a whole the evidences obtained thus far strongly favor the interpretation that serotonin directly influence metabolism of extrahepatic tissues and presumably hepatic tissue to cause a marked increase in blood lactate.

In vitro effects of serotonin in lactate release of extrahepatic tissues and on lactate uptake by liver slices as well as biochemical mechanisms involved in serotonin-induced hyperlactacidemia are currently under investigation.

SUMMARY

1. From the results now presented, serotonin-induced hyperlactacidemia occurs without participation of other endocrine factors.
2. Serotonin directly reduced the lactate utilization of perfused liver.
3. Eviscerated rats developed hyperlactacidemia in response to minute dose of serotonin.
4. It may be concluded that an increase of blood lactate caused by serotonin is due to both enhancement of extrahepatic production of lactate and retardation of hepatic utilization.

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