Pharmacology Biochemistry & Behavior, Vol. 13, Suppl. 1, pp. 67–71. Printed in the U.S.A.

Role of Hormones in the Mechanism of the Swift Increase in Alcohol Metabolism in the Rat[1]

TAKEHIKO YUKI, BLAIR U. BRADFORD* AND RONALD G. THURMAN*

Department of Pharmacology, Kyoto Prefectural University of Medicine, Kamikyo-Ku, Kyoto 602, Japan
and
**Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27514*

YUKI, T., B. U. BRADFORD AND R. G. THURMAN. *Role of hormones in the mechanism of the swift increase in alcohol metabolism in the rat.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 67–71, 1980.—Gastric intubation of female Sprague-Dawley rats (80–150 g) with one large dose (5 g/kg) of ethanol nearly doubled both hepatic oxygen uptake and ethanol metabolism within 2.5 hr in the perfused rat liver (Swift Increase in Alcohol Metabolism—SIAM). Hepatic oxygen uptake could also be elevated by direct infusion of epinephrine and glucagon into the perfused liver. Alcohol treatment produced significant increases in circulating epinephrine, norepinephrine and glucose but did not effect levels of plasma immunoreactive insulin. Administration of α- and β-adrenergic blocking agents, adrenalectomy and hypophysectomy prevented the increase in oxygen uptake due to ethanol treatment. These data suggest that catecholamines and possibly other hormones play an important role in the mechanism of the Swift Increase in Alcohol Metabolism (SIAM).

Epinephrine Glucagon Propranalol Phenoxybenzamine Ethanol metabolism

IT is well known that ethanol metabolism is increased following chronic exposure to ethanol, a phenomenon which exists in both animals [11] and humans [4]. The interest of our laboratory for a number of years has concentrated on mechanisms responsible for this adaptive increase [9,10].

Recently, we showed that administration of one large dose of ethanol (5 g/kg, IG) to the rat nearly doubled hepatic oxygen uptake as well as ethanol uptake within 2.5 hr in the perfused rat liver (Swift Increase in Alcohol Metabolism—SIAM) [12]. This SIAM was shown to involve alcohol dehydrogenase (ADH) and the mitochondrial respiratory chain since it was inhibited by 4-methylpyrazole and KCN [12]. Following alcohol treatment, rates of glycolysis were decreased significantly. Since glycolysis is coupled to ATP synthesis, slower rates of this process following ethanol treatment are probably responsible for the elevated oxygen uptake since the ADP not phosphorylated via glycolysis stimulates mitochondrial respiration and leads to an increased rate of reoxidation of NADH. This ultimately accelerates ADH-dependent ethanol oxidation.

Since these metabolic changes are rapid, it is possible that hormone action upon the liver is involved in the mechanism of SIAM. Thus, the present experiments were designed to investigate the role of hormones in the mechanism of SIAM. The data indicate that epinephrine release due to alcohol treatment accounts, in part, for the SIAM.

METHOD

Animals

Female albino, Sprague-Dawley rats (80–150 g) were allowed free access to laboratory chow and water. When indicated, some rats were starved for 24 hr before surgical preparation. Animals were given one single dose of ethanol (5.0 g/kg) 2.5 hr before surgery; control rats were intubated with 0.9% NaCl.

In some rats, the adrenal glands were removed bilaterally; controls were sham-operated. Both groups of rats were then given 0.9% NaCl for one week prior to the experiment. Hypophysectomized rats were purchased from Zivic-Miller and used within 2 weeks.

Non-Recirculating Hemoglobin-Free Liver Perfusion

The perfusion technique has been described elsewhere [7,8]. The perfusion fluid was Krebs-Henseleit bicarbonate buffer, pH 7.4, saturated with O_2/CO_2 (19/1). The perfusate flowed past an oxygen electrode before the solution was discarded and the oxygen concentration was monitored continuosly. Metabolic rates were calculated from the influent minus effluent concentration differences, the flow rate and the liver wet weight.

[1]Supported, in part, by grant AA-03624 and Career Development Award (R.G.T. AA-00033).

Collection of Blood

After anesthesia of the rat with pentobarbital to avoid stress (50 mg/kg), the femoral artery was ligated and blood samples (50 μl) were collected every 10–30 min. Blood was deproteinized with 1.5 M $HClO_4$ (30 μl), centrifuged, and neutralized with 2.5 M NaOH. Glucose was determined in the supernatant by standard enzymatic procedures [1].

Measurement of Epinephrine, Norepinephrine and Immunoreactive Insulin

Portal blood was collected from which plasma was prepared. The catecholamine concentration in portal vein plasma was determined by radioenzymatic techniques according to the method of Peuler [6]. Insulin was determined employing the dual antibody system described by Hales *et al.* [3].

Analytical

Samples of the effluent perfusate were collected every 2 min for determination of glucose, lactate and pyruvate by standard enzymatic procedures [1].

Reagents

Epinephrine, norepinephrine, glucagon and propranolol were purchased from Sigma Chemical Co. (St. Louis, MO). Phenoxybenzamine was the kind gift of Smith, Kline and French (Philadelphia, PA). All other chemicals were reagent grade from standard sources. Statistical comparisons were made with Student's *t*-test.

RESULTS

Effect of Ethanol Treatment on Oxygen Uptake in Perfused Livers

To determine the minimal time necessary for ethanol to increase hepatic oxygen uptake, rats were given ethanol (5.0 g/kg) via gastric intubation (Fig. 1). Basal rates of oxygen uptake by the ethanol-free perfused livers were between 100–110 μmol/hr/g and were unchanged by gastric intubation with saline. The oxygen uptake increased swiftly and nearly doubled 2.5 hr after ethanol treatment (Swift Increase in Alcohol Metabolism—SIAM). The respiration subsequently declined nearly to basal values at 5 hr (Fig. 1).

This treatment with ethanol also doubled alkylpyrazole-sensitive ethanol uptake by the perfused liver [12].

Effect of Epinephrine and α- and β-Blocking Agents on Hepatic Oxygen Uptake

It is well known that alcohol causes a release of epinephrine and norepinephrine from the adrenal medulla [5]. Therefore, we investigated the effect of epinephrine on oxygen uptake by the perfused rat liver. In this study, epinephrine (2 mg/kg, IP) was given 1 hr prior to liver perfusion. This treatment increased hepatic oxygen consumption by about 40%. In addition, the effects of ethanol and epinephrine were not additive, suggesting that they may have common mechanisms of action (Table 1). This hypothesis was further evaluated with α- and β-adrenergic blocking agents. Phenoxybenzamine, an α-adrenergic blocker, and propranolol, a β-blocker, were injected 30 min prior to ethanol. Both inhibitors blocked the increase in respiration

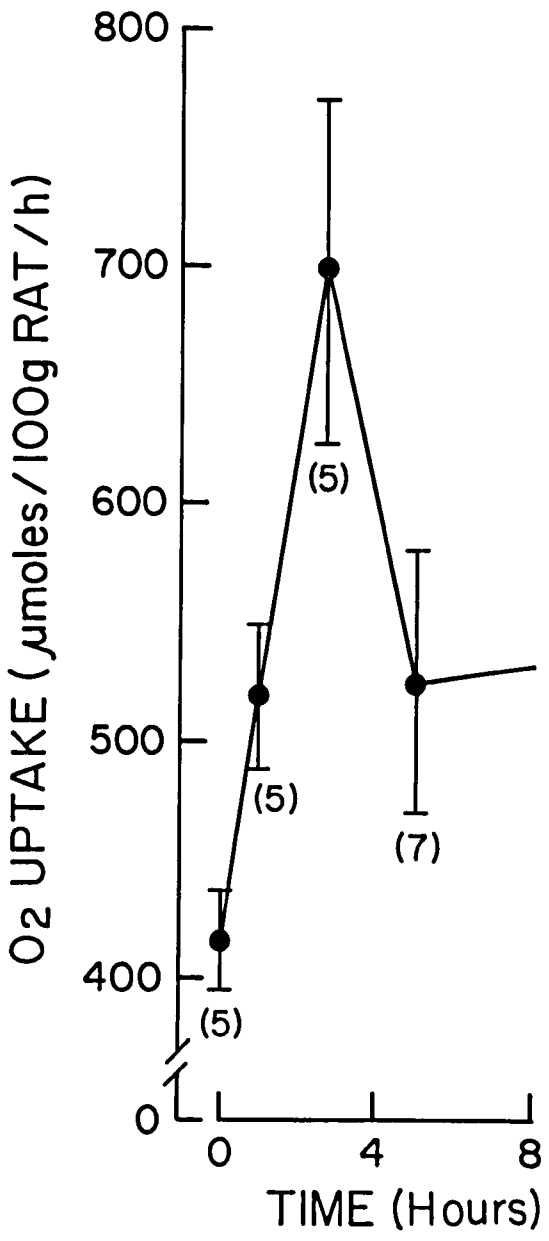

FIG. 1. Time course of the effect of ethanol treatment on hepatic oxygen uptake by perfused rat liver. Ethanol (5.0 g/kg) was given to rats via gastric intubation at zero time. Untreated rats were intubated with 0.9% NaCl. At times indicated by the filled circles, livers were perfused with ethanol-free Krebs-Henseleit bicarbonate buffer, pH 7.4. After 30 min of preperfusion, steady-state rates of oxygen uptake were calculated from the influent-effluent oxygen concentration difference, the flow rate and the liver weight. Results are means ± SEM for the numbers of experiments in parentheses.

TABLE 1

EFFECT OF ALCOHOL AND HORMONE TREATMENT ON OXYGEN UPTAKE OF PERFUSED RAT LIVERS

Treatment	Oxygen Uptake μmoles/g Liver (wet)/hr	p
	From Fed Rats	
None (8)	107 ± 8	—
Alcohol (10) (2.5 hr)	192 ± 16	<0.001
Epinephrine (9)	150 ± 5	<0.001
Alcohol + Epinephrine 2 mg/kg (7)	150 ± 18	<0.1
Alcohol + Phenoxybenzamine 40 mg/kg	116 ± 9	n.s.
Alcohol + Propranolol 40 mg/kg (5)	112 ± 11	n.s.
Alcohol + Adrenalectomy (5)	95 ± 16	n.s.
Alcohol + Hypophysectomy (5)	101 ± 8	n.s.
	From Fasted Rats	
None (8)	113 ± 19	n.s.
Alcohol (8)	157 ± 16	n.s.
Epinephrine (8)	141 ± 18	n.s.

Rats were treated with ethanol 2.5 hr prior to surgical preparation. Oxygen uptake by the perfused liver was determined polarographically as described in the Method section. Epinephrine and adrenergic blockers were injected intraperitoneally 1 and 3 hr before liver perfusion, respectively. Starved rats were deprived of food 24 hr before surgical preparation. Phenoxybenzamine, propranolol, adrenalectomy and hypophysectomy had no significant effect on oxygen uptake in the control rat. Results are means ± SEM for the numbers of experiments in parentheses. Each group was compared with its own non-treated control by Student's *t*-test. Column *p*: n.s., not significant ($p>0.05$).

due to ethanol completely (Table 1), indicating that the SIAM phenomenon involves adrenergic hormones.

Effects of Adrenalectomy and Hypophysectomy on SIAM

Both adrenalectomy and hypophysectomy prevented the increase in respiration observed after ethanol treatment (Table 1).

Effect of Ethanol Treatment on Blood Glucose

Control blood glucose levels were between 5 and 6 mM in the rat. After intubation with ethanol (5 g/kg), blood glucose concentrations increased to between 9 and 10 mM within 2.5 hr (Fig. 2). Under similar conditions, hepatic glycogen levels and rates of glycolysis were decreased by 40 to 50% (data not shown).

Effect of Glucagon and Epinephrine on Hepatic Oxygen Uptake, Glucose Production and Glycolysis in Livers from Fed Rats

It is well established that glucagon and epinephrine cause glycogenolysis and elevate blood glucose [2]. Therefore, dose-response relations between oxygen uptake, glucose output and glycolysis were established for these two hormones in the perfused rat liver. Both hormones produced step-wise increases in glucose production and oxygen uptake while decreasing rates of glycolysis (Fig. 3 and Fig. 4). However, glucagon was clearly much more potent than epinephrine on these three metabolic parameters. For example, the

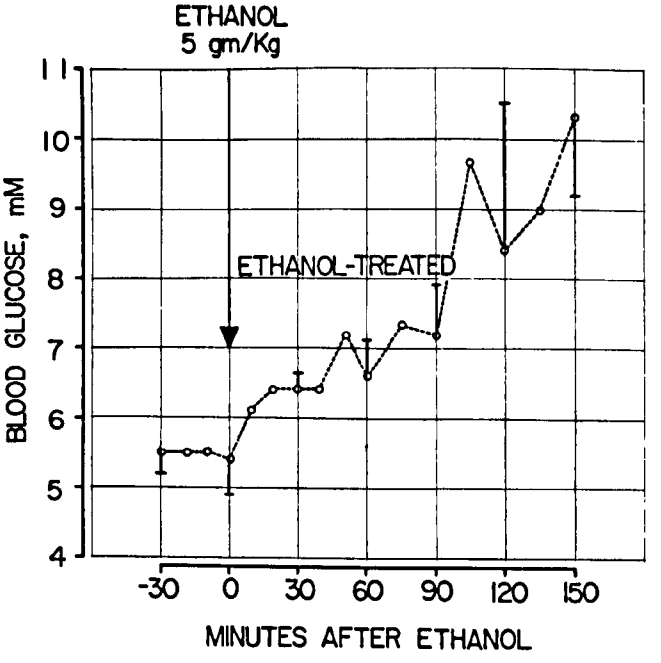

FIG. 2. Effect of ethanol treatment on blood glucose concentrations in fed rats. Rats were anesthetized lightly with pentobarbital (20 mg/kg) and secured on an animal board. An incision was made in the skin covering the right thigh and the femoral artery was exposed. Two surgical ligatures were placed around the femoral artery to secure a heparinized catheter. Ethanol (5 g/kg) was given as indicated by the arrow and blood samples (50 μl) were taken. Blood glucose was measured enzymatically in deproteinized blood samples (see Method). Results are means ± SEM (n=6–8).

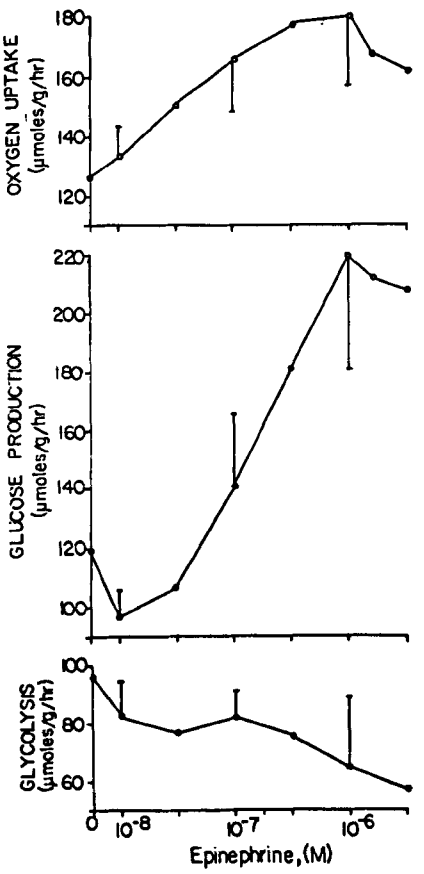

FIG. 3. Effect of epinephrine on hepatic oxygen uptake, glucose production and glycolysis in perfused livers. Livers from fed rats were perfused as described in Method. Epinephrine was infused in increasing concentration steps lasting 10 min each. Oxygen was monitored in the effluent perfusate polarographically. Perfusate was collected every 2 min and analyzed for glucose and lactate + pyruvate (glycolysis) by standard enzymatic procedures [9]. Data is expressed as mean values from the 10 min infusion. Results are means ± SEM [4].

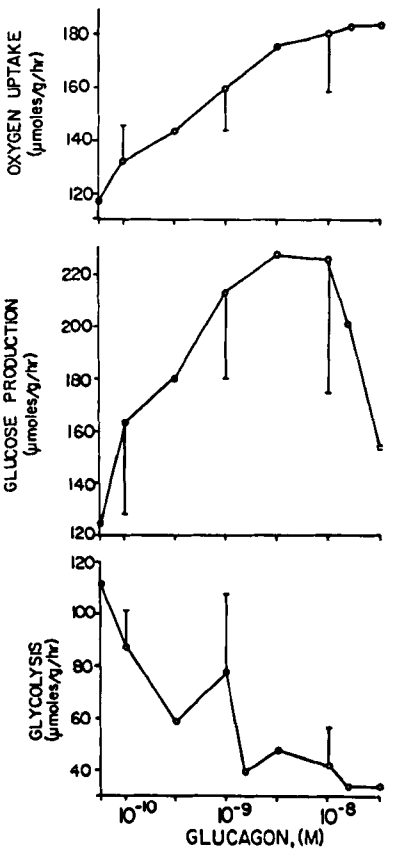

FIG. 4. Effect of glucagon on hepatic oxygen uptake, glucose production and glycolysis in perfused livers. Conditions as in Fig. 3.

effects of glucagon were half-maximal between 5×10^{-9} and 1×10^{-8} M hormone (Fig. 4). In contrast, 5×10^{-7} M epinephrine was needed for half-maximal stimulation of oxygen uptake whereas 8×10^{-6} M epinephrine was required to increase glucose production half-maximally (Fig. 3). In general, two orders of magnitude more epinephrine were required than glucagon to perturb carbohydrate metabolism in the perfused rat liver.

Effect of Ethanol Treatment on Portal Vein Catecholamine and Insulin Concentrations In Vivo

Plasma levels of epinephrine and norepinephrine were 90 and 470 pg/ml, respectively. Ethanol treatment elevated both hormones approximately 80% above control level (Table 2). On the other hand, immunoreactive insulin was not altered by ethanol treatment (Table 3).

TABLE 2

PORTAL VEIN EPINEPHRINE AND NOREPINEPHRINE CONTENTS IN CONTROL AND ALCOHOL-TREATED RATS

Treatment	Epinephrine (pg/ml)	Norepinephrine (pg/ml)
Control (5)	89 ± 18	468 ± 91
Alcohol-Treated 2.5 (5)	160 ± 32	862 ± 201

Rats were treated with ethanol 2.5 hr prior to the collection of blood. Portal blood was collected and centrifuged at 1000 × G for 5 min to isolate the plasma. 50 μl of plasma was used to determine catecholamine concentrations by radioenzymatic procedures [10]. Mean ± SEM (n).

TABLE 3

IMMUNOREACTIVE INSULIN LEVELS IN CONTROL AND ALCOHOL-TREATED (2.5 hrs) RATS

Treatment	μU/ml
Control (5)	78 ± 15
Alcohol-Treated (5)	81 ± 22

Conditions as in Table 2. Mean ± SEM (n).

DISCUSSION

Role of Hormones in the Swift Increase in Alcohol Metabolism

Recently, a Swift Increase in Alcohol Metabolism (SIAM) was described [12]. We demonstrated that the oxygen and ethanol uptake of the ethanol-free perfused liver can be nearly doubled 2 to 3 hr after one large dose (5.0 g/kg) of ethanol to the rat (Fig. 1). The activation of oxygen uptake tended to return to the baseline at 5 hr (Fig. 1), indicating that the effect of ethanol was transient. Moreover, a direct relationship between hepatic oxygen and ethanol uptake exists [9]. Enhanced oxygen uptake activates NADH reoxidation, thereby facilitating the rate-limiting step in ethanol metabolism which is the dissociation of the alcohol dehydrogenase-NADH complex.

Because the activation of oxygen uptake was rapid (2–3 hr), involvement of hormonal factors is possible. Epinephrine injected into the rat partially mimicked the increase observed with ethanol (Table 1). In addition, the increase in hepatic respiration observed 2.5 hr after treatment with ethanol could be blocked by α- and β-adrenergic blocking agents as well as by adrenalectomy and hypophysectomy (Table 1). Finally, the actions of ethanol and epinephrine were not additive, indicating that the rapid increase in hepatic oxygen uptake is triggered, at least in part, via an ethanol-mediated release of epinephrine or other hormones. Because of its potency, glucagon (Fig. 4) may also be involved in this phenomenon.

This hypothesis is supported by the observations that infusion of glycogenolytic hormones into the perfused liver (epinephrine and glucagon; Figs. 3 and 4) decreased rates of glycolysis, increased glucose output, and increased rates of oxygen uptake. Moreover, alcohol-treatment elevated levels of circulating catecholamines at times (2.5 hr) when effects on oxygen uptake were observed (Table 2).

The activation of oxygen uptake by ethanol was considerably less (about 50% as large) in livers from starved rats where glycolysis was absent (Table 1). The increase in oxygen uptake also was partially reversed by infusion of glucose. Since glycolysis is an ATP-producing reaction, the inhibition of glycolysis is equivalent to the stimulation of an ATPase. These data are consistent with the hypothesis that one factor responsible for the elevation of oxygen uptake after ethanol treatment is diminished rates of glycolysis resulting from hormone-stimulated glycogenolysis.

One possible explanation for the sequence of metabolic events responsible for SIAM follows: First, hormone-stimulated glycogenolysis leads to depletion of carbohydrate reserves which in turn causes glycolysis to decline. Glycolysis is an ATP-producing reaction, so ADP not phosphorylated via glycolysis must enter the mitochondrion and be phosphorylated by the electron transport chain at the expense of extra oxygen. As a consequence of this, NADH is reoxidized at a faster rate thereby providing more NAD^+ for the alcohol dehydrogenase reaction.

ACKNOWLEDGEMENT

We thank Dr. Hiroshi Senmaru for his technical assistance with the determination of insulin.

REFERENCES

1. Bergmeyer, H. U. *Methoden der Enzymatischen Analyse*, 2nd ed. Weinheim: Verlag Chemie, 1970.
2. Exton, J. H., G. A. Robinson, E. W. Sutherland and C. R. Park. Studies on the role of adenosine 3′–5′-monophosphate in the hepatic actions of glucagon and catecholamines. *J. biol. Chem.* 246: 6166–6177, 1971.
3. Hales, C. N. and P. J. Randle. Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.* 88: 137–146, 1963.
4. Mendelson, J., S. Stein and N. Mello. Effects of experimentally induced intoxication of ethanol-1-C^{14} in alcohol subjects. *Metabolism* 14: 1255–1266, 1965.
5. Perman, E. S. The effect of ethyl alcohol on the secretion from the adrenal medulla of the cat. *Acta physiol. scand.* 48: 323–328, 1960.
6. Peuler, J. D. and G. A. Johnson. Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. *Life Sci.* 21: 625–636, 1977.
7. Scholz, R. In: *Stoffwechsel der Perfundierten Leber*, edited by W. Staib and R. Scholz. Berlin, Heidelberg, New York: Springer-Verlag, 1968, pp. 24–47.
8. Scholz, R., W. Hansen and R. G. Thurman. Interaction of mixed-function oxidation with biosynthetic process. 1. Inhibition of gluconeogenesis by aminopyrine in perfused rat liver. *Eur. J. Biochem.* 38: 64–72, 1973.
9. Thurman, R. G., W. R. McKenna and R. B. McCaffrey. Pathways responsible for the adaptive increase in ethanol utilization following chronic treatment with ethanol: Inhibitor studies with the hemoglobin-free perfused rat liver. *Molec. Pharmac.* 12: 156–166, 1976.
10. Thurman, R. G. and R. Scholz. Ethanol metabolism in perfused rat liver at low ethanol concentrations: Involvement of alcohol dehydrogenase in the adaptive increase in ethanol metabolism due to chronic pretreatment with ethanol. *Hoppe-Seyler's Z. Physiol. Chem.* 357: 1443–1446, 1976.
11. Videla, L., J. Bernstein and Y. Israel. Metabolic alterations produced in the liver by chronic alcohol administration. Increased oxidative capacity. *Biochem. J.* 134: 507–514, 1973.
12. Yuki, T. and R. G. Thurman. The swift increase in alcohol metabolism. Time course for the increase in hepatic oxygen uptake and the involvement of glycolysis. *Biochem. J.* 186: 119–126, 1980.