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Ethanol-Induced Hyperglycemia Mediated by the Central Nervous System[1]

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ERWIN, V. G. AND J. F. TOWELL. *Ethanol-induced hyperglycemia mediated by the central nervous system.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 559–563, 1983.—A marked difference in ethanol-induced hyperglycemia was found in two lines of mice (LS/Ibg and SS/Ibg) with differential central nervous system sensitivity to ethanol. The LS/Ibg line had a greater sensitivity to the ethanol-induced hyperglycemia, but this difference was not found after administration of hypnotic doses of pentobarbital or halothane. The two lines did not differ in their response to glucose tolerance tests. Fasting, adrenalectomy, and pretreatment with α- and β-adrenergic antagonists (dibenamine and propranolol) eliminated the ethanol-induced hyperglycemic response. Results from intracerebroventricular injections of ethanol indicated that the ethanol-induced hyperglycemia was mediated by the central nervous system.

Hyperglycemia Ethanol Pharmacogenetics Central nervous system

SINCE the early studies of Tennet [22] and Klingman and Haag [15], the effects of ethanol on plasma glucose levels have become well known. These researchers suggested that ethanol produces hyperglycemia by enhancing the release of catecholamines from the adrenal gland [14]. Subsequent studies [4, 10, 16] have demonstrated an ethanol-induced release of adrenal epinephrine in several mammalian species including dog, cat, and rat, as well as man.

Ethanol-induced hyperglycemia is a consequence of increased glycogenolysis [3], and evidence suggests that this effect is most likely a result of the action of epinephrine on the pancreas. Sokal and Sarcione [20] and Exton *et al.* [12] found rat liver to be relatively insensitive to the glycogenolytic and gluconeogenic effects of epinephrine. Recently, Woodson and Potter [24] showed that epinephrine stimulates glucagon release and inhibits insulin release from pancreatic α and β cells, respectively. Also, it was shown [17] that ethanol-induced hyperglycemia was related to elevation of plasma glucagon levels and to suppression of plasma insulin levels.

Ethanol and its proximal metabolite, acetaldehyde, increase the secretion of catecholamines from isolated perfused adrenal glands [1,19]; however, relatively high (nonphysiological) concentrations of these substances were required to enhance catecholamine release. Recent studies of Brown *et al.* [9] showed that intracerebroventricular (ICV) administration of bombesin, neurotensin, and carbachol produced hyperglycemia in the rat. The data strongly indicate that these neural stimuli produce a central nervous system (CNS)-mediated secretion of epinephrine by the adrenal medulla. Consequently, it was of interest to determine whether ethanol-induced hyperglycemia is mediated by a CNS or peripheral mechanism. For these studies we chose to utilize the LS/Ibg (LS) and SS/Ibg (SS) lines of mice which have been selectively bred for differences in duration of loss of righting response following a hypnotic dose of ethanol [13]. Erwin *et al.* [11] have shown that LS and SS mice differ in CNS sensitivity to depressant effects of ethanol, and Tabakoff *et al.* [21] have reported differences in sensitivity to ethanol-induced hypothermia. This paper describes studies carried out to determine the actions of ethanol in producing hyperglycemia in LS and SS lines of mice and to characterize whether the hyperglycemia is mediated by the CNS.

METHOD

Adult male or female LS and SS mice, 70–90 days of age, weighing 20–30 g, were obtained from the Institute for Behavioral Genetics, University of Colorado, Boulder, CO, and were fed Purina Rodent Chow and water ad lib except in fasting and adrenalectomy experiments. LS and SS lines of mice represented the 32nd generation of mice selectively bred from a genetically heterogeneous population (HS/Ibg) derived from an eight-way cross of inbred strains of mice [13]. These lines were selectively bred for differences in CNS sensitivity to ethanol by measuring the duration of loss of righting response, i.e., sleep time. LS and SS represent long-sleep and short-sleep lines of mice, respectively. The animals were housed in an environment controlled for temperature (22°C) and humidity (20%) with 12 hr of light (0600–1800) and 12 hr of dark (1800–0600).

Dibenamine hydrochloride and propranolol hydrochloride were provided by Dr. J. A Ruth (School of Pharmacy, University of Colorado, Boulder, CO). All solutions for ICV injection were prepared fresh daily in artificial cerebral spinal fluid (CSF), and all solutions for intraperitoneal (IP) in-

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jection were prepared in isotonic saline. Plasma glucose concentrations were determined with a coupled hexokinase, glucose-6-phosphate dehydrogenase enzyme procedure using glucose assay kits No. 15-UV obtained from Sigma Chemical Co. (St. Louis, MO). In these analyses, plasma glucose levels were determined by measuring NADPH formation at 340 nm with a Beckman doublebeam spectrophotometer, Model 25 (Beckman Instruments, Palo Alto, CA). The ethanol solutions, in CSF, were administered in a 10-μl volume into the lateral ventricles of the brain [2] with a Hamilton microliter syringe (Hamilton Co., Reno, NV). In these experiments animals were lightly anesthetized with halothane before the injections. Ethanol was administered IP as a 30% (v/v) solution in isotonic saline and was administered ICV as a 20% (w/v) solution in CSF.

Blood samples of 50 μl were collected with heparinized microcapillary tubes by retro-orbital sinus puncture [18]. Routinely, blood samples were taken by this technique at four time periods: immediately before ethanol injection and at 30, 60, and 120 min postethanol injection. The blood samples in microcapillary tubes were centrifuged for 3 min in a Clay-Adams microhematocrit centrifuge, Model 0557 (Parsippany, NJ), and 2 μl of the plasma were used for glucose assay. The effects of ethanol and other agents on body temperature were measured by determining rectal temperature with a mouse rectal thermocouple and a Thermalert TH5 instrument (Bailey Instrument, Saddle Brook, NJ).

The experimental techniques including IP an ICV injections, blood sampling, and generalized handling of the animals can easily produce unwarranted stress resulting in a generalized sympathetic discharge [7]. Therefore, saline-treated control groups were used to determine the extent to which stress contributed to the ethanol-induced hyperglycemic responses. Experiments were carried out utilizing 6–10 animals per experimental group, and the Student's t-test was utilized for determinations of statistical significance.

RESULTS

Comparison of Ethanol-Induced Hyperglycemia in LS and SS Lines of Mice

Ethanol at doses up to 3.0 g/kg IP produced a sustained elevation in plasma glucose in LS but not in SS mice (Figs. 1 and 2). In SS mice a significant increase (ca. 45%) in plasma glucose was observed only at the highest dose (4.0 g/kg) of ethanol (Fig. 1). The peak increase in plasma glucose occurred at 30 min after ethanol in SS mice. The marked hyperglycemia induced by ethanol in LS mice was dose dependent and was sustained over a 2–3 hr period (Fig. 2). The maximum increase in plasma glucose in LS mice was obtained at approximately 60 min where the glucose levels were elevated by 2.5-fold and 3-fold at ethanol doses of 3.6 g/kg and 4.0 g/kg, respectively.

Experiments were conducted to determine whether the differential hyperglycemic effects of ethanol in LS and SS mice might be due to differences in rates of glucose clearance and insulin release. After glucose administration (1.0 g/kg), orally or IP, the plasma glucose levels increased about 50% from control values of 9-10 mM in both LS and SS mice and returned to control values at a similar rate in the two lines of mice. These results suggest that the LS and SS mice do not significantly differ in glucose clearance. Previously it was

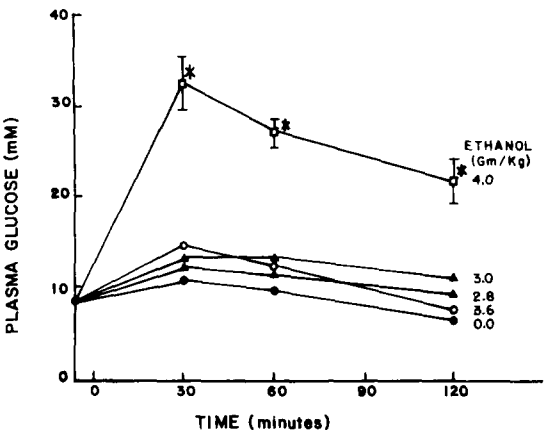

FIG. 1. Plasma glucose response of SS mice as a function of time after ethanol administration. Ethanol (30% v/v) in isotonic saline (0.9% NaCl solution) was administered IP immediately after a 0-time blood sample was taken as described in the text. Blood samples for glucose assay were collected at 30, 60, and 120 min following ethanol administration. Dosages of ethanol were as indicated, and asterisks represent values significantly different ($p<0.001$) from 0-time control (n=10). Other values are not significantly different from controls ($p<0.05$). All values are expressed as the mean±SEM.

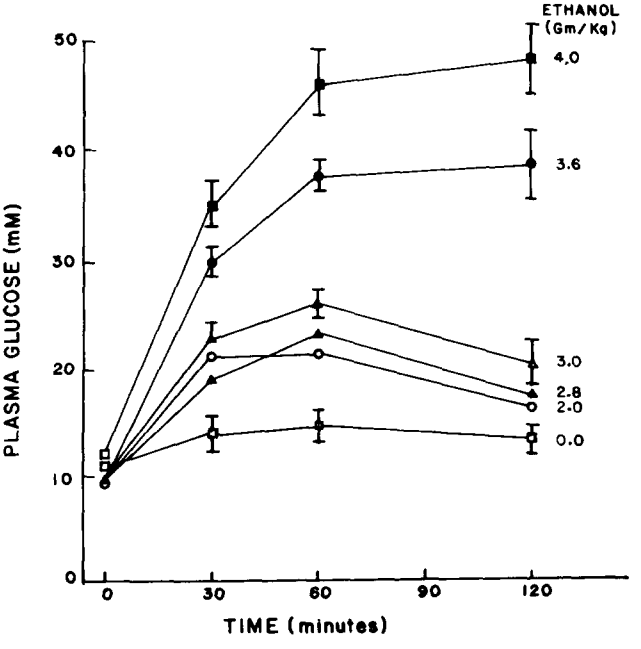

FIG. 2. Plasma glucose response of LS mice as a function of time after ethanol administration. Conditions were as described in Fig. 1, with dosages of ethanol as indicated (n=10). All values are significantly different from control ($p<0.01$).

shown that brain glucose utilization rates are similar for LS and SS mice before and after a 4 g/kg IP dose of ethanol [23].

In order to determine if the differential hyperglycemic response to ethanol in LS and SS mice was a specific action of ethanol, the effects of other hypnotic agents were deter-

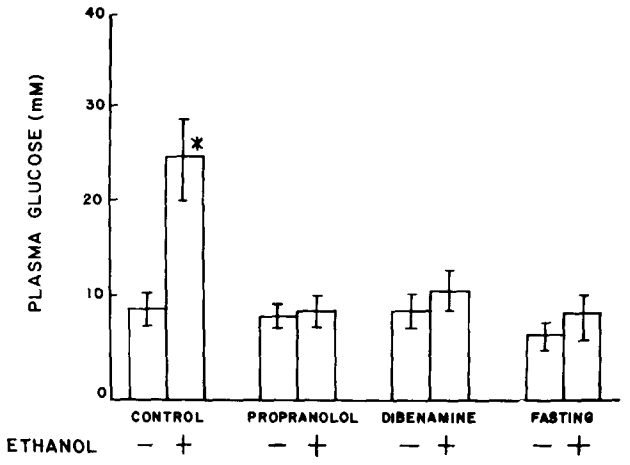

FIG. 3. Effects of propranolol, dibenamine, or fasting on ethanol-induced hyperglycemia in LS mice. Experimental conditions were the same as in Fig. 1 except that animals received ethanol (3.6 g/kg) IP with blood samples taken immediately before and 60 min after ethanol administration. Propranolol (1 mg/kg) and dibenamine (5 mg/kg) or saline (control) were administered 30 min prior to ethanol administration, and propranolol and dibenamine control animals received saline instead of ethanol. Mice, fasted as described in the text, received either saline or ethanol at 0-time, and plasma glucose was determined at 60 min. Asterisk value was significantly ($p<0.001$) different from control (n=8) in each group).

mined. Pentobarbital (40 mg/kg, IP) was without effect on plasma glucose in either LS or SS mice, and halothane anesthesia produced only a small increase in plasma glucose in both LS and SS mice.

Effects of Fasting, Adrenergic Antagonists, and Adrenalectomy on Ethanol-Induced Hyperglycemia in LS Mice

Studies were performed to determine if ethanol-induced hyperglycemia was primarily a consequence of enhanced glycogenolysis. The results presented in Fig. 3 show that ethanol (3.6 g/kg, IP) produced a 2.5-fold increase in plasma glucose in control LS mice whereas no increase in plasma glucose was observed in animals fasted for 24 hr. It should be noted that in the absence of ethanol (0-time) the plasma glucose levels in fasted animals were lower (6.0 mM) than in control animals (10 mM). Results presented in Fig. 3 demonstrate clearly that propranolol, a nonselective β-adrenergic antagonist, and dibenamine, an α-adrenergic antagonist, when administered prior to ethanol, blocked ethanol-induced hyperglycemia in LS mice. In the absence of ethanol these adrenergic blockers did not alter control glucose levels. These results indicate that ethanol-induced hyperglycemia in LS mice is produced by an adrenergically mediated increase in glycogenolysis.

Since it is well known that ethanol causes an increase in epinephrine secretion by the adrenal gland in some mammalian species [6,8], it was of interest to determine the effects of adrenalectomy on ethanol-induced hyperglycemia in LS mice. The data presented in Fig. 4 show that ethanol failed to produce an increase in plasma glucose in adrenalectomized LS mice but caused a marked increase in sham control mice.

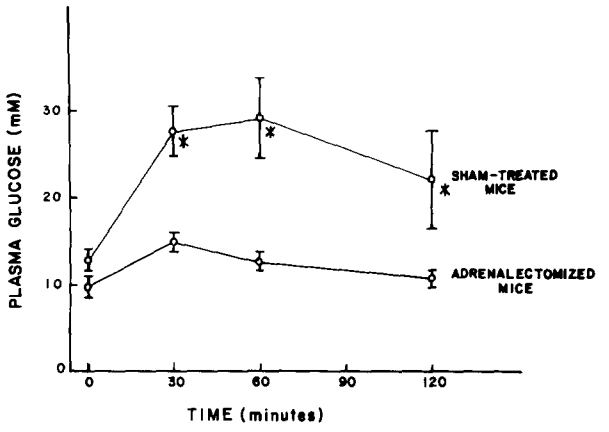

FIG. 4. Effects of adrenalectomy on ethanol-induced hyperglycemia in LS mice. Experimental conditions were as described in Fig. 1 and in the text, except that ethanol (3.0 g/kg) was administered to sham-operated and adrenalectomized LS mice. Experiments were conducted 24 hr after surgery (n=8).

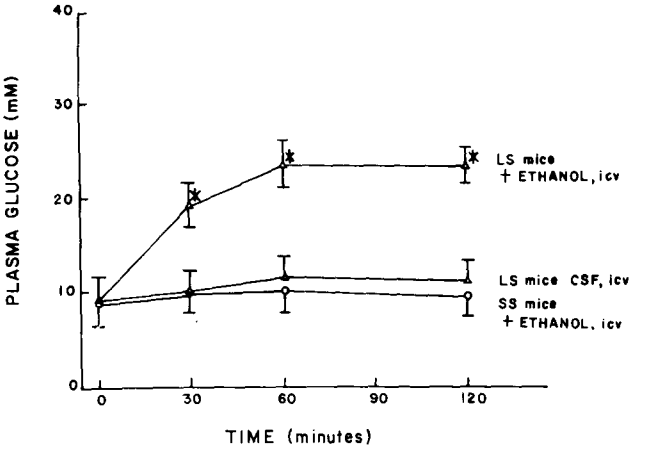

FIG. 5. Effects of ethanol, ICV, on plasma glucose levels in LS and SS mice as a function of time. Each animal was lightly anesthetized with halothane vapor, and 10 μl of ethanol (20% w/v solution) or artificial CSF were administered ICV as described in the text. Blood samples for glucose assay were collected immediately prior to ICV administration (0-time) and at 30, 60, and 120 min after ICV injection. Values represent the mean±SEM, and asterisks indicate values significantly different ($p<0.001$) from control (artificial CSF) (n=8).

Evidence That Ethanol-Induced Hyperglycemia in LS Mice Is Mediated by the Central Nervous System

Since the above results suggested that ethanol-induced hyperglycemia was caused by an increase in adrenal-medullary secretion of epinephrine, it was of interest to determine whether the hyperglycemia was centrally (brain) or peripherally (adrenal gland) mediated. Administration of 10 μl of 20% w/v ethanol ICV containing 2 mg ethanol in artificial CSF produced a long-lasting elevation in plasma glucose in LS but not in SS mice (Fig. 5). Sixty min after administration of ethanol ICV plasma glucose levels were increased

1.8- and 2.5-fold. The amount of ethanol given in the ICV injections (2 mg) was equivalent to a 0.1 g/kg dose given IP. Artificial CSF did not significantly alter plasma glucose levels in either LS or SS mice.

DISCUSSION

The results presented in this study using mice confirm earlier observations [15,22] in dogs and other species that acute administration of ethanol produces a dose-dependent, long-lasting hyperglycemia. Also, the present studies show that LS and SS lines of mice, which have been selectively bred for differences in sensitivity to ethanol-induced loss of righting reflex [11], differed in hyperglycemic response to ethanol. These findings provide prima facie evidence that the hyperglycemic action of ethanol is genetically influenced. However, whether the differential effect of ethanol on plasma glucose and the differential sensitivity to the hypnotic and hypothermic effects of alcohol are related remains to be determined. Preliminary data (unpublished) indicate a small but significant correlation ($r=0.4, p<0.01$) between duration of loss of righting response and increase in plasma glucose produced by ethanol in a genetically segregating F_2 generation of mice derived from LS and SS lines. This could indicate that a gene product which has a role in the determination of ethanol-induced sleep time also plays a minor role in a centrally mediated glucoregulatory mechanism. Thus, by breeding for a particular phenotype (such as sleep time), other neuropharmacological processes were selected. The precise relationship between these processes remains to be determined.

The data in Fig. 4 are consistent with the hypothesis that ethanol acts within the brain to stimulate sympathetic outflow resulting in secretion of epinephrine from the adrenal gland. Brown *et al.* [9] and Potter and Morris [17] have shown that in rats and dogs hyperglycemia results from elevated plasma catecholamines as a result of increased levels of glucagon and suppressed levels of insulin. Studies are in progress to determine whether ethanol ICV in LS and SS mice produces a differential effect on plasma catecholamines.

While it is known that ethanol and acetaldehyde may act directly on the adrenal medulla to enhance catecholamine secretion [1,19], it is unlikely that a direct action on the adrenal gland is responsible for the hyperglycemia caused by ethanol. This conclusion is supported by the observation that 2 mg ethanol ICV caused a marked increase in plasma glucose levels (Fig. 5), and when administered to a mouse weighing 20–30 g, this dose of ethanol produced maximum blood ethanol levels equivalent to an IP dose of approximately 0.10 g/kg. This latter dose of ethanol did not increase plasma glucose levels when administered IP.

The effects of ethanol on plasma glucose levels paralleled closely the hyperglycemic actions of the neuropeptides bombesin, β-endorphin, and neurotensin and of the cholinergic agonist, carbachol [5, 6, 8, 9]. Like these substances, ethanol administered ICV in mg quantities elicited a marked hyperglycemia with a duration far exceeding the presence of the drug in the brain. The possibility that ethanol-induced hyperglycemia may be mediated through actions on neuropeptides is under investigation in our laboratory.

Towell and Erwin [23] have observed that ethanol infusion (87 mM) into an isolated perfused mouse brain inhibits glucose utilization while equivalent blood ethanol levels in an intact mouse have no effect. It was suggested that ethanol may impair glucose utilization in mammalian brain, but it has not been observed because of compensatory mechanisms which depend on the brain's communication with the periphery. Whether the ethanol-induced hyperglycemia reported here was due to an ethanol effect on cerebral glucose utilization which in turn stimulated a response in the glucoregulatory system or whether ethanol directly perturbed the central portion of the glucoregulatory system remains to be determined.

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