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EFFECTS OF ETHANOL AND 3-MERCAPTOPICOLINIC ACID ON ISOPROTERENOL AND EPINEPHRINE-INDUCED CHANGES IN GLUCOSE HOMEOSTASIS IN NORMAL AND ALLOXAN-DIABETIC RATS*

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Abstract—The relative contribution of gluconeogenesis to the catecholamine-induced hyperglycemic response in rats remains controversial. In the present study, fasted rats were treated acutely with either ethanol or 3-mercaptopicolinic acid and then challenged later with saline, epinephrine or isoproterenol. Pretreatment with either agent suppressed the hyperglycemic response to isoproterenol more than the response to epinephrine. Isoproterenol-induced changes in blood lactate were augmented by ethanol pretreatment in normal fasted rats. In alloxan-diabetic rats, the augmented hyperglycemic response to isoproterenol was partially suppressed by pretreatment with ethanol or 3-mercaptopicolinic acid. Interestingly, pretreatment of normal, fed rats with ethanol unmasked a hyperglycemic response to isoproterenol which appeared to be the result of inhibition of insulin release. These data suggest that the hyperglycemic response to catecholamines in normal, fasted and alloxan-diabetic rats is due, in part, to stimulation of gluconeogenesis and that the response to isoproterenol in fasted rats is more dependent upon gluconeogenesis than is the response to epinephrine.

Catecholamine-induced changes in glucose homeostasis are the product of several effects including the promotion of hepatic glycogenolysis [1], the enhancement of gluconeogenesis [2], and the inhibition of peripheral glucose utilization directly [3] or indirectly via alterations in pancreatic hormone secretion [4]. However, the relative contribution of each of these mechanisms to the total hyperglycemic effect elicited by individual catecholamines is a controversial issue.

Several recent studies have suggested that the hyperglycemia produced by subcutaneously injected epinephrine originates primarily from hepatic gluconeogenesis rather than from hepatic glycogenolysis [5–7]. To investigate this hypothesis further, two inhibitors of gluconeogenesis, ethanol and 3-mercaptopicolinic acid, were tested for activity against epinephrine- and isoproterenol-induced changes in blood glucose, lactate and insulin in normal rats and in alloxan-diabetic rats controlled with protamine zinc insulin.

MATERIALS AND METHODS

Male rats of the Holtzman strain, weighing between 200–300 g, were used in these experiments. Rats were allowed free access to Purina laboratory chow and water except in those experiments utilizing fasting rats where food was withheld for 24 hr. One group of rats was made "diabetic" by i.v. injection of alloxan monohydrate, 50 mg/kg; the care and maintenance of these animals have been described in detail in a previous communication [8].

Fed or fasted, normal rats or fed, diabetic rats were divided into 5 groups and treated as follows: Group 1 and 2 received an injection of saline (0.1 ml/100 g), 1 hr before an injection of either *l*-isoproterenol (0.1 mg/100 g, base) or *l*-epinephrine (0.03 mg/100 g, base). Groups 3, 4 and 5 received an injection of ethanol (119 mg/100 g) or 3-mercaptopicolinic acid (2.5 mg/100 g), 1 hr before an injection of saline, *l*-isoproterenol, or *l*-epinephrine in the concentration indicated above. All drugs were administered by the intraperitoneal route. In those experiments where only blood glucose was determined, sampling of blood was carried out by cutting the tip of the tail.

Sampling of blood by cardiac puncture was used in those experiments involving the determination of blood lactate, insulin and glucose levels after *l*-isoproterenol; this required light anesthesia with pentobarbital (30 mg/kg i.v.). Blood samples for lactate determination were taken from fasted rats at 2 hr after injection of *l*-isoproterenol since this time coincided with the peak hyperglycemic response. Blood samples for insulin determination were taken at 3 or 4 hr after the injection of *l*-isoproterenol.

Blood or plasma glucose levels were determined by the Hoffman method [9] as adapted to the Technicon AutoAnalyzer. Lactate levels were determined enzymatically on blood previously deproteinized with 0.6 M perchloric acid [10]. The amount of NADH formed was measured at 340 nm using a Beckman spectrophotometer, and lactate levels calculated according to the formula: maximum $A_{340} \times 65.5 =$ mg/dl lacatate. Plasma levels of immunoreactive insu-

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lin were determined in duplicate using a single antibody radioimmunoassay technique with separation of free and bound tracer by adsorption of free tracer onto dextran-coated charcoal [11]. After centrifugation, aliquots of supernatant were counted by the microfuge technique [12] using a Nuclear-Chicago liquid scintillation system.

Stock solutions (1 mg/ml) of *l*-isoproterenol hydrochloride and *l*-epinephrine bitartrate were prepared daily in acidified saline (pH 4.5) and then diluted to the desired concentration. Ethyl alcohol was made up daily as a 15% v/v solution. Mercaptopicolinic acid, kindly supplied by Smith, Kline and French Laboratories, was prepared each day at a concentration of 25 mg/ml. All drug concentrations are expressed in terms of the mg of active drug per ml of solution. Protamine zinc insulin, generously supplied by Eli Lilly Laboratories, was used to maintain the colony of alloxan-diabetic rats.

Statistical comparisons were made using the Student's *t*-test.

RESULTS

Effect of pretreatment with ethanol or 3-mercaptopicolinic acid on catecholamine-induced changes in blood glucose in fasted rats. As reported previously, pretreatment with ethanol at a dose that did not produce overt hypoglycemia, suppressed the hyperglycemic response to isoproterenol more than the response to epinephrine [13]. A similar experiment using 2.5 mg/100 g of 3-mercaptopicolinic acid (MPA) produced results similar to those produced by ethanol. As shown in Fig. 1, the hyperglycemic response to isoproterenol in fasted rats was inhibited more by pretreatment with mercaptopicolinic acid than was the response to epinephrine. Mercaptopicolinic acid (25 mg/kg) produced no effect on blood glucose levels in a group of rats treated with saline.

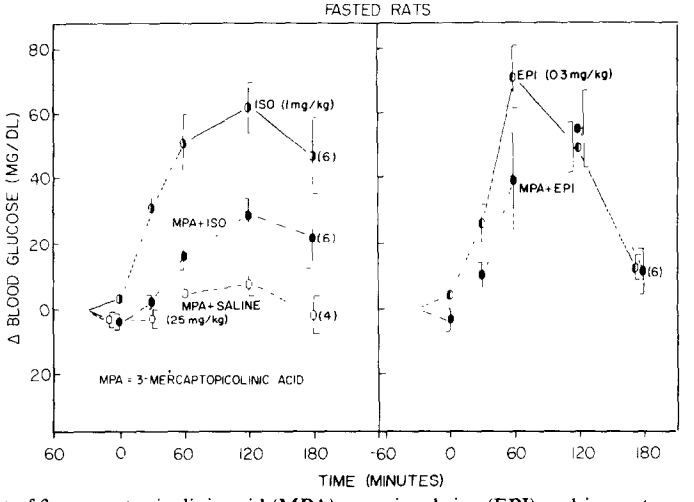

Fig. 1. Effect of 3-mercaptopicolinic acid (MPA) on epinephrine (EPI) and isoproterenol (ISO) -induced hyperglycemia in fasted normal rats. The number of animals in each group are shown in parentheses. Each point and the linit on each are the mean and the S.E., respectively.

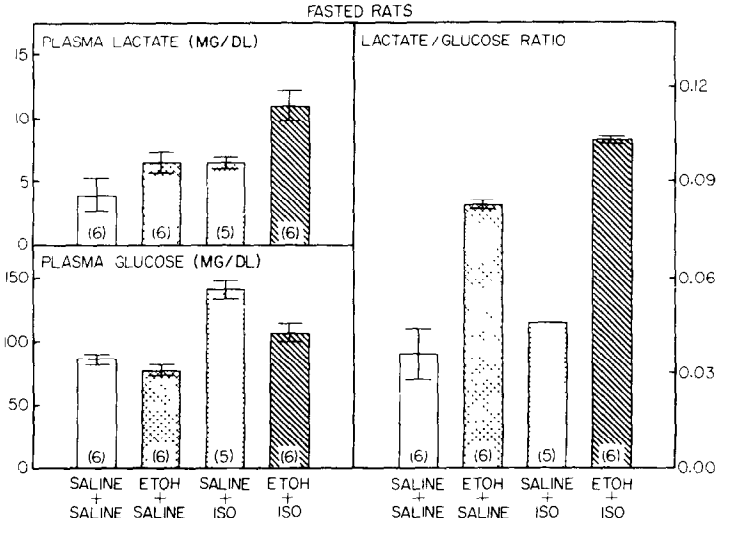

Fig. 2. Effect of ethanol (ETOH) isoproterenol-induced changes in plasma lactate and glucose levels in fasted normal rats. The mean and S.E. for each column were determined from the number of animals shown in parentheses.

Effect of ethanol on isoproterenol-induced changes in plasma lactate and glucose levels in fasted rats. Since the conversion of lactate to glucose is presumed to be important in the production of hyperglycemia by isoproterenol, fasted rats were pretreated with ethanol (119 mg/100 g) 1 hr before an injection of isoproterenol to determine if ethanol was inhibiting hyperglycemia by preventing the re-cycling of lactate to glucose. Plasma lactate levels were determined enzymatically at the time that coincided with the peak hyperglycemic effect (2 hr after injection of isoproterenol). As shown in the upper left panel of Fig. 2, lactate levels were significantly higher ($P < 0.05$) in the groups of rats pretreated with ethanol compared to the groups pretreated with saline. Although there was no difference in lactate levels between the group treated with ethanol plus saline and the group treated with saline plus isoproterenol, lactate levels were elevated significantly ($P < 0.001$) in the group of fasted rats pretreated with ethanol and then challenged with isoproterenol. The lower left panel of Fig. 2 illustrates the concurrent changes in plasma glucose levels in the four groups of rats. Whereas there was no significant change in plasma glucose levels in the saline plus saline-treated group and the ethanol plus saline-treated group, a significant difference ($P < 0.05$) in glucose levels was observed between the groups receiving saline or ethanol 1 hr prior to the injection of isoproterenol. The lactate/glucose ratio depicts these differences more clearly as shown in the right panel of Fig. 2. Pretreatment with ethanol produced a significant elevation in the lactate/glucose ratio in fasted rats treated with saline or isoproterenol, and these increases are also markedly elevated when compared to the appropriate saline-pretreatment groups.

Effect of ethanol and 3-mercaptopicolinic acid on isoproterenol-induced blood glucose changes in alloxan-diabetic, insulin-controlled rats. In experimentally-induced diabetic states, enhanced gluconeogenesis is considered an important contributor to the hyperglycemia characteristic of this disorder. In the fed, alloxan-diabetic, insulin-controlled rat, isoproterenol produced an exaggerated hyperglycemic response [8]. As shown in Fig. 3, pretreatment with ethanol (left panel) or 3-mercaptopicolinic acid (right panel) suppressed the hyperglycemic response to isoproterenol. A very modest hyperglycemic response to ethanol was observed in a group of saline-treated rats whereas 3-mercaptopicolinic produced very little change in resting blood glucose levels. The saline plus saline group (left panel) demonstrates the degree of control of blood glucose levels achieved by the schedule of treatment with protamine zinc insulin.

Effect of ethanol in isoproterenol-induced changes in plasma insulin and glucose in fed or fasted normal rats. Figure 4 shows the effect of pretreatment with ethanol on isoproterenol-induced insulin release in fasted rats. Ethanol treatment followed by saline resulted in a lowering of plasma glucose (lower left panel) that was accompanied by a decline in plasma immunoreactive insulin levels (upper left panel). Pretreatment with saline followed by isoproterenol resulted in a 2.5–3-fold increase in plasma insulin levels and modest elevation of blood glucose. Ethanol pretreatment followed by isoproterenol resulted in an increase in plasma insulin levels that was not different from saline plus isoproterenol, however, the modest increase in plasma glucose at 45 min after the injection of isoproterenol was suppressed completely by ethanol pretreatment. The expression of these values in the form of a ratio (right panel) emphasizes the changes in plasma insulin levels relative to changes in plasma glucose. Ethanol plus saline produced a fall in the insulin/glucose ratio indicative of a decline in insulin that was greater than the fall in glucose. Since the plasma insulin rose and the plasma glucose level did not change in the ethanol plus isoproterenol group, the insulin/glucose ratio was significantly higher in this group ($P < 0.05$) compared to the saline plus isoproterenol group.

Pretreatment of fed rats with ethanol resulted in a modest reduction of the hyperglycemic response to epinephrine at 30 min after injection, but there was no appreciable change in the peak response at 60 min

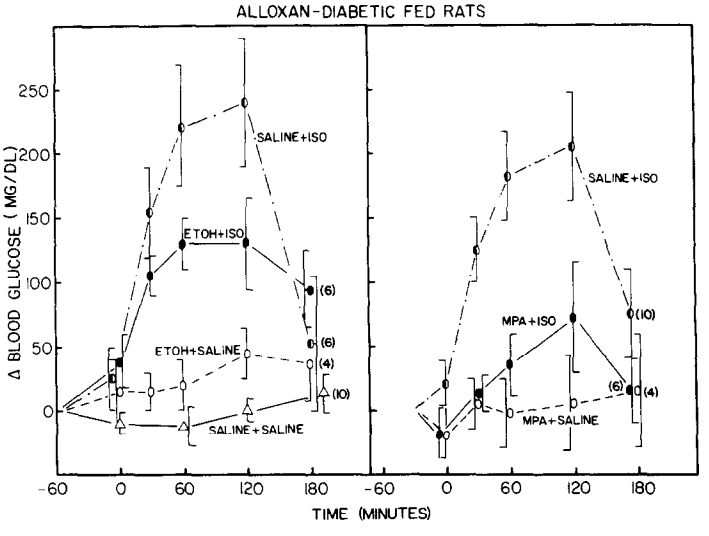

Fig. 3. Effect of ethanol and 3-mercaptopicolinic acid on isoproterenol-induced changes in alloxan-diabetic, insulin-controlled, fed rats. The values shown at 0 time illustrate the change produced by ethanol and 3-mercaptopicolinic acid alone 1 hr after treatment in each group of rats.

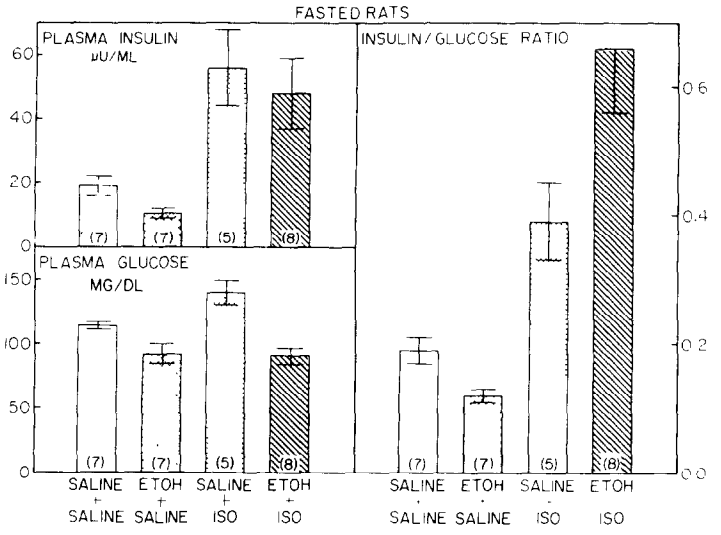

Fig. 4. Effect of ethanol on isoproterenol-induced changes in plasma insulin and glucose in fasted rats. Blood samples were taken 3 or 4 hr after injection of saline or isoproterenol. Each column is the mean ± S.E.

(Fig. 5). In fed rats, isoproterenol is virtually inactive as a hyperglycemic agent [8] but as shown in Fig. 5 isoproterenol produced a highly significant ($P < 0.001$) increase in plasma glucose levels in fed rats following pretreatment with ethanol.

Figure 6 (upper left panel) shows that ethanol pretreatment suppressed isoproterenol-induced insulin release in normal fed rats. Furthermore, as shown in the right panel the insulin/glucose ration was reduced significantly ($P < 0.05$) in the ethanol plus isoproterenol group as compared to the saline plus ethanol group. In contrast ethanol plus saline has no significant effect on basal (non-stimulated) insulin levels in fed rats.

DISCUSSION

In the present study, ethanol and 3-mercaptopicolinic acid suppressed the hyperglycemic response to isoproterenol more than the response to epinephrine in fasted rats. Moreover, lactate levels were significantly higher in ethanol-treated rats suggesting that ethanol was inhibiting the hyperglycemic response in part by reventing the re-cycling of lactate to glucose. These results are in general agreement with those of Yajima and Ui [7], who demonstrated inhibition of epinephrine-induced hyperglycemia by a series of gluconeogenic inhibitors.

Furthermore, pretreatment of alloxan-diabetic rats with ethanol or 3-mercaptopicolinic acid suppressed the augmented hyperglycemic response to isoproterenol. In diabetic rats, the exaggerated hyperglycemic response to isoproterenol is presumed to be the consequence of markedly reduced insulin release by isoproterenol [8] which allows stimulation of glucose production by the catecholamine. A portion of the

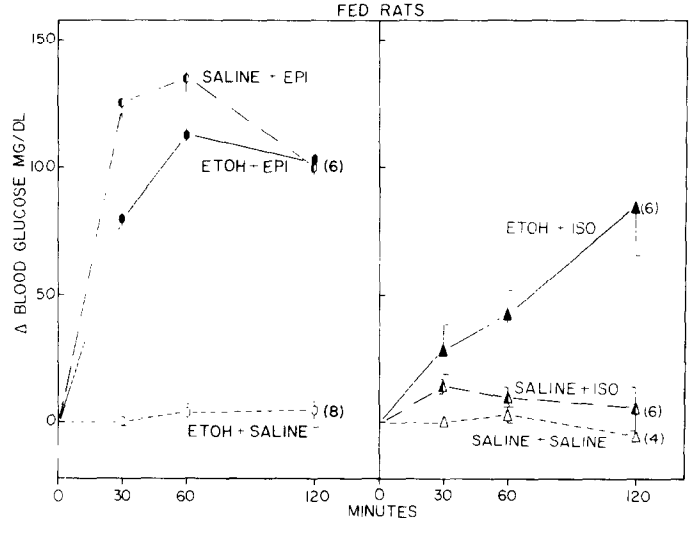

Fig. 5. Effect of ethanol pretreatment on epinephrine- and isoproterenol-induced hyperglycemia in fed, normal rats. Note that ethanol augmented the response to isoproterenol whereas the response to epinephrine was relatively unchanged. Each column is the mean ± S.E..

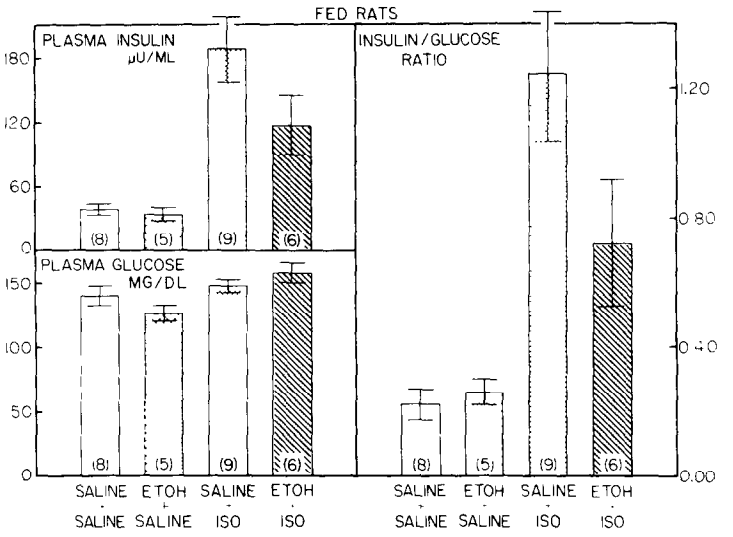

Fig. 6. Effect of ethanol on isoproterenol-induced changes in plasma glucose and insulin levels in fed, normal rats. Note that ethanol suppressed the insulin response to isoproterenol. Each column is the mean ± S.E..

increased glucose production is presumed to be the product augmentation of gluconeogenic processes.

Interestingly, pretreatment of fed rats with ethanol resulted in a hyperglycemic response to isoproterenol. Previous studies from this laboratory have demonstrated that the failure of isoproterenol to produce hyperglycemia in fed rats is the consequence of the potent insulin releasing effect of this β-adrenergic receptor agonist [8]. These observations led to the examination of the effects of ethanol on isoproterenol-induced insulin release in fasted and fed rats.

Ethanol inhibited isoproterenol-induced insulin release more in fed rats than in fasted rats. Moreover, the insulin–glucose ratio was lower in the isoproterenol-treated fed rats pretreated with ethanol than in the fasted group treated similarly. Inhibition of cyclic AMP-induced insulin release by ethanol has been demonstrated recently by Colwell *et al.* [14], and this may explain the inhibitory effects of ethanol on insulin release by isoproterenol in fed rats. Inhibition of isoproterenol-induced insulin release by ethanol would allow expression of the stimulatory effects of this catecholamine on hepatic production of glucose and thus hyperglycemia results.

In summary, ethanol and 3-mercaptopicolinic acid suppressed isoproterenol-induced hyperglycemia in fasted rats more than they suppressed epinephrine-induced hyperglycemia. As the result of inhibition of lactate utilization, ethanol pretreatment elevated lactate levels more than did saline pretreatment in isoproterenol-treated, fasting normal rats. Moreover, these inhibitors of gluconeogenesis tended to suppress the augmented hyperglycemic response to isoproterenol in alloxan-diabetic rats. In fed rats, pretreatment with ethanol suppressed isoproterenol-induced insulin release and thus isoproterenol produced significant hyperglycemia. The present study suggests that inhibition of catecholamine-induced hyperglycemia by ethanol and 3-mercaptopicolinic acid is the result of inhibition of gluconeogenesis and that the unmasking of isoproterenol-induced hyperglycemia in fed rats by ethanol pretreatment is the consequence of suppression of insulin release.

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