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Ethanol Induces Hyper and Hypoglycemia in Both Fasted and Nonfasted Rats Dependent on the Ambient Temperature[1]

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SOUZA, M. L. O. AND J. MASUR. *Ethanol induces hyper and hypoglycemia in both fasted and nonfasted rats dependent on the ambient temperature.* PHARMACOL BIOCHEM BEHAV **20**(5) 649–652, 1984.—An experiment was undertaken to characterize the influence of ambient temperature on ethanol-induced glycemic alterations in rats. Animals under two different feeding conditions (nonfasted or 48-hr fasted) were IP injected with 4.0 g/kg of ethanol. Blood glucose and body temperature were measured before, 90 and 180 min after drug administration. The rats were tested under ambient temperatures of 16, 21 and 32°C. Fed animals with a mean pre-drug glycemia of near 105 mg/100 ml presented a variation of blood glucose ranging from 50 mg/100 ml at 16°C to 140 mg/100 ml at 32°C. The glycemia from fasted rats, with a starting value of 70 mg/100 ml ranged from 20 to 115 mg/100 ml at 16 and 32°C, respectively. It was concluded that the administration of ethanol can render nonfasted as well as fasted rats hypo or hyperglycemic, depending upon the environmental temperature.

Blood glucose and ethanol Ethanol induced hyperglycemia Ethanol induced hypoglycemia
Ambient temperature and ethanol glucose alterations Body temperature and ethanol
Environmental temperature and ethanol

ACUTE doses of ethanol can cause an alteration in blood glucose levels. Oxidation of alcohol in the liver by the alcohol dehydrogenase pathway increases the NADH/NAD+ ratio [13]. One important metabolic consequence related to the changed redox state is the inhibition of hepatic gluconeogenesis. Consequently, hypoglycemia could occur in fasted, glycogen depleted organisms (e.g., [4, 9, 20, 25]). Conversely, as reviewed by Tabakoff *et al.* [24], in fed organisms ethanol may induce hyperglycemia, which has been ascribed to an increase in catecholamine release from the adrenal medulla and/or to a decrease in peripheral utilization of glucose.

An experiment designed to evaluate the glycemic response of rats under ethanol and exposed to different periods of fasting provided results in accordance with the above [22]. At high doses (3.0 and 5.0 g/kg) alcohol produced either an increase or a decrease of glucose levels depending upon the state of fasting. Starved rats showed a marked decline of blood glucose levels, while nonfasted rats, under the same doses of ethanol presented an increase in glycemia. Decreasing the dose to 1.0 g/kg abolished the glucose alterations.

However, there is further data indicating that the ethanol-induced glycemic alterations are not contingent only upon dose and feeding condition. Recently we reported that the values of blood glucose under ethanol varies accordingly to the ambient temperature in which the rats are tested [23]. The hypoglycemia observed in 48-hr starved rats injected with 4.0 g/kg of ethanol and tested in a room temperature of 21°C was partially prevented by raising temperature to 28°C. Also, hyperglycemia was more accentuated in fed rats when tested under 28°C when compared to values obtained at 21°C. Thus, besides being dose and feeding condition dependent, the ethanol-induced alterations in blood glucose are also ambient temperature-dependent.

A question to be addressed is to what extent are the ethanol glycemic alterations susceptible to variations in ambient temperature. As mentioned above, hypoglycemia was partially counteracted in fasted rats by raising room temperature from 21 to 28°C. Would it be possible, by further raising temperature, to induce hyperglycemia in fasted rats? Conversely, would the increase of blood glucose levels induced by ethanol in fed rats be shifted to hypoglycemia by decreasing ambient temperature? The present experiment was undertaken to test both possibilities.

METHOD

Subjects

Ninety-six male, Wistar rats, from our own colony, 75 days old weighing 200–250 g were used. They were main-

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tained in wire cages measuring 30×20×15 cm in groups of three animals in air conditioned laboratories at a temperature of 23±1°C in a 12 hr dark-light cycle (lights on from 7:00 to 19:00 hr).

Drugs

Ethanol (Merck Lab.). The concentration was 20% w/v in a 0.9% NaCl solution.

Blood Glucose Determinations

The glycemia was determined through the reaction of a blood drop, collected from the tail of the rat, with a Dextrostix strip using a Dextrometer Reflectance Colorimeter (Ames Div., Miles Lab.) [18, 22, 23].

Body Temperature Measurements

For the measurement of body temperature, the animals were placed in a small plastic restrainer and were inserted a vaseline-lubricated thermistor probe 2.5 cm into the rectum, until the stabilization of the measure (30 sec). A digital thermometer (Dixtal Tec. Ind. Com., Ltda., Model 128E) was used.

Procedure

In order to determine the influence of different room temperatures on the glycemic and thermic alterations induced by ethanol a single dose of 4.0 g/kg of ethanol was employed. The experiment was carried out on three different days respectively for each room temperature: 16, 21 and 32°C.

A group of sixteen animals was used for each temperature. One half of the rats was 48-hr fasted and the remaining rats were not. For this last group the food was removed in the beginning of the experiment. After two hours of acclimatization to the specified temperature, the rats were placed in plastic restrainers and the basal rectal temperature and blood glucose levels were taken. Then, the rats were injected IP with 4.0 g/kg of ethanol being afterwards returned to their home cages. New measurements of blood glucose and rectal temperature were taken 90 and 180 min after ethanol administration. All experiments were carried out between 13:00 and 17:00 hr to avoid possible diurnal effects.

The same procedure described above was followed for a control group, which received an equivalent volume of saline. Different animals were used not only for each group (ethanol or saline) as also for each room temperature (16, 21 or 32°C).

Statistical Analysis

The values of body temperature and blood glucose after drug administration were compared to the basal values (before drug) using the 0.95 confidence interval for small samples according to Hardyck and Petrinovich [7].

RESULTS

The effect of ethanol on blood glucose levels of rats is clearly dependent on the ambient temperature. Figure 1 illustrates the results obtained in fed and 48 hr-fasted rats. In both groups the dose of 4.0 g/kg of alcohol altered glucose levels. However, not only the magnitude of the alteration, but also the direction (fall or raise) was contingent upon room temperature. Thus, independently of the feeding condition, glycemic levels showed either a marked decrease or increase. In fed animals glucose levels at the lowest test temperature (16°C) declined to near 50 mg/100 ml 180 min after ethanol while at the highest temperature (32°C) the values of glucose reached 140 mg/100 ml. The results obtained with the fasted group are in the same direction. At 16°C blood glucose reached levels as low as 20 mg/100 ml when assessed 180 min after alcohol administration. Conversely, at 32°C the values were raised to 115 mg/100 ml. When comparing the glucose levels of fasted and fed rats under ethanol it has to be considered that, as expected, the baseline values for the starved ones were lower.

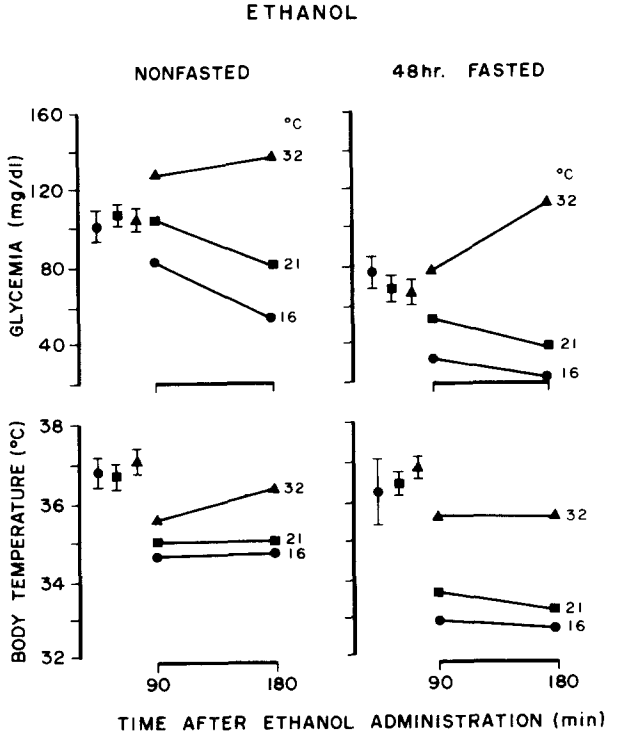

FIG. 1. Mean blood glucose levels and body temperature of nonfasted and 48 hr fasted rats after administration of 4.0 g/kg of ethanol, at 16°C (●—●), 21°C (■—■) and 32°C (▲—▲) room temperature. The isolated points indicate the mean basal values of each group, immediately before ethanol administration. Vertical bars represent 0.95 confidence intervals. Each group was formed by eight rats. Standard deviations of the post-injection value varied from 14.5 to 30.9% of the mean for glycemia and from 0.87 to 4.1% for body temperature.

The decline in body temperature under ethanol was also ambient temperature-dependent. In both feeding conditions the larger drop was observed under 16°C, the nonstarved and starved group presenting a decay of about 2 and 3°C respectively. At 32°C the decay in both groups was less accentuated, deviating about 1°C from the baseline values. It is noteworthy that although the starting points of the animals' temperatures were similar, the food-deprived rats were apparently more susceptible to the hypothermic action of alcohol when tested under 16 and 21°C. This is most likely due to the fact that acute starvation decreases the rate of ethanol oxidation in the liver [21].

Figure 2 shows that under saline, no major alterations in blood glucose levels or in body temperature were observed,

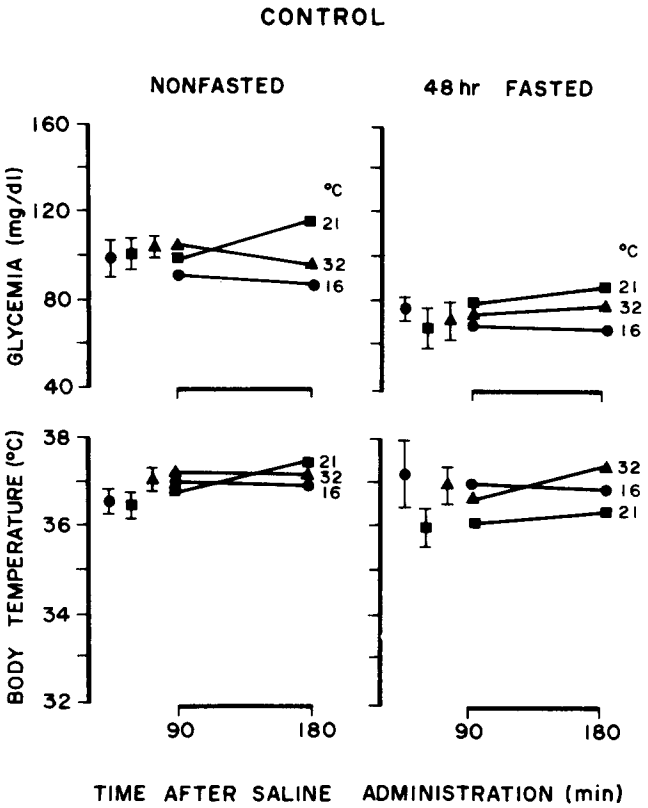

FIG. 2. To be read as in Fig. 1. Mean blood glucose levels and body temperature of nonfasted and 48 hr fasted rats after administration of control solution (saline). Standard deviations of the post-injection values varied from 2.8 to 18.8% of the mean for glycemia and from 0.62 to 1.85% for body temperature.

indicating that the effects observed with ethanol cannot be attributed either to the manipulation of room temperature or to restraint or other stressors.

No specific group was tested for controlling drug × restraint interaction, as the animals remained restrained for at most one minute (time required for performing the glucose/temperature measures). Also, at least for body temperature there is literature data indicating that there is no significant difference in response to a given dose of ethanol when restrained animals are compared to unrestrained ones [12].

DISCUSSION

The ambient temperature-dependent nature of blood glucose changes induced by ethanol is demonstrated by the present data. In our previous report [23] it was shown that the hypoglycemia induced by 4.0 g/kg of ethanol in 48-hr fasted rats could be partially counteracted by raising room temperature from 21 to 28°C. The present data show that under the same dose of ethanol, hypoglycemia presented by 48-hr starved rats can be shifted to hyperglycemia through exposure to a warmer room temperature. This is apparently a paradoxal finding. In the fasted animal there is a depletion of glycogen in the liver being blood glucose maintained from gluconeogenesis. Ethanol is known to inhibit hepatic gluconeogenesis by decreasing the $NAD^+/NADH$ ratio in the cytosol of hepatocytes (e.g., [11, 13, 24]). Thus, how can rats submitted to 48-hr of food deprivation and under a high dose of ethanol present a marked increase of blood glucose levels? Although no definitive explanation can be provided, it can be speculated on the possible role of renal neoglucogenesis. Although the liver is considered the most important organ in the control of glycemia, it was described that under special physiological circumstances the kidney cortex may have an important gluconeogenesis role [3,17]. Jones *et al.* [10] already suggested that renal glucose production could act as a compensatory mechanism preventing severe hypoglycemic responses in starved organisms after ethanol. Chan *et al.* [2] showed an increase in the activities of key enzymes of gluconeogenesis in the kidney cortex of rats after ethanol. In a recent experiment we observed that bilateral nephrectomy enhanced the hypoglycemic response to ethanol in 48-hr fasted rats exposed to a room temperature of 24°C [15], further suggesting the importance of renal mechanisms.

Several explanations have been provided in the literature to explain the refractoriness to alcohol hypoglycemia observed in non-diabetic obese subjects and in chronic malnourished alcoholics [1,19]. They are based mainly on assumptions of an increased glycogen store, a diminished glucose utilization, or a reduced activity of liver alcohol dehydrogenase [20]. It also has been suggested that hypoglycemia might occur only in subjects with an unusual susceptibility which according to Hed *et al.* [8] could be a disturbed release of insulin.

The data here reported also show that nonfasted rats presented a marked decay in blood glucose at an ambient temperature of 16°C. Thus, at least in rats, the assumption that starvation is a necessary although not a sufficient condition to promote ethanol hypoglycemia does not hold, as by manipulation of room temperature a marked decay of blood sugar was observed in fed rats.

What is the nature of the relationship between ambient temperature and alcohol induced glycemic changes? Haight and Keatinge [6] suggested that the depletion of blood glucose levels contributes to the ethanol-induced decrease in body temperature. However, there are data showing that at least in rats this causality does not hold. Thus, Myers [16] reported that pre-treating rats with glucose did not attenuate the decay in colonic temperature induced by alcohol. Also, in previous papers we have shown that hypothermia and hyperglycemia may occur concomitantly [22,23]. The data presented here show that hypo and hyperglycemia can be observed independent of the feeding condition, but contingent upon room temperature. It is also shown, confirming data of other authors, that the magnitude of the change body temperature is a function of the ambient temperature (e.g., [5, 14, 16]).

The interaction between feeding condition and ambient temperature becomes evident by analysing the present data together with results already reported [22,23]. Thus, a major hypoglycemic effect in response to ethanol was observed in fasted animals at a room temperature about 24°C [22] while for fed animals the temperature had to be decreased to 16°C as herein reported (Fig. 1). Conversely, hyperglycemia was the rule at 24°C for nonfasted rats [22] while for fasted rats a marked raise in blood glucose was observed only at 32°C (Fig. 1). It becomes clear that although the glycemic alterations induced by alcohol are extremely susceptible to variations of ambient temperature the shift of room temperature required is dependent of the feeding condition.

Another worthwhile point is related to the relationship between ethanol toxicity and ambient temperature. Malcolm and Alkana [14] reported data showing that the depression induced by ethanol and assessed through sleep-time is more evident in mice submitted to warm environmental temperature. Based on this data the authors suggest that "maintaining normal body temperature in overdose patients might enhance ethanol toxicity, whereas, holding the body temperature bellow normal may reduce toxicity." The present data indicating that an opposite conclusion would be appropriate for the hypoglycemiant effect of alcohol, show the complexity of the mechanism of action of this drug.

Myers [16] pointed out that, considering ethanol-induced body temperature changes, different results could be obtained when done in different climate conditions. In our opinion, due to the straight relationship as reported herein, between ambient temperature and the ethanol effects on blood glucose levels the same conclusion could be extended to the experiments and/or clinical observations on the glycemic alterations observed after ethanol.

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