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Does Hypothermia Play a Relevant Role in the Glycemic Alterations Induced by Ethanol?[1]

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OLIVEIRA SOUZA, M. L. AND J. MASUR. *Does hypothermia play a relevant role in the glycemic alterations induced by ethanol?* PHARMAC. BIOCHEM. BEHAV. 16(6) 903–908, 1982.—The potential role of ethanol-induced hypothermia on the glycemic alterations induced by this drug were evaluated. In Experiment 1 ambient temperature was manipulated. After 4.0 g/kg of ethanol blood glucose levels and body temperature were assessed in fed or 48 hr starved rats at either 21°C or 28°C room temperature. Hyper or hypoglycemia was observed depending on both the feeding condition and the environmental temperature. In Experiment 2, this hypothesis was tested by determining if rats tolerant to the thermic effects of ethanol would show a decreased glycemic response. The results support this assumption.

Hypothermia and ethanol Glycemia and ethanol Glycemia/body temperature relationship
Tolerance to ethanol Room temperature and ethanol

ALTHOUGH the hypothermic and glycemic alterations induced by ethanol have been extensively investigated (e.g., [1, 4, 10, 16, 22, 23]) there is a paucity of experimental data regarding a possible link between both phenomena. Haight and Keatinge [5] suggested that hypothermia occurs as a consequence of the ethanol-induced hypoglycemia. Conversely, we recently reported that in 48 hr starved rats, when hypoglycemia and hypothermia occurred, the fall in body temperature paralelled or preceded the decrease in blood glucose levels. Furthermore, it was also observed that rats not deprived of food were hyperglycemic while being hypothermic [20].

One approach to provide further data on this issue is to observe whether altering the hypothermic action of ethanol alters the glycemic reaction. This hypothesis was tested in Experiment 1 by presenting alcohol to starved and fed rats at different environmental temperatures, as this procedure has been reported to alter the hypothermic action of ethanol [3, 13, 21].

Another possibility is to observe whether the development of tolerance to the hypothermic effect of ethanol [2,18] prevents the glycemic alterations. This hypothesis was tested in Experiment 2.

METHOD

Animals

Male Wistar rats from our own colony, 75 days old at the beginning of the experiment, were used. After weaning, at 25 days of age, they were housed in wire cages measuring 30×20×18 cm, three per cage. They were fed ad lib and maintained at a room temperature of 22–24°C on a 12 hr light-dark cycle.

Drugs

For IP injections, ethanol for analysis diluted with saline to a strength of 20% (w/v) was used, while for oral consumption, a solution of 15% ethanol v/v with 0.15% saccharin mixed in tap water was employed.

Body Temperature Measurements

Rectal temperature was obtained with a Dixtal thermometer (Dixtal Tec. Ind. Com., Ltda., Model 128E) by placing the rat in a small restrainer and inserting a vaseline-lubricated thermistor probe 2.0 cm into the rectum, until the stabilization of the measure (20–30 sec).

Blood Glucose Determinations

Blood glucose was measured through the reaction of a blood drop collected from the tail of the rat with a DEXTROSTIX strip using the DEXTROMETER/DEXTROSTIX system, a digital version of the DEXTROSTIX-EYETONE method [17].

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Experiment 1: Influence of Environmental Temperature on Glycemic and Thermic Alterations Induced by Ethanol

Two groups of 32 rats were tested at a room temperature of either 21°C or 28°C. One half of the rats were 48 hr food deprived and the other remaining rats were not. These groups were further divided so that half of the rats in each of these groups received saline and the other half ethanol. Therefore, four subgroups of 8 animals each were used at each temperature. 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. Immediately after these measures, the animals were injected IP with 4.0 g/kg of ethanol or an equivalent volume of saline, and the rectal temperature and the blood glucose were again measured after 45, 90 and 180 min. All experiments were carried out between 1:00 and 5:00 p.m. to avoid possible diurnal effects. During this period the animals had no access to food.

Experiment 2: Influence of Tolerance to Hypothermic Effect of Ethanol on Glycemic Alterations in Starved Rats

For 30 days two groups of 8 rats each, received a 15% (v/v) solution of ethanol with saccharin (0.15%) as their sole fluid source and were injected daily with 2.5 g/kg of ethanol IP. The animals had free access to food. A third group of 6 rats were daily injected with an equivalent volume of saline. Their food and fluid consumption (0.15% saccharin solution) was determined on the basis of the average daily intake of the two groups receiving ethanol.

On the experimental day, the solutions of ethanol or saccharin were withdrawn at 8:00 a.m. and replaced by tap water ad lib. By 1:00 p.m., the rats were 48 hr food deprived and 4.0 g/kg of ethanol was injected IP in one of the ethanol treated group, while saline was injected into the other group. This second group was designed to test for the possibility that withdrawing alcohol from 8:00 a.m. to 1:00 p.m. could *per se* alter the parameters studied. The group injected daily with saline received 4.0 g/kg of ethanol. The same procedure described in Experiment 1 was followed in a room temperature of 23°C.

RESULTS

Experiment 1

Figure 1 shows the results obtained when ethanol was administered to the fed rats at temperatures of either 21 or 28°C. The animals were first divided according to the direction of the glycemic alterations, using as criterion the departure from the basal values, considering 0.99 confidence limits for small samples [6]. Therefore, rats that showed either no alteration or an increased glycemia were considered apart from those who presented a fall in blood glucose levels. The body temperature of the rats was, accordingly, also represented separately.

At 21°C, 6 out of 8 rats presented either no alteration or an increase of glycemia, with a mean above the basal values at 45 and 90 min after ethanol. Conversely, the remaining 2 animals showed hypoglycemia. Although all rats at 21°C presented a decrease in body temperature, there was a trend for a more pronounced decay in the two hypoglycemic rats. In this particular case, no statistical analysis was performed considering the small size of the samples (six and two). At room temperature of 28°C (Fig. 1) the glycemia of the 8 rats injected with ethanol was altered only in the direction of hyperglycemia. It is noteworthy that the fall in body temperature of these 8 rats was less pronounced than the observed with the 8 rats under 21°C, at 90 and 180 min (Student t-test, $p \leq 0.05$).

The results obtained with the 48 hr starved rats are represented in Fig. 2, using the same criterion described for Fig. 1. At room temperature of 21°C all ethanol-injected rats presented marked hypoglycemia and hypothermia. Two rats from the control group also showed a slight decrease in glucose levels, although temperature remained unchanged. Different results were observed when the room temperature was increased to 28°C. In this condition, the majority of the ethanol-injected rats (6 out of 8) showed mean blood glucose levels slightly above the basal values. Only 2 animals presented a slight decay of glycemia at 180 min after ethanol, showing also the trend for a greater fall in body temperature. Again, as occurred with the fed group, the hypothermia observed for the 8 ethanol-treated rats under 28°C was less pronounced than for the group under 21°C, at 45, 90 and 180 min (Student t-test, $p \leq 0.001$).

Experiment 2

Figure 3 shows that withdrawal from ethanol from 8:00 a.m. to 1:00 p.m. did not alter either glycemia or body temperature as the basal levels of the 3 groups are similar (Duncan's new multiple range test; $p > 0.05$). In the same figure the alterations in glycemia and body temperature induced by 4.0 g/kg of ethanol in rats either chronically treated with ethanol or receiving the drug for the first time can be observed. Partial tolerance developed to the hypothermic effect of ethanol, as the decrease of temperature in the chronically treated group was less pronounced than that observed in the animals receiving ethanol for the first time. The statistical analysis using the Duncan's new multiple-range test at a level of 0.05 shows that the ethanol chronically treated group presented body temperature values significantly different from both, the saline and the ethanol acute injected groups.

Concerning glycemia a similar trend was found, that is, the chronic plus acute ethanol-treated rats showed less alteration than the acute ethanol-injected group. The Duncan's new multiple range test at a level of 0.05 revealed that the chronic plus acute ethanol-injected group differed from the chronic ethanol plus acute saline-injected animals only at 90 min after injection, while the chronic saline plus acute ethanol-injected rats differed at 45, 90 and 180 min.

DISCUSSION

Pohorecky and Rizek [15] studying the role of ethanol-induced hypothermia on free fatty acids, corticosterone, tyrosine levels and behavioral parameters, concluded that the decrease in body temperature influences and interacts with other actions of ethanol. The data here presented show that, in rats, the glycemic response to this drug is also dependent on the magnitude of body temperature alterations.

The association between hypothermia and hypoglycemia induced by ethanol has been described in the literature, as a decline in glycemia inducing a decrease in body temperature [5]. The present results, extending observations from our previous work [20], show an inverse relationship. Thus, by altering environmental temperature (Experiment 1), a variation in both the hypothermic and glycemic responses induced by ethanol was observed. This was most evident in the

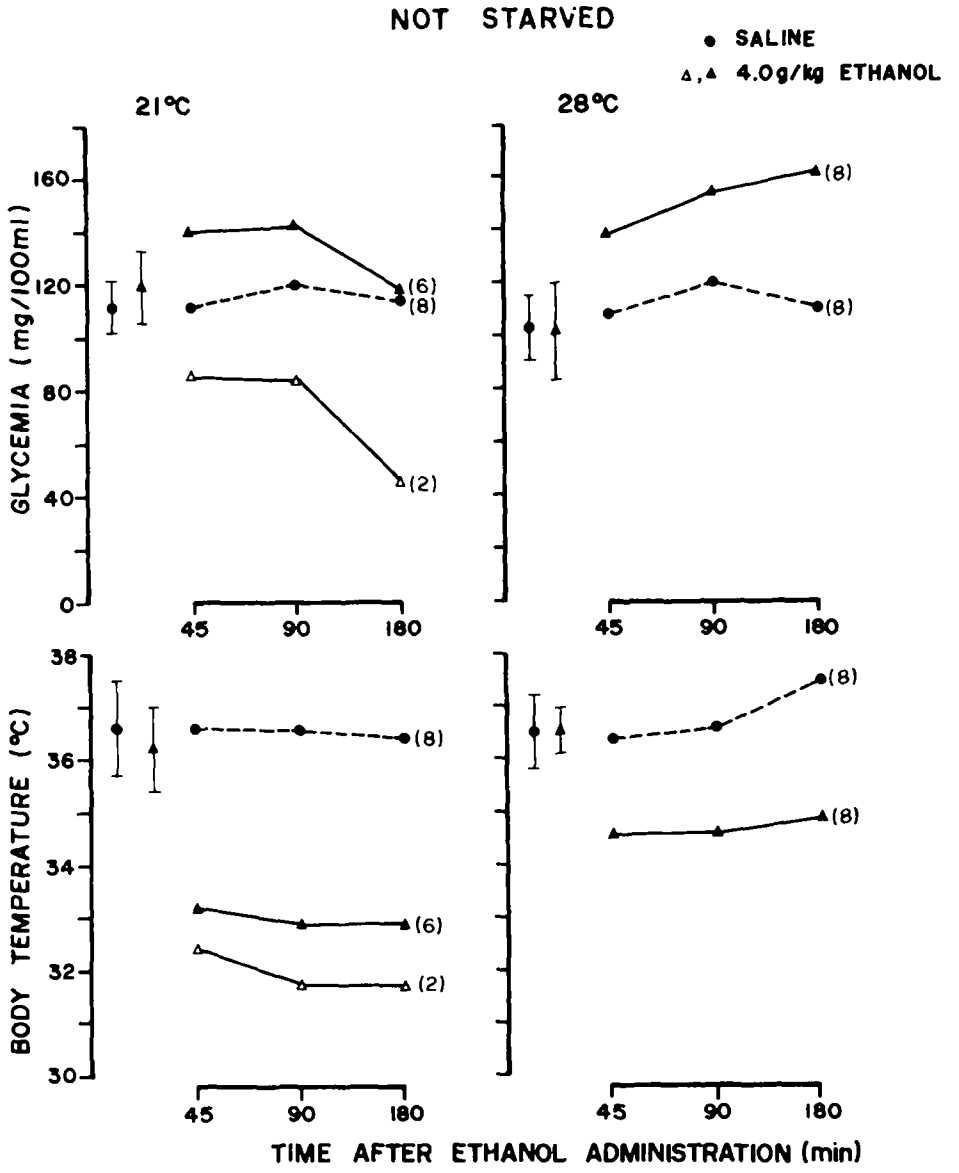

FIG. 1. Mean blood glucose levels and body temperature of normal fed rats, after saline or 4.0 g/kg of ethanol injections, at 21°C and 28°C room temperature. The isolated points indicate the mean basal values of each group, immediately before injections. Vertical bars represent 0.99 confidence limits. The animals whose mean values of glycemia, after the ethanol injections, were within or above (▲) these limits are represented apart from those which showed decreased glycemia (△). The number of animals of each of these groups is indicated within brackets. Standard deviations of the post-injection values varied from 7.5 to 25.3% of the mean for glycemia and from 1.1 to 5.6% for body temperature.

48 hr starved rats, which at 21°C were markedly hypothermic and hypoglycemic, while at 28°C they presented a less pronounced decline of body temperature along with minor glycemic alterations.

The relationship between the magnitude of the hypothermic and glycemic alterations under ethanol is strengthened by the results obtained in Experiment 2, which showed that the development of tolerance to the hypothermic effect of ethanol goes along with a decreased hypoglycemic response.

The explanation provided in the literature for the hypoglycemia induced by ethanol in food deprived subjects is based on the inhibitory effect of this drug on neoglucogenesis (e.g., [9, 11, 19]), which is the primary mechanism responsible for hepatic glucose output in food deprivation conditions [12,22]. However, if inhibition of neoglucogenesis alone is to account for hypoglycemia, raising the room temperature should not prevent the decrease in blood glucose, as actually occurred in the present experiment.

Another interesting finding in Experiment 1 was that al-

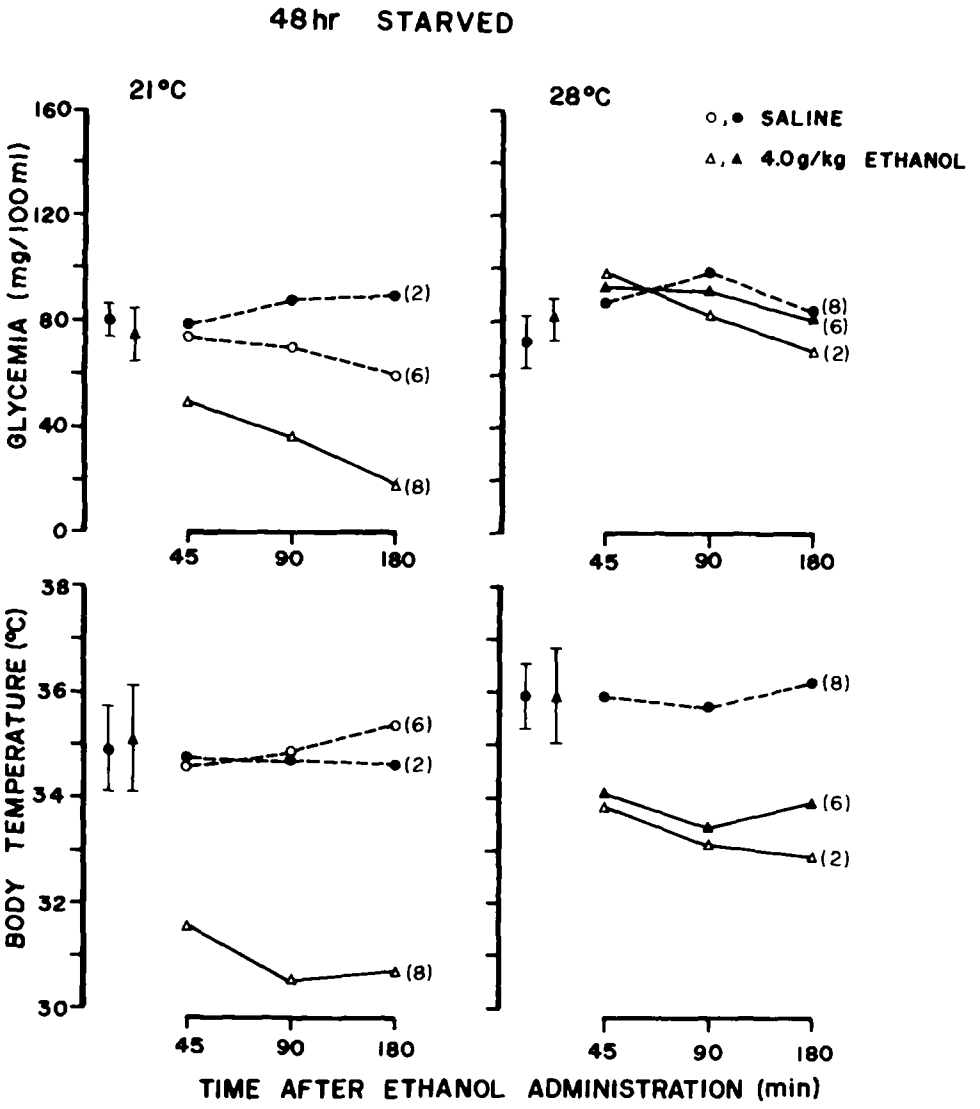

FIG. 2. Mean blood glucose levels and body temperature of 48 hr-starved rats, after saline or 4.0 g/kg of ethanol injections, at 21°C and 28°C room temperature. Standard deviations of the postinjection values varied from 0 to 33.2% of the mean for glycemia and from 0.2 to 2.6% for body temperature. To be read as in Fig. 1.

though at 21°C the prevalent glycemic response of the fed group to alcohol was either an increase or no alteration in glycemia, 2 out of the 8 rats developed hypoglycemia. As these rats were not starved how can this fall in glucose concentrations be explained? Increased rate of gluconeogenesis is a significant source of glucose in animals exposed to very low temperature (4°C) [8,14]. It is possible that these two particular animals, being specially sensitive to cold, could not rely on neoglucogenesis for glucose production, as it was impaired by ethanol. Based on this hypothesis it should be expected that at still lower room temperature than used in the present experiment an increased number of fed rats should present hypoglycemia under ethanol.

In combination our results show that ethanol-induced hypoglycemia depends not only on the state of nourishment of the organism but also on the degree of hypothermia. If hypothermia is magnified, hypoglycemia can occur even in fed animals. Conversely, by reducing the hypothermic effect, hypoglycemia can be partially prevented in starved rats.

Based on this finding it is interesting to analyse recent data in the literature showing that ethanol in young rats induced hypoglycemia whereas older rats exhibited raised glucose concentrations [7]. As suggested by the authors, a reduced glycogen storage in young organisms, may make them more sensitive to the inhibitory action of alcohol on neoglucogenesis. However, an alternative possibility could be that early in development, a more precarious thermoregula-

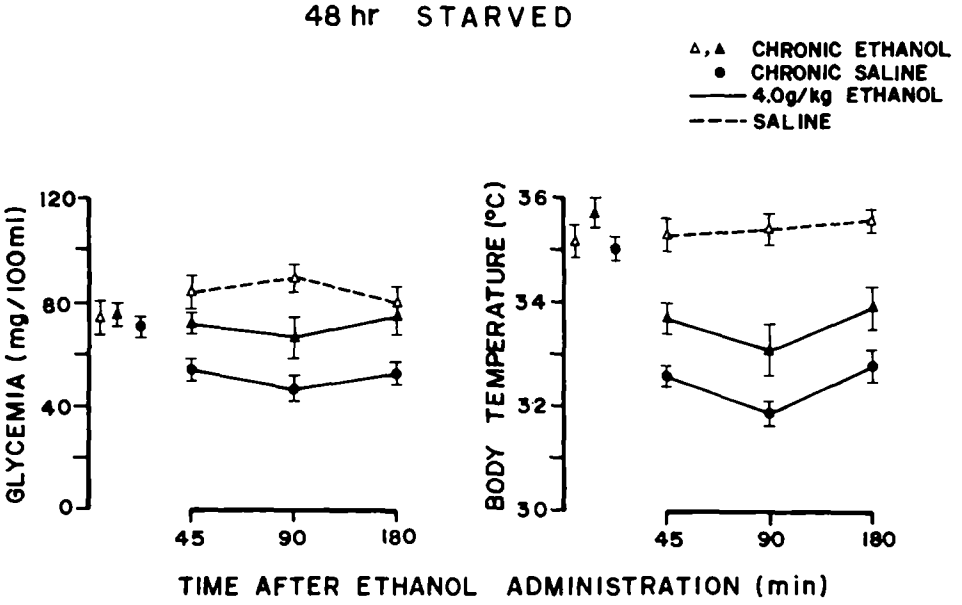

FIG. 3. Mean body temperature and blood glucose levels of 48 hr starved rats after IP saline or 4.0 g/kg of ethanol at 23°C room temperature. The animals were previously treated for 30 days with daily administration of ethanol or saline (see text for details). Vertical bars indicate S.E.M. Body temperature for the chronic plus acute ethanol-treated group was significantly different from both, the chronic ethanol plus acute saline or the chronic saline plus acute ethanol treated rats. Glycemia for the chronic plus acute ethanol-treated group was significantly different from the chronic ethanol plus acute saline-injected rats only at 90 min, while the chronic saline plus acute ethanol-injected animals differed at all intervals considered (Duncan's new multiple range test at a level of 0.05).

tory control would make rats more sensitive to the ethanol hypothermic effect, and consequently present a hypoglycemic response.

A question to be formulated concerns the relevance of this data for the clinical condition of alcohol intoxication. It is accepted that humans have a greater capacity to maintain normal body temperature than do rodents, in that humans only suffer ethanol-induced hypothermia under unusually severe environmental conditions [4]. But to what extent this greater thermoregulatory capacity functions as a protection against hypothermic and hypoglycemic effects of alcohol in undernourished human beings is an issue open to investigation.

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