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Effects of insulin-produced hypoglycemia in combination with ethanol on spontaneous motor activity in rats

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Abstract

The hazardous consequences of drinking alcohol by persons receiving insulin treatment is indicated by clinical reports, but little controlled research has investigated the combination of hypoglycemia and ethanol intoxication. Ethanol's effect on spontaneous motor activity (SMA, detected by Opto-Varimax activity meters) in hypoglycemic (HG) rats was determined over a range of ethanol doses in two experiments. Combinations of insulin and ethanol were administered intraperitoneally to moderately food-deprived rats. Blood glucose was measured before and after a 30-min SMA-monitoring period. In Experiment 1, ethanol doses of 300, 600 and 1200 mg/kg were combined with insulin at doses 0.5 and 2 U/kg. A second experiment tested a narrower range of drug doses (ethanol 600 and 1200 mg/kg, insulin 1 U/kg) under slightly different procedures. After insulin treatment, blood-glucose levels dropped to approximately 40–60% of control levels and this HG was accompanied by decreased SMA. Ethanol did not influence blood-glucose levels, nor did it potentiate the HG produced by insulin. Combination of HG and the highest ethanol dose potentiated the SMA-depressant effect in both experiments, whereas lower ethanol doses partially reversed the suppression of motor activity in HG rats.

Keywords: Ethanol; Hypoglycemia; Insulin; Rats; Spontaneous motor activity

1. Introduction

Both ethanol intoxication and hypoglycemia (HG) are conditions that disturb nervous system function. The cognitive and behavioral results of these two sources of CNS malfunction share some similarities, and in some cases, HG has been incorrectly diagnosed as ethanol intoxication (Kerr, 1993; Marks, 1981). Both conditions can cause cognitive impairment, behavioral depression, and in extreme cases, death.

The basis of CNS malfunction caused by ethanol is of course quite different from that resulting from HG. Ethanol's mechanisms of action are complex, involving either indirect agonist or antagonist effects on several neurotransmitter systems including GABA (cf. Mihic and Harris, 1997), glutamate (cf. Gonzales and Jaworski, 1997), and serotonin (cf. Lovinger, 1997), in addition to temporary structural alteration of the neuron membrane (Hunt, 1985). Glucose is the sole energy source ordinarily available for neurons (Amiel, 1995; McCall, 1993), and when plasma glucose levels fall below a critical value, progressive degrees of neuronal malfunction result from the decreased utilization of energy, including reduced synthesis of ATP (Bendtson, 1993; Benzi and Agnoli, 1987). Permanent CNS damage occurs if HG is severe and prolonged (Auer et al., 1984).

A very large body of research literature exists concerning ethanol's complex and varied behavioral and physiological effects. Some aspects of HG have also been studied extensively, including counterregulatory responses and other physiological sequelae (Cryer, 1993; Kerr et al., 1989). Self-recognition of the HG condition in human subjects has been studied (Cryer et al., 1989; Deary et al., 1993), as has the cognitive impairment and other types of performance deficits that accompany HG (Blackman et al., 1990; Heller and Macdonald, 1996). Much less attention has been given to the behavioral correlates of HG in animal subjects. Reduction in spontaneous motor activity (SMA) of HG rats

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has been described (Duncan and Koontz, 1994), as has increased time to complete trials in the radial-arm maze (Duncan and Gaffney, 2001). HG produces an interoceptive stimulus that can be discriminated by rats in the operant drug-discrimination paradigm (Duncan and Hooker, 1997; Duncan and Lichty, 1993), and Pavlovian conditioning of glycemic and behavioral responses to the onset of HG has been investigated (e.g., Siegel, 1975).

Both ethanol and HG alter CNS function with resultant behavioral and cognitive impairment, and there is considerable clinical interest in the results of ethanol administration in combination with HG (Kerr, 1993). Clinical observations dating from the 1960s have indicated that combinations of HG and ethanol intoxication may cause extremely severe CNS depression that can result in permanent disability or death (Arky et al., 1968; Berczeller, 1993). In one of the very few controlled studies dealing with the behavioral effects of this combination (Kerr et al., 1990), ethanol administration decreased the ability of both normal and diabetic humans to recognize the HG state. This "hypoglycemia unawareness" occurred even though some physiological responses associated with HG were potentiated by ethanol.

Previous work conducted in this laboratory (Duncan and Koontz, 1995) demonstrated that insulin-produced HG decreases SMA in rats prior to emergence of a more extreme state of unresponsiveness and coma. Ethanol also has a dose-related depressant effect on rat SMA (Criswell et al., 1994; Duncan et al., 2000; Masur et al., 1986), thus SMA was chosen as a relatively simple direct behavioral indicator of the depressant action of HG, ethanol, and their combination. The first hypothesis tested here was that since both drugs decrease SMA, a moderate to high ethanol dose would depress this behavior to a greater extent in HG rats, compared to euglycemic (normal blood-glucose level) animals. Under some conditions ethanol disinhibits or activates behavior (Criswell et al., 1994; Frye and Breese, 1981; Pohorecky, 1977), so a second hypothesis was that a low dose of ethanol would increase SMA suppressed by early-stage HG.

The effects of ethanol over a range of doses on SMA in euglycemic, compared to HG rats, were determined in two experiments. Blood-glucose levels were monitored in an attempt to determine whether any altered behavioral effect was due to exacerbation of HG, or to some other type of interaction between the two drug treatments.

2. Experiment one

2.1. Method

2.1.1. Animals

Thirty male rats of the Long–Evans strain were obtained from the Harlan Sprague–Dawley. These animals were 90 days of age, weighed approximately 340 g at the start of the experiment, and were housed individually in a room with light onset at 0800 h and darkness onset at 2000 h. Water was freely available, but food was restricted as described below.

The protocol for these experiments was approved by the Old Dominion University IACUC review process, which ensures compliance with ethical standards as outlined by the National Institutes of Health, and the US Department of Agriculture.

2.1.2. Drugs

Ethanol was diluted with saline to provide the following concentrations (w/v) for the corresponding doses: 6%, 300 mg/kg; 12%, 600 mg/kg; 15%, 1200 mg/kg. Injection volume for the two lowest ethanol doses was 6 ml/kg, and for the highest ethanol dose and saline injection, 10 ml/kg. Regular Iletin Insulin was diluted with distilled water to a concentration of 2 U/ml.

2.1.3. Apparatus

Blood-glucose levels were measured with a Lifescan "One-Touch" glucometer manufactured by Johnson and Johnson, and "First-Choice" reagent strips generously donated by Lifescan. A similar measuring system, designed for self-monitoring of blood-glucose by diabetic persons, compares favorably in accuracy and reliability to a research-quality glucose analyzer manufactured by Beckman instruments (Messier and Kent, 1995).

Motor activity was detected by four Opto-Varimax Mini "A" animal activity meters, manufactured by Columbus Instruments. The test enclosures were translucent plastic containers (L58 × W42 × H23 cm). Each enclosure had two linear arrays of 16 IR light sources paired with sensing photocells. The lower array was 1 cm, and the upper array was 13 cm above the floor of the test enclosure. Successive interruptions of adjacent light beams in the lower array were registered as ambulatory activity counts and any interruption of an upper-array beam registered as a "rearing" count. A computer-recorded total ambulatory and rearing counts that occurred during successive 15-min periods.

2.1.4. Experimental design and procedure

The rats were given six daily 30-min adaptation sessions in the activity test boxes, and were fed 23–25 g of rat chow each day. SMA levels during the three final adaptation sessions were used to assign rats to three insulin dose groups, resulting in similar group mean levels of baseline activity. Drug effects were determined over the course of four SMA test sessions, conducted at 48-h intervals. Twenty-four hours prior to the first test session each rat was given 8 g of food (approximately one-third the normal daily ration). Immediately after each SMA test 40–42 g were given, followed 24 h later (24 h prior to the next SMA test) by the 8-g ration. Under this feeding schedule the rats maintained a relatively stable body weight during the 8-day

period of test procedures, and when tested were at the moderate level of food deprivation necessary for a low dose of insulin to produce HG.

Insulin dose was a between-subjects independent variable, with each group of 10 rats receiving one insulin dose of 0, 0.5, or 2 U/kg prior to each SMA test. Ethanol dose was a within-subject variable, with each rat receiving all four doses (including dose 0, saline injection) during the course of the 4 test days. The sequence of ethanol dose administration was counterbalanced within each insulin dose group in a modified Latin-square design.

After an initial intraperitoneal injection of insulin or saline, rats were returned to the home cage for 20 min. At this time, a drop of blood was drawn from the tail for the first blood-glucose measurement, followed immediately by the second intraperitoneal injection (ethanol or saline), and the start of the SMA test. At the end of the 30-min activity-recording period, a second BG reading was obtained and the rat replaced in the home cage and fed as described above.

2.1.5. Statistical analyses

Data were analyzed by a factorial analysis of variance (ANOVA). Within-subject factors for analysis were ethanol dose, and time after injection (15-min blocks), and insulin dose was a between-subjects factor. Where indicated, specific pairs of conditions were compared by means of Tukey's tests (significance level, $P < .05$).

2.2. Results

2.2.1. Motor activity

The pattern of differences among group means for rearing and for ambulatory activity were very similar. The rearing-activity results are not reported here, and "SMA" refers to ambulatory (locomotor) activity counts. The means and standard error of the total SMA counts for the three insulin dose groups on the final adaptation day were: insulin 0, 436 (±53); insulin 0.5, 430 (±59); insulin 2, 475 (±61).

The effects of each drug, including the time-course of these effects on SMA were determined by a three-way ANOVA. This analysis revealed significant main effects of time periods [$F(1,27) = 18.32$, $P < .001$) and of Insulin × Time period interaction [$F(2,27) = 4.15$, $P < .03$). None of the remaining time-related interaction effects were significant, including the three-way Time × Insulin × Ethanol interaction effect. The most obvious time-related pattern revealed by the 15-min means was a marked decrease in activity in all groups under all treatment conditions, with the decrease between the first and second periods being the greatest for the no-insulin group.

Although the Ethanol × Time period interaction was not significant, the drug effects and their interaction during the entire 30-min recording session was revealed by significant main effects of insulin dose [$F(2,27) = 6.53$, $P < .005$), ethanol dose [$F(3,81) = 3.53$, $P < .02$], and Insulin × Ethanol interaction [$F(6,81) = 2.31$, $P < .05$]. The pattern of mean SMA scores (Fig. 1) in conjunction with further analysis via Tukey's tests indicate the following: Ethanol had no significant effect at any dose in the absence of insulin treatment; insulin at both doses resulted in significantly decreased SMA, but effects of the two insulin doses in the absence of ethanol were not significantly different. In the insulin 0.5 group, ethanol at 600 mg/kg produced significantly greater activity compared to the no-ethanol condition, and ethanol at 1200 mg/kg resulted in less activity than the control condition. In the insulin 2 group, ethanol at 300 mg/kg produced significantly greater activity, and at 1200 mg/kg significantly less activity, both differences in comparison to the no-ethanol condition. Although the mean of the 600 mg/kg condition was markedly lower than that of the no-ethanol control, the difference failed to reach significance due to the variability of SMA levels in this Insulin × Ethanol condition.

2.2.2. Blood-glucose levels

Insulin at both doses produced marked HG that was apparent at the 20-min postinjection BG reading, and that became more pronounced at the 50-min measurement (Table 1). The ANOVA revealed the following significant effects: main insulin dose [$F(2,27) = 122.4$, $P < .001$], main time delay [$F(1,27) = 117.2$, $P < .001$], and Time × Insulin group interaction [$F(2,27) = 38.57$, $P < .001$]. The ethanol dose main effect and the remaining interaction effects were not significant. Tukey's tests revealed that at the second time delay (50 min) the BG level of the insulin 2-mg/kg group was significantly lower than that of the insulin 0.5-mg/kg group. Both insulin dose groups' BG levels were significantly lower than that of the no-insulin control group at both postinjection delays. At the first postinjection delay the high insulin dose group mean BG level (pooled across all ethanol

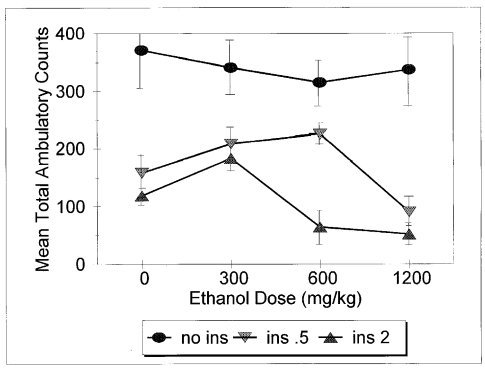


Fig. 1. Mean (±S.E.M.) total ambulatory activity counts during 30 min following ethanol injection in euglycemic (no insulin) and HG (insulin doses = 0.5, 2.0 U/kg) rats. Each insulin dose represents a separate group of 10 rats. Ethanol dose was a within-subject (repeated measures) manipulation. Insulin (or saline) injection 20 min prior to, and ethanol (or saline) injection immediately prior to start of activity recording session.

Table 1
Blood-glucose levels after insulin and ethanol treatment

		Mean (±S.E.M.) blood-glucose level (mg/dl) Post insulin-injection delay (min)	
Insulin dose (U/kg)	Ethanol dose (mg/kg)	20	50
0	0	71 (±4)	73 (±5)
	300	66 (±5)	65 (±4)
	600	67 (±4)	67 (±3)
	1200	68 (±3)	73 (±3)
Mean of all EtOH doses		68.0	69.5
0.5	0	52 (±4)	35 (±2)
	300	55 (±5)	39 (±3)
	600	56 (±4)	36 (±4)
	1200	57 (±6)	34 (±4)
Mean of all EtOH doses		55.0	36.0
2	0	54 (±3)	30 (±3)
	300	51 (±4)	27 (±1)
	600	52 (±4)	27 (±3)
	1200	51 (±3)	31 (±3)
Mean of all EtOH doses		52.0	28.8

Insulin conditions represent separate groups, $n = 10$ each.
Ethanol injected immediately after first blood-glucose reading.

doses) was 76% of the non-insulin control group, and the low insulin dose group mean was 81% of the control mean. At the second postinjection delay, these values were 42% for the high-dose and 52% for the low-dose group.

3. Experiment 2

The hypothesis that ethanol has a different dose effect on SMA in HG rats in comparison to rats with normal BG levels was supported by the results of Experiment 1. However, the generality of these results might be questioned because no depressant effect from ethanol was seen in the non-insulin control animals at 1200 mg/kg, an ethanol dose that has definite depressant action in various behavioral paradigms (e.g., Criswell et al., 1994; Duncan et al., 2000; Duncan and Baez, 1981; Duncan and Cook, 1981; Masur et al., 1986; Petry, 1998). The extensive pretest adaptation to the test apparatus and the repeated testing of the animals due to the within-subject manipulation of ethanol dose seemed likely to have contributed to the failure to observe an ethanol-produced SMA decrement (Geyer, 1990). Experiment 2 was designed to determine the effects of combinations of HG and ethanol on SMA by use of procedures intended to more clearly reveal ethanol's typical dose effect in euglycemic (normal BG) rats, including between-subjects manipulation of both drug treatments. The relative time courses of insulin and ethanol effects were also changed somewhat from the first experiment by injecting both drugs at the same time and measuring SMA from 10 through 40 min postinjection. These changes were intended to maximize the observed effect of ethanol and to further establish the generality of the interaction effects.

3.1. Methods

3.1.1. Animals

Sixty male Long–Evans rats were 150 days old with a mean weight of 370 g at the beginning of the experiment. Maintenance was as described for Experiment 1 with the exception of food availability as described below.

Apparatus and drugs were as described for Experiment 1.

3.1.2. Experimental design and procedure

The rats were weighed and adapted to handling (but not the test apparatus) by placement in an elevated plus-maze for approximately 10 min daily for 3 days. Then one 30-min adaptation session in the SMA test boxes occurred on the day before the test session. A 1-ml/kg ip saline injection was administered, and a drop of tail blood drawn immediately before this adaptation session. Based on SMA during adaptation 10 rats were assigned to each of six treatment groups with similar activity baselines. Insulin at 0 or 1 U/kg was factorially combined with ethanol at dose 0, 600 or 1200 mg/kg to provide different combinations of drug treatments for the six groups. The rats were maintained on 28 g of rat chow daily during the handling phase, but were given only 10 g 24 h before the single SMA test.

The SMA test, drug injections and the two BG tests were as described for Experiment 1, except that the insulin (or saline) injection was given immediately before the ethanol (or saline) injection, 10 min prior to the start of the SMA test.

3.2. Results

3.2.1. Motor activity

The $2 \times 2 \times 3$ ANOVA results revealed the effects of drug treatments during the entire 30-min test period (see Fig. 2), as indicated by significant main effects for insulin [$F(1,54) = 64.98$, $P < .0001$], ethanol [$F(2,54) = 14.92$, $P < .0001$], and Ethanol × Insulin interaction [$F(2,54) = 4.22$, $P < .025$]. Further analysis with Tukey's tests comparing these total-session means indicated that the SMA level of the insulin–saline group was significantly lower than that of the saline-only control group; the SMA level of the insulin + ethanol 600 group was significantly greater than that of the insulin–saline group, and the SMA of the insulin + ethanol 1200 group was significantly lower than that of the insulin–saline group. The post hoc tests also indicated that among the no-insulin groups neither the 600 nor the 1200 ethanol groups were significantly different from the saline-only control group.

The ANOVA also indicated significant main effects of 15-min time periods [$F(1,54) = 396.42$. $P < .0001$], and of

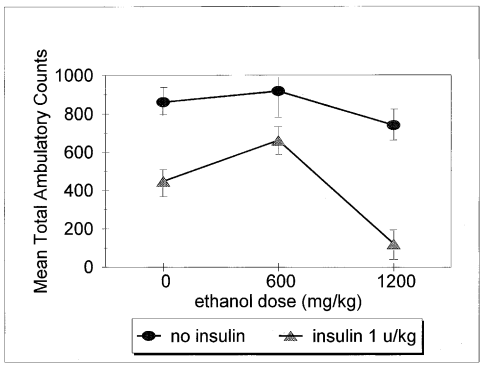


Fig. 2. Mean (±S.E.M.) total ambulatory activity counts during 30 min following ethanol injection in euglycemic (no insulin) and HG (insulin dose = 1 U/kg) rats. Each drug or drug-combination treatment condition represents a separate group of 10 rats. Both drug (or saline) injections administered 10 min prior to start of activity recording period.

Table 2
Blood-glucose levels after insulin and ethanol treatment

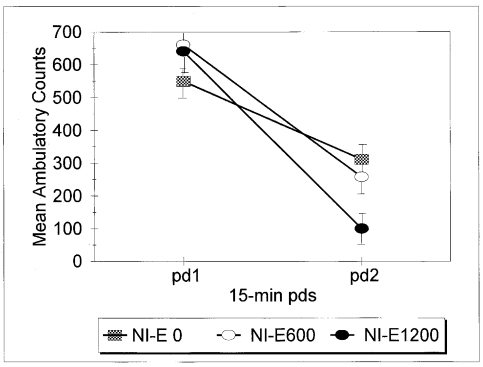
		Mean (±S.E.M.) blood-glucose level (mg/dl) Post insulin-injection delay (min)	
Insulin dose (U/kg)	Ethanol dose (mg/kg)	10	40
0	0	66 (±3)	63 (±5)
	600	66 (±4)	62 (±4)
	1200	68 (±4)	65 (±5)
Mean of all EtOH doses		66.6	63.3
1	0	58 (±6)	36 (±4)
	600	57 (±5)	35 (±3)
	1200	60 (±5)	38 (±5)
Mean of all EtOH doses		58.3	36.3

Each drug combination represents a separate group, $n = 10$ each. Ethanol injected at the same time as insulin injection.

the three-way Insulin × Ethanol × Time period interaction [$F(2,54) = 24.41$, $P < .0001$]. The remaining interaction effects were also significant, but are less important for interpretation of experimental treatments so are not reported here. Fig. 3a and b indicate the time course of SMA, and individual comparisons of pairs of groups at each time period showed the following: For the no-insulin groups (Fig. 3a) there were no significant differences among the saline control and the two ethanol dose groups during the first 15-min period, but during the second period the ethanol 1200 group was significantly lower than the saline control group. For the insulin groups (Fig. 3b) the insulin + ethanol 1200 group was significantly lower than the insulin-only group and the insulin + 600 ethanol group during both time periods. The insulin + 600 ethanol group was significantly higher than the insulin-only control group during both time periods. Finally, the insulin-only group was significantly lower than the saline–saline control group during both time periods.

3.2.2. Blood glucose

Table 2 summarizes the mean blood-glucose values for each treatment group immediately prior to and after the SMA test session. ANOVA revealed significant main effects for postinjection time [$F(1,54) = 19.14$, $P < .001$], insulin treatment [$F(1,54) = 27.55$, $P < .001$], and Insulin × Time interaction [$F(1,54) = 14.33$, $P < .001$]. The remaining main and interaction effects were not significant. Further analysis via Tukey's tests indicated that the experimental groups were not different from the saline–saline control group at the 10-min postinjection delay interval, but that all insulin groups were significantly different from the saline–saline control group at the 40-min postinjection delay. This analysis indicates that insulin caused a significant decrease in blood-glucose levels in all insulin-treated groups at 40-min, but not at 10-min postinjection delay intervals. At the 10-min delay the insulin groups' overall mean BG level was 88%, and at 40 min was 57% of the overall (all ethanol dose groups combined) mean of the non-insulin groups.



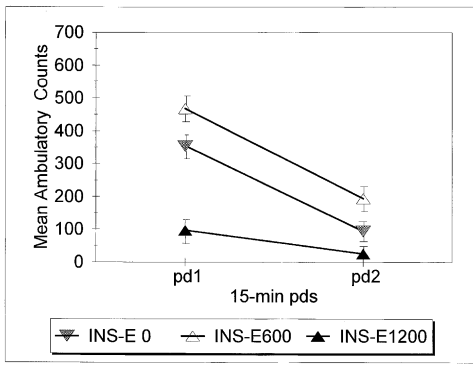


Fig. 3. (a) Mean (±S.E.M.) total ambulatory counts for two successive 15-min periods following ethanol injection in euglycemic rats (no insulin administered). (b) Mean (±S.E.M.) total ambulatory counts for two successive 15-min periods following ethanol injection in HG rats (insulin dose = 1 U/kg).

4. Discussion

The marked and rapid decrease of blood-glucose levels after insulin injection is characteristic of the drug's HG effect after administration to food-deprived rats (Duncan and Gaffney, 2001; Duncan and Hooker, 1997; Duncan and Lichty, 1993). An insulin dose effect was seen in Experiment 1, with the higher dose resulting in significantly greater HG at the 50-min postinjection BG measurement. In Experiment 2 an intermediate insulin dose and a shorter postinjection delay resulted in HG levels similar to those of the lower dose of the first experiment.

Insulin treatment reduced SMA in both these experiments, with similar decreases seen at both insulin doses in Experiment 1. Such decreased SMA is a prominent behavioral correlate of HG in rats, and these results are similar to those of a previous study utilizing insulin doses and SMA measurement much like those of the present experiments (Duncan and Koontz, 1994), and of an earlier study with much higher insulin doses (Siegel, 1975).

Ethanol administered without insulin caused a significant decrease in SMA in Experiment 2 (1200 mg/kg dose, second 15-min recording period). The failure to detect ethanol's typical depressant effect on locomotion in the first experiment was apparently due to the extensive adaptation to the test environment and the resultant low baseline SMA levels (Geyer, 1990). See Figs. 1 and 2 for comparison of SMA levels between the control conditions of the two experiments. A failure to observe SMA effects from a similar ethanol dose in rats well habituated to a test environment has been previously reported (Paivarinta and Korpi, 1993). Another factor that may have contributed to this difference in ethanol effects between the two experiments is the somewhat earlier part of the ethanol time course measured in the Experiment 1, given that a significant decrease in SMA at the higher ethanol dose occurred only during the second 15-min period (minutes 25–40 postinjection) of Experiment 2.

When ethanol at 1200 mg/kg was administered to HG rats, greater SMA reduction occurred than with either separate drug treatment. In Experiment 2 the potentiated ethanol effect emerged during the first 15-min period of the activity test (minutes 10 through 25 postinjection). In Experiment 1 SMA levels summed over the entire 30-min period also demonstrated potentiation of ethanol's depressant action, although the absence of a significant three-way interaction effect precluded analysis of the interaction during specific time periods. Comparison of the two insulin dose-groups of the first experiment suggests that a greater degree of HG (higher insulin dose) produced potentiation of the depressant effect at ethanol 600 mg/kg, whereas in the lower-dose insulin group this ethanol dose produced disinhibition.

A different type of interaction occurred at the lower ethanol doses in the HG animals. Low to moderate ethanol doses may cause increased performance or activity levels in some situations, especially in behavior that is subject to ethanol's disinhibitory effects (Criswell et al., 1994; Frye and Breese, 1981; Pohorecky, 1977). Such disinhibition was seen in HG rats here as the decrease in SMA levels was partially reversed by ethanol. As with the higher-dose interaction, similar results were found in both experiments. In Experiment 1 the pattern of mean SMA values (Fig. 1) suggests that the ethanol dose that produced the greatest SMA increase was a function of the degree of HG, with the "peak" of disinhibition occurring at the lower 300 mg/kg ethanol dose in the higher insulin dose group (which displayed slightly, but significantly more severe HG).

Disinhibition (significant ethanol-produced SMA increase) was not seen in the non-insulin rats at any ethanol dose in these experiments. Previous research with the Long–Evans rat strain has also shown an absence of behavioral activation by ethanol treatment over a range of doses in SMA (Duncan et al., 2000; Duncan and Cook, 1981; Masur et al., 1986), and in the running wheel paradigm (Duncan and Baez, 1981).

Ethanol caused no significant change in blood-glucose levels. This result is consistent with previous reports of BG levels in rats after acute ethanol administration (Oliveira de Souza and Masur, 1981; Oliveira de Souza and Masura, 1982; Tramill et al., 1981) in which ethanol did produce HG in rats, but only with combinations of greater degrees of food deprivation (48 h) and/or higher ethanol doses (3 g/kg) than were employed in the current experiments. These research reports, in addition to anecdotal clinical observations (e.g., Berczeller, 1993) indicate that food deprivation and ethanol dose interact to produce HG, which tends to emerge sooner as ethanol dose and food deprivation increase. At the moderate level of food deprivation in the present experiment, a much higher ethanol dose would have been required to produce HG, which might then emerge only after a longer postinjection delay.

Ethanol also failed to potentiate insulin-produced HG in the current experiment. Previous work (Molina et al., 1994) has shown that under some conditions ethanol may prolong the duration of HG after insulin administration. In contrast, another report (Kolaczynski et al., 1988) demonstrated that ethanol facilitated recovery from HG. Given the variability of these findings, the absence of an interaction is not inconsistent with previous studies of acute ethanol effects on insulin-produced HG.

Blood alcohol levels (BAL) were not measured in the current experiment, but there is no evidence that insulin treatment increases or prolongs blood concentration of alcohol. Some investigators found no HG-related pharmacokinetic effect on ethanol (Kerr et al., 1990; Kolaczynski et al., 1988), and there is one report (Molina et al., 1994) that HG dogs had lower BAL than did euglycemic controls.

The additive or potentiating relationship of the insulin and ethanol effects seen in both experiments at the high ethanol dose would seem to be an example of a "homergic" drug interaction (Wessinger, 1986) that occurs with a

combination of two drugs that, at some dose, cause similar effects on behavior when administered separately. Wessinger (1986) points out that for many homergic drug interactions, the actual site of interaction could involve any of several different physiological processes, and often the identification of this site of action is not possible. Since ethanol did not potentiate HG, that possible basis of the interaction can be ruled out. It also seems unlikely that ethanol pharmacokinetics were altered by HG, although that possibility should not be entirely dismissed. Further characterization of underlying processes cannot be determined from the results presented here, although the two different types of CNS alteration did combine to produce greater decreases in SMA. This potentiation of behavioral depression may be related to clinical cases of humans who experienced severe depression after drinking alcohol during HG states (Amiel, 1995; Berczeller, 1993; Kerr, 1993).

The nature of the interaction of HG and lower doses of ethanol is suggested by some well-established behavioral and subjective effects of HG in human and animal subjects. Early in the time course of HG, sympathetic nervous system activation and concomitant anxiety (subjective reports, human subjects) occur as counterregulatory processes are initiated to counteract the decline in available blood glucose (Cryer, 1993; Cryer et al., 1989; Deary et al., 1993). A recent study done in this laboratory (Duncan and Grasso, 2000) demonstrates anxiety resulting from early-stage HG as indicated by rat behavior in the elevated plus maze. Ethanol at low doses produced an anxiolytic effect in these rats, suggesting that the disinhibition of SMA seen in the current experiments may be related to ethanol's anxiolytic effects, as also demonstrated in other behavioral paradigms (Blanchard et al., 1990; Ferreira et al., 2000; Pohorecky, 1977, 1981).

In summary, it appears that low doses of ethanol disinhibit motor activity suppressed by HG and possibly by HG-related sympathetic arousal. Higher ethanol doses further depress locomotor activity already reduced by decreased glucose availability. This interpretation of the differences seen here in SMA levels after combinations of ethanol and insulin is consistent with well-established effects of ethanol, and of insulin as administered separately.

The ethanol–HG interactions seen in the present experiments may be somewhat specific to certain behavioral paradigms. Only a tendency for potentiated depression at a high ethanol dose (1500 mg/kg) occurred when ethanol and insulin treatments were combined for rats performing in the radial-arm maze (Duncan and Gaffney, 2001). In that study, drug doses and HG levels were similar to those of the present experiments, and the most apparent differences between the two paradigms are those of motivation and duration of the tested behavior. In the radial maze experiment, food-deprived animals ran for food reward, and the test duration was limited to 5 min.

The results presented here indicate that when spontaneous motor behavior is measured for at least 30 min, HG rats respond differently to ethanol than do euglycemic animals. The nature of the drug interaction depends on ethanol dose and possibly on the degree of HG, but moderate to high drug doses can result in potentiation or addition of depressant effects. Such combined effects may be of clinical significance for diabetic persons, who are at risk of experiencing HG as a consequence of strict control of hyperglycemia via insulin therapy.

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