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Neonatal Monosodium Glutamate Lesions Alter Neurosensitivity to Ethanol in Adult Mice

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CRABBE, J. C. AND D. M. DORSA. *Neonatal monosodium glutamate lesions alter neurosensitivity to ethanol in adult mice.* PHARMACOL BIOCHEM BEHAV **24**(5) 1343–1351, 1986.—A number of studies have indicated a relationship between brain peptide activity and sensitivity to the behavioral effects of ethanol. Specifically, it has been suggested that ethanol effects are mediated by changes in the endogenous opioid peptides derived from the proopiomelanocortin (POMC) precursor. Most cell bodies containing brain POMC-derived peptides are found in the arcuate nucleus of the hypothalamus. Neonatal administration of monosodium glutamate (MSG) has been reported to destroy cell bodies of the arcuate nucleus. We treated WSC strain mice on postnatal Day 4 with a single SC injection of 4 mg/g MSG or saline. When adult, MSG and control mice were challenged with an IP injection of ethanol and its effect on body temperature, open field activity, or duration of loss of righting reflex was assessed. Blood ethanol concentration (BEC) was measured and the hypothalamic content of β-endorphin like immunoreactivity (β-EP) was determined by radioimmunoassay. β-EP was markedly reduced in both females and males by MSG treatment. MSG-treated animals of both sexes showed significantly less ethanol-induced hypothermia than controls. BEC was higher in MSG-treated animals of both sexes than in controls, so the differences were not due to ethanol pharmacokinetics. β-EP was generally lower in males. Duration of righting reflex was prolonged in MSG treated animals, and the reduction in open field activity was potentiated. These latter effects may be in part attributable to the higher BECs achieved in lesioned animals. These data suggest that β-EP cell bodies in the arcuate nucleus of the hypothalamus mediate neurosensitivity to some effects of ethanol in mice, but further experiments will be necessary to implicate β-EP specifically.

MSG Hypothalamic arcuate nucleus Proopiomelanocortin β-Endorphin Hypothermia
Open field activity Loss of righting reflex Ethanol Mouse Neural peptides

ACUTE exposure to ethanol has been shown to increase plasma glucocorticoid concentrations in several species [20, 33, 43, 57]; this may be taken generally to indicate an influence of alcohol on the hypothalamic-pituitary-adrenal axis. The ethanol metabolite acetaldehyde can condense with catecholamines to form isoquinolines (TIQs) and related compounds, which bind to opioid receptors. Since initial reports of this possibility [6,11], much interest has been focussed on the speculation that TIQs may mediate some of ethanol's effects (see [40] for review). The opioid antagonist naloxone has been variously reported [17] to antagonize [2] or not to antagonize [26] some acute responses to ethanol. Ethanol also directly influences opiate receptor binding. It has been suggested that these effects are selective for delta receptors [28,29], but this is still controversial [30].

Chronic alcoholism has been reported to lead to a pseudo-Cushing's syndrome [44]. Since ACTH and beta-endorphin (β-EP) are synthesized from the same precursor, proopiomelanocortin (POMC), and both are secreted in response to stressors [1], it has been suggested that ethanol dependence may be mediated by endorphins. A number of experiments have demonstrated that pituitary or plasma concentrations of immunoreactive β-EP are usually decreased in response to chronic ethanol intake [10, 22, 23, 24, 34, 48, 49, 50, 51]. POMC-derived peptides are also synthesized in the arcuate nucleus of the hypothalamus [1, 18, 53], and we and others have found that brain β-EP also is altered by chronic ethanol treatment [4, 21, 48, 55].

In order to pursue our interest in the potential role of brain β-EP in mediating sensitivity to ethanol, we took advantage of the fact that the neurotoxin monosodium glutamate (MSG) has been reported to destroy cells containing β-EP in hypothalamus when it is administered to neonatal mice [3,36]. We reasoned that if MSG-lesioned mice were

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seen to differ in response to ethanol from control mice, the relevance of brain β-EP systems in ethanol's effects would be further supported.

One effect of ethanol that seemed a likely candidate for possible mediation by endogenous opioid peptides was the effects on thermoregulation. A plausible case may be presented that β-EP released locally within preoptic/anterior hypothalamic areas (PO/AH) affects body temperature. A review of the role of neuropeptides in thermoregulation concluded that β-endorphin is a likely candidate for an important thermoregulatory peptide, but the data are to fragmentary as yet for a clear modulatory role to be specified [5], probably due to a large number of variables such as species, route of administration, site of administration, and so forth. Under most ambient laboratory environmental conditions (18–25°C), ethanol produces hypothermia. Although the mechanism of action is not clearly understood, the thermoregulatory effects of ethanol have been reported to be nonregulated (i.e., poikilothermic) in rodents [41,42] or regulated [37,46]. We have demonstrated that β-EP is released from anterior pituitary by acute low doses of ethanol [35], although it is not known if hypothalamic endorphin is similarly affected. Thus, we sought to generate preliminary data with animals lesioned with MSG to destroy the arcuate nucleus cell bodies giving rise to PO/AH β-EP. We wished to see whether MSG-induced reduction in hypothalamic β-EP content led to an altered hypothermic response to ethanol. In addition, we monitored sensitivity of other behavioral responses to ethanol (open field activity and duration of loss of righting reflex), assessed blood ethanol concentrations (BEC), and hypothalamic content of beta-endorphin-like immunoreactivity (β-EP).

METHOD

Animals and Husbandry

Mice from the genetically heterogeneous WSC stock were used in all experiments. These animals have been bred for 16 generations in our colonies starting from a base population of HS/Ibg mice purchased from the Institute for Behavioral Genetics, Boulder, CO. Two reproductively isolated WSC lines (WSC-1 and WSC-2) are maintained, and all mice studied were F_1 hybrids from crosses between the WSC lines. Mating pairs were housed in Plexiglas cages (28×17×11.5 cm) with stainless steel lids and sawdust bedding. Ambient temperature was 25±1°C and lights were on from 0600 to 1800 hr. All behavioral testing was performed in the colony room between 0900 and 1200 hr. Food and water were available ad lib.

MSG Treatment

All females to be mated were housed in adjacent cages for 2–3 weeks to allow estrus cycles to synchronize. All mating pairs were set up on the same day, and births were recorded each morning. Approximately 80% of the births occurred 20–24 days after mating, and additional litters that were born within the following 20 days were also included in the study.

On Postnatal Day 4, each mouse was injected subcutaneously in the lower back with either 4 mg/g MSG in 0.025 cc/g 0.9% sterile saline, or saline vehicle. Sterile procedures were employed, and care was taken not to allow pups to become hypothermic during the brief separation from their dam and littermates. Half of each litter was randomly assigned to each drug injection condition without regard to sex. Drug treatment was coded by clipping a small distal segment of one toe on either a right or left paw. As animals matured, this code was sufficiently unobstrusive to allow the behavioral experiments to be conducted blind to experimental condition. The pups were then returned to their dams. All pups were weaned at 21 days of age and sexes were separately housed as described in groups of 2–6 pups per cage.

Behavioral Testing

Temperature measurements. Body temperatures were tested according to a modification of previously-published procedures [8]. One mouse was tested per minute. Ten minutes before testing, the cage lid was removed from the first cage of animals. Five minutes later, the first mouse was picked up by the tail and dragged backwards into a cylindrical restrainer with a slot along the side and a hole in the closed end to allow for probe insertion. The diameter of the restrainer was chosen to be large enough to allow freedom of movement, and mice did not generally struggle to escape while being held in the apparatus by the tail. A RET-3 copper-constantan thermocouple rigid rectal probe for mice (Sensortek: 0.06 mm diameter with a 1.5 mm ball at the distal end) was immediately inserted 1.8 cm into the animal's rectum. Temperature was recorded on a Thermalert Model TH-8 Digital Thermometer (Sensortek) to the nearest 0.1°F after the 5 second period required for stabilization. The mouse was released directly into its home cage. All temperatures were later converted to °C for analysis. Five minutes later, each mouse was picked up, rapidly weighed to the nearest 0.1 g on a Mettler PC4400 Digital Balance, immediately injected intraperitoneally with ethanol (20% v/v in 0.9% saline) at the chosen dose, and returned to its home cage. Subsequent temperature measurements were made at different times after injection as described below following the same procedure.

Duration of loss of righting reflex (LORR). Each mouse was weighed to the nearest 0.1 g and injected IP with ethanol (20% v/v in 0.9 saline). The mouse was manually restrained until it lost its ability to right when placed on its back in a V-shaped trough. First attempt to place the mouse was made after 30 seconds, and any mouse that had not lost righting reflex within 3 minutes was eliminated from the experiment. Latency to LORR was recorded and duration was calculated as the difference between LORR and regaining righting reflex. Criterion for loss or regaining righting reflex was two successful rightings within a 30 sec period.

Open field activity (OFA). Mice were weighed, injected with saline or one of two doses of ethanol (1.5 or 3.0 g/kg, 20% v/v in saline), and returned to their home cage. Fifteen minutes later, each mouse was introduced into one of two identical 61 cm diameter circular open fields (Lehigh Valley Electronics) with a solid metal floor. Beam interruptions from seven equally-spaced radially-oriented pairs of infrared photocells were automatically counted. OFA was recorded during a 5 minute period, the mouse was removed to its home cage, and the chamber wiped with a damp cloth before the introduction of the next mouse.

Chemical Measurements

Blood ethanol concentrations (BEC). At appropriate intervals, mice were restrained as described and a 20 μl blood sample was drawn from the tip of the tail. When subsequent blood samples were to be drawn, the tip of the mouse's tail was dipped in melted paraffin and then into ice water to seal the wound site and prevent blood loss. For

TABLE 1

EFFECTS OF MSG LESIONS AND ETHANOL ON BODY TEMPERATURE

Treatment	N	Baseline Temperature	Hypothermia at Thirty Minutes	Hypothermia at Sixty Minutes
MSG	25	37.8 ± 0.1	2.9 ± 0.2	3.1 ± 0.2
Control	24	38.1 ± 0.1	3.8 ± 0.1	3.3 ± 0.1

Values are mean ± SEM in °C. Baseline temperatures differed significantly, $F(1,47)=10.22$, $p<0.01$. Hypothermia is shown as difference from baseline temperature. Control mice showed significantly more body temperature reduction 30 minutes after 3 g/kg ethanol than MSG-Treated mice, $F(1,47)=14.09$, $p<0.001$, but this difference was no longer significant at 60 minutes, $F(1,47)=0.86$.

determining BEC, a previously published procedure [8] adapted from Roach and Creaven [47] was employed. Determinations were made with a Packard Model 428 Gas Chromatograph with flame ionization detector and a Porapak Q column.

Hypothalamic tissue preparations. Mice were rapidly killed by cervical dislocation and the brain quickly removed on dry ice. The hypothalamus was removed according to established procedures [25]. Peptides were extracted by boiling individual tissues in 0.5 ml 1 N acetic acid for 20 min, cooling in ice, and sonicating at 20 KHz for 20 seconds or until the tissue was totally disrupted. Aliquots of the homogenate were removed for protein determination [38]. The homogenates were then centrifuged for 20 minutes at 30000 g. Supernatants were removed and vacuum-dried in a Speed-Vac Concentrator (Savant). Dried samples were stored at −70°C. Individual hypothalamic extracts were reconstituted in 200 μl of 0.05 N acetic acid, and 100 μl aliquots were measured by radioimmunoassay.

β-Endorphin radioimmunoassays (RIAs). RIAs for β-endorphin-like immunoreactivity (β-EP) were performed as previously described [18,43]. The β-EP antiserum recognizes all molecules containing the carboxy terminus of β-lipotropin on an approximately equimolar ratio. RIAs utilize the appropriate synthetic peptide (Peninsula Laboratories) as reference standards and for radiolabeling. The sensitivity of the RIAs is approximately 10–20 pg, with assay midpoints of 70–140 pg. The β-EP antiserum employed has greater than 70% cross-reactivity on a molar basis with β-endorphin 1–27, α-N-acetyl-β-endorphin 1–27, and β-lipotropin, and less than 0.1 cross-reactivity with γ-endorphin and met-enkephalin [18].

Statistical Analyses

Most analyses were conducted with factorial analyses of variance. Significant differences were further analyzed by the post-hoc group comparisons using the method of Neuman-Keuls [56]. For some dichotomous variables, Chi-square analyses of two-way contingency tables were performed [52]. Finally, bivariate Pearson's r values were calculated to clarify the relationships between some variables. Two-tailed significance of r values were determined by Fisher's r to Z transformation [19]. In interpreting the regression relationships, we were not blindly guided by levels of statistical significance, since with large enough numbers of subjects, even correlations which account for very small amounts of total variance in a bivariate distribution can be highly significant. Rather, we scatterplotted relevant correlations and exercised our judgment as to their practical significance in reporting these data. Where means are given in the text, they are expressed as ±standard error of the mean.

TABLE 2

EFFECT OF MSG LESIONS ON ETHANOL-INDUCED LOSS OF RIGHTING REFLEX

Treatment	Sex	Number of Mice With Righting Reflex: Present	Absent
MSG	Male	12	2
Control	Male	2	10
MSG	Female	1	10
Control	Female	1	11

Male mice treated with MSG were significantly more likely to have lost righting reflex than their saline-treated littermates, $\chi^2(1)=9.77$, $p<0.01$, two-tailed. There was obviously no difference between MSG-Treated and Control females.

EXPERIMENT 1

Our initial experiment was designed to see whether we could detect differences in sensitivity to ethanol-induced hypothermia in mice treated with a single neonatal MSG injection on the fourth postnatal day of life.

METHOD

Twenty four saline-treated mice (12 of each sex) and 25 MSG treated mice (11 female and 14 male) were tested at 77 days old for their acute hypothermic response to injection of 3 g/kg ethanol as described. A baseline temperature was taken 5 minutes before injection, and post-injection temperatures were taken 30 and 60 minutes after injection. This experiment was not designed to evaluate sensitivity to ethanol's effect to induce LORR, but at the 30-minute post-injection temperature measurement, it was immediately apparent that many of the animals clearly had lost righting reflex. We therefore scored LORR as present or absent for each mouse immediately before taking the 30 minute temperature. By 60 minutes after injections, no mice scored positively for LORR.

RESULTS AND DISCUSSION

Baseline body temperatures differed significantly be-

tween treatment groups (see Table 1). Males had significantly lower baseline temperatures than females (37.8±0.1 vs. 38.0±0.1°C, respectively), $F(1,45)=5.94$, $p<0.05$, but the interaction of Treatment × Sex was not significant, $F(1,45)=0.69$. We therefore expressed hypothermic response as a difference from baseline for the sexes combined. Baseline temperatures and the reduction from baseline 30 and 60 minutes after injection are given in Table 1.

MSG-treated mice were clearly less hypothermic 30 minutes after ethanol injection than their saline-treated littermates. This difference had largely dissipated by 60 minutes after injection. Although the difference, as noted, was not statistically reliable, males seemed to show more attenuation of hypothermia than females, a finding we will return to in a later experiment. The significant difference in baseline temperature between treatment groups was small.

The LORR data were also striking. Table 2 shows the number of animals of each sex and treatment group that had lost righting reflex at the 30 minute post-injection time point. Male mice given MSG postnatally were significantly more likely to display LORR 30 minutes after ethanol injection than their saline-treated counterparts, while for females no such difference was evident.

Experiment 1 thus encouraged us that MSG lesions might prove to be an effective tool for exploring endorphinergic mediation of ethanol responsiveness. Three features of these results seemed particularly interesting. First, there was indication of a sexual dimorphism. Second, MSG treatment antagonized one acute response to ethanol while it potentiated another, suggesting that it might be possible to identify fairly specific roles for endorphin systems. Third, although differences in baseline temperature were small, they were consistent and reliable (in future experiments to be discussed below). This suggested that MSG lesions might prove to be a useful tool in assessing the postulated role of endorphins in normal thermoregulation.

However, two obvious limitations of Experiment 1 precluded any confident interpretation of the results. First, we had to determine that the MSG treatment was indeed effective in destroying arcuate nucleus perikarya containing β-EP. Second, we had to determine whether differences in sensitivity to ethanol responses were due simply to pharmacokinetic factors ancillary to the MSG treatment. For example, MSG-treated mice could have simply achieved lower blood ethanol concentrations and therefore displayed less hypothermic responses. Since they showed potentiated LORR, this argument could not be used to explain both results, but direct tests of these hypotheses were undertaken in the next experiment.

EXPERIMENT 2

Experiment 1 had suggested that MSG lesions protected mice from the hypothermic effects of an acute ethanol injection. We sought to replicate this finding, to verify that the MSG treatment indeed depleted hypothalamic content of β-EP, and to assess the role of ethanol concentration in these effects. In addition, we administered some mice ethanol chronically to see what effect MSG lesions might have on the development of tolerance to ethanol-induced hypothermia.

METHOD

Employing the same strain and basic procedures outlined above, we raised WSC mice of both sexes that had been treated on Postnatal Day 4 with 4 mg/g MSG or saline. Eight groups of 7–11 mice randomly constituted a 3-factor design, with the factors Lesion (Control vs. MSG), Sex (Male vs. Female), and Ethanol Treatment (Naive vs. Tolerant). Injections of ethanol or saline were begun when mice were 51–67 days old. Mice in the Tolerant groups were weighed (at 0900 hr) and injected twice daily (at 0900 and 1600 hr) with 3.5 g/kg ethanol (20% v/v in saline) for three days and returned to their home cages. Mice in the Naive groups received equivalent-volume saline injections.

Beginning at 0900 hr on Day 4, mice were tested for baseline temperature, were weighed and injected with ethanol 3.5 g/kg IP (20% v/v in saline), and their temperature was taken again at 45 and 90 min after injection. Both Naive and Tolerant groups received ethanol on Day 4. After the 45 and 90 min body temperature measurements, a blood sample was drawn from mice in the Naive groups for analysis of BEC.

Eight additional groups of 4–9 mice per group were similarly treated with ethanol (3.5 g/kg) or saline for three days, but on the fourth day, they were injected with 4.5 g/kg ethanol and tested for duration of LORR as described.

RESULTS AND DISCUSSION

Baseline body temperatures differed significantly. The main effect of Lesion, $F(1,69)=18.79$, $p<0.001$, reflected lower temperatures in MSG-treated than Control mice (38.1±0.1 vs. 38.6±0.1°C, respectively), in agreement with Experiment 1. The sexes also differed significantly, $F(1,69)=6.94$, $p<0.01$, with females higher than males, as in Experiment 1 (38.4±0.1 vs. 38.2±0.1°C, respectively). The factor Ethanol Treatment, $F(1,69)=3.04$, $p<0.10$, and the Ethanol Treatment × Sex interaction, $F(1,69)=3.33$, $p<0.10$, also tended toward significance. No other interactions were significant (all F values <1). We consequently analyzed hypothermia scores as differences from baseline body temperature, as in Experiment 1.

Analysis of the reduction in body temperature 45 minutes after injection revealed that all main effects were significant. MSG-treated animals had significantly less hypothermia than littermate Controls, $F(1,69)=11.15$, $p<0.001$ (2.3±0.2 vs. 2.9±0.1°C, respectively). Males displayed a greater hypothermia than females, $F(1,69)=8.52$, $p<0.01$ (3.0±0.1 vs. 2.4±0.1°C, respectively). Groups that had been given six previous ethanol injections were less hypothermic than Naive groups challenged for the first time with ethanol, indicating that they had developed tolerance, $F(1,69)=32.56$, $p<0.001$ (2.1±0.1 vs. 3.2±0.2°C, respectively). The two- and three-way interactions did not reach significance (all Fs<1) with the exception of the Lesion × Ethanol Treatment interaction, which tended toward significance, $F(1,69)=3.24$, $p<0.10$. The pattern of changes suggests that MSG lesions may have attenuated the degree of ethanol tolerance.

By 90 minutes after injection, only the Sex difference, $F(1,69)=5.08$, $p<0.05$, and the Ethanol Treatment difference, $F(1,69)=63.10$, $p<0.001$, were still significant. The MSG groups were 2.6±0.1°C hypothermic versus 2.3±0.1°C in the Control groups. Thus, the protective effect of the MSG lesion was apparent early, but no later after ethanol injection, as we had previously seen in Experiment 1.

To summarize the more important body temperature data, the results of Experiment 2 indicated that MSG-treated animals had slightly lower baseline body temperatures than their saline-treated littermates. MSG-treated

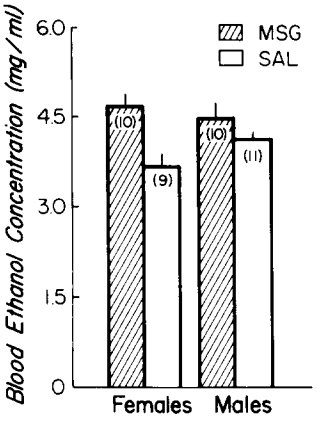

FIG. 1. Effect of MSG-Treatment and sex on blood ethanol concentrations 45 minutes after an IP injection of ethanol (3.5 g/kg) to Naive mice. MSG=mice given 4 mg/g MSG lesions subcutaneously on Postnatal Day 4. SAL=littermates given saline SC.

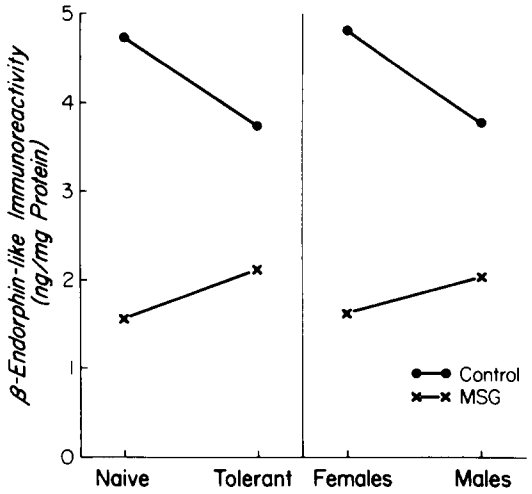

FIG. 2. Effect of MSG-Treatment and sex on β-Endorphin-like immunoreactive material in hypothalami of mice. MSG=mice given 4 mg/g MSG lesions subcutaneously on Postnatal Day 4. Control=littermates given saline SC. Tolerant=mice given twice-daily injections of ethanol (3.5 g/kg) for three days before the test day after which samples were taken. Naive=mice similarly preinjected with saline twice daily.

animals showed less hypothermia than Controls early, but not later after injection. MSG-treated animals tended to develop less tolerance to the hypothermic effects of ethanol.

BEC for the Naive groups 45 minutes after injection are shown in Fig. 1. The main effect of Lesion was significant, $F(1,36)=10.01$, $p<0.01$, reflecting higher BEC in MSG-treated groups. The effect of Sex was not significant ($F<1$), and the Lesion × Sex interaction also was statistically unreliable, $F(1,39)=2.59$, $p=0.11$. Results for the 90-minute BEC were quite similar and are not reported. We also calculated the difference in BEC for each animal (45 minute value minus 90 minute value) to derive a rough estimate of rate of ethanol metabolism. Analyses of these data also revealed no significant main effects or interactions (all $Fs\leq 1.2$). Since the MSG-treated groups had less pronounced ethanol-induced hypothermia at a time (45 min after injection) when they displayed significantly higher BEC than controls, we conclude that the difference in hypothermic response to ethanol is likely due to a difference in functional neurosensitivity. Although it is possible that brain ethanol concentrations were actually lower in MSG-treated animals than in Controls while brain ethanol concentrations were higher, this seems unlikely.

Both MSG-treated and Control groups developed significant tolerance to ethanol-induced hypothermia. Given the absence of BEC from tolerant animals, we do not know whether this represents functional or dispositional tolerance. Since the development of tolerance to ethanol-induced hypothermia can vary in rate of development as well as in maximal degree (including failure to develop at all [9]), and given the trend toward an interaction between Lesion and Ethanol Treatment, it seems premature to conclude that the MSG lesion has no effect on tolerance development.

MSG treatment was successful in reducing β-EP in hypothalamus of treated animals. Protein analyses revealed a significant main effect of Ethanol Treatment, $F(1,64)=9.27$, $p<0.01$, reflecting lower protein values in Tolerant groups (661±37 vs. 779±21 μg/mg, respectively). No other main effects or interactions were significant. Consequently, we report β-EP values per unit protein. Results are shown in Fig. 2.

The main effect of Lesion was highly significant, $F(1,64)=45.33$, $p<0.001$, while neither the main effects of Sex or Ethanol Treatment were significant ($Fs<1$). However, both the Lesion × Ethanol Treatment, $F(1,64)=5.08$, $p<0.05$, and the Lesion × Sex, $F(1,64)=4.05$, $p<0.05$, interactions were significant. The Ethanol Treatment × Sex and the three-way interactions were not significant.

Post-hoc analyses of the data from the left panel of Fig. 3 revealed that in Naive groups, MSG treatment produced a 66% reduction in hypothalamic β-EP content ($p<0.01$). This represented a 50% reduction in Naive males and a 79% reduction in Naive females. Tolerant groups showed a 43% reduction in β-EP ($p<0.05$). The interaction appeared to result principally from the 22% depletion of β-EP content in Tolerant Control animals, although this difference was not significant. The right panel of Fig. 3 shows that across Naive and Tolerant groups, the effect of the MSG lesion was more marked in females ($p<0.01$) than in males ($p<0.05$). The size of the sex difference was somewhat attenuated by the (again, non-significant) reduction in β-EP content attributable to ethanol tolerance in the Control group. Clearly, the MSG lesion was effective in reducing hypothalamic β-EP content in both sexes. In other experiments, we have seen reductions of as much as 90% in females and 75% in males. We have always seen a consistent sexual dimorphism, with naive females having higher β-EP hypothalamic content than naive males and greater proportional reductions than males after MSG treatment. In the current experiment, for example, Naive females had 5.9±0.9 versus 3.9±0.6 ng β-EP/mg protein in Naive males. Both panels clearly indicate that the MSG lesion was effective in both sexes and that the effect of chronic ethanol administration was small compared to the lesion effect.

We related the β-EP content measurements to the body temperature data and BEC data by examining bivariate Pearson's r between measures for the various groups and subgroups of animals. The strongest pattern of correlation was between β-EP content and severity of hypothermic response 45 minutes after ethanol. Over all mice, this correlation was

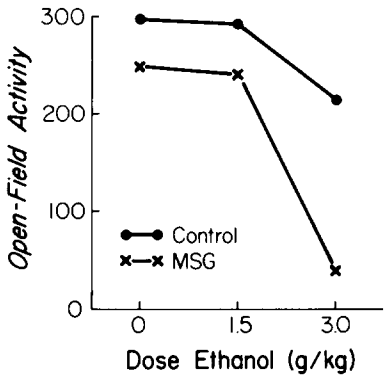

FIG. 3. Effect of MSG-Treatment and acute injection of ethanol on activity in an open field during a 5-minute test starting 15 minutes after injection. MSG=mice given 4 mg/g MSG lesions subcutaneously on Postnatal Day 4. Control=littermates given saline SC.

r=.34 (p=0.004). Within the Control group, there was no correlation (r=.08), but within the MSG group the correlation was present (r=.41, p=0.01). The correlation was evident in females (r=.52, p=0.001), but not in males (r=.16). Since chronic ethanol treatment had tended to lower β-EP, at least in Control groups, we reassessed the pattern of correlation in the Naive group only. Essentially the same pattern was found. Over all mice, β-EP content and hypothermic response at 45 minutes were positively correlated (r=.35, p=0.03). This was true within the MSG group (r=.56, p=0.01) but not within the Control group (r=−.25). In Naive group mice, both female (r=.43) and male (r=.41) correlations tended to be significant (p_s=0.07). Thus, the results of correlational analysis were consistent with the between-group findings indicating that depleted levels of hypothalamic β-EP lead to reduced sensitivity to the hypothermic effects of ethanol.

The data for mice tested for LORR revealed that MSG lesions sensitized mice to this effect of ethanol. The main effects of Lesion, $F(1,48)=59.3$, $p<0.001$, and Sex, $F(1,48)=16.3$, $p<0.001$, were significant. This resulted from longer durations in the MSG group than in the Control group (208.4±12.4 vs. 112.8±7.1, respectively) and in males than in females (180.8±11.4 vs. 130.7±13.7, respectively). The two-way interactions did not reach significance, but the three-way interaction tended toward significance, $F(1,48)=3.4$, p=0.07. The pattern of differences suggests that MSG may have attenuated tolerance in females but not in males.

Although the main effect of Ethanol Treatment did not reach significance, it was in the predicted direction (shorter LORR duration in Tolerant groups). Of the 29 mice that started the experiment in the Ethanol-Tolerant group, 6 failed to lose righting reflex on the test day, as opposed to one of the 34 mice that started in the Naive group. Data from these mice were eliminated from the analysis, but the effect was to underestimate the degree of tolerance to ethanol actually present, since those animals most tolerant were excluded from the experiment. We had indeed noticed on the second day of injections that some mice were no longer losing righting reflex, or were only losing it for a short while. On the third day of injection, we scored the mice for LORR by turning them over on their backs in their home cages after injection. On the third day, 10 of the 40 Control mice but only 1 of the 37 MSG mice failed to lose righting reflex. This difference was significant, $\chi^2(2)=6.09$, $p<0.02$, and suggests that MSG lesions antagonized the development of tolerance to ethanol-induced LORR. This led us to start testing mice on Day 4 at a dose of 4 g/kg instead of the 3.5 g/kg they had been receiving. Since the first several mice failed to lose righting reflex, we switched to a dose of 4.5 g/kg, which explains the difference in numbers of mice starting and ending the experiment. Only data from mice tested after 4.5 g/kg injections are reported.

Since our analysis of the BEC data collected from mice given 3.5 g/kg injections of ethanol had revealed that MSG-treated mice attained significantly higher levels of blood, and presumably brain, ethanol 45 and 90 minutes after injection, it is possible that the difference in sensitivity to LORR may be explained simply by differences in effective dose of ethanol. Dose-effect studies will be necessary to evaluate this possibility.

EXPERIMENT 3

Experiments 1 and 2 had indicated that MSG lesions protected mice against one effect of ethanol, hypothermia, while MSG treatment appeared to potentiate sensitivity to the LORR induced by ethanol. When MSG is given repeatedly during neonatal development to mice, it is known to influence open field activity [12,13]. In a final experiment, we studied mice neonatally lesioned with MSG as described for their open field activity.

METHOD

Groups of 8–12 mice were treated as described and allowed to grow to be 71–89 days of age before testing. Each mouse was weighed and given an IP injection of saline, 1.5 or 3.0 g/kg ethanol. Fifteen minutes later, beam interruptions in an automated open field were recorded during 5 minutes as described above. Data were analyzed by a 3-factor ANOVA (Lesion × Dose × Sex).

RESULTS AND DISCUSSION

MSG lesions and the high dose of ethanol reduced activity. The main effects of Lesion, $F(1,106)=22.7$, $p<0.001$, Dose, $F(2,106)=24.1$ $p<0.001$, and Sex, $F(1,106)=4.9$, $p<0.05$, were all significant. The sex difference was due to lower scores in males than females (206±17 vs. 245±18, respectively). The interaction of Lesion × Dose was significant, $F(2,106)=5.3$, $p<0.01$. Since no interactions involving sex were significant, the sex difference was ignored and the Lesion × Dose interaction is depicted in Fig. 3. Post-hoc tests revealed that the Control mice given 3.0 g/kg ethanol were not significantly lower in open-field activity than their saline-treated controls. MSG-treated mice tested after 3 g/kg ethanol were significantly lower in activity than MSG-treated mice given saline or Control group mice tested after 3 g/kg ethanol ($p<0.01$). Thus, the effect of the MSG lesion was to potentiate ethanol-induced decrease in activity.

As discussed for Experiment 2, it is likely that the mice lesioned with MSG had higher BEC than those saline-treated neonatally. Thus, it is possible that this behavioral potentiation was due to differences in dose of ethanol rather than differences in neurosensitivity. Nonetheless, the open field data resembled the LORR data as opposed to the hypothermia data.

GENERAL DISCUSSION

These preliminary data demonstrate that mice lesioned neonatally with a single MSG injection on Postnatal Day 4 were depleted in hypothalamic β-EP in adulthood. Females had higher levels of hypothalamic β-EP and were more sensitive to the MSG lesion than males. The β-EP depletion was accompanied by a reduced sensitivity to the hypothermic effects of acutely administered ethanol. Although we did not intentionally manipulate lesion severity, there were clearly differences among animals within the MSG-lesioned groups. We found that the amount of radioimmunoassayable β-EP was positively correlated with the degree of hypothermic response to ethanol. That is, the more complete the lesion, the more significant the protection against ethanol-induced hypothermia.

The mechanism by which MSG treatment led to a small but significant elevation in blood ethanol concentration 45 and 90 minutes after an acute injection is not known. The rate of ethanol metabolism was not significantly affected. We have been unable to think of a plausible explanation for these differences. Determination of blood and brain concentrations of ethanol at different times after injection might provide the data needed to venture an hypothesis.

Neither do we understand how MSG lesions influence thermoregulatory response to ethanol. B-EP and analogues active at opioid receptors generally mimic the effects of morphine on thermoregulation, which are variable depending upon dose, route, and site of administration, species, and a number of environmental- and handling-related variables [27]. Naloxone or naltrexone generally antagonize β-EP effects on temperature, but exceptions have been noted [5]. Two groups have independently reported that β-EP injections into rat PO/AH caused dose-related, biphasic responses of hyperthermia after low doses and hypothermia after higher doses [39,54]. Preinjection peripherally with high doses of naloxone antagonized the hyperthermic component of this response in rats [39] and PO/AH preinjection of naloxone antagonized the analogous response in rabbits [45]. In rabbits, the β-EP response was interpreted to be due to an alteration of set-point rather than modulation of the activity of thermoregulatory effectors [45]. Intraventricular injection of β-EP also caused a dose-dependent, biphasic response in mice. This effect was blocked or reversed by naloxone, and appeared to be a regulated response [32]. Intraventricular administration of β-EP to rats is usually reported to produce a dose-dependent hypothermia [31]. Given the complexity of results seen after direct administration of β-EP to brain, it seems still premature to speculate about the mechanisms underlying endogenous hypothalamic β-EP involvement in thermoregulation [27].

The single-dose MSG lesion we employed is relatively restricted to a particular region of the hypothalamus, the arcuate nucleus, that has a vulnerable blood-brain-barrier at the time of treatment. Most experiments with this system involve 5 or 10 repeated injections of MSG and result in a rather diffuse damage to circumventricular organs, severe obesity even in the face of hypophagia, lethargy, profound reductions in endocrine function, and even retinal damage (see [13] for review). R. G. Dawson, however, reported that a single injection of MSG to mice or rats on Postnatal Day 4 yielded a more restricted lesion with fewer behavioral side-effects [13,14]. While even the single MSG injection employed lesions a number of peptide and transmitter containing perikarya, there is evidence that a compensatory increase in dopamine uptake occurs in spared dopamine cell bodies [16]. More interesting, there may be separate pools of cells for a single susceptible peptide, some of which are responsive and some of which are resistant to MSG lesions [15]. Also, destruction of β-EP cell bodies with MSG has been reported to increase the number of delta opioid receptors in thalamic region of the rat brain [58]. In mice, up-regulation of opiate receptors has been reported after MSG lesions [53]. The specificity of β-EP in mediating the responses to ethanol we have reported here remains to be demonstrated more directly in future experiments employing specific antisera or opioid receptor antagonists.

In previously-published experiments, we chronically treated mice with ethanol for 24, 48, or 72 hr and then rapidly dissected brain into 7 discrete areas known to contain β-EP. Samples were then assayed for content of β-EP. We found the ethanol exerted regionally-specific effects on brain β-EP content. Of most interest in the present context, β-EP in hypothalamus was reduced after 24 hr of exposure [55]. Hypothalamic content returned to normal by 48 hr of intoxication and tended to decrease again at 72 hr. Using a high pressure liquid chromatography system capable of discriminating the six or more identifiable variants of endorphin that collectively are recognized by our antisera as β-EP, we analyzed hypothalamic content. The discrimination of these forms is important because some of them, which have been acetylated as part of the post-translational processing sequence, do not have affinity for opioid receptors. A preponderance of 1–31 beta endorphin, an opiate-active peptide, was found in hypothalamus. The reduction in β-EP after 24 hr of chronic exposure was predominantly in this opioid-active form of endorphin. The overall pattern of results we saw is consistent with the hypothesis that chronic ethanol treatment led to integrated changes in content of hypothalamus and other brain areas consistent with a shift in relative β-EP activity between the two principal projection areas [55].

The fact that mice treated with MSG had enhanced responsiveness to two other effects of ethanol, LORR and open field activity reduction, underscores that responses to ethanol are not monolithic. That is to say, each response system affected by the drug is likely to be under separate and perhaps differentiable neural and endocrine control. Different behavioural responses to ethanol have been clearly demonstrated to be under genetic control systems that are to a large degree independent [7]. Thus, the MSG lesion may prove to be a useful tool in dissecting out the neural control mechanisms underlying the development of tolerance to and physical dependence on ethanol.

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