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Flurothyl Seizure Thresholds in Mice Treated Neonatally With a Single Injection of Monosodium Glutamate (MSG): Evaluation of Experimental Parameters in Flurothyl Seizure Testing

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DAWSON, R., JR. AND G. BIERKAMPER. *Flurothyl seizure thresholds in mice treated neonatally with a single injection of monosodium glutamate (MSG): Evaluation of experimental parameters in flurothyl seizure testing.* PHARMACOL BIOCHEM BEHAV **28**(2) 165–169, 1987.—Monosodium glutamate (MSG) administration to neonatal rodents produces convulsions and results in numerous biochemical and behavioral deficits. These studies were undertaken to determine if neonatal administration of MSG produced permanent alterations in seizure susceptibility, since previous investigations were inconclusive. A flurothyl ether seizure screening technique was used to evaluate seizure susceptibility in adult mice that received neonatal injections of MSG (4 mg/g and 1 mg/g). MSG treatment resulted in significant reductions in whole brain weight but did not alter seizure threshold. A naloxone (5 mg/kg) challenge was also ineffective in altering the seizure thresholds of either control of MSG-treated mice. Flurothyl ether produced hypothermia which was correlated with the duration of flurothyl exposure; however, the relationship of hypothermia to seizure induction was unclear. Flurothyl seizure testing proved to be a rapid and reliable technique with which to evaluate seizure susceptibility.

MSG Flurothyl ether Seizures Naloxone Hypothermia

NEONATAL administration of MSG to mice results in damage to the arcuate nucleus of the hypothalamus [19] and consequent alterations in neurochemical, endocrine and behavioral measures [7, 9, 18, 20]. Parenteral administration of MSG to neonatal mice [17] or rats [3,14] produces convulsions as an acute response to its neuroexcitant properties. It has been reported that neonatal administration of MSG results in increased susceptibility to pentylenetetrazol (Metrazol; PTZ) induced seizures in adult mice [21]. Prabhu and Oester (1971), however, failed to detect any changes in PTZ seizure threshold in MSG-treated mice that were tested as adults. This apparent inconsistency in the effects of MSG treatment on seizure susceptibility prompted us to investigate seizure susceptibility in MSG-treated mice using a flurothyl ether seizure threshold screening method.

Flurothyl, a hexafluorinated nonflammable ether, is a powerful chemical convulsant [1,5] which has been used previously to produce convulsions in man [11]. Moreover, flurothyl has been used successfully as an experimental tool for seizure threshold testing in rats, mice and other species of laboratory animals [1]. Flurothyl has many advantages for this purpose including; ease of testing, a clearly defined endpoint, reliability, and characterization with regard to interaction with other drugs, age and sex effects, effects of repeated testing, and species variation [5,23]. Flurothyl is not metabolized [1,5] and flurothyl seizure thresholds are independent of body weight over a wide range of weights [23].

The present study takes advantage of the properties of flurothyl to examine the seizure susceptibility of adult mice that were administered MSG neonatally. MSG-treated mice were also challenged pharmacologically to examine the role of MSG-induced β-endorphin depletion [13,15], since endogenous opiates have been implicated in seizure disorders [12,26]. In addition, data are presented which evaluate the effects of flurothyl on body temperature regulation in mice.

METHOD

Male CF-1 mice (Charles River) were injected (SC) on postnatal day 4 with either 4 mg/g MSG, 1 mg/g MSG or 0.9% saline [9,10]. The mice were housed in groups of 5–6 after weaning in a standard, temperature controlled colony room on a 12 hour light-dark cycle. Food and water were available ad lib, throughout the study.

TABLE 1

EFFECTS OF MSG TREATMENT AND NALOXONE ADMINISTRATION OF FLUROTHYL SEIZURE THRESHOLD*

Group	N	Seizure Threshold (sec ±SE)
Control	26	330 ± 21
Control + NAL	13	315 ± 26
MSG (4 mg)	22	373 ± 33
MSG (4 mg) + NAL	16	319 ± 41
MSG (1 mg)	14	390 ± 45

*Naloxone (NAL) 5 mg/kg was administered 15 minutes prior to seizure testing. MSG was administered as a single subcutaneous injection on postnatal day 4 at doses of 4 mg/g or 1 mg/g.

TABLE 2

EFFECTS OF ENVIRONMENTAL TEMPERATURE AND MSG TREATMENT ON FLUROTHYL SEIZURE THRESHOLD AND BODY TEMPERATURE

Group	N	Environmental Temperature °C	Seizure Threshold (sec ±SE)	Body Temperature (°C ± SE) Before Flurothyl	After Flurothyl
Experiment 1					
Control	7	23	380 ± 49	37.7 ± 0.31	35.9 ± 0.48*
MSG (1 mg/g)	8	23	351 ± 71	37.3 ± 0.26	35.1 ± 0.46*
Experiment 2					
Control	6	35	318 ± 44	37.5 ± 0.33	36.5 ± 0.38*
Control	6	0	925 ± 118†	37.3 ± 0.22	31.9 ± 0.69‡

*$p<0.01$, significantly different from body temperature before flurothyl-induced seizure.
†$p<0.05$, significantly different from controls at 23.
‡$p<0.001$, significantly different from body temperature before flurothyl-induced seizure.

Seizure testing was conducted on postnatal day 60 with the exception of some mice that were retested on postnatal days 90 and 120. Seizure thresholds were determined by placing a mouse in a two-gallon desiccator jar and injecting 1.5 ml of a flurothyl [Bis(trifluoro ethyl ether) Armageddon Chemical Co.] solution (10% in 95% ethanol V/V) into the base of the jar [4] which was then immediately sealed. The desiccator jar contained a perforated porcelain plate which was 7.5 cm above the base of the jar so the mouse was never in physical contact with the flurothyl prior to it volatilization. The atmosphere of the jar was continually mixed with the aid of a large magnetic stirring bar with star-shaped fins. The time elapsed, after sealing the jar, to the development of a stereotypic clonic-tonic seizure (with loss of righting reflex), was taken as the seizure threshold. All mice were removed immediately after the recording of the first clonic-tonic seizure. A pharmacological challenge with the opiate antagonist, naloxone HCl, was performed by administering 5 mg/kg (IP) and determining seizure thresholds of MSG treated and control mice 15 minutes after the naloxone injection.

The effects of environmental temperature on flurothyl seizure threshold and body temperature were examined. These experiments were performed at environmental temperatures of 23° (ambient), 35° and 0°. Core temperature was determined using a thermometer (Yellow Springs Instrument Co. (YSI), Model 43 TK) equippped with a thermistor probe (YSI Type 402) that was inserted rectally.

The mice were sacrificed after seizure testing and wet weights of whole brain (minus the olfactory bulbs) were determined in mice randomly selected from each treatment condition.

Statistical Analysis

Nonparametric statistical analyses were employed where appropriate since the seizure thresholds of the MSG-treated mice did not exhibit a normal distribution. Comparisons between the seizure thresholds of the MSG-treated mice and those of the controls were performed using the Mann-Whitney U-test [25]. Body temperatures were analyzed by paired t-tests and brain weights by the Students t-test.

RESULTS

MSG at doses of 4 mg/g or 1 mg/g did not significantly alter the threshold for flurothyl-induced seizures (Table 1). Groups of MSG-treated (4 mg/g n=6, 1 mg/g n=6), and control (n=7) mice tested again at postnatal days 90 and 120 also did not differ in their thresholds for flurothyl-induced seizures (data not shown). Naloxone administration did not re-

TABLE 3

EFFECTS OF FLUROTHYL EXPOSURE AND ENVIRONMENTAL TEMPERATURE ON BODY TEMPERATURE

Condition	N	Exposure Duration (min)	Environmental Temperature	Body Temperature (°C ± SE) Before	After
100% Ethanol	5	10	23	37.7 ± 0.23	37.9 ± 0.10
Flurothyl	6	2.5	23	37.4 ± 0.27	37.4 ± 0.20
Flurothyl	5	3.5	23	37.3 ± 0.23	37.3 ± 0.30
No Flurothyl	4	15	0	37.4 ± 0.14	37.7 ± 0.23
Flurothyl	5	10	0	37.8 ± 0.33	35.8 ± 0.46*

*$p<0.001$, significantly different from before treatment.

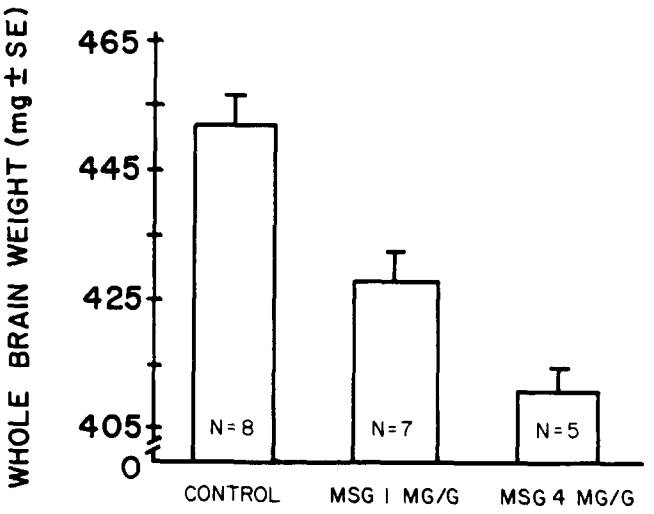

FIG. 1. MSG-induced decreases in whole brain wet tissue weight. MSG at doses of both 1 mg/g and 4 mg/g produced significant ($p<0.05$) reductions in whole brain weight.

sult in any significant alteration in the seizure thresholds of MSG-treated or control mice (Table 1). The effects of flurothyl-induced seizures on body temperature are presented in Table 2. Flurothyl-induced seizures produced a significant drop in body temperature at all the environmental temperatures examined in this study (Table 2). There was a significant increase in seizure threshold at 0°, however this may be attributable to the decreased volatility of flurothyl at 0°. The latency to seizure and the magnitude of the drop in body temperature at 0° ($r=-0.93, p<0.01$) and 23° ($r=-0.84$, $p<0.05$) were significantly correlated. No such correlation ($r=-0.72$) between exposure to flurothyl and drop in body temperature was found at 35°, although there was a significant drop in body temperature following exposure to flurothyl (Table 2). Therefore, a longer exposure of flurothyl at either 23° or 0° resulted in a greater drop in body temperature.

A series of experiments were performed to examine the effects of the ethanol vehicle, environmental temperature and flurothyl in the absence of overt seizure activity. The results of these experiments are presented in Table 3. Ethanol or 15 minute exposure to 0° did not significantly influence body temperature, however 10 minute inhalation of flurothyl at 0° resulted in a significant drop in body temperature (Table 3). Brief exposures to flurothyl also did not produce measurable hypothermia (Table 3).

A dose related decrease in whole brain weight was produced by MSG treatment (Fig. 1). The histologic and neurochemical consequences of this particular dosing regimen have been previously published [9,10].

DISCUSSION

Our experiments support the findings of Prabhu and Oester [22] in that no significant alteration in seizure susceptibility was detectable in adult mice that had been neonatally treated with MSG. There are a number of distinct methodological and procedural differences between our study and the studies of Pizzi *et al.* [20] and Prabhu and Oester [22]. We chose the flurothyl technique since it has numerous advantages over PTZ and flurothyl is thought to have a similar mode of action [1, 5, 28]. The increased susceptibility to PTZ-induced seizures reported by Pizzi *et al.* [21] may be attributable to damage outside the arcuate nucleus. Pizzi *et al.* [21] used multiple injections of MSG and the ICR strain of mouse in their study. Hippocampal damage, as well as damage to other brain regions may be severe when multiple injections of MSG are administered [16,27]. In addition, the ICR strain of mouse appears to be much more susceptible to extra-arcuate damage than are other mouse strains [16]. Pharmacokinetic factors may also contribute to the difficulty in interpreting studies that use lipophilic drugs such as PTZ to induce seizures in obese mice.

Opiates have been shown to modulate flurothyl-induced seizure thresholds [6]. A naloxone challenge was therefore used to examine endogenous opiate involvement in flurothyl-induced seizures. MSG treatment results in β-endorphin depletion [13,15] and increased naloxone-induced anorexia [8]. Naloxone administration to either MSG-treated or control mice failed to influence flurothyl seizure thresholds. Naloxone in doses of 1–10 mg/kg has previously been shown not to alter flurothyl seizure thresholds in rats [6]. The failure of MSG treatment and/or naloxone administration to alter flurothyl seizure thresholds suggests that MSG-induced β-endorphin depletion or opiate receptor blockade by naloxone has neither proconvulsant or anticonvulsant actions in this seizure testing paradigm. The failure of MSG-induced β-endorphin depletion to alter flurothyl seizure thresholds would be consistent with other studies that show β-endorphin to be involved in nonconvulsive limbic seizures [12,26]. These studies demonstrated β-

endorphin-induced EEG abnormalities originating in limbic structures but no generalized convulsions [12,26]. Thus, these data argue against a major role for β-endorphin in generalized convulsive seizures.

During the course of these studies it was determined that in addition to flurothyl's seizure producing properties, hypothermia occurs as a pharmacological consequence of exposure. This hypothermia is evident even at elevated (35°) environmental temperatures. Our results on the effects of temperature on seizure threshold are in agreement with the work of Truitt *et al.* [28]. Whether hypothermia is involved in the mechanisms of flurothyl-induced seizures is currently under investigation and appears likely since hypothermia per se results in an increased susceptibility to experimentally induced seizures [31]. Flurothyl-induced alterations in thermoregulation merit further study.

The significant reduction in adult whole brain wet weight in the mice treated with 4 mg/g or 1 mg/g of MSG is an interesting form of toxicity. Previous reports of decreased brain weight have been noted in mice [29] and rats [24] fed glutamate in the diet. Decreases in brain weight following multiple injections of MSG to neonatal mice have also been reported [2]. The single injection of MSG on postnatal day 4 results in seizures followed by somnolence. Recent evidence indicates that neonatal seizures in rats result in an arrest in neuronal mitotic activity and a consequent drop in brain weight and DNA content [30]. In the rat, however, brain weight does not remain permanently decreased (Dawson, unpublished findings) after MSG-induced neonatal seizures. Further studies are needed to characterize this form of MSG toxicity and to assess the biochemical consequences of MSG-induced decreases in whole brain weight since select populations of neurons may not develop, dependent on the time at which postnatal mitotic activity is arrested.

In conclusion, flurothyl served as a useful experimental tool with which to assess seizure susceptibility. MSG treatment or naloxone administration did not produce alterations in flurothyl seizure thresholds. Flurothyl was discovered to have hypothermia producing properties which appeared independent of environmental temperature but the magnitude of the hypothermia was dependent on the duration of the exposure. MSG treatment was found to produce permanent reductions in whole brain weight even after only a single injection. Therefore, despite the numerous morphological and biochemical deficits exhibited by MSG-treated mice, seizure susceptibility as indexed by flurothyl screening is not altered.

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