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# Effect of Aminooxyacetic Acid on Audiogenic Seizure Priming in C57BL/6J and SJL/J Mice[1]

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SIPORIN, S. AND J. L. FULLER. *The effect of aminooxyacetic acid on priming for audiogenic seizures in C57BL/6J and SJL/J mice.* PHARMAC. BIOCHEM. BEHAV. 4(3) 269–272, 1976. – An attempt was made to determine the effects of genetic and temporal factors on the inhibitory action of aminooxyacetic acid on priming for audiogenic seizures. SJL/J and C57BL/6J mice were administered 20 μg/g of AOAA subcutaneously, employing either a 2 hr or 5 hr injection-prime interval, and either a 2 day or a 9 day prime-test interval. It was found that an inhibitory effect on priming occurred only in C57BL/6J mice; this effect was significant only for the 9 day groups, and was observed when wild running was used as the criterion for seizures, but not when clonic convulsion was the criterion. Effects of AOAA are discussed in relation to theories of seizure development through disuse supersensitivity, or by chemical actions on the GABA system.

AOAA Audiogenic Seizures Priming GABA

THE phenomenon of audiogenic seizures involves an exaggerated response to an intense sound stimulus. A seizure may occur in four degrees of severity, wild running, clonic convulsion, tonic convulsion and sometimes death (presumably due to respiratory failure). The more severe seizures always are preceded by the more benign forms. Certain susceptible strains of mice, such as DBA/2J, seize consistently on their first exposure to sound; other resistant strains such as SJL/J and C57BL/6J do not. Audiogenic seizure susceptibility of mice has been correlated with the concentration in the brain of adenosine triphosphatase, glutamic acid and ACTH [12]. Drugs which decrease norepinephrine or serotonin levels usually increase seizure severity [19] and drugs increasing these amines are protective [3,4]. Drugs decreasing brain gamma-aminobutyric acid (GABA) sensitize animals to noise and to audiogenic seizures [14].

The separation of inbred strains of mice into susceptible and resistant categories is not absolute. C57BL mice, exposed to an intense sound at a critical age do not convulse at that time but will do so on a subsequent trial after a suitable induction period [13]. The same priming phenomenon has been found in SJL/J [9] and HS [3] mice. Since the form of seizures in the spontaneously susceptible and primed-susceptible mice appears identical we have here a phenocopy, at least at the level of gross behavior, of a genetic condition. Clarification of the chemical basis of priming might provide insight into the nature of developmental processes involved in spontaneous susceptibility. The general issues of relating the genetic and physiological findings have been discussed by Fuller and Collins [10].

As far as we know, the only successful attempt to prevent the occurrence of priming by pharmacological intervention was by Sze [21]. Working with C57BL/6J mice, he found that animals exposed to auditory stimulation experience a transitory decrease in brain GABA; he postulated that administration of a drug which increases GABA levels would prevent priming by deterring this decrease. Sze administered aminooxyacetic acid (AOAA), an inhibitor of GABA transaminase, subcutaneously to C57BL mice 5 hr before priming them, tested them for seizure response 9 days later at 28 days of age, and reported a significant inhibition of priming. Fuller (unpublished) at about the same time found no inhibitory effect of AOAA on the priming of SJL mice. There were potentially important differences in the time parameters of the 2 studies. The injection-priming intervals were 2 hr for Fuller and 5 hr for Sze; the priming-test intervals were 2 days and 9 days respectively. Also, the experiments were conducted

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with 2 unrelated strains. In this experiment, we tried to determine the contribution of genetic and procedural factors to the discrepancy in these 2 studies.

## METHOD

### *Animals*

Fourteen male and fourteen female SJL/J mice and a similar number of C57BL/6J mice obtained from Jackson Laboratories, Bar Harbor, Maine, were used as breeding stock from which 179 C57BL and 175 SJL offspring were obtained to serve as animals for the experiment. All mice were primed at 19 days of age, unless they were under 7.0 g; in this case, they were not tested until they had approximated this weight or attained the age of 22 days, whichever came first. (Most mice reached the desired size by 22 days.)

### *Apparatus*

A wooden box with a glass viewing screen was the seizure box. Inside the box was a circular plastic tube about 34.5 cm in dia. in which the animals were placed. A doorbell hung from the interior ceiling of the box produced the sound stimulus, which was on the order of 107 ± 2db.

### *Procedure*

Four experimental groups were used, employing injection-prime intervals of 2 or 5 hr, and prime-test intervals of 2 or 9 days. The 5h-9d and 2h-2d groups were the original Sze and Fuller groups respectively, and the 2 major control groups were a group primed without AOAA 2 days before testing, (P-2) and a group primed without AOAA nine days before testing (P-9). Four additional control groups consisted of animals which were not primed but tested at either 21 days (NP-2) or 28 days (NP-9); or which received AOAA but were not primed, and were tested at 21 days (AOAA-2) or 28 days (AOAA-9).

Experimental animals received injections of AOAA at a dosage of 20 μg/g subcutaneously at 19 days of age (subject to restrictions mentioned previously). Either 2 or 5 hr after injection, animals were primed in the seizure box by subjecting them to the bell stimulus for 60 sec. Either 2 or 9 days after priming, animals were again placed in the seizure box, and subjected to the bell stimulus until they exhibited clonic seizure or until 60 sec elapsed, whichever came first. Type of response, none (NR), wild running (WR), clonic or tonic convulsions (CN) and latencies were recorded. Control animals were tested similarly, and data were recorded.

## RESULTS

Except for a few anomalous cases (possibly due to unintentional priming caused by extraneous laboratory noise) unprimed animals did not convulse whether or not they had been administered AOAA. Injection-prime interval (2 hr or 5 hr) had no effect on seizure incidence; therefore, the 2h-2d and 5h-2d groups were combined into a single experimental 2 day group; the 5h-9d and 2h-9d groups were combined into a single 9 day group. Also, there were few tonic convulsions in some groups so that all convulsions (clonic and tonic) were combined for some statistical tests. Table 1 shows data for experimental and control mice of both strains.

TABLE 1

NUMBER OF ANIMALS FOR EACH STRAIN OF MICE DISPLAYING NO RESPONSE (NR), WILD RUNNING (WR) AND CLONIC OR TONIC CONVULSION (CN) UNDER 2 DAY AND 9 DAY PRIME-TEST INTERVALS

| | Priming Test Interval | | | | | |
|---|---|---|---|---|---|---|
| | 2-day Maximal Response | | | 9-day Maximal Response | | |
| | NR | WR | CN | NR | WR | CN |
| C57BL/6J | | | | | | |
| AOAA injected | 17 | 10 | 13 | 37 | 0 | 3 |
| Noninjected | 9 | 9 | 12 | 18 | 8 | 4 |
| SJL/J | | | | | | |
| AOAA injected | 13 | 9 | 18 | 19 | 5 | 16 |
| Noninjected | 14 | 4 | 12 | 11 | 0 | 19 |

When any type of seizure (sum of the WR and CN categories) was the criterion of susceptibility, AOAA produced a significant reduction in the effectiveness of priming in the 9-day C57BL group ($\chi^2 = 10.75$; $df = 1$; $p<0.01$). For the 2 day C57BL group the same criterion yielded no significant effect ($\chi^2 = 1.15$; $df = 1$; $p>0.20$). When the occurrence of a convulsion was the criterion for susceptibility, no effect of AOAA upon priming effectiveness was found for either the 9 day group ($\chi^2 = 0.65$; $df = 1$; $p>0.30$) or the 2 day group ($\chi^2 = 0.42$; $df = 1$; $p>0.50$). For the 9 day group, the lack of significance using a convulsion as a criterion was related to the low incidence of convulsions in the combined group of 9 day experimental and control animals (10%). Convulsions were highest in the control 2 day group (40%), whereas the 9 day control group had only a 13% incidence of clonic seizures.

Since there was a rather substantial number of tonic seizures occurring among animals in the 2 day group, a comparison was made between the number of control and experimental animals in this group with regard to incidence of tonic seizure among animals which exhibited clonic convulsions. (Table 2) It was found that, as compared with control, significantly few of the injected animals which convulsed went on to the more severe tonic seizure ($\chi^2 = 5.59$; $df = 1$; $p<0.02$).

TABLE 2

NUMBER OF C57BL/6J MICE SHOWING TONIC OR SUB-TONIC RESPONSE FOR EXPERIMENTAL AND CONTROL GROUPS IN THE TWO DAY PRIME-TEST INTERVALS. (ONLY ANIMALS EXHIBITING A SEIZURE RESPONSE ARE INCLUDED.)

| | 2 Days | |
|---|---|---|
| | Experimental (AOAA-Injected) | Control (Non-Injected) |
| Sub-Tonic | 15 | 11 |
| Tonic | 2 | 10 |

Table 1 also shows data for experimental and control SJL mice in 9 day and 2 day prime-test interval groups using all seizures (WR + CN) as the criterion. A $\chi^2$ test showed that there was no significant difference between injected and control SJL mice in the effectiveness of

priming for either the 9 day group ($\chi^2 = 0.82$; $df = 1$; $p>0.30$) or the 2 day group ($\chi^2 = 1.45$; $df = 1$; $p>0.10$). Using a convulsion as the criterion for seizure susceptibility, no significant difference was found between control and experimental groups in either the 9 day group ($\chi^2 = 3.73$; $df = 1$; $p>0.05$) or the 2 day group ($\chi^2 = 18$; $df = 1$; $p>0.50$).

Very few tonic seizures were seen among the SJL mice, and no test of significance was done on this measure.

Convulsions in control SJL mice of the 2 day group were of the same incidence as those among control C57BL mice of the 2 day group (40%); however, the incidence of clonic seizure in the 9 day group was much higher in SJL than in C57BL controls (66% for SJL vs. 13% for C57BL). The incidence of wild running as the maximal response was slightly above 50% in the P-2 group of SJLs; comparable C57BL controls showed almost 70% incidence. For the SJL 9 day group (P-9), wild running occurred about 65% of the time, as compared to 40% for comparable C57BL mice.

In general, it appeared that AOAA effects were strain and time dependent, and were significant only among C57BL/6J mice exposed to a 9 day prime-test interval.

## DISCUSSION

It seems that both Fuller and Sze were correct; AOAA is effective in inhibiting priming in C57BL/6J mice, but not SJL/J mice. The finding of strain differences in AOAA effects has also been found using C57BL/6/Bg and DBA/1/Bg mice [15]. In our experiment, the effect was shown to be on inhibition of wild running, whereas Sze found a strong effect on inhibition of clonic convulsions. He, however, found a high incidence of convulsions (73%) while we found only a low incidence (13%). Boggan *et al.* reported 61% seizures in C57BL mice treated similarly to our 9 day controls [3]. It appears that seizure elicitation is somewhat more variable than one would desire, and that small differences in procedure affect the magnitude of the effects of AOAA.

The trend toward inhibition of clonic but not wild running seizure in SJL mice is also interesting; it apparently is also replicable, as earlier work in this laboratory (Siporin, unpublished) yielded an effect of approximately the same magnitude. Since there was a high incidence of clonic seizure in the control groups, perhaps AOAA acts to inhibit seizure expression through a persistent neuromotor effect, thus yielding diminished convulsive severity, rather than actually interfering with priming per se.

Hypothesized explanations of priming include damage to the inner ear by intense noise leading to decreased neural input which in turn produces disuse supersensitivity at higher levels in the auditory pathways. Tympanic membrane destruction also leads to the development of audiogenic seizure susceptibility in BALB/c mice [6]. Since such damage causes reduction of input, it supports the disuse supersensitivity hypothesis. Disuse at the neuromuscular junction leads to increased sensitivity to ACh, as well as increased transmitter release and faster resynthesis [16]. The mechanism of supersensitivity might involve alterations in neurotransmitter receptors on the cell membrane [21]. In hippocampal preparations long lasting facilitation of a synaptic potential following tetanization was found, as well as increased postsynaptic sensitivity to transmitter [20]. Increase in cell excitability, synaptic alterations or other factors might be involved.

What might be the relationship between such concepts of disuse supersensitivity and the effect of AOAA on priming? It has been found that primed mice show altered cochlear microphonic responses [17,18]; AOAA administered to guinea pigs resulted in a reduction of the endocochlear potential, the cochlear microphonics, and the compound action potential of cochlear nerve [2]. An AOAA effect on GABA might be related to this, as Sze suggests. In fact, evidence for an inhibitory GABA system in the cochlear nucleus was found in cats [7]. However, AOAA might have effects of its own; AOAA induced depression of spinal reflexes in cats was not mediated by effects on GABA [1].

Alterations in the cochlea caused by noise stimuli have been shown in chinchillas [5]; hair cell damage was noted along with increased vacuole formation. However, it was not just the loss of cells which indicated the effect of damge, but the condition of existing cells. This is significant, because in terms of disuse supersensitivity one might be more interested in an alteration of cells rather than cell destruction per se. The duration of temporary threshold shifts [12] may also be of interest in terms of the developmental aspects of priming induced seizure development [18]. Certain temporary cell alterations may show up after exposure to noise. One significant point is that the cell deterioration may occur some time after the stimulus has ended.

AOAA may exert an effect on priming, whether mediated by GABA or not, by preventing or decreasing damage through reduction of input by means of reduced cochlear microphonics. It may also act in other ways; since there are genetic differences in response to AOAA, there may be more than one mechanism by which priming induces seizure susceptibility.

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