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Increased Susceptibility of Audiogenic Rats to Barbital Withdrawal Convulsions

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BOURN, W. M. AND R. L. GARRETT. *Increased susceptibility of audiogenic rats to barbital withdrawal convulsions.* PHARMACOL BIOCHEM BEHAV **19**(5) 839–841, 1983.—Non-responsive progeny from Sprague-Dawley derived rats genetically susceptible to sound-induced (audiogenic) convulsions (AGS-negative) and non-responsive progeny from Sprague-Dawley derived rats not genetically susceptible to audiogenic convulsions (SD-negative) were subjected to a seven-day treatment regimen of sodium barbital, 125 mg/kg, every 12 hours. This represents a lower dose and shorter treatment period than that normally used in this laboratory to induce barbiturate dependence in rats. Animals were subjected to a 115 dB sound stimulus 38 hours following the last dose of sodium barbital. SD-negative rats did not become susceptible to sound-induced convulsions, but AGS-negative rats did experience convulsions when exposed to the sound stimulus during withdrawal. These results are consistent with the hypothesis that rats generally referred to as "audiogenic" may actually suffer from differences which result in an increase of susceptibility to seizures induced by any of several means.

Audiogenicity Barbiturate withdrawal Convulsions Epilepsy

A number of investigators have reported studies involving rats which are susceptible to sound induced convulsive seizures [2, 5, 10, 13]. This susceptibility has resulted in common reference to these animals as "audiogenic rats." However, this term may be somewhat narrow as it has been shown that such animals are more susceptible to convulsive seizures induced by several means, including minimal [8] and maximal [16] electroshock, pentylenetetrazol chemoshock [15], and hyperthermia [14].

It has also been observed that rats not ordinarily susceptible to sound-induced seizures become susceptible during withdrawal from a chronic dosing schedule of sodium barbital [1, 3, 6, 7, 18]. It is interesting that these animals experience seizures which are nearly identical in appearance to the seizures experienced by audiogenic rats [19].

Another interesting aspect of animals derived from audiogenic colonies deals with the fact that some of the progeny are not susceptible to the sound stimulus. Working with such non-responsive progeny from susceptible rats, Jobe and others [11] reported that Ro4-1284, an agent which produces a rapid reduction of the brain amines NE, DA, and 5HT will cause the non-responsive progeny to become audiogenic. Furthermore, they demonstrated that these animals continued to remain susceptible to the sound stimulus long after the effects of the drug on brain amines had dissipated.

These authors then suggested that several sets of determinants exist which influence the seizure activity displayed by audiogenic rats. The evidence supportive of this suggestion includes the finding that graded monoaminergic deficits exist in animals which experience seizures of different levels of severity [12] and that cochlear deficits are present in audiogenic rats [9].

The increasing complexity of our knowledge of sound-induced convulsive seizures led to the present study in which non-responsive progeny from genetically susceptible parents (animals from a colony of audiogenic rats maintained in this laboratory) were compared with non-responsive progeny of parents from a non-audiogenic colony relative to barbiturate dependence liability.

METHOD

Animals were housed in individual stainless steel cages and given free access to water and purina lab chow pellets.

All rats used in the study were screened on 3 separate occasions by being subjected to a challenge of approximately 115 dB inside a special testing chamber [13]. This was done in order to confirm that all animals used in the study were not susceptible to the sound stimulus.

Animals were assigned to 6 treatment groups, 3 groups of non-responsive progeny from susceptible parents in the audiogenic colony (AGS) and 3 groups of non-responsive animals from a colony of Sprague-Dawley derived rats (SD) bred at the Northeast Louisiana University School of Pharmacy Animal Resource Center. One group of the AGS rats and one group of the SD rats were administered a seven-day regimen of intraperitoneal sodium barbital, 125 mg/kg every

12 hours (14 doses), followed by a continuation of the treatment regimen for 3 additional doses of barbital (AGS continued and SD continued, respectively), representing a total regimen of 17 doses. The rats were then subjected to a sound challenge 14 hours after the last dose of barbital, a time at which there appears (from behavioral observations) to be a sufficient level of activity of the barbital remaining in the animals that they may be regarded as being "maintained." Two other groups, one from the non-responsive AGS animals and one group from the SD animals, were administered the seven day (14 dose) regimen of sodium barbital and then switched to an intraperitoneal dose of normal saline every 12 hours for 3 doses. These animals were challenged with the sound stimulus 14 hours after the last dose of normal saline or 38 hours after the last dose of sodium barbital (AGSWD and SDWD, respectively). An appropriate pair of controls, tested in order to determine that other various aspects of the experiment did not result in changes of response to the sound stimulus, were treated with normal saline injections for the entire treatment period (AGSNS and SDNS, respectively). It should be noted that the barbiturate dose regimen was intentionally selected to be at a lower dose level and a shorter duration of exposure than that utilized in earlier studies [17,19]. The previously used higher dose regimen (150 mg/kg twice daily for 14 days) results in approximately 80% incidence of susceptibility to sound induced seizures in normally non-responsive animals [17].

Animals were then subjected to the test stimulus and evaluated according to the method of Jobe *et al.* [13]. This is an evaluation system in which responding animals are assigned scores on a scale of 1 to 9, with a score of 1 assigned to animals which exhibit a wild running fit, and a score of 9 assigned to animals which exhibit a running fit terminated by a convulsion involving full tonic extension. Scores between 1 and 9 are determined by a system which includes such factors as the extent of body involvement, presence of clonus or tonus, and the number of running fits preceding the convulsive phase.

Group susceptibility comparisons were made using Fisher's Exact Probability test [4].

RESULTS

The only group demonstrating a high incidence of convulsive activity in this study was the barbiturate withdrawn group of non-responsive progeny from susceptible parents (AGSWD) (Table 1). In this group 14 animals experienced some level of response out of 17 animals in the group, significantly different from the one response out of 15 animals tested in the barbiturate withdrawn Sprague-Dawley group (SDWD).

As expected, animals which had received normal saline for the entire treatment time (AGSNS and SDNS, respectively) did not show significantly elevated seizure susceptibility, although the AGSNS group did display a trend in that direction, with 5 of the 17 animals in that group experiencing seizures. Also as expected, both groups maintained on the barbiturate up to the time of testing (AGS cont and SD cont) did not demonstrate significant convulsive activity upon sound challenge.

An important consideration was whether the 5 positive responses out of 17 in the normal saline treated AGS rats represented a significant elevation of seizure susceptibility when compared to normal saline treated Sprague-Dawley rats. The probability factor in this comparison was 0.089392,

TABLE 1

SOUND INDUCED SEIZURE SUSCEPTIBILITY IN NON-RESPONSIVE AUDIOGENIC PROGENY DURING BARBITURATE WITHDRAWAL

	No. Convulsing/ No. Tested
AGS cont*	1/16§
AGSWD†	14/17
AGSNS‡	5/17
SD cont	0/13
SDWD	1/15
SDNS	1/16

*See text for description of groups.

†Different from SDWD at $p=0.000018$ and different from AGSNS at $p=0.0024$ according to Fisher's Exact Test [4].

‡Compared to SDWD and SDNS, $p=0.102429$ and $p=0.089392$, respectively.

§Number of animals experiencing a seizure with a score of one or higher/Number of animals tested.

TABLE 2

SEIZURE SEVERITY OF ANIMALS EXPERIENCING CONVULSIONS

AGS cont*	5.0 ± 0.0 (1)†
AGSWD	4.6 ± 0.6 (14)
AGSNS	3.0 ± 0.9 (5)
SD cont	—
SDWD	1.0 ± 0.0 (1)
SDNS	1.0 ± 0.0 (1)

*See text for description of groups.

†Severity score ± S.E.M.; (Number of animals responding).

not sufficiently small to indicate a significant difference. Similarly, the p-value for a comparison made between the AGS animals treated with normal saline and the barbiturate-withdrawn group of Sprague-Dawley animals was 0.102429 (Table 1). An additional question was whether the incidence of seizure susceptibility in barbiturate withdrawn AGS rats exceeded that of the normal saline-treated AGS rats. This was significantly different, showing that the increased incidence of convulsions in the withdrawn group of AGS rats was a result of the withdrawal and not produced by other factors exerting an influence on the "genetically-loaded" AGS animals (Table 1).

A further consideration is the severity of seizures experienced by the animals which did respond. Analysis of variance revealed that there were no significant differences among the various groups (Table 2).

A time course study of seizure susceptibility of the animals was conducted after the withdrawal period (Table 3). Unlike the results of Jobe's investigation in which a significant number of non-responsive progeny of susceptible parents remained susceptible as long as 19 days after being rendered susceptible with Ro4-1284 [11], responsive animals in the AGS withdrawn group in the present study did not remain susceptible to the sound challenge. In fact, by the 8th day following the withdrawal test, none of the groups displayed significant elevation of seizure susceptibility. Of some interest here is that on the 3rd day after testing, the rats

TABLE 3

TIME COURSE OF SEIZURE SUSCEPTIBILITY FOLLOWING BARBITAL WITHDRAWAL

	Days After Withdrawal					
	1	3	6	8	10	13
AGS cont*	1/16†	8/12	3/12	0/8	0/8	1/8
AGSWD	14/17	5/11	2/11	1/9	1/9	1/9
AGSNS	5/17	0/13	5/13	1/8	1/8	1/8
SD cont	0/13	1/11	1/11	1/5	1/5	2/5
SDWD	1/15	1/11	0/11	2/5	0/5	1/5
SDNS	1/16	0/10	0/10	0/5	0/5	0/5

*See text for description of groups.

†Number of animals experiencing a seizure with a score of one or higher/Number of animals tested.

which had been continued on the barbiturate during the withdrawal period, were now experiencing their own withdrawal period; that is, this test was conducted 52 hours after the last dose of barbiturate, and 8 out of 12 animals experienced seizures. As expected, the comparable Sprague-Dawley group still exhibited no increase in seizure susceptibility.

DISCUSSION

The failure of animals in the present study to remain susceptible to the sound stimulus may be related to the fact that the level of seizure severity in the AGS withdrawn animals was low compared to the mean score of nearly nine observed in the Ro4-1284-treated animals in Jobe's study [11]. The maximally severe seizures experienced by those animals may be more likely to induce a priming or kindling mechanism.

Since the non-responsive AGS animals used in the present study became responsive upon being withdrawn from a barbiturate dose regimen which did not induce withdrawal audiogenicity in Sprague-Dawley rats, we suggest that this adds further support to the hypothesis that rats generally referred to as "audiogenic" may actually suffer from differences which result in an increase in susceptibility to seizures induced by any of several means.

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