Effects of Pharmacological Agents on Acoustic Priming of Audiogenic Seizures[1] (36827)

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Acoustic priming induces a long-lasting, extremely stable susceptibility to audiogenic seizures in mice. Exposing a mouse to a loud acoustic source (*e.g.*, an electric bell) during a sensitive period of early neural development constitutes the priming situation. This alters the animal so that a subsequent exposure, days or weeks later, results in a convulsion which often ends with death. This is effective even if the subjects are anesthetized with Nembutal or ether during priming (1). In addition, severe metabolic alterations before, during, or after priming have failed to attenuate this process; electroconvulsive shock (ECS), hypothermia (reduction of brain temperature to 17°), changes in endogenous central nervous system (CNS) levels of acetylcholine, and reduction of brain protein synthesis are equally ineffective (2). Thus far (3), the only agent which has been reported to reduce the effectiveness of priming is amino-oxyacetic acid (AOAA), a pharmacological agent which increases endogenous brain levels of gamma-aminobutyric acid (GABA). In addition, Sze demonstrated (3) that priming resulted in a temporary decrease of brain GABA, although no changes were observed in the amino acids glutamate and aspartate, or in the putative neural transmitters, serotonin and norepinephrine.

To date the implication has been that GABA is centrally involved in the development of priming-induced audiogenic seizures, but other findings have cast doubt on this interpretation. Cats which were given AOAA showed a time-dependent increase in an auditory reflex threshold, and guinea pigs had decreases of cochlear potentials when given this agent (4). These data suggest that AOAA protection from the effects of priming occurs via a temporary peripheral decrease in auditory sensitivity, rather than from a developmental change resulting from a temporary increase of brain GABA.

A test of this hypothesis was performed by analyzing the effects on priming of other drugs which affect GABA metabolism or which affect the transduction of sound in the mammalian ear. Both chlorpromazine (Thorazine) and diphenylhydantoin sodium (Dilantin) have been shown (5, 6) to increase brain content of GABA in mice. In addition, both these drugs have a demonstrated anticonvulsant (7–9) activity. Pentobarbital sodium (Nembutal) also has a pronounced anticonvulsant effect, and it depresses the activity of those middle ear muscles which are responsible for the aural reflex. This involuntary uncoupling of the auditory ossicles can reduce the amplitude of a loud noise in the middle ear by 12 dB (10, 11). This agent has also been demonstrated to increase the effectiveness of acoustic priming (12).

Methods. Six hundred eighty-five C57 BL/6J mice, maintained on a lighting schedule from 7 AM to 7 PM, were acoustically primed on their sixteenth day postpartum. Six hundred subjects were randomly assigned to AOAA, Dilantin, chlorpromazine, or saline conditions. These 600 mice were injected with one of the agents either 6, 4, or 2 hr before priming; immediately before or after priming (0 hr condition); or 1 or 2 hr after priming. Injections were administered to produce the desired change (5, 13) of brain GABA; AOAA, 25 mg/kg (sc); chlorpromazine, 10 mg/kg (im); Dilantin, 25 mg/kg (ip); and isotonic saline. The remaining 85

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mice were tested for the effectiveness of Nembutal on acoustic priming. Twenty-six subjects were anesthetized with 60 mg/kg Nembutal ip 5 min before priming, and an equal number were anesthetized immediately following priming. Thirty-three saline-injected mice served as controls for the anesthetized mice.

Priming of the Dilantin, chlorpromazine and AOAA mice and their controls was effected by exposing them to 30 sec of 120 dB (re. 2×10^{-4} dyn/cm^2) noise produced by an electric bell. This stimulus intensity and duration induced a large enough susceptibility to audiogenic seizures in the mice so that any protection offered by AOAA would be apparent. The Nembutal mice and their controls were primed with a 110 dB electric bell for 15 sec, a condition which would maximize the expected enhancement of priming by this anesthetic. The mice were all overtly nonreactive during priming; *i.e.*, no audiogenic seizure was observed during this first acoustic exposure.

The test situation was merely a 30-sec reexposure to the same sound 5 days after priming. At this time, occurrence of the 4 successive stages of the audiogenic seizure syndrome (wild running, myoclonic seizures, myotonic seizures, and death) was recorded, and each component was weighted as 25% of the possible seizure severity score of 100 (14). Because of the 8-hr span involved in the AOAA-chlorpromazine–Dilantin study, the seizure severity scores of the controls were subtracted from those of the experimentals at each time of injection to remove the confounding circadian variation. The Nembutal mice and their controls were all injected within 15 min of each other, so such a transformation of the severity scores was unnecessary.

Results and Discussion. The differential seizure severity scores for the mice administered AOAA, Dilantin, or chlorpromazine are illustrated in Fig. 1. None of the agents were effective if administered at time 0 or after priming. As reported by Sze (3), AOAA resulted in a partial protection if administered before priming. The time course of this protection, however, did not correspond to the

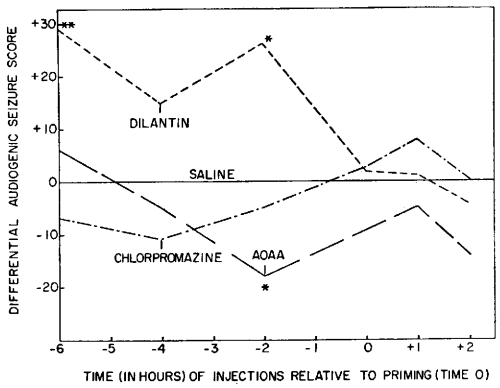

FIG. 1. Effects of various agents on ability of 120 dB sound source to prime mice for audiogenic seizures. Sze's (3) AOAA protection (*$p < .05$, 1-tailed Fisher's LSD) was replicated, and Dilantin was found to enhance priming (* $p < .05$, 2-tailed LSD; ** $p < .01$, 2-tailed LSD). Each data point represents 25 mice.

maximal elevation of brain GABA (13, 15); rather, it corresponded quite closely to the time course of the AOAA-induced protection from electroconvulsive seizures (15). It also corresponded well with the time courses of the elevation of the Preyer acoustic startle reflex threshold (4) and with the retropulsion (16) which have been observed in mice injected with AOAA. These correlations suggest that there is little reason for believing that the protection which AOAA affords on priming is causally related to its elevation of GABA.

Chlorpromazine did not differ significantly from saline injections, but Dilantin produced an unexpected increase in seizure severity if administered either 6 or 4 hr before priming. Since chlorpromazine and Dilantin both produced a 9–10% increase of brain GABA in mice (5), it would be difficult to explain opposite effects on priming by a common GABA mechanism.

The results from the mice injected with Nembutal suggest a different mechanism. When mice were injected with this barbiturate 5 min before priming, subsequent testing revealed an average audiogenic seizure severity score of 81.6. This was 31.6 units more severe than scores of mice anesthetized with Nembutal immediately after priming (t (57) $= 18.71$; $p << .0001$). In fact, mice injected

with Nembutal immediately after priming had severity scores no greater than those obtained from primed mice injected with saline (50 vs 48.5; $t < 1$).

A unitary explanation does not have to apply to the described actions of AOAA, Dilantin, and Nembutal. However, the protective effects of AOAA and the harmful effects of Nembutal could both be accounted for by their opposite effects on the transmission of acoustic information along the classical auditory pathway. AOAA decreases the cochlear potential and increases the threshold of the acoustic startle response by 40 dB in guinea pigs and cats, respectively (4). Nembutal produces a 12 dB depression of the reflexive decoupling of the middle ear ossicles in rabbits and men (10, 11), thereby removing inhibition to the auditory network. Dilantin may have a similar effect, thereby also accounting for its enhancement of the effects of acoustic priming.

Summary. Amino-oxyacetic acid (AOAA), chlorpromazine, and Dilantin were examined for their effects on acoustic priming of audiogenic seizures. AOAA administered before priming exhibited a protective effect, increasing the severity of subsequent audiogenic seizures. Dilantin had opposite effects, suggesting that the increase of brain levels of GABA provided by all three agents was not causally related to their effects on priming. Nembutal had an effect similar to that seen with Dilantin, and it was proposed that the active drugs exerted their influence by altering the threshold of the auditory transducer.

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