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GABAergic Modulation of Inferior Colliculus Excitability: Role in the Ethanol Withdrawal Audiogenic Seizures

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Abstract

The role of the inferior colliculus and GABAeric transmission within this structure in the development of susceptibility to sound-induced seizures in ethanol-dependent rats was examined. Ethanol-dependent rats with bilateral electrolytic lesions which destroyed approximately 50.0 ± 6.4% of the inferior colliculus failed to exhibit susceptibility to sound-induced seizures. However, comparable medial geniculate body lesions (82.7 ± 2.7% complete) did not alter wild running, slightly reduced tonus and actually increased clonus susceptibility in rats treated similarly with ethanol. As reported previously, bilateral injection of either muscimol (43–263 pmol/site) or racemic baclofen (520–1580 pmol/site) into the inferior colliculus also suppressed seizure susceptibility. Other studies in ethanol-naive animals found that bilateral microinfusion of(+)-bicuculline methoiodide (2 or 20 pmol/min for up to 5 min) into the inferior colliculus induced wild running and clonus closely resembling sound-induced seizure responses in ethanol-dependent rats. Although similar microinjections of (+)-bicuculline methiodide (0.4 pmol/min for 5 min) into the inferior colliculus did not induce seizure activity directly, an increased susceptibility to sound-induced seizures was observed. Electrolytic lesions of the medial geniculate body did not block wild running responses induced by (+)-bicuculline methiodide, but slightly reduced clonus. Five-minute infusions of picrotoxin (200 pmol/min), Ro5-3663 (2000 pmol/min), kainic acid (20 or 200 pmol/min), strychnine (2000 pmol/min) or carbachol 2000 pmol/min) into the inferior colliculus of ethanol-naive rats all induced bicuculline-like seizures. Seizures induced by bicuculline methiodide, picrotoxin or Ro5-3663 occurred within 5 min after the start of infusions. Seizures induced by non-GABAergic drugs were delayed 10 to 15 min after beginning the infusions. Finally, muscimol (263 pmol/site) administered into the inferior colliculus blocked sound-induced seizures in Uaz:AGS (S.D.) epilepsy prone rats and prevented seizures induced by bicuculline or kainic acid infused into this brain site. However, seizure responses after i.v. bicuculline infusion or maximal electroshock were not diminished by injection of this dose of muscimol into the inferior colliculus. These results suggest that adaptation in GABAergic inhibitory mechanisms in the inferior colliculus could play an important role in the genesis of sound-induced seizures in ethanol-dependent rats, although additional mechanisms involving excitatory processes may also be important.

The inferior colliculus is a prominent relay nucleus in the auditory pathway and appears to play an important role in modulating auditory sensory input to the cerebral cortex (Morest, 1975). Recent experimental evidence also suggests that the inferior colliculus is involved in the generation of sound-induced seizures. For example, destruction of the inferior colliculus

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(Kesner, 1966; Wada *et al.*, 1970; Ward, 1971; Henry *et al.*, 1972) prevents audiogenic seizures in sound-sensitive rats and mice. This action does not appear to be due to interuption of sensory input to the cerebral cortex because lesions of other relay nuclei such as the medial geniculate body, nucleus of the lateral lemniscus, superior olivary complex or ventral cochlear nucleus all fail to block audiogenic seizures (Koenig, 1957; Duplisse *et al.*, 1974; Willott & Su, 1980).

GABAergic neurotransmission in the inferior colliculus may play an important role in modulating susceptibility to sound-induced seizures. Duplisse *et al.* (1974) have shown that microinjection of GABA into the inferior colliculus can prevent seizures in rats genetically susceptible to audiogenic seizures. GABAergic transmission in the inferior colliculus may also play an important role in the modulation of audiogenic seizure susceptibility in ethanol-dependent rats. Microinjection of GABA agonists, such as muscimol (263 pmol/site), into the inferior colliculus blocks completely sound-induced seizures during ethanol withdrawal (Frye *et al.*, 1983). As yet, it is unclear whether the inferior colliculus plays an active role in initiation of genetic or ethanol withdrawal-induced audiogenic seizures. However, the inferior colliculus of ethanol-naive rats does appear to be uniquely sensitive to bicuculline, a GABA antagonist, that can evoke audiogenic seizure-like responses when infused there in exceedingly small amounts (Duplisse *et al.*, 1974; Frye *et al.*, 1983). In addition, a single electrical stimulation of the inferior colliculus with relatively small currents can evoke seizure responses resembling audiogenic seizures (McCown *et al.*, 1984), suggesting that this brain area may be important in initiating the audiogenic seizure response. The present studies were designed to evaluate further the role of the inferior colliculus in the development of susceptibility to audiogenic seizures in the ethanol-dependent rat, and to characterize the relative sensitivity of the inferior colliculus to a variety of pharmacologically distinct chemical excitants.

Methods

Animals and surgical treatments

Male Sprague-Dawley rats (Timco-Harlan Industries, Houston, TX and Charles Rivers Laboratory, Somerville, MA) weighing 125 to 150 g were housed under environmentally controlled conditions including a 7:00A.M. to 7:00 P.M. light cycle, 25°C and continuous access to water and Purina rat chow. For one study Uaz:AGS (S.D.) rats were reared at the University of North Carolina animal care facility after obtaining breeding stock from Dr. Paul Consroe of the University of Arizona. One day after arrival, rats were anesthetized with sodium pentobarbital (40 mg/kg i.p) and mounted in a stereotaxic apparatus fitted with blunt guinea-pig ear bars (David Kopf Instruments, Tujunga CA) to prevent tympanic membrane rupture. After opening the scalp and placing small stainless-steel anchoring screws in the skull, a pair of 26-gauge stainless-steel guide tubes were lowered into the brain through small burr holes to a depth 1 mm dorsal to the intended bilateral microinjection sites and made fast with methyl methacrylate cement as described previously (Frye *et al.*, 1983). Coordinates for microinjection into the inferior colliculus were derived from the atlas of Paxinos and Watson (1982; incissor bar, −3.0 mm; interaural +0.1; L, 1.5; and below skull surface, −3.75). Lesions of the medial geniculate body and inferior colliculus were made electrolytically in pentobarbital-anesthetized rats. A stainless-steel electrode (0.2 mm diameter), insulated except for 0.5 mm at the tip, and lowered into the inferior colliculus (same coordinates as above) or medial geniculate body (interaural, +3.2; L, 3.25; and below skull surface, −6.0). A 2 mA anodal current was then passed for 5 sec. Sham-operated controls received identical surgical treatment but no current was passed. After surgery, all animals received penicillin (im; 30,000 U of Longicil, Fort Dodge Laboratories, Fort Dodge, IA). After surgery, all rats were allowed to recover for 2 to 3 weeks. During this time some

animals were fed a liquid diet containing ethanol to induce physical dependence (see below) whereas the remainder continued to receive standard rat diets.

Chronic ethanol treatment

Immediately after stereotaxic surgery, rats selected for ethanol dependence studies were housed singly and supplied with 50 ml of a nutritionally complete liquid diet and continuous access to water as described previously (Frye *et at.*, 1983). Ethanol treatment was continued for 15 consecutive days such that the ethanol concentration in the diet was 0.07 g/ml for the first 5 days and was then increased to 0.08 and 0.09 g/ml for the last two consecutive 5-day periods, respectively, to compensate for the development of metabolic tolerance. This regimen induced daily ethanol consumption of 12 to 16 g/kg/24 hr, maintained large amounts of ethanol in the blood stream (up to 2 mg/ml) and allowed continued weight gains (1 to 2 g/day; Frye *et al.*, 1981).

Drug microinjections

All drugs were dissolved in sterile 0.9% sodium chloride solution and the pH was adjusted to 7.0. (+)-Bicuculline methiodide was purchased from Pierce Chemical Co. (Rockford, IL); muscimol, kainic acid, strychnine hydrochloride, picrotoxin, ibotenic acid, carbachol, (+)-bicuculline (free base) and (−)-bicuculline methiodide from Sigma Chemical Co. (St. Louis, MO); DL-baclofen was a gift from Ciba-Geigy Corp. (Summit, NJ); and 5-methyl-1,4-benzodiazepine (Ro5-3663) from Hoffmann-La Roche Inc. (Nutley, NJ). Microinjections into the inferior colliculus were administered through 33-gauge stainless-steel cannulas, which extended into the target tissue 1 mm beyond the tip of the permanent guidetube, as described previously (Frye *et al.*, 1983). Drug solutions (0.5 μl) were delivered to the cannula at the rate of 0.1 μl/min over a 5-min interval (unless otherwise stated) *via* PE-10 tubing attached to a 10-μl microsyringe (Precision Instruments, Baton Rouge, LA). Upon completion of the infusion, injection cannulas were left in place for an additional 30 sec. All drug doses are expressed as the amount injected into each side of a bilateral structure.

Histological evaluation

At the conclusion of each experiment the microinjected or lesioned animals were sacrificed, the brain removed gently, fixed in 10% w/v formalin for at least 2 days and then placed on a cryostat chuck and frozen. Each brain was sectioned to allow visual identification of the cannula tip placement or the extent of the lesion by a trained individual with no knowledge of the individual treatments. Data from animals with microinjector tip tracts outside the desired target area of the inferior colliculus (fig. 1) were excluded from the analysis. Only data from animals with electrolytic lesions restricted to the hatched areas shown in figure 1 were analyzed.

Assessment of seizure activity

Susceptibility to audiogenic seizures, an index of the presence of physical dependence on ethanol, was evaluated 6.5 to 8.5 hr after ethanol was removed (8:00 A.M) on the final day of ethanol diet treatment during the period of greatest audiogenic seizure susceptibility. Seizure susceptibility was determined by exposing confined individual rats to the sound of a 98 db electric bell for 1 min as described previously (Frye and Ellis, 1975; Frye *et al.*, 1981, 1983). Sound-susceptible rats whether ethanol-dependent or genetically seizure-prone, exhibited one or more wild-running episodes (at least three rapid circles of the wire cage within a 3-sec interval) often leading to loss of upright posture and clonic-tonic convulsions. Hindlimb clonus was defused as a loss of upright posture followed by rapid cycles of hindlimb extension and retraction for at least 3 sec. Hindlimb tonus was defined as either a sustained extension or retraction of both hindlimbs for a period of not less than 3 sec. Wild

running and hindlimb clonus always preceded hindlimb tonus. A trained observer, without knowledge of the treatment assignments, evaluated the seizure tests. Similar measures were used to evaluate seizures caused by infusion of excitant drugs into the inferior colliculus.

For induction of i.v. bicuculline seizures, animals were restrained in a Plexiglas animal holder and the drug was injected rapidly through the lateral tail vein (2 ml/kg). Immediately after the injections animals were removed and placed in a Plexiglas cage for observation. Seizure activity began within 5 sec after animals were removed from the holder and consisted of facial spasms, followed rapidly by loss of upright posture and clonic movements of both the fore and hindlimbs which sometimes progressed to tonic limb extension. Pilot studies indicated that a dose of (+)-bicuculline (68 μmol/kg) was just sufficient to evoke hindlimb clonus in all animals and tonic hindlimb extension in approximately 33%. This dose was used in the present experiments and the number of animals exhibiting hindlimb clonus or tonus was recorded.

Electroshock seizures were induced by passing a 0.2 sec train of 60 Hz sine waves of 50 mA amplitude from a Wahlquist stimulator (Salt Lake City, UT) through saline-moistened corneal electrodes (Swinyard, 1972). This stimulus produced generalized clonic-tonic seizures in all animals tested. Stimulation induced flexion and extension periods were timed and used to calculate E/F ratios for determining seizure severity (Swinyard, 1972).

Statistical evaluations

Effects on the frequency of various seizure components were evaluated by the Chi square test (Winer, 1971). A one-way analysis of variance followed by Dunnett's *t* statistic was used to test for differences between treatment and appropriate control groups. An *alpha* of . 05 was used for all statistical tests.

Results

Effect of medial geniculate body and inferior colliculus lesions

Chronic administration of ethanol to rats *via* a liquid diet for 15 days increased the susceptibility of the animals to sound-induced seizures markedly 7 hr after ethanol withdrawal (Frye *et al.*, 1983; fig. 2). Although none of the test animals exhibited sound-induced seizures before ethanol treatment, figure 2 shows that over 66.7% exhibited wild running which frequently progressed to hindlimb clonus and tonus. Bilateral electrolytic lesions placed within the medial geniculate body did not reduce the number of animals exhibiting wild running seizure responses significantly when compared with sham-lesioned controls. However, the incidence of clonic hindlimb responses was increased significantly by this lesion, although the frequency of tonic hindlimb extension was reduced (fig. 2). In contrast to lesions of the medial geniculate body, bilateral lesions of the medial area of the inferior colliculus prevented wild running, clonus and tonus completely in response to sound stimulation in similarly treated ethanol-dependent rats.

Differences in the seizure responses of sham-lesioned controls relative to groups lesioned in the medial geniculate body or inferior colliculus did not appear to be due to different levels of physical dependence, because the average daily ethanol consumption was not significantly different for the three groups (sham = 13.62 ± 0.44; medial geniculate body = 13.34 ± 0.43; inferior colliculus = 13.17 ± 0.45 g/kg/24 hr). In addition, differences in the extent of the two lesions did not explain the observed differences in seizure suppression. Lesions were generally more complete for the medial geniculate body (82.7 ± 2.7%) than for the inferior colliculus (50.0 ± 6.4%), eventhough the most dramatic seizure blockade occurred after collicular damage. An example of an ideal lesion, one considered to have destroyed 100% of the medial geniculate body or inferior colliculus, is depicted in figure 1.

Anticonvulsant effect of intracollicular muscimol and baclofen

Previous studies indicated that intracollicular injection of muscimol or 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol suppressed ethanol withdrawal audiogenic seizures effectively (Frye *et at.,* 1983). Because these GABAmimetic agents are more specific for $GABA_A$ receptors than for $GABA_B$ sites (Hill and Bowery, 1981), the anticonvulsant effects of baclofen, a relatively specific $GABA_B$ agonist, were studied. Bilateral microinjections of muscimol (26–79 pmol/site) into the inferior colliculus suppressed sound-induced seizures in ethanol-dependent rats undergoing withdrawal in a dose-related manner (fig. 3), as reported previously (Frye *et al.,* 1983). A dose of 79 pmol/site blocked wild running as well as clonus and tonus completely in all rats tested. Similar infusions of (±)-baclofen (158–1580 pmol/site) into the inferior colliculus also caused a dose-related inhibition of susceptibility to sound-induced seizures in ethanol-dependent rats. Complete suppression of seizure susceptibility was observed after bilateral infusions of (±)-baclofen (1580 pmol/site). A smaller dose (520 pmol/site) caused a small but significant reduction in the incidence of clonus, although neither wild running nor tonus were significantly altered. An estimated ED_{50} dose of 46 pmol/site for muscimol and 444 pmol/site for (±)-baclofen suggested that muscimol was 10 times more effective in suppressing clonic seizure responses than was the racemic baclofen.

Effect of convulsant drug infusions

In a separate study, bilateral microinfusion of (+)-bicuculline methiodide into the inferior colliculus of ethanol-naive rats at the rate of 20 pmol/ mm for up to 5 min evoked vigorous wild running-like seizure responses as reported previously (Frye *et al.,* 1983). Seizure activity usually occurred within 3 to 4 min of the start of the infusion (table 1), often forcing the termination of the infusion process before the full 5-min infusion interval was complete. Seizure responses progressed to hindlimb clonus in 71.5% of the animals (fig. 4; table 1). What appeared to be a tonic hindlimb extension was observed in approximately 50% of the animals (data not shown). In contrast to the distinct boundary between hindlimb clonus and tonus in the ethanol-dependent rat, hindlimb tonus was not as easily distinguished from hindlimb clonus in (+)-bicuculline methiodide-treated animals and so it was not scored. Similar infusions of (+)-bicuculline methiodide in animals with bilateral lesions of the medial geniculate body (fig. 1) still evoked wild running in all animals (fig. 4). The average latency to the onset of wild running in lesioned rats (3.90 ± 0.20 sec) was not different from that of unlesioned bicuculline-infused controls (3.66 ± 0.23); however, there was a small but significant reduction in the frequency of hindlimb clonus in the lesioned animals (fig. 4). Five-minute infusions of (+)-bicuculline methiodide (2.0 pmol/min) in unlesioned rats also were marginally active, evoking wild running seizure activity in 50% of the animals which progressed to clonus in 16%. A lower dose of (+)-bicuculline methiodide (0.2 or 0.4 pmol/min) failed to initiate seizure activity directly. However, three of seven animals that received a 5-min infusion of (+)-bicuculline methiodide (0.4 pmol/min) exhibited sound-induced seizures when exposed to the 98 db electric bell (as used to test ethanol-dependent rats) 2 min after the termination of the drug infusion. These seizures included initial wild running phases that progressed to hindlimb clonus that closely resembled sound-induced seizures that could be evoked during ethanol withdrawal. None of these same seven animals showed susceptibility to sound-induced seizures when tested after intracollicular saline infusions.

In order to determine the relative selectivity of the excitatory action of (+)-bicuculline methiodide in the inferior colliculus, the (−)-isomer of bicuculline methiodide was studied. Infusions of (−)-bicuculline methiodide (20 pmol/min), the reportedly less active bicuculline isomer (Collins and Hill, 1974), rapidly evoked wild running of 100% in a separate group of experimental animals (table 1) that was indistinguishable from that after (+)-bicuculline methiodide. Infusion of a lower dose of either (+)- or (−)-bicuculline methiodide (2 pmol/

min) was less effective in evoking wild running but apparently there were no significant differences between the two stereoisomers. A 10-fold lower dose of either isomer failed to evoke wild running in any of the animals, although both drugs were infused for the full 5 min. The frequency of clonus following either (+)- or (−)-bicuculline methiodide infusion was also dose-related and was not significantly different for the isomers at any dose (table 1).

In addition to the stereoisomers of bicuculline methiodide, several other chemical excitants were microinfused into the inferior colliculus to determine whether they would evoke seizure activity (table 1). Among the agents tested were picrotoxin (Olsen, 1981) and Ro 5–3663 (Harrison and Simmonds, 1983) which inhibit function of the GABA receptor-linked chloride channel, but do not directly bind to the GABA recognition site as does bicuculline. Bilateral infusions of picrotoxin (200 pmol/min) evoked seizures in three of five animals which progressed to clonus in two animals. A lower concentration of picrotoxin (20 pmol/min) caused wild running in only one animal. The rapid onset and general characteristics of picrotoxin-induced seizure activity were similar to that after either isomer of bicuculline methiodide. The benzodiazepine derivative, RO 5–3663 (2000 pmol/min; table 1), caused wild running in 100% of the animals tested which progressed to hindlimb clonus in two animals. A lower dose (200 pmol/min) was without an effect. The onset of seizures was rapid (3.25 ± 0.25 min) as had been observed with (+)-bicuculline methiodide and picrotoxin.

Five-minute bilateral intracollicular infusions of other non-GABA related agents also evoked seizures. Relatively small amounts of kainic acid, an agonist at glutamate receptors (Watkins, 1981), into the inferior colliculus evoked seizures in a dose-related manner (table 1). Although the wild running and clonic seizures resembled those induced by bicuculline methiodide, the onset of the seizures was delayed significantly (table 1). Bicuculline methiodide-induced responses occurred well before the conclusion of the 5-min infusion (mean onset 3.66 ± 0.23 min), but seizures induced by kainic acid were delayed an average of 10.00 ± 1.47 or 9.80 ± 1.20 min, respectively, after the 5-min infusion of kainic acid (20 or 200 pmol/min) began. The largest dose of kainic acid (200 pmol/min) caused wild running and clonus in 100 and 67% of the animals, respectively. Wild running seizures also occurred in over half the animals infused with kainate (20 pmol/min) but none were seen after the lowest dose (2 pmol/min). In contrast to the convulsant actions of kainic acid, bilateral infusion of ibotenic acid (2000 pmol/min), another glutamate analog (Watkins, 1981), into the inferior colliculus, failed to evoked seizure-like responses. Both carbachol (2000 pmol/min), a nonselective cholinergic agonist (Bebbington and Brimblecombe, 1965), and strychnine (2000 pmol/min), a glycine antagonist (Kuno and Weakly, 1972), infusions also evoked wild running in a small but significant number of animals; however, only carbachol induced clonus. The onset of wild running after bilateral infusions of either of these drugs was also significantly slower than that for (+)-bicuculline methiodide (20 pmol/min; table 1).

Effect of intracollicular muscimol on different types of seizures

Although intracollicular muscimol injections (79 pmol/site) suppressed sound-induced seizures in ethanol-dependent animals effectively (fig. 3), it was not clear whether this was a generalized anticonvulsive effect or one only selectively active against ethanol withdrawal seizures. For this reason, an even larger dose of muscimol was tested for anticonvulsant effects against other types of seizures. Table 2 summarizes the results of a study designed to determine the relative efficacy of bilateral intracollicular muscimol (263 pmol/site), a dose three times larger than that necessary to block ethanol withdrawal seizures completely, against other types of seizures triggered by auditory, chemical or electrical stimuli. As expected, this dose of muscimol suppressed sound-induced wild running and clonus

completely in 11 of the 12 ethanol-dependent rats tested. Next, the effects of muscimol (263 pmol/site) were tested against sound-induced seizures in genetically epilepsy-prone Uaz:AGS (S.D.) rats, which exhibited seizures that were very similar to those in ethanol-dependent animals. Although these rats exhibited wild running and clonic seizure responses these reactions normally did not progress to tonic flexion or extension as was often seen in ethanol-dependent rats. After intracollicular muscimol 30 min earlier sound-induced hindlimb clonus was completely prevented whereas the frequency of wild running was reduced to one of the six test animals. Subsequent intracollicular administration of saline to the same animals several days later did not prevent normal expression of either wild running or hindlimb clonus in response to sound stimulation in all six animals.

The next experiment was designed to determine the relative efficacy of bilateral intracollicular microinjections of muscimol to prevent chemically induced seizures. Two types of seizures were tested. The first type of chemical seizure was generated by bilateral infusions of either (+)-bicuculline methiodide (20 pmol/min) or kainic acid (200 pmol/min) over 5 min into the inferior colliculus as detailed in table 1. In this paradigm intracollicular muscimol (263 pmol/site) pretreatments 30 min earlier completely prevented the development of both wild running and clonus for 60 min after intracollicular infusions of either (+)-bicuculline methiodide (no seizures in six rats) or kainic acid (no seizures in five rats); see table 2. The second type of chemical seizure was induced by i.v. infusion of (+)-bicuculline. This response consisted primarily of clonic-tonic activity without wild running. Of seven rats which served as controls and received intracollicular saline infusions before i.v. (+)-bicuculline (0.68 μmol/kg) injection, all seven exhibited hindlimb clonus and two of the seven exhibited hindlimb tonus. Bilateral intracollicular microinjection of muscimol (263 pmol/ site) in 12 rats before an i.v. infusion of (+)-bicuculline (0.68 μmol/kg) did not reduce the incidence of either hindlimb clonus (100% of control response) or tonus (89.5% of the control response) in contrast to the suppression of inferior colliculus derived chemical seizures.

In the final experiment the effect of collicular muscimol microinjections on electroshock seizures was examined. Bilateral intracollicular microinjection of muscimol (263 pmol/site) had no significant effect on the E/F ratios of electroshock seizures as compared to the saline infused or untreated seizure responses of the same animals (untreated E/F = 8.02 ± 0.56, saline E/F = 6.57 ± 1.43 and muscimol E/F = 7.12 ± 1.02 for six animals).

Discussion

The present data suggest that auditory input to the inferior colliculus may play an important role in the generation of audiogenic seizures in the ethanol-dependent rat. Even partial lesions of this site were sufficient to inhibit seizure induction completely. It seems unlikely that the lesion of the inferior colliculus inhibits seizures by simply blocking access of auditory input to the cerebral cortex, because a relatively more complete lesion of the medial geniculate body, the next afferent auditory relay after the inferior colliculus, failed to inhibit seizure generation significantly. These results are consistent with other findings that indicate that the inferior colliculus, independent of the cerebral cortex, probably plays an important role in the generation of other types of sound-induced seizures (see the introductory section).

Several findings suggest that the inferior colliculus of normal untreated rats is unusually sensitive to excitatory stimuli. For example, the present report and earlier studies (Duplisse *et al.*, 1974; Frye *et al.*, 1983) show that infusion of picomole amounts of certain chemical excitants such as bicuculline, picrotoxin and kainic acid into the inferior colliculus can induce wild running and generalized seizures closely resembling those evoked by sound or electrical stimulation. As with sound-induced seizures, lesions of the medial geniculate body

did not present seizures induced by intracollicular infusion of bicuculline, suggesting that direct excitation of afferent auditory pathways to the cerebral cortex is not necessary for this seizure response. In addition to excitatory chemical stimulants, relatively low intensity electrical stimulation of a discrete area, best described as the confluence of the external, pericentral and central nuclei of the inferior colliculus, has been shown to effectively generate wild running responses which closely resemble those seen in ethanol-dependent rats undergoing sound-induced seizures (McCown *et al.,* 1984). The reason for the differential effect of medial geniculate body lesions on the frequency of hindlimb clonus during seizures induced by ethanol withdrawal or intracollicular (+)-bicuculline methiodide infusion is not clear but could be due to modification of descending inhibitory or excitatory influences of the cerebral cortex on the excitability of the inferior colliculus (Morest, 1975). The physiological basis for excitatory behavioral responses after stimulation of the inferior colliculus also is not clear but may be related to excitatory input to the mesencephalic reticular formation (Irvine and Jackson, 1983) where it may activate a general central nervous system alerting mechanism.

With respect to the components of the audiogenic ethanol withdrawal seizure, it appears that GABAergic mechanisms in the inferior colliculus could be linked most closely to the generation of wild running responses. The clonic and tonic components of seizures originating from stimulation of this structure probably result from a spread of seizure activity to other brain areas. Swinyard and co-workers (1972) have pointed out that regardless of the initiating stimulus, the occurrence of clonic and tonic activity are the result of increasing degrees of seizure spread (Piredda *et al.,* 1985b). This relationship has been noted in electrically induced inferior colliculus seizures where wild running was associated with epileptiform EEG spiking activity limited to the inferior colliculus, whereas EEG spiking in the forebrain occurred only after the manifestation of the tonic and myoclonic seizure components (McCown *et al.,* 1984). In the present study, intracollicular infusion of (+)-bicuculline methiodide induced a muscimol reversible episode of wild running and clonus with a rare occurrence of a poorly defined tonic activity. However, i.v. (+)-bicuculline reliably produced tonic extension and myoclonus that could not be blocked by intracollicular muscimol. Inasmuch as microinjection of muscimol (263 pmol/site) into the inferior colliculus did not alter tonic components of either i.v. bicuculline-induced or electroshock seizures it is unlikely that GABAergic mechanisms in this brain area directly influence the spread of seizure activity throughout the brain. This suggests that blockade of GABAergic inhibition in the inferior colliculus is sufficient to generate wild running and clonic activity but that without additional antagonism of GABAergic mechanisms in other areas of the brain there may not be sufficient seizure spread to produce tonic convulsions. Other studies have shown that microinjection of picomole amounts of bicuculline into the deep prepiriform cortex of the rat can induce generalized clonic seizures (Piredda *et al.,* 1985a), but apparently without inducing wild running responses like those observed after intracollicular bicuculline injections. Although several brain sites such as the medial septum (Frye *et al.,* 1983), substantia nigra (Iadarola and Gale, 1982; Frye *et al.,* 1983), striatum (Piredda *et al.,* 1985a) or neocortex (Woodbury, 1981) are significantly less sensitive to bicuculline than either the inferior colliculus or deep prepiriform cortex, seizures can still be elicited or enhanced from these areas. For example, the observation that subconvulsant doses of bicuculline can significantly enhance the responsiveness of the mesencephalic and bulbar reticular formation to sensory stimuli suggests that this brain area could be important in the induction of generalized seizures by bicuculline (Faingold *et al.,* 1983). Thus, other as yet untested brain areas may be equally or more sensitive to this drug than those regions tested so far.

A continuing question concerns whether adaptive changes in inhibitory GABAergic transmission in the inferior colliculus could play an important role in the initiation of sound-

induced seizures in the ethanol-dependent rat. Clearly, bilateral destruction of the inferior colliculus blocks seizure induction completely indicating the importance of these nuclei to the generation of audiogenic seizures. The present studies with baclofen (fig. 3) also extend previous work (Frye *et al.*, 1983) with muscimol and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol suggesting that activation of either $GABA_A$ or $GABA_B$ receptors in the inferior colliculus can suppress sound-induced seizures in the ethanol-dependent rat. The relative potency of muscimol was approximately 10 times that of the racemic baclofen for suppression of the clonic component of these seizures, which is in line with the 10-fold greater efficacy of muscimol relative to baclofen to displace [^{3}H]GABA from crude whole rat brain synaptic membranes (Bowery, 1983). Although (+)-baclofen is reported to antagonize the analgesic effects of (−)-baclofen (Sawynok, 1984), extra- and intracellular recordings from hippocampal CA1 pyramidal cells do not support the view that (+)-baclofen is an antagonist of the actions of the (−)-isomer (Haas *et al.*, 1985). Finally, the ability of (+)-bicuculline methiodide infusion into the inferior colliculus to induce either audiogenic-like seizures directly or to prime normal rats for susceptibility to sound-induced seizures could indicate a role for reduced collicular GABAergic inhibition in the generation of ethanol withdrawal sound-induced seizures. Although ethanol withdrawal seizure severity is diminished by muscimol microinjected into either the substantia nigra (Frye *et al.*, 1983;Gonzalez and Hettinger, 1984) or the inferior colliculus (Frye *et al.*, 1983), the relative insensitivity of the former brain area to seizures caused by intranigral microinfusion of (+)-bicuculline methiodide suggests that reduced GABAergic inhibition there probably does not play a major role in ethanol withdrawal seizure induction but probably reduces the spread of seizure activity.

The relative delay in the onset of seizures after infusions of various chemical excitants into the inferior colliculus of ethanol-naive rats could suggest some degree of GABAergic specificity in the basic responsiveness of this nucleus. Seizure latencies of 5 min or less were only observed after infusions of (+)-bicuculline methiodide, the GABA recognition site antagonist or picrotoxin and Ro5-3663, both of which appear to bind to the chloride channel associated with the GABA-benzodiazepine receptor complex (Olsen, 1981; Harrison and Simmonds, 1983). Somewhat surprising was the finding that (−)-bicuculline methiodide appeared to be of similar potency to (+)-bicuculline methiodide when infused into the inferior colliculus in contrast to previous findings indicating that the (+)-isomer was 400 times more potent in evoking convulsive discharges after microinjections into the cerebral cortex (Collins and Hill, 1974). Although strychnine, a glycine antagonist (Kuno and Weakly, 1972), kainic acid, a glutamate analog (Watkins, 1981), and carbachol, a nonselective cholinergic agonist (Bebbington and Brimblecombe, 1965), all evoked seizure activity, the responses were delayed (10–15 min after starting the infusion) relative to those of GABA-related drugs. Strychnine and carbachol were also less efficient in generating seizures than the GABAergic agents. The delayed seizure responses resulting from infusions of non-GABAergic drugs may have been due to their diffusion to other active brain sites; however, this would still be consistent with a relatively greater sensitivity of the inferior colliculus to excitation caused by blockade of GABA receptor function. Furthermore, in the deep prepiriform cortex small doses of carbachol (136 pmol), kainic acid (117 pmol) or bicuculline (49 pmol) all evoked bilateral clonic seizures within 3 min, whereas strychnine (450 pmol) was ineffective (Pirreda and Gale, 1985). The rapid response to all of these drugs would support the idea that the inferior colliculus is normally more sensitive to GABA antagonism. Finally, a previous study found that intracollicular injection of a large dose of ranitidine (20,000 pmol/min), an H_2 antagonist, for 5 min would also induce seizures with a latency of approximately 16 min (Trzeciakowski and Frye, 1985). However, this action probably does not involve H_2 blockade because cimetidine, a chemically distinct H_2 antagonist, at a similar dose did not cause seizures. Although these data do not provide direct evidence of GABAergic adaptation in the inferior colliculus of ethanol-dependent

rats, they suggest that manipulations which reduce GABAergic inhibition within the colliculus are very likely to initiate a sound-induced ethanol withdrawal-like seizure. Consistent with this idea are recent studies showing that diminished inhibitory GABAergic mechanisms in the inferior colliculus may also explain in part the increased susceptibility to audiogenic seizures exhibited by genetically epilepsy-prone rats (Gehlbach and Faingold, 1984; Roberts *et al.*, 1984). However, because relatively small amounts of excitatory amino acids, such as kainic acid or N-methyl-D-aspartate, also can evoke seizure activity after intracollicular infusions in normal rats (table 1; Meldrum *et al.*, 1985), it is possible that excitatory transmitter mechanisms could also be involved in ethanol withdrawal seizures.

In summary, the present results indicate that sound seizure susceptibility in ethanol-dependent rats requires an intact inferior colliculus, but does not depend on the access of sound stimuli to higher auditory centers. The results are also supportive of a role for adaptive changes in GABAergic mechanisms in the inferior colliculus in the initiation and expression of sound-induced seizures in the ethanol-dependent rat.

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ABBREVIATIONS

GABA	γ-aminobutyric acid
E/F	extensor/flexor

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Fig 1.

Sites of electrolytic lesions and drug injections in the inferior colliculus and medial geniculate body. Animals with electrolytic lesions and/or microinjector tips placed within the confines of the hatched areas were considered successful and were included in the data analysis. Values in the upper right corner of each section represent the distance in millimeters from interaural zero. Adopted from Paxinos and Watson (1982).

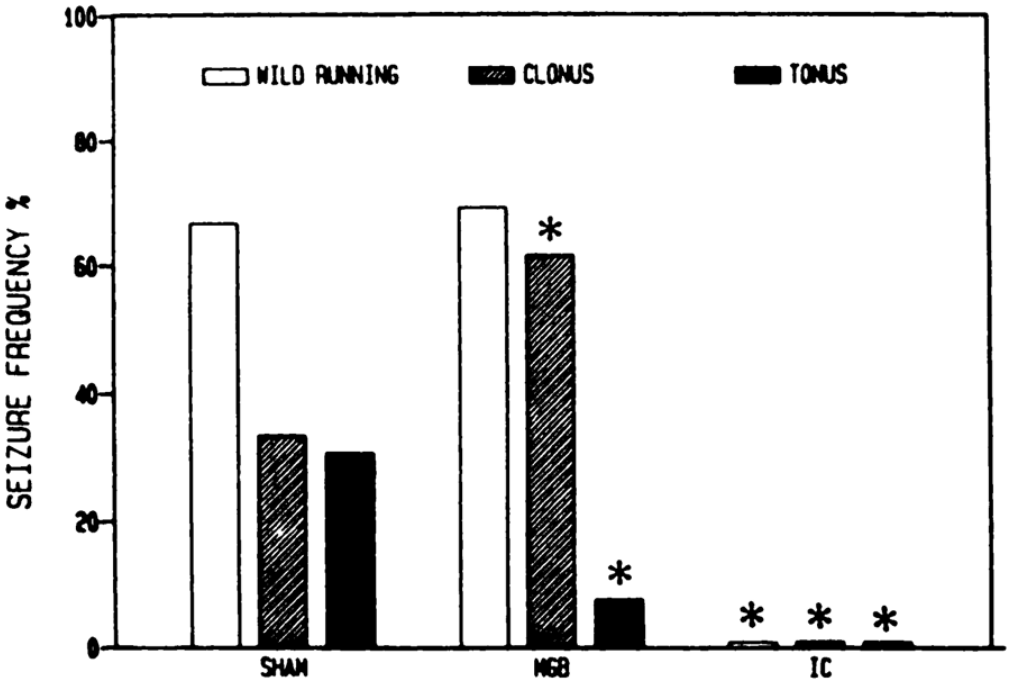

Fig 2.

Effect of electrolytic lesions of the inferior colliculus (IC) and medial geniculate body (MGB) on susceptibility to sound-induced seizures in ethanol-dependent rats. Electrolytic lesions of the IC (*N* = 17), the MGB (*N* = 26) or sham controls (IC = 16; MGB = 23) were made before inducing physical dependence. Tests for sound susceptibility were made 6.5 to 7.5 hr after ethanol withdrawal or 16 days after lesioning. Data for sham groups were not statistically different and were combined. *P < .05 when compared with sham lesioned controls.

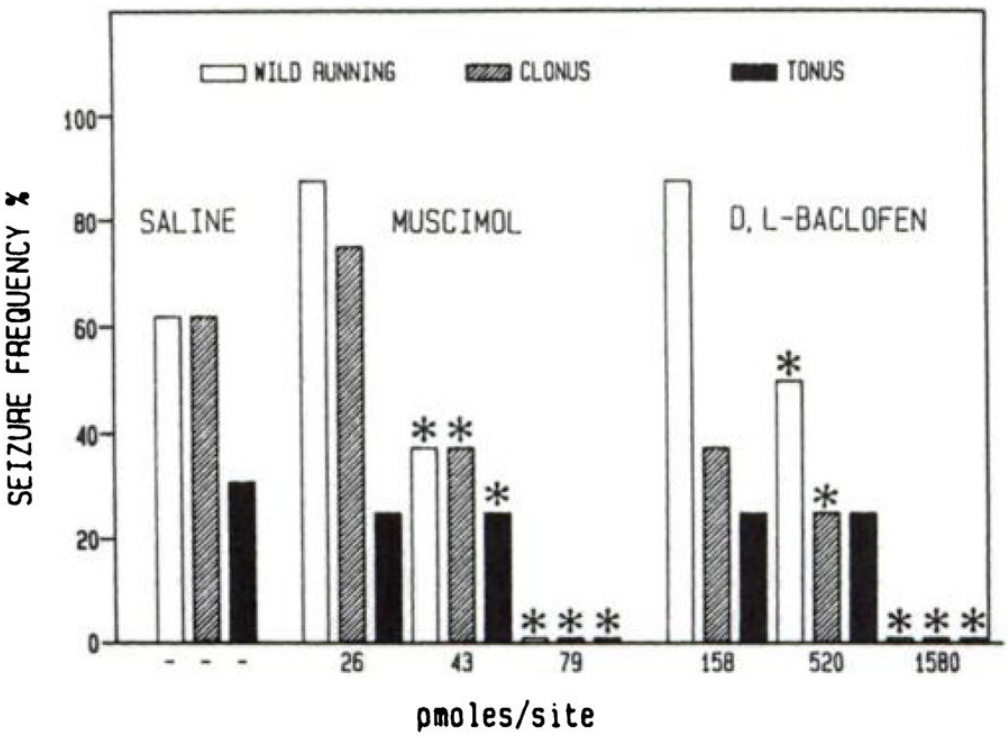

Fig 3.

Effect of intracollicular baclofen or muscimol on sound-induced ethanol withdrawal seizures. Seven hours after withdrawal ethanol-dependent rats received intracollicular injections of muscimol (26 pmol, *N* = 7); (43 pmol, *N* = 8); (79 pmol, *N* = 6) or (±)-baclofen (158 pmol, *N* = 8); (520 pmol, *N* = 8); (1580 pmol, *N* = 7). The frequency of audiogenic seizure susceptibility was tested 30 min later. *P < .05 when compared with saline microinjected controls.

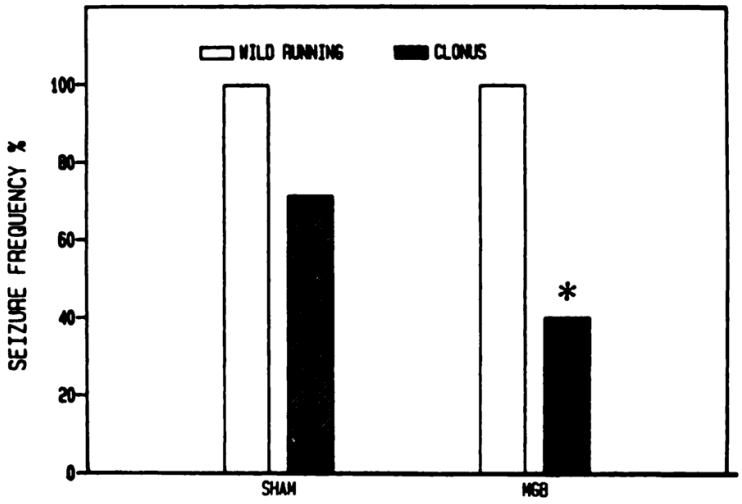

Fig 4.

Effect of electrolytic lesions in the medial geniculate body on bicuculline-induced seizures. Ethanol-naive rats received either electrolytic (five rats) or sham lesions (seven rats) of the medial geniculate body. After recovery (+)-bicuculline methiodide (20 pmol/min) was infused bilaterally into the inferior colliculus for up to 5 min. The frequency of wild running and hindlimb clonus were recorded. *P < .05 when compared with sham lesioned controls.

TABLE 1

Frequency and latency of wild running and hindlimb clonus after bilateral microinfusion of convulsant drugs into the inferior colliculus

Ethanol-naive rats received a drug infusion (0.1 μl/min) for up to 5 min or until a seizure began. The time of onset of wild running and clonus were recorded during a 30-min postinjection period. Seizure latency represents the time between the initiation of the drug infusion and onset of hindlimb clonus

Dose	Infusion-Related Seizure Frequency			*N*
	Wild running	Clonus	Seizure latency	
pmol/min	*% Responding*		*min*	
(+)-Bicuculline methiodide				
20.0	100	71.5	3.66 ± 0.23	7
2.0	50	16.7	4.25 ± 0.25	6
0.2	0	0.0		5
(−)-Bicuculline methiodide				
20.0	100	50.0	3.50 ± 0.33	6
2.0	28	14.0	5.00 ± 0.00	7
0.2	0	0.0		6
Picrotoxin				
200.0	60	40.0	5.00 ± 1.00	5
20.0	20	0.0		5
RO 5–3663				
2000.0	100	50.0	3.25 ± 0.25	4
200.0	0	0.0		4
Strychnine				
2000.0	38	0.0	15.00 ± 3.21*	8
200.0	0	0.0		5
Kainic acid				
200.0	100	67.0	9.67 ± 1.02*	6
20.0	67	17.0	10.00 ± 1.47*	6
2.0	0	0.0		5
Carbachol				
2000.0	40	20.0	13.00 ± 3.00*	5
200.0	0	0.0		5
Ibotenic acid				
2000.0	0	0.0		5

* $P < .05$ compared with bicuculline.

TABLE 2

Frequency of various seizure responses after intracollicular (i.c.) microinfusion of muscimol

Microinjections of muscimol (263 pmol/site)or saline were made bilaterally into the inferior colliculus 30 min before testing the animals in one of several different seizure tests listed below. Audiogenic seizures were evaluated in “ethanol-dependent” genetically “epilepsy-prone” rats. Seizures originating from the inferior colliculus were induced by 5-min “(+)-bicuculline methiodide (i.c.)” infusion (20 pmol/min) or by “kainic acid (i.c.)” infusion (200 pmol/min). Generalized seizures were induced by i.v. “(+)-bicuculline (i.v.)” infusion (68 μmol/kg) or by “electroconvulsive shock”

Seizure type	Seizure Response
Ethanol-dependent	Suppressed
Epilepsy-prone	Suppressed
(+)-Bicuculline (i.c.)	Suppressed
Kainic acid (i.c.)	Suppressed
Bicuculline (i.v.)	Unaltered
Electroconvulsive shock	Unaltered