Effects of (−)-baclofen, clonazepam, and diazepam on tone exposure-induced hyperexcitability of the inferior colliculus in the rat: possible therapeutic implications for pharmacological management of tinnitus and hyperacusis

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Abstract

Recent investigations in the authors’ laboratory have shown that acute tone exposure (4 kHz continuous tone, 104 dB sound pressure level (SPL), 30-min duration) induces increases in the amplitude of click-evoked potentials in the inferior colliculus (IC). These increases have been attributed to a decrease in GABA-A-mediated inhibition on IC neurons. In the present study, we examined the effects of three compounds (diazepam, clonazepam, and (−)-baclofen) that are known to enhance GABAergic inhibition on these tone exposure-induced increases and on changes in temporal integration in the IC. (−)-Baclofen was the only one of the three compounds tested that reversed in a dose-dependent manner the effects of tone exposure on both the amplitude of the click-evoked potentials recorded from the IC and on measures of the changes in temporal integration based on these potentials. Diazepam and clonazepam exhibited remarkably different effects on the click-evoked potentials recorded from the surface of the IC. Diazepam caused a dose-dependent decrease in one of the components of the IC potentials that reflects postsynaptic activity in the IC, whereas clonazepam caused a dose-dependent decrease in a peak that reflects input to the IC from the superior olivary complex (SOC). At dosages up to 40 mg/kg, neither diazepam nor clonazepam reversed the changes in temporal integration in the IC that were induced by the tone exposure; diazepam caused a small, but statistically significant, enhancement of the effects of tone exposure on this function. The results of this study show that (−)-baclofen is a potent modulator of both the excitability of neurons in the ascending auditory pathway and the processing of auditory information by IC neurons. The finding of the present study that two benzodiazepines (clonazepam and diazepam) have remarkably different effects on evoked potentials, which reflects both input to the IC and postsynaptic events in the IC neurons, suggests heterogenicity of the GABA-A receptor from one structure to another in the ascending auditory pathway. We suggest that (−)-baclofen may be clinically useful in treating disorders of the auditory system that are caused by plasticity in the ascending auditory pathway.

Keywords: Plasticity; Auditory evoked potential; Tinnitus; Baclofen; Clonazepam; Diazepam

1. Introduction

Recent investigations have yielded considerable insight into the ability of sensory systems to change their function in response to changes in neural input. This form of plasticity was first described for the somatosensory and visual cortex (Merzenich et al., 1988; Kaas et al., 1990; Kaas, 1991). Recent work has begun to focus on the question of whether this plasticity may play a role in various sensory disorders following injury to the peripheral portion of the nervous system (Woollf, 1993).

Interestingly, some studies suggest that subcortical structures of the somatosensory system also have the capacity to reorganize as a result of manipulations of their sensory input (Nicolesis et al., 1991, 1993). Although it has been known for many years that the function of specific subcortical auditory nuclei also changes following manipulations of their input, the relationship of these changes to reorganization has only recently been explored.

Alterations of sensory input to the central auditory system can be achieved using intense noise, ototoxic drugs,
and other manipulations that damage cochlear function. Noise exposure has been shown to cause a decrease in inhibition in the cochlear nucleus (Henderson and Möller, 1975), changes in the tonotopic map of the dorsal cochlear nucleus (Kaltenbach et al., 1992), an increase in acoustically evoked activity in IC neurons (Willott and Lu, 1981), an increase in the amplitude of auditory evoked potentials recorded from the inferior colliculus (IC) (Salvi et al., 1990; Popelar et al., 1994; Szczepaniak and Möller, 1995a), a decrease in GABAergic inhibition on IC neurons (Szczepaniak and Möller, 1995b), and changes in temporal integration in IC neurons (Gerkin et al., 1991; Szczepaniak and Möller, 1996). Loss of sensory input induced by complete or partial destruction of the cochlea using mechanical methods or ototoxic drugs may trigger a reorganization of the tonotopic map of the primary auditory cortex (Reale et al., 1987; Robertson and Irvine, 1989; Harrison et al., 1992).

Injury to the cochlea or auditory nerve has also been found to cause a number of other changes to subcortical auditory nuclei, including a decrease in the number of neurons and the volume of the cochlear nucleus (CN) and the superior olivary complex (SOC) (Trune, 1982a,b, Nordeen et al., 1983; Moore, 1990, 1991). Changes in both resting and acoustically evoked metabolic activity of auditory nuclei have also been observed (Taniguchi and Saito, 1978; Sasaki et al., 1980; El-Kashlan et al., 1993).

From these studies, the IC has emerged as one of the primary sites in the ascending auditory pathway that experiences changes in function following noise exposure and injury to the peripheral portion of the auditory system. Thus, we and others have hypothesized that plasticity in the ascending auditory system following either chronic noise exposure or injury to the peripheral portion of the system may be at least partially responsible for the alterations in auditory perception that occur in individuals with various disorders such as tinnitus and hyperacusis (Möller et al., 1992; Lenarz et al., 1993). This hypothesis is further supported by the results of a clinical study that indicated that hyperexcitability of the IC may be involved in the generation of some forms of severe tinnitus (Möller et al., 1992).

It is well documented that GABAergic inhibition is prevalent in the ascending auditory system and that it has a profound influence on neurons in the IC. It is also well known that benzodiazepines increase the efficacy of GABA-mediated inhibition. In addition to GABA_A receptors, GABA_B receptors are abundant in the nuclei of the ascending auditory pathway. Baclofen, which is a GABA_B agonist, has been shown to have a distinct effect on the discharges of single neurons in the cochlear nucleus (Caspar et al., 1984). Since the effect of acute noise exposure on IC neurons is a result of a decrease in GABA_A-mediated inhibition (Szczepaniak and Möller, 1995b, 1996), we chose to study the effect of three compounds that are known to modulate GABAergic inhibition.

In the present study, we investigated the effects of three compounds (diazepam, clonazepam, and (-)-baclofen) on noise-induced increases in the amplitude of click-evoked potentials recorded from the surface of the IC. The effect of each of these three drugs on noise-induced changes in temporal integration in the IC was studied by recording the responses to tonebursts with durations between 1 and 20 ms. These three drugs are currently used clinically in treating other neurological disorders for which there is overwhelming evidence that these drugs act to modulate GABAergic inhibition.

2. Methods

2.1. Animal preparation

A total of 43 adult female Wistar rats were used in this study. The animals were anesthetized with a mixture of ketamine and acepromazine (100 mg/kg and 10 mg/kg, respectively). The trachea was cannulated and the animals were artificially ventilated (Harvard Instruments, Ventilator Model 683). A skin incision was made in the neck and the jugular vein was cannulated with a polyethylene catheter for intravenous administration of the compounds to be tested. The animals were then placed in a headholder, with hollow ear bars placed over the bony ring of the tympanic membrane. A strap clamp was used to firmly hold the head in the headholder.

An incision was made in the scalp, and the skin and periosteum overlying the occipital bone were removed. A burr hole was made in the occipital bone with a hypodermic needle, and the hole was extended with rounders to the edges of the sigmoid and transverse sinuses. The dura was opened, and the cerebrum overlying the IC was removed with suction. Hemostasis was accomplished through the use of oxidized cellulose (Surgicel), and the cavity was filled with mineral oil to prevent desiccation of the IC. Care was taken to avoid injury to blood vessels on the surface of the IC at all times.

2.2. Stimulus generation

Auditory stimuli were delivered monaurally to the left ear by a miniature stereo earphone (Realistic, Radio Shack) placed in an ear specula and attached to the ear bar with polyethylene tubing in such a manner as to form a closed system. The ear bar contralateral to the stimulated ear was occluded with petroleum jelly. Toneburst stimuli (4 kHz, 1–20 ms duration, rise and fall times 2 cycles, i.e., 0.5 ms) were generated with an auditory stimulus generator (Model #10U1C1MA, Grass Instrument Co.). The onset phase of toneburst stimuli was condensation. Click stimuli were generated by a stimulator (Model #5D9, Grass Instrument Co.) set to deliver rectangular waves of 20-μs duration. All auditory stimuli were presented at a rate of 5.1 pulses

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2.3. Evoked-potential recordings

Evoked potentials were recorded from the surface of the right IC (contralateral to the stimulated (left) ear) by placing a bipolar electrode made from two Teflon-insulated silver wires (Type AG-10, Medwire Corp.) on the surface of the IC. The tips of the wires were placed 1 mm apart, and a line through the two tips of the electrode was in the ventral-dorsal plane. A negative voltage on the most ventral tip of the electrode gave an upward deflection. A ground electrode was placed in the musculature of the shoulder. The electrodes were connected to a differential amplifier (Model #P511, Grass Instrument Co.). The recordings were filtered (3 kHz lowpass, 3 Hz highpass), amplified 5000 X (Model P511K, Grass Instrument Co.), and stored on an Apollo workstation equipped with a 12-bit analog-to-digital converter and sampled at a rate of 25 kHz (40 μs). Each record consisted of 1024 datapoints and all recordings were the average of 500 individual responses.

2.4. Drug preparation

(−)-Baclofen (Ciba-Geigy) was dissolved in physiological saline to a final concentration of 5 mg/ml. Diazepam (Hoffman-LaRoche) and Clonazepam (Hoffman-LaRoche) were sonicated for 1 min in physiological saline to a final concentration of 5 mg/ml. All compounds were administered intravenously (in <1 min) through a catheter inserted in the jugular vein.
2.5. Stimulus protocol

Baseline responses to click stimuli presented monaurally to the contralateral ear were recorded for 15–20 min. After this period of acclimation, responses to toneburst stimuli of 1–20 ms duration were recorded, and click stimuli were recorded again for 5 min, after which acute tone exposure (4 kHz, 104 dB SPL, 30-min duration) was begun. In some animals, responses to click stimuli were recorded concomitantly with acute tone exposure. After 30 min the tone exposure was terminated and responses to click stimuli were recorded for 15 min (in a previous study, we showed that the increase in the amplitude of the potentials recorded from the IC following tone exposure reached maximum levels within 15 min after termination of the tone exposure (Szczepaniak and Möller, 1996). After recording the responses to clicks, the responses to tonebursts of varying durations (1–20 ms) were recorded a second time. After this, the responses to click stimuli were again recorded (for 5 min). After these recordings were completed, the compound to be tested was injected intravenously. After 5 min, recording of the responses from the IC (to click and toneburst stimuli) resumed.

2.6. Analysis

All amplitudes were measured by hand (base-to-peak) from printouts of recordings that were the average of 500 individual responses. Statistical analysis was done using commercially available software (Systat). Each datapoint, pre- and post-drug administration, was tested for statistical significance by a Student’s t test (P < 0.05).

This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pittsburgh School of Medicine.

3. Results

Both click- and toneburst-evoked potentials recorded from the surface of the IC with a bipolar electrode have four distinct peaks (labeled A–D in Fig. 1). In previous work we had determined that the 'A' peak reflects the

![Fig. 4. A: Dose–response curves of clonazepam (■), (-)-baclofen (△), and diazepam (○) on the amplitude of the 'A' wave in response to click stimulation (n = 6, SEM). Amplitudes are expressed as a percentage of pre-drug administration values. B: Amplitudes of the 'B' wave of the click-evoked ICX response under the same conditions as in (A). C: Amplitudes of the 'C' wave of the click-evoked ICX response under the same conditions as in (A). D: Amplitudes of the 'D' wave of the click-evoked ICX response under the same conditions as in (A).](image-url)
Fig. 5. Average change in the toneburst-evoked (4 kHz, 94 dB SPL) amplitude of the ‘C’ wave as a function of stimulus duration (1–20 ms) before (■) and after (▲) exposure to a 4 kHz, 104 dB SPL continuous toneburst of 30 min duration (n = 25 ± SEM). Dashed line shows amplitude at a duration of 1 ms (100%). An asterisk (+) indicates statistical significance by Student’s t test (P < 0.05).

Fig. 6. A: The relationship between the toneburst-evoked (4 kHz, 94 dB SPL) amplitude of the ‘C’ wave and stimulus duration (1–20 ms) after a 30-min exposure to a 4 kHz, 104 dB SPL continuous toneburst and before (■) and 15 min after (▲) a 40 mg/kg intravenous injection of (-)-baclofen (n = 6 ± SEM). An asterisk (+) indicates statistical significance by Student’s t test (P < 0.05). B: Curves obtained under the same conditions as in (A), but showing the effects of a 40 mg/kg intravenous injection of diazepam (n = 6 ± SEM). C: Curves obtained under the same conditions as in (A), but showing the effects of a 40 mg/kg intravenous injection of clonazepam (n = 6 ± SEM).
We investigated the effects of the same three compounds on the responses to tonebursts of different durations (1–20 ms) in noise-exposed animals. Before noise exposure, the amplitude of the ‘C’ wave decreased with increasing stimulus duration, reaching a plateau at a duration of 4 ms, when the amplitude was 70–75% of the amplitudettes at 1 ms duration (Fig. 5, □). After tone exposure the amplitude of the ‘C’ wave was less dependent on stimulus duration (Fig. 4, A). A systemic administration of a large dose of (-)-baclofen (40 mg/kg) reversed the effects of noise exposure on temporal integration in the IC (Fig. 6A), whereas an equal dose of diazepam caused a small, but statistically significant, increase in the effects of noise on temporal integration in the IC (Fig. 6B), Student’s t test, P < 0.05). An intravenous injection of clonazepam (40 mg/kg) did not significantly change this function (Fig. 6C, Student’s t test, P < 0.05). Fig. 7 shows a dose-response effect of the actions of (-)-baclofen on the reversal of the noise exposure-induced changes in the IC integration function.

4. Discussion

Previous studies have shown increases in both spontaneous and acoustically evoked activity in neurons of the IC following acute noise exposure (Salvi et al., 1975; Lonsbury-Martin and Martin, 1981; Willott and Lu, 1981; Popelar et al., 1987; Gerkin et al., 1991; Szczepaniak and Möller, 1996). These findings alone have important implications on how noise exposure-induced plasticity in the IC may lead to various hearing deficits.

It has also been shown that temporal integration in IC neurons is altered by exposure to noise (Szczepaniak and Möller, 1995b, 1996; Gerkin et al., 1991). Since temporal integration is the mechanism by which IC neurons process and encode important features of complex sounds such as sound duration (Casseday et al., 1994), it is possible that noise exposure-induced plasticity in IC neurons could also result in additional hearing impairments, such as deficits in the discrimination of complex sound patterns commonly found in speech. Deficits in speech discrimination have been found to occur in patients with tinnitus (Newman et al., 1994).

Thus, since there is evidence that the IC might be partially responsible for alterations in the auditory system that result in severe tinnitus and hyperacusis in some patients (Möller et al., 1992; Lenartz et al., 1993), the effects of a substance that is aimed as a treatment for these disorders or both the overall excitability of IC neurons as well as on temporal integration in IC neurons must be considered.

The two benzodiazepines we studied (diazepam and clonazepam) showed remarkably different effects on the amplitudes of peaks in click-evoked responses from the IC. Results from experiments in which we produced temporary inactivation of the IC with either lidocaine or a glutamate receptor antagonist have shown that the broad peaks labeled ‘C’ and ‘D’ (Fig. 1) reflect postsynaptic events that are generated by IC neurons (Szczepaniak and Möller, 1993). Interestingly, in the course of these investigations we observed that it is possible, by controlling both the microinjection site and the volume of the vehicle injected, to preferentially affect either the ‘C’ or ‘D’ wave. Thus, we have concluded that although both the ‘C’ and ‘D’ peaks are generated by postsynaptic events that occur in IC neurons, the ‘C’ and ‘D’ peaks are most likely generated by two anatomically distinct subpopulations of neurons located within the IC (Szczepaniak and Möller, unpublished observations). We are currently investigating this phenomenon. In previous work we showed that during a 4 kHz, 104 dB continuous tone exposure, the amplitudes of all peaks in the IC response decreased by more than 50% (Szczepaniak and Möller, 1996). After termination of the tone exposure, the amplitudes of the ‘A’ and ‘B’ peaks slowly returned to near-baseline levels, whereas the amplitudes of the ‘C’ and ‘D’ peaks increased above baseline levels by an average of 35% and 49%, respectively, and remained at these levels for the duration of the recording (120 min) (Szczepaniak and Möller, 1996).

Diazepam causes small dose-dependent decreases in the ‘A’ and ‘B’ peaks, which reflect input to the IC from the CN (‘A’ wave) and SOC (‘B’ wave), whereas clonazepam showed no influence on input to the IC from the CN (‘A’ wave). This indicates that there is a large degree of specificity in the neurons of the IC with regard to the structure of different benzodiazepines. The differential effects of these two compounds that were observed on peaks that reflect postsynaptic events in the IC (‘C’ and ‘D’ waves) — diazepam causing a large dose-dependent decrease in the amplitude of the ‘D’ wave and clonazepam having no effect on either waves ‘C’ or ‘D’ — also support this indication.

The difference in the actions of clonazepam and diazepam on temporal integration in the IC is another exam-
ple of the differential effects of different benzodiazepines in the ascending auditory pathway. We had previously shown that the decrease in the amplitude of the 'C' wave with increasing stimulus duration is due to an influence of GABA- mediated inhibition (Szczepaniak and Möller, 1995b). Thus benzodiazepines would be expected to enhance the GABA inhibitory influence on temporal integration in the IC, as would be reflected in a larger decrease in the amplitude of the 'C' wave with increasing stimulus duration. However, this did not occur with either diazepam or clonazepam: diazepam caused the amplitude of the 'C' wave with increasing stimulus duration to be slightly larger than in control animals and clonazepam did not significantly affect the relationship between the amplitude of the 'C' wave and stimulus duration.

Although it is unclear from these studies why the effects of diazepam indicate a suppression of GABA- mediated inhibition on temporal processes in the IC, it is possible that diazepam has a greater inhibitory effect on one or several nuclei in the ascending auditory pathway that sends inhibitory projections to the IC. We are currently investigating this possibility.

The results of the present study clearly show a differential effect of various benzodiazepines throughout the ascending auditory pathway. Differences in the binding and actions of different benzodiazepines have been shown to occur in the central nervous system and are generally believed to be the result of regional differences in GABA receptor subunit expression (for reviews see Guisti and Arban, 1993; Luddens and Korpi, 1995). Such regional differences in GABA receptor subunit expression has been found to occur in subdivisions of the IC (Gutierrez et al., 1994; Milbrandt et al., 1995). Although the physiological function of an arrangement in which distinct benzodiazepine receptor (BDR) complexes are segregated from one another in the ascending auditory pathway is unclear, it is important to note that different benzodiazepines can have entirely different effects on the processing and perception of auditory stimuli. Thus, future studies on the use of benzodiazepines as therapeutic agents for the treatment of various auditory disorders such as tinnitus and hyperacusis must evaluate each benzodiazepine compound separately, and one must not assume that data from one or several benzodiazepine compounds reflects the actions of all compounds classified as benzodiazepines.

The finding that (-)-baclofen caused a large dose-dependent decrease in all peaks of the click-evoked responses from the IC indicates that (-)-baclofen acts on many different levels of the ascending auditory pathway. Although (-)-baclofen has been shown to have large effects on overall excitability in the ascending auditory pathway (Szczepaniak and Möller, 1995a), little is known about the physiological role of the GABA B receptor in the auditory system. Autoradiographic and immunohistochemical studies have shown that the GABA B receptor is present in the cochlear nucleus (CN) and in the IC (Juiz et al., 1994; Milbrandt et al., 1994; Veenman et al., 1994), and that there is a significant age-related decrease in GABA B receptor binding in the IC in rodent models of neural presbycusis (Milbrandt et al., 1994). Further studies are needed to establish the role of GABA B receptors in the auditory system as well as in plasticity of the auditory system.

(-)-Baclofen was the only compound tested that was found to be capable of reversing the effects of tone-exposure on temporal integration in the IC. In previous studies we have shown that tone-exposure causes a decrease in the GABA A -mediated inhibitory component of this function (Szczepaniak and Möller, 1995b). The results of the present study indicate that systemic administration of (-)-baclofen is able to compensate for the tone-exposure-induced decrease in GABA A -mediated inhibition. Although (-)-baclofen could be acting either pre- or postsynaptically on IC neurons to enhance inhibition (for a review see Bowery, 1993), we currently do not know by which mechanism(s) (-)-baclofen is able to supply additional inhibition to IC neurons. We are currently investigating this phenomenon.

The results of the present study thus suggest that (-)-baclofen may be a promising treatment for auditory disorders that may at least partially be caused by plastic changes to the ascending auditory pathway. Baclofen is well tolerated, as evidenced from its use in treating several neurological disorders, and its side effects are well known.

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References

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