Benzodiazepines alter cochleo-cochlear loop in humans

N. Morand a, E. Veuillet a,*, M.C. Gagnieu b, P. Lemoine c, L. Collet a

a Laboratoire 'Neurosciences et Systèmes Sensoriels', 3 place d’Arsonval, Pavillon U, Hôpital Edouard Herriot, 69003 Lyon, France
b Laboratoire de Pharmacologie, Hôpital Edouard Herriot, Lyon, France
c Unité Clinique de Psychiatrie Biologique, Le Vinatier, Bron, France

Received 26 November 1997; revised 26 March 1998; accepted 7 April 1998

Abstract

By using otoacoustic emission, we looked for change in outer hair cell (OHC) motile activity and medial olivocochlear (MOC) system inhibition due to benzodiazepine administration, a drug that is known to produce a pharmacological effect by interacting with GABAergic inhibitory neurotransmission. No effect was observed on OHC motile activity, in contrast benzodiazepines decreased MOC system effectiveness suggesting the existence of GABAergic fibers projecting onto the MOC system.

Key words: Gamma-aminobutyric acid; Outer hair cell; Olivocochlear efferent system; Otoacoustic emission

1. Introduction

The organ of Corti is innervated by the olivocochlear efferent system (Rasmussen, 1946), in which two subcomponents have been clearly distinguished (Warr, 1992; Warr and Guinan, 1979). The lateral olivocochlear (LOC) system originates from lateral superior olivary neurons making synaptic contact with the afferent inner radial fibers of the inner hair cell system. The medial efferent system originates from the medial superior olivary complex (SOC) or trapezoid body, synapsing with the basal pole of outer hair cells (OHCs).

Various neurotransmitters seem to be involved between the medial olivocochlear (MOC) system and the OHCs, principally acetylcholine (Ach) and the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (for review see Eybalin, 1993). Concerning GABA, several studies seem to prove the presence of GABAergic synapses in the cochlea. The presence of GABA and glutamic acid decarboxylase in fibers and efferent endings synapsing onto OHC bodies has been revealed by immunocytochemistry data (Fex and Altschuler, 1984; Fex et al., 1986; Thompson et al., 1986; Eybalin et al., 1990). Furthermore, the presence of GABA A receptors in the outer cell membrane of isolated OHCs has been demonstrated using monoclonal antibodies against the α- and β-subunits of GABA A receptors (Plinkert et al., 1989). GABA A receptors are at the basal pole of OHCs. GABA uptake by efferent endings has also been demonstrated (Schwartz and Ryan, 1983; Ryan et al., 1992).

The action of GABA or agonists of GABA on OHC mechanisms has been studied, leading to contradictory conclusions. In vitro, OHC hyperpolarization was observed after GABA application; this hyperpolarization was increased by an application of benzodiazepines (Plinkert et al., 1993). GABA also decreased the electromotile response (Sziklai et al., 1996). In contrast, Evans et al. (1996) observed no OHC response during whole-
cell recording after GABA application. In vivo, OHC function can be explored by otoacoustic emission (OAE) recordings. OAEs are sounds recorded in the external ear canal in the presence of acoustic stimulation (evoked OAEs (EOAEs) and distortion products (DP)) or without acoustic stimulation (spontaneous emissions (SOAEs)). OAEs are generated in the cochlea, probably by OHC motile activity. The influence of benzodiazepines on EOAE amplitude has been studied in humans: Hauser et al. (1992) found no significant difference in EOAE amplitude under premedication with midazolam; likewise diazepam and flunitrazepam had no effect on EOAE amplitude (Delb et al., 1994). It has been shown that contralateral acoustic stimulation decreases the EOAE amplitude in humans (Collet et al., 1990) and acoustic DPs in guinea pig (Puel and Rebilard, 1990). The medial efferent system is involved in this contralateral suppression effect, which is greatly reduced in humans after sectioning of the vestibular nerve, which severs olivocochlear efferents (Giraud et al., 1995). Thus this suppression effect observed on EOAEs in the presence of contralateral acoustic stimulation is a good means of investigating the MOC system in vivo. In guinea pigs, the perfusion of the cochlea with bicuculline (GABA antagonist) abolished the $f_2-f_1$ DP reduction normally observed after contralateral stimulation in guinea pigs (Kirk and Johnstone, 1993), thus confirming a role for GABA in the OHC mechanism. So the terminal elements involved in the contralateral acoustical suppression of $f_2-f_1$ can be identified as being part of a GABAergic innervation, whether LOC or MOC system, of the apical OHCs.

Thus a majority of studies confirm the presence of GABAergic efferents on OHCs and presence of GABA$_A$ receptors on the basal pole of OHC, but there is no evidence of a real action of GABA on outer hair cell mechanisms. Furthermore the presence of GABAergic synapses in human cochlea has not been studied.

On the other hand, the olivocochlear efferent system could be under the control of central auditory pathways. The inferior colliculus (IC) receives descending input from the auditory cortex, and sends ipsilateral and contralateral projections onto the medial olivocochlear system (Huffman and Henson, 1990). So it is anatomically possible that the IC plays a role in the activity of the olivocochlear efferent system. Furthermore, physiological studies show an IC contribution to the olivocochlear efferent system. Stimulating the IC induces a decrease in compound action potential (CAP) (Dolan and Nuttal, 1988a) that is similar to the change seen after electrical stimulation of the crossed olivocochlear bundle (Dolan and Nuttal, 1988b). Furthermore, stimulating the IC protects the cochlea from noise, as does stimulation of the crossed olivocochlear bundle (Rajan, 1990).

GABA is an inhibitory transmitter which is abundant in the central auditory pathways (Wynne et al., 1995; Vater et al., 1992; Fubara et al., 1996). It has been shown that benzodiazepines increase the CAP and decrease cochlear microphonics in guinea pigs, in contrast to electrical stimulation of the crossed olivocochlear bundle. These benzodiazepine effects have been thought to reflect a central action of GABA on the efferent system (Velluti and Pedemonte, 1986).

Thus GABA may have a peripheral action on outer hair cell mechanisms, and a central action on the medial efferent system. The aim of the present study was to investigate these two hypotheses in humans by studying benzodiazepines known to produce their pharmacological effect by interacting with GABAergic inhibitory neurotransmission and their effect on EOAE amplitude and on the MOC system, by comparing EOAE amplitude with and without contralateral acoustic stimulation (Collet et al., 1990; Veuillet et al., 1991).

2. Methods

2.1. Subjects

The study was performed on 10 healthy men ranging in age from 20 to 34 years (mean 24 years, S.E. 1.23). All the subjects were volunteers and this study was carried out with their written consent and with the agreement of the local institutional Ethics Committee.

The subjects were selected according to the following acoustic criteria: normal auditory thresholds (< 20 dB loss between 250 and 8000 Hz by octave) measured by a Madsen DAIII audiometer, normal tympanometric recordings done using an Amplaid 702, presence of EOAEs (amplitude better than 5 dB SPL at 80 ± 3 dB SPL), no spontaneous otoacoustic emissions. The volunteers were all right-handed (Olfield, 1971).

A questionnaire and a medical examination further enabled selection of subjects without prior acoustic pathology, taking no medical treatment, using no hypnagogics or benzodiazepines. On the day of experimentation a urine assay for benzodiazepine was made to confirm the last of these conditions.

2.2. Otoacoustic emissions

EOAE recordings were made with subjects reclining in a sound-proof room, using an Otodynamics® ILO 88 apparatus. A probe placed in the external ear canal delivered acoustic stimulation through a loudspeaker (Knowles 1712 transducer) and recorded responses with a microphone (Knowles 1843). To be sure that the probe was always placed in the same position over successive recordings, a silicone mold of the probe and of the external ear canal was made.
Stimuli were non-filtered clicks with a duration of 80 μs and a rate of 50/s. Linear and non-linear differential cochlear echo methods were used (Kemp et al., 1990).

2.3. Procedure

During this study a double-blind administration of oxazepam versus placebo was made (the kind of molecular administered was not known either to subjects or to experimenters). So the data were collected in a blinded manner. The subjects were tested before and 1, 3, 7 and 24 h after oxazepam (20 mg) or placebo administration. The order of testing (oxazepam versus placebo) was randomized. We employed a crossover design: all 10 subjects were tested twice with a 2-week interval (once after oxazepam intake, and once after placebo intake). EOAEs were recorded in right ears.

A first recording was made with a non-linear click at 80 ± 3 dB SPL. The amplitude of the response was calculated from 2.5 to 20 ms.

Then the efferent system was investigated according to the protocol proposed by Collet et al. (1992): EOAEs were recorded with linear clicks for five ipsilateral intensities levels ranging between 60 and 72 dB SPL. Presentation of these five intensities with and without contralateral acoustic stimulation delivered by the ILO 88 apparatus was randomized. Contralateral acoustic stimulation was a 30 dB SL broadband noise (bandwidth 500–8000 Hz). The response window was set at 3.2–20 ms.

Oxazepam versus placebo was administered at the same hour of the day to all the subjects. Before each recording, a sample of blood was taken, to measure plasma oxazepam concentration.

2.4. Data processing and statistical analysis

EOAE amplitudes were studied after non-linear clicks at 80 ± 3 dB SPL. In order to eliminate stimulus artifacts, the amplitude of the response was calculated from 2.5 to 20 ms. The overall click-evoked response level, corresponding to the sum of echo power in the whole frequency range of the power spectrum, was noted.

The effectiveness of contralateral acoustic stimulation was expressed as an equivalent attenuation which is the decrease in ipsilateral stimulus inducing the same EOAE amplitude effect as the contralateral broadband noise (Collet et al., 1992; Veuillet et al., 1991).

Statistical analysis was performed using Sigmastat® software and included parametric tests (normality test being passed). ANOVAs on repeated measures, paired t-test and Pearson product moment correlation were conducted. Probabilities below 0.05 were considered significant.

3. Results

3.1. Plasma oxazepam concentration

Fig. 1 shows the mean plasma concentration of oxazepam. The highest plasma concentration was found 3 h after intake. Individual variations in maximum plasma concentration can be noted, values ranging between 0.076 and 0.4 mg/l (mean 0.185, S.E. 0.028).

Like data were collected in a double-blind manner, plasma oxazepam concentration was measured after placebo administration: no oxazepam was found in any blood samples taken after placebo administration and this for all the subjects.

3.2. EOAE amplitudes

Fig. 2 shows non-linear EOAE amplitude as a function of time. There was no significant variation in amplitude between the various recordings made on the day of placebo administration (paired t-test, P > 0.05). Response amplitude was also studied after oxazepam intake. EOAE amplitudes after oxazepam intake were compared with themselves and with EOAE amplitudes before oxazepam intake (paired t-test). These results were further compared with results obtained after a 2-week interval (paired t-test). We note that the EOAE amplitude obtained at the same time at two intervals was statistically comparable before oxazepam intake (11.7 dB, S.E. 1.17) and before placebo intake (11.2 dB, S.E. 1.17), suggesting no damage and no exposure to noise trauma during the course of the 2-week study period. No significant effect of oxazepam was observed.
3.3. Effectiveness of the MOC system

Fig. 3 shows the mean equivalent attenuation obtained before and after administration of placebo. No significant difference between the recordings was observed under placebo (one-way ANOVA, paired t-test). The effectiveness of MOC is constant across the hours of day.

The mean results obtained before and after oxazepam intake are shown in Fig. 3. The mean equivalent attenuation obtained at the same time at 2-week intervals being statistically comparable before oxazepam intake (−1.75 dB, S.E. 0.32) and before placebo intake (−1.88 dB, S.E. 0.36), it was possible to compare equivalent attenuation calculated at the same time but at a 2-week interval. So we note that the effectiveness of the MOC system is the same after the 2-week interval. To evaluate treatment effects (oxazepam and placebo) on equivalent attenuation, a two-way ANOVA for repeated measures was conducted and showed a significant treatment effect (F = 14.83, df = 1, P = 0.004).

First, we decided to observe if the equivalent attenuation is different under placebo and oxazepam. By paired t-test, there was a significant effect of the treatment 1 h after oxazepam intake (paired t-test, t = 4.9, P = 0.02). Oxazepam intake induced a decrease in equivalent attenuation. A tendency to decrease 3 h after oxazepam intake was, however, not statistically significant (paired t-test, t = 2.01, P = 0.076). So there is a decrease of MOC system effectiveness after oxazepam intake compared to placebo intake. This effect appears 1 h after oxazepam intake.

Second, we decided to observe the effect of oxazepam over time. Fig. 3 shows a variation in the effect of oxazepam with time which is not statistically significant (one-way ANOVA). We compared the results obtained after oxazepam intake with the result obtained before oxazepam intake (paired t-test). A decrease in equivalent attenuation, significant 3 h after oxazepam intake, was observed (paired t-test, t = −2.569, P = 0.030). A strong correlation was observed between mean plasma oxazepam level and mean equivalent attenuation (Pearson product moment correlation, r = 0.976, P = 0.005). At 3 h (maximum plasma concentration) a significant correlation between oxazepam concentration and the change in equivalent attenuation between the recording made without oxazepam and the recording 3 h after oxazepam intake was observed (Pearson product moment correlation, r = 0.684, P = 0.03): the higher the oxazepam concentration, the more the equivalent attenuation decreases (Fig. 4).

Perception threshold for contralateral stimulation (broadband noise) in the left ear did not change between successive recordings (ANOVA, paired t-test).

4. Discussion

The first aim of this study was to test the effect of benzodiazepines on OHC mechanisms in humans. No statistically significant changes could be detected before and after oxazepam administration with respect to non-linear EOAE amplitude. This result confirms previous studies (Hauser et al., 1992; Delb et al., 1994): benzodiazepines have no direct effect on OHC motility in humans.

Benzodiazepines are lipophilic substances and so easily cross the blood-brain barrier to penetrate brain tissue. Therefore benzodiazepines should be present in
the perilymph. Benzodiazepines act as coreceptors increasing GABA-induced chloride ion influx into the cell. Benzodiazepines thus increase the effect of GABA. Although benzodiazepines act on OHCs, GABA must be released in the synaptic gap. Thus if the medial efferent system has no tonic action on OHC, no variation in EOAE amplitudes can be observed after oxazepam intake. It may also be that the GABA
A receptors at the basal pole of the OHCs are non-functional as in vitro studies on guinea pig have shown (Evans et al., 1996). The presence of GABAergic synapses in human cochlea could also be discussed. Finally, the dose of oxazepam used (20 mg) was perhaps not high enough to influence EOAEs significantly.

Thus it can be concluded that, with the doses of oxazepam used in our study, no effect on OHC active micromechanisms was obtained.

The second aim of this study was to test, for the first time in humans, the effect of benzodiazepines on the MOC system. Our findings clearly demonstrate a decrease in contralateral suppression effect, the contralateral acoustic stimulation having less effect on OHC active micromechanisms under oxazepam. This decrease in effectiveness seems to be a true consequence of oxazepam administration, being strongly correlated with plasma oxazepam concentration. Since benzodiazepines did not modify OHC responses, the decrease in suppression can be attributed to a change in MOC system performance rather than to a modification in the OHCs. Such a change could be due to a reduction in MOC stimulation. The message carried by the auditory afferents may be modified by benzodiazepines: GABA is thought to be a LOC neurotransmitter (for review see Eybalin, 1993), presumably linked to the firing of auditory neurons; GABA
A receptors have been found by autoradiography in the cochlear nucleus of the guinea pig (Juiz et al., 1994). Thus there may be reduced auditory afferent firing after oxazepam intake. Nevertheless the contralateral acoustic threshold was measured before all the tests: a 30 dB broadband noise was always used to elicit the contralateral effect.

The decrease in MOC performance could be explained by inhibition. Benzodiazepines may act directly on the synapse between afferent pathway and efferent system. GABA would be liberated in the synapse by the afferent pathway; benzodiazepines by acting on GABA
A receptors would enhance the GABA effect and the inhibition of the MOC. These hypotheses are supported by the fact that GAD and GABA positive cells have been found in the SOC (Vater et al., 1992) and that the medial nucleus of the trapezoid body contains GABAergic endings (Suneja et al., 1995); nevertheless the nature of the neurotransmitters involved between the afferent pathways and MOC system in the SOC is unknown. The decrease in MOC system performance could also reflect some central control of the MOC, suggesting the existence of GABAergic descending fibers projecting onto the MOC system.

This study shows that benzodiazepines have an action on the MOC system in humans; some ascending or descending GABAergic projections act on the MOC which controls active OHC mechanisms.

References


Fex, J., Altschuler, R.A., 1984. Glutamic acid carboxylase immuno-


