The role of GABAergic mechanisms in the regulation of cochlear dopamine release

Thesis

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Introduction

According to the internet data of WHO, approximately 278 million people suffer from moderate or severe hearing loss worldwide. This means a significant increase compared to the 2001 data (250 million). 2/3 of this huge population lives in developing countries, however 4 percent of the people suffering from hearing loss higher that 40dB lives in the developed countries. Perceptive hearing loss has been in the focus of clinical and experimental studies since decades. Although the patomechanism has been clarified in many senses, it is still is unknown as a whole, therefore we still have to wait for the definitive clinical treatment. Ischemic hearing loss has high significance within etiological reason of the sensorineural hearing loss because the majority of old-age hearing loss can be classified into this category. The noise-induced hearing loss can be classified as a civilizational damage and the technical development increases this great number of patient pool/population. European countries spend 0,2-2 percent of their gross domestic product on the prevention of noise-induced hearing loss. (WHO, 1997).

The real receptors of hearing are the inner hair cells (IHC) from where the impulse is transmitted toward the afferent cochlear dendrite. There is a growing number of evidence that transmission between them is produced by the released glutamate. Ischemic conditions or noise exposure can result in excessive glutamate release, having excitotoxicitic effect on the afferent auditory neuron. Dopamine (DA) – among other – is released from the lateral olivocochlear (LOC) efferent terminals, the efferent arm of the short-loop feed-back between the brainstem and the cochlea. As it was shown by several studies, DA is likely neuroprotective against noise exposure and ischemic damages in the auditory organ because it can reduce the excitotoxicitic effect of glutamate.

Metabotropic glutamate receptors (mGluR)

All kind of ionotropic glutamate receptors have been found on the dendrite of the afferent nerve. AMPA/kainate glutamate receptor-antagonist (GYKI-52466) failed to modulate the release of DA from the cochlea, therefore the protective DA releasing effect of glutamate was supposed only via the short-loop feed-back mechanism. Although several studies confirmed the presence of metabotropic glutamate receptors (mGluRs) on both the spiral ganglion cells and inner hair cells, little is known about their function in the cochlea. As they have slower and longer kinetics than ionotropic glutamate receptors, they are more likely involved in modulatory actions in the cochlea. It is particularly important that group II and III mGluR agonists mediate inhibitory action and are considered as neuroprotective compounds.

Serotonin (5-HT)

In humans, 5-HT has been implicated in the regulation of eating, sleeping, sexual behaviors, circadian rhythm, various neuroendocrine functions, vascular constriction and in the etiology of numerous disease states, including depression, anxiety, social phobia, schizophrenia, and obsessive–compulsive and panic disorders. However, the functional role of serotonin and 5-HT receptors is much less understood in the cochlear neural network. Serotonin (5-HT) receptors are divided into seven distinct classes (5-HT_1 to 5-HT_7) largely on the basis of their structure and function. 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 receptors couple to Gs proteins and promote cAMP formation. Serotonergic fibers have recently been identified by immunocytochemal methods in the cochlea. These fibers innervate regions below both the inner and the outer hair cells. Their cochlear distribution suggests that they belong to the LOC system. The 5-$HT_{1/2}$ antagonist methysergide reduces the compound action potential of the auditory nerve in the guinea pig. Measurement of 5-HT metabolites, together with the identification of 5-HT receptor mRNAs in the mammalian cochlea revealed the serotonergic activity in this organ. It has been suggested that serotonergic

innervation of the organ of Corti could also modulate of the auditory process. Beside its physiological role, 5-HT seems to play an important role in tinnitus; disrupted or modified 5-HT function initiates plastic changes, which strongly influence tinnitus. It has been demonstrated that local application of the 5-HT_6 receptor antagonist SB-271046 dose dependently increased DA release in the frontal cortex.

Oxygen-Glucose Deprivation (OGD)

Several other studies also indicated the possible protective role of DA in noise or ischemia-induced cochlear damage. Using RT-PCR, $D_{2(long)}$ and D_3 receptors are suggested to transmit the action of DA in the mouse cochlea. In accordance with these findings, functional evidence indicated that the protective effect of DA is mediated via D_2 and D_3 receptors. The type and function of DA autoreceptors are, however, less understood. The role of autoreceptors on dopaminergic nerve terminals in the brain is to regulate a negative feedback control by decreasing the synthesis and release of DA and upregulating the uptake of the transmitter.

The γ-amino-butyric-acid (GABA)

GABA is the well-known inhibitory element in the central nervous system. It can be found as GABA transmitter in 20-50% of the central nervous system synapses. It was demonstrated in rat cerebral cortex that $mGluR_{2/3}$ receptors regulate the GABA release. Immonogold techniques confirmed the presence of $mGluR_3$ on GABAerg fibres indicating a possibile heterosynaptic effect. Parallel serotonin-glutamate interaction was observed in learning, memory and sensory processes. In CA3 pyramidal cells, 5-HT inhibits selectively the $GABA_B$ component of GABA-mediated inhibitory post-synaptical potential (IPSP).

Aims

DA, relased from the lateral olivochlear efferent fibers, is suggested to be neuroprotctive against ischemia and noise exposure in the mammalian cochlea because it can reduce the postsynaptic excitotoxic effect of glutamate on dendrite of the afferent auditory neuron. Our major aim was to better understand the various modulatory possibilities of DA release via receptors, as well as to explore novel potential pharmaceutical targets to prevent these damages.

1. To further investigate the intracocheal protective mechanism besides the short-loop feed back, we aimed to explore the functional role of mGluRs in the cochlea. In the light of the known neuroprotective feature of mGluRs, we assumed that cochlear DA release, which is known to be associated with protective actions, may be functionally linked to activation of mGluRs in the cochlea.

2. It has been suggested that serotonergic innervation could modulate the auditory process, however the exact mechanism is still obscure. As presynaptic release-modulating heteroreceptors represent suitable targets for pharmacological intervention, our aim was to uncover the role of 5-HT_6 and 5-HT_7 receptors in the modulation of the cochleoprotective DA release. We tested the effect of known, selective 5-$HT_{6/7}$ receptor antagonists and that of new substances, whether they can influence DA release.

3. Given the neuroprotective role of cochlear DA, we studied the direct effect of oxygen-glucose deprivation (OGD), as an *in vitro* ischemia model, on the DA release in the guinea-pig cochlea.

Materials and Methods

Experiments using *in vitro* microvolume superfusion were carried out in a perilymph-like solution as describer earlier (Gáborján és mtsai, Neuroscience, 1999). The pH was adjusted to 7.4. The osmolality was set by D-glucose and the solution was gassed continuously with 100% O_2 and thermoregulated at 37^{o}C. In a set of experiments the perfusion buffer was gassed with 100% N_2 and contained saccharose instead of glucose from the 24th minute in the OGD experiments.

Animals and tissue preparation

Male guinea pigs were used, weighing 250-350g. After decapitation, the occipital bone was cut and the bulla tympani was opened and removed, that is equal to the pars petrosa of the human temporal bone. The preparation was then placed into perilymph-like solution. The bony capsule of the cochlea was removed under stereomicroscopic guidance, the stria vascularis was stripped and the cochlea was fractured at the basis of the modiolus and removed. The preparatum contained the following elements: ganglion spirale, cochleal afferents and efferents, as well as the Organ of Corti, the outer and inner hair cells and supporting cells.

The measurement of DA release: in vitro microvolume superfusion method

The isolated cochleae were incubated for 35 min at 37^{o}C in 1 ml of the perilymph-like solution containing 10 μM [^{3}H]DA. Cochleae were than placed in plexi chambers (100 μl inside volume, one cochlea per chamber) and perfused with the perilymph-like solution at 3 ml/min rate. After one hour preperfusion the outflow was collected in 3-minute fractions for 57 minutes (19 fractions) and their DA content was determined by measuring the radioactivity of each sample. 0.5 ml aliquots of each sample were assayed with a liquid scintillation counter (Packard Tri-Carb 1900TR, Meriden, CT, USA). After the collection period, cochleae were transferred to 0.5 ml of 10% trichloroacetic acid for 24 hours then 0.1 ml was used to measure the radioactivity of the tissue.

Electrical field stimulation

Electrical field stimulation was applied through platinum electrodes, placed on top and bottom of the chambers using a Grass S88 stimulator (West Warwick, RI, USA) during the 3rd (S_1, 360 pulses) and the 13th fractions (S_2, 360 pulses) at 60V, 2 Hz, 0.5 ms duration.

Drugs

Drugs were added to the perfusion solution at the 21th or 24th minute of the collecting period and maintained till the end of the experiments. The selective $GABA_A$ antgonist bicucullin was added at the 24th minute to the perfusion solution and was perfused for 15 minute.

Calculations, statistical analysis

During the evaluation of results of the experiments the resting and electrically evoked DA release were examined. The DA content of each fraction was determined as a percentage of radioactivity of the whole tissue during one collecting period (fractional release, FR). Data are expressed as means +S.E.M. Number of experiments (n) corresponds the number of microvolume superfusion experiments (each with a single cochlea). ANOVA followed by Newman-Keuls or Tukey HSD post hoc comparisons was used to determine the statistical significance. Levels of significance were as follows: *p<0,05; **P<0,01;***p< 0,001.

Results

Examination of metabotropic glutamate receptors

In order to explore the role of mGluRs in the regulation of cochlear DA release we tested the effect of selective agonists and antagonists of group I, II and III mGluRs on the spontaneous and electrically evoked release of DA from isolated guinea pig cochleae. Perfusion with agonists and antagonists of group I mGluRs (DHPG and MPEP) failed to alter the release of DA from the cochlea. In contrast, mGluRs belonging to group II were active in modulating DA release: administration of 2R,4R-APDC increased significantly the spontaneous release of DA from isolated cochleae. The selective group II antagonist LY-341495 caused no significant effect on either the spontaneous or the evoked release of DA. Group III agonist (L-AP4) and antagonist (MSOP) caused no significant effect on the DA outflow. To further investigate the excitatory action of mGluR group II receptors on the spontaneous DA release from the cochlea, we tested 2R,4R-APDC at 100 and 300 μM concentrations. These experiments revealed that the action of 2R,4R-APDC seems to be dose-dependent. As the polarity of the modulation was opposite to that we would expect from an inhibitory receptor, such as the group II mGluR, we assumed that 2R,4R-APDC supressed the activity of an inhibitory element, presumably GABAergic inputs, which would act to depress the dopaminergic terminal. To explore the possible involvement of such disinhibitory mechanism by removing the inhibitory step, we blocked $GABA_A$ receptors by bicuculline. In the presence of bicuculline, 100 μM 2R,4R-APDC failed to increase the spontaneous release of DA.

Examinations of 5-$HT_{6/7}$ antagonists

In a collaboration with Division of Chemical Research, EGIS Pharmaceuticals Plc, we tested the effect of 5-HT_6 and 5-HT_7 receptor antagonists and the enantiomers on cochlear DA release in order to explore the role of cochlear serotonergic system. The 5-HT_6 antagonist SB-271046 (10 μM) significantly elevated the resting outflow

of DA in isolated cochleae. When both 5-HT_6 and 5-HT_7 receptors were blocked by EGIS-12233 the increase in the resting DA release was larger than in the case of selective block of 5-HT_6 receptor. Because both 5-HT_6 and 5-HT_7 receptors produce stimulatory effects on target cells, the antagonists of these receptors are expected to have either an inhibitory effect (in case of tonic endogenous stimulation of the receptor) or no effect. Our finding that the inhibition of 5-HT_6 / 5-HT_7 receptors caused excitation instead of depression in the release of DA from the cochlea raised the possibility of an indirect, disinhibitory mechanism. To address this question, we blocked the local GABAergic input by bicuculline, a selective $GABA_A$ receptor antagonist. In the presence of bicuculline (20 μM) EGIS-12233 was not able to increase the resting release of DA in the cochlea.

The effect on DA release by OGD

OGD was applied as an ischemia model: the perfusion buffer was gassed with 100% N_2 and contained saccharose instead of glucose. To explore of the function of the autoreceptor, two structurally different D_2 selective antagonists (sulpiride and L-741,626) were used, as well as a DA uptake inhibitor (nomifensine). OGD caused no detectable change in the resting release of [^{3}H]DA and did not influence the field stimulation-evoked release of DA, however in the presence of antagonist of the D_2 inhibitory autoreceptor (10 μM sulpiride, or 1 μM L-741,626) OGD induced an elevation of the basal DA level. The DA uptake blocker nomifensine (10 μM) alone significantly increased the electrical field stimulation-evoked release of DA and failed to influence the resting DA outflow. In the presence of 10 μM nomifensine, OGD plus sulpiride (10 μM) failed to induce release of cochlear DA. When nomifensine was present in the perfusate L-741,626 (1 μM) was also inactive to facilitate the release of DA during OGD.

Discussion

1. Metabotropic glutamate receptors

The mGluR agonist 2R,4R-APDC supresses the activity of an inhibitory neurons, presumably GABAergic cells, which would act to depress the dopaminergic terminal. Our neurochemical evidence also suggests that the GABA containing LOC terminals have spontaneous GABA release that keeps the cochlear dopaminergic nerve endings under tonic inhibition. Our data confirmed that these GABAergic fibers express functional $mGluR_{2/3}$. In the present study, we explored that a GABAergic disinhibitory loop underlies the DA-releasing effect of the activation of group II mGluRs. Through these mGluRs, the IHC-released glutamate is able to decrease the release of GABA, leading to the disinhibition of DA containing LOC terminals: our new findings suggests a recently described ultra- short feed-back loop.

2. Serotonin 5-$HT_{6/7}$ receptor antagonists

Our finding that the inhibition of 5-HT_6 / 5-HT_7 receptors caused excitation instead of depression in the release of DA from the cochlea raised the possibility of an indirect, disinhibitory mechanism. In the presence of bicuculline (20 μM) EGIS-12233 was not able to increase the resting release of DA in the cochlea. The present results indicate that GABAergic fibers express functional 5-$HT_{6/7}$ receptors and their antagonists are able to decrease the release of GABA, which leads to the disinhibition of DA containing LOC terminals. Although the selective 5-HT_7 antagonist was ineffective to modulate DA release, functional 5-HT_7 receptors likely exist in the cochlea as (i) the mixed 5-$HT_{6/7}$ antagonist was more effective than the selective 5-HT_6, (ii) the mixed 5-HT_7/D_4 receptor antagonist produced an even more pronounced enhancement of cochlear DA release, that also indicates the supporting role of D_4 receptors.

3. In vitro ischemic model (OGD)

Our experiements revealed that OGD, which can taken as an experimental

model of ischemia, was able to evoke the release of DA from the LOC fibers in the guinea-pig cochlea, in the presence of D_2 receptor antagonists. We found that the underlying mechanism of the cochlear DA-releasing effect of OGD involves an action on the DA transporter. As the inhibition of the reuptake would increase in itself all non-transport dependent extracellular level by the blocking of transporters, therfore DA release by OGD might be the reason for the reverse operation of the transporter. In this sense OGD induces the reverse operation of an uptake mechanism, leading DA release. As the result of the inhibition of transporter by nomifensine, such effect of OGD is blocked. D_2 receptor antagonists contributed to the electrically-evoked DA release. Their siginificant effect on field stimulation-evoked exocytotic release is probably due to disinhibition of the multiple feed-back action of D_2 autoreceptors. In conclusion, our data indicate the presence of a functional D_2 autofeedback receptor on LOC efferents in the guinea-pig cochlea. Blockade of these autoreceptors attenuated the released DA induced facilitation of the reuptake and therefore revealed the release of DA from LOC terminals due to OGD. Our neurochemical data sugges that GABAergic fibres express funcional mGluRII and excitatory 5-$HT_{6\text{-}7}$.

In conclusion, we found that the inhibitory mGluR agonist (APDC) and excitatory 5-$HT_{6\text{-}7}$ antagonist (EGIS-12233) are both able to decrease the release of GABA which leads to the disinhibition of DA containing LOC terminals. GABA containing LOC terminals maintain spontaneous GABA release that keeps the cochlear dopaminergic nerve endings under tonic inhibition. The application of mGluR agonists and the 5-$HT_{6\text{-}7}$ agonists could be target of the future therapy of sensorineural hearing loss.

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