



The role of N-methyl-d-aspartate receptors and nitric oxide in cochlear dopamine release

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Abstract

Dopamine (DA) released from lateral olivocochlear (LOC) terminals may have a neuroprotective effect in the cochlea. To explore the role of N-methyl-d-aspartate (NMDA) receptors and nitric oxide (NO) in the modulation of a cochlear DA release, we measured the release of [3H]DA from isolated mouse cochlea in response to the application of NMDA. NMDA at 100 μ M significantly increased the electrical-field stimulation-evoked and resting release of DA from the cochlea. The NO donor sodium nitroprusside enhanced the basal outflow of DA but failed to influence the evoked release. The administration of the nitric oxide synthase inhibitor N ω -nitro-l-arginine methyl ester (l-NAME) alone was ineffective, but it significantly inhibited the initial phase of the NMDA-induced elevation of DA outflow, which suggested the role of NO in the NMDA-induced DA release. The DA uptake inhibitor nomifensine increased the electrically evoked release of DA. Nomifensine failed to change the effect of NMDA on the resting or electrically-evoked DA release, which suggested that the uptake mechanism does not play a role in NMDA-evoked and NO-mediated DA release. In summary, we provide evidence that NO can modulate the release of DA from the cochlea following NMDA receptor activation, but does not affect the uptake of DA. Key words: cochlea; dopamine; uptake; lateral olivocochlear efferent; nitric oxide; NMDA

Abbreviations: DA, dopamine; FR, fractional release; IHC, inner hair cell; l-NAME, N ω -nitro-l-arginine methyl ester; LOC, lateral olivocochlear; NMDA, N-methyl-d-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; TTX, tetrodotoxin

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The inner hair cells (IHC) communicate with other cells of the cochlea by releasing glutamate. It is well known that dopamine- (DA) containing boutons of the lateral olivocochlear (LOC) efferent fibers have axo-dendritic synapses on afferent dendrites ([Eybalin 1993] and [Eybalin et al 1993]) and modulate afferent neurotransmission ([Darrow et al 2006], [Niu and Canlon 2006], [Le Prell et al 2005], [Mulders and Robertson 2004], [Guinan et al 1983], [Liberman 1980], [Liberman et al 1990], [Puel 1995] and [Pujol and Puel 1999]). Several lines of evidence suggest that DA, which is released from the LOC fibers, may have a protective effect on cochlear function ([d'Aldin et al 1995], [Oestreicher et al 1997] and [Ruel et al 2001]). In a previous study, we found that the group II metabotropic glutamate receptor agonist aminopyrrolidine-2,4-dicarboxylic acid (APDC) can increase the release of cochlear DA (Doleviczenyi et al., 2005). This result also supports the idea of dopaminergic neuroprotection in the cochlea. All types of ionotropic glutamate receptors have been identified on afferent dendrites ([Matsubara et al 1996], [Furness and Lawton 2003], [Hakuba et al 2003], [Knipper et al 1997] and [Eybalin et al 2004]). The clinical importance of glutamate receptors has been highlighted by the finding that N-methyl-d-aspartate (NMDA) and AMPA receptors play an important role in the development of cochlear damage ([Ohinata et al 2003], [Duan et al 2000] and [Puel et al 1998]). Further highlighting their importance, NMDA receptors are likely to play a role in the induction of tinnitus (Guitton et al., 2003). Extreme glutamate levels cause excessive Na⁺ and Ca²⁺ fluxes through AMPA and NMDA receptors in afferent dendrites in turn leading to water influx and swelling (Pujol et al., 1990). Ca²⁺, after entering the cells through NMDA receptors, can activate proteases and lipases that lead to the irreversible damage of the cells ([Ohinata et al 2003] and [Choi 1987]). The increased Ca²⁺ level becomes sufficient to activate the nitric oxide synthase (NOS) that is linked to NMDA receptors (Moncada and Bolaños, 2006).

In addition to being a neurotransmitter, nitric oxide (NO) plays a role in free radical production ([Ohinata et al 2003] and [López-González et al 1997]) and the inactivation of several enzymes (Snyder and Brecht, 1992). The neuronal form of NOS is connected to NMDA receptors and produces NO primarily in response to the activation of NMDA receptors (Brenman and Brecht, 1997). NO can act on nonsynaptic targets, as suggested by Kiss et al. (2004). NO can interact with the DA release in the striatum (Hanbauer et al., 1992). Different NOS isoforms were identified in the organ of Corti (Michel et al., 1999). In support of the structural data, significant NO activity was reported in the cochlea, which can be inhibited by N^ω-nitro-l-arginine methyl ester (l-NAME) (Shi et al., 2002). It has also been suggested that NO in the human cochlea could act as a neurotransmitter/neuromodulator (Popa et al., 2001). Although several aspects of NO as a transmitter have been described in the cochlea (cf. Takumida and Anniko, 2004), very little is known about the role of NO in modulating the release of cochlear neurotransmitters. In the present study, we aimed to explore the local DA releasing effect of glutamate through NMDA receptors by applying NMDA to an isolated cochlea preparation. We also made an attempt to uncover the role of NO in the action of NMDA within the cochlea.

Experimental procedures

Animals and tissue preparation

We used CD-1 male mice (from OGR, KOKI, Hungary), weighing 15–25 g for our experiments. We state that all animal experiments were carried out in accordance with the

European Communities Council Directive of 24 November 1986 (86/609/EEC) and all efforts were made to minimize animal suffering, to reduce the number of animals used. After decapitating the animals, the cochleae were removed and placed immediately in a perilymph-like solution (150 mM NaCl, 3.5 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 2.75 mM Hepes and 2.25 mM Tris at 37 °C; Gaborjan et al., 1999). Under stereomicroscopic guidance the bony capsule of the cochlea together with the spiral ligament and stria vascularis were chipped away, and the modiolus was fractured at the base of the cochlea. The preparation contains the following neural elements besides for the osseous and vascular structures: the ganglion spirale, afferent auditory fibers, axons, and axon terminals of the efferent bundles and the inner and outer hair cells. In a set of experiments, the tissue was examined to estimate the viability of the preparation. The fixative contained 4% paraformaldehyde, 0.5% glutaraldehyde in PB, and a pH of 7.4. After washing with phosphate buffer, the preparations were postfixated in 1% OsO₄ for 30 min. The tissue blocks were dehydrated in graded ethanol, block-stained with 2% uranyl acetate in 70% ethanol for 1 h, and embedded in Taab 812 (Taab, Aldermaston, Berks, UK). Ultrathin sections were examined in a Hitachi 7100 transmission electron microscope (Hitachi Corporation, Tokyo, Japan). Electron-microscopic examination proved the structural integrity of the organ of Corti in our preparation at the end of the experiments showing structurally preserved axon terminals (Fig. 1A). Previously, we also demonstrated intact ribbon synapse and surrounding neural structures in the cochlea preparation after the perfusion (Halmos et al., 2005).

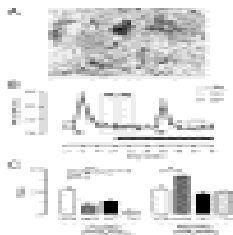


Fig. 1. Neuronal origin of DA outflow from isolated mouse cochleae. (A) Electron microscopic image of intact neural elements in a cochlea preparation placed into the fixative right after the experiment. T, nerve terminal; arrow, synapse; scale bar=1 μ m. (B) Under control conditions, electrical field stimulation evoked reversible and reproducible DA release from the cochlea. TTX (1 μ M) and Cd²⁺ (100 μ M) significantly decreased the evoked release. Electrical field stimulations (FRS1 and FRS2) are indicated by horizontal black bars. The control resting period (FRR1) and the phase of drug action (FRR2, 21–27 min of sample collection) on the resting release are shown by the dashed rectangles. Drugs were applied in the perfusion solution as the horizontal bar indicates. (C) The bar chart shows the effect of drugs on the resting (FRR2/FRR1) and electrical stimulation-evoked (FRS2/FRS1) DA release. All test solutions (Ca²⁺-free, Cd²⁺ and TTX) significantly decreased the evoked outflow. Interestingly the Ca²⁺-free solution significantly increased the resting release of DA that could be explained by a compensatory Ca²⁺-outflow from the bony structures of the preparation. Cd²⁺ and TTX did not change the basal release of DA. Data presented are means \pm S.E.M. ** P<0.01; * P<0.05 (n=5–8).

In vitro microvolume superfusion

The isolated cochleae were incubated for 35 min at 37 °C in 1 ml of the perilymph-like solution containing 0.2 μM [7,8-³H]DA (specific activity: 45.0 Ci/mmol; Amersham, USA). Cochleae were then placed in plexi chambers (100 μl inside volume, three cochleae per chamber) and superfused with the perilymph-like solution at 3 ml/min. In some experiments cochleae were perfused with Ca²⁺-free solution (150 mM NaCl, 3.5 mM KCl, 0 mM CaCl₂, 1 mM MgCl₂, 2.75 mM Hepes, 2.25 mM Tris and 100 μM EGTA). After 1 h of preperfusion, the outflow was collected for 57 min in 3-min 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. Twenty-four hours later 0.1 ml was used to measure the radioactivity of the tissue. Electrical field stimulation was applied through platinum electrodes placed on top and bottom of the chambers with a Grass S88 stimulator (West Warwick, USA) during the 3rd (S1) and 13th fractions (S2) for 3 min at 30 V, 5 Hz, and 0.5 ms duration. Treatments were applied from the 21st minute of the collection period. NMDA and glycine (10 μM) were applied in the perilymph-like solution in the absence of Mg²⁺. In some cases the DA uptake blocker nomifensine was perfused from the beginning of the experiments. All perfusion solutions were continuously saturated with 100% O₂ and kept at 37 °C.

Calculations and statistical analysis

There is a constant decline in the outflow of tritiated DA because of the decreasing amount of radioactivity in the tissue. To best describe the release of DA during one collecting period, the fractional release (FR) of the tritium-outflow was determined as the percentage of total radioactivity (³H-DA) that was present in the tissue at the time of the sample collection. The release evoked by the electrical field stimulations (S1 and S2), was determined as FRS1 and FRS2, i.e. the FR during S1 and S2 after subtracting the mean basal release (average of the resting FR values before and after the electrical stimulation). The effect of the study drug on the electrical stimulation-evoked release of [³H]DA was expressed as the ratio of FRS2/FRS1. The resting release was estimated by the calculation of the mean FR of DA within 6 min of the perfusion at three different periods (FRR1, 15–21 min; FRR2, 21–27 min; FRR3, 30–36 min). FRR2 and FRR3 were sampled in the presence of drugs but before the second electrical stimulation, FRR1 corresponds to the control, drug-free period. The effect of drugs on the resting release was expressed by the ratio of the resting FR values in the presence of the drug (FRR2 or FRR3) over the value before the drug reached the preparation (FRR1) as FRR2/FRR1 or FRR3/FRR1. Experiments with different treatments were performed simultaneously in two microchambers. The data are expressed as means±S.E.M. ANOVA followed by a Tukey post hoc comparison was used to determine the statistical significance.

Drugs

NMDA (used at 10 and 100 μM), l-NAME (used at 30, 100, and 1000 μM), nomifensine maleate (used at 10, 30, and 100 μM), nitroprusside sodium (used at 300 μM), diethylamine NONOate (DEA NONOate, used at 100 μM), cadmium (Cd²⁺, used at 100 μM), and tetrodotoxin (TTX, used at 1 μM) were purchased from Sigma-Aldrich. The drugs were freshly prepared on the same day of use.

Results

The role of Ca²⁺ and Na⁺ channels, and DA uptake, in the release of DA from the cochlea

Under the control circumstances, the electrical field stimulation of isolated cochleae produced the stable and reproducible release of DA (Fig. 1B); the mean release was $1.44 \pm 0.02\%$ of the total radioactive DA content during 3 min at rest (n=72). During the electrical stimulation the DA release reached $3.15 \pm 0.08\%$ of the DA content (n=72). The ratio of FRS2/FRS1 was close to the one (0.96 ± 0.1 , n=12) showing function stability. Between the two stimulations the outflow of DA, the resting release of DA was also stable in the control experiments as shown by the FRR2/FRR1 ratio (0.92 ± 0.03 , n=12). Omission of Ca²⁺ from the 24th minute until the end of the experiment reduced the evoked release by 60% (FRS2/FRS1= 0.39 ± 0.11 ; $P < 0.01$). Surprisingly, the resting release increased during the perfusion of the Ca²⁺-free solution (Fig. 1B), which might be partly attributable to a compensatory Ca²⁺ outflow from the bone. The presence of EGTA at a high concentration most likely enhanced this process. Perfusion of the non-selective voltage-sensitive Ca²⁺ channel blocker Cd²⁺ (100 μM) caused a 41% inhibition in the second electrical stimulation-evoked DA release (FRS2/FRS1= 0.57 ± 0.06 ; $P < 0.05$) (Fig. 1B–C). The voltage-sensitive Na⁺ channel blocker TTX (1 μM) decreased the evoked release by 88% (FRS2/FRS1= 0.11 ± 0.12 ; $P < 0.001$) (Fig. 1B–C). Neither Cd²⁺ nor TTX could significantly affect the basal DA outflow (Cd²⁺: FRR2/FRR1= 0.92 ± 0.06 ; TTX: FRR2/FRR1= 1.01 ± 0.04) (Fig. 1B–C).

To estimate the activity of the uptake system in removing the released DA, we studied the effect of its pharmacological inhibition. The DA uptake inhibitor nomifensine dose-dependently increased the electrical stimulation-evoked release of DA in a concentration range of 10–100 μM (10 μM : FRS2/FRS1= 1.99 ± 0.07 ; $P < 0.001$; 30 μM : FRS2/FRS1= 3.28 ± 0.42 ; $P < 0.001$; 100 μM : 4.45 ± 0.74 ; $P < 0.001$). Nomifensine did not change the basal DA release (Fig. 2A–B).

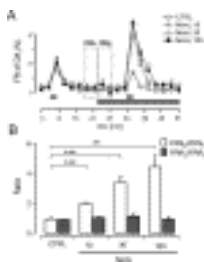


Fig. 2. Effect of uptake inhibition on the release of DA from the cochlea preparation. (A) DA release over time under control and nomifensine-treated conditions (nomifensine at 10–100 μM). Nomifensine was applied from the 21st minute as indicated by the horizontal gray line. Electrical field stimulations (FRS1 and FRS2) are indicated by horizontal black bars. (B) The summary bar chart of the effect of nomifensine given at 10–100 μM (n=9–9) on the resting and evoked DA release. Nomifensine dose-dependently increased the electric field stimulation evoked release of DA and left the

resting release unaffected. The data presented are means±S.E.M; asterisks indicate significant differences from control (n=9, *** P<0.001, ** P<0.01).

Effect of NO on the DA release: the lack of endogenous tonic nitrgic activity

To investigate the effect of NO on dopaminergic transmission, we applied the NO donor sodium nitroprusside in the perfusate. At a concentration of 300 µM, nitroprusside produced a large increase in the resting release of DA from isolated cochleae (FRR2/FRR1=1.84±0.10; P<0.001). The enhancing effect of the NO donor was restricted to the resting release; nitroprusside failed to influence the stimulation-evoked release of DA (FRS2/FRS1=1.13±0.12; P=0.51) (Fig. 3A–B). To confirm the action of NO in the cochlea, the selective NO donor DEA NONOate was applied at a 100 µM concentration (Miller and Megson, 2007). DEA NONOate also released DA from the isolated cochlea but the effect was smaller (FRR2/FRR1=1.17±0.03; P<0.01; Fig. 3A–B). Next, we studied whether the release of DA is under tonic NO modulation. The inhibition of NOS by 1-NAME (100 µM) did not influence the DA outflow (FRS2/FRS1=1.05±0.11; FRR2/FRR1=1.02±0.06), which indicated a lack of tonic NO release in this preparation (Fig. 3B).

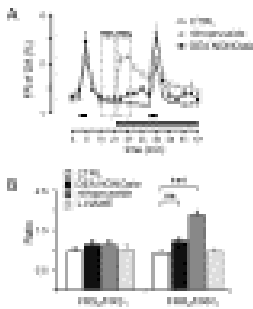


Fig. 3. Effect of exogenous NO and the lack of endogenous NO tone. (A) DA release over time under control, sodium nitroprusside (300 µM) and DEA NONOate (100 µM) conditions. Electrical field stimulations (FRS1 and FRS2) are indicated by horizontal black bars. Drugs were applied from the 21st minute as indicated by the horizontal gray line. (B) The summary bar chart shows the effect of nitroprusside sodium (300 µM), DEA NONOate (100 µM) and 1-NAME (100 µM) on the electrically evoked (FRS2/FRS1) and resting (FRR2/FRR1) outflow of DA. Sodium nitroprusside and DEA NONOate significantly elevated the resting release of DA. The data presented are means±S.E.M.; asterisks indicate significant difference from control (n=8, *** P<0.001; ** P<0.01).

NMDA-evoked NO production induces DA release

We speculated that the stimulation of NMDA receptors may induce NO production to modulate DA release from the cochlea. NMDA at 100 µM in Mg²⁺-free solution and in the presence of glycine induced a relatively small, but highly significant release of DA at rest (n=12, Fig. 4A–B). To estimate the modulatory effects on the NMDA-evoked DA

release at different times, we analyzed the resting release at the onset (0–6 min of the NMDA application, corresponding 21–27 min of perfusion; FRR2) and a later phase (9–15 min of NMDA application corresponding 30–36 min of perfusion; FRR3) of the perfusion (n=12, Fig. 4C). In the control experiments, the resting release was fairly stable as was revealed by the ratios of the FR fractions at different phases (FRR2/FRR1=0.96±0.02; FRR3/FRR1=0.92±0.03). The increase in the resting release by 100 μM NMDA was significant at both phases (FRR2/FRR1=1.12±0.04; P<0.01; FRR3/FRR1=1.11±0.04; P<0.001) corresponding to an increase of 17 and 20%, respectively. The DA mobilization by NMDA was not significantly different between FRR3 and FRR2. In addition to the direct releasing effect, NMDA also enhanced the electrically evoked DA release (FRS2/FRS1=1.36±0.09; n=10, P<0.05; Fig. 4B). In a 10 μM concentration NMDA was ineffective to modulate the resting and electrical stimulation-evoked DA release (Fig. 4A).

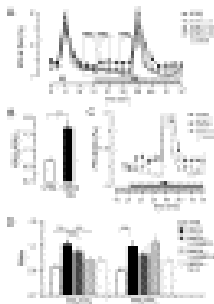


Fig. 4. NMDA receptors induce DA release in the cochlea through NO production. (A) DA release over time under control and NMDA-treated (10 and 100 μM) conditions. D-AP-5 (50 μM) was used to prove the specificity of the NMDA action. Electrical field stimulations (FRS1 and FRS2) are indicated by horizontal black bars. The control resting period (FRR1), the earlier phase of drug action (FRR2, 21–27 min of sample collection; 0–6 min of NMDA application) and the later phase (FRR3, 30–36 min, 9–15 min of NMDA application) of resting release are shown by the dashed rectangles. NMDA was perfused from the 21st minute as indicated by the horizontal gray line. (B) The summary bar chart shows the effect of NMDA (100 μM) on the electrically evoked (FRS2/FRS1) outflow of DA. NMDA significantly elevated the electrically evoked release of DA at 100 μM. Data presented are means±S.E.M.; asterisks indicate significant difference from control (n=10, ** P<0.01). (C) DA release over time under control, NMDA- (100 μM) and NMDA plus l-NAME-treated (100 μM each) conditions, focusing on the change in the resting release. NMDA was applied from the 21st minute as indicated by the horizontal dark gray bar. l-NAME was perfused from the preperfusion (light gray bar). (D) The bar chart shows the effect of l-NAME (10 and 100 μM) and D-AP-5 (50 μM) on the NMDA (100 μM) -evoked release of DA. l-NAME could significantly prevent the first phase of the release (FRR2/FRR1) induced at a 100 μM concentration by NMDA but it left the later phase intact (FRR3/FRR1). The data presented are means±S.E.M.; asterisks indicate significant difference from control (N=10, ** P<0.01; * P<0.05).

To investigate whether the NMDA-evoked DA release could be linked to NO production, the releasing effect of NMDA was tested in the presence of l-NAME. l-NAME was perfused from the preperfusion and maintained until the end of the sample collection. At a low concentration (10 μ M), l-NAME was ineffective in modulating NMDA-evoked DA release ($P=0.139$; Fig. 4D). The application of l-NAME at 100 μ M caused a significant inhibition of the NMDA-evoked release of DA during the first phase of NMDA action ($FRR2/FRR1=0.99\pm0.04$, $n=7$, $P<0.001$) but it left the later phase intact ($FRR3/FRR1=1.13\pm0.06$, $n=7$, $P=0.879$) (Fig. 4C–D). To test the specificity of an NMDA action, the selective antagonist AP-5 was used in the bath at a 50 μ M concentration from the preperfusion. AP-5 completely blocked the NMDA-evoked increase in cochlear DA release (Fig. 4D). The NMDA was unable to enhance the electrically evoked release of DA ($FRS2/FRS1$ was 1.03 ± 0.07 , which is not significantly different from the control).

Uptake is not involved in the action of NMDA

Next, we investigated the potential role of the uptake systems in the NMDA-evoked DA release. Nomifensine (10 μ M) was already present in the bath throughout the experiment and, therefore, the ratio of the evoked responses remained constant (Fig. 5A–B) in spite of its large effect on the electrical stimulation-evoked release (Fig. 2). The presence of nomifensine did not influence the DA-releasing effect of NMDA (100 μ M) indicating a lack of a transporter-mediated component in the mechanism of the action ($FRS2/FRS1=1.47\pm0.13$; $n=6$, $P=0.18$, Fig. 5A–C). The application of l-NAME (100 μ M) during the continuous perfusion of nomifensine (10 μ M) also failed to influence the resting and stimulated DA outflow, which indicated that the removal of the uptake did not interfere with the endogenous NO production ($FRS2/FRS1=1.04\pm0.11$; $FRR2/FRR1=0.97\pm0.05$, $n=5$, $P>0.01$, Fig. 5B–C).

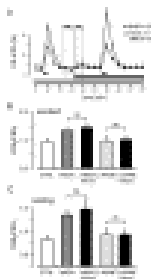


Fig. 5. Lack of effect of the uptake system to modulate the NMDA-evoked DA release. (A) DA release over time in response to the application of a 100 μ M NMDA without (solid circle) and during the application of nomifensine (10 μ M, gray triangles). NMDA was applied from the 21st minute as indicated by the horizontal dark-gray bar, and nomifensine from the preperfusion period until the end of the experiment, as shown by the light-gray bar. Electrical field stimulations (FRS1 and FRS2) are indicated by horizontal black bars. (B) The bar chart shows the effect of NMDA (100 μ M) and l-NAME (100 μ M) without and with the simultaneous application of nomifensine (10 μ M) on the electric field stimulation evoked release of DA. Nomifensine did not change

significantly the effect of NMDA or l-NAME. Data presented are means±S.E.M.; ns=not significant. (C) The bar chart shows the effect of NMDA (100 μM) and l-NAME (100 μM) without and with the simultaneous application of nomifensine (10 μM) on the resting outflow of DA. Nomifensine failed to significantly change the effect of NMDA or l-NAME. The data presented are means±S.E.M.; ns=not significant (n=5–6).

Discussion

Although we found no evidence of tonic endogenous release, NO could effectively regulate the cochlear DA levels, as shown by the releasing effect of the NO donor nitroprusside and DEA NONOate. NMDA receptor activation clearly increased the resting release of DA through NO production. It is possible that the NMDA receptors that are located outside of the cochlear dopaminergic system can contribute to the NO production and increase the resting DA release because NO is a diffusible transmitter. As NO itself did not modulate the electrically-evoked release of DA, we conclude that the NMDA-mediated increase in the evoked release was not a NO-mediated process. The DA-releasing property of NMDA suggests that a local feedback mechanism may exist within the organ of Corti: the excessive release of glutamate from the IHCs has the potential to decrease its own toxic effect by locally enhancing the release of DA, which protects the afferent nerve endings.



NO is an ideal nonsynaptic neurotransmitter because it is highly diffusible, and crosses biological membranes freely (Gally et al., 1990). Since it is released by NMDA receptor activation and inhibits the function of monoamine transporters, it can serve as a link between glutamatergic synaptic and monoaminergic nonsynaptic transmission ([Vizi et al 2004] and [Kiss and Vizi 2001]). In the inner ear, nNOS has been demonstrated in spiral ganglion cells, fiber endings below the inner and outer hair cells, IHCs and OHCs, and Deiters' cells (Takumida and Anniko, 2002). In our preparation, the electrical field stimulation did not induce NO production, or the released NO did not influence the release of DA from the LOC terminals, since l-NAME alone failed to change the FRS2/FRS1 ratio. DA release can be produced by NO donors, but this finding does not necessarily refer to the characteristics of endogenous NO action. To uncover the role of endogenous NO, we tested NMDA, which was able to induce an NO-mediated increase in the resting DA release from the cochlea. In our experiments the source of NO, which contributes to DA release following NMDA activation, is unclear. As a gaseous messenger molecule it was able to diffuse from any nearby cellular element equipped with NMDA receptors. It is known that NMDA receptors are present in cochlea, especially on the inner and outer hair cell (Knipper et al., 1997). NMDA receptor activation at these sites is likely to induce NO production; the released NO could diffuse to LOC terminals to increase the resting DA release. As DA is known to inhibit the firing rate of the primary afferent neurons, DA may play a role in the negative feedback. Our finding that NMDA application increased the resting DA release in the cochlea supports the idea that the glutamatergic transmission has a built-in protection system that prevents the connected elements from overactivation. We find that NO is produced in the early phase of NMDA receptor activation; the later phase of the NMDA action must be mediated by an unknown mechanism independent of DA uptake and NO.

In the brain, NO inhibits the function of monoamine uptake ([Lonart and Johnson 1994] and [Kiss and Vizi 2001]). Therefore, the lack of an effect of nomifensine on NMDA-evoked responses needs more explanation. The reverse operation of uptake mechanisms is known to occur during ischemic conditions in the CNS (Lonart and Zigmond, 1991) and also in the cochlea (Halmos et al., 2005). Previous experiments by Ruel et al. (2006) proved the presence and function of DA-uptake at the LOC efferent axon terminals. The lack of the effect of nomifensine on the NMDA-evoked DA response, as found in our experiments, indicates that the NO-mediated component of NMDA action on DA release was most likely a result of NO diffusion and not a transporter-mediated action. In conclusion, we provided direct evidence for the first time that the NMDA-evoked DA release is partly mediated by a NO-dependent mechanism in the cochlea. This may serve as a fast-acting feedback mechanism by which NO can protect cochlear cells against its harmful effects during various physiological and pathophysiological processes, such as NMDA receptor activation during excessive glutamate release and excitotoxicity. More information regarding these protective mechanisms may provide potential sites of action that are relevant for the development of new therapeutic drugs to protect hearing.

Acknowledgments

The authors thank Erika Tischler and Judit Őszi for their technical contributions. This study was supported in part by the Hungarian Research Fund (OTKA Ts 049868), Hungarian Medical Research Foundation (576/2006) and the Sixth Research Framework Programme of the European Union, EuroHear (contract LSHG-CT-2004-512063).

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