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165x319mm (300 x 300 DPI)
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Research Article

Betahistine metabolites Aminoethylpyridine and Hydroxyethylpyridine increase Cochlear Blood Flow in guinea pigs in vivo

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Short title: Betahistine metabolites increase cochlear perfusion

Keywords: betahistine, histamine, aminoethylpyridine, hydroxyethylpyridine, pyridylacetic acid, cochlear blood flow, Meniere’s disease

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ABSTRACT

Objective: Betahistine is a histamine-like drug that is used in the treatment of Menière’s disease. It is commonly believed that betahistine increases cochlear blood flow and thus decreases the endolymphatic hydrops that is the cause of Menière’s. Despite common clinical use, there is little understanding of the kinetics or effects of its metabolites. This study investigated the effect of the betahistine metabolites aminoethylpyridine, hydroxyethylpyridine and pyridylacetic acid on cochlear microcirculation.

Design: Guinea pigs were randomly assigned to one of the groups placebo, betahistine, or equimolar amounts of aminoethylpyridine, hydroxyethylpyridine or pyridylacetic acid. Cochlear blood flow and mean arterial pressure were recorded for 3 minutes before and 15 minutes after treatment.

Study Sample: Thirty Dunkin-Hartley guinea pigs assigned to one of five groups with six guinea pigs per group.

Results: Betahistine, aminoethylpyridine and hydroxyethylpyridine caused a significant increase in cochlear blood flow in comparison to placebo. The effect seen under aminoethylpyridin was greatest. The group treated with pyridylacetic acid showed no significant effect on cochlear blood flow.

Conclusion: Aminoethylpyridine and hydroxyethylpyridine are, like betahistine, able to increase cochlear blood flow significantly. The effect of aminoethylpyridine was greatest. Pyridylacetic acid had no effect on cochlear microcirculation.


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Introduction

When in 1861 Prospere Menière described a condition that involved repeated attacks of one-sided hearing loss, tinnitus and vertigo, he was the first to ascribe the aforementioned symptoms not to the central nervous system, but to the semicircular canals (Meniere, 1861). Despite the significant amount of time that has passed since the first description of Menière’s disease and notwithstanding the considerable prevalence in the population of up to 0.51% (Neuhauser, 2007), there is still extensive debate about its etiology, pathophysiology and treatment.

Nowadays, a wide range of treatments is administered, such as surgical approaches including surgery of the endolymphatic sac (Pullens et al., 2010), local application of gentamicin (Pullens & van Benthem, 2011) and more pharmacological therapeutic options like diuretics (Thirlwall & Kundu, 2006) or dietary restrictions (Boles et al., 1975).

The common approach in Europe is the oral administration of betahistine dihydrochloride, a structural derivative of histamine that shows inverse agonism on histamine H3-receptors and slight agonistic effects on histamine H1-receptors (Gbahou et al., 2010). Several studies suggest that betahistine might have a positive effect on the course of the disease (James & Burton, 2001; Strupp et al., 2011). Moreover, several clinical trials have found dose-dependent effects of betahistine on the frequency of attacks (Lezius et al., 2011). A sigmoid dose-response curve could also be reproduced in an animal model (Ihler et al., 2012). However, a solid and well-conducted, double-blind placebo-controlled prospective clinical study is still lacking.

So far it has been generally accepted that betahistine increases cochlear blood flow (Meyer et al., 1994; Laurikainen et al., 1998; Dziadziola et al., 1999; Lamm & Arnold, 2000). However, little is known about the pharmacokinetics of betahistine and its metabolites: A monoamino oxidase mediated strong first-pass effect has been suggested at the hepatic level. (Konzett et al., 1971; Sternson et al., 1974) It has been shown that the end product of betahistine metabolism is pyridylacetic acid, an inactive compound that can be found in both urine and plasma after oral betahistine ingestion(Chen et al., 2003; Val et al., 2010). Hypothesized degradation paths give rise to the metabolites aminoethylpyridine and hydroxyethylpyridine (Bowman et al., 1972; Sternson, Tobia et al., 1974; Chen, Zhong et al., 2003; Val, Chen et al., 2010). These metabolites have been shown to possess an affinity to histamine-receptors on their own (Fossati et al., 2001). However, the effects of these metabolites on cochlear blood flow have not been investigated so far. Moreover,
even though identified as the end product of betahistine metabolism, neither has pyridylacetic acid been investigated for its potency to alter cochlear blood flow.

Therefore, the aim of the present study was to determine if either aminoethylpyridine, hydroxyethylpyridine or the final metabolite of betahistine, pyridylacetic acid, may increase cochlear blood flow and to investigate their potency of action in comparison to betahistine.
Materials and methods

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Thirty Dunkin-Hartley Guinea Pigs purchased from Charles River Wiga Laboratories (Sulzfeld, Germany) and weighing 200-500g were included in this study. Anesthesia was induced by inhalation of 1 l O2/min, 0.5 l N2H/min and 2.5 Vol% Isoflurane in a custom-made chamber. Thereafter, it was continued by an initial intraperitoneal injection of ketamine (50 mg/kg bw) and xylazine (5 mg/kg bw) and injections of half the aforementioned dosages every 30 minutes.

Surgical preparation

Surgical preparation and intravital microscopy were performed as described previously (Canis et al., 2010). In brief, a fiberoptic pressure transducer was placed in the right femoral artery for continuous blood pressure monitoring. A catheter was placed in the right jugular vein for intravenous application of fluids, plasma markers and the agents that were to be tested. Following these initial preparations, the right auditory bulla was carefully opened and a rectangular window was incised in the second cochlear turn.

Measuring of Cochlear Blood Flow

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different vessels every minute. A mean value was calculated afterwards.

In order to calculate cochlear blood flow, the formula established by Baker & Weyland in 1974 $q = (v/1.6) \times (d/2)^2 \times \pi$ was used (Baker & Wayland, 1974). In order to correct for varying vessel diameter as well as individual differences between the examined animals, the results are presented in arbitrary units, thus reflecting the relative change of blood flow.

**Measurement of Mean Arterial Pressure**

Mean arterial pressure was measured using a Samba Fiber-Optic Pressure Measurement System by Samba Sensors AB (VästraFrölunda, Sweden) (Woldbaek et al., 2003). The tip of a fiber-optic catheter was inserted into the right femoral artery. During the experiment, the results were recorded with a Samba 201 Control Unit in millimeters of mercury (mmHg). Recording took place with a frequency of 40 Hz. The proprietary Samba 200 control software was used for later off-line analysis. To correct for differences between individual animals, changes in blood pressure are reported as arbitrary units.

**Treatment protocol**

30 animals were randomly assigned to one of five groups (betahistine, aminoethylpyridine, hydroxyethylpyridine, pyridylacetic acid or placebo) and underwent identical microsurgery as described above. After an initial picture was obtained, baseline measurements were recorded for three minutes. Subsequently, betahistine, aminoethylpyridine, hydroxyethylpyridine, pyridylacetic acid or placebo were administered over 2 minutes. From the beginning of the infusion, cochlear blood flow and arterial pressure were continuously monitored for a further 15 minutes.

**Calculation of corresponding dosages for metabolites**

A concentration of 0.1 mg betahistine per kg body weight was used since it had been calculated to be equivalent to 48-160mg of orally applied betahistine, (Ihler, Bertlich et al., 2012) which is a dosage that is commonly applied in the clinic. Moreover, it has been shown that this dose is capable of significantly increasing cochlear blood flow without causing any adverse effects (Meyer, Schmidt et al., 1994; Ihler, Bertlich et al., 2012). Aminoethylpyridine and hydroxyethylpyridine were both applied in concentrations of 0.06 milligrams per kilogram body weight, whilst pyridylacetic acid was administered in a dose of 0.08 milligrams per kilogram body weight, representing equimolar amounts between the agents and betahistine.
Statistical analysis

Statistical analysis was carried out using SigmaPlot for Windows 12.0. The statistical test applied was Two Way Repeated Measures Analysis of Variance (ANOVA) in order to compare corresponding points in time between placebo and treatment (betahistine, aminoethylpyridine and hydroxyethylpyridine respectively) groups.

In order to correct for multiple testing for multiple groups and time-points, a Bonferroni t-test was performed. A p-value of $\alpha < 0.05$ was considered to be statistically significant.
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**Results**

*The effect of betahistine and its metabolites on cochlear microcirculation*

Three of the four groups showed significantly increased levels of cochlear microperfusion in comparison to the placebo group. The group receiving betahistine showed an increase in cochlear perfusion to a peak value of $1.437 \pm 0.120$ arb. units. Significant differences from placebo values were assessed from minutes 7 to 13 and 15 to 18 ($p < 0.05$; Two Way RM ANOVA/Bonferroni t-test). Cochlear perfusion in the betahistine group remained at a constant level after approximately 10 minutes. The average for minutes 10-18 was 1.227 arb. units with a standard deviation of 0.034 arb. units. In comparison to this, the average for the same period in the placebo group was 1.012 arb. units with a standard deviation of 0.051 arb. units. (Fig 1A)

The administration of aminoethylpyridine also led to a significant increase in cochlear perfusion in comparison to the placebo group ($p < 0.05$; Two Way RM ANOVA/Bonferroni t-test). At minute 11 a peak of $1.533 \pm 0.263$ arb. units (range 2.015-1.306 arb. units) could be observed, which exceeded the values achieved in the betahistine group. After an initial steep increase, cochlear blood flow remained constant at a level of approximately 1.3 arb. units for the rest of the observation period. Overall, cochlear blood flow in the treatment group receiving aminoethylpyridine was significantly different from the values of the placebo group from minute 6 to minute 18. (Fig 2A)

The group that received hydroxyethylpyridine showed significantly increased levels of cochlear perfusion in comparison to placebo ($p < 0.05$; Two Way RM ANOVA/Bonferroni t-test). After a moderate increase in cochlear perfusion at the beginning of metabolite infusion, a peak value of $1.268 \pm 0.213$ arb. units was measured at minute 7. This peak level was below the maximum value in the betahistine group. The levels of cochlear perfusion remained steady at an average of $1.181 \pm 0.159$ arb. units from minute 7 to minute 18. Overall, cochlear blood flow was significantly elevated in comparison to placebo from minute 7 to minute 11. (Fig 3A)

The group that had been treated with pyridylacetic acid showed no significant changes in comparison to placebo. (Fig 4A)
The effect of betahistine and its metabolites on mean arterial pressure

Both betahistine and hydroxyethylpyridine groups showed statistically significant differences in mean arterial pressure compared to placebo.

The treatment group receiving betahistine displayed an initial slight drop in mean arterial pressure with the lowest value at the beginning of the infusion (mean value 0.936 arb. units with a standard deviation of 0.044 arb. units and ranging from 0.868-0.993 arb. units). After this initial and short drop, the systemic blood pressure increased to levels significantly different from placebo at minutes 7 and 8 (p < 0.001, Two Way Repeated Measures ANOVA/Bonferroni t-test), reaching a peak at minute 8 with 1.250 ±0.99 arb. units and from thereon kept constantly decreasing. (Fig 1B)

No statistical differences from placebo in terms of systemic blood pressure were noted in the treatment group with administration of aminoethylpyridine. (Fig 2B)

The group receiving hydroxyethylpyridine showed a constant increase in mean arterial pressure upon initial infusion, reaching a peak significantly different from placebo at minute 6 with 1.183 ± 0.124 arb. units (p < 0.001, Two Way Repeated Measures ANOVA/Bonferroni t-test). From then on, average values for systemic blood pressure gradually declined, eventually reaching the levels of the placebo group from minute 9. (Fig 3B).

In terms of mean arterial pressure, the group that had received pyridylacetic acid had shown no significant changes in comparison to placebo. (Fig 4B)
Discussion

The major finding of this study on the effects of three metabolites of betahistine was that two of the three compounds cause a significant increase in cochlear blood flow. Aminoethylpyridine even exerted a major effect compared to betahistine. Hydroxyethylpyridine had an additional impact on systemic blood pressure in a dimension comparable to betahistine. Pyridylacetic acid, on the other hand, was unable to alter either systemic blood pressure or cochlear blood flow.

Betahistine acts as an agonist of histaminergic H1-receptors and as an inverse agonist of H3-receptors (Gbahou, Davenas et al., 2010). It has been established in vitro that aminoethylpyridine has similar properties on the H3-receptor, whilst being a much weaker agonist of the H1-receptor (Fossati, Barone et al., 2001). Hydroxyethylpyridine is an even weaker agonist of the H1-receptor and an inverse agonist of the H3-receptor (Fossati, Barone et al., 2001). The receptor binding profile for pyridylacetic acid has not yet been investigated, however it has been repeatedly described as being practically non-existent (Botta et al., 2000; Fossati, Barone et al., 2001; Chen, Zhong et al., 2003). As histamine is an omnipresent substance in the body, it is not surprising that it has considerable effects on the cardiovascular system. Histamine H1-receptors have been reported to cause negative inotropic effects on the heart as well vasoconstriction in greater and vasodilation in smaller vessels and a general drop in blood pressure (Sakai, 1980). In this respect, H3 receptors are similar: upon activation they cause a drop in noradrenaline levels and a general decrease in blood pressure. (Malinowska et al., 1998) This is commonly viewed as the most likely mode of action of betahistine in the inner ear. Histamine receptors, including that of the H3-subtype are present in various tissues of the inner ear (Dagli et al., 2008). It seems probable that these H3-receptors modulate local noradrenaline release for the arterioles of the stria vascularis, thus altering cochlear blood flow. (Laurikainen, Miller et al., 1998) Fittingly, it has been described that α-methylhistamine, an H3-agonist with an effect directly opposite to that of betahistine at the H3-receptor, is capable of inducing a vasoconstriction in resistance vessels of prepared rat bowel. (Sun et al., 2011)

There has not been much research on betahistine metabolites so far. However, it has been shown that aminoethylpyridine is able to decrease blood pressure in mongrel dogs (Konzett, Bost et al., 1971) during a 2-minute infusion. This finding does not contradict the presented data: even though not statistically different from placebo, the guinea pigs receiving aminoethylpyridine infusions showed a drop in mean arterial pressure below basal values (Fig 2B, minutes 4 and 5).
However, apart from the initial drop in mean arterial pressure during aminoethylpyridine infusion, we could not find any data on either of the betahistine metabolites and their effect on mean arterial blood pressure.

The fact that hydroxyethylpyridine – unlike aminoethylpyridine – is able to generate a significant increase in mean arterial pressure, despite its aforementioned somewhat weaker potency at histamine H1- and H3-receptors (Fossati, Barone et al., 2001) implies that other receptors might be involved in the effects of betahistine and its metabolites on mean arterial pressure. Fittingly, it has been described that pretreatment of animals with idazoxane, an adrenergic α2- and imidazole I2-antagonist, is able to decrease the betahistine-typical changes in both mean arterial pressure and cochlear blood flow (Laurikainen, Miller et al., 1998). Hence adrenergic receptors might play a role in the mediation of the effect of betahistine and its metabolites.

Whilst we recorded mean arterial pressure in order to have a measure for the systemic effects of betahistine, it seems probable that the main effect of betahistine that is considered as beneficial takes place in the cochlear vascular network (Laurikainen, Miller et al., 1998; Laurikainen et al., 2000). It has been shown that cochlear function and cochlear microcirculation are closely related (Ihler et al., 2012; Arpornchayanon et al., 2013). Moreover, the fact that the cochlea is a circulatorily privileged organ with a strong autoregulation of its blood flow (Kawakami et al., 1991; Brown & Nuttall, 1994) suggests that the effects observed are specific to the cochlea, thus rendering this the most likely mode of action of betahistine in Menière’s disease.

To this day, there are no studies that have investigated the effect of any of the metabolites on cochlear blood flow. Yet, there have been in vitro studies that have examined the ability of all three metabolites in comparison to betahistine to decrease the resting discharge rate of prepared frog’s ampullar receptors (Botta, Mira et al., 2000; Botta et al., 2001). One major finding was in line with the results presented here: In both the aforementioned as well as in our experiment, aminoethylpyridine exerted effects that were very similar to that of betahistine on the dependent variable, whilst the effect of hydroxyethylpyridine and pyridylacetic acid was much smaller.

This study differs from earlier investigations into cochlear microcirculation and betahistine (Meyer, Schmidt et al., 1994; Laurikainen, Miller et al., 1998; Dziadziola, Laurikainen et al., 1999; Laurikainen, Miller et al., 2000) in the application of intravital microscopy for cochlear blood flow measurement. From the 1980s onwards, laser Doppler flowmetry has been the main method of measuring cochlear blood flow (Miller et al., 1983; Goodwin et al., 1984; Miller et al., 1984).
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An important limitation of laser Doppler flowmetry is that it is not selective for cochlear microcirculation because it does not exclusively assess stria vascularis vessels (LaRouere et al., 1989; Nakashima et al., 2001). However, with the assessment of cochlear perfusion, these are the relevant vessels responsible for cochlear metabolism. Instead, laser Doppler flowmetry will measure any vessel upon which it is placed and the blood flow in the vessel. Therefore, in our case, values generated with this method would include averages of the stria vascularis as well the spiral modiolar artery and vessels of the bony capsule of the cochlea (Nuttall, 1987; Canis, Arpornchayanon et al., 2010). Although superior in specificity, one considerable limitation of this method is the time for which cochlear blood flow can be measured, since after approximately 20 minutes, the vessels observed show an increasing tendency to clot and thus no valid data can be measured anymore.

One has to acknowledge the effects exerted by aminoethylpyridine, which are at least similar to betahistine, (Botta, Mira et al., 2000; Botta, Mira et al., 2001) or in the present study even greater than that observed under betahistine treatment. Hence, it is tempting to speculate whether the main therapeutic effect of medication with betahistine could be exerted by its metabolites. Could betahistine even act as a prodrug, and aminoethylpyridine or another metabolite as the main therapeutic agent? A prodrug is a partially or completely inactive precursor that is only fully converted into its active form at or near the site of action (Wu & Farrelly, 2007). In that respect, one should also acknowledge the fact that if one considers aminoethylpyridine as the major therapeutic agent, the greater the distance of the examined substance in the presumed metabolic pathway of betahistine, the smaller its effect. Moreover, the strong hepatic first-pass effect (Sternson, Tobia et al., 1974) would further support this theory. However, the exact kinetics of betahistine and its metabolites in the plasma upon ingestion are unknown to this date.

Therefore, in further investigations the temporal kinetics of betahistine and its metabolites by route of delivery as well as their effect on the actual endolymphatic hydrops in an animal model should be examined (Kimura, 1967; Kimura, 1982).

Conclusion

This study showed that the betahistine metabolites aminoethylpyridine and hydroxyethylpyridine both exert an effect on systemic blood pressure and cochlear blood flow, whilst pyridylacetic acid had no effect whatsoever. It should encourage
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further research in this particular field like chronic hydrops models and receptor studies to facilitate the investigation of betahistine metabolites and their role in the treatment of Menière’s disease.
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Figure Legends

Figure 1: Effects over time before and after infusion of betahistine. A: cochlear blood flow; B: mean arterial pressure in arbitrary units; mean ± SE. *: p < 0.05 (Two Way RM ANOVA/Bonferroni t-test)

Figure 2: Effects over time before and after infusion of aminoethylpyridine. A: cochlear blood flow; B: mean arterial pressure in arbitrary units; mean ± SE. *: p < 0.05 (Two Way RM ANOVA/Bonferroni t-test)

Figure 3: Effects over time before and after infusion of hydroxyethylpyridine. A: cochlear blood flow; B: mean arterial pressure in arbitrary units; mean ± SE. *: p < 0.05 (Two Way RM ANOVA/Bonferroni t-test)

Figure 4: Effects over time before and after infusion of pyridylacetic acid. A: cochlear blood flow; B: mean arterial pressure in arbitrary units; mean ± SE. *: p < 0.05 (Two Way RM ANOVA/Bonferroni t-test)

Acknowledgements

This work is part of the doctoral thesis of Mattis Bertlich.

Conflicts of Interest

This project was supported by funds from the Deutsche Forschungsgemeinschaft to Prof. Canis under the grant code CA 629/2-1. The authors Bertlich, Ihler, Weiss, Sharaf and Canis declare that they have no conflicts of interest. Prof. Strupp declares to have received funds in return for consulting services to Abott, Pierre-Fabre and Biogen Idec as well as having received funds for the preparation of scientific training for Abbott, Biogen Idec, CSC, Henning Pharma and GSK.
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Research Article

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different vessels every minute. A mean value was calculated afterwards.

In order to calculate cochlear blood flow, the formula established by Baker & Weyland in 1974 $q = (v/1.6) * (d/2)^2 * \pi$ was used (Baker & Weyland, 1974). In order to correct for varying vessel diameter as well as individual differences between the examined animals, the results are presented in arbitrary units, thus reflecting the relative change of blood flow.

*Measurement of Mean Arterial Pressure*

Mean arterial pressure was measured using a Samba Fiber-Optic Pressure Measurement System by Samba Sensors AB (Västra Frölunda, Sweden) (Woldbaek et al., 2003). The tip of a fiber-optic catheter was inserted into the right femoral artery. During the experiment, the results were recorded with a Samba 201 Control Unit in millimeters of mercury (mmHg). Recording took place with a frequency of 40 Hz. The proprietary Samba 200 control software was used for later off-line analysis. To correct for differences between individual animals, changes in blood pressure are reported as arbitrary units.

*Treatment protocol*

30 animals were randomly assigned to one of five groups (betahistine, aminoethylpyridine, hydroxyethylpyridine, pyridylacetic acid or placebo) and underwent identical microsurgery as described above. After an initial picture was obtained, baseline measurements were recorded for three minutes. Subsequently, betahistine, aminoethylpyridine, hydroxyethylpyridine, pyridylacetic acid or placebo were administered over 2 minutes. From the beginning of the infusion, cochlear blood flow and arterial pressure were continuously monitored for a further 15 minutes.

*Calculation of corresponding dosages for metabolites*

A concentration of 0.1 mg betahistine per kg body weight was used since it had been calculated to be equivalent to 48-160 mg of orally applied betahistine (Ihler, Bertlich et al., 2012) which is a dosage that is commonly applied in the clinic. Moreover, it has been shown that this dose is capable of significantly increasing cochlear blood flow without causing any adverseeffects (Meyer, Schmidt et al., 1994; Ihler, Bertlich et al., 2012). Aminoethylpyridine and hydroxyethylpyridine were both applied in concentrations of 0.06 milligrams per kilogram body weight, whilst pyridylacetic acid was administered in a dose of 0.08 milligrams per kilogram body weight, representing equimolar amounts between the agents and betahistine.
Statistical analysis

Statistical analysis was carried out using SigmaPlot for Windows 12.0. The statistical test applied was Two Way Repeated Measures Analysis of Variance (ANOVA) in order to compare corresponding points in time between placebo and treatment (betahistine, aminoethylpyridine and hydroxyethylpyridine respectively) groups.

In order to correct for multiple testing for multiple groups and time-points, a Bonferroni t-test was performed. A p-value of $\alpha < 0.05$ was considered to be statistically significant.
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Results

The effect of betahistine and its metabolites on cochlear microcirculation

Three of the four groups showed significantly increased levels of cochlear microperfusion in comparison to the placebo group. The group receiving betahistine showed an increase in cochlear perfusion to a peak value of 1.437 ± 0.120 arb. units. Significant differences from placebo values were assessed from minutes 7 to 13 and 15 to 18 (p < 0.05; Two Way RM ANOVA/Bonferroni t-test). Cochlear perfusion in the betahistine group remained at a constant level after approximately 10 minutes. The average for minutes 10-18 was 1.227 arb. units with a standard deviation of 0.034 arb. units. In comparison to this, the average for the same period in the placebo group was 1.012 arb. units with a standard deviation of 0.051 arb. units. (Fig 1A)

The administration of aminoethylpyridine also led to a significant increase in cochlear perfusion in comparison to the placebo group (p < 0.05; Two Way RM ANOVA/Bonferroni t-test). At minute 11 a peak of 1.533 ± 0.263 arb. units (range 2.015-1.306 arb. units) could be observed, which exceeded the values achieved in the betahistine group. After an initial steep increase, cochlear blood flow remained constant at a level of approximately 1.3 arb. units for the rest of the observation period. Overall, cochlear blood flow in the treatment group receiving aminoethylpyridine was significantly different from the values of the placebo group from minute 6 to minute 18. (Fig 2A)

The group that received hydroxyethylpyridine showed significantly increased levels of cochlear perfusion in comparison to placebo (p < 0.05; Two Way RM ANOVA/Bonferroni t-test). After a moderate increase in cochlear perfusion at the beginning of metabolite infusion, a peak value of 1.268 ± 0.213 arb. units was measured at minute 7. This peak level was below the maximum value in the betahistine group. The levels of cochlear perfusion remained steady at an average of 1.181 ± 0.159 arb. units from minute 7 to minute 18. Overall, cochlear blood flow was significantly elevated in comparison to placebo from minute 7 to minute 11. (Fig 3A)

The group that had been treated with pyridylacetic acid showed no significant changes in comparison to placebo. (Fig 4A)
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The effect of betahistine and its metabolites on mean arterial pressure

Both betahistine and hydroxyethylpyridine groups showed statistically significant differences in mean arterial pressure compared to placebo.

The treatment group receiving betahistine displayed an initial slight drop in mean arterial pressure with the lowest value at the beginning of the infusion (mean value 0.936 arb. units with a standard deviation of 0.044 arb. units and ranging from 0.868-0.993 arb. units). After this initial and short drop, the systemic blood pressure increased to levels significantly different from placebo at minutes 7 and 8 (p < 0.001, Two Way Repeated Measures ANOVA/Bonferroni t-test), reaching a peak at minute 8 with 1.250 ±0.99 arb. units and from thereon kept constantly decreasing. (Fig 1B)

No statistical differences from placebo in terms of systemic blood pressure were noted in the treatment group with administration of aminoethylpyridine. (Fig 2B)

The group receiving hydroxyethylpyridine showed a constant increase in mean arterial pressure upon initial infusion, reaching a peak significantly different from placebo at minute 6 with 1.183 ±0.124 arb. units (p < 0.001, Two Way Repeated Measures ANOVA/Bonferroni t-test). From then on, average values for systemic blood pressure gradually declined, eventually reaching the levels of the placebo group from minute 9. (Fig 3B).

In terms of mean arterial pressure, the group that had received pyridylacetic acid had shown no significant changes in comparison to placebo. (Fig 4B)
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Discussion

The major finding of this study on the effects of three metabolites of betahistine was that two of the three compounds cause a significant increase in cochlear blood flow. Aminoethylpyridine even exerted a major effect compared to betahistine. Hydroxyethylpyridine had an additional impact on systemic blood pressure in a dimension comparable to betahistine. Pyridylacetic acid, on the other hand, was unable to alter either systemic blood pressure or cochlear blood flow.

Betahistine acts as an agonist of histaminergic H1-receptors and as an inverse agonist of H3-receptors (Gbahou, Davenas et al., 2010). It has been established in vitro that aminoethylpyridine has similar properties on the H3-receptor, whilst being a much weaker agonist of the H1-receptor (Fossati, Barone et al., 2001). Hydroxyethylpyridine is an even weaker agonist of the H1-receptor and an inverse agonist of the H3-receptor (Fossati, Barone et al., 2001). The receptor binding profile for pyridylacetic acid has not yet been investigated, however it has been repeatedly described as being practically non-existent (Botta et al., 2000; Fossati, Barone et al., 2001; Chen, Zhong et al., 2003). As histamine is an omnipresent substance in the body, it is not surprising that it has considerable effects on the cardiovascular system. Histamine H1-receptors have been reported to cause negative inotropic effects on the heart as well vasoconstriction in greater and vasodilation in smaller vessels and a general drop in blood pressure (Sakai, 1980). In this respect, H3 receptors are similar: upon activation they cause a drop in noradrenaline levels and a general decrease in blood pressure. (Malinowska et al., 1998) This is commonly viewed as the most likely mode of action of betahistine in the inner ear. Histamine receptors, including that of the H3-subtype are present in various tissues of the inner ear (Daghli et al., 2008). It seems probable that these H3-receptors modulate local noradrenaline release for the arterioles of the stria vascularis, thus altering cochlear blood flow. (Laurikainen, Miller et al., 1998) Fittingly, it has been described that α-methylhistamine, an H3-agonist with an effect directly opposite to that of betahistine at the H3-receptor, is capable of inducing a vasoconstriction in resistance vessels of prepared rat bowel. (Sun et al., 2011)

There has not been much research on betahistine metabolites so far. However, it has been shown that aminoethylpyridine is able to decrease blood pressure in mongrel dogs (Konzett, Bost et al., 1971) during a 2-minute infusion. This finding does not contradict the presented data: even though not statistically different from placebo, the guinea pigs receiving aminoethylpyridine infusions showed a drop in mean arterial pressure below basal values (Fig 2B, minutes 4 and 5).
However, apart from the initial drop in mean arterial pressure during aminoethylpyridine infusion, we could not find any data on either of the betahistine metabolites and their effect on mean arterial blood pressure.

The fact that hydroxyethylpyridine – unlike aminoethylpyridine – is able to generate a significant increase in mean arterial pressure, despite its aforementioned somewhat weaker potency at histamine H1- and H3-receptors (Fossati, Barone et al., 2001) implies that other receptors might be involved in the effects of betahistine and its metabolites on mean arterial pressure. Fittingly, it has been described that pretreatment of animals with idazoxane, an adrenergic α2- and imidazole I2-antagonist, is able to decrease the betahistine-typical changes in both mean arterial pressure and cochlear blood flow (Laurikainen, Miller et al., 1998). Hence adrenergic receptors might play a role in the mediation of the effect of betahistine and its metabolites.

Whilst we recorded mean arterial pressure in order to have a measure for the systemic effects of betahistine, it seems probable that the main effect of betahistine that is considered as beneficial takes place in the cochlear vascular network (Laurikainen, Miller et al., 1998; Laurikainen et al., 2000). It has been shown that cochlear function and cochlear microcirculation are closely related (Ihler et al., 2012; Arpornchayanon et al., 2013). Moreover, the fact that the cochlea is a circulatorily privileged organ with a strong autoregulation of its blood flow (Kawakami et al., 1991; Brown & Nuttall, 1994) suggests that the effects observed are specific to the cochlea, thus rendering this the most likely mode of action of betahistine in Ménière’s disease.

To this day, there are no studies that have investigated the effect of any of the metabolites on cochlear blood flow. Yet, there have been in vitro studies that have examined the ability of all three metabolites in comparison to betahistine to decrease the resting discharge rate of prepared frog’s ampullar receptors (Botta, Mira et al., 2000; Botta et al., 2001). One major finding was in line with the results presented here: In both the aforementioned as well as in our experiment, aminoethylpyridine exerted effects that were very similar to that of betahistine on the dependent variable, whilst the effect of hydroxyethylpyridine and pyridylacetic acid was much smaller.

This study differs from earlier investigations into cochlear microcirculation and betahistine (Meyer, Schmidt et al., 1994; Laurikainen, Miller et al., 1998; Dziadziola, Laurikainen et al., 1999; Laurikainen, Miller et al., 2000) in the application of intravital microscopy for cochlear blood flow measurement. From the 1980s onwards, laser Doppler flowmetry has been the main method of measuring cochlear blood flow (Miller et al., 1983; Goodwin et al., 1984; Miller et al., 1984).
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An important limitation of laser Doppler flowmetry is that it is not selective for cochlear microcirculation because it does not exclusively assess stria vascularis vessels (LaRouere et al., 1989; Nakashima et al., 2001). However, with the assessment of cochlear perfusion, these are the relevant vessels responsible for cochlear metabolism. Instead, laser Doppler flowmetry will measure any vessel upon which it is placed and the blood flow in the vessel. Therefore, in our case, values generated with this method would include averages of the stria vascularis as well the spiral modiolar artery and vessels of the bony capsule of the cochlea (Nuttall, 1987; Canis, Arpornchayanon et al., 2010). Although superior in specificity, one considerable limitation of this method is the time for which cochlear blood flow can be measured, since after approximately 20 minutes, the vessels observed show an increasing tendency to clot and thus no valid data can be measured anymore.

One has to acknowledge the effects exerted by aminoethylpyridine, which are at least similar to betahistine, (Botta, Mira et al., 2000; Botta, Mira et al., 2001) or in the present study even greater than that observed under betahistine treatment. Hence, it is tempting to speculate whether the main therapeutic effect of medication with betahistine could be exerted by its metabolites. Could betahistine even act as a prodrug, and aminoethylpyridine or another metabolite as the main therapeutic agent? A prodrug is a partially or completely inactive precursor that is only fully converted into its active form at or near the site of action (Wu & Farrelly, 2007). In that respect, one should also acknowledge the fact that if one considers aminoethylpyridine as the major therapeutic agent, the greater the distance of the examined substance in the presumed metabolic pathway of betahistine, the smaller its effect. Moreover, the strong hepatic first-pass effect (Sternson, Tobia et al., 1974) would further support this theory. However, the exact kinetics of betahistine and its metabolites in the plasma upon ingestion are unknown to this date.

Therefore, in further investigations the temporal kinetics of betahistine and its metabolites by route of delivery as well as their effect on the actual endolymphatic hydrops in an animal model should be examined (Kimura, 1967; Kimura, 1982).

Conclusion

This study showed that the betahistine metabolites aminoethylpyridine and hydroxyethylpyridine both exert an effect on systemic blood pressure and cochlear blood flow, whilst pyridylacetic acid had no effect whatsoever. It should encourage
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further research in this particular field like chronic hydrops models and receptor studies to facilitate the investigation of betahistine metabolites and their role in the treatment of Menière’s disease.
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**Figure Legends**

Figure 1: Effects over time before and after infusion of betahistine. A: cochlear blood flow; B: mean arterial pressure in arbitrary units; mean ± SE. *: p < 0.05 (Two Way RM ANOVA/Bonferroni t-test)

Figure 2: Effects over time before and after infusion of aminoethylpyridine. A: cochlear blood flow; B: mean arterial pressure in arbitrary units; mean ± SE. *: p < 0.05 (Two Way RM ANOVA/Bonferroni t-test)

Figure 3: Effects over time before and after infusion of hydroxyethylpyridine. A: cochlear blood flow; B: mean arterial pressure in arbitrary units; mean ± SE. *: p < 0.05 (Two Way RM ANOVA/Bonferroni t-test)

Figure 4: Effects over time before and after infusion of pyridylacetic acid. A: cochlear blood flow; B: mean arterial pressure in arbitrary units; mean ± SE. *: p < 0.05 (Two Way RM ANOVA/Bonferroni t-test)

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**Conflicts of Interest**

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