Effects of betahistine on the vestibular receptors: binding sites

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Abstract

Betahistine has been used to treat several vestibular disorders of both central and peripheral origin. The objective of this work has been to study the betahistine action mechanism at the peripheral level. Experiments were carried out in wild larval axolotl (*Ambystoma tigrinum*). Multiunit extracellular recordings were obtained from the semicircular canal nerve using a suction electrode. Betahistine (10 µM to 10 mM, n = 32) inhibited the basal spike discharge of the vestibular afferent neurons with an IC_{50} of 600 µM. To study if betahistine action on the afferent nerve discharge was somehow related to nitric oxide (NO) generation, betahistine 1 mM (n = 5) was co-administered with N^G-nitro-L-arginine 3 µM. The action of betahistine remained as in control experiments. To determine the influence of betahistine on the efferent innervation of hair cells its interactions with carbachol (200 µM, n = 5), and with cholinergic antagonists: atropine (10 µM, n = 3) and d-tubocurarine (10 µM, n = 3) were also studied. Betahistine 1 mM reduced the excitatory action of carbachol in a 30 ±3.4%. Cholinergic antagonists did not modify betahistine actions. Postsynaptic actions of betahistine were analized on the basis of its capability to interact

with kainic acid (10 to 100 µM, n = 7). Betahistine reduced kainic acid excitatory action in 46 and 35% respectively. These results corroborate that betahistine has a peripheral inhibitory action in the spike discharge of the afferent neurons in the vestibule. This action seems to involve neither NO production nor modifications in the release of acetylcholine from the efferent fibers. The inhibitory action of betahistine seems to be due to a postsynaptic binding site on the afferent neurons which reduces their sensitivity.

Keywords: hair cells, vestibular system, *Ambystoma tigrinum*, betahistine, histamine, nitric oxide, kainic acid, Meniere, vertigo.

Introduction

Various evidences support the notion that histamine plays a significant role in the sensory coding in the vestibular periphery. Histamine and other imidazole containing substances like L-histidine and carnosine were found to increase the spike discharge of the semicircular canal afferent neurons in the frog (Housley, Norris and Guth, 1988). The inhibition of the histamine sintethizing enzyme L-histidine decarboxilase produces a significant inhibitory action on the afferent neurons. Pharmacological evidences indicate that H_1 , H_2 and H_3 histamine receptors exist in the vestibular periphery (Housley, Norris and Guth, 1988; Tomoda et al., 1997). Based on these data, it has been proposed that histamine may function as a neuromodulator of the synaptic transmission in hair cells.

In isolated hair cells from the guinea pig, it has been found that histamine increases intracellular Ca^{2+}, and that H_1, H_2, and H_3 receptors mediate the actions of histamine (Tomoda et el., 1997). Particularly, H_3 receptors were found to inhibit histamine-induced intracellular Ca^{2+} concentration increase when applied at the nanomolar range, suggesting that its action is mediated by very specific receptor interactions.

Betahistine (N-α-methyl-2-pyridylethylamine), a histamine-like substance, was introduced as an active drug in the treatment of certain vascular and vasomotor disorders (Horton and von Leden, 1962). Later it was used in the treatment of vertigo, motion sickness and various vestibular disorders of central and peripheral origin (Oosterveld, 1984; Kingma, 1997; Gordon and Shupak, 1999; Lamm and Arnold, 2000). Its activity may be explained by its direct action on histamine receptors on which, betahistine has a complex action: as a partial agonist of postsynaptic H_1 and H_2 receptors (Arrang et al.,

1985), and as an antagonist of presynaptic H_3 receptors (Arrang et al., 1985; van Cauweberge and de Moor, 1997).

At the medial vestibular nucleus, betahistine had little excitatory action when applied by its own, but significantly reduced the excitation caused by histamine (Wang and Dutia, 1995). This effect of betahistine was consistent with a partial-agonist action at H_1 receptors, reducing the excitatory response to histamine presumably by occupying these receptors in competition with histamine (Wang and Dutia, 1995). Betahistine significantly attenuated the barrel rotation induced by kainic acid microinjection into the medial vestibular nucleus (O'Neill et al., 1999), indicating that it reduces the excitability of vestibular neurons. Although the effectiveness of histamine-related drugs in the treatment of peripheral and central vestibular disorders may be explained by its action on the histamine receptors in vestibular nuclei, it has also been suggested that betahistine anti-vertigo effects can also take place at the peripheral level. It has been proposed that betahistine actions were due to an improvement on the microcirculation of the labyrinth (Martinez, 1972; Lamm and Arnold, 2000). Also, its effectivity in the treatment of Meniere`s disease was attributed to a reduction in the endolymphatic pressure (Martínez, 1972).

Using an isolated semicircular canal preparation of the frog, it has been shown that the resting basal discharge and not the mechanically-evoked responses of ampullar receptor was very sensitive to betahistine. Drug concentrations as low as 100 nM were sufficient to produce a significant reduction in the resting firing rate, whereas the mechanical response of the semicircular canal was affected only by the highest concentrations used (Botta et al., 1998).

Pharmacokinetic studies showed that betahistine is transformed, mainly at the hepatic level, in aminoethylpyridine (M_1) and hydroxyethylpyridine (M_2), then excreted with the urine as pyridylacetic acid (M_3) (Konzett et al., 1971). In the frog posterior canal, the effects of betahistine metabolites were tested on both resting and mechanically-evoked responses of ampullar receptors. Results demonstrated that, whereas M_2 and M_3 exerted no effect, M_1, at concentrations higher than 10 µM, was able to reduce the resting discharge of ampullar receptors without affecting their responses to mechanical stimuli (Botta et al., 2000). On the basis of these results authors proposed that the action of betahistine is at first sustained by betahistine itself and then prolonged in time by M_1, and that, anti-vertigo effect of betahistine can be due to its capability to reduce the vestibular sensory input.

Since the action mechanism of betahistine at the peripheral vestibular system is not yet clear, we decided to examine the effects of this drug on

neurotransmission in the isolated inner ear of the axolotl with the objective to determine its binding sites and mechanism of action.

Material and Methods

Experiments were carried out in wild larval axolotl (*Ambystoma tigrinum*, 30 to 60 g body weight) as reported previously (Soto and Vega, 1988; Soto et al., 1994). The animals were anaesthetized by immersion in 3-aminobenzoic acid ethyl ester (0.1% in water) and subsequently decapitated. The otic capsule was opened ventrally and the inner ear structures identified (Figure 1). The nerve fibers of the anterior and lateral canals were dissected up to the brainstem. The cartilaginous otic capsule was cut and isolated from the cranium. The isolated inner ear was transferred to a recording chamber

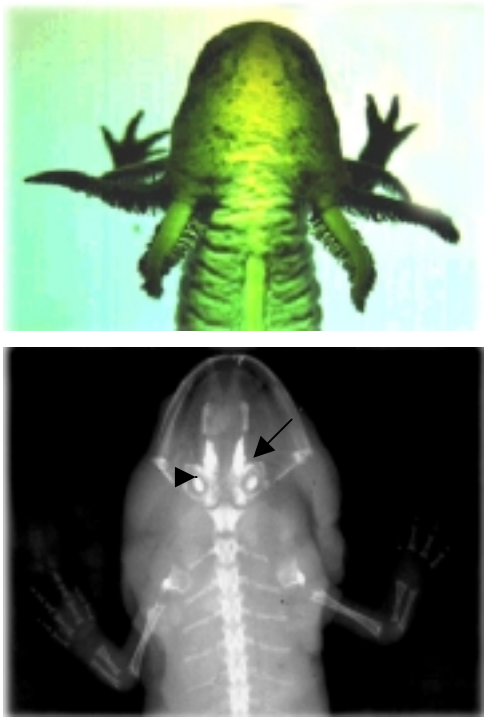


Figure 1.
The axolotl inner ear has the main structures which constitute the mammalian vestibule. In fact, it has been shown that various vestibular disorders have their counterpart in lower vertebrates. Amphibians are capable of developing signs indicating that they are also susceptible to functionally complex vestibular disorders such as motion sickness (Wassersug et al., 1993). Therefore, supporting the notion that, despite important specific evolutionary adaptations, vestibular system fundamental mechanisms have a notable phyllogenetic constancy. Lower panel depicts an X ray of the head and upper limbs of an axolotl. Otic capsule can be cearly identified (arrow). Saccular otolith is the most conspicuous structure of the axolotl´s inner ear (arrowhead).

and continuously perfused with Ringer solution of the following composition (in mM): KCl 2.5, NaCl 111, $CaCl_2$ 1.8, $MgCl_2$ 1.0, glucose 10, HEPES 5, pH 7.4. Multiunit extracellular recordings were obtained from the semicircular canal nerve using a suction electrode. Electrical activity amplified by means of a conventional AC amplifier was filtered at cutoff frequencies of 100 and 2000 Hz and monitored in an oscilloscope. The signal was led to a magnetic tape recorder and to a window discriminator, whose output was connected to a microcomputer for on-line analysis of discharge rate (Soto and Vega, 1987; Soto, Manjarrez and Vega, 1997). In some of the experiments the preparation was mechanically stimulated. For this, the recording chamber, the manipulators, and the amplifier were mounted on a rotating table driven by a DC-controlled motor (Figure 2). A function generator output was fed to the DC amplifier to produce sinusoidal accelerations (0.2 Hz, 440 degrees s^{-1} peak velocity) of the rotating table (Soto et al., 1994). Typically, the preparation was stimulated during thirty-second periods in control conditions and 1, 5 and 10 min after drug administration.

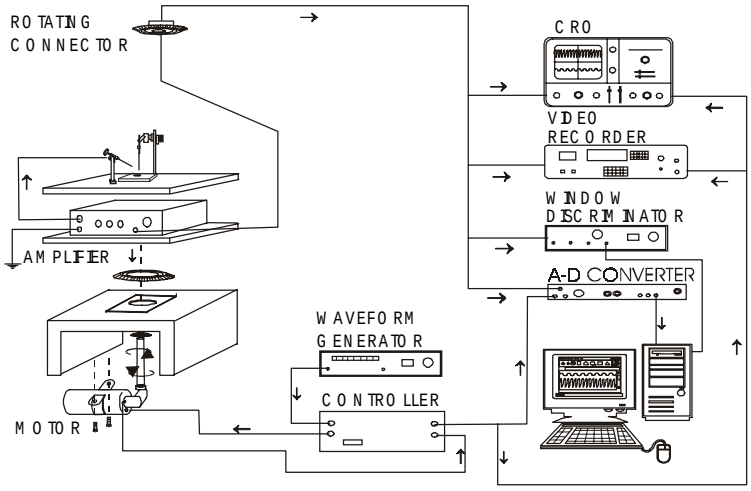


Figure 2.
Experimental setup for recording and mechanical stimulation. The preparation and the recording amplifier were mounted in a rotating table. Record was led out through a rotating connector, displayed in an oscilloscope (CRO) and stored on a tape recorder. The spike discharge frequency of semicircular canal nerve afferent neurons was analyzed on line by means of a window discriminator and a computer. The rotating table was driven by a servo-controlled DC motor. The mechanical stimulus control signal was derived from a function generator. Tachometer output from the rotating table was used as a control signal, and was digitized by using an analog-digital converter.

Drugs were either bath-, or locally applied as indicated in each case. For bath application, the desired drug concentration was added to the perfusion Ringer and the recording chamber bath completely replaced. For local application, 20 µl of the drug were pressure ejected from a pipette (100 µm tip diameter, flow rate 7-10 µl / s) positioned close to the ampula at less than 500 µm from the origin of the afferent fibers.

Atropine, d-tubocurarine, carbachol, L-arginine, N^G-nitro-L-arginine (L-NOARG), and Kainic acid were all obtained from Sigma Chemicals Co. (St. Louis, MO). Betahistine was obtained from Formenti (Italy).

To construct the concentration-response relationship, the spike discharge was normalized as a percentage of change with respect to control conditions, and the mean and standard error calculated from these values. Comparisons of the mechanical responses were done by obtaining the mean of the peak response in at least three cycles of the sinusoidal stimulus period. To determine the statistical significance of drug effects, a U-Mann-Whitney ($p > 0.005$) statistical test was performed as previously described (Soto, Echagüe and Vega, 1989).

Results

Betahistine

Betahistine was tested in concentrations ranging from 10 µM to 10 mM (n = 32). At the lowest doses used, betahistine showed a facilitatory action on the mechanical response of the inner ear afferent fibers. At higher concentrations betahistine inhibited the basal spike discharge of the vestibular afferents with an IC_{50} of 600 µM, and also produced a much less potent inhibition of the mechanically-evoked activity. A typical example of betahistine effect is shown in Figure 3. It can be noted that the drug produced a significant reduction in the basal discharge of the afferent fibers, whereas the response to mechanical stimuli decreased in a minor proportion. The inhibitory action of betahistine was reversible after bathing the preparation with Ringer solution for 20 min.

Betahistine and nitric oxide (NO) production

To study if betahistine action on the afferent nerve discharge is somehow related to nitric oxide production N^G-nitro-L-arginine (L-NOARG), an antagonist of the nitric oxide sinthetizing enzyme (NOS), was used. L-NOARG 3 µM was perfused for 10 min prior to betahistine administration. This concentration of L-NOARG was selected because previous studies from our laboratory had shown that this is the IC_{50} of L-NOARG on the discharge

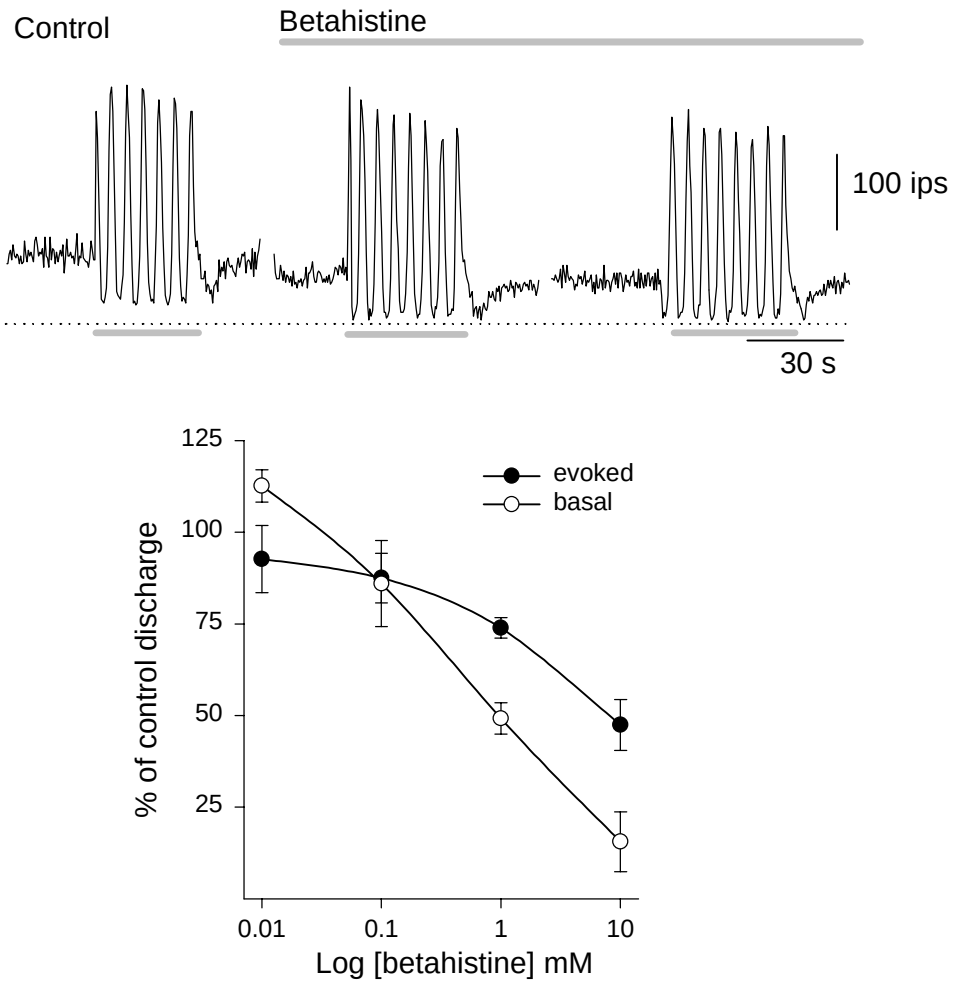


Figure 3.
Betahistine (BH) action on the electrical discharge of vestibular afferent neurons. In A, frequency plot showing the basal (spontaneous) and the mechanically-evoked discharge after 5 and 10 min of betahistine perfusion (impulses per second: ips). Bath application of betahistine 1 mM diminished the basal and the mechanically evoked activity in a 51% and 22% respectively. Gray bars represent the periods during which mechanical stimulation was applied. In B, concentration-response relationship of drug actions on the basal and the mechanically-evoked spike discharge. Data were taken after ten minutes of drug perfusion in the bath. Each point represents the mean of 5 experiments ± standard error.

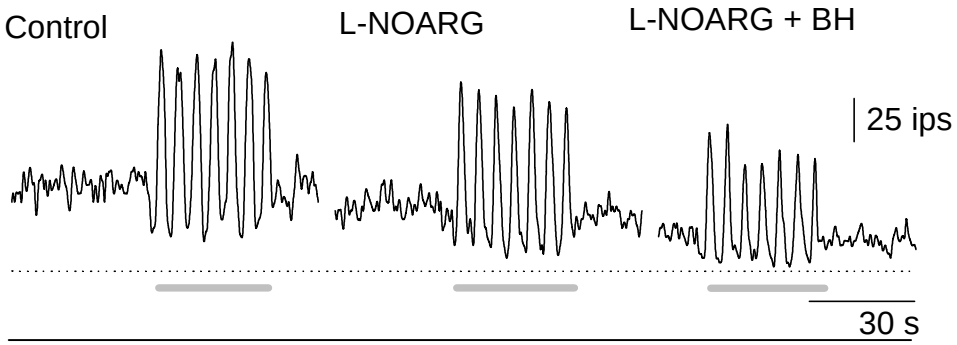


Figure 4.
L-NOARG administration prior to betahistine did not modify the betahistine inhibitory potency. Consecutive records showing the control activity, after 10 min of L-NOARG 3 µM bath perfusion, and then after 10 min betahistine 1 mM was also added to the bath. Gray bars represent the periods during which mechanical stimulation was applied.

of the afferent fibers (Flores et al., 1996). Figure 4 shows a typical example of L-NOARG inhibitory action in the electrical activity of vestibular afferent fibers. Bath perfusion of L-NOARG (n = 5) decreased the basal discharge in 20.7 % and the mechanically-evoked responses in 8.2 % (Table I). Experiments in which betahistine 1 mM (n = 5) was administered after the bath was perfused with L-NOARG for 10 min, showed that the action of betahistine remained the same as in control experiments in which no L-NOARG preceded its application (Table I).

In order to create conditions which favor the generation of NO in our *in-vitro* experimental circumstance, the preparation was bathed with a Ringer to which glycine 1 µM and L-arginine 1 µM were added. Glycine has been shown to be a co-agonist of the NMDA receptors in the central nervous system and in the inner ear (Johnson and Ascher, 1987; Soto et al., 1994). L-arginine is the natural substrate for the NOS and it has been shown to have an excitatory action in the afferent resting activity in the cephalopod statocyst neurons (Tu and Budellmann, 1999). The perfusion of the preparation with these aminoacids had no obvious action upon the basal discharge of the afferent fibers; although the responsiveness and stability of the preparation increased. In this case the inhibitory action of L-NOARG 3 µM (n = 4) increased twofold (table I). Betahistine 1 mM (n = 4) inhibitory effect either prior or after L-NOARG, did not significantly change by perfusion with a Ringer solution to which L-arginine and glycine have been added (Table I).

TABLE I. Betahistine (BH) and L-NOARG actions on the vestibular nerve discharge

Normal Ringer	**n**	**basal**	**evoked**
L-NOARG, 3 µM	5	19 ±4.4 %	11.2 ±4.4 %
BH, 1 mM	5	43 ±8.8 %	28.2 ±5.5 %
BH after L-NOARG	5	46 ±9.2 %	30.8 ±8.2 %
Ringer with glycine and L-arginine			
L-NOARG, 3 µM	4	37.3 ±11.2 %	17 ±4.7 %
BH, 1 mM	4	45.3 ±6.2 %	29.8±4.7 %
BH after L-NOARG	4	46 ±6.9 %	26 ±6.8 %

Betahistine actions on the efferent input

To test the hypothesis that the inhibitory action of betahistine was due to an influence of this drug on the efferent terminals innervating hair cells, we studied the interaction between cholinergic muscarinic and nicotinic antagonists with betahistine. The idea behind these experiments was that, if betahistine acts by modifying the release of acetylcholine (ACh) from the efferent terminals, then ACh antagonists should modify the action of betahistine. Atropine (10 µM) and d-tubocurarine (10 µM) were applied by bath perfusion. As it has been previously reported (Caston and Roussel, 1984), these drugs showed no effect on the afferent nerve activity except in those experiments in which they were applied in the first 15 minutes after obtaining the isolated inner ear preparation. The lack of activity of ACh antagonists in the isolated inner ear preparation indicates that efferent axons became inactive after they have been severed from the cell body. Although there seems to be no efferent activity in the isolated inner ear preparation, ACh could be released from efferent terminals under various influences, and ACh agonists such as carbachol may activate the ACh receptors associated with the efferent synapse. Therefore, interaction experiments were performed by perfusing atropine (10 µM, n = 3) or d-tubocurarine (10 µM, n = 3) for 10 min, and then betahistine 1 mM was also added to the bath perfusion (Figure 5). Betahistine inhibitory action did not change due to prior application of cholinergic antagonists, indicating that very probably this drug is not capable of influencing ACh release from the efferent axons.

To activate ACh receptors, carbachol was applied by microperfusion in the range between 10 to 300 µM (n = 20). Characteristically, carbachol produced a long lasting (about 3 min) increase in the basal spike discharge of the

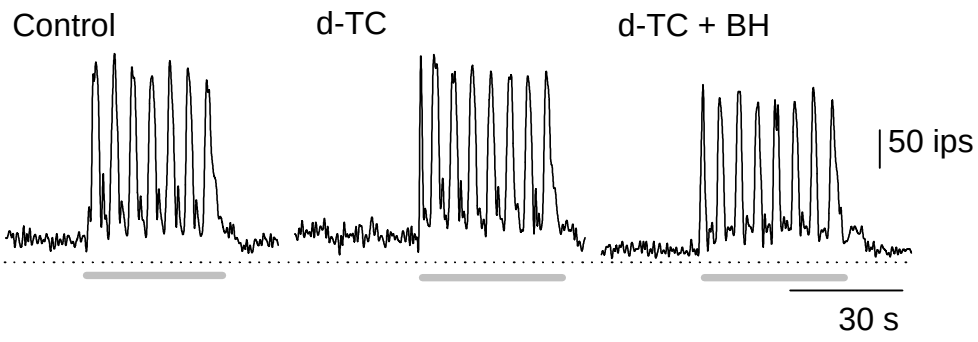


Figure 5.
Records show a typical example of d-tubocurarine and betahistine interactions. After a control record, application of d-tubocurarine (d-TC, 10 µM) for 10 min produced no significant change in either the basal or the mechanically-evoked discharge (gray bars). Betahistine (1 mM) applied for 10 min along with d-tubocurarine produced the same effect as that caused when it was applied alone.

semicircular canal nerve. In contrast, it did not induce any significant effect in the mechanically-evoked discharge at all the concentrations tested. Bath perfusion with betahistine 1 mM for 10 min preceding the carbachol 200 µM application (n = 5) reduced the excitatory action of carbachol in 30 ±3.4% (Figure 6).

Betahistine actions on the afferent synapse
To study the influence of betahistine on the afferent neurons synaptic activation, kainic acid (KA) which is one of the most potent excitatory amino acid (EAA) receptor agonists, was applied by microperfusion (20 µl) in concentrations of 10 µM (n = 4) and 100 µM (n = 3). Typically, KA induced a very strong excitatory action in the basal spike discharge of the semicircular canal nerve. The excitatory action of KA is usually followed by a postexcitatory inhibition of the spike discharge. Bath perfusion for 10 minutes with betahistine 1 and 10 mM prior to the KA application produced a reduction of 54 and 65% respectively of the kainic acid excitatory action (Figure 7).

Discussion

In this work we have tested various hypotheses in relation to the action mechanism of betahistine at the vestibular peripheral end organs. Very

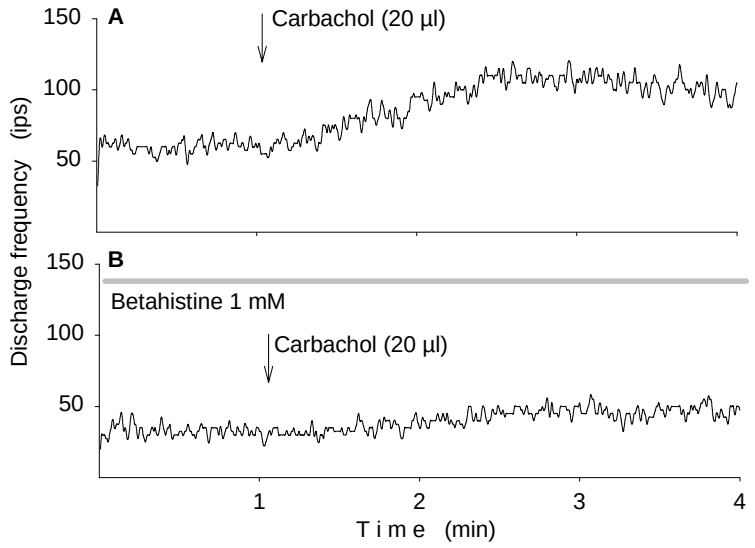


Figure 6.
Betahistine decreases the excitatory action of carbachol. In A, typical record of the excitatory action of carbachol (200 µM) microperfusion on the semicircular canal nerve discharge. Carbachol induced a long lasting excitatory effect of the basal discharge of the afferent neurons, with no change in the peak response to mechanical stimuli. In B, after 10 min of betahistine 1 mM bath perfusion, the excitatory action of carbachol is reduced in about 30%, while its time course remains the same.

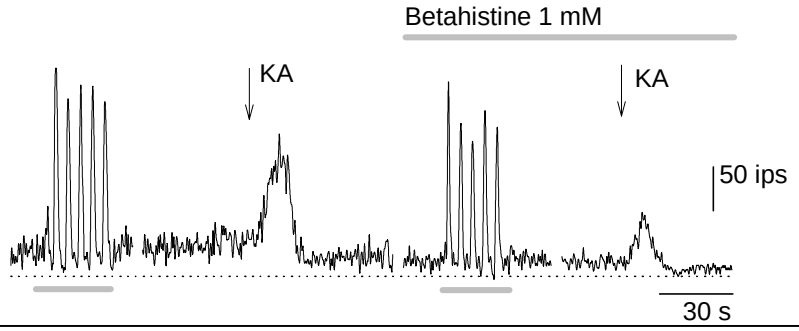


Figure 7.
Interactions between betahistine and kainic acid responses. Records show the control basal discharge and mechanically-evoked (gray bar) response of the semicircular canal afferent neurons. Then control response to kainic acid (100 µM) microperfusion is shown. After 10 min washing of the preparation, betahistine 1 mM was applied. It produced an inhibitory action in the basal and mechanically-evoked response. After 10 min that the preparation has been in contact with betahistine kainic acid (100 µM) was applied. Betahistine produced a clear cut reduction in the KA excitatory action on the afferent neurons.

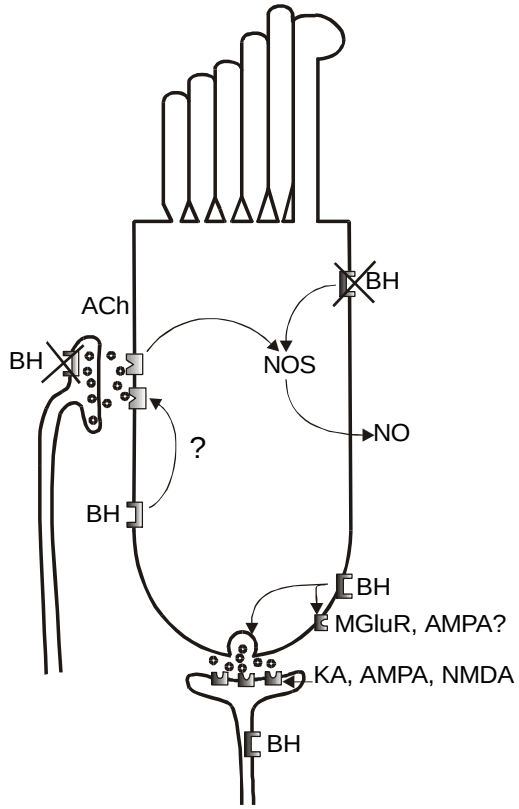


Figure 8.
Hypotheses tested about the betahistine site of action at the vestibular periphery. The most plausible explanation of our results implies that betahistine has a binding site on the afferent neurons that may explain its inhibitory action and its capability to inhibit the response of afferent neurons to KA acid. Influence of betahistine on the efferent release of neurotransmitter and on the generation of NO were discarded (crossed binding sites). Existence of binding sites at the hair cell basolateral membrane that may affect the action of ACh and of EAA presynaptic receptors could not be discarded and should be considered as an alternative possibility.

probably this compound is acting at various levels including the hair cells and the afferent neurons. Betahistine produced a significant inhibitory action on the basal discharge of the afferent neurons as has been previously reported (Botta et al., 1998). We found that betahistine exerted also a slight inhibitory action on the mechanically-evoked responses of the ampullar receptors.

The inhibitory actions of betahistine were observed only after the drug was in contact with the preparation for several (5-10) minutes. The low potency and the latency of its effects argue in favor of betahistine having a complex action, probably involving second messengers. Betahistine was active only at high doses at which receptor specificity is very dubious. However, it should be taken into account that we are using an *in-vitro* preparation of the inner ear, and that experiments were performed in an "acute"-like condition. Therefore, our results hint at the action mechanism of the drug. Nevertheless, some type of "chronic" preparation seems desirable to better define the action of betahistine when chronically administered.

On the basis of our previous observations we have proposed that hair cells may produce NO under the influence of the efferent fibers (Flores et al.,

1996). Also NO production from hair cells may be modulated through an autoreceptor for the afferent excitatory amino acid (EAA) neurotransmitter (Prigioni et al., 1990; Guth et al., 1998b). NO generated at the hair cell level might affect both the efferent and the afferent neurons, and can also play a role in the homeostasis of the blood flow in the sensory epithelia (Flores et al., 1996).

Previous reports have put forward the hypothesis that peripheral action of betahistine is due to a modification of the local microcirculation in the labyrinth (Martinez, 1972). Using laser Doppler flowmetry, it has been shown that betahistine increases the vestibular blood flow in the posterior canal ampulla of guinea-pig (Dziadziola et al., 1999). We studied the effects of this drug in the presence of NOS antagonist L-NOARG. The hypothesis to be tested was that betahistine may somehow modulate NO production in the inner ear, and consequently induce a local modification of the blood flow. Our results indicate that blockade of NOS did not significantly modify the betahistine action on the afferent neuron electrical discharge (Figure 8). Therefore, at least as judged on the basis of the action of NO in the electrical activity, it seems that betahistine has no influence on the NO production pathway. It should be taken into account that in our *in-vitro* preparation there is no blood flow; thus, the evaluation of NO actions in this work is exclusively based on its influence in the afferent nerve electrical activity. The probability that sources of NO which are not capable of influencing afferent nerve discharge may be affected by betahistine and mediate some of its clinical actions remains open. In fact, it has been described that vasculature of the dark cells region of the cristae ampullaris in guinea-pigs, is innervated by trigeminal fibers which constitute a system for vasodilatatory responses not related to the VIII nerve innervation of the vestibular periphery (Vass et al., 1998).

In relation to the efferent system, the hypothesis tested was that betahistine may modify the release of neurotransmitter from the nerve terminals, and in this form, produce a modification of the vestibular sensory input to the central nervous system. We studied the interaction between muscarinic and nicotinic antagonists with betahistine. The fact that ACh antagonists atropine and d-tubocurarine did not modify the inhibitory action of betahistine implies that this drug is not capable of inducing the ACh release from the severed efferent axons in the isolated inner ear preparation (Figure 8). In these experiments the influence of other substances, apart from ACh, that can be potentially released from the efferent neurons was not analyzed (Scarfone et al., 1996; Vega et al., 1991; Guth et al., 1998a; Andrianov and Ryzhova, 1999).

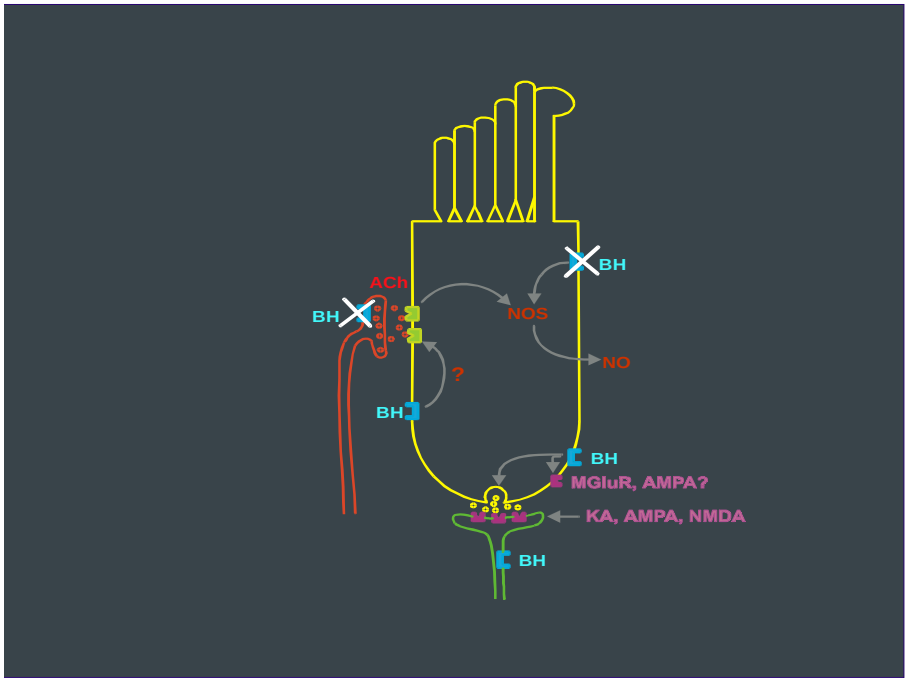
An explanation of the mechanism of action of histamine that has been put forward is that this agent may interact with cholinergic receptors at the hair

cell level (Housley, Norris and Guth, 1988). In fact, it has been found that atropine was capable of antagonizing histamine´s excitatory action in the semicircular canal afferent neurons, clearly indicating an interaction of histamine with ACh receptors (Housley, Norris and Guth, 1988). Betahistine may also be acting at the ACh receptor level upon the hair cells (Figure 8). In this work, we found that betahistine reduced the response to carbachol. However, a direct interaction of betahistine with the ACh receptors seems very unlikely. In fact, the lack of effects of ACh antagonists on betahistine actions support this notion. It remains the possibility that some indirect interaction between histamine and ACh receptors could occur at the hair cell level, particularly considering that histamine receptors are of the G type family coupled to second messenger systems (Hill et al., 1997). Another explanation for the reduction of carbachol excitatory effect is that betahistine diminishes the output of the vestibule consequently restraining any type of facilitatory action exerted at the presynaptic level.

Excitatory amino acids have been shown to be the neurotransmitters at the synapse between the hair cells and the afferent neurons. It has been found that N-methyl-D-aspartic acid (NMDA), KA and α-amino-3-hydroxi-5-methil-isoxazole-4-propionic acid (AMPA) receptors are located at the postsynaptic level in the afferent neurons, and probably also the AMPA and the metabotropic EAA receptors at the presynaptic level (Soto and Vega, 1988; Zucca et al., 1992; Soto et al., 1994; Guth et al., 1998a, b). The fact that betahistine diminishes the action of exogenously applied EAA agonists such as KA constitutes a strong evidence indicating that its inhibitory effect is taking place at the postsynaptic membrane (Figure 8).

It remains also possible that betahistine may bind to histamine receptors that interact with presynaptically located EAA receptors, thus modifying the response to KA. However this explanation seems not very feasible because it implies that presynaptic EAA receptors have a key role in the response to exogenously applied EAA agonists.

The use of amphibians as a model to study pharmacological agents of clinical interest can be questioned. Nevertheless, the discovery of the action mechanisms, and of new drugs for the treatment of vestibular disorders has been hampered by the lack of adequate animal models. Due to the very evident phylogenetic constancy of the peripheral vestibular end organs, it seems germane to argue that amphibians constitute a model to study the action of several drugs. Particularly, studies intended to define the action mechanism of a substance could be developed in very simplified animal models; predictions and hypotheses based on the information so obtained, can be precisely tested, and specific experiments performed in higher animals and in humans.



One aspect that should be solved in the animal models is the definition of where endogenous histamine comes from. In pathological conditions accompanied by an inflammatory process histamine may be generated by mast cells (Guth et al., 1998a; Tomoda et al., 1997; Gordon and Shupak, 1999); but in normal conditions it is not clear where histamine may come from and what the histamine receptor function may be.

We have addressed the problem of defining the betahistine interactions with known neurotransmitter systems in the vestibular organ. In conclusion, our results indicate that betahistine acts in the vestibular periphery modifying the output of the system at the afferent neurons. This, in no way excludes some other actions of the drug which may be exerted at the hair cell level or in non-neural structures of the sensory organ. These results give further support to the idea that betahistine anti-vertigo action may be due to a decrease in the sensory input form the vestibular system.

Abbreviations

ACh: acetylcholine.
AMPA: α-amino-3-hydroxi-5-methil-isoxazole-4-propionic acid.
BH: betahistine.
d-TC: d-tubocurarine.
EAA: excitatory amino acid.
KA: kainic acid.
L-NOARG: N^G-nitro-L-arginine.
mGluR: metabotropic glutamate receptors.
NMDA: N-methyl-D-aspartic acid.
NO: nitric oxide.
NOS: nitric oxide sinthase.

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References

Andrianov, G. and Ryzhova, I. (1999) *Opioid receptors as possible neuromodulators of the afferent synaptic transmission in the frog semicircular canal.* Neuroscience. 93: 801-806.

Arrang, J.M., Garbarg, M., Quach, T.T., Dam Trung Tong, M., Yeramian, E. and Schwartz, J.C. (1985) *Action of betahistine at histamine receptors in the brain.* European J. Pharmacol. 111, 73-84.

Botta, L. Mira, E., Valli, S, Benvenuti, C., Fossati, A., Soto, E., Guth, P.S. and Valli, P. (2000) *Effects of betahistine metabolites on frog ampullar receptors.* Bárány Society 21st Meeting, June 4-7, Uppsala, Suecia.

Botta, L., Mira, E., Valli, S., Perin, P. Zucca, G. and Valli, P. (1998) *Effects of betahistine on vestibular receptors of the frog.* Acta Otolaryngol. 118, 519-523.

Caston, J. and Roussel, H. (1984) *Curare and the efferent vestibular system.* Acta Otolaryngol. 97, 19-26.

Dziadziola, J.K., Laurikainen, E.L. Rachel, J.D. and Quirk, W.S. (1999) *Betahistine increases vestibular blood flow.* Otolaryngol. Head Neck Surg. 120, 3400-3405.

van Cauweberge, P.B. and de Moor, S.E.G. (1997) *Physiopathology of H_3 receptors and pharmacology of betahistine*. Acta Otolaryngol. Suppl. 526, 43-46.

Flores, A., León-Olea, M., Vega, R. and Soto, E. (1996) *NADPH histochemistry and role in the amphibian inner ear.* Neurosci. Letters 205, 131-134.

Gordon, C.R. and Shupak, A. (1999) *Prevention and treatment of motion sickness in children.* CNS Drugs. 12, 369-381.

Guth, P.S., Perin, P., Norris, C.H. and Valli, P. (1998a) *The vestibular hair cells: post-transductional signal processing.* Prog. in Neurobiol. 54: 193-247.

Guth, P.S., Holt, J.C., Perin, P., Athas, G., Garcia, M., Puri, A., Zucca, G., Botta, L. and Valli, P. (1998b) *The metabotropic glutamate receptors of the vestibular organs.* Hearing Res. 125: 154-162.

Hill, S.J., Ganellin, C.R., Timmerman, H., Schwartz, J.C., Shankley, N.P., Young, J.M., Schunack, W., Levi, R. and Haas, H.L. (1997) *International Union of Pharmacology. XIII. Classification of histamine receptors.* Pharmacol. Revs., 49, 253-278.

Horton, B.T. and von Leden, H. (1962) *Clinical use of beta-2-pyridiylalkylamines.* I. Mayo Clinic Proc. 37, 692-702.

Housley, G.D., Norris, C.H. and Guth, P.S. (1988) *Histamine and related substances influence neurotransmission in the semicircular canal*. Hearing Res. 25, 87-98.

Johnson, J. W. and Ascher, P. (1987). *Glycine potentiates the NMDA response in cultured mouse neurons*. Nature 325, 529-531.

Kingma, H., Bonink, M., Meulenbroeks, A. and Konjinenberg, H. (1997) *Dose-dependent effect of betahistine on the vestibulo-ocular reflex: a double blind, placebo controlled study in patients with paroxysmal vertigo.* Acta Otolaryngol. 117, 641-646.

Konzett, H., Bost, R.G. Bowman, F.J., Bowman, E.R. and McKennis, H. (1971) *Betahistine its metabolites and vascular responses in the forelimb of the dog.* J. Pharmacol. Exp. Therap. 178, 122-129.

Lamm, K. and Arnold, W. (2000) *the effect of blood flow promoting drugs on cochlear blood flow, perilymphatic pO2 and auditory function in the normal and noise-damaged hypoxic and ischemic guinea pig inner ear*. Hearing Res. 141, 199-219.

Martinez, D.M. (1972) *The effect of Serc (betahistine hydrochloride) on the circulation of the inner ear in experimental animals.* Acta Otolaryngol. Suppl 305, 29-47.

O'Neill, A.B., Pan, J.B., Saullivan, J.P. and Brioni, J.D. (1999) *Pharmacological evaluation of an in vivo model of vestibular dysfunction in the rat.* Methods Find. Clin. Pharmacol. 21, 285-289.

Oosterveld, W.J. (1984) *Betahistine dihydrochloride in the tratment of vertigo of peripheral vestibular origin. A double blind placebo study*. J. Laryngol. Otol. 98, 37-41.

Prigioni, I., Russo, G., Valli, P. and Masetto, S. (1990) *Pre- and postsynaptic excitatory action of glutamate agonists on frog vestibular receptors.* Hearing Res. 46, 253-260.

Scarfone, E., Ulfendahl, M. and Lundeberg, T. (1996) *The cellular localization of the neuropeptides substance P, neurokinin A, calcitonin gene-related peptide and neuropeptide Y in guinea-pig vestibular sensory organs: a high resolution confocal microscopy study.* Neuroscience. 75, 587-600.

Soto, E. and Vega, R. (1987) *A Turbo Pascal program for on line spike data acquisition and analysis.* J Neurosci. Meth. 19, 61-68.

Soto, E. and Vega, R. (1988) *Actions of excitatory aminoacid agonists and antagonists on the primary afferents of the vestibular system of the axolotl (Ambystoma mexicanum).* Brain Res. 462, 104-111.

Soto, E., Echague, J.V. and Vega, R. (1989) *A computer program for statistical non parametric Mann Whitney U analysis of neuronal spike activity.* Comput. Progr. Meth. Biomed. 28, 197-200.

Soto, E., Flores, A., Erostegui, C. and Vega, R. (1994) *Evidence for NMDA receptor in the afferent synaptic transmission of the vestibular system.* Brain Res. 633, 289-296.

Soto, E., Manjarrez, E. and Vega, R. (1997) *A microcomputer based expert system for neuronal spike train data analysis.* Int. J. Med. Informat. 44, 203-212.

Tomoda, K., Motoki, N., Harada, N., Iwai, H. and Yamashita, T. (1997) *Effect of histamine on intracellular Ca^{2+} concentration in guinea pig isolated vestibular hair cells.* Acta Otolaryngol. 528, 37-40.

Tu, Y. and Budelmann, B. (1999) *Effects of L-arginine on the afferent resting activity in the cephalopod statocyst.* Brain Res. 845, 35-49.

Vass, Z., Shore, S.E., Nuttall, A.L. and Miller, J.M. (1998) *Direct evidence of trigeminal innervation of the cochlear blood vessels.* Neuroscience 84, 559-567.

Vega, R., Soto, E. and Pérez, M.E. (1991) *Opioid receptors neuropharmacology in the vestibular afferents.* Neuroscience Abstracts. 17, 317 (128.14).

Wang, J.J. and Dutia, M.B. (1995) *Effects of histamine and betahistine on rat medial vestibular nucleus neurones: possible mechanism of action of anti-histaminergic drugs in vertigo and motion sickness.* Exp. Brain Res. 105, 18-24.

Zucca, G., Botta, L., Milesi, V., Dagani, F. and Valli, P. (1992) *Evidence for L-glutamate release in frog vestibular organs.* Hearing Res. 63,52-56.

Wassersug, R.J. Izumi-Kurotani, A., Yamashita, M. and Naitoh, T. (1993) *Motion sickness in amphibians.* Behav. Neural Biol. 60, 42-51.