Neuropharmacological targets for drug action in vestibular sensory pathways

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Introduction

Dizziness may be caused by many disturbances in central and peripheral neural function. Vertigo, dizziness, and dis-equilibrium consistently rank among the most common complaints experienced by individuals with vestibular dysfunction. Moreover, vestibular deficits appear to be among the most common causes of dysequilibrium symptoms requiring medical intervention. Decreased function of the peripheral vestibular system can result in decreased visual acuity during head movements and a degradation of postural control. The management of dizziness related symptoms has mostly involved the use of pharmacological agents during the early acute phase of symptom onset. The common objective of drug treatment is to suppress vestibular sensory input. This is thought to reduce conflicting sensory input, control undesirable perceptions, and improve the quality of life of patients.

Vestibular signals originate from the sensory organs of the three semicircular canals and two macular organs within the petrous portion of the temporal bone (Fig. 1). The vestibular neuroepithelium including hair cells, supporting cells as wells as afferent and efferent neurons are thought to interact with a diverse number of neuroactive substances (neurotransmitters and neuromodulators), which bind to specific receptors at the postsynaptic membrane. During normal chemical synaptic transmission, these neurotransmitters and modulators are synthesized and stored in presynaptic vesicles and are released into the synapse to bind and act on postsynaptic cell receptors during the transmission and processing of sensory information. Glutamate (Glu or a glutamate-like excitatory amino acid...
Pharmacological Targets for Vestibular Disorders

(EAA)) and acetylcholine (ACh) are widely accepted as being the primary neurotransmitters released by vestibular hair cells and efferent neural terminals respectively [1,2]. In the peripheral vestibular system, there are a variety of other neuroactive molecules that are thought to be present and may act as modulators at these synapses including γ-amino butyric acid (GABA), substance P, calcitonin gene-related protein (CGRP), and opioid peptides. These neuromodulators may affect the response of neurons by facilitating or inhibiting the effect of the primary neurotransmitters or by altering patterns of release. Neuroactive substances bind with numerous corresponding postsynaptic and presynaptic receptors to mediate neural transmission or modulate the response of postsynaptic neurons. Pharmacologic agents can act at these receptors to alter synaptic messaging. In this way, pharmacological agents have a diverse array of cellular targets that provide a means for modifying sensory transmission. In Fig. 2, many of the possible targets of pharmacological agents in the peripheral vestibular sensory neuroepithelium are summarized.

Neuroactive Substances and Receptors

Molecular biological, biochemical, and electrophysiologic evidences suggest that glutamate, ACh, histamine, enkephalins, GABA, CGRP, and substance P are all released to some extent as neurotransmitters or modulators in the peripheral vestibular system [3,4]. In central vestibular circuits, the vestibular nuclei integrate vestibular, somatosensory, proprioceptive, and visual inputs. These diverse synaptic inputs to the nuclei are also represented by glutamatergic, GABAergic, histaminergic, serotoninergic, and dopaminergic neurons [4]. These neural projections to vestibular nuclei arise mainly from vestibular afferent neurons, spinal afferents, cerebellum, and more rostral descending visual and motor control systems [5]. The following provides an overview of neurotransmitter and neuromodulator receptors identified in the peripheral vestibular neuroepithelium and ganglion as well as in central vestibular nuclei.

Glutamate receptors

In the vestibular system, glutamate is an EAA and the major neurotransmitter of the vestibular sensory hair cells [1].

Fig. 1. Schematic illustration of the vestibular end organ (top), which includes the neuroepithelium of the crista ampullaris (lower left) and vestibular macula (lower right). The membranous labyrinth is fluid-filled with endolymph (blue color on top panel) and perilymph (red color). The vestibular system is composed of five organs; three semicircular canals also called “crista sensors” to detect angular accelerations of the head and two otolith organs also called “macular sensors” to detect the linear acceleration of the head. In the crista, kinocilia and stereociliary bundles of hair cells extend into the gelatinous cupula. During angular acceleration of the head, surrounding endolymph lags and moves in the opposite direction in response to rotational acceleration. In the macula, dense calcium carbonate (called “otoconia”) are embedded within the otocional layer, which is greater density than surrounding fluid and tissues of the maculae. During linear acceleration of the head, high density of otocional layer causes the otocional layer to shift position relative to the neuroepithelium producing a movement of stereociliary bundles of receptor hair cells.
Glutamate interacts with ionotropic receptors such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartic acid (NMDA), and kainic acid (KA) as well as metabotropic receptors such as metabotropic glutamate receptor 1-8 (mGluR1-8) [1,6]. Ionotropic and metabotropic glutamate receptors are located on the vestibular primary afferent dendrites. These receptors mediate the input signals from vestibular hair cells and also between primary afferents and second order vestibular nuclei neurons (see below).

AMPA is a major glutamate receptor in the inner ear. The AMPA response to glutamate produces a postsynaptic increase in intracellular cations including especially Na⁺ postsynaptically; these cations thus play a functional role in AMPA-mediated depolarization of vestibular afferent neurons [7]. Immunohistochemical studies have suggested that AMPA receptor subunits GluR1, GluR2-3, and GluR4 are expressed in mammalian vestibular hair cells and calyx afferents [3,7]. The application of a specific AMPA/KA antagonist (1.5 mM DNQX) to the mouse vestibular afferents blocked AMPA responses [7]. The neurons of the vestibular nuclei also express AMPA EAA receptors. Popper, et al. [8] used immunohistochemistry in the vestibular nuclei of the chinchilla and found ionotropic AMPA receptor subunits (GluR2-3) in the most neurons, (GluR1) in some neurons, and (GluR4) in the fewest number of neurons.

There is evidence in support of the presence of the NMDA receptors on both vestibular hair cells and afferent neurons. The NMDA receptor (NMDAR) subunits NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C, and NMDAR2D are reportedly expressed in rat vestibular afferent neurons [6]. Electrophysiological studies have suggested that the application of NMDA antagonists [2-amino-5-phosphonopentanoic acid (AP5) and 7-chloro-kyneurenic acid (7ClKyn)] decreased spontaneous spike discharge in vestibular afferents [9]. In addition, an electrophysiological study using whole-cell patch clamp methods in the brainstem reported NMDA receptors contribute to afferent synaptic transmission in the medial vestibular nucleus (MVN) of the rat [10]. These studies suggest that NMDA receptors contribute to afferent neurotransmission in both the peripheral and central vestibular system.
Evidence for KA glutamate-receptors in the vestibular system is far less compelling, and glutamate affinity at ionotropic KA receptors seems to be low. There are five KA receptor subunits (GluR5-7 and KA1-2); however, GluR5, GluR6, KA1, and KA2 were only immunocytochemically expressed in mammalian vestibular ganglia [6]. Several pharmacological studies have suggested that KA receptors like other glutamate receptors act during afferent neurotransmission in mammalian vestibular neurons. Trimetazidine, a potent antagonist of KA receptor, blocked kainate-induced excitatory responses in the rat vestibular afferent neurons [11]. AMPA/KA receptor antagonists, CNQX and NBQX, were delivered to the intersitial fluids of the inner ear through perilymphatic perfusion and the effect on vestibular compound action potentials using linear vestibular sensory evoked potentials (VsEPs) showed VsEP response amplitudes were significantly reduced [12]. Evidence for metabotropic glutamate receptors in the vestibular pathways is far less compelling, with a limited number of studies. Numerous studies have shown that the afferents innervating vestibular hair cells are triggered by responses to the activation of glutamate receptors, and glutamate is a major afferent neurotransmitter at both peripheral and central afferent synapses.

ACh receptors

The primary neurotransmitter of vestibular efferent synapses is acetylcholine [13,14]. In mammals including humans, the activity of choline acetyltransferase (ChAT), which synthesizes ACh, and acetylcholinesterase (AChE), which inactivates ACh, was found in numerous bouton terminals at the basal surface of the vestibular hair cells [13,15]. Two major classes of ACh receptors found in the vestibular neuroepithelium are nicotinic ACh receptor (nAChR) and muscarinic ACh receptor (mAChR). Over the last decade, Holt and his colleagues have focused on addressing the function of the vestibular efferent system and reported that vestibular efferent control of mAChRs may provide a means to modulate primary afferent response characteristics [20].

In the vestibular nuclei, specific nAChR and mAChR subunits were not clearly identified. Even in the vestibular efferent neurons of brainstem, ChAT (responsible for the synthesis of ACh) is found only in some of the vestibular efferent neurons [13] suggesting that some of these neurons may not be mediated by nAChR and mAChR. Intracellular recordings of rat MVN have shown that nACh and mACh receptor agonists produced membrane depolarization [21]. Extracellular recordings in all four major vestibular nuclei (i.e., medial, lateral, inferior, and superior) of the rat vestibular nuclear complex have indicated that nACh and mACh receptor agonists (carbachol and muscarine) increased spontaneous firing rates and established that the effect of ACh agonists was highest in the MVN [22]. These studies indicate that both nACh and mACh receptors are present and influence the excitability of the mammalian vestibular nucleus neurons.

GABA receptors

The role of GABA in the vestibular system has been widely debated, but a clear consensus has not been reached. GABA receptors are divided into three types [23]: 1) GABA<sub>A</sub> receptor is an ionotropic receptor controlling a ligand-gated CI
Histamine receptors

Histamine and its four receptors (H1, H2, H3, and H4) have been identified in the vestibular sensory pathway: H1, H2, and H3 receptors in the vestibular hair cells [29]; H3 and H4 receptors in the vestibular afferents [30,31]. All histamine receptors are metabotropic, with four different G-protein coupled receptors acting through a second messenger intracellularly. Evidence for histamine receptors in the vestibular system suggests that they may perform an important role in the control of vestibular function.

Even though H1-3 receptors are expressed and their activation induces an influx of Ca\(^{2+}\) in vestibular hair cells, intracellular recordings of guinea pig vestibular hair cells have shown that H3 receptors may mediate a notably larger increase in Ca\(^{2+}\) permeability (larger influx of Ca\(^{2+}\)) compared to H1 and H2 receptors [29]. Recently, H4 antagonist JNJ7777120 has been studied in Scarpa’s ganglion of the rat and electrophysiological recordings showed reversible inhibitory effects elicited by the H4 receptor antagonist without subsequent excitatory action [31]. However, this effect may not be shared by central vestibular nucleus neurons. The findings suggest that H4 receptors mediate an excitatory action in vestibular primary afferent neurons. Histamine also modulates the neural activities of the second order neurons in the vestibular nuclei and its various effects on the vestibular nuclei have been reported in several electrophysiological studies. Dimaprit, a selective H2 receptor agonist, produced an excitatory action on rat MVN neurons, and its effect was blocked by ranitidine, a selective H2 receptor antagonist, blocked its effect [32]. Betahistine (strong H3 receptor antagonist and weak H1 receptor agonist), produced a weak excitatory action, but significantly reduced the excitatory effect elicited by histamine on the MVN neurons [32,33]. Thus, the effectiveness of histamine receptor antagonists on motion sickness, vertigo and dizziness can be explained by their action in reducing the excitatory actions in the mammalian vestibular nuclei and/or in the vestibular periphery.

Opioid peptide receptors

The opioid receptors preferentially activated by the enkephalins largely comprise three receptor subtypes (μ, κ, and δ-receptors; G-protein coupled receptors) based on their pharmacological characteristics [34]. In addition to three major receptor subtypes, the opioid-like orphan (ORL1) receptor is another member of the opioid receptor family and is specifically activated by nociceptin. Enkephalins and opioid receptors were found in the vestibular sensory pathway: μ- and κ-opioid receptors in the vestibular hair cells and afferent neurons; δ-opioid and ORL1 receptors in the MVN neurons [4,13,35]. Opioid peptides may be candidates for modulating synaptic transmission in the vestibular afferent neurons. Whole-cell patch clamp recordings of amphibian vestibular hair cells showed that vestibular afferents were excited by μ-receptor.
agonists at the postsynaptic level (postsynaptic activation of afferent neurons) and inhibited by k-receptor agonists at the presynaptic level (inhibition of hair cells), reducing Ca²⁺ currents in the hair cells [36]. This study suggests that the vestibular afferents are regulated by the complex of opioid receptors. Electrophysiological data from mammalian MVN neurons indicated that the 6-opioid receptor agonists inhibited rotation- and glutamate-induced firing rates [37] and ORL1 receptor agonists also inhibited the spontaneous discharge rates [35]. These results suggest that enkephalins modulate afferent transmission at both the peripheral and the central vestibular levels.

Dopamine receptors

Dopamine receptors have been divided into two families: a D1-like family of dopamine receptors including D1 and D5 as well as a D2-like family including D2, D3, and D4 (all G-protein coupled receptors) [38]. Several findings support a possible involvement of dopamine as a modulator of excitatory neurotransmission at postsynaptic afferent terminals [38,39]. In the mammalian vestibular neuroepithelium, immunohistochemical evidence has indicated that dopamine D1 and D2 receptors are expressed in both type I and type II vestibular hair cell membranes [40]. Pharmacological studies using extracellular recordings showed that frog vestibular afferents were excited by EAA agonists and the excitation was suppressed by co-administration of D1 or D2 agonists [39]. This result suggests that the responses of D1 and D2 receptors act not only to modulate glutamate receptors postsynaptically, but also may have a protective function in the vestibular dendrites. In the vestibular nuclei, guinea-pig MVN neurons were depolarized only by D2 agonists; D1 receptors were not involved, suggesting the presence of D2 receptors in the mammalian MVN [41]. In clinical applications, dopaminergic receptor antagonists such as prochlorperazine, promethazine, and domperidone [27] might exert a modulatory action on vestibular afferent neurons.

CGRP receptors

Immunohistochemical studies found that ChAT and CGRP probably co-exist in vestibular efferent neurons [13,42]. Electrophysiological data confirmed the function of CGRP receptor and showed that CGRP released from efferent neurons increased vestibular afferent activities [3]. Sewell and Starr [43] argued that CGRP released from efferent neurons produces an increase in spontaneous discharge rate and this increases the probability of excitatory postsynaptic potentials. Recently, mice with loss of αCGRP gene (-/-) showed a significant reduction in Vestibular Ocular Reflex (VOR) gain, suggesting CGRP dependent effects in peripheral and/or central vestibular pathways [42]. Taken together, the results of these studies regarding CGRP suggest that efferent fibers making contact with both calyx afferents and type II hair cells contain CGRP and CGRP may exert modulatory effects on ACh receptors to increase the excitability of peripheral vestibular afferents.

Immunocytochemistry studies have reported the presence of substance P in mammalian vestibular hair cells and Scarpa’s ganglion [3,4,44] and investigated the coexistence of substance P and CGRP in the rat vestibular end organs using immunocytochemistry. Usami, et al. [44] revealed three different types of immunoreactivities localized within as well as beneath the sensory epithelia in rats. Since most immunoreactivity data clearly indicate that CGRP is associated with efferent fibers, the finding of substance P also supports the coexistence of the two and suggests a possible role for substance P in modulating the peripheral vestibular system.

Conclusion

The current review of the literature reveals that basic research on the pharmacological targets of drugs and medications has provided many answers to questions about the biochemical, molecular and functional nature of neural signaling in the vestibular sensory pathways. If we are to understand the action of drugs and medications, we must first understand the target receptors through which drugs and medications act to produce clinical outcomes. A considerable amount of effort has been carried out to identify neurotransmitters and modulators in the vestibular system using in vitro animal preparations. Medications work by mimicking, suppressing or otherwise altering these normal signaling processes. One may speculate and infer that the lessons learned from study of target processes and mechanisms, although often discovered in vitro, also apply to drug actions in the intact animal. However, in many cases particularly in the case of new and promising medications, drug actions in the intact animal are not fully understood. Therefore, developing reliable direct in vivo assessment tools for the study of vestibular sensory pathways will be important to accurately quantify drug effects on vestibular function. Clinically, in vivo research on vestibular pharmacological targets may aid the development of more accurate and effective intervention strategies for the treatment of dizziness and vertigo.

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Conflicts of interest

The authors have no financial conflicts of interest.

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Pharmacological Targets for Vestibular Disorders

