The metabotropic glutamate receptors of the vestibular organs

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Abstract

This research sought to test the presence and function of metabotropic excitatory amino acid receptors (mGluR) in the frog semicircular canal (SCC). The mGluR agonist ±1-aminocyclopentane-trans-1,3-dicarboxylate (ACPD) produced an increase in afferent firing rates of the ampullar nerve of the intact posterior canal. This increase was not due to a stimulation of cholinergic efferent terminals or the acetylcholine (ACh) receptor, since atropine, in concentrations which blocked the response to exogenous acetylcholine, did not affect the response to ACPD. Likewise, ACPD effects were not due to stimulation of postsynaptic NMDA receptors, since the NMDA antagonist D(-)-2-amino-5-phosphonopentanoate (AP-5) did not affect the response to ACPD, reinforcing the reported selectivity of ACPD for mGluRs. When the SCC was superfused with artificial perilymph known to inhibit hair cell transmitter release (i.e. low Ca-high Mg), ACPD failed to increase afferent firing. This suggests that the receptor activated by ACPD is located on the hair cell. Pharmacological evidence suggested that the mGluRs involved in afferent facilitation belong to Group I (i.e. subtypes 1 and 5). In fact, the Group III agonist AP-4 had no effect, and the ACPD facilitatory effect was blocked by the Group I mGluR antagonists (S)-4-carboxyphenylglycine (CPG) and (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA). Additional pharmacological evidence supported the presence of Group I mGluRs. Interestingly, the mGluR antagonists, AIDA and 4CPG, by themselves did not affect the resting firing rates of ampullar afferents. This may suggest that the mGluRs are not involved in resting activity but perhaps only in evoked activity (as suggested in Guth et al. (1991) Hear. Res. 56, 69–78). In addition, the mRNA for the mGluR1 has been detected in hair cells of both SCC, utricle, and sacculle. In summary, the evidence points to an mGluR localized to the hair cell (i.e. an autoreceptor) which may be activated to produce a positive feedback augmentation of evoked but not resting transmitter release and thus affect afferent activity. © 1998 Elsevier Science B.V. All rights reserved.

Key words: Semicircular canal; Metabotropic glutamate receptor; Vestibular

1. Introduction

Valli et al. (1985) reported that glutamate (Glu) exerted both pre- and postsynaptic effects on the hair cell-afferent synapse of the isolated semicircular canal (SCC) of the frog. Prigioni et al. (1990), using excitatory amino acid agonists, suggested that Glu, as the premier hair cell neurotransmitter candidate, might act presynaptically in a positive feedback manner. Similarly, Starr and Sewell (1990) offered evidence that Glu

antagonists act presynaptically on saccular hair cells to reduce the amount of neurotransmitter released by sound stimulation. This evidence also supported the possibility of an excitatory amino acid autoreceptor on vestibular hair cells that could mediate enhanced neurotransmitter release. Devau et al. (1993) were in accord with this suggestion.

Guth et al. (1991) presented data from several laboratories, including their own, that the release of the primary hair cell neurotransmitter is differentially modulated in the stimulated vs. the unstimulated or resting mode. These authors proposed that the excitatory amino acid autoreceptor may be a part of the
differential modulation in that it may only be activated in the mechanically-evoked mode. Hence, when the sensory epithelium is mechanically stimulated, Glu released by the hair cells may act on a hair cell autoreceptor that enhances neurotransmitter release and thereby greatly increases afferent firing. Such a Glu-mediated positive feedback would be unnecessary and even undesirable in the resting condition.

A presynaptic Glu receptor, whose activation augments transmitter release, has been reported in other structures and seems to be a common feature of glutamatergic synapses (Herrero et al., 1992; Hu and Storm, 1991; Jones and Roberts, 1990; Vazquez et al., 1995). The Glu receptor involved seems to be metabotropic in that it is activated by ±1-aminocyclopentane-1,3-dicarboxylate (ACPD) (Vazquez et al., 1995) and that this activation involves the phospholipase C (PLC)/protein kinase C (PKC) cascade which ultimately results in either the inhibition of a potassium channel or the increase of intracellular free calcium concentrations by affecting both Ca mobilization and influx (Barrie et al., 1991; Vazquez et al., 1995; Glaum et al., 1990). Both of these processes may be involved in Glu’s actions on the hair cell. The observation that Glu and Glu antagonists may act on a presynaptic receptor is not a new one (Colton and Freeman, 1975; Cotman et al., 1986; Thieffry and Bruner, 1978; Usherwood and Machili, 1966). Nevertheless, such a positive feedback is unusual for transmitters as the majority of them exert a negative feedback control over their own release (Vizi, 1979).

In summary, there is the developing notion of an excitatory amino acid autoreceptor on vestibular hair cells which modulates hair cell transmitter release. We have examined the possibility that such an autoreceptor could be of the metabotropic variety by employing pharmacological agents known to act on such receptors as well as molecular biological techniques testing for the presence of appropriate receptor messages.

2. Materials and methods

2.1. Experimental setup

All electrophysiological recordings were made using the isolated labyrinth of R. pipiens. This preparation has been previously described in detail (Guth et al., 1986; Norris et al., 1988). Briefly, the non-stimulated firing of multiple afferent units in the ampullar nerve was recorded from the whole labyrinth using a suction electrode (Fig. 1). This permits detection of changes in the firing rate of much of the afferent fiber population. Resting or non-stimulated firing is considered to be due to activation of the afferents by the tonic, spontaneous release of endogenous transmitter (Guth et al., 1991).

The labyrinth was placed in a 15-ml bath and continuously perfused with artificial perilymph (AP) (NaCl 95 mM; KCl 1.7 mM; NaHCO₃ 3.4 mM; Na₂HPO₄ 2.5 mM; NaH₂PO₄ 0.5 mM; CaCl₂ 1.8 mM; MgCl₂ 0.8; glucose 5 mM, saturated with 95% O₂/5% CO₂, pH adjusted to 7.25 with 1.0 N NaOH) at a flow rate of 3–5 ml/min. A preparation was considered viable if unstimulated firing rates were within the range of 200–500 spikes/s after equilibration in the AP.

All agonists and antagonists used were obtained from Sigma or Tocris-Cookson. The following drugs were employed: agonists: ±1-aminocyclopentane-trans-1,3-dicarboxylate (ACPD), t(+)-2-amino-4-phosphonobutyrate (AP-4), trans-azetidine-2,4-dicarboxylic acid (t-ADA), N-methyl-D-aspartate (NMDA), sodium glutamate (Glu), quisqualate, acetylcholine iodide (ACH); antagonists: (S)-4-carboxyphenylglycine (4CPG), (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA), d(−)-2-amino-5-phosphonopentanoate (AP-5), atropine sulfate. Drugs were applied either by bath substitution or by close injection (4–30 s/50 μl/min) through a multi-barrel perfusion pipette linked to a mechanical syringe pump. This drug delivery pipette was routinely placed about 0.5 mm from the neuroepithelium. Agonists were generally applied by close injection and antagonists by bath substitution or co-injection with the agonists. Acetylcholine was made freshly every day to avoid hydrolytic degradation. trans-ACPD, 4CPG, t-ADA, AP-4, AP-5 and AIDA were dissolved in 200 mM NaOH to a stock concentration of approximately 100 mM; after addition of the drugs to AP, the pH was corrected with 200 mM HCl. As a control, NaOH and HCl in the same concentration used for drug dissolution were added to AP; injection of this
solution did not normally evoke any response. (Note: a response to the control solution was seen when the pH was monitored with an electrode that leaked K⁺. Such electrodes were successfully avoided.) As a control for other drugs, normal Ringer solution was injected.

Standard responses of the SCC were tested by a series of control applications of substances from a pipette. First, several control injections of AP were made. A response to AP would indicate a mechanical stimulation by the injection method or the presence of some active contaminant. If this occurred the pipette would be cleaned, changed or moved until minimal or no response was seen. When the preparation showed only minimal responses to these injections then an injection of ACh (0.1–1 mM solution) was made as a test of diffusional continuity and synaptic accessibility. The response to ACh exhibited marked tolerance if the ACh was injected at short intervals. Thus, to avoid tolerance, all injections of ACh were made at intervals of no less than 10 min. ACh was used because its response is so reliable. However, the preparation was discarded if problems persisted after repositioning of the pipette, failure of 1 mM ACh to elicit any response, or if the baseline exhibited significant spontaneous sloping.

2.2. aRNA methodology

This procedure used to amplify RNA from a single cell combines reverse transcription of the cellular mRNA population into cDNA and re-amplification of RNA from the cDNA template (Van Gelder et al., 1990; Eberwine et al., 1992). This process is made possible by use of an oligo-dT/T7 primer. The oligo-dT/T7 primer consists of oligo-dT residues extended at their 5’ end with the DNA sequence containing the promoter site for the T7 RNA polymerase. The oligo-dT/T7 primer is used to synthesize single stranded cDNA from the cell’s mRNA population using reverse transcriptase (RT); thus the T7 sequence becomes part of the cDNA. After 2nd strand synthesis, the T7 promoter is also made double stranded creating a functional T7 promoter site and the means necessary to reamplify RNA.

Vestibular hair cells were isolated and electrophysiological recordings were made (data not shown). The micropipette (containing internal buffer and amphoter- icin only) remained attached to the cell for about 5–35 min for electrophysiological recordings. The cellular contents were then aspirated and placed in a microfuge tube containing internal buffer, pH 8.3, 2.5 mM each dNTP, 5 ng/ml oligo-dT/T7 primer, and 10 units Avian Myeloblastosis Virus RT (AMVRT; Seikagaku America). First strand cDNA synthesis followed at 37°C for 1 h. This was followed by second strand synthesis, S1 nuclease treatment to remove any hair-pin loops formed during the cDNA synthesis, blunting of the ds cDNA with the Klenow fragment and drop dialysis to remove excess salt using standard methodologies (Sambrook et al., 1989). RNA was made from one-fifth of the recovered volume from dialysis. Using 2000 units T7 RNA polymerase (Epicenter Technologies), aRNA was made off the T7 promoter binding site which had been incorporated into the cDNA using NTPs and radioactive CTP. The aRNA product was phenol/chloroform extracted and ethanol precipitated and then reamplified through a second round of cDNA synthesis followed by aRNA amplification. Size distribution of aRNA transcripts was checked by autoradiography following denaturing agarose gel electrophoresis. The level of incorporation of radioactivity was determined using TCA precipitation to determine the amount of aRNA made. Equivalent amounts of aRNA were added to slot blots containing linearized plasmid clones to determine an expression profile for each cell.

2.3. Expression profiling from individual hair cells

Expression profiling (Athas et al., 1997) uses the radiolabeled aRNA population from a single cell as a probe to hybridize to cloned cDNAs of various candidate genes which have been immobilized on nitrocellulose via a slot blot apparatus. The relative levels of the corresponding mRNAs are reflected by the intensity of the hybridization signal of each of the blotted cDNAs. These plasmid cDNA clones are in vectors of similar sizes, i.e., pUC-based vectors, so equal amounts of each clone (1 µg) were added to each slot well. Pertinent plasmid clones used in this study were: ATCC #65934, GluHI, GenBank accession #M642752 (AMPA receptor clone); ATCC #78526, HHCOP63, GenBank accession #M79013 (mGLuR1); ATCC #194065, HBCV94, GenBank accession #T33422 (mGluR2); ATCC #84920, HBBB25, GenBank accession #T08384 (mGluR3); ATCC #85570, HBBB092, GenBank accession #T09090 (mGluR4).

Plasmid clones (all RNase free) were first linearized with restriction enzymes, denatured, and added to nitrocellulose in slot wells (BIO-DOT SF, Bio-Rad) at 1 µg/200 ml of 10× SSC. The cDNA clones were fixed to the nitrocellulose using a Bio-Rad GS Gene Linker UV Chamber. Prehybridization and hybridization solutions consisted of 50% formamide, 5× SSC, 5× Denhardt’s solution, 1 mM NaPPi and 100 mg/ml salmon sperm DNA and were made with DEPC-treated water. Hybridization proceeded for 48 h at 42°C in a Belco microhybridization oven. Washes were also done under RNase free conditions and consisted of two low stringency washes (2× SSC/0.1% SDS at 42°C×20 min×2) and one high stringency wash (0.1× SSC/0.1% SDS at 50°C×1). After washing, filters were exposed either to film (X-Omat AR5, Kodak) or to a Fuji Imaging Plate for use in a BAS 1000 Bio Imaging Analyzer. We present relative levels of mRNA species in Fig. 7; how-
ever, with further refinement of the technique, quantitative RNA levels may also be determined.

3. Results

3.1. The effects of mGluR agonists in the SCC

ACPD is an mGluR agonist active at both Groups I and II receptor subtypes. Application of ACPD to the neuroepithelium of the posterior SCC produced increases in firing rates of ampullar afferents (see Fig. 2A). Fig. 2B depicts the relationship between concentrations of ACPD applied and its facilitatory effects on afferent firing. The EC$_{50}$ is approximately 40 µM.

Quisqualate, thought to be a poorly selective glutamatomimetic has some agonistic effects at mGluRs, particularly Group I (mGluR$_1$ and mGluR$_5$) (Littman et al., 1995). It causes an increase in afferent firing in the SCC (Annoni et al., 1984; Prigioni et al., 1990). t-ADA, weakly selective for mGluR$_5$ over mGluR$_1$, produced modest facilitation of afferent firing at 1–3 mM (data not shown).

3.2. Site of action of ACPD

In an attempt to determine the site of action of ACPD, the whole labyrinth was bathed in AP containing 0.1 mM Ca and 10 mM Mg. This alteration in ionic composition of the bathing medium is designed to inhibit hair cell transmitter release (Annoni et al., 1984; Valli et al., 1985; Bobbin et al., 1985; Soto and Vega, 1988; Guth and Drescher, 1990). Under these conditions, resting afferent activity ceased (Fig. 3) underscoring the idea that the afferents themselves are silent unless stimulated by the hair cell transmitter (as reviewed in Guth et al., 1991). ACPD was now incapable of causing increases in ampullar afferent activity indicating that its site of action was probably the hair cell and not the afferent neurons per se. To demonstrate that the afferent neurons were still capable of responding, injections of AP with 1 mM Glu were made. These produced a rapid and dramatic rise in afferent firing rates.

**Fig. 2.** A: This trace shows a typical resting firing rate recording from the ampullar nerve of the posterior semicircular canal of the frog. The application of the mGluR agonist ACPD produced a dose-dependent increase in resting multi-unit afferent discharge. The response to 100 µM ACPD lasted 7 ± 1.5 min (n = 8). Con: control. The arrows mark the beginning of drug injections. B: Dose-response curve for ACPD facilitation of afferent discharge. Applications of 100 µM ACPD reversibly increased spontaneous firing rate to 132 ± 8.9% (n = 8) of control values (baseline noise was 8 ± 2.9% in the absence of agonist). The EC$_{50}$ for ACPD action was 35.4 ± 8.5 µM; the dose-response saturated above 300 µM (n = 4). Nfr: nerve firing rate.

**Fig. 3.** The trace represents resting firing rate recording from whole ampullar nerve; recording was interrupted during substitutions of the bath for at least 30 min. The perfusion of the labyrinth with a low-Ca high-Mg solution is known to block transmission at the afferent synapse (Valli et al., 1985). While blocking transmitter release with a low-Ca high-Mg Ringer solution (horizontal arrow), the effects of 100 µM ACPD on the afferent discharge became negligible; however, 1 mM glutamate (Glu) was still able to increase the resting discharge (Glu was applied for 20 s in the first application and for 10 s in the second application). This suggests that the site of ACPD action is presynaptic. The high resting discharge observed in the presence of Ca is due to neurotransmitter released by hair cells in resting conditions; accordingly, the block of neurotransmitter release produces a dramatic drop in the resting firing rate.

**Fig. 4.** This trace shows a continuous recording from a single semicircular canal; interruption of the traces represents washing periods. Bath-applied atropine (3 µM) completely suppressed the response to 300 µM ACh, but it did not affect the response to 100 µM ACPD. The horizontal arrow indicates the duration of atropine perfusion. Con: control.
3.3. Testing the possibility that ACPD could be acting at non-mGluR sites

3.3.1. Acetylcholine (ACh) receptors

Acetylcholine, the primary efferent transmitter, has been shown to cause an increase in ampullar afferent firing rates by an action on hair cell ACh receptors (Bernard et al., 1985; Guth et al., 1986; Norris et al., 1988; Housley et al., 1990; Guth et al., 1994; Guth and Norris, 1996). Since ACPD has been shown to increase transmitter release (Schrader and Tasker, 1997) and it mimics ACh in the SCC in regard to facilitation of afferent firing, it seemed reasonable to test whether ACPD was acting indirectly through ACh. In the SCC, atropine at low concentrations (e.g. 3 μM) antagonizes the facilitatory effect of applied ACh (reviewed in Guth and Norris, 1996). Therefore, atropine (3 μM) was applied by bath substitution. The responses to ACh and ACPD on ampullar afferent activity before and after atropine were compared (Fig. 4). While this concentration of atropine completely blocked the effect of ACh, no interaction between atropine and ACPD was seen. This suggests that ACPD neither acts by releasing ACh from efferent endings nor by acting on muscarinic receptors in the SCC.

3.3.2. Non-mGluR glutamate receptors

Other so-called selective agonists for mGluRs such as quisqualate and 2,3-dicarboxycyclopropylglycine have been seen to have measurable activities at AMPA and NMDA receptors (Schoepp, 1994; Wilsch et al., 1994). Both AMPA and NMDA receptors appear to exist in the SCC (Prigioni et al., 1990; Prigioni et al., 1994; Zucca et al., 1993; Soto et al., 1994; Cochran and Correia, 1995; Devau et al., 1993). To eliminate the possibility that ACPD, used as an mGluR test-probe, was acting on NMDA receptors, either directly or in concert with the transmitter (see Conn and Pin, 1997) the NMDA selective antagonist N(-)-2-amino-5-phosphonopentanoic acid (AP-5) was employed. Fig. 5 depicts the antagonism of NMDA-induced facilitation of SCC afferent firing by AP-5. Note that the ACPD-induced facilitation is unaffected. This suggests that the ACPD is not acting on or influencing an NMDA-type
receptor, at least at the concentrations used. The enhancement of the responses to ACh and ACPD after AP-5 was unexpected but is reproducible and as yet unexplained.

3.4. Antagonism of ACPD by mGluR antagonists

Two different mGluR antagonists were pitted against the facilitatory effect of ACPD. One was (S)-4-carboxyphenyl-glycine (4CPG) and the other was (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA). Both are said to be Group I-selective mGluR antagonists (Sekiyama et al., 1996; Pelliciari et al., 1995). 4CPG antagonized ACPD in a dose-dependent manner (Fig. 6) and at 1 mM completely blocked the effect of 100 μM ACPD. AIDA was partially effective at 1 mM and completely effective at 3 mM against 100 μM ACPD (data not shown). These results suggest, if the reported selectivity of these antagonists is to be trusted, that ACPD may be activating a subset of Group I mGluRs. This subset may be the mGluR<sub>1</sub> (Sekiyama et al., 1996) since both 4CPG and AIDA affect these receptors and t-ADA (data not shown), which was weakly effective, prefer mGluR<sub>3</sub> to mGluR<sub>1</sub> (Conn and Pin, 1997). When applied alone, neither mGluR antagonist, 4CPG (Fig. 6) or AIDA, affected resting activity.

3.5. Expression profiling of individual vestibular hair cells

The focus of this study has been on elucidating the phenotype of metabotropic glutamate receptors in the vestibular system. Using expression profiling, we have investigated the expression of metabotropic glutamate receptors (mGluR<sub>1–4</sub>) from individual hair cells from the three vestibular organs. Fig. 7 shows representative slot blots using radioactive aRNA from the three cell types as probes (SCC and sacculo: n = 3; utricle: n = 1). Note the strong hybridization signal for mGluR<sub>1</sub>. Since the blots were subjected to a high stringency wash and the negative control plasmid (pUC18) shows no signal, we can be confident that the strong hybridization signal for mGluR<sub>1</sub> is true. No probe for mGluR<sub>3</sub> was available at the time of this study. The mGluR subtypes 2 and 3 are members of the Group II mGluRs and subtype 4 is a member of the Group III mGluRs. Thus evidence is presented only for the presence of Group I mGluRs in type II vestibular hair cells.

4. Discussion

The SCC provides information to the brain about movement of the head no matter which way the head turns and which way the stereocilia are bent. This is accomplished by having the hair cell release its transmitter continuously at rest so that the afferents fire under non-stimulated conditions, thus allowing bi-directional modulation of the resting activity by the graded release of hair cell transmitter in response to graded mechanical stimulation. However, a problem arises precisely from this continuous resting activity. When an important excitatory stimulus is to be transmitted to the afferents its signal-to-noise ratio (S/N) is compromised by the constant background activity. If, however, a mechanically-evoked signal of importance could cause a disproportionately large release of hair cell transmitter then the S/N ratio could be enhanced. The solution to the enhanced transmitter release in the evoked but not the resting mode could be solved by a positive feedback autoreceptor. This function could be served by a Group I metabotropic glutamate receptor (mGluR) as it is in the central nervous system (Nicoletti et al., 1996).

The mGluR family is composed of eight subtypes, subdivided into three groups according to structures, second messengers involved and pharmacology. Group I consists of sub-types 1 and 5; Group II of sub-types 2 and 3; and Group III of sub-types 4, 6, 7 and 8. mGluR<sub>1</sub> also has splice variants a, b, c and d and mGluR<sub>3</sub> has splice variants a and b. The pharmacology of all the mGluR<sub>1</sub> variants is identical (Conn and Pin, 1997). Group I mGluRs are coupled negatively to K<sup>+</sup> channels (Gluam and Miller, 1995) and positively to L-type calcium currents in cerebellar granule cells (Chavis et al., 1995). There is also such a receptor coupled to phosphoinositide hydrolysis whose activation leads to increased intracellular Ca<sup>2+</sup> and increased Glu release (Herrero et al., 1992). Lee and Boden (1997) adduced evidence that the mGluR Group I agonist used in this research, ACPD, causes depolarization by activating a Ca-Na exchanger. The activation of the other two groups of mGluRs involves inhibition of cAMP and is generally thought to inhibit transmitter release via negative coupling to voltage-operated Ca channels (Nicoletti et al., 1996).

The recruitment of the hair cell mGluRs in evoked but not the resting mode could be accomplished in several ways. Either the mGluRs could have low affinities or they could be located extrasynaptically (as occurs in some brain glutamatergic synapses (Nicoletti et al., 1996; Lujan et al., 1996; Baude et al., 1993), so as not to respond to the lower concentrations of transmitter released in the resting mode. Because the affinity of glutamate for the mGluRs is about an order of magnitude greater than for the AMPA receptor (the dominant glutamate receptor on vestibular afferents; see Guth et al., 1997) the latter possibility seems most likely. The work of Lujan et al. (1997) also favors the latter possibility in that Group I mGluRs were localized to a perisynaptic annulus within 60 nm of the synapse in the hippocampus. These authors suggest that the
distinct patterns of mGluR distribution may reflect specific spatial requirements for different effector mechanisms (authors’ note: such as resting and evoked activity). Thereby, the distant mGluRs would not be stimulated in the resting mode with its limited transmitter release but only when synaptic transmitter concentrations reached higher, critical levels able to diffuse to the distant receptor in effective concentrations as in the stimulated mode. This view is supported by the work of Scanziani et al. (1997) in brain who demonstrated activation of mGluRs only when the release of glutamate was enhanced by increased frequency of stimulation. In our preparation, the mGluRs are probably not involved in resting mode activity as attested by the inability of the mGluR antagonists, 4CPG and AIDA, to affect resting firing rates.

ACPD clearly increases afferent firing rates of the SCC in a dose-dependent manner. Its site of facilitatory action is the hair cell as shown by its lack of effect when hair cell transmitter release is inhibited in low Ca-high Mg solution. ACPD does not seem to work indirectly through NMDA receptors. Finally, ACPD’s effect is blocked by the mGluR antagonist 4CPG. These findings strongly support the hypothesis of an mGluR on hair cells of the SCC which may be involved in the evoked-mode activation of the afferents. It bears mentioning that even though the mGluRs may be recruited physiologically only in the evoked mode, they are still available for activation by exogenous mGluR agonists in the resting mode as was done in this research.

The hypothesis under consideration is that there are functional, positive-feedback autoreceptors on vestibular hair cells which are activated physiologically in the stimulated but not the unstimulated state (Fig. 8). The use of pharmacological agents alone rarely provides incisive testing of such hypotheses. The lack of incisiveness of drugs as hypothesis testers lies in their selectivities or lack thereof. Only rarely does a drug influence only one receptor or enzyme and the selectivity is always dose-dependent. Pharmacologists have learned that a substance’s selectivity is inversely proportional to the number of publications in which it appears. All the above notwithstanding, drugs are often very useful guideposts and easily used hypothesis-testing tools. The results of pharmacological experiments urge or inhibit the investigator from more incisive tests of the hypothesis. To this end we have performed molecular biological testing which found the presence of the mRNA for mGluR

Fig. 8. This diagram depicts the putative role of mGluRs in semicircular canal afferent discharge. Glutamate (small dots) released by the hair cell acts both on the ionotropic receptors (both NMDA and non-NMDA) present on the afferent terminal (producing EPSPs) and on the mGluRs probably located on hair cells themselves. The activation of mGluRs induces a facilitation of glutamate release, through an uncharacterized mechanism (the arrows with a question mark indicate possible interactions with presynaptic Ca or K channels or modulation of the synaptic apparatus) thus producing a positive feedback at the afferent synapse. The positive feedback induced by mGluR₂ activation probably increases the gain of afferent synapses. Since the Glu concentration at the afferent synapse is most likely lower at rest than under mechanical stimulation of hair cells, presynaptic facilitation may be more important in the latter condition, therefore providing a rationale for the differential modulation of spontaneous and evoked discharge of the ampullar nerve (Guth et al., 1991).

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HEARES 3109 29-10-98
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