Differential modulation of spontaneous and evoked neurotransmitter release from hair cells: some novel hypotheses

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It has been generally accepted that even in the absence of mechanical stimulation of the transductional elements, a resting depolarizing current exists which is ultimately responsible for the spontaneous release of neurotransmitter. Movement of the transductional elements modulates this resting current and thereby the evoked release of neurotransmitter occurs. Recent data from our laboratory and others have led us to question whether the relationship between spontaneous and evoked neurotransmitter release is as simple as stated. Indeed, a variety of experimental manipulations appear to influence the two modes of release differently. Examination of our results and the results of others has led us to four hypotheses:

1. the two modes of neurotransmitter release are processed differently by the hair cells;
2. cyclic AMP is involved in spontaneous but not evoked neurotransmitter release;
3. there is a positive feedback step involving an excitatory amino acid and its receptor on the hair cell in evoked neurotransmitter release;
4. different pools of calcium are involved according to the mode of release.

Accordingly, there may be several biochemical steps between the transductional movement of the stereocilia at the apex of the hair cells and the ultimate release of the neurotransmitter at the base of these cells. Some of these biochemical steps are different depending on whether the mode of release is spontaneous or evoked. These biochemical steps may amplify or at least interact with the biophysical processes previously described in the hair cells.

Hair cell; Neurotransmitter; Octavolateralis; Semicircular canal; Glutamate; Calcium

Introduction

The production of action potentials in the afferent neurons depends completely on transmitter release from hair cells.

The neurosensory epithelium of the inner ear organs contains hair cells which synapse on their basolateral (perilymphatic) sides with afferent and efferent neurons. In the absence of stimulation of the neurosensory epithelium the afferent fibers innervating the hair cells of the semicircular canal (SCC), and most probably the other acousticolateralis organs, exhibit a spontaneous firing rate (spontaneous activity) which is believed to be entirely dependent on a 'basal' release of the neurotransmitter from the hair cells. The evidence supporting the assertion that the afferent neurons themselves are not spontaneously active but that their firing depends entirely on a basal release of neurotransmitter is as follows:

1. Manipulations designed to inhibit neurotransmitter release from hair cells such as replacement of normal perilymph with a solution containing high Mg2+ and low Ca2+ (SCC-Valli et al., 1985; Cochlea-Siegel and Relkin, 1987) or cobalt application (Lateral line-Sewell, 1990) cause the afferent fibers innervating these hair cells to become silent. However, the role of charge screening caused by these divalent cations in the silence of the afferents must be considered.
2. Application of excitatory amino acid antagonists (eg. kynurenate) (Annoni et al., 1984; Soto and Vega, 1988) or spider venom (Cousillas et al., 1988) or spider venom (Cousillas et al., 1988) can cause complete cessation of firing of the afferents.
3. In the absence of hair cells, apparently normal cochlear afferents exhibit no spontaneous activity (Durham et al., 1989).
4. Afferent fibers became silent when deprived of hair cell input by acute (Bernard, 1983) or chronic (Norris et al., 1990) aminoglycoside treatment.
5. The frequency of afferent firing in type I neurons goes to zero when the hair cells are transductionally hyperpolarized (Precht, 1976).
6. Passing D.C. currents across the neuroepithelium of the SCC (Fig. 2) in which endolymph is negative with regard to the perilymph can cause the afferents to become silent when the current amplitude is sufficiently high (Ricci et al., 1991). These currents are...
thought to act only on the hair cells for several reasons. First, passage of hyperpolarizing currents affects spontaneous and mechanically-evoked afferent firing differently. If the current were acting on the afferents themselves both spontaneous and mechanically-evoked transmission might be equally affected. Second, during the passage of hyperpolarizing current, the afferents may be caused to fire by the application of a solution containing 10 mM potassium.

7. When endolympathic K\(^+\) is replaced by Na\(^+\) and tetraphenylboron is added to the endolymph, while perilymph is kept unchanged, the afferents become silent (Valli et al., 1985).

8. \(\alpha\)-tubocurarine in endolymph (not perilymph) can completely reduce spontaneous and evoked afferent neural activity (Valli et al., 1974) presumably by interfering with transduction.

9. Electrical stimulation of the efferents which end primarily, if not totally, on hair cells of the lateral line (Russell and Roberts, 1972) or the frog semicircular canal (Valli et al., 1986) can cause total suppression of spontaneous afferent firing. Therefore, the recording of the afferent neuronal activity provides a dependable means of monitoring the release of neurotransmitter from the hair cells.

**Differential modulation of ‘spontaneous’ and ‘evoked’ afferent activity**

Classically, a resting current across the transductional elements is believed to account (through the release of neurotransmitter) for the generation of the spontaneous afferent activity. Modulation of this current by the mechanical stimulation of the sensory epithelium is responsible for the modulation of the firing rate which is called evoked activity. This current is mainly carried by the entry of K\(^+\) (Valli et al., 1979; Hudspeth, 1983) and is accompanied by the entry of Ca\(^{2+}\) (Ohmori, 1989) from endolymph. Thus movement of the stereocilia toward the kinocilium induces a depolarization of the hair cells (transductional depolarization) which is accompanied by an increase of the firing rate of the afferent neurons presumably by an increase of the release of the neurotransmitter from the hair cells. In contrast, the decrease in the firing rate recorded when the stereocilia are displaced in the opposite direction is caused by a hyperpolarization of the hair cells (transductional hyperpolarization) and apparently a reduction of the hair cell neurotransmitter release.

These events involve the interactions of biophysical mechanisms such as entry of K\(^+\) through transduction channels and subsequent electrical modulation of voltage-sensitive Ca\(^{2+}\) channels, calcium-dependent K\(^+\) channels and K\(^+\) channels. In this paper attention is drawn to the possible interactions of membranal and intracellular biochemical mechanisms with the above-mentioned biophysical processes. This paper suggests that the modulation of the release of neurotransmitter under spontaneous and mechanically evoked conditions is achieved by different although complementary processes and that these processes are largely biochemical.

**Differences between ‘spontaneous’ and ‘evoked’ afferent activity**

A series of observations, by ourselves and others (Bernard, 1980; Drescher and Drescher, 1987; Starr and Sewell, 1990; Gleich et al. 1990), has led to the conclusion that there are differences between spontaneous and mechanically evoked afferent activity as regards mechanisms responsible for the release of the hair cell neurotransmitter. These observations are that evoked and spontaneous neurotransmitter release can be differentially affected by a variety of chemical and physical manipulations.

The observations given in the Tables I and II illustrate several general differences between spontaneous and evoked neurotransmitter release.

Note that evoked and spontaneous activities may be independently modulated to cause either an increase or a decrease in the release of the hair cell neurotransmitter. Note also that either evoked or spontaneous release of transmitter may be the more readily affected. Taken together the observations of Tables I and II strongly suggest that the spontaneous and evoked modes of neurotransmitter release are modulated by different mechanisms and that these mechanisms operate presynaptically in the hair cells. Any change in the afferent dendrite would likely affect both spontaneous and evoked activity similarly. Of particular clarity is the demonstration that a D.C. current flowing across the neuroepithelium of the SCC can, depending on its intensity and duration, cause a complete abolition of evoked neuronal activity but only a partial inhibition of

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**Table I**

<table>
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<th>Description</th>
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<tr>
<td><strong>Evoked neurotransmitter release is affected more than spontaneous.</strong></td>
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<tr>
<td>A. Glutamate antagonists reduced evoked more than spontaneous firing.</td>
<td>1. Lateral line (Bledsoc et al., 1988)</td>
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<td>2. Cochlea (Coussillas et al., 1988)</td>
<td>3. Saccula (Starr and Sewell, 1990)</td>
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<td>B. In the cochlea, perfusions of glutamate caused a reduction in tone-evoked activity without a change in spontaneous rate (Gleich et al. 1990).</td>
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<td>C. In the semicircular canal electrical D.C. currents across the neuroepithelium, in which the endolymph was negative relative to the perilymph, decreased evoked more than spontaneous afferent activity (Fig. 2). When of sufficient amplitude the current can completely inhibit all afferent activity.</td>
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<td>D. In the lateral line, evoked activity has been shown to be more affected than spontaneous with decreasing calcium and increasing magnesium concentrations (Drescher and Drescher, 1987).</td>
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A. In the semicircular canal an increase of cyclic AMP (cAMP) produced an increase in spontaneous, but not evoked firing rates (Fig. 3).
B. In the semicircular canal Ach application produced an increase in spontaneous, but not evoked firing rates (Fig. 4).
C. In the semicircular canal application of a calcium ionophore caused an increase in spontaneous but not evoked activity. (Aubert et al., 1991).
D. In the semicircular canal 4-aminopyridine (4-AP) caused a decrease in spontaneous which was much greater than the decrease in evoked activity (Fig. 5), (Ricci, et al., 1991).
E. In the lateral line, salicylate decreased spontaneous more than evoked activity (Puel et al., 1989).
F. 1. In the semicircular canal, (Fig. 6) and in cochlea (Siegel and Relkin, 1987) substituting low Ca\textsuperscript{2+}, high Mg\textsuperscript{2+} solutions for perilymph caused a marked reduction in spontaneous activity before the subsequent reduction in evoked activity occurred.
2. Likewise, diltiazem or streptomycin (thought to act also on Ca\textsuperscript{2+} channels) both caused spontaneous activity to fail before evoked activity (A. Ricci-Personal communication).
3. In the lateral line, spontaneous activity is affected more than evoked for 2-4 mM calcium (Drescher and Drescher, 1987).
G. In the semicircular canal, D.C. currents across the neuroepithelium in which the endolymph is positive to the perilymph increased spontaneous activity much more than evoked (Ricci et al., 1991).

Methods

A schematic of the experimental preparation is given in Fig. 1. The posterior semicircular canal of the frog, *Rana pipiens*, was isolated from the labyrinth. This canal was then mounted in a two chambered bath in which the endolympathic and perilymphatic spaces could be maintained independently. The endolymphatic solution contained: 75 mM KCl, 50 mM NaCl, 2 mM CaCl\textsubscript{2}, 5 mM NaHCO\textsubscript{3}, and 5 mM NaH\textsubscript{2}PO\textsubscript{4}. The artificial perilymph solution contained: 2.5 mM KCl, 105 mM NaCl, 2 mM CaCl\textsubscript{2}, 5 mM NaHCO\textsubscript{3}, 5 mM Na\textsubscript{2}HPO\textsubscript{4}, and 5 mM NaH\textsubscript{2}PO\textsubscript{4}. All solutions were bubbled to saturation with a gas mixture of 95% O\textsubscript{2} and 5% CO\textsubscript{2}. The pH of each solution was adjusted to 7.2 with NaOH (Norris et al., 1988, Valli et
which the equipment responded linearly. The gain of all afferent firing data were normalized as percent of calibration. This allowed for increases in mechanically- or chemically-stimulated firing to occur, over a range in mechanical stimulator was set at the beginning of the experiment, the window was first set above the electronic noise level and then raised so that the afferent nerve firing never exceeded the upper limits of the calibration. This allowed for increases in mechanically- or chemically-stimulated firing to occur, over a range in which the equipment responded linearly. The gain of the mechanical stimulator was set at the beginning of each experiment so that a sub-maximal firing rate of the afferent neurons was achieved, thus allowing for either increases or decreases in firing to be recorded. All afferent firing data were normalized as percent of the control data. No experimental data were included in the analysis if the effects of the drugs applied were not reversible by washing to within 95% of control values. Measurements were made as follows: spontaneous activity was averaged over one minute prior to any drug application. Mechanically evoked activity was measured as the increase in frequency of firing over spontaneous activity. Five cycles of stimulus were averaged once the activity had stabilized. Similar measurements were made during a cAMP-altering drug application once the response had equilibrated. Measurements were made again after each drug was washed out. All changes in firing were compared using a two-tailed Student's t-test with significance chosen to be at the P = 0.05 level. The transepithelial potential was measured as the potential difference between the endolymphatic and perilymphatic solutions. The change in transepithelial potential induced by mechanical stimulation is a measure of the electrical state of the hair cell. It was measured differentially between silver/silver chloride electrodes placed in the endolymph and perilymph respectively. The signal was taken across a Grass amplifier (1000×) and displayed on an oscilloscope and strip chart recorder. The two parameters of transepithelial potential observed were a change in baseline and a change in magnitude of the response induced by the mechanical stimulus. Only relative changes were recorded in the transepithelial potential.

In all experiments ACh was applied to the neuroepithelium of the semicircular canal as an injection of 10 μl of a 300 μM solution over 10-s period. A threaded syringe attached to a glass pipette was utilized for the administration of the bolus. Artificial perilymph was administered as the control injection.

All other drugs were applied by bath substitution to the perilymph solution at the indicated concentrations. Forskolin was first dissolved in 95% ethanol and then diluted in artificial perilymph. Controls of ethanol in perilymph were carried out and no effect was noted in the parameters studied at the ethanol concentrations (0.5%) present in artificial perilymph at the final drug dilutions. 3-isobutyl-1-methylxanthine (IBMX) and Dibutyl cAMP were dissolved directly in perilymph. All chemicals were purchased from Sigma Chemical Company.

Results

D.C. electrical stimulation

Fig. 2 depicts the differential effect of electrical stimulation with D.C. currents as mentioned in Table 1B. Such electrical stimulation completely inhibits the response of the afferent neuron to mechanical stimulation, but spontaneous activity remains, albeit at a reduced level. This result not only demonstrates the primary point of this paper (i.e. differential modulation of spontaneous and evoked transmitter release) but suggests that such D.C. electrical stimulation acts primarily on the hair cells and not on the afferent neurons. If it acted primarily on the postsynaptic element, the afferent neuron, then both spontaneous and evoked activity might be equally affected or even that spontaneous activity might be the more affected because there might be less transmitter release under the spontaneous than under the evoked condition. It is worthy of note that as the current is increased, it is the
Fig. 3. Three drugs known to cause increases in cAMP were applied. This figure illustrates the effect of drugs expected to cause an increase in cyclic AMP concentrations. The responses to all three such drugs—forskolin, IBMX and dibutyryl cyclic AMP were all qualitatively similar. This preparation is as depicted in Fig. 1. The upper trace depicts the transepithelial potential measured across the neuroepithelial layers of the isolated semicircular canal and at least partially reflective of hair cell activity. The lower trace is of multiunit afferent firing rate recorded from the cut ampullar nerve. Spontaneous afferent firing is increased by applying cAMP-elevating drugs. However, the response to intermittent mechanical excitation (M.E.) (shown in the lower trace as tall regular fluctuations in the afferent firing rate) and the response to acetylcholine (20 μl of a 1 mM solution applied by injection close to the neuroepithelium indicated by Ach) are not modified. The frequency is in multiunit afferent impulses per second. The dashed horizontal line in the lower trace indicates basal spontaneous activity.

depolarizing (upward) response to mechanical stimulation that is inhibited first. The hyperpolarizing (downward) response is maintained until the 40 μA current level is reached.

Elevation of cyclic AMP

Only the response to forskolin, the stimulator of adenyl cyclase is shown (Fig. 3). However, all the drugs used to produce an elevation of cAMP (dibutyryl cAMP and the cAMP phosphodiesterase inhibitor IBMX) caused similar changes in the transepithelial potential and in the spontaneous rate of afferent firing. Note that while the transepithelial potential is falling and the spontaneous firing rate is increasing, there is no change in the response to Ach or mechanical stimulation (as noted in Table II A).

Differential effect of Ach on evoked and spontaneous activity

It can be seen from Fig. 4 that Ach application increased spontaneous afferent activity without increasing mechanically evoked activity. It may even be

Fig. 4. Effect of acetylcholine on spontaneous and mechanically evoked afferent activity. The preparation is as depicted in Fig. 1 and described in Methods. The trace is of multiunit activity recorded from the cut end of the ampullar nerve and is expressed as impulses per second (frequency). Ach indicates the response to an injection of 20 μl of a 1 mM solution of acetylcholine close to the neuroepithelium. Evoked indicates the mechanical stimulation of the semicircular canal. The evoked activity, marked by the dot, points out a cycle of mechanical stimulation of higher volume than the other cycles. This was done in order to illustrate the ability of the preparation to increase in rate and the electronics to record such increase. The dashed curve emphasizes the response of the spontaneous afferent activity to Ach application during mechanically-evoked afferent activity. This trace demonstrates that the response to Ach increases spontaneous activity but does not affect mechanically evoked activity (or even decreases it). Frequency is measured in impulses per second.
argued that Ach caused a reduction in evoked activity suggesting that the response to Ach and mechanical stimulation somehow compete or arc physiological antagonists. Ach is thought to act on the hair cell by means of two receptors, one atropine-preferring and the other strychnine-preferring (Norris et al., 1988). The atropine-preferring receptor acts to depolarize the hair cell by reducing the hyperpolarizing calcium-dependent potassium current (Housley et al., 1990). The site of this competition might be a pool of calcium required for transmitter release. This competition is also suggested by Fig. 6. That is, in the face of a high Mg\(^{2+}\)-low (10 mM) Ca\(^{2+}\) external (0.1 mM) solution, despite the fact that spontaneous activity has already failed, hair cell transmitter can still be released to cause afferent firing either by Ach or mechanical stimulation. But, when the effect of either Ach or mechanical stimulation is exhausted, the as-yet-unapplied stimulus also becomes ineffective. This parallel exhaustion suggests that both stimuli operate on similar intra-hair cell mechanisms, for instance intracellular calcium pools. Other agonists such as glutamate when injected increase spontaneous activity without affecting evoked activity (Carlos Erostegui, personal communication).

**Fig. 6.** Usage of the procedure previously described (Fig. 1). The perilymphatic solution was replaced with a high magnesium, low calcium solution (10 mM, Mg\(^{2+}\); 0.1 mM, Ca\(^{2+}\)). These results clearly demonstrate that the response to mechanical stimulation evoked is much less susceptible to changes in extracellular calcium than the spontaneous neurotransmitter release which rapidly goes to zero. Ach at the arrow indicates a 10 \(\mu\)l bolus of acetylcholine (1 mM) applied to the perilymphatic side of the ampulla. Similar results to those seen in high magnesium-low calcium were obtained using the calcium channel blockers diltiazem, cadmium, cobalt or streptomycin. Frequency is measured as multiple unit afferent impulses per second.

The differential effect of 4-AP

An entire publication describing the effects of 4-AP on the SCC is forthcoming (Ricci et al., 1991). These data (Fig. 5) are only included to demonstrate the entire dose-response curves of the differential effects of this agent on spontaneous and evoked activity as in Table IID.

**Fig. 5.** These dose-response curves were obtained from application of 4-aminopyridine (4-AP) into the perilymph of the preparation depicted to Fig. 1. Data has been normalized as percentages of the control and errors bars represent standard errors. These curves clearly demonstrate that spontaneous and mechanically evoked firing can be regulated independently.

**The role of cyclic adenosine monophosphate (cAMP)**

The expected increases of cAMP levels caused by applying dibutyryl cAMP, stimulating adenylyl cyclase
with forskolin or by inhibiting cAMP catabolism by mean of the phosphodiesterase inhibitor IBMX (3-isobutyl-1-methylxanthine) all led to increases in spontaneous activity with no change or even slight reductions in evoked activity (Fig. 3). This result suggests a role for cAMP in the control of the spontaneous but not the evoked release of the afferent neurotransmitter (Ricci et al., 1991c). The effect of manipulations designed to increase cAMP levels is probably exerted on the hair cells. For, if the effect were to take place postsynaptically then both evoked and spontaneous firing would likely be affected.

A putative glutamate autoreceptor on the hair cell

Hair cells have been shown to release glutamate in a calcium-dependent manner (Jenison et al., 1985; Drescher et al., 1987b). Although Drescher et al. (1987) found that glutamate release was not inhibited by a supranormal magnesium concentration (10.1 mM). Several groups have reported that both glutamate antagonists and spider toxins, known to oppose the action of glutamate, reduced the evoked afferent activity more than the spontaneous (Bledsoe et al., 1988) (recording from \textit{Xenopus laevis} lateral line afferents) Cousillas et al. (1988) (recording from the cochlear afferents; spider toxin study); and Starr and Sewell (1990) (recording from goldfish saccular afferents). Valli et al. (1985) found that glutamate and one of its antagonists, alpha-amino-adipate, exerted presynaptic effects on the release of neurotransmitter from the hair cells of the semicircular canal. Prigioni et al. (1990) using excitatory amino acid agonists not only confirmed the earlier finding (Valli et al., 1985) of both pre- and postsynaptic effects of excitatory amino acids but suggested that

![Fig. 7. Schematic illustrating the different events involved in the spontaneous (left) or evoked (right) release of the neurotransmitter from the hair cells of the frog semicircular canal according to the novel hypotheses presented in this paper. The solid lines and arrows point out generally accepted processes while the dashed lines and arrows correspond to the novel hypotheses reported in this paper. (Left) In the absence of mechanical stimulation of the neurosensory epithelium, a resting influx of $K^+$, through the transductional elements (1), is believed to be responsible for the activation of voltage-dependent calcium channels (2), located on the basolateral side of the hair cells. The resulting influx of calcium into the sensory cells is in turn responsible for the spontaneous release of the neurotransmitter (3). The possible role of a calcium influx from the endolymph (4), in the spontaneous mode of neurotransmitter release, has not yet been determined. The spontaneous release of the neurotransmitter is enhanced by an increase in cAMP levels or by a local application of acetylcholine (Ach, 5). Although Drescher et al. (1987)

![Fig. 7. Schematic illustrating the different events involved in the spontaneous (left) or evoked (right) release of the neurotransmitter from the hair cells of the frog semicircular canal according to the novel hypotheses presented in this paper. The solid lines and arrows point out generally accepted processes while the dashed lines and arrows correspond to the novel hypotheses reported in this paper. (Left) In the absence of mechanical stimulation of the neurosensory epithelium, a resting influx of $K^+$, through the transductional elements (1), is believed to be responsible for the activation of voltage-dependent calcium channels (2), located on the basolateral side of the hair cells. The resulting influx of calcium into the sensory cells is in turn responsible for the spontaneous release of the neurotransmitter (3). The possible role of a calcium influx from the endolymph (4), in the spontaneous mode of neurotransmitter release, has not yet been determined. The spontaneous release of the neurotransmitter is enhanced by an increase in cAMP levels or by a local application of acetylcholine (Ach, 5). Although Drescher et al. (1987)
these amino acids may function presynaptically in a positive feedback manner. Starr and Sewell (1990) suggested that glutamate antagonists act presynaptically on the saccular hair cells to reduce the amount of neurotransmitter released by sound stimulation. These data, indicating the existence of a glutamate receptor on the hair cell, suggest that there is normally a glutamate-mediated recruitment of the afferent neurotransmitter during the evoked release but not during the spontaneous release mode. Guth et al. (1988) have in fact produced evidence that glutamate does not appear to be involved in the spontaneous release of the neurotransmitter. These authors showed that of two manipulations of the SCC leading to a loss in response to applied glutamate neither caused a change in the spontaneous multiple unit afferent firing rate. These two manipulations were: applications of glutamate in quick succession to cause glutamate desensitization and bath application of the enzyme glutamate decarboxylase to catabolize extracellular glutamate. Likewise, Gleich et al. (1990) found that glutamate application to the point of desensitization did not affect the spontaneous rate in cochlear afferents. In contrast, Rebillard and Bryant (1989) employed the enzyme glutamate dehydrogenase in perfusions of the cochlea to test whether catabolism of glutamate would alter cochlear electrophysiology. Unfortunately, these authors did not examine spontaneous activity but only sound-evoked compound action potentials. Their finding of a shift to the right of the input-output curve to compound action potential comports with the suggestion that glutamate may be involved in evoked activity. Thus, when the sensory epithelium is mechanically stimulated, glutamate release by the hair cells may act on a hair cell autoreceptor that potentiates neurotransmitter release and thus greatly increases afferent firing. Such a glutamate-mediated positive feedback is unnecessary and even undesirable in the spontaneous condition.

The observation that glutamate and glutamate antagonists may act on a presynaptic receptor is not a new one (Usherwood and Machili, 1966; Florey and Woodcock, 1968; Colton and Freeman, 1975; Thieffry and Bruner, 1978; Cotman et al., 1986; Forsythe and Clements, 1988). Nevertheless, such a positive feedback is unusual for transmitters, the majority of them antagonists may act on a presynaptic receptor as described in the cochlea (Bobbin and Thompson, 1978; Jenison et al., 1986) and SCC (Valli et al., 1985) and such an action by glutamate or other excitatory amino acid cannot be ruled out.

The roles of various calcium sources in the spontaneous and evoked modes of transmitter release

The manipulations of $\text{Ca}^{2+}$ concentrations and fluxes listed in Tables I and II suggest not only that $\text{Ca}^{2+}$ is involved in both spontaneous and evoked release of transmitter but that its involvement in the two modes of release is different. Calcium is probably in the final common path to transmitter release in both the spontaneous or evoked modes. However, the source of the calcium in the two modes may be different. The most parsimonious explanation for the results seen with calcium channel blockers and with low $\text{Ca}^{2+}$-high Mg$^{2+}$ (Fig. 3) (i.e. that spontaneous fails before evoked activity) is that spontaneous activity is more dependent on the entry of extracellular calcium through ion channels whereas the evoked mode can cause the mobilization of intracellular stores, at least for a time. It is important to emphasize that the two sources of calcium, intra- and extracellular, are both parts of a calcium circuit (i.e. $\text{Ca}^{2+}$ flows into the cell through ion channels from which it may be mobilized and it may be transported back across the cell membrane to the extracellular fluid). The intracellular pools, being much smaller than the extracellular source, are ultimately dependent on the extracellular source (i.e., if extracellular calcium is removed, intracellular calcium will in time be reduced or depleted). Thus the intra- and extracellular pools are interdependent and a change in one pool may ultimately cause a change in the others. Therefore, experimental manipulations designed to influence one mode of transmitter release may over time, affect the others. The evidence implicating one or another of these pools as proximate sources of $\text{Ca}^{2+}$ for spontaneous or evoked transmitter release in the intact organ is at the moment indirect.

Moreover, the existence of an influx of calcium from the endolymphatic compartment, previously described by Ohmori (1989), could be the biochemical stimulus responsible for the mobilization of intracellular stores of calcium. This influx of calcium may act directly or via second messengers to release intracellular stores of calcium (as reviewed in Mayer and Miller, 1990). The effect of an increase of intracellular $\text{Ca}^{2+}$ concentration on $\text{Ca}^{2+}$ mobilization from intracellular pools is biphasic. Indeed, as reviewed by Yamamoto and Karaide (1990) low concentrations of $\text{Ca}^{2+}$ promote and higher concentrations inhibit intracellular cell mobilization.

In any case, it seems clear that mechanisms responsible for the afferent neurotransmitter release during spontaneous and evoked activities are different and should be the focus of new research activity. A note of caution—the majority of studies cited and illustrated are those carried out in the SCC. It is not yet clear the
extent to which these hypotheses may be generalized to other hair cell-bearing organs.

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References


