Is GABA an afferent transmitter in the vestibular system?

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This study was undertaken to determine the possible role of GABA as an afferent transmitter in the vestibular system of the axolotl. We studied the effects of GABA, muscimol, bicuculline and picrotoxin on the spontaneous spike discharge of the afferent fibers of the sacculi lagena and anterior semicircular canal. It was found that GABA and muscimol produce a very weak excitatory effect which does not mimic either the temporal course or the amplitude of the response of vestibular afferents to physiological stimuli. The GABA antagonist bicuculline has no significant effect on these fibers, and picrotoxin partially blocks the spontaneous activity in 33% of the fibers studied. These results indicate that GABA is probably not an afferent transmitter in the vestibular system as has previously been proposed.

GABA; Muscimol; Bicuculline; Picrotoxin; Hair cell; Vestibular system

Since the original proposal of Flock and Lam (1974), that GABA might be the afferent transmitter in hair cell sense organs, several papers have supported this hypothesis. It has been found that GABA has an excitatory effect on the vestibular fibers in the cat (Felix and Ehrenberger, 1982), and that picrotoxin inhibits vertigo in man (Ehrenberger et al., 1982). It has also been shown that glutamic acid decarboxylase (GAD) and specific GABA-binding sites exist in the vestibular system of the chick (Meza et al., 1982, 1985; Meza, 1984).

In contrast, it has been reported that GABA has no effects on the cochlear afferent fibers of the cat (Klinke and Oertel, 1977), nor in the semicircular canals of the frog (Annoni et al., 1984; Guth and Norris, 1984). Finally GABA has been described to exert an inhibitory effect on the lateral line of Xenopus laevis (Bobbin et al., 1985a).

In an attempt to elucidate these controversial results, we have studied the effect of GABA, and some of its agonists and antagonists, on the spontaneous discharge of vestibular afferents of the axolotl (Ambystoma mexicanum). To determine if there are several pharmacologically different classes of primary afferents (Ryan and Schwartz, 1983), as has been postulated for the efferent fibers (Schwartz and Ryan, 1983), we studied the effect of the above listed drugs on fibers of different origin: sacculi, lagena and semicircular canal.

Experiments were performed in wild Axolotls weighing from 25 to 30 g. The animals were decapitated and the otic capsule reached through the cartilaginous palate. The fibers from the inner ear were cut proximally to the brain, and their anterior and posterior bundles carefully detached. Once the origin of the fibers was identified the bundle selected for recording was held in a steel tweezer which operates as an active electrode. The bundle was further dissected until a record in which a single unit discharge was clearly distinguishable from the background noise (signal-to-noise ratio greater than 2) was obtained. Extracellular electrical activity of afferent fibers was recorded by means of an AC amplifier, the output of which was connected to an oscilloscope, a window discriminator and a tape recorder for standard off-line processing the afferent activity as frequency histograms (Glaser and Ruchkin, 1976). The whole preparation was mounted on a
manual tilting table in order to study the vestibular afferents response to tilts (Budelli and Macadar, 1979) (Fig. 1). Tilts of 45° were done in less than 1 s and the table maintained in the new position for more than 1 min.

The results presented here correspond to fibers recorded for no more than 5 h, during which time the basal discharge of the fibers remains stable (in fact, we were able to follow the activity of a fiber for as long as 15 h). Usually we studied the effect of one drug on a fiber more than once.

The preparation was perfused with axolotl Ringer (115 mM NaCl, 3 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂ and 5 mM Hepes, pH 7.4; Bracho and Budelli 1978). Drugs in pH adjusted Ringer's solution were bath applied dropping them from a pipet in the vicinity of the fiber's origin. Ringer's solution was removed during recording leaving just the cartilaginous otic capsule as the bath, so the volume of the drug applied suffices to completely change the Ringer in contact with the synaptic area. Time was allowed between drug applications for a complete washing of the drugs; normally no change was detected in the response after subsequent application of the same drug. GABA 10⁻², 10⁻⁴ and 10⁻⁵ M; muscimol 10⁻⁴, 10⁻⁵, 10⁻⁶ M; bicuculline methiodide 10⁻², 10⁻⁴, 10⁻⁵ M and picrotoxin 10⁻² and 10⁻⁴ M (all from Sigma Chemical Co.) were applied. Effects on basal spike discharge were considered significant when there was at least a 20% change of the basal discharge frequency.

Effects of GABA superfusion were studied in 42 experiments (in 8 units more than one concentration was tried), in concentrations ranging from 10⁻² to 10⁻⁴ M (lower doses of GABA did not produce detectable changes in all the fibers studied). It was found that GABA is unable to evoke any effect in the majority (about 70%) of the fibers studied (Fig. 2 and table I). In two
Fig. 2. Frequency histograms of two different fibers. Each row shows the application of various drugs to the same fiber. Interruptions between each drug application are due to processing disconnection. The upper histogram of a fiber from the sacculi shows the effects of consecutive application of normal Ringer (NR), GABA, $10^{-2}$ M (G), and bicuculline, $10^{-2}$ M (B). (Second row) Effect of the same manipulations in a fiber from the semicircular canal. (Third row) A fiber from the sacculi is subjected to the application of muscimol, $10^{-5}$ M (M) and then (NR) as control. (Fourth row) Fiber from the semicircular canal; basal discharge after Ringer application and response to muscimol, $10^{-4}$ M. Calibration bars are 1 min and 10 c.p.s.

occasions it produced an excitatory effect of up to a five-fold increase in basal frequency (Fig. 3), but generally not more than a 30–50% increase in basal frequency was observed. It seems worth while to note that some cells do not change their firing frequency in the presence of GABA, even if they show a change in their discharge pattern. This is particularly notorious in the more irregular cells, in which GABA induces them to discharge in trains separated by silent periods (Fig. 3). No significant differences were found between the response of fibers from different origins.

![Fig. 2](image1)

![Fig. 3](image2)

**TABLE 1**

<table>
<thead>
<tr>
<th>Firing frequency:</th>
<th>Canal</th>
<th>Sacculi-lagena</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>=</td>
</tr>
<tr>
<td>GABA $10^{-2}$</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>GABA $10^{-4}$</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Muscimol $10^{-5} - 6$</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Muscimol $10^{-4}$</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Bicuculline $10^{-3} - 4$</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Picrotoxin $10^{-3} - 4$</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

Muscimol application was studied in 21 experiments (16 fibers), in concentrations ranging from $10^{-4}$, $10^{-5}$ and $10^{-6}$ M. The cells were practically unresponsive to muscimol (see Table I). This drug produced an excitatory effect in only two fibers at the maximal dose tried (Fig. 2). Bicuculline methiodide application in concentrations of $10^{-2}$ and $10^{-4}$ M was studied in 18
fibers from different origins. It had some effect in only one fiber at $10^{-2}$ M (Fig. 2 and Table I).

Picrotoxin in concentrations of $10^{-2}$ and $10^{-4}$ M was applied to 21 preparations. It produced a decrement in the afferent discharge in 33% of the fibers, and no effect in the remaining, whatever their origin and response to tilts.

Our experimental results do not give further support to the hypothesis of GABA being an afferent transmitter in hair cell sensory systems. However, GABA and muscimol (at high concentrations) may have an excitatory effect on afferent activity. This effect is very weak when compared with the one produced by excitatory amino acids (Bobbin, 1979; Comis and Leng, 1979; Annoni et al., 1984; Dechesne et al., 1984; Bobbin et al., 1985b; Soto and Vega, 1986), and it does not reproduce neither the amplitude nor the time course of the response to mechanical stimuli (Fig. 3). Additionally GABA antagonists bicuculline and picrotoxin, do not show the expected inhibitory effects, picrotoxin being active only at high concentrations. These negative results contrast with the effect of excitatory amino acid agonists and antagonists which produce a notorious modification in the frequency of discharge of these fibers (Fig. 3, bottom).

These results lead us to speculate that GABA may probably have a modulatory role in afferent transmission in hair cell systems, a suggestion supported by the observed modification in the pattern of discharge of some afferent fibers in the presence of GABA. Since our results do not fully substantiate this hypothesis, further analysis is being done in our laboratory in order to test it.

The possibility of a non-uniform afferent vestibular system innervation is not supported by our results. Instead, they point towards homogeneous pharmacological properties of the vestibular primary afferents, regardless of their origin and response characteristics.

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