Fast, Slow, and Steady-State Effects of Contralateral Acoustic Activation of the Medial Olivocochlear Efferent System in Awake Guinea Pigs: Action of Gentamicin

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Background: The function of the medial olivocochlear efferent system can be achieved by electrical stimulation of the OC bundle (OCB) on the floor of the fourth ventricle (Galambos 1956) or of the round window (RW) of the contralateral (CL) ear (Rajan and Johnstone 1983). It is also more physiologically activated by acoustic stimulation of the CL ear. During or briefly after CL acoustic stimulation, a suppression of the ipsilateral cochlear activity has been observed in single afferent nerve fibers responses to ipsilateral sounds (Büño 1978), in tone-pip-evoked VIIIth nerve compound action potentials (CAPs) (Liberman 1989), in otoacoustic emissions (Guinan 1986; Puel and Rebillard 1990), and in the ensemble background activity (EBA) of the VIIIth nerve (Popelar et al. 1994, 1997). The first described OC effects occurred within time constants for onset and offset of suppression in the 50- to 100-ms range (Wiederhold and Kiang 1970). More recently, an additional slow suppression of cochlear responses has been described with a decay time constant of 25–50 s; this was termed the “slow” effect (Sridhar et al. 1995), in comparison with the “fast” effect originally described. These different fast and slow effects were observed on cochlear potentials after repeated electrical stimulation of the OCB at the floor of the IVth ventricle in anesthetized, tracheotomized, and artificially ventilated guinea pigs (GPs).

INTRODUCTION

The medial efferent nerve fibers seem to regulate the contractile state of the outer hair cells (OHCs) and thus modulate the mechanical properties of the cochlea (Brownell et al. 1985; Fex 1967; Kemp 1978; Mountain 1980; Siegel and Kim 1982; Weiderhold 1986). Activation of this medial olivocochlear (OC) efferent system can be achieved by electrical stimulation of the OC bundle (OCB) on the floor of the fourth ventricle (Galambos 1956) or of the round window (RW) of the contralateral (CL) ear (Rajan and Johnstone 1983). It is also more physiologically activated by acoustic stimulation of the CL ear. During or briefly after CL acoustic stimulation, a suppression of the ipsilateral cochlear activity has been observed in single afferent nerve fibers responses to ipsilateral sounds (Büño 1978), in tone-pip-evoked VIIIth nerve compound action potentials (CAPs) (Liberman 1989), in otoacoustic emissions (Guinan 1986; Puel and Rebillard 1990), and in the ensemble background activity (EBA) of the VIIIth nerve (Popelar et al. 1994, 1997). The first described OC effects occurred within time constants for onset and offset of suppression in the 50- to 100-ms range (Wiederhold and Kiang 1970). More recently, an additional slow suppression of cochlear responses has been described with a decay time constant of 25–50 s; this was termed the “slow” effect (Sridhar et al. 1995), in comparison with the “fast” effect originally described. These different fast and slow effects were observed on cochlear potentials after repeated electrical stimulation of the OCB at the floor of the IVth ventricle in anesthetized, tracheotomized, and artificially ventilated guinea pigs (GPs).

Recording the ongoing bioelectrical signal at the RW allows the study of the EBA of the VIIIth nerve and provides a useful tool for measuring medial OC function in awake GPs (Popelar et al. 1994, 1997). The spectrum of this background bioelectrical noise, in the absence of any ipsilateral acoustic stimulation, presents a peak at ~1.0 kHz that is supposed to derive mainly from partly synchronized ongoing activities of auditory nerve fibers (particularly fibers with high characteristic frequencies), either spontaneous or induced by endogenous internal noise (Cazals and Huang 1996; Dolan et al. 1990; Lima da Costa et al. 1997c). A low-level CL broadband noise (CLBN) significantly reduces the EBA, similar to what is observed for tone-pip-evoked CAPs and otoacoustic emissions. These suppressive effects are commonly accepted as being mediated by the CL activation of the medial efferent system innervating the OHCs of the ipsilateral cochlea.

Studying the EBA of the auditory nerve presents several...
advantages: 1) it can be easily performed in awake GPs; 2) it allows long duration and repeated recordings; 3) it does not require any ipsilateral acoustic stimulation, which might activate ipsilateral efferents; 4) ipsilateral activity may be measured simultaneously to the CL stimulation; and 5) the CL OC efferents are activated by a low-level broadband noise, which is a typical physiological condition. Thus this approach is well suited for the detailed study of medial OC function, particularly its time course, under physiological activation, in normal awake conditions. To further analyze these fast and slow effects, we studied their changes after a single injection of gentamicin (GM), which has been shown to selectively and reversibly block the medial efferent system effect without affecting OHC function (Avan et al. 1996; Smith et al. 1994).

METHDS

Experimental animals were female pigmented GPs chronically implanted with an indwelling platinum ball electrode on the RW membrane of the left ear and reference screw electrodes on the skull. Implantation was realized under general anesthesia with an intramuscular injection of xylazine (8 mg/kg) and ketamine (33 mg/kg). The wires from the electrodes terminated in a five-pin electronic connector fixed with the screws and methacrylate dental cement onto the skull. Details of the implantation technique can be found in Aran and Erre (1979). The animals were allowed to recover for ≥1 wk before normal cochlear function was assessed by measuring the VIIIth nerve CAP thresholds to ipsilateral activity may be

CLBN stimulation

Electrophysiological measurements were performed in the GPs in an acoustically attenuated chamber (IAM model AC-2, New York, NY) placed in a quiet laboratory room. The signal-generating and recording equipment was situated in an adjacent room, except for a preamplifier that was placed inside the soundproof room. Each animal was placed in a standing-yoke tube with the animal’s head fixed by a sliding ring placed over the nose.

Broadband noise stimuli were generated with the use of a PC-based waveform generator (Tucker and Davis Technologies, Gainsville, FL) and delivered by a Sennheiser loudspeaker (HD 480 II, Wedemar, Germany) mounted in an animal housing. The sound was delivered to the right ear via a 7-cm Silastic tube, which was sealed in the external auditory meatus of the right ear with surgical glue (Histoacryl, Braun, Melsungen, Germany). The sound pressure level and the spectrum of the broadband noise were measured with a calibrated probe connected to a Knowles microphone (BT-1751) near the tympanic membrane in 10 lightly anesthetized GPs (Popelar et al. 1997). The level and the frequency spectra varied little between GPs or in the same GP with several repeated placements of the tube in the ear canal. The primary components of the broadband noise were in the frequency range of 1.5–13.0 kHz; the maximum difference between GPs did not exceed 5 dB for frequencies <9.0 kHz and did not exceed 10 dB for frequencies >9.0 kHz. The CL noise level used was typically 55 dB SPL, which is in the range of maximum suppression effect, supposedly without any risk of cross talk from one ear to the other (Avan et al. 1996; Popelar et al. 1997; Smith et al. 1994).  The left ear was occluded with cotton to further attenuate any outside sound. The most commonly studied durations were 1 s, 1 and 1.5 min, and ~2 h. Each CLBN had 2.5-ms rise-fall times.

Recording and analysis of the EBA

The signal from the RW/vertex electrodes was amplified 5,000 times with a custom-made, low-noise preamplifier (band pass 3 Hz to 10.0 kHz, 6 dB/octave, 0.5-µV RMS noise in the 3-Hz to 10.0-kHz band). After further amplification (3 or 10 times), the signal was recorded and analyzed through two different procedures, depending on the duration of the recorded epoch.

Recordings during intermittent CLBN stimulation

SHORT-DURATION CLBN (1 S). For short-duration recordings, the signals were recorded in a PC-based CED data acquisition system (Cambridge Electronic Design, Cambridge, UK, model 1401-plus, 12-bit A/D conversion) and sampled at 12,000 Hz. Single recordings consisted of a 2-s epoch made with a 1-s CLBN stimulation digitally triggered 0.5 s after the start of the recording. Successive recordings were separated by intervals close to 20 s. Recordings that included muscular movement or electrical artifacts were rejected. The raw signals were analyzed off-line (LabView software, National Instruments, Austin, TX, Fig. 1). Namely, the signal was digitally filtered (Butterworth, order 2) between 0.5 and 2.5 kHz. Then the filtered signal was squared and the mean power was calculated every millisecond by averaging over 12 successive points. The resulting power signal was visualized on a video screen and stored for further analysis (Fig. 1).

The time course of EBA changes induced by 1-s CLBN was examined after averaging 100 successive 2-s power epochs in three awake GPs. In one of these GPs, 400 additional recordings were obtained, allowing the averaging of 500 2-s power epochs to get a clearer representation.

An example of the end result of this procedure is shown in Figs. 2 and 3. The power value before, during, and after the CLBN is clearly represented with a 1-ms time resolution. With this procedure we observed an increase of the power during a few milliseconds after the onset of the CLBN, followed by a decrease below the initial level. We could demonstrate, by averaging the signal itself over the same time range, that this early peak corresponded to an auditory evoked potential (AEP) recorded by the RW-vertex electrodes in response to the onset of the CLBN (Fig. 2). At the offset of the CLBN, only a very small, slow evoked potential could be observed. These evoked potentials contaminate the recordings and alter the accurate measurement of the power of the signal at the RW. To eliminate this response, we alternately reversed the polarity of the recorded signal and added together the pair of successive recordings of reverse polarity. In this summed signal, the AEP, as well as any potential that would repeat itself at each sweep, is canceled out, whereas the amplitude of the noise signal is amplified by √2. Next we calculated, as before, the power of this sum signal per millisecond and averaged successive individual 2-s pairs. The resulting power signal, thus averaged over half the number of sweeps, presented without the peak at the onset of the CLBN, but with a fluctuation/√2 times larger than without this AEP cancellation procedure.

For evaluating suppression coefficients, the mean AEP power values during the 1-s periods with and without the 1-s CLBN, respectively, were calculated after removal of the first 50 ms after the onset and the offset of the CLBN, eliminating the periods of fast EBA changes. Suppression was expressed in percent as the ratio of the change in EBA from without to with CLBN to its value without CLBN.

LONG-DURATION CLBN (≥1 MIN). Because the awake animals would not stay quiet enough over longer duration periods (in the range of min), for recording they had to be sedated with one
intramuscular injection of xylazine (≈12 mg/kg) and their body temperature had to be maintained at ≈38°C. In a previous study we demonstrated that in such conditions the EBA power value and the CL suppression were not affected (Lima da Costa et al. 1997c).

Typical recordings consisted of a 2- or 5-min epoch made with a 1- or 1.5-min CLBN stimulation, respectively, presented 30–40 s after the start of the recording (7 and 12 different GPs, respectively). Thus the interval between two successive CLBN was ≈1 min. Because the duration of the CLBN was determined by an analog pulse generator, its duration could not be accurately fixed; its onset triggering, however, was precisely determined by the data acquisition computer.

The power of the RW signal in the 0.5- to 2.5-kHz band was measured with the use of a different procedure, so that storing long-duration signals with the 12,000-Hz sampling period (i.e., 24,000 points for a 2-s epoch) was avoided. After amplification, the signal was filtered in an analog active filter (Kemo, Type VBF8, Beckenham, Kent, 48 dB/octave) in the same 0.5- to 2.5-kHz band. This filtered signal was subsequently passed through a Hewlett-Packard 3400A RMS voltmeter, the DC output of which was sampled by the CED data acquisition device with a 100-ms sampling period. No AEP cancellation procedure was used because of the small contribution of the evoked potentials to the power value within the 100-ms sampling period. Because of this long sampling period, averaging only a series of three to five signals from the voltmeter output was sufficient to obtain a clear averaged power signal. Sporadic artifacts brought about by slight animal movements despite sedation were removed from the records.

The amount of suppression was expressed as suppression coefficients: the mean EBA power value was calculated over 1 s (10 points) before, at specific times during, and after the onset and offset of the CLBN.

Recordings during continuous CLBN stimulation

Measurements of EBA power values were also performed during continuous CLBN (up to 2–3 h after an intramuscular injection of NaCl 0.9% or GM) in three GPs with the use of series of three to five 2-s-average recordings. With these short-duration recordings, it was not necessary to sedate the animals; signals with movement artifacts were manually rejected.

Before the injection, a series of 2-s recordings with 1-s CLBN, as described earlier, was performed. Then a CLBN was installed for 1–2 min and a 2-s recording was obtained at the end of this CLBN. Such procedure was repeated three to five times. After these preliminary measurements, GPs received a single intramuscular...
injection of NaCl 0.9% (1 ml/kg). The continuous CLBN was then installed and maintained during 2–3 h, and series of three to five 2-s recordings were performed every 10 min. After the end of the continuous noise, a series of 2-s epochs with 1-s CLBN was recorded again.

Unless otherwise stated, regardless of subsequent experimental protocol, changes in EBA amplitude were examined in details at the onset and offset of the CLBN. Fits of exponentials were obtained with the use of Prism software (Graphpad, San Diego, CA). Statistical analyses were performed with the use of the paired Student’s t-test, comparing power levels and suppression coefficients before and after the injection. The level of significance accepted was \( P < 0.05 \). Unless otherwise stated, the values presented are means ± SD.

Experiments with GM

Most of the GPs used in the above experiments subsequently received a single intramuscular injection of GM (Gentamicin, Dakota Pharm, Créteil, France). In five of the GPs used in the 1-min CLBN normative study, similar recordings (2-min recordings with 1-min CLBN) were performed before and after a 150-mg/kg GM injection. The 12 GPs used in the other experiment (5-min recordings with 1.5-min CLBN) were divided into three groups that received an intramuscular injection of GM at doses of 150, 200, and 250 mg/kg (6, 3, and 3 GPs, respectively) and were similarly tested. In both cases, between the series of recordings (10–15 min intervals) there was no acoustic stimulation. Changes in CL suppression were followed during the 2–8 h after the injection. Measures were repeated 24 and/or 48 h afterward. The three GPs submitted to the >2-h continuous CLBN, received, a few days after the serum injection, a 150-mg/kg GM injection and were subsequently followed as after the serum injection and 24 and 48 h afterward.

Results

1-s CLBN

Within a few milliseconds after the onset of the 55-dB SPL CLBN, the EBA amplitude exhibited a reduction of 38.0 ± 4.2% (mean ± SD, \( n = 3 \), Figs. 2 and 3). The latencies of the fast onset and offset of suppression following the CL stimulation were typically between 5 and 10 ms (Fig. 3). The decay and recovery of the EBA power amplitude, obtained after averaging 100 recordings, could be approximated by nonlinear regressions to exponential functions, with time constant of 13.1 ± 1.0 ms (onset) and 15.5 ± 2.9 ms (offset).

\( CLBN > 1 \text{ min} \)

When the CLBN was presented for ≈1 min, the EBA first showed a fast decrease followed by a slower decrease for ~30 s, and then the EBA amplitude remained constant until the end of the CLBN. At the offset of the CLBN, EBA recovery also displayed fast and slow components. However, the complete return of the EBA to the pre-CLBN level did not occur before 30 s and thus was measurable only on the 5-min recordings, which lasted ≥2 min after the end of the CLBN. A schematic representation of the time courses and amplitudes of EBA changes during and after the CLBN is represented in Fig. 4, and individual recordings taken from different animals before the injection of GM (0 h) can be seen in Figs. 5–7. Several levels of EBA amplitude are distinguished: before CLBN (EBA\(_0\)), just after the fast effect at the onset of the CLBN (EBA\(_1\)), and after the following slow effect (EBA\(_2\)). Most of the time a delay (\( d \)) of several seconds can be clearly observed between the fast effect and the beginning of the slow effect. Then the offset of the CLBN is followed by a partial fast recovery of EBA amplitude to a third level (EBA\(_3\)), usually different (larger) than EBA\(_1\), and a slow progressive return to a post-CLBN level EBA\(_4\) identical to the pre-CLBN level (EBA\(_0\)). These different steps can be expressed as different suppression effect coefficients in relation with the CLBN pre-
sentation: a fast and slow ON suppression ($S_1$ and $S_2$, respectively) and similarly a fast and slow OFF recovery ($S_3$ and $S_4$, respectively). The global suppression ($S$) is thus the sum of $S_1 + S_2$ ($S_{ON}$) or $S_3 + S_4$ ($S_{OFF}$, Fig. 4). The slow ON and OFF effects could be fitted with exponential functions providing time constants $\tau_{ON}$ and $\tau_{OFF}$. Mean values calculated from measurements in the 12 GPs with the 5-min recording epochs and 1.5-min CLBN are summarized in Table 1 and Fig. 4. Although there are no statistically significant differences between the ON and the OFF amplitudes of the fast and slow effects, the ON and OFF slow time constants are significantly different ($P < 0.01$): the slow OFF time constant ($\tau_{OFF} = 32.1 \pm 8.1 \text{ s}$) is about twice the slow ON time constant ($\tau_{ON} = 16.1 \text{ ms} \pm 5.0 \text{ s}$).

Similar values for $S, S_1, S_2, d,$ and $\tau_{ON}$ were observed in the seven GPs studied with the 1-min CLBN and 2-min recording, respectively ($S = 49.7 \pm 9.5\%$, $S_1 = 33.9 \pm 9.4\%$, $S_2 = 15.8 \pm 5.2\%$, $\tau_{ON} = 18.4 \pm 7.4 \text{ s}$).

**Continuous CLBN**

Three GPs were submitted to different set of measurements before and after an intramuscular injection of NaCl 0.9% (see METHODS and Fig. 8) used as a control for further experiments in which the effects of a single injection of GM in the same GPs were investigated (see below). The mean EBA power value without CLBN (EBA$_0$) was found to be $63.7 \pm 7.4 \mu V^2$ (Table 2). After the fast effect (EBA$_1$), measured with 1-s CLBN, the mean EBA power value fell to $40.5 \pm 9.6 \mu V^2$. Then, the CLBN was presented for 1 min and the mean EBA power value decreased further to a stable, low value (EBA$_2$) of $29.7 \pm 7.1 \mu V^2$. The suppression coefficients, relative to the power level without CLBN, were $S = 53.7 \pm 7.3\%$ (global suppression), $S_1 = 37.0 \pm 8.7\%$ (fast component), and $S_2 = 16.7 \pm 4.2\%$ (slow component). Then the continuous CLBN was applied for $>2$ h. As shown in Fig. 8, the power level (EBA$_2$) during the $>2$-h continuous CLBN remained constant. The global suppression ($S$) was relatively unchanged from the beginning ($1 \text{ min}$ after the onset) and throughout the continuous $>2$-h stimulation period. After the end of the continuous CLBN, although EBA$_0$ was slightly smaller than before, both fast ($S_1$) and slow ($S_2$) suppression levels were also unchanged (Table 2, Fig. 8).

**FIG. 6.** Same as in Fig. 5 for another GP (GP 301) after injection of 200 mg/kg GM.
Effects of GM

DURING CONTINUOUS CLBN. The same three GPs (Table 2) were further submitted to the same set of measurements, except that a few days later the intramuscular injection of NaCl 0.9% was replaced by an intramuscular injection of GM (150 mg/kg). During continuous CLBN stimulation, we observed an increase of the EBA values (EBA0, i.e., a decrease in CL suppression), which started <30 min after the injection and continued progressively until the end of the 2- to 3-h CLBN (Fig. 8). After termination of the continuous CLBN, EBA0 (without CLBN) was slightly larger than before, the fast effect (S1) was almost completely suppressed (it completely disappeared in 1 GP, Fig. 8); the slow effect (S2), however, was unchanged. In other words, GM seemed to affect only the fast component, but not the slow one. Such observations were consistent in the three GPs studied (Table 2). For each GP, fast and slow ON effects were examined 24 and 48 h after GM injection and a complete recovery was observed (see Fig. 8).

DURING INTERMITTENT CLBN. The fast, slow, and global components of the CL suppression were also observed in five GPs with the use of intermittent 1-min CLBN stimulations (during the 2-min recording epochs) before and at different times (10-min intervals) after a single intramuscular injection of GM (150 mg/kg). Initially, the global suppression was S = 51.6 ± 8.3%, and the fast and slow suppression effects were S1 = 36.8 ± 9.3% and S2 = 14.8 ± 4.8%, respectively. Between 30 min and 1 h after the GM injection, the EBA amplitude during the CLBN started to increase (i.e., the suppression effect decreased) and maximal decrease in suppression was observed within 1.5–3 h postinjection; then suppression started to slowly increase and was completely back to preinjection value 24 or 48 h later. At time of maximal effect of GM, S was reduced to 25.2 ± 5.9%. The fast component of the suppression (S1) was significantly reduced to 11 ± 3.5%, whereas the slow component (S2) was unchanged (14.2 ± 3.3%). However, at this time, as well as at any other time, particularly at 24 and 48 h, EBA amplitude without CLBN (EBA0) was not significantly different from its value before the injection, and CAP thresholds did not change significantly.

The 12 GPs from the study in which longer-duration CLBN (1.5-min) over longer recording epochs (5-min) was used were treated later with a single GM injection at doses of 150 mg/kg (6 GPs), 200 mg/kg (3 GPs), and 250 mg/kg (3 GPs) and were investigated with the use of the same experimental protocol. Typical examples of changes in EBA suppression before and after the GM injection are presented for each dose in Figs. 5–7, respectively. With 150 mg/kg, the results are similar to those obtained in the previous experiments, particularly the decrease of the ON fast effect and the persistence of the ON slow effect. However, even when the ON fast effect had completely disappeared, one could still observe a significant fast component in the OFF condition (Fig. 5). With the doses of 200 and 250 mg/kg, we observed in all cases a rapid and complete disappearance of the ON fast effect, but then the ON slow effect also progressively decreased and the global suppression was finally completely canceled (at 250 mg/kg). Moreover, at the time of maximum effect of GM, between 2 and 5 h after the GM injection, not only had the fast suppression effect disappeared completely, but in some cases it seemed to reverse polarity: one could observe a slight increase in the

### Table 1. Mean parameter values

<table>
<thead>
<tr>
<th>EBA0, µV²</th>
<th>S1, %</th>
<th>d, s</th>
<th>S2, %</th>
<th>S3, %</th>
<th>S4, %</th>
<th>SON, %</th>
<th>SOFF, %</th>
<th>τON, s</th>
<th>τOFF, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>41.0 ± 13.2</td>
<td>32.0 ± 4.7</td>
<td>9.8 ± 1.3</td>
<td>15.6 ± 3.1</td>
<td>34.9 ± 4.6</td>
<td>13.1 ± 3.7</td>
<td>47.8 ± 5.8</td>
<td>48.1 ± 5.9</td>
<td>16.1 ± 5.0</td>
<td>32.1 ± 8.1*</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 12) of ensemble background activity amplitude before the 1.5-min contralateral broadband noise (EBA0), of the different suppression coefficients (S1–S4), the global suppression calculated from the ON and OFF measurements (SON and SOFF, respectively), the delay (d) between the fast and slow ON effects, and the slow time constants τON and τOFF (see Fig. 4). * Statistically significant difference between τON and τOFF (P < 0.01).
EBA amplitude during the CLBN, with rather fast time constants. Still a slow decrease of EBA could be seen, superimposed on this global slight increase at 200 mg/kg (at 2, 2.5, and 3 h in Fig. 6, GP 301). With the higher dose of 250 mg/kg, the disappearance of the slow effect revealed, alone, this kind of fast “excitatory” response to the CLBN. The means ± SD of the global suppression (S<sub>ON</sub>) and of the fast and slow effects (S<sub>1</sub> and S<sub>2</sub>) before and at the time of maximum effect of GM have been calculated, not taking into account this slight increase, which, as explained in the DISCUSSION, is likely due to a residual ipsilateral noise from an incomplete transcranial attenuation of the CLBN. The values are summarized in bar graph form in Fig. 9. Again, whatever the dose, no CAP threshold changes were detected after the GM injection and there was no significant statistical difference between EBA values in silence (EBA<sub>0</sub>) before GM, at the times of maximum effects of GM, and 24 or 48 h after.

DISCUSSION

In the present study we demonstrate, in the awake animal, that CL acoustic stimulation triggers fast and slow suppressions of basal ipsilateral cochlear activity followed by fast and slow return to prestimulation levels after the end of the CLBN. We characterized these different effects in terms of amplitudes, latencies, and time constants. Interestingly, we showed that the fast and slow ON effects led to a steady-state level that remained stable for hours. Furthermore, we showed that after a single injection of GM the fast effect only was suppressed at 150 mg/kg, whereas both the fast and slow effects were completely suppressed at higher doses (250 mg/kg). These effects were all completely reversible within 24–48 h, and the afferent function, as assessed by CAP thresholds and EBA amplitude without CLBN (EBA<sub>0</sub>), was never significantly affected.

The effect of a CL sound on ipsilateral cochlear activity is thought to exclusively involve the medial OC fibers that cross the midline. This is quite well established for the suppression effect that has been observed with similar characteristics on otocoustic emissions (Puel and Rebillard 1990). As for the slow effect, Sridhar et al. (1995), on the basis of the action of numerous cholinergic antagonists, tended to demonstrate that this is also related to activation of the medial efferent system. Indeed, with their CAP recordings as well as with our observations on the EBA of the VIIIth nerve, there is no direct evidence that the lateral system controlling single auditory nerve fiber activities is not involved. However, in the monitoring of the EBA of the VIIIth nerve during CL acoustic stimulation, without any ipsilateral acoustic stimulation, there is no involvement of the numerous ipsilateral lateral efferents; and only the crossed efferents from the CL side, thus most exclusively medial efferents (Warr and Guinan 1979), are activated by the CLBN.

Because neither anesthesia nor electrical stimulation of OCB is required, the use of low-level CL acoustic stimulation in awake animals is probably the best physiological condition for studying the OC function. Low-level broadband noises supposedly avoid cross talk from one ear to the other and should trigger moderate activation of efferent fibers. Maintenance of the global effect (steady-state effect) of efferent stimulation supports this assumption.

The time constants of the fast and slow effects reported in this study are of the same order of magnitude as those described in the literature: a fast reduction of the EBA in the range of tens of milliseconds (13.1 ± 1.0 ms, Figs. 2 and 3) and a further additional slow reduction of tens of seconds (16.1 ± 5.0 s), with a delay of 9.8 ± 1.3 s (Table 1, Fig. 4). These time constants are shorter than those reported previously after OCB electrical stimulation, i.e., 50–100 ms (Wiederhold and Kiang 1970) or 50–250 ms (Brown and Nuttall 1984) for the rapid effects. The 25- to 50-s time

| **Table 2.** EBA power values and coefficient suppressions before and 2–3 h after the intramuscular injection of NaCl 0.9% and GM (after 2–3 h of continuous CLBN stimulation) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **NaCl 0.9%** | **EBAs, µV<sup>2</sup>** | **EBAl, µV<sup>2</sup>** | **EBAu, µV<sup>2</sup>** | **S<sub>1</sub>, %** | **S<sub>2</sub>, %** | **S, %** |
| Before injection | 63.7 ± 7.4 | 40.5 ± 9.6 | 29.7 ± 7.1 | 37.0 ± 8.7 | 16.7 ± 4.2 | 53.7 ± 7.3 |
| End of continuous CLBN | 58.9 ± 8.6* | 38.9 ± 5.1 | 30.6 ± 5.3 | 33.8 ± 1.3 | 14.3 ± 5.1 | 48.1 ± 4.1 |
| **GM** | **EBAs, µV<sup>2</sup>** | **EBAl, µV<sup>2</sup>** | **EBAu, µV<sup>2</sup>** | **S<sub>1</sub>, %** | **S<sub>2</sub>, %** | **S, %** |
| Before injection | 55.8 ± 2.7 | 34.1 ± 4.8 | 24.1 ± 5.3 | 39.1 ± 5.7 | 18.0 ± 2.1 | 57.1 ± 7.6 |
| End of continuous CLBN | 68.6 ± 0.6* | 60.6 ± 7.1* | 48.1 ± 6.7* | 11.8 ± 9.6* | 18.2 ± 3.8 | 30.0 ± 9.0* |

Values are means ± SD, n = 3. GM, gentamicin; CLBN, contralateral broadband noise; for other abbreviations see Table 1. * Significant difference between values before and at the end of the continuous CLBN (P < 0.05).
constant for the slow effect reported by Sridhar et al. (1995) is the slow decay time constant after the end of the OCB stimulation; it compares well also with our finding of a slow OFF time constant ($\tau_{\text{OFF}}$) of 32.1 s. The fact that our measurements provide generally shorter time constants might be attributed to differences in the general condition of the experimental animals (awake vs. anesthetized). Indeed, anesthetic agents are known to alter the physiology of the peripheral auditory system (Dodd and Capranica 1992) and, more specifically, to slow down the sound-evoked efferent activity (Brown 1989; Liberman and Brown 1986; Lima da Costa et al. 1997c). This should lead to an overestimation of time constants. Another source of overestimation, especially for the fast effect, might arise from the examination of offset suppression instead of onset. Also, as suggested by our values, fast and slow ON suppression effects (13.1 ms and 16.1 s) appear to be shorter than fast and slow OFF recovery effects (15.5 ms and 32.1 s). Moreover the delay we observed in the development of the ON slow effect after the fast (9.8 ± 1.3 s) is similar to that already reported by Sridhar et al. (1995) and also remarkably identical to the delay recently observed by Dallos et al. (1997) in the change in the electromotile response of isolated OHCs after the application of acetylcholine (ACh).

Using electrical stimulation at the floor of the IVth ventricle, Sridhar et al. (1995) reported fast components in CAP suppression that were twice as large as the suppression coefficients we found in this study. This might be explained on the basis of the two fundamental differences in the methods employed. First, the measures are different: Sridhar et al. measured synchronized responses to ipsilateral stimulation, whereas we measured spontaneous and/or only partly synchronized activities (Cazals and Huang 1996; Lima da Costa et al. 1997c), i.e., supposedly without any ipsilateral stimulation. Along these lines, our results are similar to those of Guinan and Gifford (1988), who measured efferent suppression of single-unit spontaneous activity. They found that, during the 1st s of crossed medial OC electrical stimulation, the spontaneous firing rate was depressed by up to 35%. Second, the electrical stimulation is expected to produce a larger release of ACh at the efferent-OHC synapses than that induced by the low-level activation of the efferent bundle by the 55-dB SPL CLBN.

The magnitudes of the slow effects reported during electrical stimulation of the floor of the fourth ventricle are similar to those we measured here. During prolonged CL acoustic stimulation (NaCl group), however, there was no desensitization of the global suppression, even after 2 h of continuous CL acoustic stimulation. This finding is contrary to the observations of Reiter and Liberman (1995) and Sridhar et al. (1995), who reported that the effect could be maintained only for 1–2 min. Besides the different physiological condition of the animal (anesthetized vs. awake), it is likely that the differences might arise from the difference in the activation, and its consequences at the synaptic level, of the medial OC fibers.

Electrical stimulation is thought to trigger massive release of ACh (Sridhar et al. 1995), which may lead to desensitization of OHCs’ ACh receptors. Accordingly, in the 300-s continuous shock paradigm presented by Reiter and Liberman (1995, their Fig. 4c), and in the 250-s shock train displayed by Sridhar et al. (1995, their Fig. 5c), there was a net reduction of the total suppression after 50–100 s, but it included a decrease of both the rapid and slow components. Otherwise, electrical stimulation rates commonly used to stimulate the OCB are ~300–400 shocks per second, whereas monaural stimulation with tones induces efferent fiber activities that rarely exceed 60 spikes/s (Liberman and Brown 1986; Robertson and Gummer 1985). Even high-level sound noise stimulation does not induce efferent activity at rates >140 spikes/s (Liberman 1988). Thus it is possible that electrical stimulation of the OCB for a few minutes causes an exhaustion of ACh contents at the efferent terminal level, preventing the adequate refill of ACh stores in the face of the sustained electrical stimulation. However, no efferent fatigue was seen under continuous acoustic stimulation up to several hours in awake or lightly sedated GPs. In other words, long-duration CL acoustic stimulation did not alter the effectiveness of the medial efferent system. We presume that no “depletion” is imposed on the efferents, and that, consequently, the cellular homeostasis is sustained and all the further molecular steps are preserved, comprising a continuous and regular release of ACh from presynaptic sites. Thus low-level CL acoustic stimulation appears to be a more physiological method of activating the medial efferent system. Such long-lasting effects are also consistent with the assumption that OC efferents might protect the ear against acoustic overstimulation (for review see Rajan 1992), nota-
The hypothesis that slow suppression effect could be produced by a slow increase in the OCB rate of discharge during and after repeated electrical stimulation has been ruled out by Sridhar et al. (1995). Those researchers sectioned the OCB immediately after a train of shocks and observed that the magnitude of the lingering suppression of the CAP and the time required for recovery were similar to control responses with an intact OCB. Our results support this idea, because GM at 150 mg/kg seems to produce a progressive pharmacological inhibition of the medial efferent fast effect, whereas the magnitude of the slow effect remained unchanged. This effect of GM also suggests that the slow effect is not due, in our experimental conditions, to mobilization of additional efferent fibers or to any increase in ACh concentration at the efferent synapses over time.

Pharmacological in vivo experiments by Sridhar et al. (1995) also suggest that fast and slow effects are mediated by the same ACh receptor, because both are similarly blocked by a variety of cholinergic antagonists. Because of the widely varying time scales between the two effects, the authors proposed that fast and slow effects are produced by different intracellular mechanisms, possibly mediated by second-messenger systems. First, binding of ACh to the receptor would induce a small calcium influx that activates nearby calcium-activated potassium channels (Blanchet et al. 1996; Óróség et al. 1994; Evans 1996; Fuchs and Murrow 1992; Housley and Ashmore 1991). The hyperpolarizing potassium current generated might be responsible for the fast effect. Sridhar et al. (1995) suggested that the calcium entry associated with the fast effect is also responsible for generating the slow effect by activating distant calcium-activated potassium channels either directly or indirectly. Recent reports (Dallos et al. 1997; Sridhar et al. 1997) argue in favor of calcium as the second messenger involved in the slow effect. It is even suggested that a calcium-induced calcium release from intracellular stores amplifies the signal. We agree that calcium influx through ACh receptor channels may be responsible for the slow effect. We do not believe, however, that this slow effect is mediated by additional calcium-activated potassium channels, because in isolated OHCs, the ACh-induced current does not increase, on a time scale of a few minutes, but desensitizes (Nenov et al. 1996). We agree that calcium, possibly after amplification, triggers the slow calcium-dependent lengthening of OHCs (Dulon et al. 1990) and the decrease of their longitudinal stiffness (Dallos et al. 1997). These phenomena can be slow and would take time to develop. They could afford the delay of ~10 s and the low time constant of the on slow effect. The absence of delay and the longer time constant of the slow off effect that we report here would correspond to the slow clearing out of free calcium and a progressive decrease of its effects on the lateral wall membrane of the OHC after the end of the CLBN, with a slow relaxation of the contractile proteins likely involved in this muscular-like mechanical mechanism. However, according to Murugasu and Russel (1996), these changes in length and stiffness do not seem to modify the tuning of the basilar membrane, but they decrease its sensitivity and shift downward the CF. Basilar membrane velocity has also been shown recently to increase during OCB stimulation (Dolan et al. 1997). However, these effects might not appear in our global recordings. Still, this hypothesis may explain the protection by the efferent slow effect against temporary threshold shifts induced by high-level tones (Sridhar et al. 1997).

At high doses (250 mg/kg), GM completely and reversibly inhibits the CL suppression, both the fast and slow components. The excitatory phase occasionally observed during the CLBN, at the time of maximum effect of GM, has been identified as an EBA response to a residual noise from incompletely attenuated CLBN from the CL ear. Several simple experiments support this interpretation: we performed similar recordings in a normal GP after destruction of the CL ear by a local application of sisomicin (Dupont et al. 1993). Indeed, no suppression effect was observed and a similar slight increase of ipsilateral EBA could be recorded, particularly with higher levels of CLBN (60 dB SPL), whereas usually with 50 dB SPL no such EBA increase could be evoked. Moreover, when we applied the same broadband noise to the ipsilateral ear, a similar small EBA increase could be obtained with ipsilateral broadband noise levels as low as a few dB SPL. Thus it appears that the 55-dB SPL level chosen for the CL stimulation was slightly over the interaural attenuation. A 45- or 50-dB CLBN would have been more appropriate, because the suppression effect would still be quite evident: it can be observed with levels of CL stimulation as low as 20 dB SPL (Popelar et al. 1997), and there would be practically no chance of interaural cross talk. Thus in these experiments the presumed absence of ipsilateral sound was not exactly true. However, we do not believe that this slight cross talk significantly altered the results: 1) the EBA before and after the CLBN (EBAo) was indeed typically without any ipsilateral acoustic stimulation; 2) the CL suppression in the normal condition was so large that it completely masked the eventual slight excitatory response; 3) in any case the suppression measured in our experimental conditions was thus only underestimated; and 4) it demonstrates the sensitivity of the EBA recordings!

The progressive actions of GM on the fast and slow components are useful for studying the underlying mechanisms of these different effects. In 1994, Smith et al. were the first to show that a single injection of a high dose of GM (150 mg/kg) selectively and reversibly blocks the suppression of ipsilateral activity, measured on CAP responses to low-level 8-kHz ipsilateral pips, in a forward CL masking paradigm. This effect was soon confirmed, with the use of similar protocols and GM dose, on the evoked otoacoustic emissions (Aran et al. 1994), the distortion products acoustic emissions (Avan et al. 1996), and more recently the EBA of the auditory nerve (Popelar et al. 1997). Looking at the experimental parameters used in these different studies, particularly the duration of the CL stimulation, it appears that most of them observed the fast effect only, whereas the study on distortion product otoacoustic emissions, because of the continuous CL pure tone acoustic stimulation, must have investigated the global fast and slow effects (Avan et al. 1996). For the first time to our knowledge, we describe the evolution of the fast and slow components of medial OCB effects over time following a GM injection. Moreover, using different doses, we demonstrate that the effect is dose dependent. In another study (Lima da Costa et al. 1997a), we...
investigated the effects of lower GM doses (60, 90, 120, and 150 mg/kg) on the fast suppression effect. No changes were observed with 60 and 90 mg/kg; a slight but significant effect was observed at 120 mg/kg, although smaller than with 150 mg/kg. The results with the dose of 150 mg/kg are interesting in that the fast effect only appears affected, whereas the slow effect is apparently unchanged. However, at the higher dose the slow effect also disappears.

Two mechanisms of GM blockade may be hypothesized to explain this progressive effects. Both are based on the presumption that the fast effect of efferent stimulation is mediated by the ACh-induced potassium hyperpolarization of OHCs and that the slow effect is dependent on calcium entry through ACh receptors. The simplest explanation for the GM effect at the dose of 150 mg/kg would be that it inhibits calcium-activated potassium channels, but not cholinergic receptors directly. Hyperpolarization and therefore the fast effect would be inhibited, whereas the slow effect would not be disturbed because calcium influx would not be impaired. However, because the slow effect is also affected at higher doses, it is more likely that, at the low 150- mg/kg dose, GM induces a partial and reversible blockade of the calcium entry necessary to elicit the potassium hyperpolarization (Eróstegui et al. 1995). In this case, small and decreasing amounts of calcium would enter into OHCs, leading to a smaller hyperpolarization and so to a reduced fast effect. This calcium entry, however, could be sufficient to ensure slow OHC movements dependent on calcium if an amplifier mechanism, such as a calcium-induced calcium release from intracellular stores, is involved. Indeed at higher doses GM would completely inhibit the entry of calcium and no effect whatsoever could develop. This blockade of calcium entry could be due to the repulsion at the ACh receptor site of Ca\(^{2+}\) ions by the positively charged GM molecules.

This particular effect of GM on the medial efferent system appears to be very specific to GM. In particular, it was not possible to find such a clear effect with other aminoglycosides of the same family (Lima da Costa et al. 1977b). Several aminoglycosides, as for instance amikacin, which is highly cochleotoxic, did not have any action on the medial efferent system, even at high doses and although the concentration in perilymph was significant; whereas other aminoglycosides, such as netilmicin, which shows the least ototoxicity, did have some effect. Also, it was observed that, in the course of a chronic treatment with lower doses of GM, the effect did not appear before the development of ototoxicity (Lima da Costa et al. 1977a). These different observations tend to indicate that there is no direct relation between the action of the aminoglycosides on the medial efferent system and their ototoxicity.

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REFERENCES


LIBERMAN, M. C. Response properties of cochlear efferent neurons: monau-


