

# Alterations in Average Spectrum of Cochleoneural Activity by Long-Term Salicylate Treatment in the Guinea Pig: A Plausible Index of Tinnitus

Y. CAZALS, K. C. HORNER, AND Z. W. HUANG

*Laboratoire d'Otologie et NeuroOtologie, Institut National de la Santé et de la Recherche Médicale, Université de la Méditerranée Aix-Marseille II, Faculté de Médecine Nord, 13916 Marseille Cedex 20, France*

**Cazals, Y., K. C. Horner, and Z. W. Huang.** Alterations in average spectrum of cochleoneural activity by long-term salicylate treatment in the guinea pig: a plausible index of tinnitus. *J. Neurophysiol.* 80: 2113–2120, 1998. Salicylate, one of the most widely used drugs, produces at repetitive high doses reversible tinnitus and hearing loss. Neural correlates of hearing loss have long been established, whereas they remain elusive for tinnitus. The average spectrum of electrophysiological cochleoneural activity (ASECA), a measure of spontaneous auditory nerve activity, was monitored in guinea pigs over weeks of salicylate administration. Auditory nerve compound action potential (CAP) was also recorded to monitor acoustic sensitivity. In the first days of treatment, ASECA decreased acutely during hours after salicylate administration; after several days this decrease could be reduced. Over weeks of treatment the level of ASECA increased progressively. No change in CAP threshold was observed. The ASECA decrease induced by a contralateral broadband noise remained unchanged. At the end of treatment, acoustic tuning of ASECA showed a partially decreased sensitivity. After cessation of treatment the ASECA level returned progressively to initial values. In control animals delivery of an ipsilateral acoustic noise could reproduce the ASECA increase observed in long-term salicylate-treated animals. This white noise was of moderate sound pressure level and it elevated slightly CAP thresholds at high frequencies. These data provide evidence for salicylate-induced ASECA alterations without changes in CAP thresholds, in accord with clinical reports of tinnitus being the first subjective sign of salicylate ototoxicity. The similarities in occurrence, development, reversibility, frequency content, and acoustic level support the idea that ASECA changes, which indicates alterations of spontaneous eighth nerve activity and reflects the presence of salicylate-induced high-pitch tinnitus.

## INTRODUCTION

There is little evidence of the physiological bases of tinnitus either from clinical observations or from animal experiments. The most common experimental approach to produce tinnitus is administration of high doses of salicylate. It has long been known from clinical studies that daily high doses of aspirin can, after several days, induce tinnitus and a moderate hearing loss, both being reversible at cessation of treatment (McCabe and Dey 1965; Myers and Bernstein 1965). Tinnitus often appears as the first subjective symptom and sounds like a high-pitch noise (Day et al. 1989; Mongan et al. 1973). Physiological experiments established that salicylate affects the cochlea (Boettcher and Salvi 1991; Silverstein et al. 1967; Stypulkowski 1990), two major sites of action being the vascular supply (Cazals et al. 1988;

Didier et al. 1993; Hawkins 1976) and the outer hair cells (Dieler et al. 1991; Kakehata and Santos-Sacchi 1996; Long and Tubis 1988; McFadden and Plattsmier 1984; Murugasu and Russell 1995; Tunstall et al. 1995). Hearing loss can readily be assessed with sound-evoked physiological responses and thus was most studied, whereas physiological alterations possibly reflecting tinnitus are scarce. Various degrees of increase in spontaneous activity of auditory neurons after salicylate administration were reported at the level of the auditory nerve (Evans and Borerwe 1982; Evans et al. 1981; Kumagai 1992; Stypulkowski 1990), the inferior colliculus (Chen and Jastreboff 1995; Jastreboff and Sasaki 1986; Manabe et al. 1997), and the auditory cortex (Ochi and Eggermont 1996). An ensemble measure of auditory nerve spontaneous firings can be obtained from the average spectrum of recordings in silent conditions from a gross electrode on the nerve or on the cochlear round window (Cazals and Huang 1996; Dolan et al. 1990; Schreiner and Snyder 1987). This average spectrum measure was found to be altered after a single high dose of salicylate (Martin et al. 1993; Schreiner and Snyder 1987). Similar measures performed in humans at the occasion of eighth nerve surgery indicated that in several patients affected with tinnitus the average spectrum of activity of the auditory nerve was altered (Feldmeier and Lenarz 1996; Martin et al. 1995). Most physiological observations from animal experiments were obtained after a single high dose of salicylate and in acute conditions; this obviously differs from humans for whom high doses of salicylate were repeatedly administered over days. In animal studies with repetitive daily doses of salicylate (Boettcher and Salvi 1991; Crifo 1975; Gold and Wilpizeski 1966) very small hearing losses, measured electrophysiologically or behaviorally, were reported. There is evidence that tinnitus in animals progressively develops over days of salicylate treatment (Jastreboff and Sasaki 1994). The use of chronically implanted electrodes and the testing of awake animals allows long-term monitoring and avoids alterations of the average spectrum of cochlear activity induced by acute conditions (Cazals and Huang 1996). This study was undertaken with chronically implanted animals to monitor both the average spectrum of cochlear activity and the hearing sensitivity of animals undergoing days of treatment with salicylate. An objective of this study was to determine whether alterations of spontaneous cochleoneural activity would somehow parallel the progression of tinnitus

over days of salicylate treatment as described in humans and observed from animal behavior.

## METHODS

Adult pigmented guinea pigs with body weights  $\sim 250$ – $300$  g at the beginning of the experiments were used in this study. They were chronically implanted with a platinum-ball electrode on the round window of the cochlea and two brass screws at the frontal cortex, which served as reference and ground electrodes. A detailed description of the implantation and other recording techniques can be found in a previous publication (Cazals and Huang 1996). All animals were given at least 1 wk rest after the operation, and all subsequent experimental measures were taken from awake chronically implanted animals.

During recording sessions of average spectrum of electrophysiological cochleoneural activity (ASECA) and compound action potential (CAP) the animals were kept in a double-wall soundproof cabin (Industrial Acoustic or Amplifon). To maintain constant acoustic conditions and to keep the animal quiet for electrophysiological recordings, the animal was placed in a restraining box with its head gently maintained with a neck and a nose ring to which all animals became quickly accustomed. For ASECA measurements electrophysiological signals from the round window were amplified 1,000 times filtered between 1 and 10 kHz and digitized through a 16-bit A/D converter at a sampling rate of 22,050 Hz and stored in a microcomputer. A rejection level of  $30 \mu\text{V}$  peak at the electrode was used, and several 10-s recordings were obtained. Then fast Fourier transforms were performed on 300 partitions of 2,048 points, which served for spectral averaging. Thus a frequency resolution of 10.7 Hz was obtained. For each spectral point a magnitude value was computed and expressed in decibels with the formula  $20 \log(M/R)$  in which  $M$  is the computed rms magnitude and  $R$  is a reference taken as  $1 \text{ nV rms}/\sqrt{\text{Hz}}$ . To examine possible changes of ASECA at frequencies  $>10$  kHz, some recordings were made at a sampling rate of 44,100 Hz. The CAP of the auditory nerve was used to assess hearing thresholds. An earphone was placed at 1 cm in front of the ear to be tested to deliver acoustic stimuli. The acoustical calibrations were performed by placing a  $\frac{1}{2}$ -in. condenser microphone in place of the animal's ear, and levels were measured and expressed in decibels of SPL. To evoke CAPs, tone pips of various frequencies were used having an envelope of 2-ms linear rise and fall times and no plateau. Tone pips frequencies were chosen from 0.5 to 16 kHz in octave steps and in third octave steps for the highest 20 and 25 kHz. Averaging of CAP was performed by using  $\sim 200$  sweeps. Threshold detection was visually determined after averaging and the reproducibility thus obtained was around 3 dB. During salicylate treatment CAP thresholds were measured two to three times per week. Ipsilateral stimulation with continuous broadband noise or pure tones was used to examine their effects on ASECA or CAP. Examining effects of contralateral noise is a way of assessment of the medial olivocochlear efferent action; contralateral broadband noise was delivered through a tube glued to the external auditory canal allowing interaural attenuation of  $\sim 50$  dB as described previously (Cazals and Huang 1996).

For salicylate treatment, salicylic acid sodium salt (Sigma-Aldrich) was dissolved in saline at a dose of 200 mg/ml. Salicylate was administered to the animals through intramuscular injection. Doses of 200 mg/kg were delivered twice a day at  $\sim 0900$  and  $1800$ . In a first experiment a treatment of 21 days was designed, and one group of three treated animals and one group of four control animals were constituted. In a second experiment a shorter 14-day treatment was administered to four animals while two control animals were monitored in parallel. The control animals received an equivalent injection of saline. During all experiments,

the electrophysiological testing session started each morning before the first injection of salicylate. Electrophysiological recordings consisted either in a short session of recordings before the first injection to assess progression of long-term treatment or could last several hours to monitor the acute effects of salicylate injection. Lidocaine is a drug known to be able to temporarily alleviate tinnitus in humans. In these experiments, lidocaine (Sigma) was dissolved in 50% ethanol at a dose of 1 g/10 ml and injected intramuscularly. In pilot experiments on control animals several doses were tried; the dose of 100 mg/kg sometimes resulted in abnormal behavioral signs after  $\sim 30$  min. In salicylate-treated animals lidocaine was used at a dose of 40 mg/kg.

## RESULTS

As previously described, ASECA recordings at the round window in silent conditions and from awake guinea pigs exhibited a similar pattern from all animals with a first peak of maximal energy at the lowest frequencies up to  $\sim 100$  Hz followed by a valley  $\sim 500$  Hz and a broad peak centered  $\sim 1$  kHz. Examples of ASECA from three animals are given in Fig. 1, where it can be seen that the spectral profile at the lowest frequencies could vary somewhat from animal to animal, whereas other parts of the average spectrum were quite similar between animals.

The changes in ASECA during salicylate treatment con-

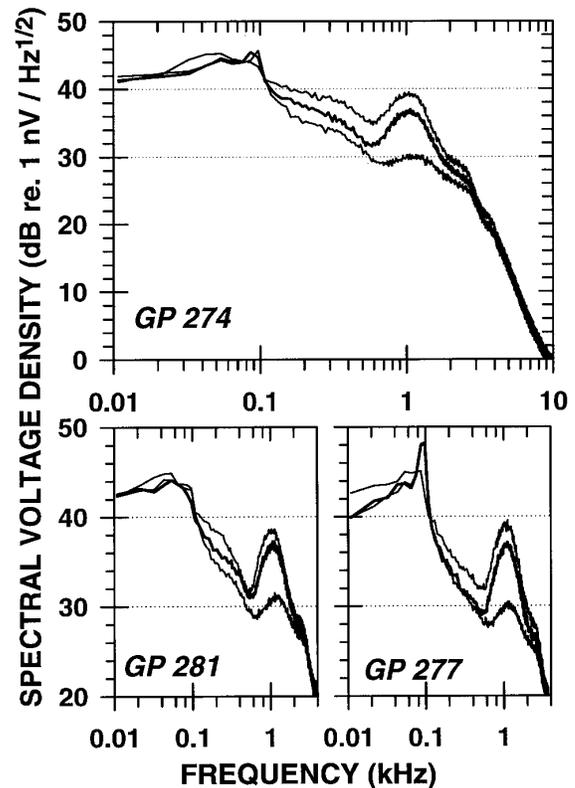


FIG. 1. The acute and long-term changes of average spectrum of electrophysiological cochleoneural activity (ASECA). ASECA obtained for 3 guinea pigs treated for 3 wk. Each panel of curves corresponds to 1 animal whose number is given in the bottom left corner. Top panel, wideband spectrum (up to the highest frequencies recorded); bottom panels, spectral span of actual change; middle curve, before beginning of treatment; bottom curve, after 1 wk of treatment and 2 h after the first daily salicylate injection; top curve, after 3 wk of treatment and 2 h before first daily injection. Horizontal dotted lines are given as visual guides to assess amplitude changes.

sisted of an acute decrease during hours after the administration of salicylate and, in contrast, in a moderate and progressive increase over days of treatment. In Fig. 1 are presented for each of three animals a decreased ASECA as obtained 2 h after administration of salicylate and an increased ASECA as observed after 3 wk of treatment. It can be seen that the changes occur mostly and consistently at frequencies between 100 and 2 kHz and that the maximum of variation is observed at the 1-kHz peak. The amplitude of this peak was thus used as a measure of variations in all subsequent experiments. All animals presented at initial measure, before starting treatments, differences of a few decibels in the absolute value of the ASECA-1-kHz peak; consequently the data were expressed for each animal as a change from its own initial value.

The time course of ASECA-1-kHz changes over 9 h after an injection of salicylate shows that at the first day of treatment a strong decrease was observed, whereas after 2 and 3 wk of treatment this decrease could be considerably attenuated; examples from three animals are given in Fig. 2. In all cases no change in CAP threshold was detected during these 9-h sessions.

In contrast in these same animals the level of ASECA-1 kHz increased progressively over days of salicylate treatment. As can be seen in Fig. 3 this increase amounted to ~1-1.5 dB after 1 wk, ~2 dB after 2 wk, and for two animals reached ~3 dB at the end of the 3 wk of treatment.

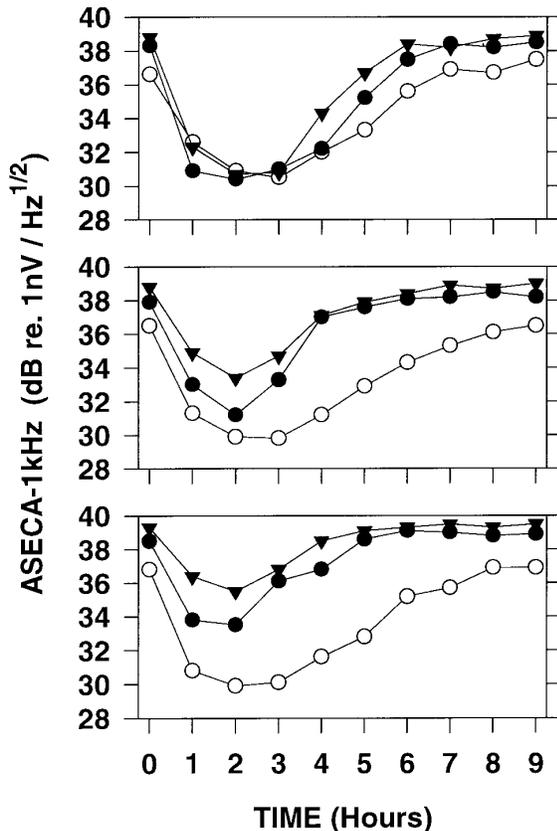


FIG. 2. Reductions in acute decreases of ASECA-1-kHz peak over weeks of salicylate treatment. ASECA decreases during 9 h after the first daily salicylate injection for 3 guinea pigs. ○, at first day; ●, at end of 2 wk; ▼, at end of 3 wk.

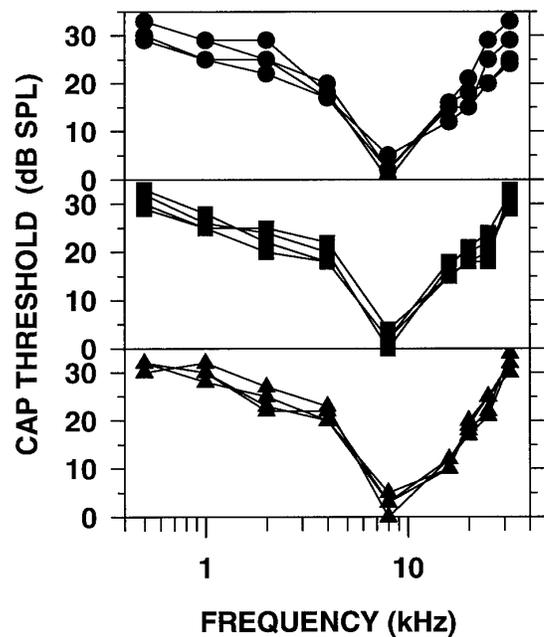
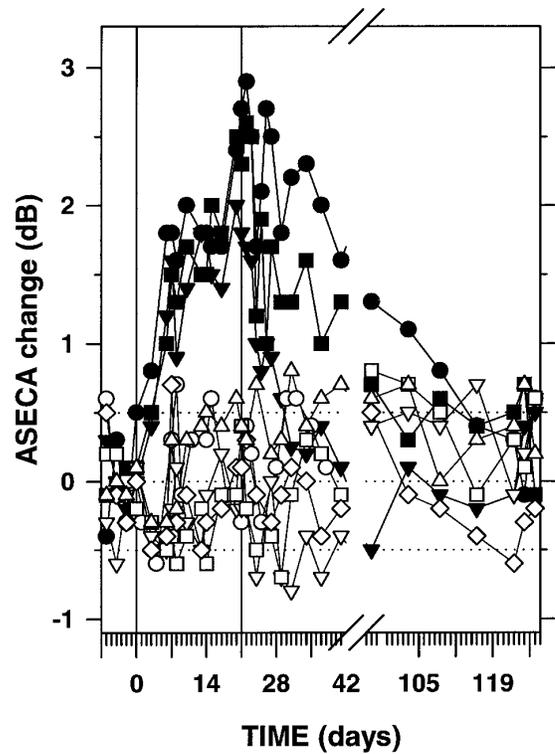


FIG. 3. Progression of increases and recoveries of ASECA-1-kHz peak together with stability of CAP thresholds for a 3-wk salicylate treatment. Open symbols: control nontreated animals. Filled symbols: salicylate treated animals. Animals are the same as in Fig. 1. *Top panel*, ASECA changes monitored over 19 wk. Vertical lines indicate beginning and end of 3-week treatment. Horizontal dotted lines are given as visual help to assess amplitude changes. Each of the 3 bottom panels corresponds to 1 treated animal, each panel displays 4 compound action potential (CAP) threshold curves obtained at days 0, 21, 42, and 119 after beginning of treatment.

The two animals showing the largest increase are those that also showed the most diminished acute effects after 3 wk. In the control saline-treated animals no such change was observed; the values for the various animals fluctuated ran-

domly within  $\sim 0.5$  dB, as can be seen in Fig. 3. After 3 wk of treatment no change in CAP threshold was detected for all but one animal. For the animal that showed the highest increase in ASECA at the end of 3 wk of treatment a very small CAP threshold elevation of  $< 10$  dB was detected at the two highest frequencies, 25 and 32 kHz. At cessation of treatment the ASECA-1 kHz returned progressively to initial values. As can be seen in Fig. 3, the animal that presented the least increase recovered in  $\sim 1$  wk, whereas the animal with the largest increase took  $\sim 14$  wk to recover.

To further document the reversibility of the ASECA increase a second group of animals was treated for 2 wk. Results presented in Fig. 4 show that ASECA increased quite similarly to the previous experiment but did not reach so high levels as the treatment was limited to 2 wk, and for all animals a return to normal was observed  $\sim 2$  wk after cessation of salicylate treatment.

ASECA is known to increase in response to an ipsilateral broadband acoustic stimulus for normal cochleas. At the end of the treatment period, the control animals of the first group were presented with an ipsilateral white noise at various sound pressure levels. The acoustic level of the noise was increased progressively until the ASECA-1-kHz level was increased by  $\sim 2$  dB, as seen in the salicylate-treated animals. This external acoustic noise produced an increase in the ASECA profile affecting the frequencies between 100 and 2 kHz similarly to changes induced by long-term salicylate treatment. This is illustrated in the *middle panel* of Fig. 5, which presents for the same animal ASECA obtained in silence before treatment, in silence at the end of a 3 wk salicylate treatment, and with a 55-dB SPL ipsilateral broad-

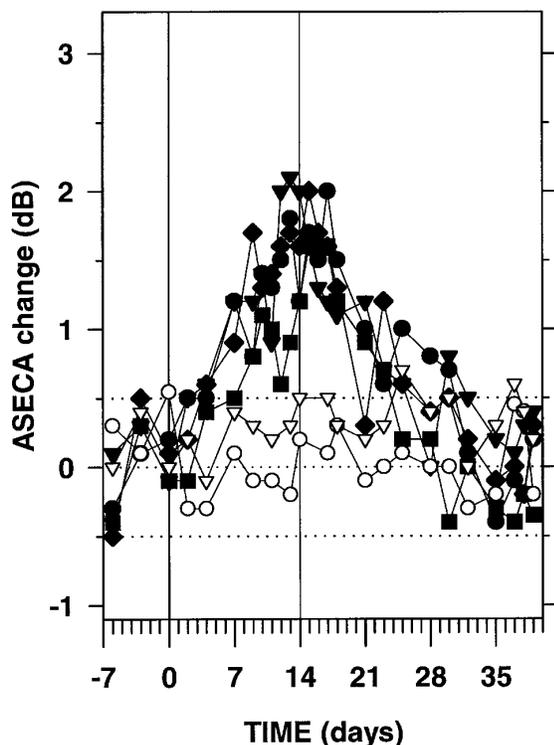


FIG. 4. Increases and recoveries of ASECA-1-kHz peak for guinea pigs treated for 2 wk with salicylate. Open symbols, control nontreated animals; filled symbols, salicylate-treated animals.

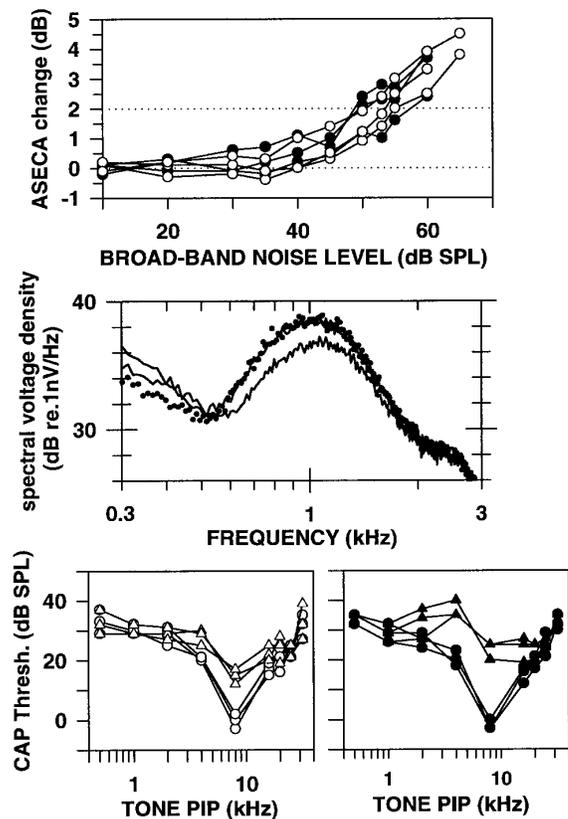


FIG. 5. Effects of an ipsilateral broadband noise on ASECA-1-kHz peak and CAP thresholds for control and treated animals. *Top graph*: increases of ASECA-1 kHz as a function of ipsilateral broadband noise level. Open circles: control nontreated animals. Filled circles: animals at the end of a 3-wk salicylate treatment. *Middle graph*: comparison of ASECA increases for the same animal as observed after 3 wk of salicylate treatment (top curve continuous line) and after complete recovery after cessation of treatment with ipsilateral broadband noise at 55 dB SPL (dotted line). Reference ASECA obtained before beginning of treatment, lower continuous line. *Bottom graphs*: CAP threshold elevations induced by a broadband noise at 55 dB SPL. Circles, thresholds in silence; triangles, thresholds with ipsilateral noise. *Left graph*: control animals. *Right graph*: animals at the end of a 3-wk salicylate treatment.

band noise 3 mo after the end of treatment. For comparison the effects of an external noise was also measured in animals at the end of the 3-wk treatment. The increase of ASECA-1 kHz with external acoustic noise was similar to that observed in control animals, as illustrated in the *top graph* of Fig. 5. A level of  $\sim 55$  dB SPL was found necessary to further elevate the ASECA-1 kHz by  $\sim 2$  dB. The effects of this noise on CAP threshold elevation showed that it induced a moderate threshold elevation of  $\sim 15$  dB at 8 kHz in control animals; in treated animals a somewhat greater threshold elevation was seen at 8 kHz, with an additional significant elevation at 4 kHz. All the above-mentioned results are presented in Fig. 5.

It was well established that in normal cochleas presentation of ipsilateral pure tones produces decreases of the ASECA-1 kHz with a maximal sensitivity at 16-20 Hz. At the end of the 3 wk of salicylate treatment, the effects of various pure tones were tested on the salicylate treated and on the control animals. It was found that salicylate-treated animals presented considerably less ASECA-1-kHz de-

crease. Data obtained from the different animals are presented in Fig. 6. A decrease of 3 dB was taken as criterion to draw tuning curves of ASECA-1-kHz reduction. From control animals these tuning curves exhibit a best sensitivity  $\sim 16$ –20 kHz; in salicylate-treated animals they appear broader, with elevated thresholds and with a best sensitivity shifted toward 8 kHz. For comparison with the previously mentioned results of ipsilateral broadband noise, the control animals were presented with a broadband ipsilateral noise increasing ASECA-1 kHz by  $\sim 2$  dB, and then, in the presence of this continuous noise, effects of pure tones were measured. The  $-3$  dB tuning curve obtained in these conditions was quite similar to that obtained from the same normal cochleas without a continuous noise as shown in Fig. 6.

The efferent cochlear innervation of the medial olivocochlear bundle is known to have an influence on ASECA as was previously shown by effects of contralateral broadband noise. These effects were examined in animals treated with salicylate for 3 wk; no change was observed over the 3 wk of treatment compared with effects measured before treatment. An example of data obtained from one animal is given in Fig. 7.

Lidocaine has been shown to be rather effective in reducing or suppressing tinnitus in human. Lidocaine was injected intramuscularly in control animals at various doses, and ASECA and CAP were monitored for 6 h after injection. When a dose of 100 mg/kg was used some abnormal behavioral signs were observed after  $\sim 30$  min; the animals pre-

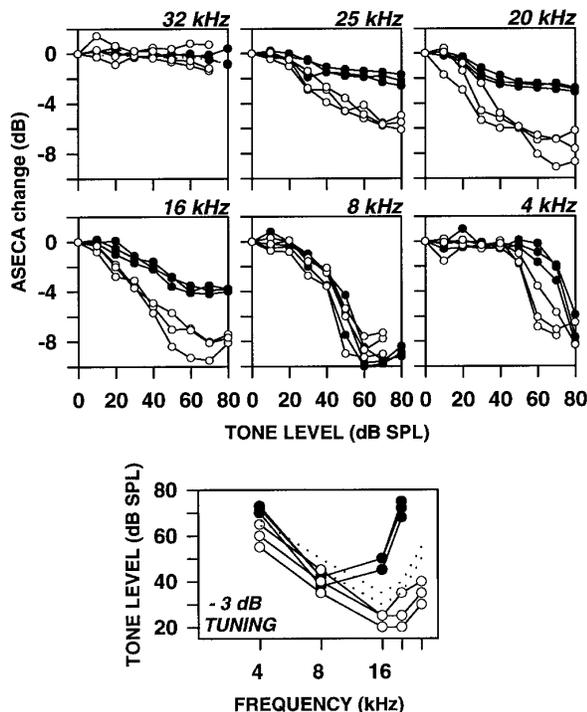


FIG. 6. Changes of the ASECA-1-kHz peak sensitivity to pure tones at the end of a 3-wk salicylate treatment. *Top graphs*: decreases of ASECA-1 kHz induced by various pure tones at different sound pressure levels obtained in control animals (open circles) and in animals at the end of a 3-wk salicylate treatment (filled circles). *Bottom graph*: plot from above results of tuning curves for a  $-3$  dB decrease. Dotted line: data obtained from control animals when an ipsilateral broadband noise at 55 dB SPL was simultaneously applied.

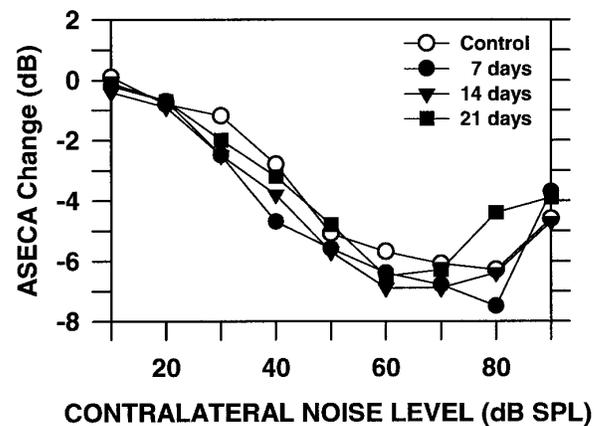


FIG. 7. Stable effects of a contralateral noise during a 3-wk salicylate treatment. Decreases of ASECA-1 kHz as a function of contralateral broadband noise level in 1 guinea pig before treatment ( $\circ$ ) and after 1 ( $\bullet$ ), 2 ( $\blacktriangledown$ ), and 3 ( $\blacksquare$ ) wk of salicylate treatment.

sented some general muscular contraction with an extension of their back. No change in ASECA or CAP threshold was observed at any dose. In salicylate-treated animals at the end of the 3-wk treatment a dose of 40 mg/kg was injected intramuscularly. No change in ASECA or CAP was detected over the following 4 h.

#### DISCUSSION

The results of this study revealed two main findings. The long-term salicylate treatment induced alterations of ASECA without modification of CAP. The characteristics of ASECA changes appeared remarkably similar to the characteristics of salicylate-induced tinnitus in humans and animals. These two aspects are first discussed, and then possible underlying physiological mechanisms are discussed.

#### *ASECA more sensitive than CAP thresholds to incipient salicylate ototoxicity*

Previous studies in the guinea pig showed that ipsilateral pure tones, at levels warranting no acoustic cross-talk to the contralateral ear, only induce decreases of the ASECA-1-kHz peak (Dolan et al. 1990), whereas ipsilateral noises produce increases of the ASECA-1-kHz peak at least when their bandwidth exceeds 1.5 kHz (Cazals and Huang 1996). In view of properties of ASECA responses to various sounds compared with known response properties of nerve fibers, there is compelling evidence that the ASECA-1-kHz peak in the guinea pig originates from a restricted tonotopic area corresponding to the high frequencies of 16–20 kHz and that it should correspond to a synchronized spontaneous firing of fibers (Cazals and Huang 1996). Consequently, ASECA-1-kHz decrease as seen in acute effects of salicylate during hours postinjection should indicate decrease of synchronized nerve activity; reciprocal increase of ASECA-1 kHz seen in the long term should represent increase in synchronized nerve activity.

The observation that these salicylate-induced ASECA alterations occurred without changes in CAP thresholds indicates that ASECA was a more sensitive measure of incipient

cochlear pathophysiology. The absence of CAP threshold elevation observed in this study is in line with previous reports that showed somewhat variable but very small hearing losses for long-term salicylate treatment. By using click-evoked brain stem potentials and 2 wk of salicylate treatment in the cat and the guinea pig, a statistically nonsignificant loss of  $\leq 4$  dB was observed (Gold and Wilpizeski 1966). By using behavioral audiometry in the guinea pig an average of 10 dB hearing loss appeared between the beginning of treatment and after 3 wk (Crifo 1975); in the chinchilla after 2 wk of salicylate treatment an average loss of  $< 8$  dB was reported (Boettcher and Salvi 1991).

The decrease of ASECA seen over several hours after salicylate injection shows a minimum after  $\sim 2$  h and then a progressive recovery in  $\sim 8$  h; this time course is similar to that often reported for CAP threshold changes in previous reports (Cazals et al. 1988; Didier et al. 1993; Gold and Wilpizeski 1966; Myers and Bernstein 1965). In this study no change in CAP threshold was observed during hours after an injection of salicylate, certainly because the dose of each injection (1/2 daily injections) was only 200 mg/kg, less than used in the previously cited studies. The similar time course probably reflects a similarity in underlying physiological processes, and ASECA measures there also appear more sensitive than CAP thresholds to incipient pathophysiology. The reduction of acute postinjection ASECA decrease after 2 and 3 wk of treatment was stronger in animals that showed the higher long-term increase of ASECA. Also dependent on the amount of ASECA increase observed at the end of the treatment was the duration of return of ASECA to initial control values after cessation of treatment. The reversibility is in correspondence with the reported simultaneous reversibility of tinnitus and hearing loss always observed in humans when salicylate treatment is discontinued. Recovery durations from human data were rarely followed and were measured only for hearing loss; recovery periods range from a few days to 2 wk and show large interindividual variability with no apparent relation to the duration of treatment, serum salicylate level, or amount of hearing loss (Long and Tubis 1988; Myers and Bernstein 1965).

#### *Similarities between ASECA changes and tinnitus induced by salicylate*

Tinnitus is the perception of a sound in the absence of a corresponding similar sound in the subject's external environment; most often it is clearly perceived in silent conditions and disappears in even moderately noisy conditions. Neurophysiologically tinnitus must be expressed by some abnormal spontaneous neural activity in auditory structures. ASECA recorded in silent conditions is known to reflect spontaneous activity of the auditory nerve (Cazals and Huang 1996; Dolan et al. 1990; Schreiner and Snyder 1987; Zheng et al. 1996). Depending on animal species, electrode position, and pathological condition, ASECA can present two main peaks, a relatively narrow one  $\sim 200$  Hz and a broader one  $\sim 1$  kHz. The 200-Hz peak was inconsistently found in control cats (Martin et al. 1993; Schreiner and Snyder 1987) and not found in guinea pigs (Cazals and Huang 1996; Dolan et al. 1990). After one high dose of salicylate in cats an emergence of the 200-Hz peak was seen

together with a decrease of the broader peak at 1 kHz (Martin et al. 1993; Schreiner and Snyder 1987). In agreement with these observations this study on guinea pigs showed a decrease of the 1-kHz peak during hours after an injection of salicylate; it also showed that this decrease can occur without elevation in CAP thresholds. Recordings of ASECA from the auditory nerve in humans during surgery provided evidence of ASECA alterations in patients suffering from tinnitus similar to those earlier reported from animal experiments after an injection of salicylate (Martin et al. 1995). Clinical reports indicate that during long-term salicylate treatment in humans tinnitus occurs after several days, becomes louder as treatment is continued, and sounds as a high-pitch noise (Day et al. 1989; McCabe and Dey 1965; Mongan et al. 1973). Behavioral experiments with rats also give evidence of tinnitus occurring after several days of treatment, increasing in loudness with duration of treatment, and having a high pitch (Jastreboff and Sasaki 1994). The ASECA increase observed in this study also occurs after several days of salicylate treatment, increases progressively as treatment is continued, and can be mimicked in control animals by presentation of an ipsilateral white noise, which affects the high frequencies as shown by induced CAP threshold elevations. The loudness of salicylate-induced tinnitus over long-term salicylate treatment was matched by humans to pure tone acoustic levels ranging from  $\sim 20$  to 60 dB SPL (Day et al. 1989); in rats behavioral measures of tinnitus level was found equivalent to a high-frequency pure tone ranging from 20 to 60 dB SPL (Jastreboff and Sasaki 1994). In these experiments mimicking the salicylate-induced ASECA increase required white noise levels of  $\sim 55$  dB SPL. The occurrence of ASECA alterations without changes in CAP thresholds appears in correspondence with the occurrence of tinnitus as a first subjective symptom of salicylate ototoxicity reported by humans (Day et al. 1989; McCabe and Dey 1965; Mongan et al. 1973). These converging similarities in occurrence, development, reversibility, frequency content, and acoustic level support the idea that ASECA changes reflect the presence of salicylate-induced tinnitus.

An ipsilateral broadband noise produced an ASECA-1kHz increase similar to that induced by salicylate elevated CAP thresholds, whereas no CAP threshold elevation was induced by salicylate treatment. The threshold elevations induced by the ipsilateral broadband noise in salicylate-treated animals were greater than those in control animals. This is to be expected in the hypothesis of salicylate inducing an internal noise increasing ASECA to which presentation of an external noise would add and produce a larger threshold elevation. In control animals the ASECA-1-kHz peak decrease induced by ipsilateral pure tones shows a relatively broad tuning with a best sensitivity of  $\sim 16$ – $20$  kHz. In salicylate-treated animals the decrease induced by pure tones was found reduced for tones above 8 kHz. The ASECA-1-kHz peak can be thought of as representing some synchronous spontaneous firing of nerve fibers innervating the 12.5–25 kHz tonotopic area (Cazals and Huang 1996). Accordingly, in response to a tone a number of fibers will be driven by the tone and will no longer participate to the synchronous spontaneous firing resulting in a decrease of the ASECA-1-kHz peak. It is known that pure tones at middle or high sound pressure levels will produce some lateral inhibition,

akin to the well-known two-tone suppression, which would result in a decrease of the ASECA-1-kHz peak; this may be the main mechanism for tones at 8 and 4 kHz, which start inducing ASECA decrease at 30–50 dB SPL. It can be speculated that in salicylate-treated animals the ASECA-1-kHz increase reflects strongly increased synchronized activity that would be more resistant to disruption by pure tones at  $\geq 16$  kHz, whereas tones at 8 kHz and possibly at 4 kHz would still induce lateral inhibition and thus decrease the ASECA-1-kHz peak. This explanation does not involve the efferent cochlear innervation, which does not play a role in lateral inhibition (Kiang 1968), and is in line with the unaltered effect of a contralateral noise observed in salicylate-treated animals.

Lidocaine is the only drug with well-established suppressive effects on tinnitus in humans (Murai et al. 1992). It diminishes or suppresses tinnitus in a majority of patients, but in  $\sim 15\%$  of cases it has no effect or it worsens tinnitus. In addition, the tinnitus-suppressing effects of lidocaine seem to depend on the pathological state of the cochlea, patients with normal hearing being least responsive (Martin and Colman 1980; Melding et al. 1978). In humans lidocaine was most effective when given intravenously; in our experiments on awake animals intravenous injection is difficult. Thus an intramuscular route was adopted. This could explain why we did not observe effects of lidocaine in our experiments. It could also be that the absence of lidocaine effect might be related to the absence of threshold elevation. In previous animal experiments the effect of lidocaine was tested only on acute salicylate-induced alterations, and few data are available. At the level of the auditory nerve, with a dose of lidocaine equivalent to that effective in humans, little effect was observed (Evans et al. 1981); with a dose four times larger a reduction of the spectral peak previously increased by salicylate was observed when lidocaine was given intravenously (Martin et al. 1993; Schreiner and Snyder 1987) but not when given intracochlearly (Lenarz et al. 1990). At the inferior colliculus a clinical dose of lidocaine clearly reduced the increase in spontaneous activity produced by a previous administration of salicylate in about one-half of the tested neurons (Manabe et al. 1997). The effects of lidocaine on tinnitus may thus occur in the CNS and not in the cochlea, in agreement with its classification as a CNS depressant (see Murai et al. 1992). The absence of effects at the cochlear level seen in this study is in line with this proposition.

#### *Physiological mechanisms associated with ASECA alterations*

The kinetics of salicylate concentration in plasma and in the cochlea during hours after a single injection of salicylate show a time course quite similar to that previously reported in animal experiments for hearing loss and that observed for ASECA decrease. After a single injection, salicylate concentration in the plasma and in the cochlea reaches a peak level after  $\sim 1$ – $3$  h and decays slowly in the following hours (Ishii et al. 1967; Jastreboff and Sasaki 1991). The reduction of ASECA decrease observed over weeks of salicylate treatment suggests that some kind of saturation was reached in the cochlea after 2 and 3 wk of salicylate treatment. To our knowledge repetitive daily salicylate injections were studied

in only two animal experiments (Boettcher et al. 1989; Jastreboff et al. 1988); plasma concentrations of salicylate were found of the same order as in humans (Day et al. 1989). A follow-up study indicated no accumulation in plasma or perilymph (Jastreboff et al. 1988). Data from humans indicate that for repetitive daily doses the plasma salicylate concentration does not show saturation over a wide range; interestingly the amount of hearing loss presents a rather linear relation with plasma concentration; however tinnitus intensity appears to saturate at higher salicylate concentrations (Day et al. 1989). Over the same time course of several hours after one salicylate injection animal experiments indicate a concentration of salicylate in the stria vascularis (Ishii et al. 1967), alterations of cochlear blood supply (Cazals et al. 1988; Didier et al. 1993), and decrease of prostaglandins with increase of cochlear leukotrienes levels (Jung et al. 1993). One physiological basis of acute salicylate ototoxicity is thus likely to originate from altered arachidonic acid metabolism, possibly mediating blood supply deficiencies (Jung et al. 1993). No biochemical data are available concerning repetitive salicylate treatment as in this study, but it can be speculated that habituation over days of treatment could occur in catecholaminergic control of blood supply.

An involvement of outer hair cell in salicylate ototoxicity is indicated by studies on otoacoustic emissions in humans (Long and Tubis 1988; McFadden and Plattsmier 1984), in *in vitro* experiments on outer hair cells (Dieler et al. 1991; Kakehata and Santos-Sacchi 1996; Tunstall et al. 1995), and in *in vivo* experiments (Murugasu and Russell 1995; Stypulkowski 1990). Interestingly the comparative study of salicylate-induced changes in basilar membrane mechanics, cochlear microphonics, and CAP in the guinea pig indicates that sensorineural alterations far outlast mechanical modifications (Murugasu and Russell 1995). This may correspond to the dissociation of ASECA and CAP observed in this study.

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Address for reprint requests: Y. Cazals, Laboratoire d'Otologie et Neuro-Otologie, INSERM, Université de la Méditerranée Aix-Marseille II, Faculté de Médecine Nord, Boulevard Pierre Dramard, 13916 Marseille Cedex 20, France.

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