Hearing Research, 45 (1990) 295–312
Elsevier

HEARES 01362

Effects of L-Glutamate on auditory afferent activity in view of its proposed excitatory transmitter role in the mammalian cochlea

Otto Gleich, Brian M. Johnstone and Donald Robertson

Department of Physiology, The University of Western Australia, Nedlands, Australia

(Received 17 July 1989; accepted 3 December 1989)

This report describes the effects of L-glutamate (Glu) introduced into the perilymph of scala tympani on the spontaneous and tone-evoked activity of guinea pig single primary auditory afferents. Concentrations below 2 mmol/l were in general ineffective, while a concentration of 5 mmol/l caused a marked decrease of the neural activity. At 2 mmol/l, roughly 60% of the Glu-perfusions were effective and produced a variety of changes. The most prominent effect was a reduction of the tone-evoked activity without a change in spontaneous rate. Indeed, in some cells, the tone-evoked activity could be almost totally abolished without affecting the spontaneous activity. More rarely observed was a moderate, generally transient increase of the spontaneous activity which was occasionally followed by a decrease in both tone-evoked and spontaneous firing rate. The increase in firing rate was always small relative to the maximum discharge rate evoked by tone stimuli. Desensitization of the Glu-evoked response without an obvious change in the spontaneous activity was also found. In a few cells Glu caused a reduction of the discharge rate below the spontaneous firing rate during loud tone presentation. Higher Glu doses generally caused a reduction of spontaneous and tone-evoked activity without any sign of a preceding increase. Thus, the effects of Glu in the mammalian cochlea appear to be complex and on balance seem inconsistent with the effects predicted for an excitatory transmitter. The findings argue against the hypothesis that Glu is *the* afferent transmitter released by inner hair-cells. However, the results do not exclude an involvement of Glu as a neuromodulator or co-transmitter.

Cochlea; Hair cell; Auditory afferents; Synapse; Transmitter; L-Glutamate

Introduction

The nature of the transmitter between the inner hair cells and the primary afferent neurones in the mammalian cochlea is still unclear. A number of substances can be excluded (for review see Klinke, 1986) and initial screening experiments indicate that Glu might be a possible transmitter candidate because it reduces the compound action potential with little change in cochlear microphonics (Bobbin and Thompson, 1978; Klinke and Oertel, 1977). Concentrations of 10 mmol/l Glu were necessary to produce clear effects.

In subsequent single fibre studies Bobbin (1979) and Comis and Leng (1979) showed that Glu introduced into the perilymph was able to excite afferents. However, according to Bobbin (1979) 'large increases in firing rate were only observed at 50 mM'. Despite possible dilution of the test solution, caused by the fact that the bulla was completely filled with artificial perilymph in these experiments, this concentration seems extremely high. The mean increase caused by perfusion with the 50 mmol/l solution was about 10 sp/s. Comis and Leng (1979) report that perfusions with concentrations of 2–7 mmol/l Glu produced significant effects. They described an increase of the spontaneous activity after 5 perfusions, a slight increase followed by a dramatic decrease after 2, and only a decrease after 5 perfusions. Unfortunately they did not quantify the increase caused by Glu, but the examples they show indicate only a moderate increase compared to the increases in action potential firing rate caused by loud sound

Correspondence to: Otto Gleich, Department of Physiology, University of Western Australia, Nedlands, 6009 WA, Australia

stimuli. In addition the increase of afferent firing rate caused by the Glu agonist quisqualate (Jenison and Bobbin, 1985) was only comparatively moderate despite the fact that a high concentration of 1 mmol/l was tested. Thus in cochlear afferents, only small increases of the discharge rate were caused by high concentrations of Glu and quisqualate. This might be explained by active cellular uptake mechanisms. However, much lower concentrations caused larger increases in other vertebrate preparations (eg. fish electroreceptor; 0.4 mmol/l Glu and 2 μmol/l quisqualate; Okano and Obara, 1988).

Inconsistencies in the action of Glu and its presumed role as the excitatory afferent transmitter have been reported recently for the frog semicircular canal (Guth et al., 1988; Valli et al., 1985), the electroreceptor of the catfish (Nagai et al., 1984), the mammalian hippocampus (Fagni et al., 1983) and the mammalian olfactory cortex (Braitman, 1986; Hori et al., 1982).

The present experiments were performed to study further the effects of Glu on spontaneous and evoked activity of mammalian auditory afferents in more detail and to evaluate further its presumed role as the afferent transmitter.

Methods

Experiments were performed on pigmented guinea pigs weighing 240–510 g. They received a subcutaneous injection of 0.065 mg atropine sulphate prior to anaesthesia which was induced by intraperitoneal administration of 25 mg/kg pentobarbitone sodium (Nembutal) followed by 0.05–0.08 ml Hypnorm (approximately 0.06 mg/kg fentanyl citrate and 2 mg/kg fluanisone). In addition a local anaesthetic (2% lignocaine hydrochloride, 0.5–1 ml) was applied to all incision points. Depth of anaesthesia was checked by the hindlimb withdrawal reflex upon pinching and additional doses of Nembutal and Hypnorm were injected if necessary.

Details of the surgery are described by Robertson and Manley (1974). Animals were artificially respirated with carbogen (5% CO_2, 95% O_2) and rectal temperature was maintained at 38°C. Access to the basal turn of the left cochlea was obtained through an opening in the bulla. The scala tympani of the basal turn was opened using a surgical blade, following which a small piece of the thin bony wall overlying the spiral ganglion was removed to allow recordings from the ganglion cells. Microelectrodes pulled from filament glass filled with 2 mol/l Na-acetate solution having final resistances of 40–120 MΩ were introduced into the ganglion to record the activity of single ganglion cells. Even after several hours of single cell recordings histological evaluation of 3 control cochleae revealed no sign of afferent dendrite swelling in the organ of Corti, which could have influenced our results.

Microelectrode recordings were made with the animals located in a sound-attenuating room. Measurements were made before during and after perfusion of scala tympani with the test solutions using a method similar to that of Robertson and Johnstone (1979). Test solutions were introduced into scala tympani by means of a glass capillary with a tip diameter of approximately 50 μm connected to a microliter syringe. These solutions, as well as perilymph, which was continuously produced to a varying degree by most animals, leaked through the hole in scala tympani into the bulla. These fluids were removed from the bulla with wicks of paper tissue to keep the middle ear dry. The middle ear was regularly checked after a cell was lost (when perfusions had been performed) to make sure that fluid had not filled the bulla. As only a single barrel perfusion pipette was used it was not possible to 'wash' after Glu had been perfused. However, ongoing perilymph overflow leaking out of the hole in the cochlear wall provided a means of removal of excess Glu, although perilymph flow might have varied between animals and during the course of the experiment.

The tip of the perfusion pipette was placed as close as possible to the basilar membrane near the tip of the recording electrode. However, the exact geometric configuration of recording site, perfusion electrode and basilar membrane varied somewhat between experiments.

The control solution contained 133.5 mmol/l NaCl, 4.5 mmol/l KCl, 0.4 mmol/l $MgCl_2$, 1.8 mmol/l $CaCl_2$ and 20.0 mmol/l $NaHCO_3$. The solution was bubbled/with CO_2 and the final pH was adjusted to 7.4 with NaOH. L-glutamate (Glu) was dissolved in the control solution to obtain the

test solution which was freshly prepared before each experiment. The pH of the solution was not changed by the addition of up to 5 mmol/l Glu and remained stable for at least several hours. Two different methods were used for the perfusions. In a first series of experiments a fast injection into scala tympani of 0.5–20 μl of the test solution within 2–6 s was used. In a second series the test solutions were introduced continuously over several minutes with perfusion rates of 0.16–5.0 μl/min generally until an effect was observed. For the rest of this paper the first type of perfusion will be referred to as 'fast perfusion' while the second type will be referred to as 'continuous perfusion'. We do not know the actual concentrations reached in scala tympani with these perfusions due to the dilution by the perilymph. However, considering that the crossectional area of scala tympani near the recording site is roughly 1 mm^2 (Fernandez, 1952) 1 μl of the perfusate corresponds to the perilymph content of 1 mm of scala tympani. Thus one might expect that the Glu concentration at the recording site immediately after fast perfusions of more than 2 μl approaches the nominal concentration of the solution used.

Acoustic stimuli were delivered via a closed sound system with the output not varying more than +/− 3 dB between 10 and 23 kHz and +/− 10 dB between 5 and 10 kHz. The maximum output at 0 dB attenuation was approximately 100 dB SPL. Gated noise or tone stimuli had a rise and fall time of 0.5 ms. Stimulation and data acquisition were performed by an automated system. The Characteristic Frequency (CF; the most sensitive frequency) was determined for each cell from a frequency-threshold curve, generally using 1 kHz frequency steps. Most CFs were between 14 and 18 kHz.

The effects of perfusions on the spontaneous and tone-evoked activity were determined in several ways. In one series of experiments the neural activity was recorded continuously over 1 or 5 s periods and stored for subsequent analysis. The measurements with 1 and 5 s resolution were checked for fast transient changes of the spontaneous discharge rate in response to perfusions. Averaged discharge rates over periods of 30 s were then calculated from the data to reduce the scatter caused by the irregular pattern of the spontaneous activity. The influence of the perfusion on the tone-evoked activity in these experiments was tested generally by a 10 kHz tone of 80 or 100 dB SPL which was switched on for 30 s (eg. Fig. 1). Perfusions were accepted as effective if the spontaneous or the tone-evoked rate changed 20% or more (30 s averages) after a perfusion compared to the pre-perfusion rate. With fast perfusions, the neural activity was monitored for at least a 4 min post-perfusion period to determine their effects. The neural activity was monitored in cases of

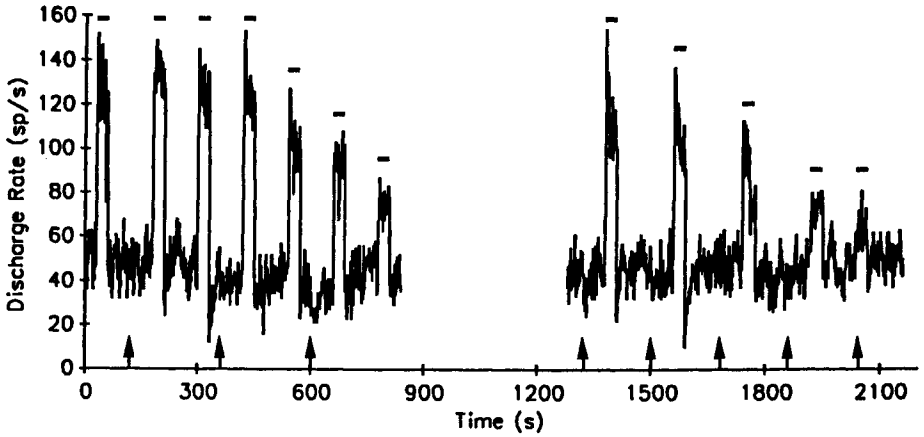

Fig. 1. Effect of repeated Glu perfusions (6 μl, 2 mmol/l indicated by the arrows below the recording trace) on spontaneous and tone-evoked discharge rate. Bars above the recording trace indicate loud 30s tone stimuli (10 kHz 80 dB SPL). Resolution is 1 s per bin.

continuous perfusions for at least 10 min after the onset of the perfusion to determine their effectivity.

Another way to assess changes of spontaneous and driven activity was the repeated measurement of input-output functions, including sound pressures well below threshold. These functions were determined in response to tone bursts (50 ms, 4/s) presented at 5 kHz and at the CF using 5 or 20 dB steps with 10 repetitions for each frequency-sound pressure combination. Determination of the functions at the two frequencies was generally completed within 1.5 min including storage of the data for off-line processing. Peri-stimulus-time-histograms (PSTHs) of responses to long tones (10 s, 3/min) were measured in a few cells at selected frequency-sound pressure combinations.

Results

A total of 56 cells from 21 animals were tested either with control or Glu-perfusions. Two lines of evidence suggest that the mechanical effects of perfusion were insignificant. First a number of perfusions (4) were performed using artificial perilymph without Glu. These perfusions had no detectable effect on the firing rate of primary afferents, in one particular cell even after 30 min continuous perfusion with 1.25 µl/min. Second the effect of Glu perfusions was dose-dependent: some Glu-perfusions caused no obvious effects within at least 4 min for the fast and 10 min for the continuous perfusions.

Glu injections into scala tympani of the basal turn produced a variety of effects which could be classified in basically 4 different categories: 1) decrease of the tone-evoked activity without a change in the spontaneous rate; 2) increase of the spontaneous activity, occasionally followed by a decrease; 3) reduction of the spontaneous activity without a preceding increase, accompanied by decreased evoked activity; 4) reduction of the tone-evoked discharge rate below the spontaneous rate.

Reduction of tone-evoked activity without changes in spontaneous activity

Fig. 1 shows a recording of the discharge rate of a cell in which repeated Glu-perfusions (6 µl, 2 mmol/l) gradually reduced the evoked response to a loud 30 s tone pip, but did not change spontaneous activity. The record of the discharge rate is interrupted between perfusion 3 and 4 because PSTHs were obtained during this time. The evoked response had almost recovered 7 min after the third perfusion. This recovery clearly shows that the reduced tone evoked activity was not caused by fluid leaking into the middle ear or deterioration of the preparation. Further perfusions caused a similar reduction of the evoked response, but did not affect the spontaneous rate. Qualitatively similar results were obtained in 18 cells.

Fig. 2 shows examples of PSTHs in response to 10 s tones obtained before (A) and after Glu-perfusion (B). Both histograms are drawn to the same scale. The time elapsed between the collection of the two PSTHs is 36 min. During this time 5 fast

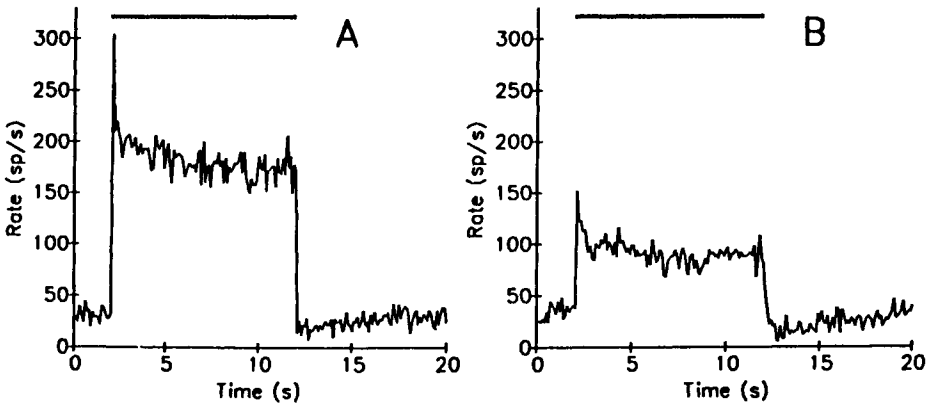

Fig. 2. PSTHs obtained in response to 15 kHz 80 dB SPL stimuli (10 repetitions; indicated by bars above recording trace). Control (A) and 36 min later (B), after perfusion with 2 mmol/l Glu. Resolution is 0.1 s per bin.

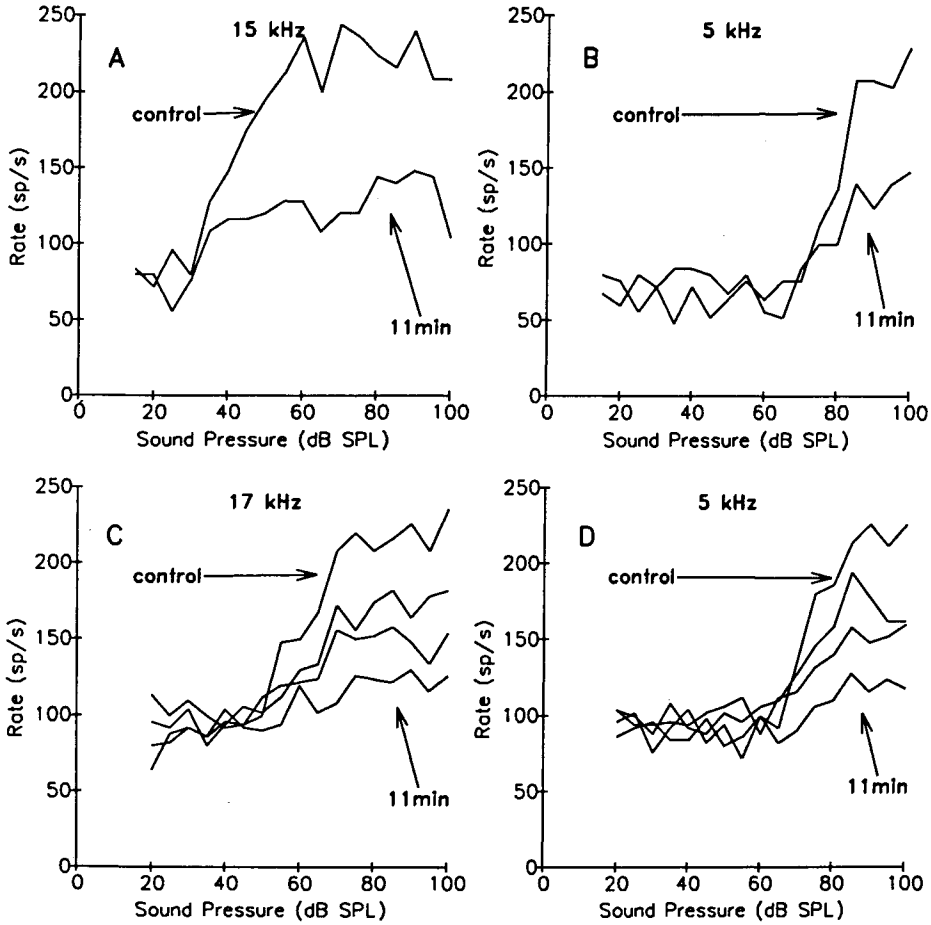

Fig. 3. Effect of Glu perfusion on input-output functions at CF (A = 15 kHz, C = 17 kHz) and at 5 kHz (B,D). Functions obtained before (control) and 11 min after perfusion with 0.31 μl/min 2 mmol/l Glu are shown for the first cell (A,B), while the second example illustrates the control as well as functions obtained 7, 9 and 11 min after perfusion with 1.25 μl/min 2 mmol/l Glu (C,D).

perfusions (4 × 4 μl and 1 × 8 μl, 2 mmol/l) were performed. After the perfusions the tone-evoked rate is reduced to about 50% compared to the control, while the spontaneous rate was unchanged.

The reduction of the maximum evoked response without a change in spontaneous activity was also obtained with 50 ms tone pips. Fig. 3 shows representative input/output functions from 2 cells, obtained at the CF (A, C) and at 5 kHz (B, D). The data for the first cell (A, B) were obtained before and 11 min after a perfusion (0.3 μl/min, 2 mmol/l), while those of the second cell (C, D) were collected before and 7, 9 and 11 min after the onset of a perfusion (1.25 μl/min, 2 mmol/l). These functions clearly demonstrate a decrease of the maximum firing rate, while the spontaneous rate and thresholds are virtually unchanged. The effect of the Glu-perfusion in these examples is thus a limitation of the maximum firing rate, both at the CF and at 5 kHz, with no obvious change in threshold. In 11 other cells, where the effects were determined at the respective CF of the cell and at 5 kHz, there were no systematic differences between the responses to the two test frequencies (e.g. the maximum rates declined in parallel when tested at the two frequencies without an obvious change in thresholds).

Fig. 4 compares the spontaneous rate to the firing rate produced by a saturating CF-tone (17 kHz, 100 dB SPL). Data were obtained from successively measured input/output functions over

a long period of perfusion. The fine and heavy lines in the upper left indicate the Glu-perfusion (0.63 μl/min and 1.25 μl/min, respectively). Although the maximum tone-evoked rate was reduced to 50% of its initial value within 30 min, the spontaneous activity was virtually unchanged during this period. Perfusion was stopped after 33 min when the spontaneous activity had also dropped, and 15 min later both evoked and spontaneous activity had recovered almost to initial values. Again, the recovery of the evoked rate to the pre-perfusion level showed that the effect was not due to a deterioration of the preparation or fluid in the middle ear.

In 3 other cells the decrease of the tone-evoked activity was accompanied by a very slight increase in the spontaneous activity which developed over several minutes. One example is shown in Fig. 5. Here, the driven rate decreased by about 50% within 13 min, but the spontaneous rate increased slightly. Subsequently both rates declined rapidly.

Increase of spontaneous activity

A fast, transitory increase in spontaneous firing rate was observed in 11 cells after 12 fast perfusions (from a total sample of 96 fast perfusions performed on 30 cells). This increase occurred within 5 s to 1 min after the perfusion and generally lasted between 1–3 min (Fig. 6). In 4 other

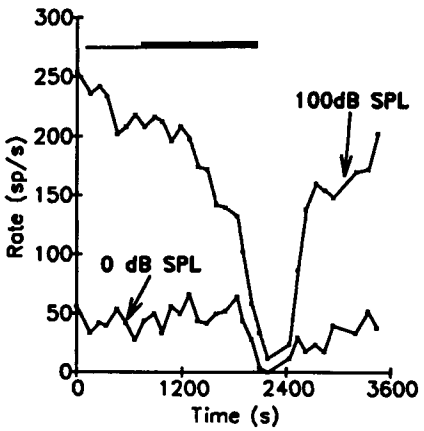

Fig. 4. Effect of Glu perfusion (0.63 μl/min and 1.25 μl/min, light and heavy bar in the upper left) on the response to a saturating CF tone (17 kHz, 100 dB SPL) and a subthreshold (17 kHz, 0 dB SPL) tone (resembling spontaneous activity). Data were obtained from repeatedly measured input-output functions (50 ms tone bursts, 10 repetitions).

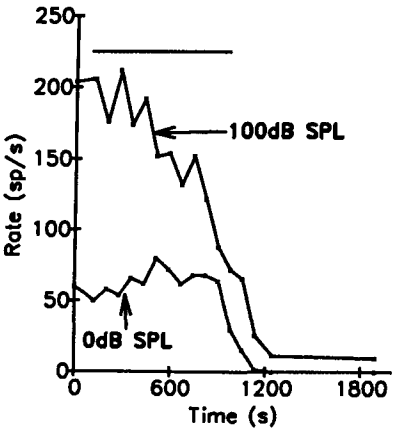

Fig. 5. Effect of Glu perfusion (0.63 μl/min light bar in the upper left) on response to a saturating CF tone (17 kHz, 100 dB SPL) and a subthreshold (17 kHz, 0 dB SPL) tone (resembling spontaneous activity). Data were obtained from repeatedly measured input-output functions (50 ms tone bursts, 10 repetitions).

cells that showed an increase with fast perfusions, this increase in discharge rate occurred more than 1 min after the perfusion and lasted more than 5 min. The two examples in Fig. 6 show the two largest increases obtained in this series of experiments. Even in these examples the maximum rates elicited by the Glu-perfusions were considerably lower than those evoked by 30 s high level tones (indicated by bars).

The cell in Fig. 6A is the only case where a second increase of the discharge rate could be elicited by another perfusion. This response to the second perfusion was, however, much smaller than that caused by the first, while the spontaneous activity before and after the Glu-evoked increases was virtually unchanged. The decrease of the tone-evoked rates, which is obvious in Fig. 6 has already been described in detail. In 5 cells where a perfusion had caused an increase of the discharge rate, a second identical perfusion after the rate had approximately reached pre-perfusion level had either no effect (4 cells) or decreased the spontaneous activity (1 cell). The perfusion that caused an increase was not necessarily the first perfusion tested in a particular cell, but it was the first perfusion that was effective in that cell.

This fast transitory increase of the spontaneous activity produced by fast Glu-perfusions was not found with continuous perfusions. In 10 cells

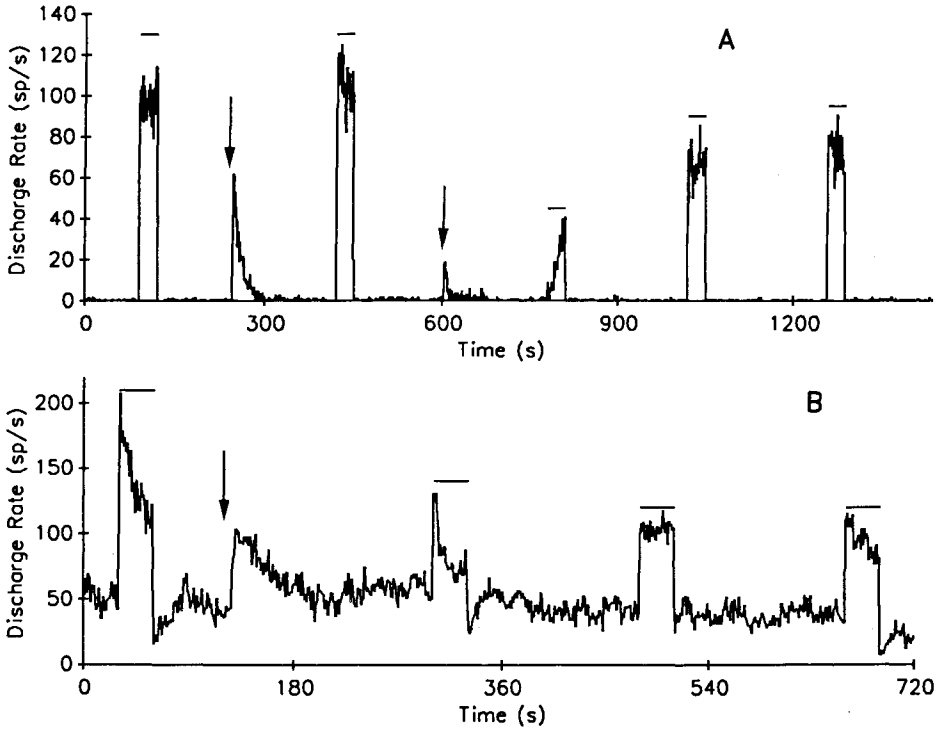

Fig. 6. Effect of Glu perfusions on spontaneous and evoked activity. Bars above recording trace indicate 30 s tone stimuli (A: 15 kHz, 0 dB SPL; B: 10 kHz, 0 dB SPL). Arrows above recording trace indicate Glu perfusions (4 μl, 2 mmol/l). Resolution is 1s per bin.

where the rate was measured continuously, only 6 showed any effect during continuous perfusion, and this was either a decrease of the spontaneous or evoked activity. In all other experiments with continuous perfusions, firing rates were determined from repeatedly measured input/output functions and, in these cases, a transient increase might not have been detected. Three cells did show a slight and gradual increase of their spontaneous activity over several minutes during the continuous perfusion, but this was much lower than tone-evoked activity (Fig. 5) and was followed by a marked decrease of spontaneous and evoked discharge rate.

Cells that showed an increase of the spontaneous activity in response to Glu-perfusions had pre-perfusion rates between < 1 to 70 sp/s (38 ± 22). The pre-perfusion rate and the increase after 10 identical perfusions (4 μl 2 mmol/l Glu) were not significantly correlated ($N = 10$, $r = 0.54$, $P > 0.05$). In 5 of the fast and all of the continuous perfusions that caused an increase, this was subsequently followed by a decrease of the spontaneous activity (e.g. Fig. 5).

The maximum increase caused by Glu perfusion was generally quite small when compared to tone-evoked rates and ranged from 4 to 30 sp/s when measured over a 30 s period. The mean peak increase measured in this way was 16.9 ± 6.5 sp/s ($N = 12$), while saturating 30 s tones (80 or 100 dB SPL) presented before the first perfusion caused much higher increases above spontaneous activity (81 ± 25 sp/s, $N = 11$). Using a 1 s bin width to detect high transient discharge rate increases also showed that the rates evoked by high level tones were higher than those caused by Glu-perfusions. This is clearly evident even in the example with the highest transient increase found in this series of experiments (Fig. 6A). Thus, in all cells tested, Glu-perfusions failed to cause an increase of the discharge rate comparable to that evoked by a saturating test tone.

Reduction of spontaneous activity

A marked reduction of spontaneous activity without a previous increase was observed after 45 perfusions in 35 cells. In cells in which the tone-

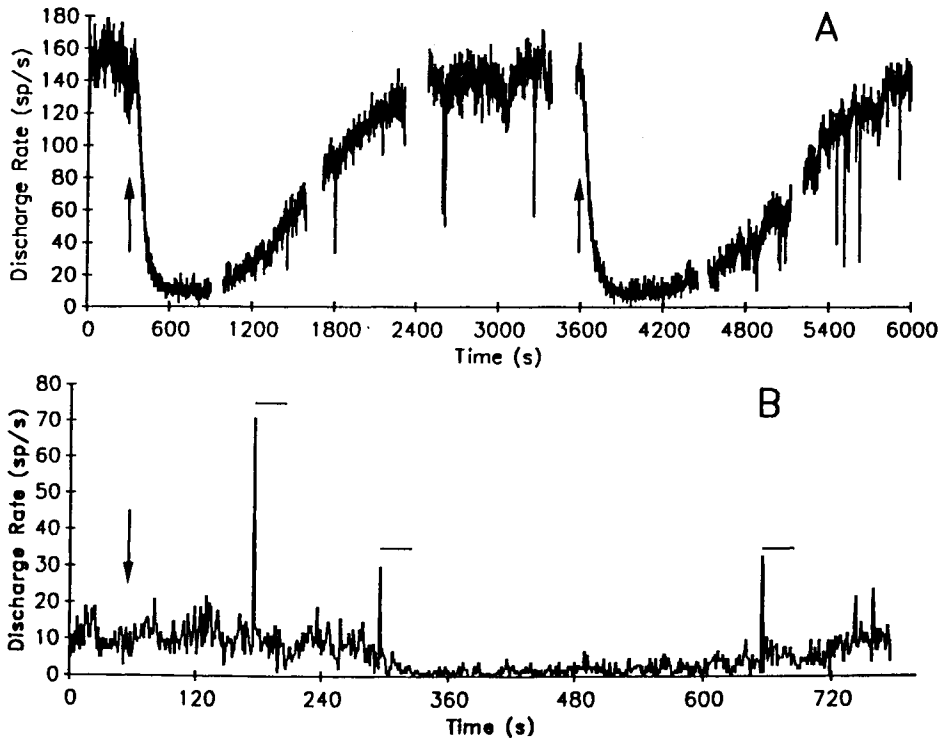

Fig. 7. Effect of Glu perfusion on spontaneous (A,B) and tone-evoked (B) activity. Arrows indicate Glu perfusions (A: 10 μl, 5 mmol/l; B: 4 μl, 2 mmol/l), bars indicate 30 s tone stimuli (B: 15 kHz, 100 dB SPL). Resolution is 1 s per bin.

evoked rate was tested it was reduced before the spontaneous rate declined. The spontaneous rates of these cells before the perfusions varied between 10 and 150 sp/s (76 ± 36). Two examples are shown in Fig. 7; the spontaneous rates of the cells before the perfusion were 150 and 10 sp/s respectively. The spontaneous discharge rate of the cell in Fig. 7A started to decline within 1 min after perfusion (10 μl 5 mmol/l Glu) and fell to 10 sp/s within 3 min, while the cell with the low spontaneous rate (Fig. 7B) started to decline within 4 min after the perfusion (4 μl 2 mmol/l) and the rate declined to below 1 sp/s. The response to loud 30s test tones (100 dB SPL, 15 kHz) in this cell was reduced to a 1–2 s transitory on-response without an increased discharge rate during the ongoing stimulus as shown by the peaks in the discharge rate in response to the tone bursts indicated by the bars. The spontaneous activity of these 2 cells had reached pre-perfusion levels within 30–35 (Fig. 7A) and 12 min (Fig. 7B). It was possible to test a second perfusion in the cell shown in Fig. 7A which caused a response similar to the first perfusion. The latency between the perfusion and the onset of the decline varied between approximately 1 and 5 min for fast perfusions. The amount of reduction, as well as the rate of recovery (if observed) varied between cells and experiments. Despite the variability, for fast perfusions, the decrease of the spontaneous rate to 50% of the pre-perfusion rate was on average more rapid with high Glu doses compared to lower doses ($N = 13$, $r = -0.59$, $P < 0.05$).

Reduction of evoked discharge rate below spontaneous activity

An unusual transient effect of Glu-perfusions on tone-evoked activity was a reduction of the discharge rate below spontaneous activity during tone presentation. This effect was observed in 7 cells. Fig. 8A shows a continuous record of the discharge rate of a cell after a perfusion (10 μl 2 mmol/l) that had caused a reduction of the initial spontaneous rate (75 sp/s) which almost re-

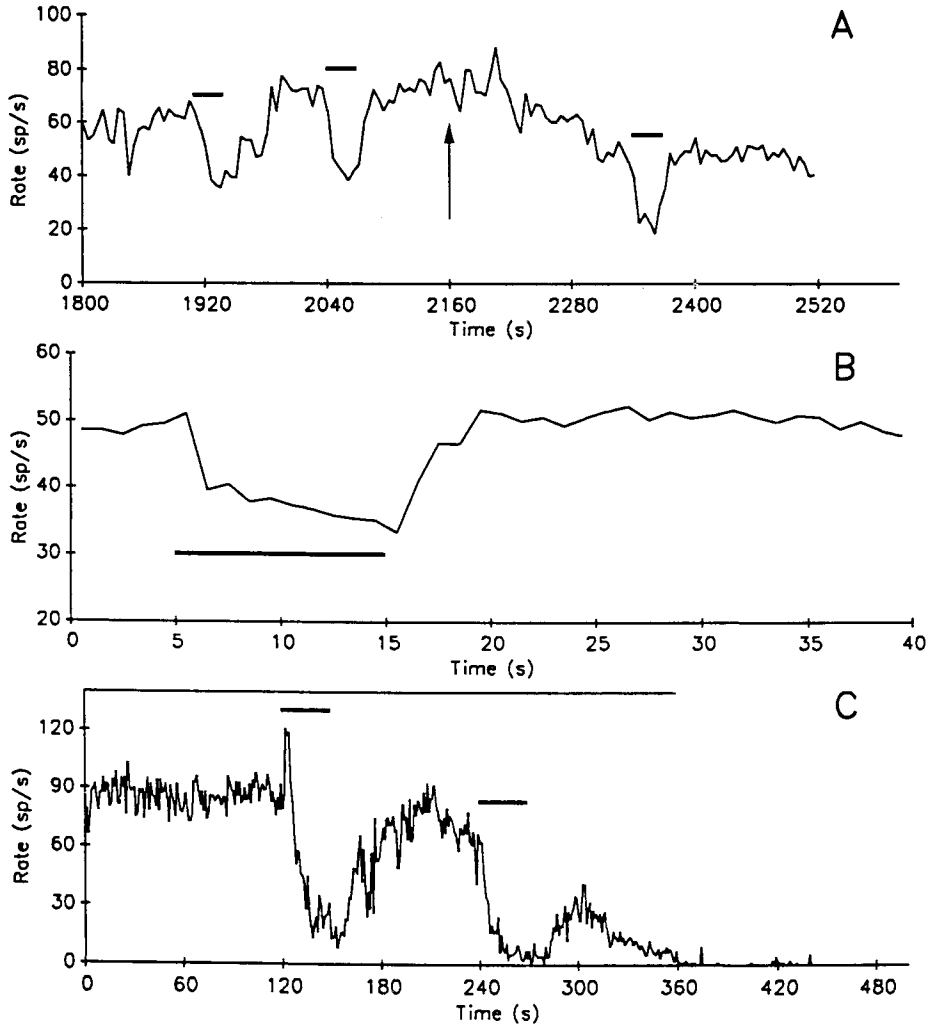

Fig. 8. Effect of Glu perfusion on tone-evoked activity; continuous records (A,C) and a PSTH (B). Bars above (A,C) and below (B) the recording trace indicate tone bursts (A,B: 13 kHz, 100 dB SPL; C: 10 kHz, 80 dB SPL). Glu perfusions are marked (A: arrow below the recording trace, 10 μl, 2 mmol/l; C: bar in the upper left, 1.25 μl/min, 2 mmol/l). Data were recorded with a 1 s (B,C) and 5 s (A) resolution. The PSTH (B) was constructed in response to 50 repetitions of the stimulus.

covered within 30 min. The 30 s tones (13 kHz, 100 dB SPL, indicated by bars) clearly caused a reduction of the discharge rate below the spontaneous level. Another perfusion (6 μl 2 mmol/l) decreased the spontaneous rate, but even with the lower spontaneous rate a tone decreased the rate further. However, it was difficult to assess this effect quantitatively (e.g. PSTHs) because the decrease had a latency in the order of seconds and varied over time. Fig. 8B shows a PSTH obtained in another cell where this effect was quite stable; however, the 1 s sampling time resulted in a poor temporal resolution. The rate during the tone is clearly reduced well below the level of spontaneous activity. Fig. 8C shows another example obtained during continuous perfusion (1.25 μl/min, 2 mmol/l). No change of the spontaneous activity was detectable during the first 2 min of perfusion. The onset of a tone caused a phasic increase which was followed by a drastic decrease of the rate within a few seconds. The rate remained low during the tone and recovered within 20 s after the tone was switched off to almost the initial value. Then the spontaneous rate started to

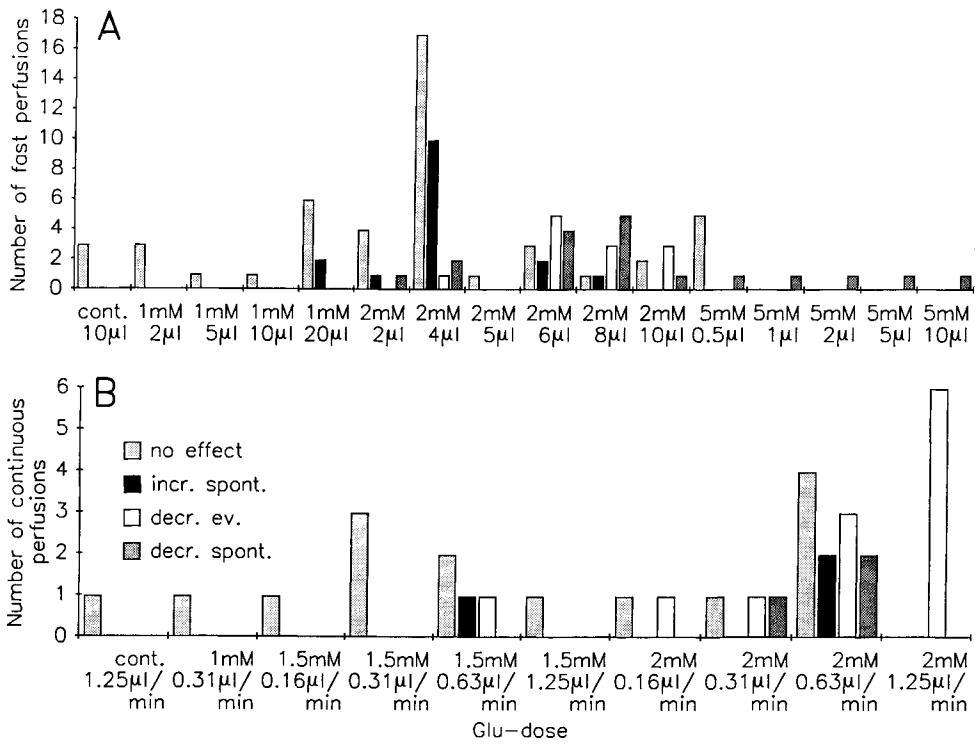

Fig. 9. Dose and concentration dependence of the Glu effects for fast (A) and continuous (B) perfusions. Height of the bars indicates the number of perfusions performed. Light dotted bars indicate no effect within the observation time of at least 4 min after (A) or at least 10 min during (B) the perfusion. Filled black bars indicate an increase of the spontaneous activity and white bars represent a decrease of the maximum tone-evoked rate without a change in spontaneous rate. The decrease of maximum tone-evoked rate was followed by a decrease of the spontaneous rate with the continuous perfusions. The heavy dotted bars indicate a decrease of the spontaneous rate after (A) or during (B) perfusions where no previous increase of the spontaneous rate and no decrease of the maximum tone-evoked rate could be detected.

decline. A second tone caused a similar decrease to the first. Thereafter the spontaneous rate decreased to almost 0 sp/s within 6 min.

Effects of different Glu concentrations

Fig. 9A summarizes the effects of single fast perfusions (more than one perfusion was performed on several cells) with different Glu concentrations and amounts perfused, while Fig. 9B shows the effects obtained with continuous perfusions.

Although, our perfusion technique does not allow to study detailed dose response relations because we do not know the precise concentration of Glu in scala tympani, Fig. 9 clearly demonstrates a dose and concentration dependence of Glu effects. Only 15% (4 out of 27) of the perfusions with concentrations below 2 mmol/l were effective while in about 60% (59 of 98) of the perfusions with higher concentrations one or another of the effects described above was observed. The difference between the effectiveness of low and high concentrations is highly significant ($\chi^2 = 17.45$, $P < 0.001$). The dose dependence is also demonstrated by the finding that for a given concentration low injected volumes or perfusion rates were less likely to produce an effect than higher ones. Higher doses or perfusion rates at a concentration of 2 mmol/l (6–10 µl and 0.63–1.25 µl/min) were significantly more effective (37 of 47) compared to the lower ones (2–5 µl and 0.16–0.31 µl/min; 18 of 42, $\chi^2 = 12.09$, $P < 0.001$).

Discussion

The experiments reported here were performed to investigate in detail the effects of Glu introduced into the perilymph on the spontaneous and tone-evoked discharge rate of primary auditory afferents.

The lack of effects in the cases of perfusions with control solutions without Glu, the dose-dependent effects demonstrated in Fig. 9 and the recovery observed after many perfusions (e.g. Figs. 1, 4, 7) clearly show that the observed effects were specifically caused by Glu. The recovery observed in many cells as well as the finding that all the described effects were obtained at least in one animal during the first perfusion show that these effects were specific, and not merely due to a deterioration of the preparation. Thus, in order to increase the data base, it seemed justified to test more than 1 cell with Glu-perfusions in each animal.

We observed a considerable variability in the response to similar Glu perfusions. Two factors could have influenced the effects of perfusions applied to the region of the organ of Corti from which recordings were obtained. One is that the position of the recording site relative to the tip of the perfusion pipette was variable, and the second factor is that perilymph production, which was used as a means of terminating the exposure to Glu, might have varied. Both factors are likely to influence the time course and concentrations effective at the recording site. However, despite these variations, there is a clear concentration dependence of the effects (Fig. 9).

The results confirm the previous finding that Glu can excite auditory afferents (Bobbin, 1979; Comis and Leng, 1979). They are also consistent with the report that Glu caused only a decrease of the spontaneous rate without a previous increase in a considerable proportion of the cells tested (Comis and Leng, 1979). However, a more detailed analysis of the Glu-effects was performed in order to evaluate the possible role of Glu as the afferent transmitter in the mammalian cochlea.

Increase of the spontaneous discharge rate

The increase of the spontaneous discharge rate caused by externally applied Glu (Bobbin, 1979; Comis and Leng, 1979) as well as the excitatory action of the Glu agonists quisqualate (Jenison and Bobbin 1985) and kainic acid (Bledsoe et al., 1981a) have been interpreted as evidence that Glu or a Glu like substance is the afferent transmitter in the mammalian cochlea. However, we found only a moderate excitatory effect in roughly half of the cells tested with near 'threshold' concentrations of Glu. This is consistent with the results of Comis and Leng (1979). Application of higher Glu concentrations caused a reduction of the spontaneous discharge rate without a preceding increase. Our findings indicate that an increase of the discharge rate requires a fast change in Glu-concentration which was provided with the fast but not our continuous perfusions. Although Bobbin (1979) used a perfusion paradigm similar to our continuous perfusions he achieved a fast increase of Glu-concentrations by using a test solution containing 50 mmol/l Glu. At lower concentrations (10 and 25 mmols/l) he found no large increases in firing rate.

It is worth stressing that the increase of the spontaneous activity caused by Glu-perfusions was only moderate (16.9 ± 6.5 sp/s) when compared to the increase caused by tone stimuli (81 ± 25 sp/s) even when the increase was followed by a drastic decline of the discharge rate below pre-perfusion level. In all cells tested, the response to a saturating tone was considerably larger than the Glu-response. This is consistent with the reported mean increase of less than 10sp/s upon perfusion with a 50 mmol/l Glu-solution (Bobbin, 1979). Unfortunately Comis and Leng (1979) do not report quantitative data on increases that they obtained by Glu-perfusions. The examples they show in their Fig. 4, however, indicate only a small increase. The Glu-agonist quisqualate has also been reported to cause increases of the spontaneous activity upon perfusion with a concentration of 1 mmol/l (Jenison and Bobbin, 1985). Although an average increase of about 500% relative to spontaneous rate looks very big, it represents only a comparatively small increase when given as absolute rate (9–13 sp/s for the 4 examples where an absolute rate can be deduced) and when compared to the increases caused by loud sound (e.g. Figs. 1, 2 and 6). The sample of Jenison and Bobbin (1985) was obviously biased

towards cells with a low spontaneous activity: the highest spontaneous pre-perfusion rate they found was only 30 sp/s, which is clearly less than the average spontaneous rate of about 60 sp/s determined for guinea pig auditory nerve fibers (this study; Manley and Robertson, 1976). Thus quisqualate at a concentration of 1 mmol/l causes only a small increase in the discharge rate of auditory afferents while it severely reduces the compound action potential at concentrations as low as 15 μmol/l (Jenison et al., 1986).

The finding that Glu-perfusions reduce the compound action potential (CAP) without interfering with other cochlear potentials (Klinke and Oertel, 1977; Bobbin and Thompson, 1978) was interpreted as a result of 'desynchronization' due to the increase of the spontaneous discharge rate (Bobbin, 1979). It seems unlikely that a mere increase of the spontaneous activity by an average of 10 sp/s as a result of perfusion with 50 mmols/l Glu (Bobbin, 1979) or 1 mmols/l quisqualate (Jenison and Bobbin, 1985) could reduce the CAP dramatically. For example, Leng and Comis (1979) showed that the threshold of auditory afferents hardly changed, despite high increases in spontaneous activity caused by high potassium perfusions. Our results suggest that at the effective Glu-concentrations (10 mmols/l) used, the reduction of the CAP was rather due to a reduction of spontaneous and evoked neural activity than to the increasing asynchronous activity above spontaneous rates.

Thus, although Glu, in this and other studies, and its agonist quisqualate, show some excitatory potency, they never elicit high sustained discharge rates comparable to those caused by the natural transmitter with loud sounds. This is in marked contrast to the electroreceptor of a fish in which perfusion with Glu and quisqualate in concentrations as low as 0.1–1 mmol/l and 1–10 μmol/l respectively caused a dose-dependent increase of afferent firing resembling that found with natural stimulation (Okano and Obara, 1988). Similar results have also been obtained in the semicircular canal and lateral line of the frog (Valli et al., 1985; Bledose et al., 1983), the lateral line of the catfish (Katsuki, 1973) and in cultured cerebellar neurones (Geller and Woodward, 1974).

Access of externally applied Glu to the synaptic receptors

Some form of specific barrier (e.g. effective cellular uptake mechanisms) could be present around synaptic areas of the auditory afferents which would prohibit the access of externally applied Glu to the post-synaptic membrane and its receptors in the experimental conditions we used. Assuming that Glu is the transmitter released by the hair cells, such a barrier might explain the necessity of large increases in Glu-concentrations applied externally to induce an increase of the discharge rate in the fibers. It might also explain the only moderate increase in discharge rate obtained with Glu or its agonists.

In most studies investigating the role of Glu its agonists and antagonists in the cochlea (e.g. Bledsoe et al., 1981a; Bobbin, 1979; Bobbin and Ceasar, 1987; Comis and Leng, 1979; Jenison and Bobbin, 1985; Jenison et al., 1986; Klinke and Oertel, 1977) it was tacitly assumed that these substances, if introduced into scala tympani reach the post-synaptic receptors of the auditory afferents. Indeed, experiments with radioactive labelled Glu have shown that it has ready access to the structures of the organ of Corti within minutes if introduced in μmol/l concentrations into scala tympani because hair cells and efferent fibers beneath the hair cells were labelled (either by Glu or its metabolites) under these experimental conditions (Eybalin and Pujol, 1983; Schwartz and Ryan, 1983).

Sewell et al. (1978) showed that the afferent postsynaptic membrane is not shielded against external application of 'ANAS' (auditory nerve activating substance) in the frog amphibian papilla, and they even suggested that diffusion away from the synaptic site might be a means of terminating the transmitter action. These findings do not support the speculation that the postsynaptic membrane of mammalian auditory afferents might be shielded against externally applied agonists, antagonists or the natural transmitter.

Loss of Glu-induced increases without change in background firing

In only 1 out of 6 cells that showed a transient increase in firing with Glu-perfusion, another

identical perfusion resulted in a second increase. This example is shown in Fig. 6A and shows that the second perfusion was clearly less effective. Noteworthy is the fact that spontaneous and evoked firing rates before and after the first Glu-induced increase are virtually unchanged although the increase in firing caused by the second perfusion is reduced to less than 20% compared to the first perfusion. In 5 other cells where a perfusion had caused an increase in discharge rate and where the spontaneous rate had returned to the pre-perfusion level, a second perfusion was either ineffective (4 cells) or caused simply a reduction of the spontaneous rate (1 cell). Thus in some cells there was apparently a desensitization to Glu applications without an effect on transmission caused by the natural transmitter. A similar desensitization of the Glu-response without a decrease of the spontaneous activity has been reported for afferents in the semicircular canal of the frog (Guth et al., 1988). Desensitization to Glu without interference with the synaptic transmission was also observed in hippocampus (Fagni et al., 1983) and the olfactory cortex (Braitman, 1986). These authors argue cogently that this is strong evidence for a difference in the action of Glu and that of the natural transmitter.

Reduction in spontaneous activity without preceding increase in firing

In 35 cells a marked reduction in spontaneous activity was clearly observed without a preceding increase. This effect has also been described by Comis and Leng (1979). 5 mmol/l Glu generally caused simply a reduction of the spontaneous discharge rate while a 1 mmol/l concentration was ineffective. At 2 mmol/l almost 40% of the perfusions were ineffective while 30% caused a decrease of the spontaneous activity without a previous increase.

A decrease of the discharge rate has been observed upon application of Glu in a number of other preparations (Okano and Obara, 1988; Valli et al., 1988; Geller and Woodward, 1974) and upon high potassium application in the cochlea (Leng and Comis, 1979). It was suggested that this decrease is the result of desensitization of the respective receptors and/or excessive depolarization of the fibres (Baudry, 1986). In contrast to Glu-application in the cochlea, these other cases show generally a substantial increase of the discharge rate before it declines. Thus the effects of Glu application in the cochlea differ from those seen in a number of other vertebrate, presumably glutamatergic systems, despite the resistance of auditory afferents to desensitization in response to the natural transmitter.

Reduction of tone-evoked activity without changes in spontaneous rate

Comparative information dealing with the effects of Glu on the tone-evoked activity of single afferents is scanty in the literature. Bobbin (1979) mentions a decrease of the evoked activity accompanied by an increased spontaneous rate for 1 cell and Fig. 4 in Comis and Leng (1979) indicates a decline of the tone-evoked activity associated with the Glu-perfusions before a decline in the spontaneous activity.

A reduction of maximal tone-evoked discharge rate without a change in the spontaneous rate was found to be the primary effect of Glu perfusion in a considerable number of cells (eg. Fig. 1–4). The input/output functions (Fig. 3) demonstrate that the maximum firing rate is decreased without a threshold increase; normalizing the curves relative to the respective maximum and minimum would, despite an increased scatter, result in virtually identical functions. If Glu acted on outer hair cells this should influence the CF region of the cells, where the active process causes the sharp tip of the tuning curve, but not the tail region (e.g. 5 kHz; Patuzzi and Robertson, 1988). However, neither the input/output functions at CF nor at 5 kHz show any increase in threshold, thus indicating that Glu neither interferes with the actual transduction nor with the active micromechanical processes (e.g. outer hair cells) in the cochlea. This is consistent with earlier reports that Glu had no effect on the cochlear microphonics (Bobbin and Thompson, 1978; Klinke and Oertel, 1977).

The reduction of tone-evoked activity without a change in spontaneous activity is difficult to ascribe to a classical post-synaptic action of a transmitter. If Glu caused a desensitization of the synaptic receptors thus causing almost an abolition of the response to high level tones (eg. Figs. 1, 3 and 4) one might also expect a decline of the

spontaneous rate, especially if it is relatively high as in the examples illustrated (40–100 sp/s). Alternatively one could postulate that evoked and spontaneous activity are separable phenomena. Drescher and Drescher (1987) demonstrated for the frog lateral line that the spontaneous activity decreased with increasing concentrations of Mg^{2+} and Ca^{2+}. In the absence of Ca^{2+} and the presence of Mg^{2+} cells exhibited spontaneous, but no evoked activity. In the mammal, however, elevating Mg^{2+} as well as lowering Ca^{2+} concentration reversibly reduced spontaneous activity and increased the threshold of cochlear ganglion cells (Robertson and Johnstone, 1979; Siegel and Relkin, 1987). This indicates that the spontaneous activity in the mammalian cochlea is due to the background release of the excitatory transmitter from the hair cells and depends on the extracellular Ca^{2+} concentration.

Anomalous tone-evoked response patterns

A variety of bizarre tone-evoked response patterns in single afferents was found after some Glu perfusions. In the cell shown in Fig. 6A for example, the response to a 30s tone burst after the second perfusion showed a build up in the response over the duration of the tone, instead of the usual phasic-tonic pattern seen normally in primary auditory afferents. This build up is difficult to explain in terms of desensitization. If the postsynaptic receptors were desensitized by the previous Glu-perfusions, then how should an increased transmitter release from the hair cells, caused by the tone stimulus, lead to the build up response seen. Instead one might expect further desensitization of the receptors and thus a blockage of response spikes.

In several other cases, presentation of a loud tone during Glu perfusion actually caused a drop in the mean discharge rate, a result never seen under normal circumstances. We have no explanation for these anomalous responses, other than to suggest that they may result from effects of Glu perfusion on the transmitter storage and release mechanisms within the inner hair cell rather than from actions on postsynaptic membrane receptors.

Application of Glu

Application of the afferent transmitter to the synaptic areas might be expected to induce increased firing of the fibres in a dose dependent manner, with sufficiently high transmitter-concentrations causing high discharge rates with subsequent desensitization and/or depolarization block. This does not resemble the effects we found after Glu-applications. Nevertheless, one has to consider the difference between external application of Glu and the release of the natural transmitter from the hair cells. The pre-synaptic site releases transmitter in individual quanta and close to the specialized postsynaptic site while external application is neither quantal nor (especially in the case of perfusions or bath applications) restricted to the postsynaptic sites. Thus, the failure of most perfusions to increase the discharge rate, the decrease of spontaneous activity without a preceding increase and the reduction in the tone-evoked rate without a change in spontaneous rate might be explained by the unnatural way of applying Glu (although iontophoretic as well as bath application of Glu has been shown to cause substantial excitation in a variety of vertebrate preparations).

Perfusion of scala tympani with Glu using our parameters might cause a random activation and subsequent desensitization of individual receptors. The random, non-synchronous activation of the postsynaptic receptors might fail to increase the discharge rate before it decreases. The average spontaneous discharge rate in the non-pathological guinea pig preparation is quite high, on average about 60 sp/s with some cells exceeding 150 sp/s (this study; Manley and Robertson, 1976). Even these high spontaneous rates are maintained by the natural transmitter without any sign of desensitization. In addition even higher evoked rates are maintained for many seconds (eg. Figs. 1 and 2). It has also been reported, that afferents driven by sound stimuli at high rates respond to an incremental increase in the stimulus by an increase in the discharge rate above the already high driven rate (Smith and Zwislocki, 1975). These examples demonstrate that auditory nerve fibers show only little desensitization in response to their natural transmitter. From this point of view, the slow perfusions might be expected to mimic the continuous spontaneous release as well as the release in response to long tones of the

natural transmitter from the hair cells more realistically than perfusions which cause very high increases of the Glu-concentrations over a few seconds.

With external application of Glu one has also to consider the effect of possible extrasynaptic excitatory amino acid receptors. Such receptors have been shown in cultured astrocytes which were depolarized by excitatory amino acids (Bowman and Kimelberg, 1984). Thus extrasynaptic excitatory amino acid receptors might be present at pre- and/or postsynaptic sites in the cochlea and could be responsible for the effects seen after perfusions with the respective agonists or antagonists. Possible extrasynaptic receptors might even dominate the response in perfusion experiments by inducing unspecific effects. This would prohibit the interpretation of results obtained by perfusions with excitatory amino acids, their agonists and antagonists.

Evaluation of Glu as the excitatory transmitter in the mammalian cochlea

In order to establish a substance as transmitter, one of the basic requirements is to show that application of the substance has the same effects as those caused by the natural transmitter (e.g. Eccles, 1964; Klinke, 1986). Spontaneous release of the natural transmitter from the hair cells induces spontaneous discharge while tone stimulation induces an increase of the transmitter-release which in turn increases the discharge rate of the afferents. In the mammalian cochlea we found that Glu applied at mmol/l concentrations induces only small maximum increases in discharge rate in a small number of cells while in a substantial portion of cells it reduces the spontaneous rate without a previous increase. In addition we found a reduction of the tone evoked rate without a change in spontaneous rate in a big portion of our sample. Desensitization to Glu without accompanying changes in the spontaneous activity was also observed. These effects do not resemble those expected to be caused by application of the afferent transmitter, although some of the effects might be explained in terms of desensitization, artificial external application and activation of extrasynaptic receptors. Considering that bath application of Glu in other vertebate synapses (Okano and Obara, 1988; Geller and Woodward, 1974) causes the effects predicted for an excitatory transmitter a straightforward explanation for the observed differences between the action of the natural transmitter and Glu in the cochlea is that they are not identical.

Another point in identifying a transmitter is to demonstrate the metabolic chain involved in its synthesis. It has been suggested that the metabolism of Glu and glutamine in the cochlea might be similar to that in the central nervous system (Eybalin and Pujol, 1983; Ryan and Schwarz, 1984) where presynaptically released Glu is taken up by supporting structures and converted to glutamine. The glutamine is then again released from the supporting structures, taken up by the presynaptic terminals and converted to Glu to restore the transmitter pool. Although hair cells show a pronounced preferential uptake of glutamine the presence of the enzymes glutaminase (Fex et al., 1985) and aspartate aminotransferase (Fex et al., 1982) could not be shown in hair cells despite the fact that immunoreactivity to both enzymes was found in afferent and efferent fibers. However, one might argue that Glu in hair cells might be produced via another biosynthetic pathway. Nevertheless, the failure to demonstrate the presence of enzymes responsible for the conversion of glutamine (which is the most important precursor of Glu in the brain, Hamberger et al., 1979) to Glu despite a presumably high transmitter turnover does not provide support for the hypothesis that Glu is the afferent transmitter.

A third criterion in identifying a transmitter is to show its release from the presynaptic sites. Attempts to demonstrate a sound-related increase of Glu have not been convincing (Melamed et al., 1982; Sewell et al., 1978). Drescher et al. (1983) did show an increase of perilymph Glu-concentration from 3.6 μmol/l to 7.5 μmol/l with exposure to noise at 115 dB SPL, but found no increase at lower sound levels. There is only one preliminary report by Bledsoe et al. (1981b) that describes an increase of Glu-levels upon stimulation with a 0.6 kHz 80 dBSPL tone, without giving any further detail of their findings. Over all, the failure to demonstrate increased levels of Glu correlated with the intensity of sound exposure (although they have been demonstrated for GABA; Drescher et

al., 1983) might be explained by very effective breakdown or reuptake mechanisms which prevent leakage into the perilymph. However, the bioassay used by Sewell et al. (1978) showed that 'ANAS' (auditory nerve activating substance) is present and effective in perilymph collected during sound exposure despite Glu-concentrations which are about 250–500 times lower (Drescher et al., 1983) than those necessary to produce an effect in the mammalian cochlea. Jenison et al. (1985) showed that cochlear perfusion with solutions containing high-potassium and normal calcium concentrations caused a significant increase of aspartate, Glu, arginine, taurine + GABA, taurine and isoleucine release. The release of these substances was not increased during high-potassium low-calcium perfusions. Baseline levels of Glu were not reduced in the low-calcium trials, although this might be expected as a result of the reduction of spontaneous transmitter release. Over all, the release experiments do not provide clear support for the hypothesis that Glu is the excitatory afferent transmitter in the cochlea.

Conclusion

The data relating to the hypothesis that Glu might be the afferent transmitter which are available at present are equivocal and may be subjected to different interpretations. However, the data and arguments presented in this paper point to a number of inconsistencies of the Glu-hypothesis in the mammalian cochlea which lead us to the conclusion that Glu is not *the* excitatory afferent transmitter, although it might somehow be involved in synaptic transmission at this site perhaps as a modulator or co-transmitter.

Acknowledgements

This research was supported by N.H. and M.R.C. and The University of Western Australia. We thank G.A. Manley, R. Patuzzi and G.K. Yates for helpful discussion.

References

Baudry, M. (1986) Functions and regulations of glutamate receptors. In: P.J. Roberts, J. Storm-Mathisen and H.F. Bradford (Eds.), Excitatory amino acids. The Macmillan Press Ltd, London, pp. 301–321.

Bledsoe, S.C. Jr., Bobbin, R.P. and Chihal, D.M. (1981a) Kainic acid: an evaluation of its action on cochlear potential. Hear. Res. 4, (1981), 109–120.

Bledsoe, S.C., Bobbin, R.P. and Chihal, D.M. (1981b) Technique for studying sound-induced release of endogenous amino acids from the guinea pig cochlea. Abstr. 4th ARO Meet. 24–25.

Bledsoe, S.C., Chihal, D.M., Bobbin, R.B. and Morgan, D.N. (1983) Comparative actions of glutamate and related substances on the lateral line of *Xenopus laevis*. Comp. Biochem. Physiol. 75C, 119–206.

Bobbin, R.B. (1979) Glutamate and aspartate mimic the afferent transmitter in the cochlea. Exp. Brain Res. 34, 389–393.

Bobbin, R.B. and Ceasar, G. (1987) Kynurenic acid and g-D-glutamylaminomethylsulfonic acid suppress the compound action potential of the auditory nerve. Hear. Res. 25, 77–81.

Bobbin, R.P. and Thompson, M.H. (1978) Effects of putative transmitters on afferent cochlear transmission. Ann. Otol. Rhinol. Laryngol. 87, 185–190.

Bowman, C.L. and Kimelberg, H.K. (1984) Excitatory amino acids directly depolarize rat brain astrocytes in primary culture. Nature 311, 656–659.

Braitman, D.J. (1986) Desensitization to glutamate and aspartate in rat olfactory (prepyriform) cortex slices. Brain Res. 364, 199–203.

Comis, S.D. and Leng, G. (1979) Action of putative neurotransmitters in the guinea pig cochlea. Exp. Brain Res. 36, 119–128.

Drescher, D.G. and Drescher, M.J. (1987) Calcium and magnesium dependence of spontaneous and evoked afferent neural activity in the lateral line organ of *Xenopus laevis*. Comp. Biochem. Physiol. 87A, 305–310.

Drescher, M.J., Drescher, D.G. and Medina, J.E. (1983) Effect of sound stimulation at several levels on concentration of primary amines, including neurotransmitter candidates, in perilymph of the guinea pig inner ear. J. Neurochem. 41, 309–320.

Eccles, J.C. (1964) The physiology of synapses. Springer Verlag, Berlin.

Eybalin, M. and Pujol, R. (1983) A radioautographic study of [^{3}H]L-Glutamate and [^{3}H]L-glutamine uptake in the guinea pig cochlea. Neuroscience 9, 863–871.

Fagni, L. Baudry, M. and Lynch, G. (1983) Desensitization to glutamate does not affect synaptic transmission in rat hippocampal slices. Brain Res. 261, 167–171.

Fernandez, C. (1952) Dimensions of the cochlea (guinea pig). J. Acoust. Soc. Am. 24, 519–523.

Fex, J., Altschuler, R.A., Wenthold, R.J. and Parakkal, M.H. (1982) Aspartate aminotransferase immunoreactivity in cochlea of guinea pig. Hear. Res. 7, 149–160.

Fex, J., Kachar, B., Rubio, J.A., Parakkal, M.H. and Altschuler, R.A. (1985) Glutaminase-like immunoreactivity in the organ of corti of guinea pig. Hear. Res. 17, 101–113.

Geller, H.M. and Woodward, D.J. (1974) Responses of cultured cerebellar neurons to iontophoretically applied amino acids. Brain Res. 74, 67–80.

Guth, P.S., Norris, C.H. and Barron, S.E. (1988) Three tests of the hypothesis that glutamate is the sensory hair cell trans-

mitter in the frog semicircular canal. Hear. Res. 33, 223–228.

Hamberger, A.C., Chiang, G.H., Nylen, E.S., Scheff, S.W. and Cotman, C.W. (1979) Glutamate as a CNS transmitter. I. Evaluation of glucose and glutamine as precursors for the synthesis of preferentially released glutamate. Brain Res. 168, 513–530.

Hori, N., Auker, C.R., Braitman, D.J. and Carpenter, D.O. (1982) Pharmacological sensitivity of amino acid responses and synaptic activation of in vitro prepyriform neurons. J. Neurophysiol. 48, 1289–1301.

Jenison, G.L. and Bobbin, R.P. (1985) Quisqualate excites spiral ganglion neurons in the guinea pig. Hear. Res. 20, 261–265.

Jenison, G.L., Bobbin, R.P. and Thalmann, R. (1985) Potassium-induced release of endogenous amino acids in the guinea pig cochlea. J. Neurochem. 44, 1845–1853.

Jenison, G.L., Winbery, S. and Bobbin, R.P. (1986) Comparative actions of quisqualate and *N*-methyl-D-aspartate, excitatory amino acid agonists on guinea pig cochlear potentials. Comp. Biochem. Physiol. [C] 84, 385–389.

Katsuki, Y. (1973) The ionic receptive mechanism in the aoustico-lateralis system. In: A.R. Møller and P. Boston (Eds.), Basic mechanisms in hearing. Academic Press, New York, pp.307–334.

Klinke, R. (1986) Neurotransmission in the inner ear. Hear. Res. 22, 235–243.

Klinke, R. and Oertel, W. (1977) Amino acids - putative afferent transmitter in the cochlea? Exp. Brain Res. 30, 145–148.

Leng, G. and Comis, S.D. (1979) The effect of potassium on the activity of auditory nerve fibres of the guinea pig cochlea. Acta Otolaryngol. 87, 39–46.

Manley, G.A. and Robertson, D. (1976) Analysis of spontaneous activity of auditory neurones in the spiral ganglion of the guinea pig cochlea. J. Physiol. 258, 323–336.

Melamed, B., Norris, C. Bryant, G. and Guth, P. (1982) Amino-acid content of guinea pig perilymph collected under conditions of quiet or sound stimulation. Hear. Res. 7, 13–18.

Nagai, R., Obara, S. and Kawaf, N. (1984) Differential blocking effects of a spider toxin on synaptic and glutamate responses in the afferent synapse of the acousticolateralis receptors of *Plotosus*. Brain Res. 300, 183–187.

Okano, K. and Obara, S. (1988) Effects of bath applied L-glutamate and related chemicals on the afferent synapse of the *Plotosus* electroreceptor. Brain Res. 457, 79–88.

Patuzzi, R. and Robertson, D. (1988) Tuning in the mammalian cochlea. Physiolog. Rev. 68, 1009–1082.

Robertson, D. and Johnstone, B.M. (1979) Effects of divalent cations on spontaneous and evoked activity of single mammalian auditory neurones. Pfügers Arch. 380, 7–12.

Robertson, D. and Manley, G.A. (1974) Manipulation of frequency analysis in the cochlear ganglion of the guinea pig. J. Comp. Physiol. 91, 363–375.

Schwartz, I.R. and Ryan, A.F. (1983) Differential labeling of sensory cell and neural populations in the organ of Corti following amino acid incubations. Hear. Res. 9, 185–200.

Sewell, W.F., Norris, C.H., Tachibana, M. and Guth, P.S. (1978) Detection of an auditory nerve-activating substance. Science 202, 910–912.

Siegel, J.H. and Relkin, E.M. (1987) Antagonistic effects of perilymphatic calcium and magnesium on the activity of single cochlear afferent neurons. Hear. Res. 28, 131–147.

Smith, R.L. and Zwislocki, J.J. (1975) Short-term adaptation and Incremental Responses of single auditory-nerve fibers. Biol. Cybernetics 17, 169–182.

Valli, P., Zucca, G., Botta, L., Casella, C. and Guth, P.S. (1985) The effect of glutamate on the frog semicircular canal. Brain Res. 330, 1–9.