GLUTAMATE RECEPTORS IN THE DEVELOPING COCHLEAR GANGLION

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The cochlear ganglion contains the primary afferent neurones of the vertebrate auditory system and it constitutes the first synaptic relay after sound transduction. The developmental expression of voltage-activated ionic channels in chick cochlear neurones has been described in some detail (Valverde et al., 1992; Jiménez et al., 1996), however, little is known about the expression of ligand operated channels. Most evidence indicates that glutamate is the neurotransmitter between transducing hair cells and the cochlear neurones (see Eybalin, 1993 for a review). We have studied the developmental pattern of expression of the response of cochlear neurones to glutamate by analyzing intracellular Ca^{2+} transients with microfluorescence methods.

Cochlear neurones were isolated from chick embryos between days 6 and 11 of incubation (E-6, E-11). For fluorescence measurements, the cell-coated coverslips were washed with standard medium containing (in mM) : NaCl, 140; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 1; glucose, 10; sodium-HEPES, 10, pH, 7.4, and loaded with fura-2 by incubation with 5 µM fura-2/AM at room temperature for 1 h. The value of intracellular free Ca^{2+} concentration was estimated from the ratio of the fluorescence values excited at 340 and 380 nm (Grynkiewicz et al., 1985). Calibration was performed by comparison with fura-2 standards. For single-cell measurements, the coverslips coated with fura-2-loaded cells were mounted under a microscope (Nikon Diaphot) in a chamber thermostated at 36°C and epi-iluminated alternately at 340 and 380 nm. Light emitted above 520 nm was recorded by an extended ISIS-M camera (Photonic Science, Robertbridge, UK) and analysed using an Applied Imaging Magical image processor (Sunderland, UK). For routine experiments, four video frames of each wavelength were averaged by hardware, with an overall time resolution of about 3 sec for each pair of images at alternate wavelengths.

Cell depolarisation with high K^+ (50 mM) induced an increase in the concentration of intracellular Ca^{2+} (Fig. 1, traces labelled K^+). The response consisted of a rapid and transient component that reached a peak in about 15 sec, to relax to a steady-state value throughout the K^+-pulse. This response was similar to that described in most neuronal populations and reflects most probably the influx of Ca^{2+} through voltage-dependent Ca^{2+}-channels (Núñez et al., 1996). The early expression of type N and type L Ca^{2+}-channels in cochlear neurones has been recently documented by Jiménez et al., (1996). The response to high K^+ was used to asses the viability of the neurones in the preparation. The experiment was accepted only if at least 50% of the neurones responded to high K^+. The fraction of cells responding to high K^+, between developmental stages E-6 and E-11 was from 80 to 85%. This fraction did not change for the different developmental stages, indicating that cell viability was not dependent on the stage selected for the study. The increase in intracellular Ca^{2+} concentration induced by high K^+ was between 250 and 400 nM and did not show a particular trend for the different developmental stages.

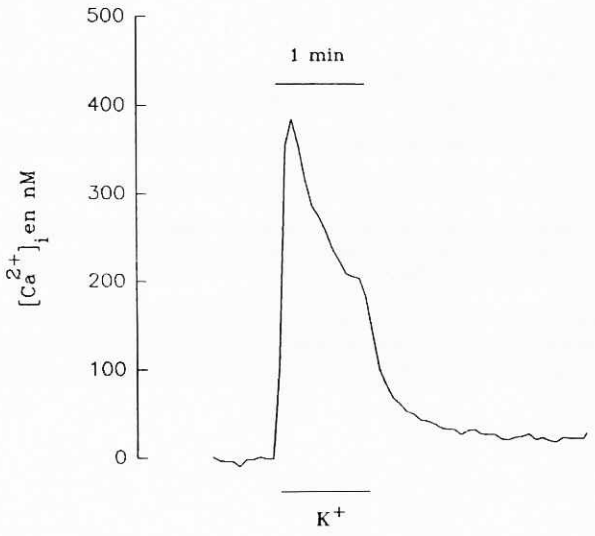

Figure 1. Increase of $[Ca^{2+}]_i$ induced by depolarization with high-K+ (50 mM) solution. The trace shown is the average of 88 neurons present in the same microscope field.

After testing responses to high-K^+, cells were superfused with a solution containing 100 µM glutamate and 10 µM glycine. The response elicited by glutamate was similar to that evoked by K^+ but of smaller amplitude. The fraction of cells responding to both, high K^+ and glutamate was between 40 and 80% (62±7%, n=91, in E-6 and 60±5%, n=46, in E-11). The increase in the concentration of Ca^{2+} induced by glutamate was about 200-300 nM and was similar throughout development. The response to glutamate was present as soon as E-6 in the development of cochlear neurones.

Glutamate receptors in the mature cochlear neurones are of the NMDA type (Yamaguchi & Ohmori, 1990). In order to make a preliminary characterization of early occurring glutamate receptors, cells were isolated from E-7 and E-8 embryos and challenged with a 100 µM NMDA solution. At both stages the majority of neurones responding to K^+ and glutamate, responded also to NMDA. Average values were 78±6% (n=4) for E-7 and 93±2% (n=4) for E-8. Therefore, early glutamate receptors were, like in mature neurones, of the NMDA type.

Neurotrophic factors are known to allow survival and differentiation of target neurones, within the cochlear neurones pool. The NGF-family of neurotrophins modulate the expression of intermediate filament molecules (San José et al., 1996) and Ca^{2+} ionic channels (Jiménez et al., 1996). We decided to study whether neurotrophins were also able to modulate glutamate receptor expression. Cells were isolated from E-7 cochlear ganglions and cultured for 48 h in the presence of 2 ng/ml NT-3. Neurones cultured with NT-3 developed typical neurites, whereas most of control neurones did not survive. Responses to high K^+ and glutamate were analyzed as described above. The fraction of neurones cultured with NT-3 that responded to high K^+ in these experiments was 94±2%, n=117. From these cells, 91±3%, n=106, responded also to glutamate. These figures are higher than the maximum values measured from freshly isolated neurones (see above). Cells rescued by NT-3, therefore, were excitable, responded to high-K^+ with Ca^{2+} transients, and also expressed functional glutamate receptors.

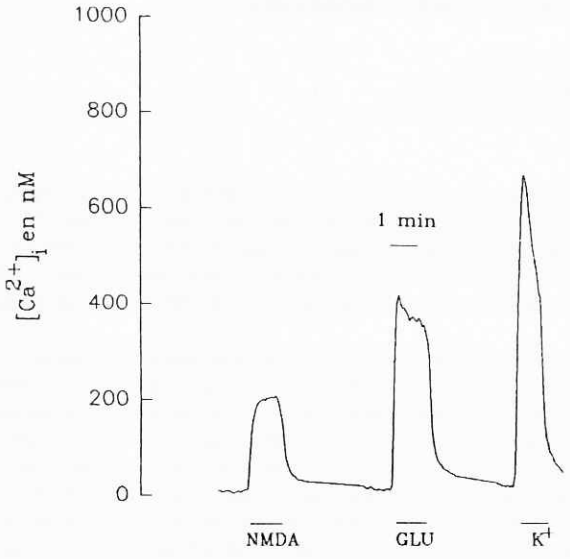

Figure 2. Response of cochlear neurons to sequential stimulation with NMDA (100 µM), L-glutamate (100 µM) and high-K^+ (50 mM) solutions. The trace shown is the average of 47 single neurons present in the same microscope field and selected by responding to all the three stimuli.

The results show the early expression of glutamate receptors in developing cochlear neurones. These receptors are of the NMDA type and they are rescued, along with cell survival, by the neurotrophin NT-3. It is not yet possible to establish the precise relation between NT-3 and the functional expression of glutamate receptors. NT-3 may regulate directly the expression of glutamate receptors but it may be also possible that the effect is not specific on receptor expression regulation but secondary to cell survival. The phenotype of cochlear neurones may be highly specified at the stage when they adquire their dependence of neurotrophins and the action of NT-3 could be only to allow survival and, as a consequence, the development of their differentiation program.

At stage E-7, when we first detect glutamate receptors, connections between hair cells and cochlear neurones do not yet exist. We face a situation where the expression of the physiological neurotransmitter anticipates the establishment of the actual synaptic communication. This is interesting because it suggests that glutamate may have a role in the innervation of the cochlea, which is not synaptically mediated. During recent years it has been show that glutamate displays pleiotropic responses, including trophic and plastic effects on developing neurones (McDonald & Johnston, 1990). Our results suggests that this may be the case in cochlear neurones. We propose that both glutamate and NT-3 may act as target-derived signals and interact to regulate the survival and differentiation of cochlear neurones.

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