Streptomycin blocks the afferent synapse of the isolated semicircular canals of the frog

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This study aimed to define the acute electrophysiological effects of the perilymphatic perfusion of streptomycin in the sensory apparatus of the semicircular canals of the frog. The ampullary DC potential, the vestibular nerve multiunit discharge, the nerve DC potential and the unitary EPSP activity were recorded in isolated semicircular canals of the frog (Rana esculenta L). The results demonstrated that perilymphatic microperfusion of streptomycin (0.1, 0.3, 1 and 3 mM) reduced both resting and mechanically evoked afferent discharge, while the response of the hair cells remains unchanged. Intracellular recordings from single afferent axons showed that the reduction of the afferent discharge was mainly due to a reduction of the amplitude, but not the frequency, of the EPSPs. These results indicate that streptomycin, when applied in the fluid bathing the synaptic pole of the sensory cells, can act as an antagonist of the vestibular afferent transmitter at the postsynaptic level.

Vestibular system; Streptomycin; Aminoglycoside; Hair cell; Inner ear; Glutamate receptor; Excitatory amino acid

Introduction

Although a great deal is known about the long term effects of aminoglycoside intoxication, less is known about their short term effects on the electrical activity of hair cells systems. Most previous experimental works have focused on the biochemistry, morphology and physiology of the hair cells. It has been shown that aminoglycoside antibiotics interfere with phospholipid metabolism in these cells (Schacht, 1986; Williams et al., 1987). Aminoglycosides damage the membrane of hair cells, altering the cilia, and on the long term, produce the cell death (Hawkins, 1976). It has also been shown that streptomycin blocks the mechano-electrical transduction channels of the sensory cells (Kroese and van den Bercken, 1980; Anderson, 1981; Ohmori, 1985; Kroese et al., 1989). These effects seem to take place in the apical region of the neuroepithelium, where transduction channels are located (Kroese et al., 1989, Hudspeth, 1982). However, perilymphatic administration of aminoglycosides affects hair cells systems with a short latency, in a manner which is not compatible with an action at the apical endolymphatic side of the epithelia (Matsuura et al., 1968; Anderson, 1981; Soto et al., 1991; Pérez et al., 1991). Further, in the axolotl inner ear, streptomycin postsynaptically blocks the excitatory effects of kainic and quisqualic acid on the vestibular afferents (Soto et al., 1991). Thus, although there exists clear evidence of aminoglycosides acting at the hair cells level, there are data indicating that they also affect some other physiological processes. In fact, aminoglycosides have well known blocking effects upon the neuromuscular synaptic transmission (for review see Pittinger and Adamson, 1972), and upon ionic membrane currents (Sokabe, 1983; Suarez-Kurtz and Reuben, 1987; Nomura et al., 1990).

We have extended these observations by investigating the short term effects of perilymphatic perfusion of streptomycin on the isolated semicircular canal of the frog. Our results demonstrate that streptomycin reversibly diminishes the basal activity of the afferent fibers, and their response to mechanical stimulation, without affecting the response of the sensory cells, thus indicating a synaptic site of action of the drug. These effects seem to be due to a postsynaptic blockade of the synaptic transmission between the hair cells and the afferent fibers, since the amplitude of the intracellularly recorded EPSPs is clearly decreased.

Materials and Methods

Adult frogs (Rana esculenta L) were used for the experiments. After decapitation, the lower jaw was removed and the head cut in two halves. The prepara-
Fig. 1. Experimental set-up. The isolated posterior semicircular canal was placed between two compartments (endo- and perilymphatic). Sp, stimulating device used to produce fluid displacements inside the canal. Ip, pipette for drug microperfusion. Adc, recording of the transepithelial potential. IR, electrodes and amplifier for intracellular recording from the afferent fibers. Np, suction electrode and AC-DC amplifier for recording of the afferent nerve multiunit spike activity (Nfr) and slow nerve DC potential (Ndc). F/V, frequency to voltage converter.

Streptomycin was given in a single bolus, close to the sensory organ at the level of origin of the ampullary nerve fibers. The drug (20 μl) was ejected by pressure at a rate of 5 μl s⁻¹, through a pipette of approximately 100–150 μm tip diameter. Since the bath volume is of 5 ml, the drug dilutes, following an exponential decay with a time constant in the range of a few ms (Waud, 1968), to a concentration which is 0.2% of the originally applied (for 1 mM it reaches a concentration of 2 μM). Further, the bath is continuously being perfused at a rate of 5 ml min⁻¹, thus diminishing the final drug concentration. We have calculated that the bath flow further dilutes the drug to 1/1000 of its initial concentration in 6 min.

The nerve multiunit firing rate (Nfr) and a slow electrical DC mass potential (Ndc) were recorded by means of a suction electrode. The ampullary transepithelial potential (Adc) was recorded between two Ag-AgCl pellets located in the endo- and the perilymphatic compartments (Fig. 1). The nerve spike activity was derived to a window discriminator and a frequency to voltage converter and continuously displayed in an oscilloscope and a pen recorder along with the Ndc and the Adc. Data were also recorded in magnetic tape for further analysis. In some experiments, intracellular recordings of single axons were also obtained by using glass microelectrodes of 40–60 MΩ resistance, filled with 3 M KCl inserted in the nerve of the canal about 500 μm distant from the synapse.

Results

Resting and evoked electrical activity were readily recorded from the isolated semicircular canal. The preparation remained in adequate condition, as judged by its regular basal discharge and its response to mechanical stimuli, during long periods (more than 8 h). This allowed us to test various drug concentrations and stimulation periods in a given preparation.

Streptomycin was ejected in the perilymphatic bath in concentrations of 0.1, 0.3, 1 and 3 mM. It consistently produced a dose dependent decrease of the spike resting basal activity of the afferent fibers and of the slow nerve DC potential (Ndc). Streptomycin did not significantly modify the transepithelial potential (Adc). The inhibitory effect of streptomycin was characterized by a rapid decrease of the nerve resting discharge, which reached its minimal level about 30 s after the drug application. The basal resting activity and Ndc potential returned to the control levels after approximately 10 min of washing with normal perilymphatic solution.

We also studied the effect of streptomycin upon the response of the preparation to sinusoidal mechanical
Fig. 2. Effect of streptomycin 1 mM on the semicircular canal response to sinusoidal stimulation. Adc, ampullary transepithelial potentials; Ndc, slow nerve DC potentials; Nfr, nerve firing rate. Stim, stimulus. A, control recording; B, after 30 s of streptomycin perfusion; C, after 10 min of streptomycin perfusion. Streptomycin 1 mM induced a significant decrease of the Ndc and Nfr response to mechanical stimuli, while the Adc remains unaltered.

Stimuli. Streptomycin consistently diminished the mechanical sensitivity of the semicircular canal. At concentrations greater than 0.1 mM it produced a concentration dependent decrease of the afferent fibers response. At 1 mM (Fig. 2), streptomycin produced an 80% reduction of the Nfr. The decline of the Nfr was accompanied with a decrease in the amplitude of the mass DC nerve potentials (Ndc) similar in amplitude and time course to the Nfr reduction. Streptomycin did not modify the amplitude of the Adc. Since the hair cells transducer current generate the Adc, this result demonstrates that, in our experimental conditions, the drug did not affect the hair cells transducer mechanism. Or the contrary, postsynaptic potentials (which generate the Ndc) and the afferent firing rate (evidenced by the Nfr) were both clearly reduced, indicating that streptomycin blocked the synaptic transmission between the hair cells and the afferent fibers.

Fig. 3 shows the dose response graphs of streptomycin on the electrical variables recorded. At all the concentrations tested streptomycin produced a very low, less than 5% reduction of the Adc (Fig. 3A). On the contrary, it induced a clear concentration dependent reduction of the Ndc and the Nfr (P < 0.001) (Fig. 3B and C). It is worth noting that the most evident effect of streptomycin was upon the resting spike activity (Fig. 3D), which declined to 10% of the control level with streptomycin 1 mM. The time course of the inhibitory effects of streptomycin were similar for the basal and the mechanically evoked responses.

Intracellular recordings of single axons showed that afferent fibers were continuously subjected to an excitatory input which determined its resting EPSP activity. A clear correlation, between subthreshold activity and afferent fibers basal discharge was observed. In order to understand if streptomycin actions are pre- or postsynaptic, we studied its effects upon the intracellularly recorded EPSP activity of the afferent fibers. Streptomycin 0.1 and 1 mM induced a clear decrease in the amplitude of intracellularly recorded spontaneous EPSPs (P < 0.005, exact test of Fisher) (Fig. 4). No change in the mean interval of the EPSPs was noted. The change in the EPSPs amplitude was temporally correlated with the change observed in the firing frequency of the afferent fibers. These results suggest that strepto-
tomycin did not significantly modify the transmitter release from the hair cells.

Discussion

Our results indicate that perilymphatic perfusion of streptomycin blocks the afferent synapse between the hair cells and the afferent fibers. Interference with synaptic transmission may take place at either the pre- or postsynaptic level. In our experiments, streptomycin induced a decrease of the intracellulary recorded EPSPs amplitude, with no apparent change of its mean frequency. This indicates that this drug produces a postsynaptic decline of responsiveness to the afferent transmitter, with no apparent changes in the transmitter release from the hair cells. Although, a presynaptic effect of the drug on the transmitter release mechanisms could not be completely discarded, particularly because the EPSPs recorded are not unitary miniature EPSP, thus it is not possible to precisely define if EPSP amplitude changes are due to a decrease in the quantal content in the postsynaptic response to the chemical transmitter. However, it has been previously shown that the Adc is due to the electrotonic sum of the receptor potentials of the hair cells, and that the Ndc amplitude is produced by the electrotonic addition of the EPSPs in the afferent fibers (Valli and Zucca, 1976). Thus, the lack of effect of streptomycin upon the Adc, implies that the response of the sensory cells to mechanical stimuli was of normal amplitude, and that streptomycin effect takes place at the synaptic level. The Ndc amplitude diminution indicates that streptomycin blocks the response of afferent fibers to the transmitter released by the hair cells. The nerve firing rate (Nfr) decrease seems to be a consequence of the alteration in the mean EPSP amplitude.

As previously described in the methods section, in our experiments the drug was applied in volumes of 20 μl. Thus it dilutes quickly to reach a final concentration which is less than 0.2% of that originally applied. Continuous perfusion of the bath and the existence of diffusion barriers further diminish the chance for the drug to reach the basal pole of the sensory epithilia. Hence, the concentration at the synaptic cleft should be very low. This gives support to the idea that streptomycin effects are not due to non-specific pharmacological actions, and practically exclude the possibility of streptomycin significantly diffusing into the endolymphatic compartment.

Concerning the mechanisms by which streptomycin might produce a blockage of synaptic transmission, there have been many reports dealing with its actions at the cholinergic neuromuscular transmission (for review see Pittinger and Adamson, 1972); also, streptomycin blocks the glutamate mediated neuromuscular transmission in the crayfish (Onoder and Takeuchi, 1977). It has been shown that streptomycin exerts both pre- and postsynaptic actions. The presynaptic effect of streptomycin has been explained on the basis of a displacement of Ca\(^{2+}\) ions, and reduction of Ca\(^{2+}\) currents associated with transmitter release (Pittinger and Adamson, 1972). The postsynaptic actions of streptomycin are less understood, and we are not aware of any comprehensive hypothesis proposed to explain them. Previous results obtained by some of us (Soto et al., 1991; Pérez et al., 1991), suggest that the blocking action of streptomycin upon the vestibular system afferent activity could be due to a blockage of postsynaptic excitatory amino acid receptors. In fact, it has been shown that streptomycin postsynaptically blocks the excitatory effects induced by kainic and quisqualic acids on the vestibular primary afferents of the axolotl inner ear (Soto and Vega, 1988; Soto et al., 1991; Pérez et al., 1991).

Since the luminal and basal surfaces in epithelia are usually functionally and morphologically different, drugs could affect these cells depending on whether they were applied from the intra or extraluminal side. The acute effects of streptomycin on the vestibule seem to be completely different when it is applied luminally or extraluminally. At the luminal endolymphatic side, it has been reported that streptomycin causes an immediate abolition of hair cell receptor potentials (Wersäll and Flock, 1964; Matsuura et al., 1968), due to a blockage of cationic channels carrying the transducer receptor current (Ohmori, 1985; Kroese et al., 1989). Our results show that extraluminal perilymphatic application of streptomycin, causes a decrease of the discharge rate of the afferent fibers, without modifying the response of the hair cells to mechanical stimuli. This indicates that the extraluminal effect of streptomycin is due to an interference with the afferent synaptic transmission and is very probably caused by a postsynaptic blockage of neurotransmitter action.

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References


Wersäll J. and Flock, Å. (1964) Suppression and restoration of the microphonic output from the lateral line organ after local application of streptomycin. Life Sci. 3, 1151–1155.