EFFECTS OF STREPTOMYCIN, KANAMYCIN, QUININE, AND OTHER DRUGS ON THE MICROPHONIC POTENTIALS OF GOLDFISH SACCLUDUS

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Summary The effects of streptomycin and kanamycin on the goldfish’s saccular microphonic potentials were examined following their local application with a perfusion technique. The results obtained were compared with those of other drugs.

Streptomycin and kanamycin suppressed microphonics only when they were administered intraluminally to the sacculus but showed almost no effect when administered extraluminally.

Metabolic inhibitors (cyanide, azide, DNP), quinine, and procaine also suppressed microphonics, but, unlike streptomycin and kanamycin, there was no difference in their effects if administered to the luminal side or to the extraluminal side. Namely, cyanide and azide at $5 \times 10^{-4}$ g/ml, DNP at $5 \times 10^{-3}$ g/ml, quinine at $1 \times 10^{-4}$ g/ml and procaine over $2 \times 10^{-3}$ g/ml suppressed microphonic potentials through either route of administration. Tetrodotoxin and salicylates showed no effect on microphonics.

The site and the possible mechanism of action of streptomycin and kanamycin on hair cells were discussed by comparing their effects with those of other drugs.

It is well known that streptomycin and kanamycin suppress cochlear and vestibular functions and inflict cellular damage selectively on sensory hair cells (DeWeese and Sounders, 1968; Duvall and Wersäll, 1964; Goodman and Gilman, 1970; Hawkins, 1959; Hawkins and Engström, 1964; Wersäll and Hawkins, 1962). Although ototoxic effects appear only slowly following systemic administration, an immediate suppression of microphonics is produced in the lateral line organ when streptomycin is administered directly to the canal organ even at a very low concentration (Wersäll and Flock, 1964). The antibiotics
are also known to suppress the sensory discharges of the excised semicircular canal organ of the frog (Harada et al., 1967).

This paper describes a study, using a microperfusion technique, of the effects of streptomycin and kanamycin on the activity of hair cells in the goldfish sacculus, with microphonic potentials used as an indicator of hair cell activity. Special attention was directed to the different effects of streptomycin and kanamycin following their luminal and extraluminal administration to the sacculus, in comparison with the effects of other drugs such as quinine, local narcotics, and metabolic inhibitors. Streptomycin and kanamycin suppressed the microphonics only when administered intraluminally, in sharp contrast with ouabain which suppressed the microphonics only when administered extraluminally (Matsuura et al., 1968, 1971). On the other hand, other drugs showed no marked difference depending on the route of administration.

METHODS

Experiments were performed on anesthetized and immobilized goldfish about 12 cm long. Perfusion procedures for intra- and extraluminal administrations of drugs were essentially the same as described in a previous paper (Matsuura et al., 1971). Intraluminal administration was performed through a micropipette inserted into the saccular cavity. Drugs to be applied were dissolved in an artificial endolymph (KCl: 79 mM; NaCl: 53 mM) or in 120 mM KCl (Matsuura et al., 1971). The perfusion rate was generally from 0.1 to 0.3 µl/min. The perfusate was changed by using a new pipette containing a new perfusate. For extraluminal application, 0.03-0.1 ml of test fluid was dropped from a small pipette onto the base of the skull so as to submerge the saccular macula under the fluid. To prevent dilution of the test fluid, the fluid was replaced with a new one every 5 min. Before applying drugs, perfusion was usually made with solution used for dissolving the drug to be tested. Usually such a control perfusion had no significant effect on the size of the microphonics (Matsuura et al., 1971). As an index of hair cell activity, negative microphonic potentials were recorded with a microelectrode filled with 3 M KCl (Furukawa and Ishii, 1967). The amplitudes of 80 microphonics were averaged on a computer (Nihonkohden ATAC-401). For sound stimulus, tone pips at about 1,000 Hz (duration: 30-40 msec) were delivered from a loudspeaker placed about 30 cm from the fish.

RESULTS

Effects of streptomycin and kanamycin

Sulfate compounds of dihydrostreptomycin and kanamycin were used. Perfusion of intra- and extraluminal spaces was carried out with drug solutions under the same condition as the control. Figure 1 shows an experiment in
Fig. 1. Effects of kanamycin (A) and dihydrostreptomycin (B) on goldfish saccular microphonic potentials. Drugs were applied, first extraluminally, then intraluminally, to the same preparations for the period denoted by continuous and broken lines, respectively. A and B were obtained on different preparations.

which dihydrostreptomycin or kanamycin at a concentration of $2 \times 10^{-4} \text{ g/ml}$ was administered successively extra- and intraluminally to the same preparation. There was a marked difference in the effects of the drug between its extra- and intraluminal administrations. It was found that extraluminal administration of either dihydrostreptomycin or kanamycin did not produce any appreciable effect on the microphonics. This finding was confirmed in further experiments where a much higher concentration of the drug was used; $5 \times 10^{-3} \text{ g/ml}$ for dihydrostreptomycin and $2 \times 10^{-2} \text{ g/ml}$ for kanamycin (see Table 1). By contrast, intraluminal administration resulted in a marked reduction in the size of the microphonics. The reduction started about 3 or 4 min after administration of the drug. The initial rapid fall of the potential was followed by a gradual decline, reaching a steady low level in about 30 min. In Fig. 1, microphonic potentials were reduced to about 40 and 30% of their initial amplitude by perfusing with kanamycin and dihydrostreptomycin, respectively.

In the experiments shown in Fig. 2, a drug at two different concentrations was applied successively to the same preparation. As can be seen very clearly, suppression of the microphonics by either drug depended on its concentration. In
Fig. 2. Effects of different concentrations of streptomycin (A) and kanamycin (B) administered intraluminally to the sacculus. A and B were obtained on different preparations.

the case of dihydrostreptomycin shown above, the microphonics were reduced to about half their control size by the drug at $5 \times 10^{-5}$ g/ml but were almost completely abolished when a solution of much higher drug concentration was perfused. The results obtained by perfusion with kanamycin solution showed similar tendencies (Fig. 2B). One marked difference was that the microphonics showed an initial increase when perfused with $1 \times 10^{-4}$ g/ml of kanamycin. A similar initial increase in the microphonic size was sometimes observed also after perfusion with a low concentration of dihydrostreptomycin, but the increment was low, less than 10% (see Table 1). The microphonic potential once reduced in size by perfusion with a drug solution showed some recovery when the perfusion was suspended or when the perfusion was switched to an isotonic KCl. Recovery from the effects of kanamycin was more prompt than that from dihydrostreptomycin, but in either case, the recovery was incomplete once a concentrated solution of either drug had been administered.

Quinine

Quinine may produce clinical disturbances in hearing when administered in a large dose or for a prolonged period of time (DeWeese and Sounders, 1968; Rüedi et al., 1952). It is also known to produce in animal experiments cellular damage of outer hair cells and of the stria vascularis (Hennebert and Fernández, 1959).
In the present experiments, quinine was found to suppress microphonics irreversibly when administered either extra- or intraluminally. Some typical time courses of the experiments on quinine are shown in Fig. 3. When $5 \times 10^{-4}$ g/ml of quinine was administered extraluminally, the microphonics decreased in size soon after its application, reaching about 10% of the control within 20 min (Fig. 3A). In 30 min, quinine was removed by several washings with fresh normal Ringer's solution, but no sign of recovery was observed. The results of intraluminal administration of quinine at the same concentration were similar, but the development of the effect was a little slower, as shown in Fig. 3B. The effects of quinine were studied on 15 cases. Quinine produced a marked reduction in the
Fig. 3. Effects of quinine administered extra- and intraluminally to the sacculus. Results on two different preparations.

Fig. 4. Effects of cyanide (A, B) and DNP (C, D) administered intra- and extraluminally to the sacculus. Results on four different preparations.
microphonic potentials in all cases, so long as the concentration was $1 \times 10^{-4}$ g/ml or more (Table 1).

**Salicylate and aspirin**

The ototoxicity of salicylate and aspirin in systemic medication has been reported previously (DeWeese and Sounders, 1968; Wittmaack, 1916). In the present study, however, salicylate and aspirin showed no depressing effect on saccular microphonics even at high concentrations such as $3 \times 10^{-3}$ and $1 \times 10^{-3}$ g/ml (Table 1).

**Metabolic inhibitors**

Metabolic inhibitors such as cyanide, 2,4-dinitrophenol (DNP), and azide were found to suppress the microphonics when administered either intra- or extraluminally. As shown in Fig. 4A, perfusion of the saccular space with $5 \times$

<table>
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<tr>
<th>Drugs</th>
<th>Route of administration</th>
<th>Concentration (g/ml)</th>
<th>Total number of cases</th>
<th>Change in microphonic size</th>
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10⁻⁴ g/ml of sodium cyanide produced a gradual reduction in the microphonic potential after a lapse of 3–5 min, with the potential finally reaching a steady level within about 40 min after the start of drug application. When 5 × 10⁻⁴ g/ml of cyanide was applied extraluminally, the microphonics decreased to about 60% of the control (Fig. 4B). The effects of cyanide were more or less irreversible, although a complete suppression of the microphonic potential was not observed even at 1 × 10⁻³ g/ml of cyanide (Table 2).

The effects of DNP were similar to those of cyanide (Fig. 4 C, D), but a little weaker. For example, at a concentration of 5 × 10⁻³ g/ml, the microphonics were depressed to 55–65% of the control (Table 2).

Tetrodotoxin and procaine

It has been reported that tetrodotoxin has a very specific depressing action on nerve excitation, but it does not usually interfere either with the receptor processes or with the postsynaptic potentials, while procaine shows diversified effects besides its local narcotic action (FURUKAWA et al., 1959; KATSUKI et al., 1966; KONISHI and KELSEY, 1968b; NARAHASHI et al., 1964).
The above finding was confirmed in the present study. Namely, tetrodotoxin applied through either route of administration had almost no influence on saccular microphonics even at as high a concentration as $5 \times 10^{-4}$ g/ml (Fig. 5A, B). The potential remained within 90% of the control size in all 10 cases studied (Table 2). In the experiment shown in Fig. 5C, procaine at a concentration of $2 \times 10^{-3}$ g/ml was successively administered extra- and intraluminally to the same preparation. The microphonics were reduced in size reversibly by procaine when it was applied through either route of administration. The effects of procaine were milder than those of quinine or cyanide, for the maximum reduction of the microphonics was only 33% even at a concentration of $2 \times 10^{-3}$ g/ml (Table 2).

**DISCUSSION**

It was clearly demonstrated in the present study that streptomycin and kanamycin suppress the saccular microphonics only when administered intraluminally. This is not necessarily a new finding, since WERSÅLL and FLOCK observed in 1964 that the microphonic output from the lateral-line organ was reversibly suppressed following local administration of streptomycin. HARADA and others have also shown (1967) that streptomycin and kanamycin promptly and reversibly suppress spontaneous and evoked discharges from the frog semicircular canal organ. In both of these experiments, streptomycin and kanamycin were directly applied to the hair-bearing surface of hair cells. On the basis of such findings, it was proposed that the primary site of action of the drug would be the surface membrane of hair cells (WERSÅLL and FLOCK, 1964; HARADA et al., 1967). No statement was made, however, as to whether the whole surface of hair cells or only the membrane on the hair-bearing surface is susceptible to streptomycin and kanamycin. Thus, the present results seem to be of some value in pinpointing the site of action of streptomycin and kanamycin on the hair-bearing surface of hair cells, since extraluminal administration of the drugs had no effect on the microphonics even at much higher concentrations. Such a special, unilateral effectiveness of streptomycin and kanamycin is quite unique, being comparable only to the effects of ouabain, which suppresses the microphonics only when administered extraluminally (MATSUURA et al., 1971). It should also be noted that other drugs tested in the present experiment showed no marked difference in their effects depending on their route of administration.

The ototoxic effects of streptomycin and kanamycin have usually been studied following their systemic administration. Systemic administration is characterized by a slow development of the drugs' effects; for example, with a daily intramuscular injection of dihydrostreptomycin in the guinea pig at a dose of 250 mg/kg/day, hearing damage is detected after the 7th or the 10th day (DUVALL and WERSÅLL, 1964; HAWKINS and ENGSTRÖM, 1964). Such a slow development of the drugs' effect may be attributed to the fact that streptomycin and kanamycin
enter the endolymphatic space very slowly. Since the excretion of streptomycin and kanamycin from the endolymphatic space takes place much more slowly, the drugs that enter the endolymphatic space tend to accumulate there following repeated daily administration, reaching a concentration as high as $10^{-5}$ g/ml on the 10th day after administration (Stupp et al., 1966).

By studying cochlear damage resulting from intratympanic application of neomycin, Kohonen and Tarkkanen (1969) state that the drug probably reached the organ of Corti via the endolymphatic space. Namely, their results indicated that the drug could enter the endolymphatic space only from the scala vestibuli by crossing Reissner’s membrane, but not from the scala tympani by crossing the reticular lamina. Other morphological studies by Duval and Wersäll (1964), Hawkins and Fngström (1964), and Kohonen (1965) disclosed that the earliest sign of hair cell degeneration during streptomycin intoxication is a swelling and peculiar disarrangements of sensory hairs on the outer hair cells, resulting in a dislocation of the W-pattern of sensory hairs and so forth. Such a morphological finding seems to indicate strongly that the primary site of action of streptomycin and kanamycin may be the membrane on the hair-bearing surface of hair cells. Therefore, the results obtained with systemic as well as intratympanic administration of streptomycin and kanamycin agree with the present finding that the antibiotics can suppress microphonics only when administered to the endolymphatic space.

It must be noted that many studies on the ototoxicity of streptomycin and kanamycin have been carried out with the aim of clarifying the nature of the subtle differences in susceptibility between hair cells located in the base and those located near the apex of the cochlea, and between the vestibular and cochlear hair cells (Kohonen and Tarkkanen, 1969). Without doubt, these differences prove to be very significant in clinical application of antibiotics. Here it is noteworthy that similar differences in susceptibility have been reported with systemic as well as with local administration of quinine, whose toxic action seems much less selective than that of streptomycin and kanamycin, as can be expected from the fact that the ototoxic effects of quinine are attributable to its protoplasmic toxicity (Harada, 1970; Hennebert and Fernández, 1959; Rüedi et al., 1952).

In the present study, the effects of drugs other than streptomycin and kanamycin antibiotics were studied chiefly in order to clarify the uniqueness of the effect of the latter. None of the former showed any difference in effects between intra- and extraluminal administration. Also, the results obtained were similar to those on the guinea pig cochlea as reported by many authors (Katsuki et al., 1966; Konishi and Kelsey, 1968a, b; Tanaka and Brown, 1970). But the material used in the present study has a certain advantage over the cochlea of higher forms in that its structure is much simpler than the latter. For example, in the guinea pig cochlea, a decrease in the endocochlear DC potential brings about a reduction in microphonic size even when the hair cells remain unaffected.
Interpretation of the results is much simpler in the present material because it lacks an endolymphatic DC potential.

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