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EFFECTS OF EFFERENT STIMULATION ON THE SACCULE OF GOLDFISH

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SUMMARY

1. The effect of single or repetitive stimulation applied to efferent nerve fibres on afferent nerve activity and microphonic potentials was studied in the saccule of goldfish.

2. The sound-evoked excitatory post-synaptic potentials (e.p.s.p.s) recorded intracellularly from afferent eighth nerve fibres were reduced in size or completely abolished by efferent stimulation. The maximum inhibitory effect produced by repetitive efferent stimulation was equivalent to reducing the sound intensity by 10–25 db. Spontaneous miniature e.p.s.p.s were also suppressed by efferent stimulation.

3. The effect of single efferent stimulation appeared with a delay of 6–7 msec and lasted for about 40 msec, reaching its peak at about 12 msec. The slow and prolonged time course makes a sharp contrast with the very fast time course of afferent synaptic action.

4. The application of hyperpolarizing current through the recording micro-electrode revealed no sign of a post-synaptic increase in membrane conductance during inhibition. Hence, the inhibition was mostly attributable to a presynaptic action, i.e. to a suppression of transmitter release from hair cells.

5. Individual e.p.s.p.s were evoked in response to each wave of sound without any change in latency, but reached their peak much earlier during inhibition than in the control period. A likely explanation for this finding is that transmitter is released from hair cells during inhibition only in the early part of the stimulatory phase of the sound wave.

6. The extracellularly recorded microphonic potentials showed a slight increase in amplitude during efferent stimulation.

7. The nature and site of action of efferent nerve action are discussed along with some drug effects.

INTRODUCTION

The effects of stimulation of efferent inhibitory fibres on hair cell organs have been fairly extensively studied in the cochlea (Galambos, 1956; Desmedt, 1962; Fex, 1962, 1967*a*, *b*; Wiederhold & Kiang, 1970; Wiederhold, 1970; Desmedt & Robertson, 1975), the vestibular organs (Llinás & Precht, 1969; Klinke & Schmidt, 1970; Rossi, Prigioni, Valli & Casella, 1980) and the lateral line canal organ (Russell, 1968; Flock

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& Russell, 1973, 1976). In most of these studies, however, this inhibitory action on afferent nerve activity was studied by recording the latter extracellularly. In the present study, potentials were recorded intracellularly from single afferent eighth nerve fibres and the effects of efferent stimulation on these fibres were studied within the saccule of goldfish. Since the effect of efferent stimulation was observed as changes in the sound-evoked excitatory post-synaptic potentials (e.p.s.p.s), the mechanism and the site of action of the inhibition could be explored in more detail than by simply observing the effect on afferent discharge. The results of this study indicated that inhibition in the present preparation was mostly attributable to a presynaptic action, i.e. to a suppression of transmitter release from hair cells. The e.p.s.p.s were reduced in amplitude during efferent inhibition and in addition their time course was cut short without any change in the synaptic delay.

METHODS

Experiments were performed in a sound-proof room on goldfish about 12 cm long. Animals were anaesthetized with meta aminobenzoic acid ethylester methanesulphonate (MS 222, Sandoz). The drug was dissolved in about 1 l. of water which was aerated and made to circulate through the gills by means of a pump. The rate of flow was about 100 ml./min and the concentration of MS 222 was 50–80 μg/ml. Fish were usually kept in a good condition for a few hours, but the concentration of MS 222 had to be carefully controlled according to the season and the condition of the fish. In some later experiments ketamine hydrochloride (Ketalar 50, Parke, Davis & Sankyo, 1 mg/g body wt., I.M.) was used for anaesthesia. Since ketamine at even this high dose was insufficient to suppress the movement of fish completely, spinal transection was made at the junction with the medulla.

The operative techniques and other procedures to record intracellularly from single afferent eighth nerve fibres have been reported elsewhere (Furukawa & Ishii, 1967; Furukawa & Matsuura, 1978). Potentials were recorded mostly from large S-1 fibres, although some records were also obtained from small S-2 fibres. (For the distinction between S-1 and S-2 fibres, see Furukawa & Ishii, 1967; Fay, 1978.)

To activate efferent fibres electrical stimulation was delivered through a small bipolar electrode to the saccular nerve near its exit from the medulla. The electrode was made from a pair of electrolytically polished iridium–platinum wires (tip diameter, about 30 μm, interpolar distance, 150 μm) which were insulated with lacquer except for the tip. Usually ten short pulses (pulse width, 0·2 msec) were delivered with an interval of 5 msec. The sound stimulus was applied through a loud-speaker placed in front of the animal at a distance of about 30 cm. Potentials were recorded on a FM-magnetic recorder (TEAC 410) and processed subsequently. In some experiments about fifty records were averaged electronically (ATAC 350).

RESULTS

Fig. 1 illustrates the inhibitory effects that appear in afferent eighth nerve fibres on efferent activation. Parts *A* and *B* of this Figure are the same records displayed at two different speeds. As shown in the top trace of *A*, intracellular potential changes evoked by sound (440 Hz, 80 db SPL) consist of e.p.s.p.s and spikes. But when preceded by a train stimulus delivered to the saccular nerve trunk (see Fig. 1*B* for shock artefacts) these responses to an identical sound stimulus were suppressed, as shown in the middle trace. Spikes were abolished leaving some small e.p.s.p.s.

The effectiveness of inhibitory stimulation depended on several different factors. First, a train of stimuli was more effective than a single pulse, although the latter produced a detectable inhibitory effect (see Fig. 2). In the present study, ten shocks were delivered, usually at a rate of 200/sec, and their effect was tested after

termination of the stimulus train. Secondly, as might be expected, changes in the position of the stimulating electrode produced large differences in the observed effect. The electrode was usually placed at the location where a marked inhibitory effect could be produced with a relatively weak stimulus intensity. It was noted that the inhibitory effect always increased in a graded manner upon increasing the stimulus intensity. This seems to indicate that hair cells are multiply innervated by efferent fibres. At a certain electrode position the impaled afferent fibre may be driven

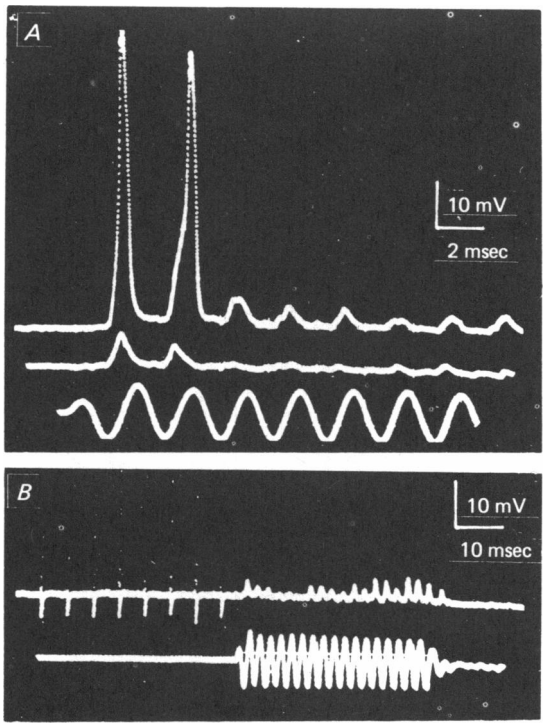

Fig. 1. Responses to sound stimulus recorded from single afferent eighth nerve fibre (S-1 fibre). *A*, top, control; middle, response during efferent inhibition; bottom, stimulus sound. *B*, temporal relationship between efferent stimulation and sound stimulus. Eight shocks were delivered at 200/sec before the sound stimulus in this case. Sound stimulus, 440 Hz and 80 db SPL. Upward deflexion positive.

antidromically during stimulation. But antidromic activation was not related in any way to the appearance of the inhibitory effect.

As pointed out by Desmedt (1962), a quantitative measure for the efferent inhibitory effect can be obtained by relating it to the intensity of the sound stimulus. In Fig. 1, for example, small e.p.s.p.s remained during the inhibition. When tested with a weaker sound, however, responses were completely eradicated. The intensity of inhibition can be estimated by comparing the effect produced by the inhibitory stimulation with the effect of reducing the sound intensity (see Fig. 6). In the present study the maximum effect was equivalent to reducing the sound intensity by 15–25 db. This figure is in the same range as values reported for stimulation of the crossed olivocochlear bundle, i.e. equivalent to a reduction of 12–26 db for gross auditory

nerve response in the cat (Desmedt, 1962; Wiederhold & Peake, 1966) and 1–25 db for effects on single axons (Wiederhold, 1970; Teas, Konishi & Nielsen, 1972).

Time course of inhibition after a single efferent stimulation

In the experiment shown in Fig. 2 the inhibition produced by a single efferent shock was tested by delivering click sounds at various intervals thereafter. The click intensity was adjusted to threshold for evoking a spike potential (Fig. 2*B*). As can

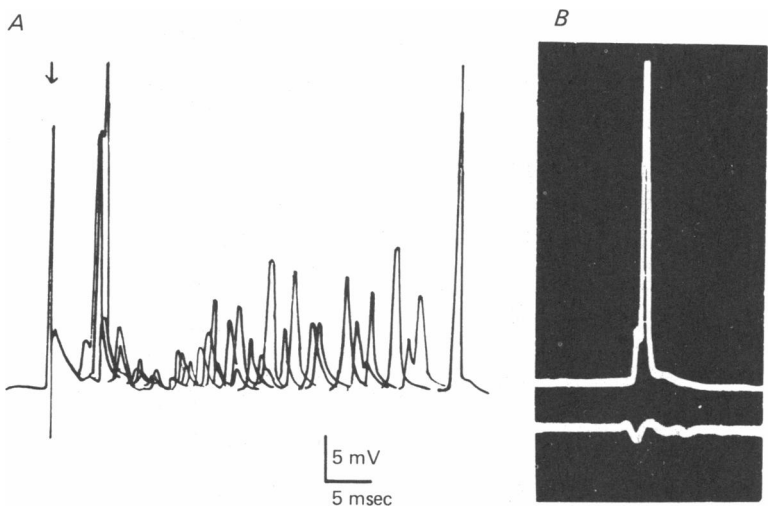

Fig. 2. Inhibitory effects produced by single efferent stimulation. *A*, time course of the inhibition as demonstrated by delivering click sounds at various intervals following single efferent stimulation (arrow). *B*, sample response to the test click under a control condition. Intensity of the click was barely enough to set up a spike. Click sound: one cycle of 420 Hz at 79 db SPL.

be seen in Fig. 2*A*, an inhibitory effect appeared with a delay of about 6 msec and reached its peak at about 13 msec after the efferent stimulus. The maximum inhibition was equivalent to about 6 db, being much weaker than that produced by delivering a train stimulus, which was equivalent to about 17 db in this case. The initial delay of 6 msec includes the conduction time in the efferent fibres of 2–3 mm. As will be apparent by comparing the result of Fig. 2 with Fig. 1, the duration of the inhibitory effect was not so different whether produced by single or by train stimulation of the nerve. Thus, the rate of dissipation of the inhibitory effect after the end of efferent stimulation was greater in the present preparation than in the cat, where inhibition produced by the crossed olivocochlear bundle stimulation has been shown to decline exponentially with a time constant of 90–200 msec (Desmedt, 1962).

The discharge of spontaneous miniature e.p.s.p.s was often very marked when afferent fibres were impaled at points close to their terminal. It was found that efferent stimulation abolished this activity and resulted in noise-less records as shown in Fig. 3.

Changes in the amplitude of the microphonic potentials during efferent stimulation

It is well known that the amplitude of the cochlear microphonic potentials increases during efferent inhibitory action (Fex, 1959, 1967*a*; Desmedt, 1962). In the present study an attempt was made to test whether similar increases could be obtained on different types of microphonic potentials recorded in the goldfish sacculus (Furukawa & Ishii, 1967; Ishii, Matsuura & Furukawa, 1971; Furukawa, Ishii & Matsuura,

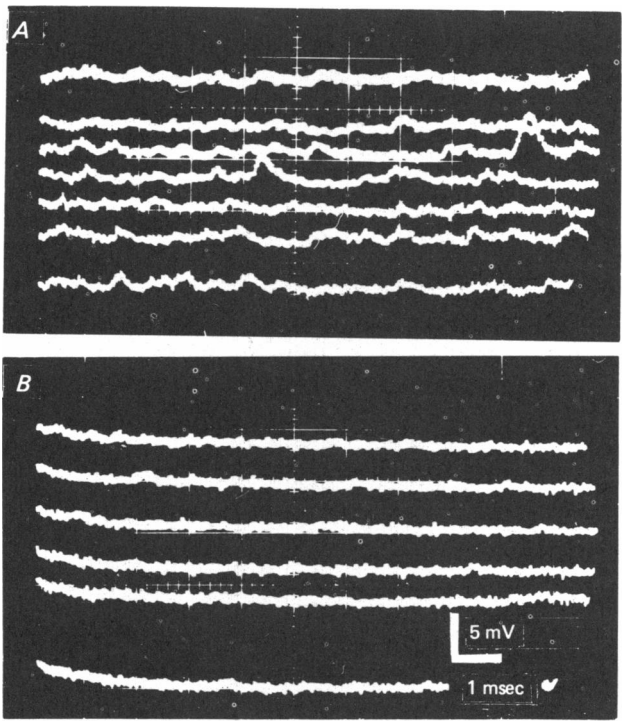

Fig. 3. Suppression of miniature e.p.s.p.s during efferent inhibition. Each trace in *B* was preceded by efferent stimulation.

1972*a*). First, an increase in the amplitude during efferent stimulation was readily demonstrated in the case of the negative microphonic potentials recorded from the endolymphatic side of the saccular macula, although the magnitude of the change was not great (about 10% in three cases).

Secondly, an increase was observed in the amplitude of the coupling potentials, i.e. the component of the microphonic potentials recorded intracellularly from afferent eighth nerve fibres (Ishii *et al.* 1971). In the case shown in Fig. 4 the coupling potential was increased during efferent inhibition by about 13% (arrowhead in the middle trace). As shown in this Figure, efferent inhibition also revealed the presence of coupling potentials throughout the whole period of sound stimulation, for it suppressed the e.p.s.p.s and spikes which otherwise masked them. All the deflexions except for the one indicated by the arrow in the middle trace in Fig. 4 can be interpreted as coupling potentials. They cannot be e.p.s.p.s because they were evoked

with time relationships different from those for e.p.s.p.s in the control record. These results can be observed more clearly in the averaged record shown in Fig. 5.

Traces *A 1* and *A 2* of Fig. 5 are the same records as Fig. 4. Traces *B 1* and *B 2* show averages of fifty responses. Only the first coupling potential is shown under control conditions (*B 1*) because later deflexions were scaled out. On the other hand, the detailed effect of efferent inhibition is clearly illustrated in *B 2*. The coupling potential was augmented during the inhibitory period by about 13 %. Although trace

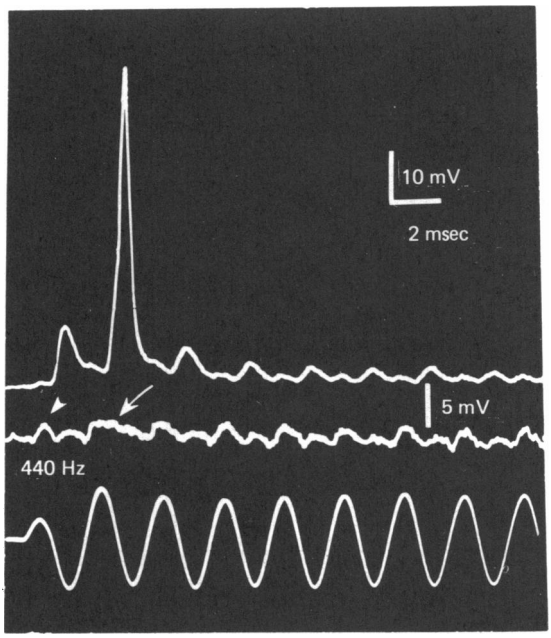

Fig. 4. Augmentation of coupling potentials during efferent inhibition. Top, control response; middle, response during efferent inhibition. Augmentation is most readily observed with the first coupling potential (arrowhead). Note the difference in the amplification. The deflexion arrowed is a sound-evoked e.p.s.p., but all other deflexions in the middle trace are coupling potentials. Sound stimulus, 440 Hz and 95 db SPL.

B 2 demonstrates the presence of coupling potentials throughout the period of the sound stimulus, it contains another interesting finding in relation to the seemingly irregular course of trace *B 2* which occurred in the middle of the record. With the assumption that a regular succession of coupling potentials continues on that part of the trace, the component attributable to the coupling potential was subtracted from the observed curve. As shown in trace *B 3*, the resultant potential change was very simple and its time course coincided with that of the unitary e.p.s.p. (Furukawa, Ishii & Matsuura, 1972*b*). Moreover, it was evoked with a regular synaptic delay of about 0·4 msec following the generation of the coupling potential. Although it might seem surprising that such a small residual e.p.s.p. during inhibition arose without incurring any prolongation of the synaptic delay, it is best explained as being produced by a special property of the efferent inhibitory mechanisms in this preparation (see next section). However, no increase in the amplitude of the

intramacular microphonic potentials was demonstrated during efferent inhibition. (Intramacular microphonic potentials are distinct positive microphonic potentials which can be recorded from around the base of the saccular hair cells: Furukawa *et al.* 1972*a*.) The reason for this failure is not clear, but may be related to the recording condition necessary to observe this type of microphonic potential.

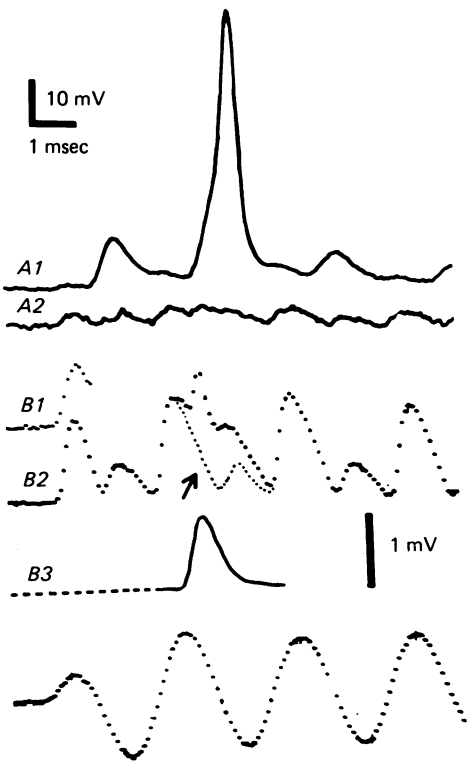

Fig. 5. Analysis of the coupling potentials by averaging; results from the same fibre as used in Fig. 4. *A*, sample records of non-inhibited and inhibited responses. *B*, averages of fifty non-inhibited and inhibited responses (*B 1* and *B 2* respectively). The supposed time course of the coupling potential is drawn with dotted line (arrowed in *B 2*). *B 3*, time course of the small e.p.s.p. as obtained by subtracting the time course of the coupling potentials from the actual record. See text for further details. Sound stimulus, 440 Hz and 95 db SPL.

Effects of efferent inhibition on the timing of generation of the e.p.s.p.s

Although the e.p.s.p.s in the afferent fibres were greatly reduced in amplitude during efferent inhibitory action, there was no detectable change in the timing of their generation. In Fig. 6, traces 1 and 2 show responses to the same intensity of the sound recorded with and without preceding inhibitory stimulation. It is seen that the small e.p.s.p.s in trace 2 were set up with the same timing as the much larger responses in trace 1. The peak of the e.p.s.p.s during inhibition came much earlier than the peak of the e.p.s.p.s in the control record. However, the timing of generation of the e.p.s.p.s was very different when responses of similar amplitude were obtained by reducing

the sound intensity. Namely, the e.p.s.p.s in trace 4 were set up with a greater delay than those in trace 2, giving an impression that the responses in trace 4 were shifted *in toto* to the right by 0·4–0·5 msec compared with responses in trace 2. In contrast, the peak position of the e.p.s.p.s generally stayed unchanged for different sound intensities (compare traces 1 and 4). Similar results were obtained practically in all the cases in which this point was carefully studied (ten in total). The difference was so marked that averaging was not needed.

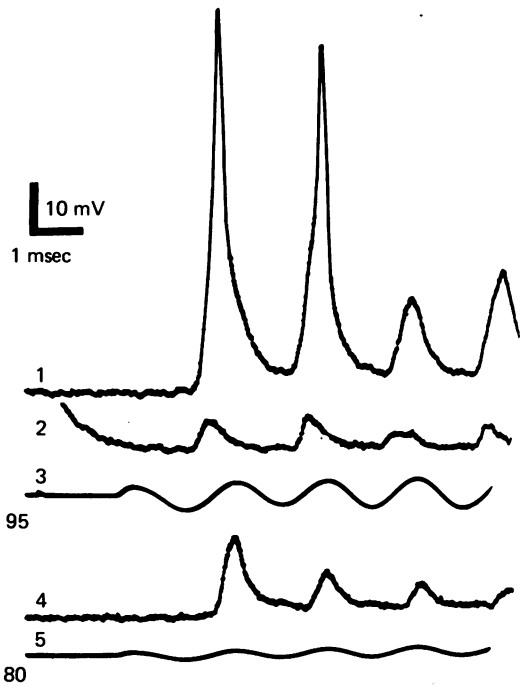

Fig. 6. Timing of generation of small e.p.s.p.s during efferent inhibition. 1 and 2, non-inhibited and inhibited responses to a sound at 95 db SPL; 4, non-inhibited response to a sound at 80 db SPL. Note the difference in the timing of generation between two responses of comparative magnitude. Sound stimulus, 530 Hz.

Observations on S-2 fibres

Different results were obtained in S-2 fibres. First, the effects of efferent inhibition varied in different S-2 fibres. For example, it was observed in some fibres that a slight increase in the intensity of inhibitory stimulation abolished all the responses, whilst in other cases an increase in the intensity of the inhibitory stimulation evoked a graded increase in the inhibitory effect. In such instances, however, it was often observed that inhibitory stimulation brought about an increase in the delay in addition to the suppression of the response (Fig. 7). In contrast to S-1 fibres, which reach hair cells without any branching or after making a single bi- or trifurcation (Furukawa, 1978), S-2 fibres have repeated bifurcations before they terminate on hair cells (T. Furukawa, unpublished observation). Therefore, it is not clear whether the nature of the small responses in Fig. 7 is really the e.p.s.p. or a blocked spike.

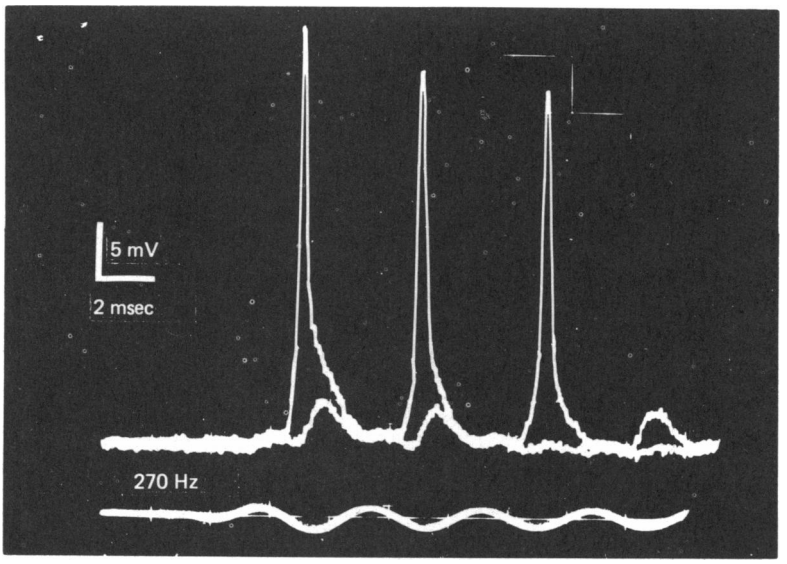

Fig. 7. Delayed initiation of e.p.s.p.s during efferent inhibition in a small afferent fibre (S-2 fibre). Stimulus sound, 270 Hz and 64 db SPL.

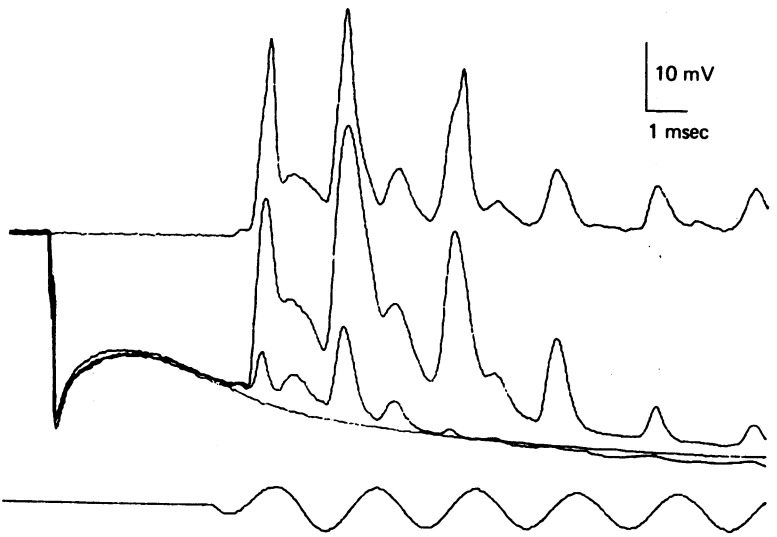

Fig. 8. Effects of hyperpolarizing current applied intracellularly to the afferent fibre through the recording electrode. Top trace, control response; middle trace, effects of hyperpolarization on non-inhibited and inhibited responses. Base line was drawn without preceding efferent stimulation. The amplitude of non-inhibited e.p.s.p.s was greatly increased during hyperpolarization, but spikes were blocked. If inhibition accompanies an i.p.s.p., the latter should appear as a big depolarizing potential during hyperpolarization.

The absence of the i.p.s.p.

Even when the effect of efferent inhibition was marked, the base line stayed flat in every instance. There was no indication of the presence of i.p.s.p.s (see Figs. 1, 5, 6 and 7). However, an absence of the i.p.s.p. might simply indicate that its reversal potential was located close to the resting membrane potential. Therefore, it was thought desirable to investigate whether the post-synaptic membrane was hyperpolarized, by passing a current through the recording micro-electrode (Furukawa *et al.* 1972*b*). Fig. 8 shows the result of such a trial. Under these circumstances hyperpolarization augmented the e.p.s.p.s by 1·5–1·8-fold, indicating that afferent fibre terminals were hyperpolarized by approximately 50 mV, i.e. much more than the amount indicated in the Figure as a shift of the base line. However, no evidence suggesting the presence of a depolarizing i.p.s.p. has been observed. Similar results were obtained in three other cases.

Effects of curare, strychnine and other drugs on efferent inhibition

In work on the goldfish, immobilization of the animal can most easily be obtained by an I.M. injection of D-tubocurarine or gallamine triethiodide (1–2 μg/g body wt.). But these drugs cannot be used in experiments in which the effects of efferent stimulation are to be studied, for they readily block the inhibitory action. Also, strychnine (1·3 μg/g body wt.) injected I.M. blocked efferent inhibition within 10–15 min. These results generally conformed to the results on cat and other animals (Desmedt & Monaco, 1962; Desmedt, 1975; Bobbin & Konishi, 1974; Russell, 1971; Rossi *et al.* 1980). Effects of methylneostigmine was also studied in five cases. An injection of a very large dose (2 μmole/g or 4 μg/g body wt., I.M.) was found to prolong the duration of the inhibition in three cases, but the effect was not very marked. In another two cases no prolongation was observed.

DISCUSSION

It was found in the present study that efferent inhibition in the goldfish's saccule takes place predominantly presynaptically, i.e. inhibition exerts its effects directly on hair cells and not on afferent nerve terminals. This finding generally conforms to the results of histological studies, which indicate that efferent fibres in goldfish's saccule, although partly distributed on afferent endings, terminate mainly and directly on the base of hair cells (Hama, 1979; Nakajima & Wang, 1974). Any post-synaptic inhibitory action may not be strong enough to be detected with current electrophysiological techniques.

However, the situation in the mammalian cochlea is more complicated. The crossed olivocochlear bundle terminals establish extensive synapses on the base of the outer hair cells. In the inner hair cells, by contrast, many synaptic contacts are found between efferent terminals and the short radial cochlear dendrites below the inner hair cells (Smith & Rasmussen, 1963; Spoendlin, 1969, 1973). Moreover, almost all afferent fibres in the cochlear nerve originate from inner hair cells (Spoendlin, 1969, 1973). Therefore, it is expected that the inhibition as detected in the afferent cochlear nerve would be mediated predominantly post-synaptically.

Questions arise as to the difference between the two types of efferent inhibition in

hair cell organs. Desmedt & Robertson (1975) argue that post-synaptic inhibition may be more effective than presynaptic inhibition, since the inhibition observed in the pigeon's cochlea (which is mediated presynaptically) was much weaker than that observed in the mammalian cochlea (Desmedt & Delwaide, 1965). The present results do not support this inference, since the inhibition in the goldfish ear, though presynaptic in origin, was comparable to the inhibition in the mammalian cochlea.

It was observed in the present study that sound-evoked e.p.s.p.s, though much reduced in size, were set up without any change in latency during efferent inhibition.

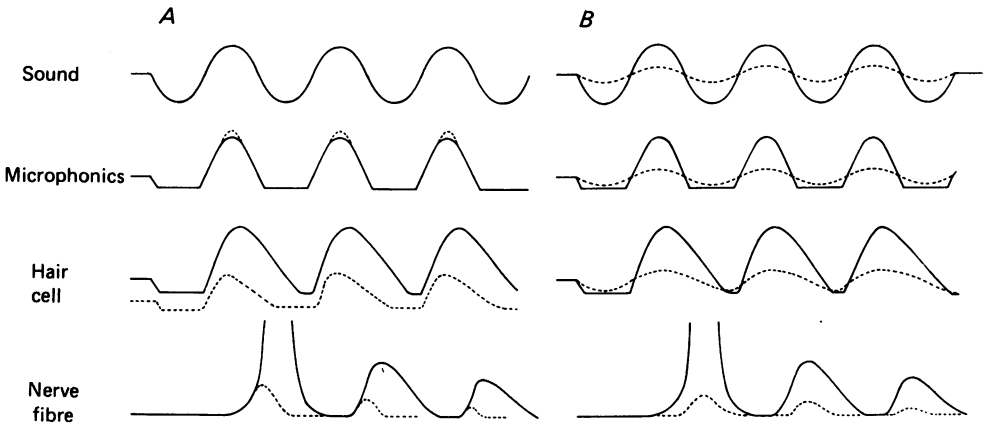

Fig. 9. Schematic diagrams to explain the difference in the timing of generation of e.p.s.p.s between responses during inhibition and those produced by a weak sound. *A*, effects of efferent inhibition. Responses under inhibitory effects are drawn with broken lines. *B*, changes produced by a reduction in sound intensity. Responses to weak sound are drawn with broken lines.

This is in sharp contrast to the effect of reducing the sound intensity, which resulted in an increase in the latency of individual e.p.s.p.s. The mechanism underlying this observation has not been clarified in detail, but is most probably related to a hyperpolarization and decrease in the time constant of the hair cell membrane during efferent inhibition (Flock & Russell, 1976). The presence of a hyperpolarization is indicated by the suppression of spontaneous miniature e.p.s.p.s during efferent inhibition (Fig. 3). Presumably, the small size and short duration of the e.p.s.p.s during efferent inhibition (Figs. 5 and 6) may be due to the reduced effective size and brief time course of hair cell depolarization. Fig. 9*A* is a schematic diagram to explain this point, and Fig. 9*B* shows in contrast the effect of reducing sound intensity. In *A* and *B*, control responses without inhibition have been drawn with continuous lines, while responses during efferent inhibition or to a reduced intensity of sound have been indicated by broken lines. Due to the presence of membrane capacitance, the hair cell's intracellular potential in the control state (third from the top) would be expected to change with a time course much slower than the time course of the current which enters the cell during each excitatory phase of sound (Russell & Sellick, 1978; Corey & Hudspeth, 1979). As the membrane time constant would decrease during efferent inhibition, the time course of the potential change in hair cells should become shorter, as drawn with the broken line in the third trace from top in Fig. 9*A*. The

line is shifted downwards to represent a hyperpolarization. These changes do not happen when the sound intensity is reduced.

Working on olivocochlear inhibition in the cat, Desmedt & Robertson (1975) have reported that, when the N_1 response to a click in the cat was inhibited by crossed olivocochlear bundle activation, its latency was unaffected, while reduction in the intensity of the click resulted in an increase in the latency. Their results are analogous to the present findings in that sound-evoked responses were reduced in amplitude during efferent inhibition without any concurrent increase in latency. Desmedt & Robertson have used this as support for their interpretation that the inhibition they observed was of a post-synaptic origin. If the suggestions advanced in the present paper are tenable, however, Desmedt & Robertson's argument could no longer be used in support of the post-synaptic origin of the inhibitory action. The results of the present study seem to suggest that the efferent inhibition in hair cell organs, be it either post-synaptic or presynaptic in origin, produces a very similar type of inhibitory action.

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