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INHIBITION OF AUDITORY NERVE ACTION POTENTIALS BY ACETYLCHOLINE AND PHYSOSTIGMINE

BY

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When the tract of Rasmussen (olivo-cochlear bundle) is stimulated electrically it produces a decrease in the amplitude of the auditory nerve action potential. This paper describes experiments undertaken to test the hypothesis that the olivo-cochlear bundle exerts its inhibitory influence by release of acetylcholine or a related substance at the olivo-cochlear-auditory nerve junction. This hypothesis grew from the data of Churchill, Schuknecht & Doren (1956) and Schuknecht, Churchill & Doran (1959) which demonstrated that the complement of acetylcholinesterase found in the inner ear is due almost entirely to the presence of olivo-cochlear fibres in the inner ear. It has also been demonstrated that the hair cells of the auditory apparatus are innervated by nerve endings containing synaptic vesicles (Engström, 1958 ; Spoendlin, 1958 ; Engström, 1960), which have come to be regarded as storage, protective or transport structures for neurotransmitter substances. Engström (1960) demonstrated that the endings containing synaptic vesicles decrease in number after sectioning the olivo-cochlear bundle. Finally, Martini (1941) demonstrated the presence of a factor in the inner ear which exhibited acetylcholine-like activity on smooth muscle assay preparations. Thus, both the synaptic vesicles and acetylcholinesterase have been shown to be associated with the olivo-cochlear bundle and an acetylcholine-like factor has been demonstrated in the inner ear. Therefore it seems reasonable to suggest that the inhibition of auditory nerve potentials achieved by olivo-cochlear bundle stimulation may be dependent on the release of a neurotransmitter and that the neurotransmitter might be acetylcholine.

METHODS

As a first approach, we decided to test the effects of injected acetylcholine and drugs related to it on the electrical activity recorded from the round window of the inner ear. For this purpose cats were anaesthetized with either sodium pentobarbitone (35 mg/kg) or Dial-urethane (Ciba) (0.75 ml./kg). Silver electrodes were placed on the left round-window niche. In some experiments the muscle tendons of the left middle ear were cut to remove the possibility that the drugs might cause auditory changes by acting on the middle ear muscles. The left axillary artery was cannulated and the cannula advanced so that it was roughly at the origin of the vertebral artery in order that the drug injections might be made close to the internal auditory artery.

Auditory nerve action potentials (usually designated N_1 when recorded from the round window, see Fig. 1) of between 100 to 250 μV amplitude were obtained in response to clicks (0.01 to 0.07 msec

duration) 15 to 30 db more intense than those producing a visually just detectable N_1 (see Fig. 1). Systemic arterial blood pressure was monitored.

RESULTS

In the majority of cats, administration of acetylcholine (10 to 20 μg/kg) *via* the axillary arterial cannula produced a brief decrease in N_1 amplitude which was rapid in onset (Fig. 2). In 21 cats responding to intra-arterial injection of 10 μg acetylcholine, the average maximal inhibition of N_1 amplitude was 40%, with a range of 15 to 100%, and the average duration of response was 3 min, with a range of 40 sec to 10 min. Factors

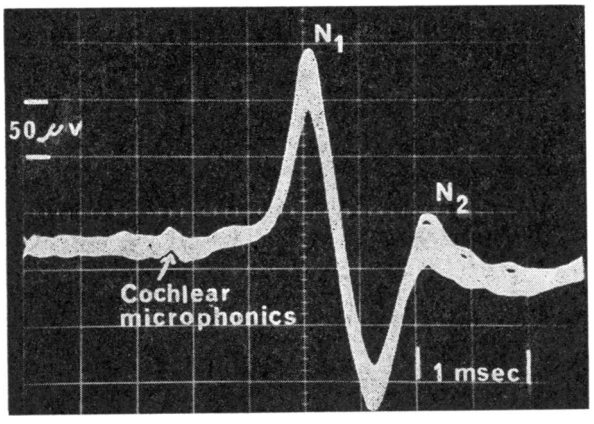

Fig. 1. Thirty superimposed electrical responses recorded from round window electrodes. These responses were evoked by 0.04 msec clicks applied 2/sec at 10 db above visual detection threshold.

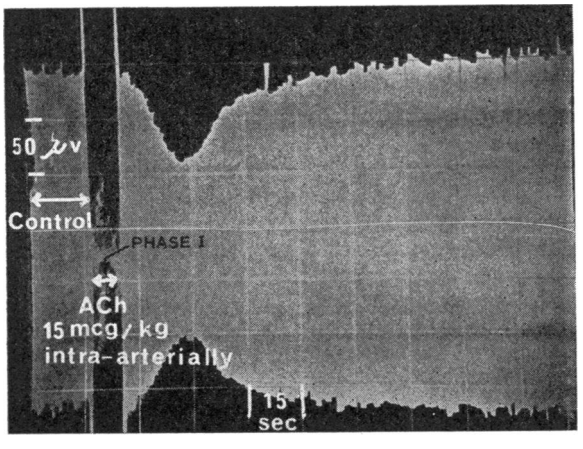

Fig. 2. Effect of 15 μg/kg ACh intra-arterially on N_1 amplitude. Responses were evoked 2/sec, causing essentially single vertical deviations on a slowly moving oscilloscope trace. These responses were stored on a memory oscilloscope face allowing comparison of N_1 amplitudes over time. At maximal inhibition (phase I), N_1 amplitude is about 60% of control amplitude.

such as speed of injection and depth of anaesthesia are probably important factors influencing intensity and duration of action. A similar inhibition, designated phase I inhibition, was produced by intra-arterial injection of physostigmine (0.3 to 0.6 mg/kg) in most cats, but in addition, physostigmine produced a secondary inhibition of slow onset (15 to 20 min) and of several hours duration (Fig. 3). The secondary inhibition produced by physostigmine is designated phase II inhibition. Some cats (about 25%) did not respond to drug injections, possibly because the placement of the cannula was faulty or because of vascular anomalies.

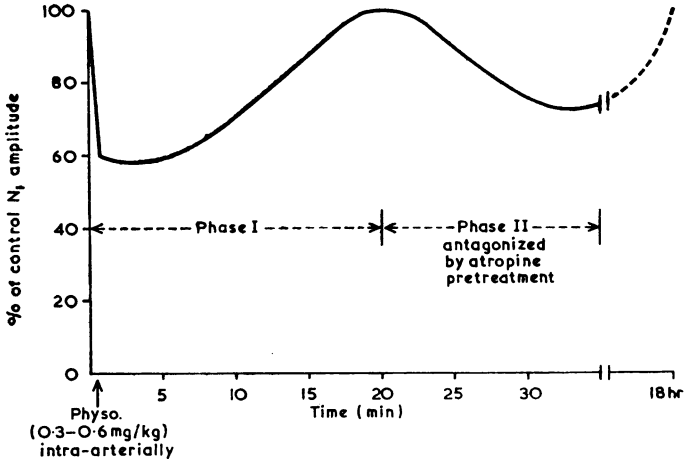

Fig. 3. Graph of Phase I and II inhibitions of N_1 amplitude produced by administration of physostigmine, 0.3 to 0.6 mg/kg intra-arterially, indicating approximate time relationships.

In 12 cats, the intra-arterial doses of acetylcholine or of physostigmine producing inhibition before the muscle tendons of the middle ear were severed produced the same or a larger inhibition after these tendons were cut, indicating that the drug effects were not the result of actions on middle ear muscles.

Intravenous injection of acetylcholine (10 to 20 μg/kg) was without effect on N_1 amplitude, and intravenous injection of physostigmine (0.3 to 0.6 mg/kg) produced only a phase II inhibition.

In each of seven cats in which atropine (0.5 to 2 mg/kg) was injected, doses of acetylcholine that had previously been effective no longer produced inhibition. Atropine also abolished the phase II inhibition produced by physostigmine but did not block the phase I inhibition produced by this agent. Thus the phase I inhibition produced by physostigmine appeared to differ from that produced by acetylcholine.

DISCUSSION

Olivo-cochlear bundle-induced inhibition is said to cause an increase in amplitude of the cochlear microphonic potentials (Fig. 1) while inhibiting N_1 (Besmedt, 1962; Fex, 1962; Sohmer, 1963). No obvious enhancement of cochlear microphonics occurred in response to acetylcholine or physostigmine injections, but neither were they depressed.

Thus the question is raised whether acetylcholine or physostigmine are really mimicking olivo-cochlear bundle stimulation. It is certainly possible that these agents are acting outside the cochlear to cause inhibition of N_1, and we are currently studying this possibility by sectioning the olivo-cochlear bundle between drug injections.

Gisselsson (1952) reported a change in the latency of cochlear potentials following injections of physostigmine, an effect we have not particularly studied but have occasionally observed. Other reports have appeared concerning the effects of acetylcholine on cochlear potentials (Katsuki, Tanaka & Miyoshi, 1965 ; Guth, Gonzales & Amaro, 1965 ; Brown & Daigneault, 1965 ; Tanaka & Katsuki, 1966). Katsuki *et al.* (1965) and Tanaka & Katsuki (1966) applied acetylcholine iontophoretically in the vicinity of the hair cells and found a diminution in both the cochlear microphonic and N_1 responses. In an earlier publication, Sohmer & Feinmesser (1963) reported that acetylcholine, physostigmine, and atropine were without effect on the cochlear potentials of cats and guinea-pigs. This discrepant result might arise from the different modes of drug administration.

The slowly developing phase II inhibition produced by physostigmine appears to be related to acetylcholinesterase inhibition and acetylcholine accumulation, because, like the inhibition produced by acetylcholine itself, it is blocked by atropine. The mechanism of the phase I inhibition produced by physostigmine is unknown. It differs from that produced by acetylcholine in that it is not blocked by atropine. Its rapidity of onset suggests a direct effect rather than an indirect one arising from enzyme inhibition and acetylcholine accumulation.

The reported results do not negate the hypothesis that the inhibition of N_1 induced by olivo-cochlear stimulation is cholinergic.

SUMMARY

1. Intra-arterial administration of acetylcholine (10 to 20 μg/kg) resulted in a decrease in amplitude of the VIII nerve action potential as recorded from the round window of the cochlea. This effect of acetylcholine is abolished by pretreatment with atropine.

2. Intra-arterial administration of physostigmine (0.3 to 0.6 mg/kg) resulted in both rapidly appearing, short-lived and late-appearing, prolonged decreases in amplitude of VIII nerve action potential. The late-appearing, prolonged decreases may be prevented by pretreatment with atropine.

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