Journal of Medicinal and Pharmaceutical Chemistry

VOL. 4, No. 3 (1961)

VI. The Peripheral Actions of the Hemicholinium Compounds*

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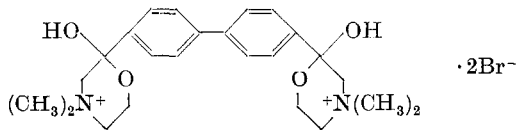
Introduction

Long and Schueler[1] reported a series of biphenyl derivatives that were potent cholinesterase inhibitors. One of the compounds was noted to be an extremely toxic material following intraperitoneal injection in mice; this compound was, however, also demonstrated to be devoid of action at the neuromuscular junction. These studies were conducted using sciatic gastrocnemius muscle preparations in the cat. The nerve was stimulated once every ten seconds using single shock. Studies to be discussed later in this paper demonstrate that the compound tested does have activity at this site when higher frequencies of stimulation are used; it has also been demonstrated by MacIntosh and co-workers[2,3] that the compounds reduce the amount of acetylcholine in a perfused ganglion as well as virtually depleting the acetylcholine in the perfusate obtained from the ganglia. More recent studies indicate that the compound also produces transmission failure at the postganglionic parasympathetics and may also have a depressive action on the postganglionic sympathetic nervous system. The mechanism of action of these substances remains to be further clarified, but the work of MacIntosh and co-workers certainly suggests that these compounds may interfere with the synthesis of acetylcholine or that they may be involved in preventing the release of acetylcholine from the nerve terminals.

Chemistry of the Hemicholiniums

Long and Schueler[1] synthesized the original compound, and later[4] the structure was shown to contain hemiacetal rings (I).

* Supported in part by a grant from United States Public Health Service (Grant No. B-1396).



(I)

On the basis of this structure Schueler[4] suggested the name hemicholinium. From a chemical point of view this is a rather unusual structure. Although rearrangements with the tertiary analogues have been reported, this is perhaps the first report of a rearrangement involving a quaternary derivative. The material is obtained as a white crystalline solid, and, depending on the solvent used for recrystallization, there are materials of two different melting points that can be obtained. One form melts at 180°. These different materials may represent different isomers that are possible; biological assay, however, indicates no difference in the activity of these two compounds.

Toxicity of Hemicholinium

A. Toxicity. Intravenous toxicity in animals has been studied in a fairly wide variety of species. In mice the LD_{50}'s reported vary from 20 to 75 μg/kg. In larger species of animals the compound appears to be less toxic. The LD_{50} in rabbits is 260 μg/kg. In cats the approximate LD_{50} is 300 μg/kg and in the dog it is 750 μg/kg. The appearance of toxicity symptoms is rather interesting in laboratory animals. In all species the onset of action is rather slow. For example, in the rabbit, the animals appear normal for about 10 to 15 min following the intravenous administration of the compound. Then there is a gradual depression of the respiration and difficulty in locomotion. If the animals have received a lethal dose of the compound, death usually occurs in about 45 min following the administration of the compound. If the animal survives, recovery is rather slow and two to three hours are required before the animals appear normal. In larger species of animals, for example the dog, the onset of action is even slower and the time for death is much more delayed than it is for the smaller species. Giovinco[5] demonstrated that motor

activity influenced the LD_{50} in mice. Increasing motor activity in the animals increased the LD_{50} in mice, while depression of the animals by pretreatment with barbiturates decreased the LD_{50}.

B. Antidotes for toxicity. Schueler[4] reported that choline was an effective antagonist for hemicholinium poisoning. Reitzel and Long[6, 7] confirmed this and studied a series of choline analogues for their ability to antagonize hemicholinium toxicity. Various analogues of choline were shown to be ineffective as antagonists to hemicholinium-induced toxicity, and various esters of choline antagonized hemicholinium toxicity. If, however, the animals were pretreated with a cholinesterase inhibitor, thus preventing the hydrolysis of the choline ester, these compounds would not antagonize hemicholinium toxicity. Therefore, it is suggested that the choline moiety is relatively specific as an antidote. Choline was capable of preventing death in dogs that received 60 mg/kg of hemicholinium. Physostigmine has some antagonistic effect against hemicholinium toxicity.[5] This marked antagonism by the choline analogues suggests that perhaps these compounds may act by interference with the choline portion of the cholineacetylase system, and that they may interfere with choline transport into the enzyme system.

Pharmacological Studies of Hemicholinium

A. Action of the compound on the central nervous system. Schueler *et al.*,[8] Schueler,[4] and Kase and Borison[9] have reported that the compound produces a depression of the respiratory centre within the central nervous system, but this site of action for hemicholinium has been questioned by Longo.[10]

B. Action at the myoneural junction. The hemicholiniums do not produce failure in transmission when nerves are stimulated at slower frequencies; when the nerves are stimulated with higher frequencies or with interrupted tetanic stimulation, however, the hemicholinium is an extremely active neuromuscular blocking agent. The ED_{50} for neuromuscular blockade in the rabbit sciatic nerve gastrocnemius muscle preparation is approximately 40 μg/kg. The onset and duration of action closely parallels that reported above in the section on toxicity. Using single-shock stimulation with a frequency of 1/sec, the time required for onset

of action would be approximately 10–15 min following the intravenous administration of hemicholinium. There would be a gradual depression in the muscle contraction until approximately 60 min following the compound's administration, when maximal blockade would occur, and then there would be a gradual return to control contractions. With interrupted tetanic stimulation the activity of the compound was markedly increased. Using interrupted tetanic stimulation with a frequency of approximately 250/sec, the ED_{50} was approximately 3 μg/kg. Under these conditions the duration of action was much longer and often the preparation required at least 7–8 h to return to control levels. Additional studies indicated that very large doses of these compounds, that is 2–4 mg/kg produced almost immediate neuromuscular blockade. This was reversed readily by neostigmine bromide, indicating that the mechanism involved with these large doses was a 'curare' type of action. Choline was observed to be a very effective antagonist at the neuromuscular junction. The higher alkyl analogues of choline appeared to be relatively poor or inactive as antagonists to hemicholinium-induced neuromuscular blockade.[7] The compound was further studied using the sciatic gastrocnemius muscle preparation in the chicken, where it decreased induced contractions and gave a response which looked quite similar to that obtained with *d*-tubocurarine chloride. The contracture induced by the intravenous administration of acetylcholine, however, is not antagonized by hemicholinium. This is further evidence that the mechanism of action is different from that of the classical competitive neuromuscular blocking agents. This conclusion is reached also when the frog rectus abdominus muscle preparation is used; the hemicholinium did not produce contraction in the muscle nor was there antagonism or potentiation of the contracture obtained with acetylcholine.

C. Action on the parasympathetic nervous system. Wilson and Long[11] studied the ability of the compound to inhibit saliva formation following chorda tympani nerve stimulation. The ED_{50} in this preparation was approximately 10 μg/kg. With higher frequencies of stimulation the compound became more effective in producing transmission failure. Intravenous administration of postganglionic parasympathetic stimulants, such as acetyl β-methylcholine, produced just as much saliva following

hemicholinium as was produced prior to the administration of the compound. In this respect the action on the parasympathetic nervous system appears to be similar to that discussed above for the somatic nervous system. The muscarinic receptors appear to be unaltered by the compound. On these preparations the antagonistic action of choline was not as definite as for the myoneural junction. Large doses of choline were required to produce antagonism and with these dosages the saliva formation that was observed could be due to the direct stimulating action of choline, and not to antagonizing hemicholinium activity. The compound shows similar responses in blocking contractions following vagal stimulation of the small intestine, and in blocking the contraction of the urinary bladder following hypogastric nerve stimulation. The compound has been evaluated for its activity in the eye and it has been demonstrated that it is capable of blocking the miotic effects of postganglionic ciliary nerve stimulation. The compound on topical application in the eye is an effective mydriatic agent. The general appearance is similar to that observed for atropine sulphate. The postganglionic parasympathetic stimulating agents are, however, extremely effective in antagonizing the mydriasis observed with the hemicholiniums. In a series of 10 rabbits hemicholinium was capable of lowering the intraocular pressure that is observed following the instillation of atropine sulphate. A recent report by Rand[12] indicates that the compound has the ability to block the postganglionic sympathetic nervous system. In at least some of these preparations the compound's effects were antagonized by choline, but in others choline was not particularly effective in antagonizing the action of hemicholinium.

D. Lactobacillus plantarum has been demonstrated to have the ability to synthesize acetylcholine. Various studies in this laboratory have failed to demonstrate any inhibition of acetylcholine synthesis. The compound in concentrations of approximately 10^{-5}M has the ability to produce degeneration in planaria. It has approximately the same activity as atropine sulphate in producing degeneration in this species. In concentrations of approximately 10^{-5}M, tadpoles and various species of fish become paralyzed after several hours' exposure to the compound. It has been observed that frogs can tolerate very large doses of the compound (up to 1·0 mg/kg) without any apparent adverse effect.

E. Analogues of hemicholinium. Schueler[4] and Marshall and Long[13] synthesized various analogues of hemicholinium. These structural modifications have involved both alterations in the cationic heads and modification of the biphenyl nucleus. Schueler[4] demonstrated that half of the molecule is inactive and also that alterations in the cationic head tended to reduce activity. Marshall and Long[13] prepared the diphenylmethane, the diphenyl ether, and benzidine analogues of hemicholinium, and these compounds were less active. Also, it was demonstrated that with the other agents there was an increase in cholinesterase-inhibiting activity in comparison with the parent compound. Therefore it appears that the original compound is still the most active of the compounds that represent this class of agents. Further studies are required in this general area in attempting to obtain compounds that are more selective in their site of action. Therapeutic possibilities, of course, exist for compounds that would be relatively selective for the somatic nervous system. If a compound could be obtained that would be relatively selective in blocking the parasympathetic nervous system, this would yield some interesting therapeutic possibilities.

(*Revised Manuscript Received 18 May, 1961*)

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