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PRELIMINARY COMMUNICATION

THE QUESTION OF CHOLINERGIC ANTAGONISM

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The theory of a 2-fold drug-receptor interaction developed from the observations of Langley (1) that an increased concentration of nicotine could overcome the blocking effects of curare on the striated muscle. This theory states that there is an initial physical interaction of a drug with a discrete macromolecular component of the excitable membrane, followed by a conformatioral change in the molecular components of the membrane altering its permeability. A functional inhibition could thus be observed by either blocking the initial drug-receptor interaction or by preventing the necessary molecular conformational change from occurring. Neither the occupation theory of Stephensen (2) nor the rate theory of Paton (3) considers this possibility, but they suggest that the biologic response is a function of only the drug-receptor interaction. Based on their hypotheses, the competitive inhibition of acetylcholine by atropine is caused by atropine occupying the receptor and preventing acetylcholine from binding. This occupation is a function of the relative affinity constants of acetylcholine and atropine for the receptor, and thus the inhibition could be reversed by simply increasing the concentration of acetylcholine. For the most part, this theory appears to hold, but several observations seem to contradict this view.

Clark (4) found that high concentrations of acetylcholine failed to accelerate the recovery of the heart from the effects of atropine. Clark suggested that acetylcholine does not neutralize the effects of atropine nor does acetylcholine appear to replace atropine from the heart. He reasoned that atropine and acetylcholine appear to combine at different receptor sites in the heart, and thus their antagonism appears to be one of effects rather than direct combination.

Some forty years later, Ariens (5) and Ellenbroek et al.(6) found that parasympathomimetic drugs and their antagonists interact only partially with a common receptor in the rat jejunum. Ariens and Simonis (7) suggested that receptors can be inhibited competitively in a functional manner by an antagonist even though the antagonist does not physically prevent the interaction of the agonist with the receptor.

More recently, Podleski and Changeux (8) have suggested that the "receptor-ionophore complex" exists in at least two conformational states: the "resting" state which has a high affinity for antagonists and the "active" state which has a high affinity for agonists.

In our continuing study of the cholinergic receptors, we prepared a spin-labeled analog of acetylcholine, 4-[N,N-dimethyl-N-(ethan-2'-olacetate)amino]-2,2,6,6-tetramethylpiperidine-1-oxyl iodide (I)(Fig.1)(9,10). We noted, at that time (11), that this agent was a potent

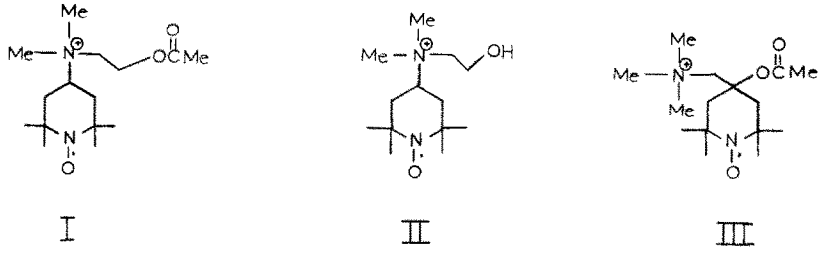

Fig. 1. Structures of the spin-labeled probes used in this investigation.

antagonist of the muscarinic cholinergic receptors of the isolated frog heart but had minimal cholinolytic activity against the nicotinic receptors of the frog satorius muscle. X-ray crystallographic analysis indicated that this probe was in the muscarinic conformation (12). We report, herein, that this spin-labeled analog of acetylcholine has no cholinomimetic nor cholinolytic activity at the muscarinic receptor sites of the guinea pig ileum but that this agent blocks the action of atropine at these receptor sites.

The guinea pig ileum was prepared by conventional methods (13,14). Contractions of the ileum were measured isotonically with a Harvard heart/smooth muscle transducer under a tension of 1 g. Contractions were elicited at equilibrium by injecting acetylcholine into the tissue bath every 2 min.

Figure 2 shows a dose-response curve for the guinea pig ileum with varying concentrations of acetylcholine (line a) and indicating that atropine (2×10^{-9} M) antagonizes competitively the action of acetylcholine (line b). When the spin-labeled acetylcholine analog (I) (2×10^{-8} M) is tested in combination with acetylcholine (line c), a greater response for the combination is elicited than from acetylcholine alone. This apparent increase in the activity of acetylcholine is probably due to the inhibition of the ileal acetylcholinesterases, since I is an inhibitor of this system (15). Second, Fig. 2 shows that I does not possess any cholinolytic activity against the acetylcholine receptors of this tissue. This was surprising, since we earlier reported that I was a potent muscarinic antagonist on the isolated frog heart ($K_d = 1 \times 10^{-8}$ M) (see Ref. 11). This observation might suggest that, like the beta-adrenergic

system, muscarinic cholinergic receptors might possess slightly different conformations from tissue to tissue and that a specific agent, like I, can easily distinguish such differences in conformation. Third, if at this point the dose-response curve is repeated using the combination of atropine (2×10^{-9} M) and I (2×10^{-8} M), competitive inhibition of the cholinergic receptors is decreased by 50 per cent as compared to atropine alone. Furthermore, if I (2×10^{-8} M) is added after incubation with atropine, while generating line b, the dose-response curve depicted by line d is obtained when equilibrium is reached. If atropine (2×10^{-9} M) is added after incubation with I (2×10^{-8} M), line d is also obtained at equilibrium.

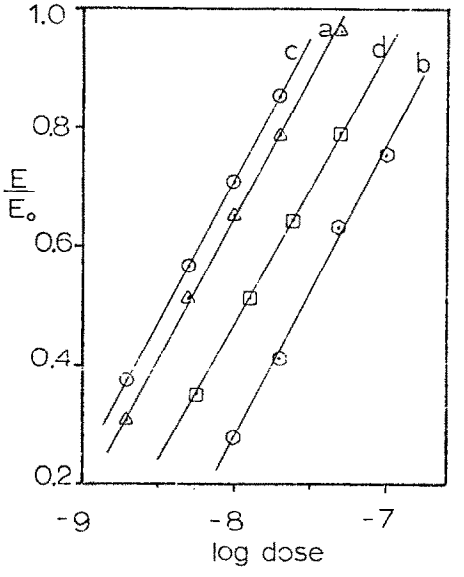

Fig. 2. Log concentration-response curve for the contraction of the guinea pig ileum by acetylcholine (line a); in the presence of atropine (2×10^{-9} M, line b); in the presence of the spin-labeled acetylcholine analog (I) (2×10^{-8} M, line c); and in the presence of atropine (2×10^{-9} M) and I (2×10^{-8} M) (line d).

In order to determine if the acetyl group of I is required for activity, the spin-labeled analog of choline, 4-[N-(2-hydroxyethyl)-N-dimethylamino]-2,2,6,6-tetramethylpiperidinooxy iodide (II), which contains only an anionic center, was found to possess no cholinergic activity. It may be noted that II is an inhibitor of acetylcholinesterase but is without effect at either the muscarinic cholinergic receptors of the isolated frog heart or at the nicotinic cholinergic receptors of the frog satorius muscle. Thus, for I, it appears that both the anionic and esterophilic centers are required for cholinergic activity.

It is known that I is in the muscarinic conformation and it is thought that this conformation is necessary for muscarinic cholinergic activity since we found that a related analog, 4-trimethylaminomethyl-4-acetoxy-2,2,6,6-tetramethylpiperidinoxyl iodide (III), which cannot assume the muscarinic conformation, has no cholinergic activity.

Our observations suggest that atropine and acetylcholine do not bind at the same receptor site but perhaps at adjacent or overlapping sites. With the guinea pig ileum, at least, atro-

pine can no longer be considered a specific competitive inhibitor of acetylcholine binding. These results indicate that an antagonism of an agonist must be considered to include a functional inhibition as well as a direct competition with the agonist at the receptor site. Antagonism of this kind presented here may be analogous to effects of inhibitors on enzymes. We found that the antagonism of atropine by I occurred with other parasympathomimetic drugs, e.g. carbachol and methacholine.

We are presently conducting experiments to determine more about the nature of the atropine receptor and to determine if the antagonism exhibited by I is a general phenomenon.

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