Isologous Oxygen, Sulfur, and Selenium Compounds as Probes of Acetylcholine Receptors

HENRY G. MAUTNER

From the Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510

ABSTRACT Evidence is presented that while the conformations of acetylcholine and acetylthiolcholine are different, acetylthiolcholine and acetylscelenolcholine are structurally and conformationally very similar. Experiments with sulfur and selenium isologs of acetylcholine, choline, and local anesthetics suggest that the active sites of receptors of the electroplax and of electric eel acetylcholinesterase are different, but are compatible with the postulate that acetylcholine receptors of axonal and synaptic excitable membranes are similar.

It has been postulated for a long time that acetylcholine plays a crucial role in the conduction of the nerve impulse both at synaptic and at axonal sites. The proposal has been made that it acts as a trigger inducing a conformational change in excitable membranes during electrical activity, altering the membranes’ permeability to cations (Nachmansohn, 1953, 1959). In view of the great biological importance of acetylcholine and the simplicity of its structure (Fig. 1), very numerous analogues of this ester have been prepared and studied. Such modifications have been centered on the onium group, the ester group, and the alkyl chain linking these groupings (Barlow, 1964). However, while a great many acetylcholine analogues have been synthesized and their pharmacological actions investigated, this approach makes the assumption, common among scientists working with antimetabolites, that the modification of the molecule will be localized at the point where it is carried out. This assumption, which has been referred to as the “stick-toy fallacy” (Mautner, 1967), ignores the fact that molecules are not stick-toys and that replacement of one atom by another may alter electron distribution and conformation throughout the molecule being modified.

In an attempt to minimize this problem, our laboratory has been investigating the physico-chemical and biological properties of analogous oxygen, sulfur, and selenium compounds. While the atomic radii of oxygen and sulfur are rather different, the radii of sulfur and selenium are very similar with the result that, while the structures of oxygen and sulfur isologs tend to differ, the structures of crystals of sulfur and selenium isologs have been found to
be extremely similar in all cases studied. X-Ray diffraction studies have shown the crystal structures and packing patterns of rigid sulfur and selenium isologs (such as 2-thiouracil and 2-selenouracil (Tsernoglou, 1966) or 2,4-dithiouracil (Shefter and Mautner, 1967) and 2,4-diselenouracil) to be virtually identical. More recently, it could be shown, by means of X-ray diffraction, that even flexible molecules of isologous sulfur and selenium compounds have essentially identical shape, size, and conformation, different from those of their oxygen analogs.

X-Ray diffraction studies of crystalline acetylcholine have shown that the $\text{--}^+\text{N}--\text{C}--\text{C}--\text{O}--$ grouping follows the gauche arrangement (Canepa, Pauling, and Sörum, 1966; Chothia and Pauling, 1968; Pauling, 1968), while in acetylthiolcholine (Shefter and Mautner, 1969) and acetyltrimethionolcholine (Shefter and Kennard, 1966), the trans arrangement is seen (Fig. 2). Studies of Sundaralingam (1968) have shown that in 12 molecules containing the $\text{--}^+\frac{3}{4}\text{C}--\text{C}--\text{O}--$ grouping, the oxygen was always gauche to the nitrogen atom. In the case of acetylcholine, it could be demonstrated by means of proton magnetic resonance studies in deuterium oxide that the gauche conformation prevails in solution (Culvenor and Ham, 1966), while it could be shown recently that the trans configuration of acetylthiolcholine and acetylttrimethionolcholine are also retained in solution (R. J. Cushley and H. G. Mautner, unpublished data).

Acetylcholine and acetylthiolcholine exhibit considerable differences in their activities in a variety of biological preparations (Mautner, Bartels, and Webb, 1966; Scott and Mautner, 1967). It was difficult to determine whether these differences were due to the fact that acetylcholine is in the gauche and acetylthiolcholine in the trans conformation or whether they were due to differences in electron distribution. However, when the biological actions of acetylthiolcholine and acetyltrimethionolcholine were compared and again found to be different it could be assumed that, in this case, electronic rather than configurational factors had to be responsible for the differences in activity noted, since, as can be seen in Fig. 3, the conformation of these molecules is almost identical.

It should be added, as a note of caution, that rotational barriers in molecules of this kind are likely to be slight (Liquori, Damiani, and De Coen, 1968), and that the possibility exists that flexible molecules of different conformation might be induced to fit a common receptor.
The fact that replacement of either of the oxygen atoms of acetylcholine or of local anesthetics by sulfur or by selenium greatly modifies the activity of these compounds, while the replacement of the oxygen of choline by sulfur or by selenium converts a compound devoid of depolarizing ability into an effective depolarizing agent, has been useful for approaching some of the questions surrounding the roles of acetylcholine in nerve conduction.

For instance, it has been suggested that acetylcholinesterase and the bio-polymer carrying the acetylcholine receptor of excitable membranes might be identical (Belleau, 1964). If these receptor polymers were identical, then sterically closely related compounds would exhibit similar relative abilities to interact with the active sites of acetylcholinesterase and with the active sites of the receptor polymer of excitable membranes. No such parallelism could be detected. It can be seen in Table I that replacement of the side-chain oxygen of acetylcholine progressively lowers depolarizing activity in the electroplax (Scott and Mautner, 1964; Webb and Mautner, 1966). On the other hand, while the hydrolysis product of acetylcholine, choline, is completely inactive, its thiol analog is a potent depolarizing agent. Oxidation
of cholinethiol to choline disulfide yields a compound which blocks depolarization induced by carbamylcholine. In spite of the quantitative and even qualitative differences in the depolarizing activities of these molecules, all the above compounds are bound to acetylcholinesterase to a similar degree (G. R. Hillman and H. G. Mautner, unpublished data). Although comparisons of events taking place on the membranes of living cells with events involving binding to purified enzymes are dangerous, these findings, as well as the findings of Podleski and Nachmansohn (1966) and Changeux et al. (1967) suggest very strongly that the active sites of the membrane receptor and of

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>(Average molar concentration to depolarize Electroplax to 45 mv)</th>
<th>Acetylcholinesterase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>$3 \times 10^{-6}$</td>
<td>$1.0 \times 10^{-4}$ $K_m$</td>
</tr>
<tr>
<td>Acetylthiolcholine</td>
<td>$5 \times 10^{-4}$</td>
<td>$0.6 \times 10^{-4}$ $K_m$</td>
</tr>
<tr>
<td>Acetylselenolcholine</td>
<td>$1 \times 10^{-4}$ (to 65 mv)</td>
<td>$0.3 \times 10^{-4}$ $K_m$</td>
</tr>
<tr>
<td>Choline</td>
<td>$2 \times 10^{-4}$</td>
<td>$4.0 \times 10^{-4}$ $K_i$</td>
</tr>
<tr>
<td>Cholinethiol</td>
<td>$1 \times 10^{-5}$</td>
<td>$5.0 \times 10^{-4}$ $K_i$</td>
</tr>
<tr>
<td>Choline disulfide</td>
<td>$1 \times 10^{-4}$ (blocks depolarization)</td>
<td>$3.0 \times 10^{-4}$ $K_i$</td>
</tr>
</tbody>
</table>

Figure 4. Isologs related to local anesthetics.

acetylcholinesterase are different, although it is likely that these receptors are related both functionally and structurally.

Studies of isologs of this type have also proved to be useful for throwing light on the drug-receptor complex formed when compounds related to acetylcholine interact with the active sites of membrane receptor or acetylcholinesterase. Thus, the observation that cholinethiol ($pK_a = 7.7$) exhibits higher depolarizing activity at pH's below the $pK_a$ than at pH's above the $pK_a$ of this molecule (Mautner, Bartels, and Webb, 1966), coupled with the observation that methylation of its sulfur atom increases depolarizing activity even further, emphasizes the importance of hydrophobic bonding and the lack of importance of hydrogen-bonding in the depolarizing action of these compounds.
Invited Discussions

Systematic variations carried out on simple local anesthetics have provided a useful approach to the question whether synaptic and axonal acetylcholine receptors are similar. The compounds synthesized (Chu and Mautner, 1968) are summarized in Fig. 4: Unfortunately, the selenocarbonylesters did not prove stable enough for biological work.

When activities of these compounds were tested in axonal (giant axon of the squid, *Loligo pealei*) and synaptic (electroplax, *Electrophorus electricus*) preparations, a striking parallelism in the relative activities of the various compounds could be seen (Rosenberg and Mautner, 1967; and G. D. Webb and H. G. Mautner, unpublished data) (Table II). In both preparations replace-

| TABLE II |
| BLOCKING ACTIVITY OF 2-DIMETHYLMINOETHYL BENZOATE, 2-DIMETHYLMINOETHYL-THIONOBENZOATE AND OF THEIR THIOLESTER AND SELENOLESTER ISOLOGS IN THE ELECTROPLAX PREPARATION AND THE GIANT AXON OF THE SQUID |

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>Blocking action in electroplax</th>
<th>Blocking action, giant axon of squid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% inhibition (10^-4 M solution)</td>
<td>% inhibition (3X10^-4 M solution)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>S</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>0</td>
<td>Se</td>
<td>99</td>
<td>100</td>
</tr>
</tbody>
</table>

- **Esters**
- **Thionoesters**

<table>
<thead>
<tr>
<th></th>
<th>% inhibition (10^-4 M solution)</th>
<th>% inhibition (3X10^-4 M solution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>57</td>
<td>50</td>
</tr>
<tr>
<td>S</td>
<td>63</td>
<td>55</td>
</tr>
<tr>
<td>S</td>
<td>Se</td>
<td>99</td>
</tr>
</tbody>
</table>

ment of the side-chain oxygen of the substituted benzoate by sulfur and selenium resulted in a progressive increase in blocking action. On the other hand, in both preparations, replacement of the side-chain oxygen of the thionobenzoate by sulfur had only a negligible effect, while replacement of this oxygen by selenium increased blocking activity significantly. Parallelism of this kind does not prove the identity of synaptic and axonal receptors, it is, however, compatible with the postulate that these receptors are similar.

The observation that reduction of disulfide groups to thiol groups inactivates electrical activity in the electroplax as well as in axonal preparations, a process that can be reversed by reoxidation of the thiol groups, has been used by Karlin (1968) as a guide for the successful design of active-site-directed compounds for inactivating thiol groups near the “anionic sites” of the electroplax. The finding that some selenoesters such as succinoylselenol-
choline or benzoylselenolcholine block both synaptic and axonal receptors irreversibly (Goodyer and Mautner, 1967; Rosenberg and Mautner, 1967), coupled with the observation that selenolesters are capable of acylating both thiol and disulfide groups (Mautner and Günther, 1961; Günther and Mautner, 1965) suggests that these compounds may also act as active-site-directed inactivators of membrane receptors.

It is interesting to note that in axonal preparations, the action of sulfhydryl reagents may be potentiated by electrical stimulation (Hillman and Mautner, 1968). It may be postulated that electrical stimulation might increase the nerve's permeability to sulfhydryl reagents, or it might result in the uncovering of "buried" thiol groups or in cleavage of disulfide groups. It is hoped that continuation of these studies may prove to be useful for approaching the problem of a possible link between electrical activity and configurational changes of excitable membranes during nerve conduction.

It is a pleasure to acknowledge the interest and the frequent, generous hospitality of Professor David Nachmansohn and his collaborators at Columbia University.

This work was supported by grants from the National Science Foundation (GB-6835) and the National Institute of Neurological Diseases and Blindness (NB-07853).

REFERENCES


Invited Discussions