replacement by the acetyl group results in abolition of the activity, (Table 1). The present findings are not in parallel with the conclusions of Brown et al (1) that the piperazine base and its simple salts are more effective against S. obvelata than are more complex substituted compounds. Further, substitution of a methyl group in the phenyl ring at ortho position enhances the activity, while substitution at the para position, decreases the activity. However, with the increase in the anthelmintic activity there is also an increase in toxicity of the compounds as is evident from the column 3 of Table 1.

REFERENCES

DIRECT INHIBITORY EFFECT OF HEMICHOLINIUM-No. 3 ON ACETYLCOLINE RELEASE

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Accepted February 24, 1975

It is well known that hemicholinium No. 3 (HC-3) reduces the synthesis of acetylcholine (ACh) in both central (1-7) and peripheral (1, 8-10) nervous tissues in vitro. It was demonstrated that the reduced synthesis of ACh was due to inhibition of the transport of choline into neuronal cells (7, 11-13). It was also found that in brain slices HC-3 inhibits uptake of ACh in the presence of an inhibitor of cholinesterase (14, 15). These findings suggest that HC-3 reduces the release of ACh from nervous tissue indirectly by reducing the ACh content of nervous tissue. However, in this study HC-3 was found to have a direct inhibitory effect on the release of ACh from brain slices under certain experimental conditions.

Sprague-Dawley strain rats, weighing 200-300 g were decapitated, the brains removed and then chilled in cold Krebs Ringer. The Krebs Ringer contained 134 mM NaCl, 5.4 mM KCl, 1.34 mM MgSO4, 1.34 mM CaCl2, 20 mM sodium phosphate buffer, pH 7.4 and 10 mM glucose. In experiments on the effect of anticholinesterase, 25 mM eserine sulfate was added to the Krebs Ringer solution. Three cerebral cortex slices were cut from each brain hemisphere using a Stadie-Rigg's slicer: two from the apical surface and one from the lateral surface. The slices (about 160-200 mg wt.) were weighed on a torsion balance, suspended in 4 ml of Krebs Ringer, and then preincubated at 37°C for 30 min in Krebs Ringer with or without eserine sulfate, or in a medium of high K+ concentration containing 90 mM NaCl and 50 mM KCl and other ions at the same concentrations as in Krebs
Ringer. After rinsing with cold Krebs Ringer the slices were incubated further at 37°C for 20 min with 4 ml of medium of high K⁺ concentration. To test the effect of HC-3, 20 μM HC-3 was added to the incubation medium.

For assay of total ACh of the slices, they were homogenized with 5 ml of Frog Ringer containing 25 μM eserine sulfate and boiled at pH 4.0 for 10 min and centrifuged. The supernatant was neutralized with NaOH and then the ACh concentration was assayed on frog rectus muscle by the method of Chang and Gaddum (16). Measurement of acetylcholinesterase (AChE) activity was carried out according to the procedure of Hestrin (17). Microsomal fraction obtained from rat brain homogenate was used as the enzyme preparation and it was preincubated with or without inhibitors at 37°C for 20 min.

Fig. 1. (A) Effects of high K⁺ ion concentration on ACh release from slices preincubated with or without eserine. (B) Effects of high K⁺ ion concentration in presence of HC-3 on ACh release from slices preincubated with or without eserine.

--- Krebs Ringer, --- Krebs Ringer containing eserine,
--- High K⁺ medium

Values are means ± S.D. and the numbers of experiments are shown in brackets.
There were significant increases in the amount of ACh in the slices, which were preincubated in Krebs Ringer containing 25 μM eserine sulfate in comparison to value on normal Krebs Ringer medium (Fig. 1, A). This finding is in agreement with results of Birks and MacIntosh (8) and of Collier and Katz (18) using cat’s superior cervical ganglion and of Potter (10) using the nerve diaphragm preparation. The extra ACh accumulated by presence of AChE inhibitor such as eserine or tetraethylpyrophosphate was termed “Surplus ACh” by Birks and MacIntosh (8). On the other hand, high K⁺ concentration is widely known to cause a release of ACh from the brain slices resulting in a reduction of ACh content in the slice. Effect of HC-3 was examined here on the K⁺-induced reduction of ACh content in the slices preincubated in the presence and absence of eserine.

As shown in Fig. 1, A and B, reduction of ACh content by high K⁺ concentration from slices preincubated in normal Krebs Ringer was not significantly influenced by HC-3. But its reduction by K⁺ in the ACh content of slices which had accumulated “Surplus ACh” by preincubation in medium containing eserine was depressed by HC-3 significantly (P<0.01). Our results, shown in Fig. 1, indicate that when slices containing “Surplus ACh” were transferred to high K⁺ medium, their ACh content was higher in presence of HC-3 than in its absence (8.8 ± 1.6 nmol ACh/g slices, mean ± S.D.; in presence of HC-3 and 6.5 ± 1.8 in its absence). This effect of HC-3 cannot be explained by its known inhibitory influence on ACh synthesis.

As the higher ACh content of slices in the presence of HC-3 might possibly be due to an inhibitory effect of HC-3 on AChE activity, the influence of HC-3 on AChE activity of rat brain microsomes was examined. As shown in Table 1, HC-3 slightly inhibited AChE. However, in the experiments described above on release of ACh, 20 μM HC-3 was used. Therefore, even if HC-3 readily enters the brain slices (19) it seems unlikely that the higher level of ACh in the slices observed in the presence of this concentration of HC-3 is due to the weak inhibitory effect of HC-3 on AChE. On the other hand it is known that HC-3 has no significant effect on activity of choline acetyltransferase (2) and an inhibitory effect on uptake of choline into brain slices (7, 11-13), but these effects of HC-3 do not indicate the possibility of an increase in ACh content of the slices. A more likely explanation of the effect of HC-3 is that it may inhibit release of ACh from the so-called “Surplus-ACh Pool” in the brain slices. In the small intestine also, HC-3 has been shown to inhibit

<table>
<thead>
<tr>
<th>Conc. of inhibitors</th>
<th>Inhibitory effect (%)</th>
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<tbody>
<tr>
<td></td>
<td>HC-3</td>
</tr>
<tr>
<td>10⁻³ M</td>
<td>21, 31, 28</td>
</tr>
<tr>
<td>10⁻¹ M</td>
<td>6, 9, 5</td>
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<tr>
<td>10⁻² M</td>
<td>7, 8, 7</td>
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<tr>
<td>10⁻⁸ M</td>
<td>2, 0, 4</td>
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<tr>
<td>10⁻⁷ M</td>
<td>5, 0, 1</td>
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</tbody>
</table>

Data are represented as per cent of inhibition to enzyme activity without inhibitors.
spontaneous and stretch-induced release of ACh (20).

The nature of "Surplus ACh" is not clear, but it may represent ACh in a free form in the cytoplasm. If this is so and if its release from the slices is inhibited by HC-3, release of "Surplus ACh" may be a carrier mediated transmembrane transport phenomenon, having a close relation with uptake of ACh by the slices which is also known to be inhibited by HC-3 (14, 15). Several workers postulate (21–23) that the ACh in the pool available for release is in a free form in the cytoplasm, not a bound form in the synaptic vesicles. In this meaning, release of "Surplus ACh" which was inhibited by HC-3 may have rather important physiological significances, though there is a report that high K⁺ concentration increased release of "Surplus ACh" from the sympathetic ganglion while electrical stimulation did not (18).

REFERENCES