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ACETYLCHOLINE RECEPTORS IN SCHISTOSOMA MANSONI: VISUALIZATION AND BLOCKADE BY HYCANTHONE

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Hycanthone is one of the very few drugs known which can be used therapeutically for the treatment of schistosomiasis.[1,2] In view of the high degree of effectiveness of this drug in treating infections with Schistosoma mansoni or hematobium, it would be very desirable to know how the drug exerts its lethal effects on schistosomes. However, most of the recent research on hycanthone has dealt with its possible effects on the host,[3-10] rather than its mechanism of action on the parasite.

It has been reported[11] that hycanthone stimulates the uptake of serotonin by schistosomes. However, our laboratory has suggested that hycanthone may act by blocking acetylcholine receptors.[12,13] This work was based largely upon measurements of the motor activity of schistosomes, in which we found that the paralytic effect of carbachol was blocked by hycanthone. In the present report we describe experiments of an entirely different kind which provide further support for the theory of an anticholinergic action of hycanthone.

DNS-chol, a dansylated choline derivative, has been used previously as a fluorescent probe of acetylcholine receptors.[14] This compound binds to the acetylcholine receptors of electric organs, permitting their identification by fluorescence measurement.[15] We have applied DNS-chol to intact schistosomes, providing visual localization of binding areas which we believe to be regions of high density of acetylcholine receptors. Our DNS-chol was synthesized according to the method described in the literature.[14]

Paired S. mansoni were recovered from infected mice and placed in Fischer's cell culture medium (FM) (Grand Island Biological Co., Grand Island, N.Y.) for one-half hour.

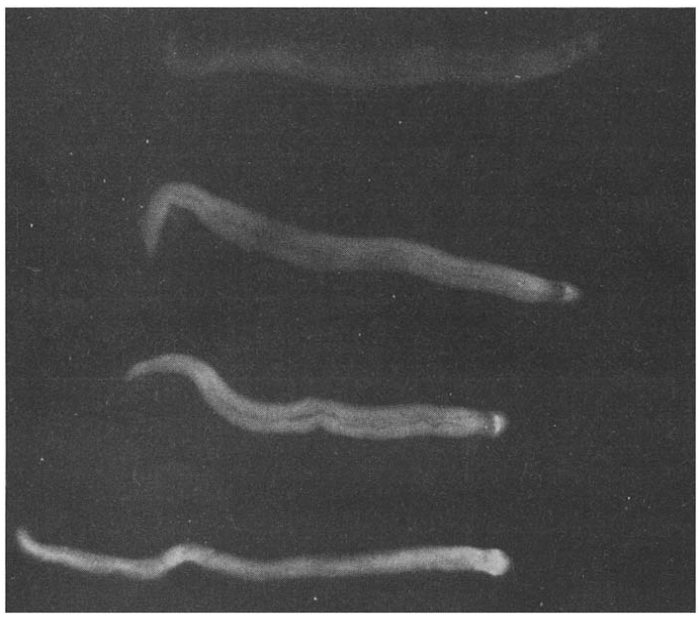

Fig. 1. Effect of hycanthone on DNS-chol fluorescence. All worms were treated with 10^{-4}M DNS-chol, and with (top to bottom) 10^{-5}M, 10^{-6}M, 10^{-7}M, and zero hycanthone.

This medium contained the drug to be tested, or no drug (control). The worms were then transferred to FM containing 10^{-4}M DNS-chol, plus the drug being tested, and were incubated for one hour. At the end of this time they were rinsed briefly with saline and placed on the stage of a stereomicroscope. Illumination was provided with a Zeiss mercury arc lamp with a Type III (UV-transmitting) filter. Fluorescence could be seen easily, and was photographed through the eyepiece of the microscope with a Nikkormat camera. Major variations in fluorescence intensity could be recognized by the eye; work is in progress to provide quantitative fluorometric measurements.

While a small amount of fluorescence is distributed throughout the body of the worm, most of it corresponds to the known anatomy of the nervous system. In particular, the central ganglion in the head appears as a bright, well-defined structure, resembling that seen when biogenic amines are visualized by fluorescence microscopy.[16] Most detail is seen in the male worms; females display only a small amount of fluorescence. No fluorescense is seen without DNS-chol staining, and only a very slight degree of diffuse fluorescense is seen when worms are treated with other dansylated compounds, such as dansyl glycine and sodium dansylate. Hycanthone has no fluorescence, and when tested in a spectrofluorometer, does not alter the fluorescent properties of DNS-chol.

To test the hypothesis that DNS-chol was binding to acetylcholine receptors, we treated with DNS-chol in the presence of atropine, as described above. Atropine greatly reduces the DNS-chol fluorescence, leaving only a trace of brightness.

The site of binding of DNS-chol was further explored by recording the compound's effects on the motor activity of schistosomes. The instrument used in these studies is a small-scale monitor which has been described previously;[17] photocell crossings by S. mansoni are counted over 2-minute intervals. In this system acetylcholine or carbachol causes paralysis of the worms. DNS-chol at 10^{-4}M caused a stimulation of motor activity, and partially blocked the paralytic action of carbachol: 10^{-4}M carbachol caused complete paralysis in the absence of DNS-chol, but only about 50% paralysis in its presence.

The effect of hycanthone, present at three concentrations during DNS-chol treatment, is shown in Fig. 1. Almost all fluorescent staining is prevented. A graded reduction in fluorescence could be seen when worms were treated with 10^{-5}, 10^{-6}, and 10^{-7}M hycanthone. This observation suggests that hycanthone and DNS-chol may act competitively at the same binding site.

The fluorescent staining and the blockage by hycanthone were also seen using a sample of DNS-chol obtained from the Pierce Chemical Co., Rockford, Ill. A higher concentration of the commercial DNS-chol was needed to produce equivalent fluorescent labelling of the worms.

Other nonspecific drugs which are toxic to schistosomes did not produce this same effect. Antimony tartrate at 10^{-5}M, or 10% ethanol, caused indistinct staining of the head ganglion by DNS-chol, but no overall reduction in fluorescence intensity was seen.

Since the lethal effect of hycanthone on schistosomes is seen several days after a single dose is administered to the host, we injected mice with 80mg/kg of hycanthone, recovered the worms three days later, and treated them with DNS-chol. While some fluorescence could be seen, the brightness was less than that of the control worms.

These findings suggest that hycanthone is an inhibitor of acetylcholine receptors. The fact that hycanthone increases, rather than reduces, motor activity, demonstrates that the receptor block could not be due to a release of stored acetylcholine by hycanthone. Since we have previously reported that hycanthone lacks anticholinergic action in mammalian systems;[12,13] it is possible that these findings point to a basis for the selective antischistosomal effectiveness of this drug.

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