THE ACTION OF CURARE ON THE MOTILITY OF NON-INNERVATED SMOOTH MUSCLE AND ITS RELATIONSHIP TO ACETYLCHOLINE * †

JOHN FERGUSON, PH.D.

Omaha, Nebraska

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THE action of curare on the motility of smooth muscle has practical as well as theoretical interest. Gross and Cullen (1) found that curare depressed tonus and rhythmicity in the isolated intestine of the rabbit. Ikeda (2) reported an increased motility in a similar preparation after the application of curare. Everett (3) used the isolated intestinal strip method and observed augmented motility after small doses of *d*-tubocurarine. In 1928 Baur (4) applied a preparation of curare, which was available at that time, to the avian amnion, but his results were so erratic that no conclusion could be reached. McIntyre intimated (5) that *d*-tubocurarine, except with large doses, does not inhibit the normal action of acetylcholine on the intestine of the rabbit. Mautner and Luisada (6) interpreted their results to indicate that *d*-tubocurarine blocked vagal impulses.

It appeared that unequivocal results could be obtained by using aneural smooth muscle. The amnion of the chick is a convenient source of such material. This is a sac-like structure surrounding the developing bird. It contains muscle fibers but is thought to be devoid of nervous elements of any description (7, 8, 9). This work was undertaken to determine the direct effect of *d*-tubocurarine on the motility of smooth muscle and to make observations on a possible antagonism between *d*-tubocurarine and acetylcholine.

PROCEDURE

By a technic described elsewhere (9) the amnion of the developing chick was removed and suspended as a strip in 100 cc. of warmed aerated Sollmann-Rademaekers' solution and the motility of the muscle recorded on a smoked drum. In this work fertile hen's eggs which had been incubated for nine to seventeen days were used, and about 80 per cent of the preparations made from the best of these developed

* From the Department of Physiology and Pharmacology, Creighton University School of Medicine, Omaha, Nebraska.

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rhythmicity when suspended in the solution. This rhythmicity continued in a uniform manner for about twenty-five minutes; it rarely persisted longer than sixty minutes. After normal activity was recorded, the drug was added.

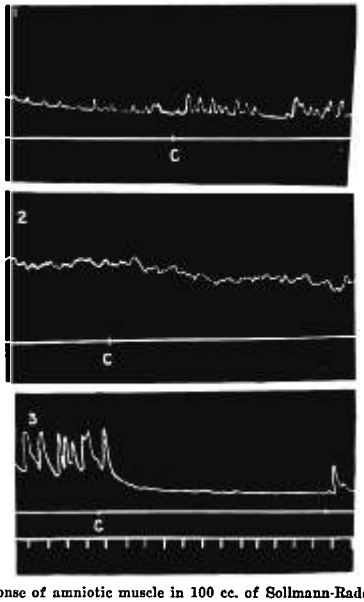

FIG. 1. The response of amniotic muscle in 100 cc. of Sollmann-Rademaekers' solution to *d*-tubocurarine chloride is illustrated. Tracing 1 shows the response of a muscle to 1.5 mg. of the drug (a concentration of 1:66,000 approximately). Tracing 2 illustrates the type of response obtained when 100 mg. was used (a concentration of 1:1000). Tracing 3 shows loss in rhythmicity and tonus when exposed to 150 mg. (a concentration of 1:660 approximately). Upstroke, contraction; downstroke, relaxation. Time 0.5 minute intervals.

RHYTHMIC MUSCLE AND *d*-TUBOCURARINE CHLORIDE ‡

To determine the effects of *d*-tubocurarine chloride, hereafter called curare, on the motility of nerve-free smooth muscle, 24 spontaneously contracting amnions were used. The drug was employed in concentrations of 1:166,600 to 1:500 approximately (0.6 to 200 mg. in 100 cc. of solution).

No significant effects were manifest at a concentration lower than 1:66,600. At 1:66,600 concentration, 11 active amnions were used. In

‡ The *d*-tubocurarine chloride was supplied through the courtesy of Messrs. Burroughs Wellcome & Co. (U.S.A.) Inc.

15 determinations (4 of which were after washing the muscle) it was found that in 5 instances (one of which was after washing) an increased motility followed the application of the drug. When a reactive muscle was washed off and suspended in fresh solution it was possible to obtain a response similar to the initial one. Figure 1, number 1 is a tracing of an amnion which had been washed off, suspended in fresh solution, and exposed to a concentration of 1:66,600 curare. Curare was applied in concentration of 1:66,600 to 5 nonrhythmic amnions but it failed to initiate activity in any of them.

A concentration of 1:10,000 had no effect on motility. A slight decrease in amplitude of contraction followed the application of 1:4,000 curare, but 1:3,300 curare had no effect on the rhythmicity of an additional amnion which had fully recovered from a response to acetylcholine.

At a concentration of 1:1000 curare, 3 spontaneously active amnions manifested a loss of tonus but little change in rhythmicity in each case. Figure 1, number 2, illustrates a typical response. In one instance the loss in tonus was preceded by an increased activity of a transient nature.

At a concentration of 1:660 the rhythmicity ceased in each of 3 amnions used and tonus was lost in 2 cases. In the third instance the muscle may have been fully relaxed before the drug was applied (figure 1, number 3).

An amnion which had been exposed to a concentration of 1:500 exhibited a drop in tonus, a decreased amplitude of contraction and a rhythmicity which faded away gradually.

These experiments with 1:1000 and 1:660 curare suggest that tonus and later rhythmicity are lost with increasingly high concentrations of this drug. Moreover, after loss of both rhythmicity and tonus, rhythmicity is the first to return (figure 1, number 3). Franck, Grandpierre and Arnould (10) found that tonus in the intestine of the dog might be increased or decreased after curare but that the amplitude and frequency of contraction were depressed under both circumstances.

After a muscle had been exposed to 1:66,600 or to 1:10,000 concentration of curare it still responded to stretch and release by increased amplitude of contraction in both instances. When exposed to 1:660 concentration, the quiescent muscle responded by one or more contractions.

ACETYLCHOLINE BROMIDE AND *d*-TUBOCURARINE CHLORIDE

Eighteen rhythmic amnions were used in experiments to determine the possible antagonism of acetylcholine bromide (referred to as acetylcholine) and curare. Acetylcholine is excitatory to amniotic muscle (9), but sensitivity to this drug varies somewhat among different amnions. Euler (11) noted a variation in response of different placental vessels to acetylcholine. Placental vessels are nerve-free, as is the amnion of the chick.

The response of each of 8 amnions to acetylcholine after curare was recorded. Acetylcholine in concentration of 1:100,000 evoked a response in 2 instances out of 2 trials after 1:10,000 curare, a slight response after 1:4000 curare, but no response after 1:660 curare. However, acetylcholine in concentrations of 1:16,600 and 20,000 was effective after 1:660 curare. In one case, an amnion was not reactive to 1:40,000 acetylcholine after 1:1000 curare, although with another preparation, 1:40,000 was effective after 1:500 curare.

In 10 preparations acetylcholine was added to each muscle before and after curare and the two graphs compared as far as was practicable. However, amniotic muscle may respond to acetylcholine by an increase in rate of contraction, amplitude of contraction or tonus. This variability in the response of the individual preparation to successive applications of acetylcholine complicated the interpretation of results. As 1:100,000 acetylcholine is an adequate concentration to evoke a response in practically every amnion, this or a greater concentration was used in each experiment. In concentration of 1:100,000 acetylcholine was applied to 8 amnions before and after curare. Curare in 1:100,000 was applied to 2 of them—one responded feebly to the acetylcholine both before and after curare, and the other responded vigorously in both instances. With 2 amnions 1:20,000 curare was used—the graphs formed by the action of each muscle before and after the curare were the same height above the base line. There was no perceptible variation from a normal response to acetylcholine after 1:10,000 curare was applied to 1 amnion. After 1:4000 curare the response to acetylcholine was decreased and after 1:3,300 curare it was very slight. After 1:2000 curare there was no response to 1:100,000 acetylcholine although there was a very good response before the curare was added. One preparation which had been treated with 1:25,000 acetylcholine before and after 1:2000 curare exhibited slight activity to the second application of acetylcholine, and another manifested marked activity to 1:25,000 acetylcholine after 1:3,300 curare.

COMMENT

A review of the literature on the relationship of curare to motility in innervated smooth muscle suggests a duality of action. Gross and Cullen (1) found that large doses depress motility, and Everett (3) reported that small doses increase amplitude of contraction. My results with noninnervated smooth muscle corroborate both reports in a general way. Baur (4), experimenting with nerve-free muscle, observed somewhat similar pharmacologic properties for atropine—small doses often stimulated, while large doses depressed. My work with atropine lends some support to this observation (9). Further similarities in the pharmacologic actions of curare and atropine have been advanced by Dutta (12). Explanations offered for polyphasic actions of a drug on plain muscle are somewhat uncertain (13). We are dealing with a complex

system. For instance, curare has probably more than one pharmacologic action. In addition to its characteristic effect of blocking the myoneural junction, there is considerable evidence that it can produce effects similar to those of histamine (14). It is assumed that curare liberates preformed histamine from the tissues. Rocha e Silva and Schild (15) found the maximal rate of release of histamine at a concentration of curare of 1:1000 or more. A lesser concentration resulted in a decreased rate of release of histamine. In previous experiments with histamine a concentration of 1:1,250,000 in the saline bath evoked a well-marked, augmented motility in amniotic muscle (16).

SUMMARY

Different concentrations of *d*-tubocurarine chloride (curare) were applied to 24 spontaneously motile preparations of noninnervated smooth muscle (amnion of the chick). No results were obtained below 1:66,600. At 1:66,600, 5 instances of increased activity occurred in 15 determinations. At this concentration, 5 nonrhythmic preparations did not respond. At 1:10,000 no effect was apparent. At 1:4000 a slightly decreased amplitude occurred in one, but no effect occurred in another at 1:3300. At 1:1000, 3 amnions exhibited a loss of tonus but little change in rhythmicity. At 1:660 rhythmicity was lost in 3, and tonus was lost in 2. At 1:500, loss of tonus and gradual loss of rhythmicity occurred.

Histamine, liberated by curare, may account for the dual response with different concentrations.

Amnions exposed to 1:66,600, 1:10,000 or 1:660 responded to stretch and release by increased motility.

Possible antagonism of curare and acetylcholine was studied on 18 amnions but the variable type of response to acetylcholine presented difficulties in interpretation. Curare in 1:100,000, 1:20,000 or 1:10,000 had no discernible effect on the response to acetylcholine bromide in 1:100,000. Curare in 1:4000 to 1:500 decreased or abolished the response to 1:100,000 acetylcholine. The quantitative relationship was not clear.

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