Colchicine IV. Neuromuscular Transmission in Isolated Frog and Rat Tissues

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Colchicine was found to inhibit neuromuscular transmission in the isolated frog sciatic nerve–sartorius muscle preparation but not in the phrenic nerve-diaphragm preparation of the rat. The blockade in the frog was readily reversible by washing, was antagonized by neostigmine, but was not antagonized by physostigmine. Dose-response comparisons indicate that in the frog preparation *d*-tubocurarine is 10,000 times as potent as colchicine; ED_{50} concentrations were 3×10^{-8} *M* and 3×10^{-4} *M* for tubocurarine and colchicine, respectively.

DURING STUDIES on the effects of colchicine in various animal systems, effects on the neuromuscular apparatus of the rat, cat, and frog were observed (1, 2). These effects were suggestive of blockade of nerve–muscle transmission. This effect has been studied in detail, both in frogs, and in a mammalian preparation.

METHODS

The amphibian experiments utilized the frog (*Rana pipiens*) sciatic nerve–sartorius muscle preparation. One muscle was dissected free with the nerve and pelvis attached. The pelvic girdle was fixed in a special holder and a tie attached to the tendinous end of the muscle. The muscle was then immersed in electrolyte solution (sodium chloride, 6.5; potassium chloride, 0.14; calcium chloride, 0.12; and sodium bicarbonate, 1.2 Gm./L.), gassed with 95% oxygen and 5% carbon dioxide, of approximately 40-ml. volume at 20°. The tendon tie was attached to a strain gauge for recording. Stimuli from a square-wave generator were delivered electively to the muscle (from electrodes at each end of the muscle) or to the nerve. Parameters from muscle stimulation ranged from 2 to 6 v. and 1 to 3 msec. and for the nerve from 0.5 to 1.2 v. and 0.01 msec. The frequency in both cases was 0.1/sec.

Mammalian experiments were done on the rat phrenic nerve–diaphragm preparation in a modification of Bulbring's method (3). The muscle was isolated as a wedge-shaped piece approximately 5–7 mm. wide at the point of attachment of the nerve. A tie fixed to the connective tissue at the base of the muscle served to anchor it to the holder, and another tie around the tendon at the apex of the wedge was attached to the strain gauge. The tissue was immersed in oxygenated bicarbonate–buffered electrolyte solution (sodium chloride, 7.0; potassium chloride, 0.42; calcium chloride, 0.24; magnesium chloride, 0.2; sodium bicarbonate, 2.1; and glucose, 1.8 Gm./L.) of 40-ml. volume and held at 37°. Nerve stimulation was employed exclusively, with parameters of 0.1 to 1.0 v. and 0.01 msec. Frequency of stimulation was 0.1/sec.

The strain gauge was activated by a Brush carrier-wave amplifier and the gauge output amplified and displayed on an oscillograph.

Thirty-minute controls were taken before the experiment was started, for it was observed that a muscle which remained stable during this period would function satisfactorily for several hours.

Drugs were added to the bath by dissolving the desired amount in 1 ml. of the nutrient solution and adding the resultant solution to the bath. A like amount was withdrawn after the addition to maintain a constant level of solution in the holder. Washing consisted of removing the solution from the bath, rinsing, and replacing with fresh nutrient solution.

The colchicine used was U.S.P. grade from commercial sources. This was purified chromatographically (4) before use. The final crystalline material had a melting point (Fisher-Johns) of 154–156° and an optical rotation of −124° (C = 1.021 Gm.% in chloroform).

RESULTS

In the frog preparation, colchicine blocked contractile responses to nerve stimulation in concentrations which were without effect on responses to direct stimulation of the muscle (Fig. 1). A concentration of 3×10^{-6} *M* to 3×10^{-4} *M* was a threshold amount, while complete inhibition occurred at 1×10^{-3} *M* to 4×10^{-3} *M*. Effects seen were qualitatively similar to those produced by *d*-tubocurarine. Dose response comparisons

Fig. 1.—Frog sciatic nerve-sartorius muscle preparation. Stimulation of muscle (M) and nerve (N). Colchicine introduced at arrows in molar concentration shown. Control force is 12.5 Gm.; stimulation at 0.1/sec.

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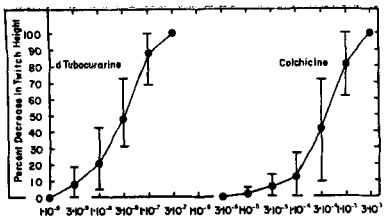

Fig. 2.—Dose-response curves obtained with frog preparation. Each point is an average of four experiments; the ranges are given by the vertical bars.

Fig. 3.—Frog nerve–muscle preparation. Neostigmine antagonism of tubocurarine (T) and colchicine (C) at the 50% level of blockade. Control forces 10 and 9 Gm., respectively.

showed, however, that colchicine was far less potent than *d*-tubocurarine. The ED_{50} of colchicine was 10,000 times that of *d*-tubocurarine (Fig. 2). Although the curves are widely separated, there is good agreement between them with respect to form and slope. In each case, the effect of the drugs was readily reversible by washing, and the recovery time after washing was essentially identical for the two drugs. Prior exposure of the preparation to *d*-tubocurarine, even though washing was thorough and recovery complete, caused a reduction in the concentration of colchicine required to produce blockade. Colchicine, however, did not affect the response of the neuromuscular apparatus to *d*-tubocurarine.

Neostigmine, added to the bath in the presence of test drugs, antagonized the paralytic effect of colchicine, as it did that of curare (Fig. 3). Concentrations of drugs sufficient to produce approximately a 50% reduction in the contractile response could be antagonized completely by neostigmine; the usual concentration required of neostigmine was about 5×10^{-4} *M*, despite wide differences in the concentrations of the agonists. However, complete blockade of transmission by either colchicine or tubocurarine could not be antagonized completely in this preparation. Attempts to produce antagonism of *d*-tubocurarine and colchicine with physostigmine were wholly unsuccessful. In fact, the usual result was enhancement of pre-existing partial blockade.

In the rat nerve–diaphragm preparation, curare again produced neuromuscular blockade. The concentrations required were slightly higher than those needed in the frog experiments, with contractile response reduction first appearing at a level of 3×10^{-9} *M* to 3×10^{-8} *M* and blockade usually becoming complete at a concentration of about 1×10^{-6} *M*. However, no action of colchicine at the neuromuscular junction could be demonstrated; concentrations ranging from 1×10^{-4} to 6.4×10^{-3} *M* were examined and none produced inhibition of twitch response. Higher levels were not tested because at a colchicine concentration of about 4×10^{-3} *M* the contractile response to either nerve or muscle stimulation increased, and a concentration of 6.4×10^{-4} *M* produced contracture. It would thus be impossible to detect any changes in transmission at the neuromuscular junction.

DISCUSSION

The effects of colchicine and *d*-tubocurarine on the amphibian preparation appear to be identical, the only exception being the dose required for equivalent effects. It is suggestive that the two drugs have the same mode of action since the dose-response curves manifest good parallelism, both are similarly antagonized by neostigmine, and neither shows any overt evidence of stimulation of the neuromuscular junction. Although the failure of physostigmine to antagonize the effects of these two drugs is surprising, there is still no evidence of a difference between the drugs in this regard, as neither was antagonized.

There is apparent species-specificity to the curariform action of colchicine, for no similar action could be observed in the rat diaphragm (at least not with drug concentrations up to the level which enhanced muscle contractility or produced contracture) nor in previous studies in the cat sciatic nerve-gastrocnemius muscle preparation (1). This stimulatory action on the rat muscle is apparently separate from that on the neuromuscular junction and is not species-specific. It occurred in frog preparations, as previously noted (2), and has also been observed in different types of isolated mammalian tissues (unpublished data). These actions on muscle are the subject of continuing investigation.

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