REFERENCES

(1) A. F. Hofmann and D. M. Small, *Ann. Rev. Med.*, **18**, 333 (1967).

(2) D. M. Small, *Advan. Intern. Med.*, **16**, 243(1970).

(3) J. L. Thistle and L. J. Schoenfield, *J. Lab. Clin. Med.*, **74**, 1020(1969).

(4) R. G. Danzinger, A. F. Hofmann, L. J. Schoenfield, and J. L. Thistle, *N. Engl. J. Med.*, **286**, 1(1972).

(5) W. I. Higuchi, F. Sjuib, D. Mufson, A. P. Simonelli, and A. F. Hofmann, *J. Pharm. Sci.*, **62**, 942(1973).

(6) J. L. Pope, *J. Lipid Res.*, **8**, 146(1967).

(7) D. M. Small, M. C. Bourges, and D. G. Dervichian, *Biochim. Biophys. Acta*, **125**, 563(1966).

(8) G. A. Bray, *Anal. Biochem.*, **1**, 279(1960).

(9) K. H. Keller, E. R. Canales, and S. I. Yun, *J. Phys. Chem.*, **75**, 379(1971).

(10) D. N. Saraf, P. A. Witherspoon, and L. H. Cohen, *Science*, **142**, 955(1963).

(11) H. Dam, I. Kruse, H. E. Kallehauge, O. E. Hartkopp, and M. K. Jensen, *Scand. J. Clin. Lab. Invest.*, **18**, 385(1966).

(12) W. M. Sperry and M. Webb, *J. Biol. Chem.*, **187**, 97(1950).

(13) K. Juniper, Jr., *Mod. Treatment*, **5**, 480(1968).

(14) W. Nernst, *Z. Physik. Chem.*, **47**, 52(1904).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 5, 1972, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48104*

Accepted for publication January 26, 1973.

▲ To whom inquiries should be directed.

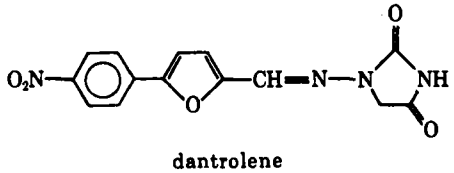
Dantrolene, a Direct Acting Skeletal Muscle Relaxant

K. O. ELLIS▲, A. W. CASTELLION, L. J. HONKOMP, F. L. WESSELS, J. F. CARPENTER, and R. P. HALLIDAY

Abstract □ Dantrolene causes skeletal muscle relaxation in animals without prominent CNS actions, and it has little or no measurable effect on smooth or cardiac muscle. Dantrolene reduces rigidity in decerebrate cats. Unlike centrally acting muscle relaxants, it has no preferential effect on polysynaptic flexor reflexes and still produces its maximum effect on the muscle twitch in an isolated, neurally intact, perfused hindlimb. Dantrolene blocks the twitch response when stimulated through the motor nerve in a way different from the action of tubocurarine or decamethonium. It inhibits direct twitch responses in denervated muscle and is equally effective in attenuating the direct and indirect twitch responses. It is hypothesized that dantrolene causes skeletal muscle relaxation by a direct action on muscle at a site beyond the neuromuscular junction.

Keyphrases □ Dantrolene—effects on skeletal muscle, CNS, and smooth or cardiac muscle □ Skeletal muscle relaxants, potential—dantrolene, proposed site of action

The synthesis of a series of 1-[(5-arylfurfurylidene)-amino]hydantoins and their identification as skeletal muscle relaxants were reported by Snyder *et al.* (1). One member of this series, dantrolene, 1-{[5-(*p*-nitrophenyl)furfurylidene]amino}hydantoin, subsequently received further evaluation (2, 3). This report details the general pharmacology of dantrolene and the proposed site of action as a skeletal muscle relaxant[1].



dantrolene

[1] Sodium dantrolene (Dantrium, Eaton Laboratories, Division of Norwich Pharmacal Co., Norwich, N. Y.) is now undergoing clinical trials as a skeletal muscle relaxant.

EXPERIMENTAL

Methods—*Gross Observational Evaluation*—Male albino mice[2], weighing 20–27 g., were used. The rating scale was similar to that described by Irwin (4). Dantrolene, suspended in 1% carboxymethylcellulose, was administered intraperitoneally and orally in logarithmically spaced doses.

Muscle Incoordination—A rotarod test similar to that reported by Dunham and Miya (5), except that the rod rotated at 20 r.p.m., was employed. The test drugs were administered intraperitoneally in groups of six previously trained mice (maximum of three training trials per animal), and the rotarod tests were conducted 15, 30, 60, and 180 min. following drug administration. The end-point for muscle incoordination was the animal's inability to stay on the rotarod for 30 sec. An ED_{50} (the dose of drug that caused 50% of the animals to fall off before 30 sec.) was estimated by probit analysis (6).

Flexor-Reflex Studies—Cats of either sex, weighing 2–4 kg., were anesthetized with an α-chloralose–urethan solution (50 and 500 mg./kg., respectively) in 50% propylene glycol with water administered intraperitoneally. Flexor-reflex preparations were prepared by a method similar to that of Berger (7), using the tibialis anticus muscle under a resting tension of 50 g. Sensory reflex muscle contractions were elicited by electrical stimulation of the central end of the cut tibial nerve. Motor nerve-induced contractions of the same muscle were obtained by electrical stimulation of the ipsilateral peroneal nerve. A pool of warm mineral oil bathed the nerves to prevent drying. Stimulus train durations of 50 msec. with a frequency of 100 Hz., a duration of 0.5 msec., and a voltage of 7–12 v. were applied to the tibial nerve. Motor nerve stimulation parameters were a duration of 1 msec., a frequency of 0.1 Hz., and a voltage adjusted so that the contraction response was comparable in magnitude to the tibial nerve-induced contraction. Nerve excitation was accomplished with bipolar platinum electrodes connected through a stimulus isolation unit[3] to a stimulator[4]. Contractions were recorded with a force-displacement transducer[5] on a polygraph[6].

[2] Taconic Farms.
[3] Grass SIU-5.
[4] Grass S88.
[5] Grass FT 10.
[6] Grass 7B.

Cross-Circulation Studies—Mongrel dogs of either sex were anesthetized with sodium pentobarbital (35 mg./kg. i.v.), tracheotomized, and prepared for trunk-to-limb cross-circulation studies similar to these described by Margolin *et al.* (8). Contractions of the tibialis cranialis muscle in the perfused hindlimb were induced and recorded as described for the cat flexor-reflex studies. Drugs were administered: (*a*) to the recipient animal to evaluate the central effects of the compounds, and (*b*) to the donor animal to test peripheral inhibition. Drugs administered to the recipient dog reached only the spinal cord, while drug administered to the donor reached the perfused hindlimb with its motor nerve and contracting muscle.

Indirect versus *Direct Muscle Stimulation*—Adult Sprague-Dawley male rats were anesthetized with allobarbital-urethan[7], 1 ml./kg. i.p., and artificially respired. Carotid blood pressure was monitored, and an external jugular vein was cannulated for drug injections. The Achilles tendon was isolated and detached with a piece of the calcaneous bone at its insertion, and the gastrocnemius muscle was freed from surrounding tissue. The free tendon was connected with a ligature to a force-displacement transducer[8], and muscle contractions were recorded on a dynagraph[9]. Indirect stimulation of the muscle was accomplished through the peripheral stump of the severed sciatic nerve. Square-wave stimuli of 10-msec. duration, 0.33 Hz., and at supramaximal voltage were delivered by the apparatus described in the cat flexor-reflex studies. The muscle was stimulated directly with stainless steel needle electrodes[10] inserted directly into the muscle, with the anode in the belly and the cathode in the Achilles tendon. Stimulus parameters were as already stated, but the voltage was adjusted so that magnitude of the contraction response approximated that produced with indirect stimulation. The nerve and muscle were kept moist with frequent applications of warm (37°) mineral oil saturated with Krebs-Ringer's solution.

Denervated Muscle (*9*)—Under ether anesthesia the right sciatic nerve in rats (adult Sprague-Dawley male) was severed and the animals were allowed to recover. Fifteen days later, gastrocnemius muscle contractions were elicited by direct muscle stimulation as already described.

Decerebrate Cat (*10*)—Cats were anesthetized with ether, and a midline incision was made from the base of the nose to the occiput. After trephination the skull bone was removed to expose the right cerebral hemisphere and, by using the tentorium as a guide, the brain stem was sectioned at this level with a spatula. The right hemisphere and then the left were removed, and the cranial vault was lightly packed with cotton.

Decerebrate rigidity ensued when the animal was positioned with its anterior-posterior axis vertical to the horizon and the head was allowed to fall backward.

Other Neuropharmacological Tests—Hexobarbital, 80 mg./kg. i.p., and paraldehyde, 0.8 ml./kg. i.p., "sleep" times were evaluated in mice by the methods of Cook *et al.* (11) and Iyer (12), respectively. Maximal electroshock seizures, produced by a method similar to that described by Swinyard *et al.* (13), and pentylenetetrazol- or strychnine-induced convulsions (14) were utilized as anticonvulsant tests in mice.

Local anesthetic activity was evaluated by the procedures of Wielding (16) on rabbit cornea and by an intradermal method in guinea pigs (17).

Autonomic Pharmacology—Anesthetized dogs (sodium pentobarbital, 35 mg./kg. i.v.) were tracheotomized and placed on artificial respiration, and a thoracotomy was performed through the fifth intercostal space. Coronary blood flow was measured with an extracorporeal flowmeter probe[11], and myocardial contractile force was evaluated with a strain gauge arch[12] in the usual manner. A femoral artery and vein were cannulated for recording of blood pressure and the injection of drugs, respectively. Doses of acetylcholine chloride (1 mcg./kg.), histamine phosphate (2 mcg./kg.), or epinephrine bitartrate (4 mcg./kg.) were administered several times before and at various times after dantrolene or vehicle (polyethylene glycol 300).

Nonvascular smooth muscle activity was evaluated on the GI tract of dogs by the procedures of Goldenberg and Burns (18).

[7] Dial-Urethane, Ciba Pharmaceutical Co.
[8] Grass FT 03.
[9] Type R. S., Beckman.
[10] Grass subdermal E-2.
[11] Medican.
[12] Walton-Brodie.

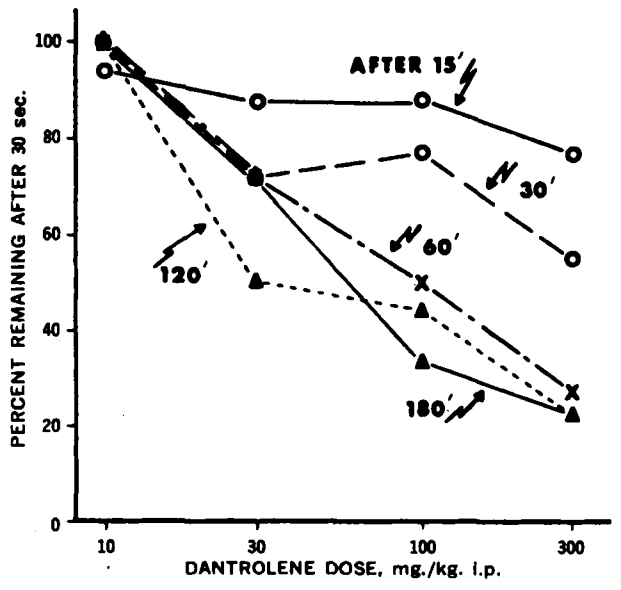


Figure 1—*Dantrolene effects on muscle incoordination in mice using the rotarod (20 r.p.m.), 18 trials per dose-time interval.*

Drugs—The compounds used were: acetylcholine chloride[13], physostigmine salicylate[13], allobarbital with urethan[7], α-chloralose[14], barium chloride[15], paraldehyde[15], dantrolene[16], decamethonium bromide[17], tubocurarine[17], histamine phosphate[17], epinephrine bitartrate[18], sodium hexobarbital[18], mephenesin[19], sodium pentobarbital[20], pentylenetetrazol[21], and strychnine sulfate[22].

RESULTS AND DISCUSSION

Dantrolene, at doses from 10 to 1600 mg./kg. i.p. in mice, induced dose-dependent decreases in spontaneous motor activity, muscle tone (leg and abdomen), and responsiveness to external stimuli. Movements became slow and occurred only when the

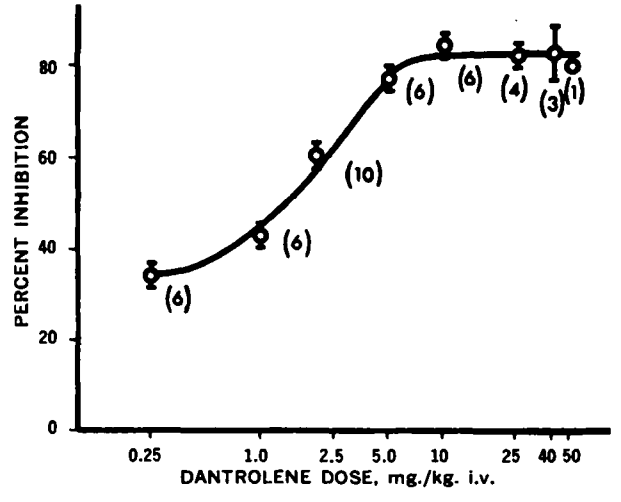


Figure 2—*Dantrolene inhibition of indirectly induced twitch contractions of rat gastrocnemius muscle. Shown are mean values and standard deviations (vertical bars); the number of animals is given in parentheses.*

[13] Merck and Co.
[14] Fisher Scientific Co.
[15] Matheson, Coleman and Bell.
[16] Norwich Pharmacal Co.
[17] Burroughs Wellcome Co.
[18] Winthrop Laboratories.
[19] E. R. Squibb and Sons.
[20] Abbott Laboratories.
[21] Knoll Pharmaceutical Co.
[22] Mallinckrodt Chemical Works.

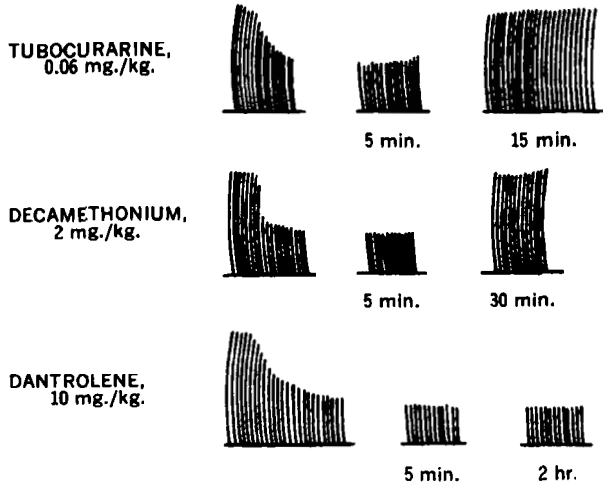


Figure 3—*A comparison of tubocurarine and decamethonium inhibition of the indirect twitch response in the rat with the inhibition caused by dantrolene.*

animals were prodded. Muscle relaxation at doses >200 mg./kg. was present for longer than 8 hr., and at doses >400 mg./kg. it was present for >18 hr. No deaths occurred after 72 hr. The only autonomic effects observed were increased frequency of urination and defecation. The urine was dark yellow following >40 mg./kg. i.p., and there were indications of chromodacryorrhea at >80 mg./kg. i.p. Similar effects in the whole animal were seen with other routes of administration (oral and intravenous) and in other species of animals (rats, cats, and dogs).

Dantrolene effects on motor coordination were evaluated using the rotarod (Fig. 1). The percentage of animals remaining on the rotarod for 30 sec. was plotted for four doses at time intervals up to 180 min. The time to peak drug effect was 60 min.; at that time, when the dose range was expanded, the ED_{50} for muscle incoordination was calculated as 153 mg./kg. i.p. with 19/20 confidence limits of 86 and 271 mg./kg. This ED_{50} for muscle incoordination was considerably above the intraperitoneal dose producing observable muscle relaxation (10–20 mg./kg.) and indicated that these two effects were readily separable.

Dantrolene's production of dose-dependent decreases in spontaneous motor activity, responsiveness to external stimuli, and skeletal muscle relaxation characterized a "sedative-like" profile. However, this profile was atypical in that sedation was secondary to muscle relaxation rather than the reverse, as characterizes sedative and hypnotic drugs. Muscle relaxation was the most pronounced and consistent feature. Unlike the centrally acting muscle relaxants and sedative and hypnotic drugs, dantrolene had no effect on the corneal and pinna reflexes and caused no loss of righting reflex.

Dantrolene (5–100 mg./kg. p.o.) increased the duration of hexobarbital and paraldehyde sleep times at doses >25 mg./kg. Since both methods are of a nonspecific nature and measure only the return of righting reflex, a long acting skeletal muscle relaxant would be expected to prolong such sleep times. Dantrolene (500–1500 mg./kg. p.o.) inhibited maximal electroshock seizures only 40% at 1500 mg./kg., but it had no effect on pentylenetetrazol-

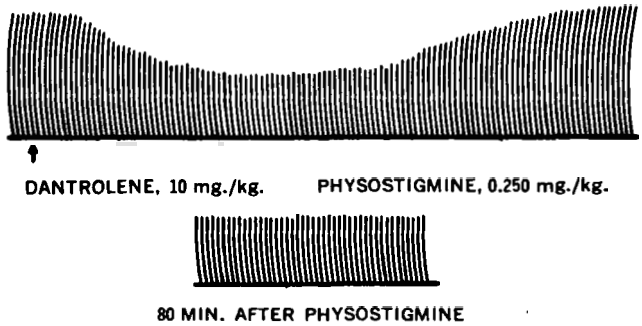


Figure 4—*Physostigmine effects on dantrolene depression of the indirect twitch response in the rat.*

Table I—Dantrolene Effects on Muscle Contractions in the Perfused Hindlimb of the Dog

Drug[a]	*n*	Percent Inhibition
Dantrolene (mg./kg.):		
1.0, monosynaptic peripheral response	3	63
2.5, polysynaptic central response	4	4
2.5, monosynaptic peripheral response	1	84
Mephenesin (mg./kg.):		
30, polysynaptic central response	4	75.2

[a] Dissolved in polyethylene glycol 300, all volumes <1.5 ml./kg.

or strychnine-induced convulsions at doses up to 500 mg./kg. p.o. or i.p. Decreased muscle strength was also observed in the rat analgesic test with 100–500 mg./kg. i.p. Although these doses produced significant muscle relaxation and slowed the reaction time for the tail withdrawal, the response was never blocked. No local anesthetic activity was observed with dantrolene (2–600 mg./ml.) as evaluated on rabbit cornea or by intradermal injections in guinea pigs.

No effect on blood pressure, coronary blood flow, heart rate, or contractile force was elicited by dantrolene (1–10 mg./kg. i.v.) in anesthetized dogs. Moreover, the cardiovascular responses induced by the various autonomic drugs were not affected by dantrolene. Only a slight increase and decrease of short duration in the contractile activity of the duodenum and colon, respectively, were observed after dantrolene (0.5–5 mg./kg. i.v.). There was no effect on the ileum. A slight antagonism of barium chloride-induced spasticity (19) of the ileum and colon was produced by dantrolene.

Dantrolene's muscle relaxant activity led to its being evaluated specifically as a skeletal muscle relaxant. Decerebrate cats that evidenced rigid extension of their legs and catatonic contraction of the trunk musculature were treated with dantrolene intravenously. At a dose of 1.8 mg./kg., the extensor rigidity was markedly reduced for a duration of 35 min., while 4.0 mg./kg. had the same effect for 85 min. Decerebrate rigidity was inhibited totally with 5 mg./kg. in two animals, whereas reduction in the rigidity and tremors, but not complete abolition, was observed in three other cats treated with the same dose. All animals treated with dantrolene (1.8–5 mg./kg.) exhibited at least some reduction in rigidity.

Centrally acting skeletal muscle relaxants are well known (7) for their preferential inhibition of polysynaptic reflex functions. This type of action is readily demonstrated in flexor-reflex preparations in which drug effects on monosynaptic and polysynaptic reflexes can be determined simultaneously. Dantrolene (0.25–10 mg./kg. i.v.) was studied in such preparations, and the results indicated that dantrolene had no preferential effect on polysynaptic reflex responses.

It was further demonstrated that dantrolene produced its effect on skeletal muscle without central actions (inhibition of polysynaptic transmission or otherwise) in the isolated perfused hindlimb of the dog in the cross-circulation preparation. Dantrolene (2.5 mg./kg.) was administered to the recipient dog and found to produce no effect (Table I) on either the sensory- or motor nerve-induced contractions. Mephenesin (30 mg./kg.) administered to the recipient dog for comparison inhibited contractions elicited *via* the polysynaptic pathway by 75%. Following mephenesin, when muscle contractions had returned to control levels (after 1 hr.), dantrolene (1.0 mg./kg.) was administered to the donor dog (periph-

Table II—Effect of Dantrolene, Tubocurarine, and Decamethonium on Denervated Rat Gastrocnemius Muscle

Compound	Dose, mg./kg. or ml./kg.	*n*	Percent Inhibition ± *SE*
Dantrolene	2.5	6	39.1 ± 15.
Polyethylene glycol 300 control	1.0	3	9.7 ± 1.9
Tubocurarine	0.05	6	4.0 ± 1.1
Decamethonium	1.0	6	61.3 ± 2.8
Saline control	1.0	3	0

eral) and the muscle contractions were reduced in the recipient dog by 63 and 84% at 1.0 and 2.5 mg./kg., respectively. This lack of effect in the recipient dog and the marked effect in the donor animals clearly demonstrated that only the peripheral elements of the twitch response were affected by dantrolene.

The peripheral elements of the twitch response were studied in the gastrocnemius muscle stimulated through its motor nerve (Fig. 2). There was a wide effective dose range that caused twitch inhibition, and the dose–response relations were unique for this type of preparation in that the dose–response curve plateaued at less than 100% inhibition of twitch. Increasing the dose of dantrolene to 50 mg./kg. (five times the maximally effective dose) did not increase the level of twitch inhibition.

The inhibition of the indirect twitch response produced by dantrolene was not unlike that produced by neuromuscular blocking agents. This was studied further by comparing dantrolene (0.3–15.0 mg./kg.) with the competitive antagonist tubocurarine (0.01–0.1 mg./kg.) and the depolarizing blocker decamethonium (0.5–15 mg./kg.). The responses at roughly equieffective doses (producing 40–60% level of twitch inhibition) are illustrated in Fig. 3. The time course of the development of the twitch inhibition was similar for all three agents, but the duration of action of the dantrolene was much greater (>2 hr.) than that produced by either tubocurarine (15 min.) or decamethonium (30 min.). Complete abolition of the twitch response was never accomplished with dantrolene at any dose, whereas it was readily accomplished with curare and decamethonium at 0.075 and 15 mg./kg., respectively.

Anticholinesterase agents potentiate the twitch response and antagonize the twitch inhibition induced by competitive neuromuscular blockers such as tubocurarine (20). They do not antagonize the effects of depolarizing blocking agents such as decamethonium (20). The twitch inhibition produced by dantrolene was found to be similar to that of tubocurarine in that physostigmine (an anticholinesterase agent) reversed the twitch depression for a short time (Fig. 4). After the addition of physostigmine, the twitch response steadily increased for 2 min., at which time the twitch height was at the predantrolene level. The physostigmine effects lasted for 80 min., when the twitch response returned to the dantrolene-treated level.

Physostigmine antagonism of dantrolene twitch depression indicated that dantrolene did not produce a depolarizing blockade like decamethonium and implied that dantrolene had anticholinergic properties. However, this anticholinergic character was not substantiated when the effects on denervated muscle were studied. Dantrolene and decamethonium inhibited the twitch response, while curare did not (Table II). (Vehicle control values are included for comparison because of the extreme sensitivity of the preparation to twitch inhibition by a variety of chemical agents.)

The effectiveness of dantrolene in denervated muscle indicated that it did not require neuromuscular transmission to produce its effects and that the reversal of the twitch depression by physostigmine was a physiological type antagonism. Greater quantities of acetylcholine, causing twitch potentiation, masked the dantrolene depression until the effects of the anticholinesterase had disappeared after 80 min. and dantrolene twitch depression was again evident.

The physiological antagonism by physostigmine and the lack of neuromuscular blocking properties pointed to a site of action of dantrolene that was beyond the neuromuscular junction. This post-neuromuscular junction action of dantrolene was tested in experiments with direct and indirect twitch responses in the same preparation. At a dose of 2.5 mg./kg. i.v., dantrolene inhibited the direct twitch response by 56.8 ± 7.6%, while the indirect twitch responses were attenuated by 57.1 ± 3.0%. Although stimulation of the muscle undoubtedly released acetylcholine from some nerve terminals, the responses were >70% direct (75–80% of the direct response remained in the totally curarized muscle). Equal inhibition of both responses confirmed that the blocking action of dantrolene was beyond the neuromuscular junction.

CONCLUSIONS

Dantrolene is a new type of skeletal muscle relaxant. It has a specific action on skeletal muscle that is beyond the neuromuscular junction. It represents a new class of skeletal muscle relaxants and should be a useful tool for studying skeletal muscle contraction mechanisms.

REFERENCES

(1) H. R. Snyder, C. S. Davis, R. K. Bickerton, and R. P. Halliday, *J. Med. Chem.*, **10**, 807(1967).

(2) S. B. Chyatte, J. H. Birdsong, and B. A. Bergman, *S. Med. J.*, **64**, 180(1971).

(3) S. B. Chyatte and J. H. Birdsong, *ibid.*, **64**, 830(1971).

(4) S. Irwin, *Psychopharmacologia*, **13**, 222(1968).

(5) N. W. Dunham and T. S. Miya, *J. Amer. Pharm. Ass., Sci. Ed.*, **46**, 208(1957).

(6) D. J. Finney, "Statistical Methods in Biological Assays," 2nd ed., Charles Griffin and Co., Ltd., London, England, 1964, p. 437.

(7) F. M. Berger, *J. Pharmacol. Exp. Ther.*, **96**, 213(1949).

(8) S. Margolin, O. J. Plekss, and E. J. Fedor, *ibid.*, **140**, 170 (1963).

(9) L. Wislicki, *Arch. Int. Pharmacodyn. Ther.*, **132**, 413(1961).

(10) L. J. Pollack and L. Davis, *Arch. Neurol. Psychol.*, **10**, 391(1923).

(11) L. Cook, J. J. Toner, and E. J. Fellows, *J. Pharmacol. Exp. Ther.*, **111**, 131(1954).

(12) S. K. Iyer, *Indian J. Physiol. Pharmacol.*, **8**, 68(1964).

(13) E. A Swinyard, W. C. Brown, and L. S. Goodman, *J. Pharmacol. Exp. Ther.*, **106**, 319(1952).

(14) F. M. Berger, *ibid.*, **112**, 413(1954).

(15) O. L. Davies, J. Raventos, and A. L. Walpole, *Brit. J. Pharmacol.*, **1**, 255(1946).

(16) S. Wielding, *Acta Pharmacol. Toxicol.*, **8**, 117(1952).

(17) E. Bülbring and I. Wajda, *J. Pharmacol. Exp. Ther.*, **85**, 78(1945).

(18) M. M. Goldenberg and R. H. Burns, *Eur. J. Pharmacol.*, **18**, 1(1972).

(19) A. Linder, H. Selzer, V. Classen, P. Gans, O. R. Offringa, and J. M. A. Zwagenakers, *Arch. Int. Pharmacodyn. Ther.*, **145**, 378(1963).

(20) "The Pharmacological Basis of Therapeutics," 3rd ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N. Y., 1964, p. 601.

ACKNOWLEDGMENTS AND ADDRESSES

Received November 1, 1972, from the *Research and Development Department, Norwich Pharmacal Company, Division of Morton-Norwich Products, Inc., Norwich, NY 13815*

Accepted for publication January 16, 1973.

▲ To whom inquiries should be directed.