thus far observed and reported are at the limits of resolution for light microscopy and are best seen by means of interference contrast or scanning electron microscopy. Figure 3a shows a dorsal epitract bearing many wall canals that are concentrated mainly near the apex of the specimen. Figure 3b shows several wall canals, each about 0.2 μm in diameter, penetrating the test wall, which is about 0.5 μm thick.

In living motile stages, such wall canals are often associated with trichocysts—the canals are called trichocyst pores. Trichocysts have a neck that contains fibrillar strands and a body that consists of proteinaceous material. The purpose of trichocysts is not fully known, although they may function as sensing devices, defense mechanisms, or mechanisms of attachment. The positions of a few trichocysts are shown on *G. pavillardi* (Fig. 2a). Trichocyst pores are also numerous on *Ceratium hirundinella* (O. F. Muller) Schrank. In this species, the pores penetrate the test wall but are lined laterally and basally by at least one membrane (*5*). Because of this lining, the pores do not actually penetrate to the cell interior (Fig. 2b). Therefore, the wall canals in *Dinogymnium* tests may never have allowed uninhibited communication between the cell interior and the outside water; they may have been trichocyst pores lined laterally and basally by impermeable or semipermeable membranes. The trichocyst pores of *C. hirundinella* are also numerous at the lateral cell margins and on the horns (*5*). Wall canals on *Dinogymnium* tests are often concentrated near the apex (Fig. 3a) and antapex, a distribution that is similar to that found in *C. hirundinella*. The purpose of such concentrations is not known.

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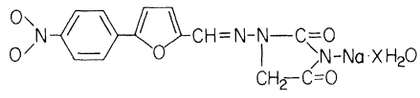
Calcium Release from Skeletal Muscle Sarcoplasmic Reticulum: Site of Action of Dantrolene Sodium?

Abstract. *The muscle relaxant dantrolene sodium acts directly and specifically on skeletal muscle, unlike other pharmacological agents which affect the central nervous system or act at the neuromuscular junction. Dantrolene sodium markedly suppresses the release of calcium previously sequestered by skeletal, but not cardiac, muscle sarcoplasmic reticulum. No effect in the total amount of calcium accumulated was found. In situ, the drug may reduce the amount of calcium necessary for muscle contraction.*

In 1967 Snyder and co-workers (*1*) reported the synthesis of a compound which acted as a skeletal muscle relaxant. This compound, dantrolene sodium (Dantrium, Norwich Pharmacal Corp.),



1-{[5-(*p*-nitrophenyl)furfurylidene]amino}hydantoin sodium hydrate, has been found to be very beneficial in the treatment of spasticity of varying origins and degrees of muscular involvement. Patients suffering from spasticity due to stroke (*2*), multiple sclerosis (*3*), and other disorders of the central nervous system manifesting themselves in some degree of involuntary muscle spasm (*2*, *4–6*) have, in a majority of cases, responded favorably to dantrolene sodium therapy (*7*). The drug appears to be a favorable choice in such maladies because it acts only on skeletal muscle (*8–10*), with no effect on the central nervous system (*5*, *6*, *10*, *11*) (such as sedation or impair-

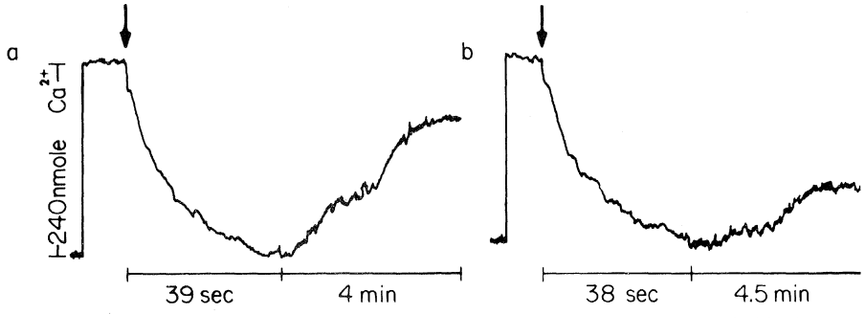
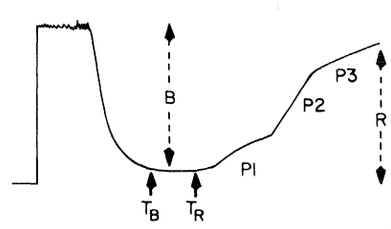


Fig. 1. Dual-wavelength spectrophotometric traces of cat tibialis anterior SR Ca^{2+} binding and release. Note that the release is spontaneous (*22*, *23*). (a) Control preparation trace. (b) Trace in the presence of $10^{-5}M$ dantrolene sodium. Reaction cuvettes contained 1.5 mg of SR, 100 m*M* KCl, 10 m*M* $MgCl_2$, 40 m*M* tris-maleate, *p*H 6.8, and 0.3 m*M* murexide in a final volume of 3 ml. Calcium, here 300 nmole, was added to the cuvette (initial upward sweep), followed by the rapid addition of Na_2ATP (arrow) to a final concentration of 0.25 m*M*. The reaction took place at 30°C and was monitored in an Aminco-Chance dual-wavelength spectrophotometer with wavelength settings at 507 and 542 nm.



	B	T_B	T_R	PI	P2	P3	R
Control	197	28	39	36.5 [.186]	79.8 [.405]	15.9 [.08]	78
Dantrolene sodium	187.5	29	36	16.5 [.08]	63 [.33]	8.2 [.04]	45

Fig. 2. Diagrammatic representation of traces from a dual-wavelength spectrophotometric recording of Ca^{2+} sequestration by SR. The numerical data represent a typical control and experimental study. Abbreviations: *B*, total Ca^{2+} bound (nanomoles per milligram of SR protein); T_B, time in seconds required for peak Ca^{2+} binding; T_R, time in seconds for initiation of Ca^{2+} release; and *PI* to *P3*, rates of Ca^{2+} release at the three phases expressed in nanomoles per milligram. Figures in brackets represent the amount of Ca^{2+} released per phase expressed as a function of the amount bound (*R*/*B*); *R* is the percentage of bound Ca^{2+} released. The values for control and dantrolene sodium Ca^{2+} sequestering studies are representative of eight separate experiments of rabbit and cat muscle SR fractions.

ment of motor coordination) or the cardiovascular system (*9–11*).

Numerous experimental studies have been made with dantrolene sodium and various mammalian and nonmammalian models in efforts to determine the site of action of this spasmolytic agent. The consensus is that the drug does not act on the central nervous system (*6, 10, 12–14*), the neuromuscular junction (*2, 8, 10, 11, 14*), or the muscle sarcolemma (*10, 12, 15, 16*). Because of its generally accepted role in the modulation of intracellular Ca^{2+} for muscle contractility through excitation-contraction coupling, the sarcoplasmic reticulum (SR) has been suggested as a site at which dantrolene sodium may act to reduce the amount of Ca^{2+} released from membranes for the subsequent contractile event (*7, 13, 15, 17–20*).

We have isolated SR from a number of mammalian sources (including canine heart) and examined the effect of dantrolene sodium on the Ca^{2+}-sequestering parameters of these fractions. The SR was isolated from rabbit back muscle; cat soleus, caudofemoralis, and tibialis anterior; and dog heart by a modification of the procedure of DeMeis and Hasselbach (*21*) using a Polytron PT20 for the initial homogenization. The capacities of the SR fractions for Ca^{2+} binding and uptake (in the presence of oxalate) were monitored by a rapid assay technique employing dual-wavelength spectrophotometry, as previously described (*22, 23*). In this system, Ca^{2+} movements, such as sequestration and release by SR vesicles, are reflected in absorbance changes in the presence of the Ca^{2+}-sensitive chelometric dye murexide. Adenosine triphosphate (ATP)–dependent Ca^{2+} binding to SR results in a downward deflection of the trace (see Fig. 2) followed by a spontaneous release of the bound Ca^{2+} (upward deflection). The reaction conditions are given in the legend to Fig. 1.

Two consecutive Ca^{2+} binding experiments (in the absence of oxalate) are shown in Fig. 1. The effect of dantrolene sodium is on the release phase only of the reaction, which is shown quantitatively in Fig. 2. Not only are the three release phases altered with respect to rates, but, more importantly, the total amount of Ca^{2+} released is much lower than in the control preparations. It is interesting to note that complete abolition of muscle twitch cannot be effected with dantrolene sodium (*11, 19*). Similarly, using higher doses in the in vitro experiments does not completely inhibit Ca^{2+} release from the SR. It is evident that none of the binding parameters, such as the amount of Ca^{2+} bound or the time required for binding, are affected by dantrolene sodium. Furthermore, the rate of Ca^{2+} uptake (in the presence of oxalate) is not significantly altered by dantrolene sodium (data not shown). It is of significance that the Ca^{2+} binding and release phases of cardiac SR (*24*) are not affected by the drug. This is the first report, to my knowledge, of such a specific difference between skeletal and cardiac muscle SR.

It may be extrapolated from the in vitro findings reported here and the recent electrophysiological data of Morgan and Bryant (*25*) that, in the intact skeletal muscle, dantrolene sodium acts at a site on the SR which suppresses but does not completely inhibit the release of Ca^{2+} necessary for the activation of the contractile apparatus. This drug would thus seem to be useful, not only clinically but as an experimental tool for probing SR energetics and differences in Ca^{2+} modulating systems in fast, slow, and cardiac muscles.

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Photochemotherapy: Identification of a Metabolite of 4,5′,8-Trimethylpsoralen

Abstract. *A compound, 4,8-dimethyl,5′-carboxypsoralen (DMeCP), has been identified in mouse urine as a major metabolite of the photoactive drug, 4,5′,8-trimethylpsoralen (TMeP). This drug is widely used in the treatment of vitiligo and psoriasis. DMeCP is fluorescent, and nonphotosensitizing when tested on guinea pig skin. DMeCP also occurs in the urine of human patients receiving TMeP orally.*

Psoralens, which are particular isomers of furocoumarins, are a ubiquitous group of naturally occurring compounds which include certain potent photosensitizers such as psoralen, 8-methoxypsoralen (8-MOP), and 4,5′,8-trimethylpsoralen (TMeP) (Fig. 1) (*1*). In the presence of long-wave ultraviolet light (320 to 400 nm) these photoactive compounds stimulate tanning (melanogenesis) of the skin (*2*). Some of these compounds are present in common food plants such as citrus fruits, celery, and figs (*1*). Extracts of other psoralen-containing plants, such as *Psoralea corylifolia* (Leguminosae) and *Ammi majus* (Umbelliferae), have been used for centuries in India and Egypt in conjunction with sunlight for treating vitiligo, a clinical condition of depigmentation in which parts of the body lose melanocytes and melanin pigmentation of the skin (*3*). Recently, psoralens as photochemotherapeutic agents have received attention in Western countries because they are useful in the treatment of psoriasis, vitiligo, and mycosis fungoides when used in combination with long-wave ultraviolet light (*4*). In the United States, the two most commonly used psoralens are 8-MOP and TMeP.

Little is known of the metabolism of psoralens in man or other animals (*5*). We have now isolated and identified a major metabolite of TMeP from mouse urine, and have found the same material