BINDING OF DANTROLENE SODIUM TO MUSCLE INTRACELLULAR MEMBRANES

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1. Introduction

Dantrolene sodium (1-{[5-(*p*-nitrophenyl)furfuryl-idene]amino}hydantoin sodium was synthesized in 1967 [1] and later shown to act as a depressant of muscle contractility. It has proven to be effective in the treatment of various forms of spasticity [2] as well as in the prophylaxis and treatment of the potentially lethal malignant hyperthermia muscle disorder in swine [3] and in human patients [4]. The drug appears to act preferentially on skeletal muscle [5] but has also effect in smooth muscle [6]. In particular, it has no effect on neuromuscular transmission [7] nor on the cardiovascular system [8]. Various suggestions have been made as to the possible of action of dantrolene sodium, but one hypothesis that has gained momentum in recent years is that of an inhibition of the release of calcium from muscle intracellular stores, particularly the sarcoplasmic reticulum. On the basis of pharmacological evidence, the existence of an intracellular compartment able to bind dantrolene sodium with high affinity was postulated [9].

In this work, the binding of [^{14}C]dantrolene sodium to intracellular membranous systems from heart and skeletal muscle has been studied. It will be shown that heart and skeletal muscle sarcoplasmic reticulum and skeletal muscle mitochondria bind the drug with low affinity. Skeletal muscle sarcoplasmic reticulum however possesses a separate class of sites which binds dantrolene sodium with high affinity.

2. Methods

Sarcoplasmic reticulum was preprared from the skeletal muscle (*m. gracilis*) and the heart muscle from pigs according to [10]. Mitochondria were prepared from pig skeletal muscle (*m. gracilis*) according to [11]. The protein concentration in the suspension was determined with the biuret procedure [12]. The binding of [^{14}C]dantrolene sodium (Norwich-Eaton Pharmaceuticals) was measured with the equilibrium dialysis system [13], using a high-permeability membrane (cut off mol. wt 10 000) and 1 ml cells. The standard medium contained 50 mM KCl, 0.5 mM $CaCl_2$, 0.5 mM EGTA, 5 mM K_2-ATP, 10 mM Hepes (pH 7.0), 1 mg sarcoplasmic reticulum or mitochondrial protein and various amount of labelled dantrolene sodium dissolved in DMSO. The cells were rotated at 12 rev./min for 60 min at 37°C. Aliquots of the suspension were counted in a Philips scintillation counter using Instagel as the scintillation fluid.

3. Results and discussion

Fig.1 shows a Scatchard plot of dantrolene sodium binding to skeletal muscle sarcoplasmic reticulum. The plot clearly demonstrates the existence of 2 classes of binding sites, differing widely in their affinity for the drug. Most likely the low affinity leg of the plot of fig.1 and also of the plots of fig.2 and 3 reflect the saturation of the phospholipids of the membranes with dantrolene sodium. Fig.2 and 3 show analogous Scatchard plots of the binding of dantrolene sodium to heart sarcoplasmic reticulum, and to skeletal muscle mitochondria. In both cases it is evident that the high affinity class of binding sites is absent. The high affinity of skeletal muscle sarcoplasmic reticulum for dantrolene sodium (K_d = 5 nM, fig.1) suggests a specific site of action for this drug. The functional relevance of the low affinity class of binding sites iden-

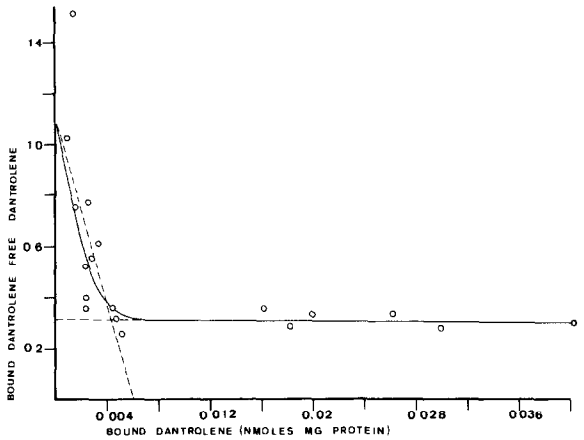

Fig.1. A Scatchard plot of dantrolene sodium binding to skeletal muscle sarcoplasmic reticulum, showing a high-affinity class of binding sites (K_d = 5 nM, capacity 6 pmol/mg protein) and a low affinity class of binding sites (K_d = 10 μM, capacity 3.3 nmol/mg protein).

tified in the three membrane systems is less evident. The data further indicate a molecular difference between heart and skeletal muscle sarcoplasmic reticulum, and offer a very promising tool for investigating this difference. We are now working on the identification of the component which specifically binds dantrolene sodium in skeletal muscle sarcoplasmic reticulum. Preliminary evidence obtained in liposomes containing reconstituted purified Ca^{2+}–Mg^{2+}-ATPase indicate that the dantrolene sodium receptor may be the ATPase.

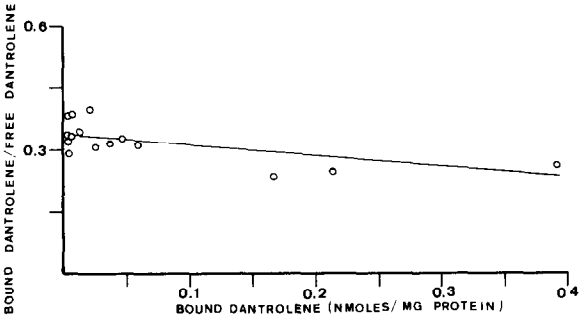

Fig.2. A Scatchard plot of dantrolene sodium binding to heart sarcoplasmic reticulum showing a low affinity class of binding sites (K_d = 2 μM, capacity 1.2 nmol/mg protein).

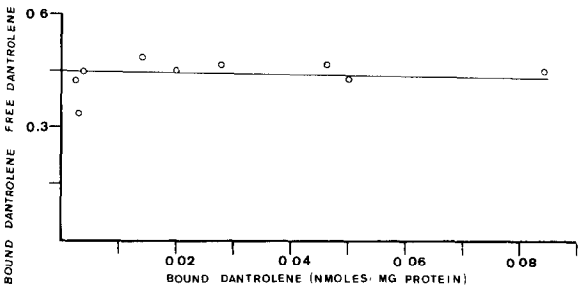

Fig.3. A Scatchard plot of dantrolene sodium binding to skeletal muscle mitochondria, showing a low affinity class of binding sites (K_d = 2.5 μM, capacity 0.8 nmol/mg protein).

Acknowledgements

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