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Effect of dantrolene sodium on [^{3}H]nitrendipine binding to rat cardiac plasma membranes

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Dantrolene sodium (Dantrium) has antiarrhythmic activity in addition to its direct-acting skeletal muscle relaxant activity. Dantrolene sodium exerts its skeletal muscle relaxant action by reducing Ca^{2+} release for sarcoplasmic reticulum. The mechanism by which dantrolene sodium produces its antiarrhythmic effects is not well defined. The effects of dantrolene sodium on [^{3}H]nitrendipine binding to rat cardiac plasma membranes were, therefore, investigated to determine whether the antiarrhythmic action involves an interaction with calcium channels. Whereas 1,4-dihydropyridines maximally inhibited [^{3}H]nitrendipine binding with IC50 values less than 1 nM, verapamil and gallopamil (D 600) inhibited the binding not more than 70% with IC50 values at μM concentrations. Dantrolene sodium caused only minimal inhibition at concentrations up to 100 μM. Thus, the antiarrhythmic action of the drug probably involves a mechanism(s) other than an interaction with the nitrendipine binding site of the slow inward calcium channel.

Dantrolene sodium (Dantrium, Norwich Eaton Pharmaceuticals, Inc.) is a direct-acting skeletal muscle relaxant that is indicated for the symptomatic management of chronic skeletal muscle spasticity. It exerts its skeletal muscle relaxant action either by reducing calcium release from the sarcoplasmic reticulum (Ellis & Carpenter 1972; Desmedt & Hainaut 1977) or by inhibiting calcium movement across the membrane (Putney & Bianchi 1974; Mahmoudian et al 1981).

Dantrolene sodium is also indicated for the prevention and treatment of malignant hyperthermia. Clinical observations indicate that the life-threatening ventricular arrhythmias prevalent during a malignant hyperthermia crisis appear to be reversed by dantrolene sodium (Roewer et al 1984a), indicating an antiarrhythmic effect of dantrolene sodium on the heart. Recent animal studies in-vitro (Lascault et al 1982; Salata & Jalife 1982; Roewer et al 1984b) and in-vivo (Butterfield et al 1983; Brooks et al 1984) further suggest that dantrolene sodium may be useful as an antiarrhythmic agent. The mechanism of antiarrhythmic action, however, is not well defined. The drug has been shown to prolong the duration of both the action potential and the refractory period in cardiac Purkinje fibres and in ventricular and atrial muscles (Lascault et al 1982; Salata & Jalife 1982: Salata et al 1983; Roewer et al 1984b). These findings suggest that dantrolene sodium may decrease the intracellular free calcium concentration (Salata et al 1983) possibly by impeding the slow inward calcium current in a manner similar to that of calcium channel blockers (Salata & Jalife 1982). Dantrolene sodium has also been shown to inhibit the isoprenaline-induced slow response action potentials in potassium depolarized canine Purkinje fibres (Salata & Jalife 1982). The slow response action potential is believed to be largely the result of slow inward current (Isi) (Aronson & Cranefield 1973) and has been used to assess the effectiveness of calcium antagonists to inhibit inward movements of calcium across the cell membranes (Fleckenstein et al 1972). The results further support their contention that dantrolene sodium may be similar in action to other slow channel blocking agents, such as verapamil (Salata & Jalife 1982). However, Hiraoka et al (1984) have shown that dantrolene sodium does not affect the slow inward current in guinea-pig ventricular muscle.

In this study, a direct interaction between dantrolene sodium and the slow inward calcium channel was evaluated as a possible mechanism explaining dantrolene sodium's antiarrhythmic action. This was done by comparing its effects with reference calcium channel blockers on the binding of [^{3}H]nitrendipine to rat cardiac plasma membranes.

Methods

The binding assay method of Bolger et al (1982) was followed with modification. The following steps were performed at 0–4 °C unless otherwise stated and all steps involving the light-sensitive nitrendipine were performed in the dark. Hearts from male Sprague-Dawley rats were homogenized in 20 times their volume of ice-cold 50 mM Tris buffer, pH 7·4, with a Brinkman Polytron PT10 (setting 6, 20 s). The homogenates were centrifuged at 1000*g* for 10 min and the resulting supernatants were then centrifuged at 48 000*g* for 30 min. The final pellets were resuspended in 20 times their volume of Tris buffer.

Aliquots of 100 μl [^{3}H]nitrendipine (final concentrations 0·2–0·6 nM) were added to disposable culture tubes. Tris buffer (total binding) (100 μl), 100 μl of cold nitrendipine (final concentration 1 μM, non-specific binding), or 100 μl of the test compounds were added to the appropriate tubes. Resuspended tissue homogenates (800 μl) were added last so that the tubes contained

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a total sample volume of 1 ml. Total-binding samples and non-specific-binding samples were assayed in triplicate; other samples were assayed in duplicate. The tubes were vortexed and then incubated at 37 °C for 60 min. After incubation, 3 ml of cold Tris buffer was added to each tube, and the solution was filtered rapidly under vacuum through Whatman GF/C filters. The tubes and vacuum filtration wells were each rinsed three times with 3 ml of cold Tris buffer. The filters were then transferred to scintillation counting vials containing 10 ml of Liquiscint (National Diagnostics, Somerville, NJ). The vials were vortexed and radioactivity was determined by counting for 10 min in a liquid scintillation counter (Isocap/300, Tracer Analytic, Inc., Elk Grove Village, IL) with counting efficiency of about 40%. Specific binding was defined as the difference between total-binding and non-specific-binding. Percentage of inhibition was calculated according to the following formula:

$$1-\left(\frac{\text{Experimental d min}^{-1}-\text{non-specific binding d min}^{-1}}{\text{Specific binding d min}^{-1}}\right)\times 100$$

IC50 values (concentrations causing 50% inhibition of specific binding) were obtained from experiments containing four to six drug concentrations.

[^{3}H]Nitrendipine (70–80 Ci mmol^{-1}) was purchased from New England Nuclear Corp., Boston, MA. Nifedipine and nitrendipine were prepared according to a general procedure described by Loev et al (1974). The synthesized products were identical to the reference standards, as evidenced by conventional analytical chemical procedures and by their effects on the [^{3}H]-nitrendipine binding assay. The reference standards, nifedipine and nitrendipine, were generously supplied by Pfizer and Miles Laboratories, respectively. Nicardipine, felodipine, and verapamil and gallopamil (D 600) were kindly donated by Syntex, A. B. Hässle, and Knoll Pharmaceutical Co., respectively. Dantrolene sodium was synthesized at Norwich Eaton Pharmaceuticals, Inc.

Nitrendipine was dissolved in absolute ethanol as a 1 mM stock solution and diluted with Tris buffer to a final concentration of 1 μM before use in non-specific-binding samples. Dantrolene sodium was dissolved in dimethylformamide. Other test compounds were prepared in Tris buffer, PEG 400, or methanol. The volume of the organic solvent in the final incubation medium was less than 1%. At this volume, the organic solvents had no appreciable effect on the binding assay.

Results and discussion

The 1,4-dihydropyridine derivatives such as nitrendipine are among the most potent of the known calcium-channel blockers. These compounds are known to alter the calcium influx through the voltage-dependent and receptor-operated calcium channels. Their extreme potency made them prime candidates for directly identifying calcium channels through radioligand binding techniques. The [^{3}H]nitrendipine binding assay has thus been introduced for directly studying the interaction of drugs with the calcium channels at the molecular level (Bellemann et al 1981). This binding assay is characterized by nanomolar potencies of the 1,4-dihydropyridines and by micromolar potencies of the other calcium-channel blockers such as verapamil and gallopamil (Bolger et al 1982; Ehlert et al 1982; Murphy et al 1983).

Table 1. Effect of dantrolene sodium and reference calcium channel blockers on [^{3}H]nitrendipine binding to rat cardiac plasma membranes.

Compound	IC50 (nM)	IC50 (μM)	Concn (μM)	Percent inhibition Mean (s.e.m.)[a]
Nitrendipine	0·72			
Nifedipine	0·31			
Nicardipine	0·21			
Felodipine	0·29			
Verapamil		~0·1	0·01	21·4[b]
			0·1	51·8[b]
			1·0	63·0 (6·9)
			10	64·7 (11·8)
			100	67·0 (12·4)
Gallopamil		~10	0·01	17·0[b]
			0·1	40·6[b]
			1·0	46·8 (3·0)
			10	50·2 (3·8)
			100	52·0 (1·6)
Dantrolene sodium	Not obtainable		0·1	16·6 (2·0)
			1·0	9·0 (3·3)
			3·0	8·1 (4·0)
			10·0	6·4 (2·8)
			30·0	4·3 (4·2)
			100	5·4 (11·6)

[a] Values are means of at least 3 separate experiments unless otherwise stated.
[b] Only one experiment.

Nitrendipine and other 1,4-dihydropyridines, i.e., nifedipine, nicardipine and felodipine, maximally inhibited [^{3}H]nitrendipine binding with IC50 values less than 1 nM, as shown in Table 1. Verapamil and its methoxy derivative gallopamil inhibited the binding about 50% at 1–100 μM concentrations with maximal inhibition of 67 and 52%, respectively. The concentration-response curves for verapamil and gallopamil were flat rather than sigmoid as characteristically demonstrated by the 1,4-dihydropyridines (Fig. 1). Verapamil and gallopamil appear to bind at a site allosterically linked to the [^{3}H]nitrendipine binding site (Murphy et al 1983). Non-specific binding accounted for about 12% of the total binding. The results obtained in this study are consistent with those reported in the literature (Bolger et al 1982; Ehlert et al 1982; Murphy et al 1983).

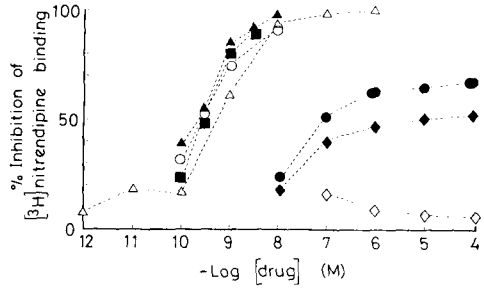

FIG. 1. Effects of dantrolene sodium and reference calcium channel blockers on [3H]nitrendipine binding to rat cardiac plasma membranes. Key: (△) nitrendipine, (○) nifedipine, (▲) nicardipine, (■) felodipine, (◆) gallopamil, (●) verapamil, (◇) dantrolene sodium.

Dantrolene sodium caused only minimal inhibition of a non-dose-related nature over the concentration ranges tested (0·1–100 μM), as shown in Table 1. Because of the limited solubility of dantrolene sodium, concentrations higher than 100 μM were not tested. Our results show that dantrolene sodium does not inhibit [3H]nitrendipine binding to rat cardiac plasma membranes. Therefore, it is inferred that an interaction with the nitrendipine binding site of the slow inward calcium channel does not play a significant role in the antiarrhythmic action of dantrolene sodium. This study supports the recent electrophysiological studies of cardiac muscle which concluded that the slow inward current was not affected by dantrolene sodium (Hiraoka et al 1984; Roewer et al 1984b). The antiarrhythmic action of dantrolene sodium may be attributable to a reduction of K-permeability or intracellular calcium release from the sarcoplasmic reticulum (Hiraoka et al 1984; Roewer et al 1984).

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