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Effect of the Skeletal Muscle Relaxant Dantrolene Sodium on the Isolated, Perfused Heart (40384)

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Ten years ago Synder, Davis, Bickerton, and Halliday synthesized 1-5-*p*-nitrophenyl furfurylidene amino hydantoin sodium hydrate, or dantrolene sodium (Dantrium, Norwich-Eaton Pharmaceuticals) and reported its skeletal muscle relaxing activity (1). Early clinical applications showed gratifying results in relieving the spasticity in hemiplegia (2), athetoid and spastic cerebral palsy (3), multiple sclerosis (4), amyotrophic lateral sclerosis (5), spinal cord injuries (6), tetanus (7) and malignant hyperpyrexia (8). The rapidly growing interest in long-term and controlled clinical investigations is due to the fact that this unique muscle relaxant has no evident effects on the central nervous system (9–12). It apparently also has only negligible effects on the cardiovascular system of anesthetized and awake dogs (10, 12–14). In these experiments no substantial changes in arterial or venous blood pressures, cardiac output, or electrocardiogram were noted, therefore, no cotraindications for the clinical use of dantrolene sodium were found. However, the integration of cardiovascular function in the body is of such complexity that we felt the testing of this interesting drug on the isolated perfused heart preparation might add to its evaluation.

Methods. Adult rats, without consideration of breed or sex, were anesthetized with sodium pentobarbital ip (30 mg/kg) and artificially ventilated through tracheal cannulae. The chest was opened through a midline incision and the ascending aorta cannulated *in situ.* Before ligation of the descending aorta, a stab incision into the left ventricle through the apex was made to provide a vent of the left ventricle to prevent overloading. The heart was rapidly excised and connected to the perfusion instrument. Success in obtaining a stable preparation depends inversely on the ischemic period of the heart. Continuous gravity flow under a pressure of 80 torr was immediately started. The temperature was kept constant at 37° by a plastic coil heat exchanger close to the aortic cannulae. The perfusion fluid in the elevated reservoir was bubble oxygenated (100% oxygen) and defoamed by a plastic sponge coated with Dow-Corning Antifoam. The perfusion fluid consisted of mammalian Ringer's solution adjusted to a pH of 7.4 with Tris buffer and containing 3.5% (W/V) bovine albumin. The solution was filtered through a 0.2 micron Millipore filter before use.

Left ventricular isovolumic pressures were measured with a transducer (Statham P23AA) and DP/DT with a differentiator (Biotronex Laboratory Differentiator BL 622). The force of contraction was measured by a displacement transducer (Grass Force Displacement Transducer FT 03C with a maximum working range of 0.05 kg) attached to a fish hook at the apex of the heart with a constant load of 1 g. The velocity of force, DF/DT, was obtained with a differentiator (Biotronex Laboratory Differentiator BL 622). The flow was measured at the outlet of the reservoir with an infrared counter (15) inside a sleeve around a standard intravenous infusion set dropper. After 16 drops, or 1 ml, the counter activated a marker on the recorder. All tracings were recorded on a direct-writing eight-channel thermal recording system (Hewlett Packard 7722-09A).

Dantrolene sodium was added to the perfusion fluid at the maximally soluble amount of 14 mg/liter, and stirred for one hour at room temperature. This solution was also filtered through a 0.2 micron Millipore filter and put into a second elevated reservoir at the same height as the control solution.

In nine experiments, measurements of cardiac function were made at 2-min intervals during ten minutes of the control perfusion period and during ten minutes of saturated dantrolene sodium solution perfusion. The eight parameters of cardiac function measured were; coronary flow, heart rate, devel-

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oped pressure, DP/DT, developed force, DF/DT, active tension, passive tension and total tension. The mean statistics of all of the actual data parameters were tested for significance ($P < 0.05$). The significance of the saturated dantrolene sodium solution data to the control perfusion data was tested by the difference of means t-test employing a two-tailed significance table.

Results. The means and standard deviations of the eight parameters tested are presented in Table I and Fig. 1. All mean statistics of actual data parameters were significant ($P < 0.05$). Compared to the control period the addition of dantrolene sodium to the perfusion fluid did not produce any statistically significant ($P < 0.05$) differences in coronary flow, heart rate, developed pressure, DP/DT, developed force, DF/DT, active tension, passive tension and total tension. During the perfusion of the isolated rat hearts the passive tension, increased slightly and progressively throughout the entire experimental period. Since no absolutely stable control period of the isolated heart's resting length, or passive tension, could be obtained without constant baseline adjustment, the changes in passive tension were measured separately from the active tension developed by the contractile force. Together they add up to the total tension. Although in these experiments the progressive rise of passive tension did not prove to be statistically sig-

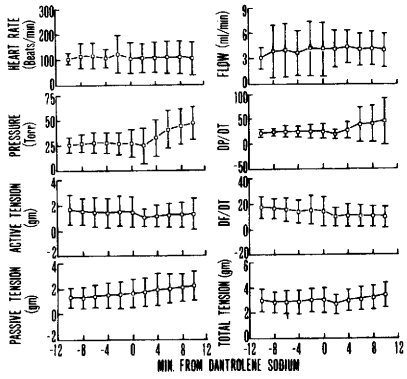

FIG. 1. Heart rate, coronary flow, intraventricular pressure and its first derivative (DP/DT), force or active tension and its first derivative (DF/DT), resting or passive tension and total tension before and during perfusion with dantrolene sodium (Mean ± SD) $P < 0.05$.

TABLE I. EFFECTS OF DANTROLENE SODIUM (14 MG/LITER) ON ISOLATED, PERFUSED RAT HEARTS.[a]

Perfusion time with dantrolene sodium	HR	FLOW	LVIP	DP/DT	AT	DF/DT	PT	TT
10′ Pre Drug	104.6	3.06	25.6	19.6	1.64	16.7	1.30	2.94
n = 7 ±SD	21.8	1.30	7.8	8.4	1.17	8.6	.77	.87
8′ Pre Drug	114.2	3.85	27.1	21.2	1.49	16.1	1.28	2.77
n = 8 ±SD	49.9	3.05	8.7	7.2	1.05	7.8	.80	.81
6′ Pre Drug	114.2	3.95	27.9	22.4	1.42	14.9	1.39	2.81
n = 8 ±SD	50.6	3.19	10.0	10.9	1.14	9.4	.88	.88
4′ Pre Drug	103.5	3.59	27.4	21.9	1.35	13.6	1.45	2.80
n = 8 ±SD	36.9	2.66	10.1	10.7	1.16	9.0	.98	.92
2′ Pre Drug	121.4	4.10	26.2	22.2	1.46	14.9	1.46	2.91
n = 9 ±SD	75.2	3.21	11.3	13.8	1.20	11.0	1.04	.89
0′ Control	105.1	4.01	26.8	23.0	1.37	13.6	1.56	2.92
n = 9 ±SD	60.5	3.16	14.0	14.2	1.22	11.3	1.12	.97
2′ During	104.0	4.06	23.7	16.3	.94	9.3	1.64	2.59
n = 9 ±SD	58.3	2.16	17.7	9.0	.68	7.4	1.13	.74
4′ During	107.3	4.27	31.5	24.3	1.06	10.3	1.83	2.90
n = 9 ±SD	61.0	1.94	18.3	16.0	.85	8.9	1.23	.84
6′ During	107.1	3.94	39.9	35.9	1.13	9.9	1.89	3.02
n = 9 ±SD	63.3	1.88	19.2	34.0	.93	8.3	1.14	.90
8′ During	107.1	4.06	42.4	37.0	1.11	9.4	2.00	3.11
n = 9 ±SD	64.7	1.97	17.6	35.4	.98	8.2	1.12	.92
10′ During	101.3	3.83	45.8	41.7	1.18	8.8	2.11	3.29
n = 9 ±SD	65.6	1.99	16.3	45.1	1.25	8.2	1.12	.98

[a] The mean values ± the standard deviation of mean: HR = Heart Rate (beats per min); FLOW = Coronary Flow (ml/min); LVIP = Left Ventricular Isovolumic Pressure (torr); DP/DT = First Derivative of Left Ventricular Pressure; AT = Actual Tension, force of contraction (g); DF/DT = First Derivative of Active Tension, velocity of force; PT = Passive Tension, resting tension (g); TT = Total developed Tension (g).

nificant, it is essential that for longer test periods the components of the length-tension relationship be known.

Discussion. Cardiac abnormalities have been found in 85 patients, documented as susceptible to malignant hyperthermia by skeletal muscle histology and caffeine contracture (16). Such patients may show electrocardiographic abnormalities, have myocardial perfusion defects in the absence of pyrexia and present cardiac symptoms, including sudden ventricular fibrillation. Thus it is of clinical importance to be assured that a therapeutic agent like dantrolene sodium does not produce any myocardial depression under stringent experimental conditions.

The mechanism of the dantrolene sodium activity has been explored in a series of elegant experiments (12, 17–19). They excluded the possibility that the site of action could be the central nervous system, monosynaptic or polysynaptic reflex responses, the motor nerve, the neuromuscular junction and the muscle membrane (12). Since the locus of the block of skeletal muscle contraction had to be one step beyond that of depolarization of the surface membrane, it was assumed, for many years, that the intracellular calcium transfer may be involved. Specifically the sarcoplasmic reticulum has been identified as being too slow in the release of calcium for the subsequent contraction of the contractile proteins (17–19). Recently calcium accumulation by isolated fragments of sarcoplasmic reticulum from mammalian skeletal muscles was measured by a rapid assay technique employing dual-wave length spectrophotometry and although calcium binding was normal, its release was markedly diminished by dantrolene sodium (20).

Considering the biological principles of muscle contraction, one would have expected that dantrolene sodium may have also affected the calcium release needed for myocardial contraction. Out of a world literature of about 175 papers, only four reports are devoted to this subject. In these investigations only negligible effects on arterial blood pressure, central venous pressure, heart rate, cardiac output and myocardial contractility were found in dogs and sheep (10, 12, 13, 21).

These experiments on whole organisms differ from those on isolated organs. In isolated curarized soleus muscles, spontaneously beating or electrically driven atria and segments of tracheal smooth muscle from guinea-pigs, dantrolene sodium caused a depression (22). The maximum depression of the contractions of spontaneously beating guinea-pig atria was 55% and continued in the presence of adrenaline. It also produced a negative chronotropic affect of 16%. In another previous study on the isolated perfused rat heart (23) dantrolene sodium produced a long lasting, 76% reduction of contractility at the maximum concentration of 15 mg/liter. These results are difficult to interpret since the rate and contractility of these hearts had to be maintained by a continuous adrenaline infusion for 90 minutes and control values could not be reestablished after the washing out of dantrolene sodium. Our studies with known resting tension confirm not only the observations on the heart *in situ* (12, 20, 24), but also the chemical studies which show that dantrolene sodium does not interfere with the physiological rate of intracellular calcium turnover of the heart.

Summary. The literature indicates that dantrolene sodium has produced negligible cardiovascular changes in intact dogs and in patients, but caused depression in the isolated perfused heart. In nine consecutive experiments rat hearts were perfused by means of a modified Langendorff preparation with 3.5% bovine albumin in buffered, mammalian Ringer's solution. Perfusion pressure and temperature were kept constant. Flow was measured with an infrared drop counter. Continuous recordings were made of heart rate, coronary flow, resting length, intraventricular pressure, force displacement and their first derivatives. Statistical analysis of all parameters before and during the perfusion with dantrolene sodium solution showed no significant changes for the same period of time. These results show that dantrolene sodium has no effect and do not confirm recent publications which reported a marked myocardial depression by dantrolene sodium.

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