Strain Differences in Subcellular Calcium Distribution in Striated Muscle of the Mouse (37144)

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Calcareous pericarditis has been reported to occur spontaneously in hearts of several strains of laboratory mice, particularly in DBA/2J mice (1–3). The present authors initiated an investigation into the subcellular distribution of calcium in hearts and skeletal muscle of mice of this strain which did and did not exhibit macroscopic evidence of the calcareous lesion. In the course of this investigation, marked differences were noted in the subcellular cardiac calcium distribution between normal mice of this strain and C57BL/6J mice which were employed as genetically unrelated controls. Subsequent investigation in other available strains revealed additional differences which are reported here.

Materials and Methods. Five strains of mice were selected to represent divergent genetic groups. The strains used were DBA/2J, C57BL/6J, CBA/J, SJL/J and CAW:CF-1. These will be referred to as DBA, C57, CBA, SJL and CF-1 in the the remainder of this paper. DBA mice which showed areas of myocardial calcification will be referred to as DBA(cal). All mice were 90 days old and about 20 g in weight (range 17.5–23.8 g), and appeared active and healthy. Female virgin mice of each strain were used.

Mice were anesthetized with ether and injected via the sublingual vein with exactly 0.10 ml (412,000 cpm) of $^{45}CaCl_2$ in distilled water. After exactly 1 hr, each mouse was sacrificed by cervical dislocation. The thorax was immediately opened and the entire heart was removed, blotted and placed in an ice-cold beaker for weighing. A sample of thigh muscle was also removed from each mouse, chilled and weighed. Heart and skeletal muscle pools were homogenized in a volume of iced buffer equal to 10 times the original weight of tissue. The buffer contained 0.25 *M* sucrose, 10 m*M* Tris HCl, 0.2 m*M* Na_2 ATP and 100 m*M* KCl at pH 6.8. Homogenization consisted of 20 secs in Sorvall Omnimixer at top speed followed by two passes in a TenBroeck all-glass homogenizer. Tissue fractions were separated by centrifugation at 0–4°. The "coarse" fraction consisted of the pellet obtained by centrifugation at 1000*g* for 10 min and probably contained connective tissue, contractile proteins, nuclei and cell membrane fragments. The second fraction was the pellet obtained by centrifugation at 10,000*g* for 30 min and was assumed to be largely composed of mitochondria. A third or microsomal fraction was the pellet obtained from centrifugation at 105,000*g* for 60 min and was considered to consist chiefly of fragments of sarcoplasmic reticulum. Finally, the supernatant solution from the last centrifugation contained all soluble material including extracellular fluid, and was designated as the "soluble" fraction. Since the objective of this study was to reveal similarities or differences between the several strains, and since the fractionation procedures of all samples were identical, no attempt was made to correct for mitochondria trapped in the coarse fraction or for microsomal membranes in the mitochondrial fractions. These errors are probably significant (4) but constant in this work.

The sedimented fractions were resuspended in 2 *N* NaOH and digested at 80° for 30 min before counting and protein determination. Measured aliquots of each fraction were neutralized, decolorized with 2 drops of 30% H_2O_2, and counted in 10 ml of Aquasol (New England Nuclear Corp.) with a Nuclear Chicago Unilux II liquid scintillation count-

er. A 0.1 ml aliquot of each fraction was taken in duplicate for protein determination by the method of Lowry *et al.* (5).

Hearts which had visible deposits of white calcification on the ventricles were separated and treated as a distinct group in determining calcium isotope distribution. The calcareous nature of these deposits was confirmed by pathological examination.

Statistical analysis of the accumulation of isotope by subcellular fractions was performed by using the F test for differences between the mean of each strain and the pooled estimate of means of all animals, and its estimate of variance (6).

Results. Some variation in heart weight was noted between mice of different strains but heart weights were uniform within any one strain (Table I). Mice of the DBA(cal) strain in which the hearts showed gross evidence of calcareous pericarditis had the heaviest hearts. Of the five normal strains observed, DBA mice differed significantly ($p < 0.05$) from the CBA and C57 in this respect.

The subcellular distribution of ^{45}Ca was determined in pooled hearts and skeletal muscle from 6 normal mice of each strain and from 8 DBA(cal) mice. The amount of ^{45}Ca found per gram of tissue differed between strains (Fig. 1). Skeletal muscle from

TABLE I. Strain Differences in Heart Weight of Mice.

Strain	N	Heart wt (mg/100 g body wt ± SD)
CBA	12	457.5 ± 10.0
C57	12	455.4 ± 10.3
SJL	12	478.4 ± 12.8
CF-1	12	481.6 ± 12.3
DBA	14	509.4 ± 10.1[a]
DBA (cal)	8	554.9 ± 16.8[a]

[a] Significantly different from CBA and C57 ($p < .05$).

CBA mice retained significantly less ^{45}Ca at one hour following injection than muscle from mice of other strains. Hearts of CBA and C57 mice contained significantly less ^{45}Ca per gram of tissue than hearts from other strains of mice. Although the binding of isotope by skeletal muscle of DBA(cal) mice was not significantly different from the pooled mean, markedly more of the isotope was found in hearts of this group.

Table II shows the protein content and isotope distribution of the several fractions from hearts and skeletal muscles. The bulk of ^{45}Ca was found in the coarse fractions of normal hearts from each strain, while the microsomal fraction contained the least amount of isotope. The protein content of each fraction gives an indication of the quantity of insol-

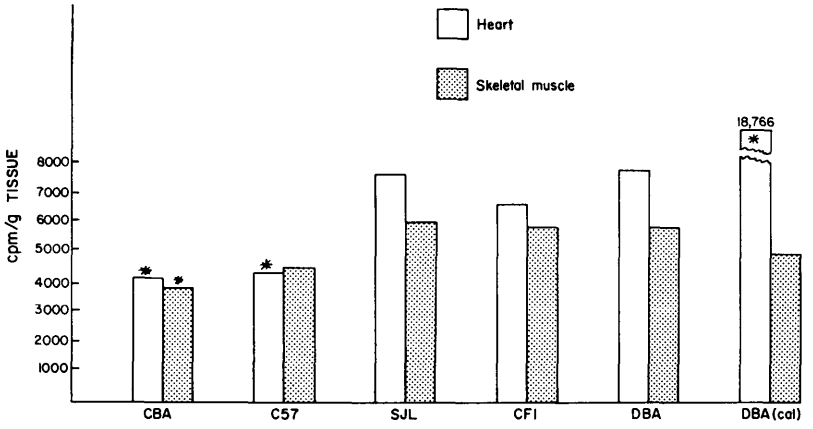

FIG. 1. Total ^{45}Ca taken up by heart and skeletal muscle 1 hr after intravenous injection of 412,000 cpm of the isotope in mice of different strains. * Significant differences from the pooled mean. The pooled mean and estimate of variance of "normal" tissues was 6010 ± 1761 cpm/g tissue for hearts and 5127 ± 964 cpm/g tissue for skeletal muscle.

TABLE II. Distribution of ^{45}Ca in Homogenate Fractions from Hearts and Skeletal Muscles of Mice, 1 hr After iv Injection of the Isotope (412,000 cpm).

	Hearts			Skeletal muscle		
Strain	cpm/g tissue	Protein concn (mg/g tissue)	cpm/mg protein	cpm/g tissue	Protein concn (mg/g tissue)	cpm/mg protein
Coarse fraction						
CBA	2970[a]	98[a]	30	1320[a]	105	13
C57	3040	107	28[a]	1430	103	14
SJL	5800	113	51	1680[a]	90[a]	19[a]
CF1	4610	106	44	1380	114	12
DBA	6160[a]	115[a]	54[a]	1550	115	14
DBA (cal)	8480[a]	113	75[a]	1770[a]	95	19[a]
Mitochondria						
CBA	371[a]	7.5	50[a]	144[a]	5.5	26[a]
C57	635	10.1[a]	63	439	3.8	116[a]
SJL	841	5.7	148[a]	1070[a]	12.4	86
CF1	838	6.3	133	308	3.7	83
DBA	619	6.8	91	656	13.5[a]	49
DBA (cal)	121[a]	3.9[a]	31[a]	160[a]	3.6	44
Microsomes						
CBA	172	19.2	9	463[a]	10.0	46[a]
C57	214	23.5[a]	9	677	13.2	51
SJL	362	13.2	27	1358	10.0	136[a]
CF1	452[a]	13.0	35[a]	1857[a]	17.2[a]	108
DBA	217	10.5	21	924	8.5	109
DBA (cal)	295	8.7[a]	34[a]	156[a]	3.7[a]	42[a]
Soluble fraction						
CBA	550	56	10	1840	43	43
C57	340[a]	50[a]	7[a]	1900	40[a]	48
SJL	580	55	10	1810	49	37[a]
CF1	620	54	12	2200	49	45
DBA	660	54	12	2630[a]	48	55[a]
DBA (cal)	9870[a]	64[a]	154[a]	2830[a]	47	60[a]

[a] Significantly different from the pooled mean of each fraction from tissues of normal mice ($p < .05$).

uble material analyzed. When the ^{45}Ca content of each fraction is expressed as cpm/mg protein, it becomes obvious that equivalent quantities of protein in the mitochondrial fraction bind more isotope than any other fraction, but that this affinity varies considerably from strain to strain. In hearts of DBA(cal) mice, the mitochondria had lost a large portion of their Ca-binding capability. The greater part of the isotope appeared in the coarse and soluble fractions of these hearts.

The coarse fraction of skeletal muscle was less active in the binding of ^{45}Ca than the corresponding fraction from hearts (Table II). A possible reason for this finding is that skeletal muscle fibers have larger diameters than heart fibers and thus have less cell membrane per unit volume of fiber. Considerable differences were found between strains in ^{45}Ca binding by mitochondria and the microsomal fractions, both in terms of quantity of isotope per gram of tissue and in terms of protein content. In DBA(cal) mice the protein content of the mitochondrial fraction was only 27% of the corresponding fraction of normal DBA skeletal muscle, and although a comparable amount of ^{45}Ca was bound per mg protein, the absolute amount of isotope bound was much reduced. Comparison of mi-

crosomes from skeletal muscle of DBA and DBA(cal) mice reveals reduction of both the cpm/mg protein and cpm/g tissue in the latter strain, paralleling the reduced protein content of this fraction. Protein content of this fraction in DBA(cal) muscle was 44% of the same fraction from DBA mice. Coarse and soluble fractions showed similar protein concentrations in both groups.

In general, microsomal binding of ^{45}Ca in skeletal muscle was higher than in myocardium. Perhaps this is a reflection of the more important role of sarcoplasmic reticulum in the coupling of excitation and contraction in skeletal muscle (7). In any case, marked strain differences were observed in this fraction as well.

Discussion. Differences in the total myocardial calcium content of mice of different strains have been noted by other workers. Clower, Williams and Matheny (8) examined the calcium-depleting effects of reserpine in four strains of mice, including DBA and strain C, which is closely related to CBA. They found the total calcium content in hearts of untreated mice to be high in the former strain and low in the latter, an observation which is in accord with our data. Further comparisons with the data from these workers is not possible because of differences in methods.

The data cited here show that normal mice tend to have some variation in the subcellular distribution of radioactive calcium which, with 1 hr of equilibrium time, is in part dependent on the kinetics of calcium flux. Shelburne, Serena and Langer (9) and Langer (10) have extensively examined the kinetics of calcium exchange in mammalian hearts and found that the slowest exchange compartment had a rate constant for calcium of 0.015 min^{-1} in rabbits (9) and 0.004 min^{-1} in dogs (10). Obviously, if these constants are applicable to mouse hearts, many hours would be required to reach a steady state of isotope concentration. Thus at 1 hr, the differences in subcellular isotope distribution will be determined by the affinity of these structures for calcium which will in turn determine the absolute quantity of calcium bound and the turnover rates. The important observation in this work is that strains of mice do vary considerably at least in the rate of exchange and possibly in the affinity of subcellular structures for the calcium ion.

Mice with calcareous pericarditis bound remarkably little isotope to mitochondria in view of the well-known affinity of these structures for the calcium ion (11). The protein content of this fraction was significantly reduced from that found in normal mice of the same strain. This suggests that the myocardium of the abnormal mice contained much fewer mitochondria or that their structure was abnormal in some respect, and were thus unable to take up normal quantities of calcium ion. Perhaps this deficiency is at least partly responsible for the very high isotope content of the soluble fraction in this group. The high isotope content of the coarse fraction is probably due to deposits in the plaques which sedimented early in the centrifugation.

Page, Kessler and Vessell (12) have demonstrated important strain differences in the uptake, pool size and turnover rates of norepinephrine in nine strains of mice. That these differences may be due to differences in the calcium turnover rates and/or binding is suggested by the findings of Clower, Williams and Matheny (8) and the present data. Horst, Kopin and Ramey (13) showed a distinct relationship between norepinephrine uptake and the concentrations of sodium and calcium in the perfusion media of isolated rat hearts. The release of norepinephrine from sympathetic nerves appears also to be related to the calcium content of the tissue (14).

Summary. The distribution of ^{45}Ca to the subcellular components of the heart and skeletal muscle of five strains of mice varies to a remarkable degree in the first hour after intravenous injection. Tissues from CBA and C57 bound less isotope than SJL, CF-1 or DBA. Most of the bound ^{45}Ca was found in the heaviest fraction of heart homogenates while distribution was more uniform in the centrifugal fractions of skeletal muscle. The mitochondria in hearts of DBA mice which showed visible areas of calcification bound much less ^{45}Ca than the same fraction of normal DBA mice. Skeletal muscle from these

mice also differed from that of normal mice of the same strain in the extent of isotope binding.

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