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## Identification of a $\text{Ca}^{2+}$ -ATPase in cerebellar Purkinje cells

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The expression of a sarcoplasmic reticulum (SR)-like  $\text{Ca}^{2+}$ -ATPase was studied in the adult chicken cerebellum. A monoclonal antibody, CaS/C1-IgG, specific for the cardiac/slow-twitch skeletal muscle SR  $\text{Ca}^{2+}$ -ATPase, was used as a probe of protein expression. An immunoblot analysis showed that CaS/C1-IgG recognized similar size polypeptides in adult chicken heart and cerebellum. CaS/C1-IgG recognized fragments of similar size after limited tryptic digestion of cardiac and cerebellar membranes. A two-dimensional  $\alpha$ -chymotryptic peptide map analysis demonstrated that the cardiac and cerebellar  $\text{Ca}^{2+}$ -ATPases were structurally very similar. Immunofluorescence microscopy localized the cerebellar  $\text{Ca}^{2+}$ -ATPase to Purkinje cell bodies and dendritic trees. These results suggest that the well-known  $\text{Ca}^{2+}$  uptake system of skeletal and cardiac muscle SR has a remarkably similar counterpart in some neurons.

### INTRODUCTION

Calcium sequestration and subsequent release from intracellular stores play important roles in neuronal  $\text{Ca}^{2+}$  homeostasis<sup>3,19</sup>. By analogy with non-neuronal systems<sup>6,7,25</sup>, the endoplasmic reticulum (ER) has been implicated as a major source and/or sink of neuronal calcium<sup>15,22,28</sup>. Cerebellar Purkinje cells possess abundant ER and subsurface ER-like cisterns in their cell bodies and dendrites and distinct cisternae in their dendritic spines<sup>12,14,24</sup>, that may contain stores of calcium<sup>5,10,15</sup>. Recently receptors for inositol 1,4,5-thrisphosphate ( $\text{IP}_3$ ), an intracellular messenger which in many other systems causes release of calcium from intracellular stores<sup>4,23,26</sup>, have been localized to Purkinje cells<sup>29</sup>.

A complete picture of  $\text{Ca}^{2+}$  regulation must also include calcium transport systems. Intracellular  $\text{Ca}^{2+}$ -ATPases have been identified in a neuronal cell line<sup>11</sup>, and in the brain<sup>13,18</sup>. There is a brain  $\text{Ca}^{2+}$ -ATPase that appears to be sarcoplasmic reticulum (SR)-like. Specifically, a 100 kDa polypeptide

present in a microsomal fraction of rabbit cerebellum was found to be immunoreactive with a polyclonal antiserum generated against the cardiac/slow-twitch  $\text{Ca}^{2+}$ -ATPase<sup>18</sup>. In addition, a cDNA clone isolated from a rat brain library has been shown to encode an alternately spliced product of the cardiac/slow-twitch  $\text{Ca}^{2+}$ -ATPase gene<sup>13</sup>. However, correlations between molecular identity and localization have not been made for these  $\text{Ca}^{2+}$ -ATPases.

In this study we used a monoclonal antibody (mAb) to localize an abundant  $\text{Ca}^{2+}$ -ATPase to Purkinje cell bodies and dendritic trees. We present immunological and biochemical evidence that this  $\text{Ca}^{2+}$ -ATPase is a form of the  $\text{Ca}^{2+}$ -ATPase expressed in the SR of cardiac myocytes and slow-twitch skeletal muscle fibers.

### MATERIALS AND METHODS

#### *Preparation of one-dimensional peptide maps*

Homogenates of adult chicken heart and cerebel-

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D6A56;A526;0299/<162@.;1 12;1?6A2@6;.116A6<; A<  
B;6>B29F;2B?<;9 @=6;2.==.?AB@2@6; A526;12;1?6H  
A60@=6;2@ FA<052.60.9 A205;6>B2@9<0.A2 0.9H  
06B: 6; A52@2@A?B0AB?2@ 9 .;1 6; 3.0A 2920A?<;H  
=?</2 ,H?F .;9F@6@5. @12;<;@A?A21 A5.A@<<A5  
' 06@A2?;26; &B?86;72 0299@=6;2@C619F .00B:BH  
9.A2 0.906B: B=<; 12=<9.?6G.A6<; )52 .  
)&.@2 D2 5.C2 612;A63621.:F /2 =.?A <3 A52  
<:920B9.? .:056;2?F D5605 @2>B2@A20006B: 6;  
<;2 <?<?2 <3 A52@2B?<;9 0<:=.?A:2;A@ +5605  
0<:=.?A:2;A@ 0<;A.6; A52 . H)&.@2 0.; /2@A2  
?2@<9C21F 6::B;<2920A?<; :60?<@0<=F  
5.@.9@</22; 12;<;@A?A21 A5.A&B?86;72 0299  
@<:<A5 06@A2?;.216@=9.F. @=206.96G296;8.42 D6A5  
A52=9.@.: :2?/?.:2C ? )52@2 7B;0A6<;@==2?  
@6:69.?A<A5<@2E6@A6;AD22; =9.@.: :2/?.:2  
.;1 A52(' <3 ! '%# :B@092 2;8.?A 2A.9  
@B442@A2A5.A A52 7B;0A6<;@6; ! '%# :B@092  
:216.A2 2E06A.A6<;H0<;A?0A6<0<B=96;4 .;1 =?<H  
=<@21 A5.A A52 <?=<9<460.99F @6:69.? ;2B?<;9  
@A?B0AB?2@ =?<C612 . 0<::B;60.A6<; 96;8 /2H  
AD22; 6;A2?;9 .;1 @B?3.02 :2/?.:2@ 0<B=96;4  
12=<9.?6G.A6<; A<C.?6<B@0299B9.?0A6C6A6A6?<B45  
A52 ?292.@2<3 0.906B: 6<;@ F .;9<4F D6A5;<;H  
<2B?<;9 @F@A2:6A @22:@ 96829FA5.A 6;<@6A<9H H  
=5<@=5.A2 & .:F /2 .;<A52? @64;9 D5605 ?2H  
92.@2@.906B: 3?<: 6;A?.0299B9.?;2B?<;9 @A<?2@  
'202;A9F ?16<9./2921 & 5.@ /22; B@21A<9<0.96G2

' '\$ (

;1?2D@ ( "2.::; ' ".:16@ # .;1  
'22@2)( 0A6C6AFH12=2;12;A00B:B9.A6<; <3 0.906B:  
6; &B?86;72 029912;1?6A60@=6;2@ " \$  
H  
.6;/?6142 ! #6992? .;1 &.78@ % .906B:H  
/6;16;4 =?<A26;16@A?6BA66; A52?A./?6; " #  
H  
9.B@A26; # & .906B: A?;@=<?A1 /B332?6;4 6; ;2BH  
?<;@ " # "%# H  
B?42@@ # <13?2F && #0!6;;2F ( 2??6142  
#9 ?C6;2 ' .;1 &BA;2F + )52 @20<;1:2@2;42?  
96;86;4 ?202=A<?0A6C.A6<A<6;A2?;9 . ?292.@26; 96C2?  
\$%" H  
B?4<F;2 ' ?F .;1 ??<; FA<052.60.9  
9<0.96G.A6<<3 0.906B: 6; A5212;1?6A60@=6;2.==.?AB@<3  
A5202??/?9 0<?A2E;1 .A @F;=A60@6A6@A5202??/299?  
0<?A2E \$ H  
.:=/299 !& ; # " ;A.; .;1 + \*.; +6;892  
1@ " ! # \$ % % # # ( # ( #  
"## = H  
.?3<96 ;A?.0299B9.? 0.906B: 5<2<@A.@6@ %  
& H  
296< #' .;1 26G.;; + .906B:H/6;16;4 =?<H

?202=A<?@6A2@?A 02??/299B: .;1 6; =.?A60B9.?A52  
&B?86;72 0299/<162@.;1 12;1?6A2@ )52 9<0.96G.H  
A6<; <3 5645 92C29@3 . H)&.@2 A<A52@2@.:2  
?246<;@6@<;@6@A21?6A5A52C62DA5.AA52@22:H  
/?.;2H/<B;1 0<:=.?A:2;A@ /<A5 @2>B2@A2;1 ?2H  
92.@2;2B?<;9 0.906B:  
)56@ @AB1F12;A6362@ . H)&.@2 6; 02??/299?  
&B?86;72 0299@)52 09<@2@A?B0AB?9. :A642;60  
@6:69.?6A62@A5202??/299.? .;1 0.?16.0 @9<DHAD6A05  
( ' . H)&.@2@@B442@A5.AA52@22 2;0<121 /F  
A52 @.:2 42;2 .;1 6;160.A2 A5.A A52 D299H8;<D;  
. HB=A.82@F@A2<3 @8292A.9;1 0.16.0 :B@092  
( ' 5.@ . ?2.:78./9F @6:69.? 0<B;A2?=.?A 6; @<2  
;2B?<;@

!\$%+" # \$(

+2 A5.;8 29<?2@ (<:2?C6992 3<? A205;60.9 .@ @6@H  
A.;02 ? '605.?1 # "2/<C6AG3<? 529= 6; 0?F<@20H  
A6<;6;4 ? (56; "6; @ 9./<?A<?F 2=.?A:2;A <3  
6<=5F@60@3<? .@ @6@A.;0? =?2=.?6;4 AD<H16:2;H  
@6<;9.H05F:<A?F=A60.:=@ .;1 <B? 9/ 0<992.4B2@  
3<? @A6:B9.A6;416@0B@@<6<@ D<B91 .9@<9682 A<  
A5.;8 ? <@5B. ' (.;2@ 3<? 529=3B9@B442@A6<;@  
-! .;1 #2 D2?? @B==<?A21F \$ 9 =?2H  
1<0A<?9 A?6;6;4 4?;A@ .;1 A52 ?2@2.705 D.@ @B=H  
=<?A21/F . 4?;A 3?<: A52 \$ "

A26;=.?C.9/B:6; .@. ;2B?<;9 .:782? \$%"

H  
.C6@ % .;1 2;;2AA \* B.; 2?FA5?<0FA09.A5?6;  
.;1 09.A5?6;HB:0<.A6;4?<A26;  
H  
638<C. #.785.; .;1 29.F ' .906B: 6;  
A52 @=6;2.==.?AB@ <3 12;1?6A60@=6;2@6; A52 12;A.A2  
<:920B9.? 9.F2? " H  
699 " .;1 5B25 (H ; 6;A?.0299B9.?)&  
#4 H12=2;12;A 0.906B: =B:= D6A56;A52\$ H ;2BH  
?<;9 029996;2 H  
?F E<H@<.A60.;1 E<H12;1?6A6@F;=@2@A52  
02??/?9 0<?A2E.; 2920A?<;60?<@0<=2@AB1F \$  
H  
B;A2@86H .:/96; H# ?22/ .;1 (5B99  
<C29 . =B:= 2E=?2@@@?;/?6; 861;2F .;1 @A<.:05  
6@;0<121 /F .; .9A2?;A6C2A?;@0?6=A3 A52 @9<DHAD6A05  
:B@092 @.70<=9.@:60?2A60B9B;.H)&.@2 42;2  
H  
2;8.?A # & ".:16@ # .;1 '22@2)( (6:69.?H  
6A?3 7B;0A6<;@AD22; =9.@.: :2/?.:2@ .;1 2;1<=9.@H  
:60 ?2A60B9B.6; :B@092 .;1 ;2B?<;@  
H  
2;8.?A # & '22@2)( .;1 ?6;92F E9 ;1<=9.@:60  
?2A60B9B:@2>B2@A20006B: 6; A52 @>B6146.;A E<;

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