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# Adaptive expression pattern of different proteins involved in cellular calcium homeostasis in denervated rat vas deferens

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**Abstract**

The activity and protein expression of plasma membrane and sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPases and ryanodine receptors were investigated in surgically denervated rat vas deferens. The function of thapsigargin-sensitive but not thapsigargin-resistant ($Ca^{2+}$–$Mg^{2+}$)ATPase (from sarco(endo)plasmic reticulum and plasma membrane, respectively), evidenced by enzyme activity and $Ca^{2+}$ uptake experiments, was significantly depressed by 30–50% when compared to innervated vas. Western blots showed that such reduction in sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase performance was accompanied by a decrement of similar magnitude in sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase type 2 protein expression, without any significant change in plasma membrane ($Ca^{2+}$–$Mg^{2+}$)ATPase expression. Finally, [$^3$H]ryanodine binding revealed that the density of ryanodine binding sites was reduced by 45% after denervation without modification in affinity. The present findings demonstrate that sarco(endo)plasmic reticulum proteins involved in intracellular calcium homeostasis are clearly down-regulated and brings further evidence of a modified calcium translocation in denervated rat vas deferens.


*Keywords:* Denervation; Vas deferens; Sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase; Plasma membrane ($Ca^{2+}$–$Mg^{2+}$)ATPase; Ryanodine receptor; Calcium

## 1. Introduction

The maintenance of relatively low concentrations of cytosolic free calcium at basal conditions and the sudden rise of its concentration in order to elicit contraction are essential for muscle cell physiology and pharmacology (Jurkiewicz et al., 1975), and are governed by different systems. These include the plasma membrane ($Ca^{2+}$–$Mg^{2+}$)ATPases and $Ca^{2+}$ channels, and the intracellular ($Ca^{2+}$–$Mg^{2+}$)ATPases and $Ca^{2+}$ channels linked to ryanodine or to inositol 1,4,5-trisphosphate receptors located in sarco(endo)plasmic reticulum membranes (Strehler and Treiman, 2004). There are also other intracellular $Ca^{2+}$–ATPases present in Golgi complex (Wootton et al., 2004) and in nuclear membranes (Gerasimenko and Gerasimenko, 2004) regulating cytosolic free calcium concentrations as well as $Na^+/K^+$–ATPase, which indirectly affects the activity of the plasmalemmal $Na^+$–$Ca^{2+}$ exchanger (Sanders, 2001). The imbalance of the expression or activity of one or more of these components may cause an impact on calcium gradient and myocyte function as a whole (Prasad et al., 2004).

We have previously shown that in the rat vas deferens the density ($B_{max}$) of dihydropyridine-sensitive voltage-dependent $Ca^{2+}$ channels is decreased by about 60%, 7 days after denervation (Jurkiewicz et al., 1994). In similar conditions, a down-regulation of $Na^+/K^+$-ATPase $\alpha_2$ isoform has also been found (Quintas et al., 2000). However, it is still unknown whether or not other agents involved in calcium translocation, such as plasma membrane and intracellular ($Ca^{2+}$–$Mg^{2+}$)ATPases, and ryanodine receptors, are altered by denervation of the vas deferens.

From the functional standpoint, it is known that several changes occur after various denervation procedures of the rat

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vas deferens, such as the development of spontaneous rhythmic contractions, an enhancement of maximal contraction and a leftward shift of the concentration–response curve to agonists (Kasuya et al., 1969; Lee et al., 1975; Jurkiewicz et al., 1977, 1992; Quintas et al., 2002), the latter change coined as adaptive supersensitivity (Fleming, 1975; Fleming and Westfall, 1988).

Although a straight correlation between partial depolarization and adaptive supersensitivity has been demonstrated in the reserpinized guinea pig vas deferens (most probably due to a decreased density of $Na^+/K^+$-ATPase $\alpha_2$ isoform) (Goto et al., 1978; Wong et al., 1981; Hershman et al., 1993), this appears not to be true for the rat vas, since the resting potential of the smooth myocyte membrane is not modified even when supersensitivity is fully established (Goto et al., 1978).

Thus, because of the lack of more information about the role of innervation as a regulatory system for calcium translocation, we have here investigated whether or not surgical denervation of the rat vas deferens modifies the activity or expression of ($Ca^{2+}$–$Mg^{2+}$)ATPases and intracellular $Ca^{2+}$ channels (ryanodine receptors), to obtain additional data for a better understanding of the above reported functional changes.

## 2. Materials and methods

### 2.1. Surgical denervation

The experimental procedures used in this work were approved by the Ethics Committee of Universidade Federal de São Paulo. Ipsi and contralateral vas deferens from male Wistar rats (4–6 months old) of the 2BAW colony were denervated as previously reported (Kasuya et al., 1969; Quintas et al., 2000). After 14 days vasa deferentia were excised and submitted to $10^{-4}$ M tyramine challenge in order to reject reactive organs (contraction above 5% of the control response). Fresh weight was not altered by denervation (control 70.0±7.5 mg, and denervated 70.4±6.8 mg, represented as means±S.E.M., $n=5$). Afterwards, vasa deferentia from denervated and sham-operated (control) rats were kept at −80 °C.

### 2.2. Vas deferens preparation

Organs from denervated and control rats (approximately 20 organs for each preparation) were minced and homogenized in Ultraturrax (20,500 rpm) in 0.25 M sucrose/5 mM Tris–HCl buffer (pH 7.4) plus 0.2 mM phenylmethylsulfonyl fluoride and 2 mM dithiothreitol, followed by 10 strokes in a glass Potter homogenizer, vacuum filtration through gauze and ultracentrifugation (108,000 ×$g$/1 h). The pellet was resuspended in the same buffer (without dithiothreitol) and stored at −80 °C. The amount of protein was measured by the method of Lowry et al. (1951) and was not significantly different between groups (control 61.8±7.7 mg/g tissue, and 55.4±6.3 mg/g tissue, represented as means±S.E.M., $n=5$, $P>0.05$ Student's $t$-test).

### 2.3. ($Ca^{2+}$–$Mg^{2+}$)ATPase activity

In a medium containing 5 mM $Na_2ATP$, 0.3 mM EGTA, 4 mM $MgCl_2$, 10 mM $NaN_3$, 5 μM A23187 (a $Ca^{2+}$ ionophore), traces of [$\gamma^{32}$P]ATP and 50 mM HEPES–Tris buffer (pH 7.4), with or without 3 μM thapsigargin (specific inhibitor of sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$) pumps) and with or without 10 μM free $Ca^{2+}$, the ATPasic reaction started with the addition of 20 μg protein. After incubation for 2 h at 37 °C, ATPase activity was determined by counting the radioactivity derived from the released [$^{32}$P]Pi. Subtraction of the basal ATPase activity (without $Ca^{2+}$) from the total ATPase activity (with 10 μM free $Ca^{2+}$) resulted in the ($Ca^{2+}$–$Mg^{2+}$)ATPase activity. The thapsigargin-sensitive ($Ca^{2+}$–$Mg^{2+}$)ATPase activity (sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$) pumps) was determined subtracting the thapsigargin-resistant ($Ca^{2+}$–$Mg^{2+}$)ATPase activity (assumed to be due to plasma membrane ($Ca^{2+}$–$Mg^{2+}$) pumps) measured in the presence of saturating concentration of thapsigargin from the total ($Ca^{2+}$–$Mg^{2+}$)ATPase activity measured in the absence of the drug (Cunha et al., 1992, 1996; Scaramello et al., 2002). Stock solutions of 5 mM thapsigargin were prepared in 100% dimethyl sulfoxide. Final concentrations of dimethyl sulfoxide (0.06 %) were without effect in ATPase activity as well as active $Ca^{2+}$ uptake.

### 2.4. Measurement of [$^{45}Ca^{2+}$] uptake

In a medium containing 5 mM $Na_2ATP$, 0.3 mM EGTA, 4 mM $MgCl_2$, 10 mM $NaN_3$, 5 μM A23187, traces of $^{45}CaCl_2$ and 50 mM HEPES–Tris buffer (pH 7.4), with 10 μM free $Ca^{2+}$ and in the presence or absence of 3 μM thapsigargin, the uptake started with the addition of 50 μg protein. A rapid filtration of the medium was performed after 90 min incubation at 37 °C and the filters were washed twice with 10 ml of a cold solution (2 mM $LaNO_3$, 100 mM KCl and 20 mM 3-(*N*-morpholino) propanesulfonic acid, MOPS, pH 7.0) before counting of the retained radioactivity by a Tri-Carb liquid scintillation (Cunha et al., 1996). The specific $Ca^{2+}$ uptake was calculated subtracting the uptake measured in the presence of 5 μM A23187 (blanks) from the total uptake.

### 2.5. [$^3$H]Ryanodine binding

The protein (150 μg) was added in a medium containing 1.5 M KCl, 0.8 mM $CaCl_2$ (107 μM free $Ca^{2+}$), 10 mM $Na_2ATP$, 0.3 nM [$^3$H]ryanodine, 10 mM HEPES–Tris buffer (pH 7.4) and various concentrations of unlabeled ryanodine (0–4.7 nM), and was incubated for 2 h at 37 °C. Nonspecific binding was determined in the presence of 10 μM unlabeled ryanodine (Silva et al., 1998).

### 2.6. Sarco(endo)plasmic reticulum and plasma membrane ($Ca^{2+}$–$Mg^{2+}$)ATPase immunoblotting

In these experiments, sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase type 2 was investigated since it is the predominant pump isoform in several smooth muscles (Strehler and

Table 1
Thapsigargin-sensitive (sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase or SERCA) and thapsigargin-resistant (plasma membrane ($Ca^{2+}$–$Mg^{2+}$)ATPase or PMCA) ($Ca^{2+}$–$Mg^{2+}$)ATPase activity and $Ca^{2+}$ uptake for control and denervated rat vas deferens

| | Specific ($Ca^{2+}$–$Mg^{2+}$)ATPase activity (μmol Pi/mg protein/h) | | Specific $Ca^{2+}$ uptake (nmol $Ca^{2+}$/mg protein) | |
|---|---|---|---|---|
| | Control | Denervated | Control | Denervated |
| SERCA | 2.12±0.39 | 1.11±0.31[a] | 5.50±1.69 | 3.96±1.89[a] |
| PMCA | 1.96±0.48 | 2.26±0.64 | 2.56±0.94 | 3.28±1.31 |

Data shown are means±S.E.M. of 5 different preparations. All assays were done in parallel and in triplicate. [a]Significantly different ($P<0.05$) from its respective control (paired Student's *t*-test).

Treiman, 2004) including the rat vas deferens, that contains most probably type 2b (Cunha et al., unpublished results). Samples (40 μg protein/slot) were separated on a 7.5% sodium dodecyl sulfate-polyacrylamide gel and electrotransferred to nitrocellulose filter papers. After incubation for 1 h in 5% non-fat dry milk dissolved in Tris-buffered saline solution containing 0.1% Tween 20, nitrocellulose sheets were washed with the same solution and then incubated for 1 h at room temperature with monoclonal mouse IgG anti-plasma membrane ($Ca^{2+}$–$Mg^{2+}$)ATPase (clone 5F10, Sigma, USA) or anti-sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase type 2 (Calbiochem, USA). Blots were rinsed and further incubated for 1 h with donkey anti-mouse antibodies peroxidase-conjugated (Promega Corporation, USA). Immunoreactivity was detected using enhanced chemiluminescence (ECL, Amersham Biosciences, England) by exposure to hyperfilm-ECL (Amersham Biosciences, England). In these conditions, the signal intensity was proportional to the quantity of protein (data not shown).

### 2.7. Data analysis

Values from equilibrium binding experiments (dissociation constant, $K_d$, and maximal number of binding sites, $B_{max}$) were calculated assuming the existence of one class of specific binding sites in the range of concentrations used, by means of nonlinear regression analysis (Prism, GraphPad Software Inc., USA). Since all experimental procedures were done in parallel, paired Student's *t* test was employed to give the statistical significance of the difference between the means of both groups for plasma membrane and sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$) ATPase activities and [$^3$H]ryanodine binding (Motulsky, 1995).

Protein blot images were captured by Bio-Rad Imaging Densitometer (model GS-700, Bio-Rad Laboratories, USA) and analyzed using the Quantity One imaging system software (Bio-Rad Laboratories, USA). For comparing the amount of each ATPase, densitometric values of bands of denervated subcellular preparation with its respective control (3 or 4 groups) were submitted to paired Student's *t*-test.

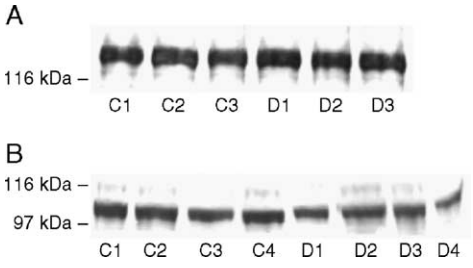


Fig. 1. Western blots of plasma membrane (A) and sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase SERCA2 (B) using specific monoclonal antibodies. The same amount (40 μg protein/lane) of control (C) and denervated (D) rat vas deferens of 3 or 4 distinct preparations was used for comparison. Each number after (C) or (D) indicates a different preparation. The images were analyzed by the Quantity One imaging system software and the corresponding densitometric values presented in Table 2.

## 3. Results

### 3.1. Effect of denervation on sarco(endo)plasmic reticulum and plasma membrane ($Ca^{2+}$–$Mg^{2+}$)ATPase activity and $Ca^{2+}$ uptake

Maximum ($Ca^{2+}$–$Mg^{2+}$)ATPase activity was achieved utilizing saturating concentrations of free $Ca^{2+}$ and $Mg^{2+}$ in our assays. In order to discriminate the presence of sarco(endo) plasmic reticulum and plasma membrane ($Ca^{2+}$–$Mg^{2+}$) ATPases we used a high thapsigargin concentration, which is able to specifically inhibit all the sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase activity in different species (Cunha et al., 1996; Scaramello et al., 2002). Compared to control rat vas deferens, denervation did not significantly alter plasma membrane ($Ca^{2+}$–$Mg^{2+}$)ATPase activity. In contrast, sarco(endo) plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase activity was largely depressed, reaching around 50% of the control activity (Table 1). Likewise, $Ca^{2+}$ accumulation ascribed to sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$) pumps in denervated group was significantly reduced whereas thapsigargin-resistant uptake of $Ca^{2+}$ was unaffected by nerve removal (Table 1).

### 3.2. Effect of denervation on sarco(endo)plasmic reticulum and plasma membrane ($Ca^{2+}$–$Mg^{2+}$)ATPase protein expression

Western blots using a monoclonal antibody specific, but not isoform-selective, for PMCA showed well-defined bands with similar relative optical density (Fig. 1A and Table 2). On the other hand, the amount of sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase protein (detected as type 2 isoform) in denervated organs was 60% lower than in control (Fig. 1B and Table 2). These results match with biochemical data and indicate that the

Table 2
Densitometric values of sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase type 2 (SERCA2) and plasma membrane ($Ca^{2+}$–$Mg^{2+}$)ATPase (PMCA) evaluated in control and denervated rat vas deferens

| | Relative optical density (arbitrary units) | |
|---|---|---|
| | Control | Denervated |
| SERCA2 | 8.03±0.19 | 3.26±0.47[a] |
| PMCA | 9.70±0.90 | 8.20±1.11 |

Data shown are means±S.E.M. of 3–4 different preparations. [a]Significantly different ($P<0.05$) from its respective control (paired Student's *t*-test).

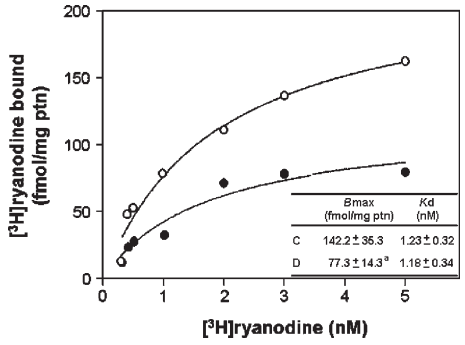


Fig. 2. Typical saturation curves for specific [$^3$H]ryanodine binding in crude membrane preparations of control (○) and denervated (●) rat vas deferens. Each point represents the mean of triplicate determinations. Inset: $B_{max}$ and $K_d$ of control (C) and denervated (D) groups which correspond to means±S.EM. of 9 different preparations. [a]Significantly different ($P<0.05$) from control (paired Student's *t*-test).

partial loss of sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$) ATPase function is due to down-regulation of its cellular expression.

### 3.3. Effect of denervation on specific [$^3$H]ryanodine binding

Saturation experiments with radiolabeled ryanodine generated representative hyperbolic saturation curves with control and denervated rat vas deferens crude preparations (Fig. 2). The binding parameters obtained revealed that affinity ($1/K_d$) was preserved after the denervation procedure; nonetheless, the density of binding sites ($B_{max}$) was reduced to a similar extent as seen for sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase activity, $Ca^{2+}$ uptake and protein blotting in denervated vasa (Fig. 2).

## 4. Discussion

Our present results demonstrate that denervation of the rat vas deferens alters the activity and expression of different proteins involved in intracellular calcium handling. While plasma membrane ($Ca^{2+}$–$Mg^{2+}$)ATPase activity and expression remained constant after sympathectomy, sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase activity and expression and ryanodine receptor density decreased by about 30–50% compared with normal vasa.

The absence of neurotransmission has already been reported to regulate both ryanodine receptor and sarco(endo) plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase activity and mRNA/protein expression, although in skeletal rather than in smooth muscle. For instance, in the case of sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$) pump, slow- and fast-twitch skeletal muscle of rats present both a reduction of their respective isoforms 2a and 1a (Schulte et al., 1994). Enzyme activity is also depressed following 2 weeks of denervation (Brazaluk et al., 1994). The same profile is observed in rabbit muscles (Salvatori et al., 1988; Zorzato et al., 1989; Nozais et al., 1996), even though Hämäläinen and Pette (2001) have evidenced an up-regulation of sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$) ATPase type 1a—paralleled with a down-regulation of type 2a—in rabbit slow-twitch muscle denervated for longer periods of time (72 days). Thus, our findings indicate that the behavior of sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$) ATPase after denervation of the vas is in general similar to that described for other muscle types. On the other hand, data concerning ryanodine receptor expression pattern after denervation seem to be more confusing and is difficult to compare with the present results. In rat slow-twitch skeletal muscle, 10 day surgical denervation decreases (Ray et al., 1995) or does not affect (Tarroni et al., 1997) ryanodine receptor mRNA levels, whereas long-term denervation (25 and 50 days) produces an elevation in expression (Péréon et al., 1997). Interestingly, when the nerve was present but its function was blocked by tetrodotoxin, transcriptional activity and protein expression of ryanodine receptors were up-regulated, suggesting the importance of spontaneously released nerve-derived factors (Ray et al., 1995). Neither ryanodine receptor mRNA (Péréon et al., 1997) nor protein (Tarroni et al., 1997) and ryanodine binding density (Delbono and Chu, 1995) from rat fast-twitch skeletal muscle were affected by lack of innervation, even though an increment in ryanodine binding sites were seen in rabbit muscle (Zorzato et al., 1989).

In relation to the present experiments, one could argue that sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase or ryanodine receptor reduction might be due to their removal together with the nerve terminals. This seems not to be the case because, as shown by electron microscopy analysis, the nerve mass in the rat vas deferens is much smaller than the muscle mass (about 0.5%, Westfall et al., 1975). Moreover, we have previously reported the absence of $Na^+/K^+$-ATPase $\alpha_3$ isoform, considered a neuronal marker (Cameron et al., 1994), in similar preparations (Quintas et al., 2000). Finally, the amount of PMCA—and $Na^+/K^+$-ATPase $\alpha_1$ subunit isoform (previously reported in Quintas et al., 2000)—as well as its activity did not change after denervation. These facts would also imply that adaptation is specific for some but not all proteins involved in homeostatic calcium regulation in such smooth muscle.

Besides the changes discussed above, a number of biochemical and pharmacological alterations are also known to occur in vas deferens after denervation (Kasuya et al., 1969; Jurkiewicz et al., 1977; Goto et al., 1978; Fleming and Westfall, 1988; Jurkiewicz et al., 1991,1992). It is well established that removal of hypogastric nerve triggers in the vas deferens an adaptive phenomenon characterized by an increased effect of diverse unrelated agonists (Kasuya et al., 1969; Fleming and Westfall, 1988) and the occurrence of spontaneous contractions which are correlated with supersensitivity (Lee et al., 1975). In relation to the mechanisms underlying supersensitivity, in guinea pig vas deferens this process has been associated to a partial membrane depolarization (Goto et al., 1978) due to a specific down-regulation of $Na^+/K^+$-ATPase $\alpha_2$ subunit isoform (Hershman et al., 1993, 1995). For the rat vas deferens, however, the mechanism(s) is still unclear. Goto et al. (1978) did not detect any variation of the resting membrane potential and, though we have demonstrated a 40% reduction of the same isoform ($\alpha_2$) in denervated rat vas (Quintas et al.,

2000), this indeed does not seem to result in supersensitivity (Quintas et al., 2002). The hypothesis of a change in $\alpha_1$-adrenoceptor for explaining supersensitivity has probably to be discarded since no alteration of density or affinity was observed after surgical or chemical sympathectomy (Abel et al., 1985; Nasseri et al., 1985). However, we have to be cautious in this respect since a change in receptor subtype ($\alpha_{1A}$ to $\alpha_{1D}$) is controversial (Pupo et al., 1997; Campos et al., 2003; Cleary et al., 2004). Furthermore, noradrenaline-stimulated inositol phosphate and diacylglycerol production was shown not to be enhanced by denervation as well as protein kinase C-induced contraction by phorbol ester (Minneman et al., 1988; Abraham et al., 2003).

In face of the lack of a comprehensive explanation for the mechanisms related to supersensitivity and rhythmic effects, modifications of density or activity of proteins (pumps or channels) involved in intracellular calcium handling might account for a possible mechanistic explanation of the phenomenon. In fact, pharmacological blockade of ryanodine receptors and sarco(endo)plasmic reticulum ($Ca^{2+}$–$Mg^{2+}$)ATPase interferes in rat vas deferens contraction, specially in the phasic response (Burt et al., 1998; Vesperinas et al., 1989). Moreover, previous reports showed much lower levels of L-type $Ca^{2+}$-channels in denervated rat vas deferens (Jurkiewicz et al., 1994; Quintas et al., 2002). Recently, Abraham et al. (2003) have reported that denervated vasa are more resistant to nifedipine inhibitory effect on noradrenaline contractile response when compared to controls, denoting a lesser dependency on external calcium entry. One of the hypotheses they postulated, and that is in agreement with our results, is that calcium sensitization of contractile proteins—a condition that promotes a given contraction using less free cytosolic calcium concentration—may be related to adaptive supersensitivity in this species. Such adjustment, whose importance has been demonstrated in guinea pig vas deferens (Ramos et al., 1986), deserves further investigation in the denervated rat organ.

In conclusion, we have here shown that an important entity for calcium translocation, namely sarco(endo)plasmic reticulum but not plasma membrane ($Ca^{2+}$–$Mg^{2+}$)ATPase had its expression and activity strikingly decreased, leading to a reduction of calcium uptake. This result, together with the additional finding that ryanodine receptor density was reduced by about 45%, and to the previous demonstration that the activity or density of $Na^+/K^+$-ATPase $\alpha_2$ isoform and L-type $Ca^{2+}$ channel are also decreased (Jurkiewicz et al., 1994; Quintas et al., 2000; Quintas et al., 2002), indicates that calcium movements may be remarkably altered after denervation of the vas deferens. Taken together, and considering that calcium is an agent sine-qua-non for drug-induced contraction, these results might help to clarify the mechanism by which the changes on calcium translocation and the changes previously described for contraction are interconnected after denervation.

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