# Differential Effect of Articaine on Sarcoendoplasmic Reticulum Calcium Adenosine Triphosphatase of Medial Pterygoid Muscle

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**Aims:** To determine the effect of articaine on sarcoendoplasmic reticulum calcium adenosine triphosphatase (SERCA) isoforms of the medial pterygoid muscle. **Methods:** Native SERCA from the medial pterygoid muscles of 24 rabbits was isolated by ultracentrifugation, and its isoforms were purified by chromatography and assessed by enzyme-linked immunosorbent assay (ELISA). SERCA activity and calcium transport capability were determined by using colorimetric and radioisotopic methods. The mean ± standard deviation (SD) half maximal inhibitory concentration (IC50) of articaine was determined for each isoform, and these values were compared by using analysis of variance (ANOVA) ($P < .05$). **Results:** The native SERCA preparation consisted of 34% SERCA1a, 53% SERCA2a, 10% SERCA2b, and 3% combined SERCA3 and SERCA1b. Articaine caused inhibition of activity and calcium uptake in the native SERCA preparation and in each of the purified isoforms. The IC50 (mM) values for enzymatic activity were: SERCA1a 22.0 ± 2.3 > SERCA2a 16.4 ± 2.4 > SERCA2b 11.3 ± 1.9, and 15.1 ± 2.1 for native SERCA. For calcium transport, IC50 values were: SERCA1a 31.1 ± 3.3 > SERCA2a 24.8 ± 1.8 > SERCA2b 21.5 ± 1.5, and 25.2 ± 3.2 for native SERCA. IC50 values for inhibition of enzymatic activity were significantly different among the purified isoforms, but only the value obtained for SERCA1a was significantly different compared to native SERCA. For inhibition of calcium transport, IC50 values for both SERCA2a and SERCA2b differed significantly compared to SERCA1a, and the value for SERCA1a was significantly different compared to native SERCA. The most articaine-sensitive isoform was SERCA2b, and the native preparation showed sensitivity similar to SERCA2a. **Conclusion:** The differential inhibition of articaine on medial pterygoid SERCA isoforms is evident at concentrations lower than used in current dental practice (125 mM) and accounts for anesthetic myotoxicity. Muscle relaxation likely becomes impaired as a result of increased calcium levels in the myoplasm due to the decreased activity and calcium transport caused by the inhibition of SERCA. *J Oral Facial Pain Headache 2017;31:e21–e28. doi: 10.11607/ofph.1835*

**Keywords:** *articaine, local anesthetics, masticatory muscles, medial pterygoid muscle, SERCA*

The muscles of mastication have a heterogenous composition of fast and slow fibers.[1,2] These muscles play a remarkable role in several functions of the stomatognathic system, including mastication, deglutition, phonation, and positioning of the mandible.[3,4] One differential characteristic of the masticatory muscles is their insertion on the mandible, which allows them to exert a direct action on bone. From a functional viewpoint, they can be classified into main and accessory muscles of mastication and include mandibular elevator, depressor, and stabilizing muscles. The medial pterygoid belongs to the group of elevator muscles of the mandible and is considered a powerful and key muscle of mastication in adults.[2,5] Besides elevating the mandible, this muscle is also active during protrusive movements and lateral positioning of the jaw. Also noteworthy is that the pterygomandibular space, where local anesthetics are injected to effectively block the inferior alveolar nerve, is bounded medially by the medial pterygoid muscle.[6]

Sanchez et al[7] reported the characterization of the functional properties of sarcoendoplasmic reticulum calcium adenosine phosphatase (SERCA) in the medial pterygoid. SERCA is an intrinsic membrane protein capable of actively transporting calcium from the muscle cell cytoplasm to the lumen of the sarcoplasmic reticulum (SR), allowing for muscle relaxation.[8,9] The SERCA acronym applies to a family of structurally related enzymes, and each member of this family has a distinctive pattern of tissue expression.[8] SERCA1 is expressed only in fast-twitch skeletal muscle and has two subfamilies known as SERCA1a and SERCA1b.[10] SERCA1a is expressed in adult fast-twitch muscle, and SERCA1b is found in neonatal fast-twitch muscle. SERCA2 is expressed in adult slow-twitch skeletal muscle and in cardiac muscle tissue[11,12] and also has two subfamilies: SERCA2a and SERCA2b. While SERCA2a is mainly expressed in cardiac or slow-twitch skeletal muscle, SERCA 2b is found in either smooth muscle or nonmuscle tissues. Another known member of the SERCA family is SERCA3, which is mainly expressed in nonmuscle tissues.[13] An essential characteristic of these SERCA isoforms is that their transmembrane topologies and tertiary structures are alike. In addition, functional assays have provided evidence for the adenosine triphosphate (ATP)–dependent calcium transport capability of each isoform and for the inhibition of this capability by thapsigargin, a potent SERCA inhibitor.[14] In their comprehensive description of SERCA expression in the muscles of mastication, Sánchez et al[15] described the combined expression of SERCA2a, SERCA1a, and SERCA2b in the medial pterygoid, with SERCA2a being predominant, which contrasts with the absolute predominance of SERCA1a in fast-twitch skeletal muscle.

Local anesthetics are intrinsically myotoxic drugs in clinically used concentrations.[16,17] They are also myotoxic when released from a wide variety of drug-release systems, even though the release systems themselves are minimally toxic.[18,19] Detailed mechanisms of the toxic effects on muscle tissues are unknown, but an increase in intracellular calcium levels is suspected to be the most important trigger. In vitro dantrolene attenuates the myotoxicity of local anesthetics, presumably by modifying sarcoplasmic calcium release.[20] Neurotoxic effects have also been described for local anesthetics[21]: Lipid protein particles containing local anesthetics increase in vivo myotoxicity[22] and may cause an inflammatory response in nerves that lasts longer than the nerve blockade.[16,17] It is known that local anesthetics used in dentistry are able to inhibit SERCA in fast-twitch skeletal muscle.[23–26] Previous research reports have given account of the inhibitory effects of amide-type local anesthetics on calcium (Ca)-ATPase activity and transport in the masticatory muscles, including the masseter, medial pterygoid,[27] and temporalis[28] muscles.

Articaine is a commonly used local anesthetic in clinical dentistry.[29,30] Local infiltration or topical administration of articaine has proved to be suitable for dental procedures requiring anesthesia with a fast onset and a short to intermediate duration of action. In this context, buccal infiltration of articaine is used to enhance the effectiveness of lidocaine-produced inferior alveolar nerve block.[31] Previous studies have demonstrated the inhibitory properties of articaine on SERCA enzymatic activity and calcium transport capability in both fast-twitch muscles[25,26] and the medial pterygoid,[32] although it is known that the latter is not composed mainly of fast-twitch fibers.[33,34] Half maximal 50% inhibitory concentration (IC50) values of articaine reported so far for masticatory muscles have been generally lower, revealing higher sensitivity to articaine compared to fast-twitch muscles. Bearing this background in mind, it was hypothesized that those lower IC50 values are caused by the combined expression of SERCA isoforms, each of which has a different sensitivity to the drug. Therefore, this work was aimed at determining the effect of articaine on SERCA isoforms of the medial pterygoid muscle. By providing evidence for the inhibition of the SERCA isoforms and consequently assuming an increase in myoplasmic calcium levels, this study will contribute to the elucidation of the mechanism through which local anesthetics exert myotoxic effects.

## Materials and Methods

### Ethical Approval

This research was conducted as recommended by the guidelines set by the National Institute of Health (USA) and by national laws regarding the use of laboratory animals. The protocol was reviewed and approved by the Ethics Commission of the School of Dentistry of the University of Buenos Aires.

### SERCA Membrane Isolation

The medial pterygoid muscles were dissected from 24 male New Zealand rabbits (6 months old, 2 kg). The sampled muscles were homogenized using a tissue homogenizer and later subjected to ultracentrifugation as described by Champeil et al.[35] This procedure yields native SR membranes as sealed vesicles with calcium accumulation capability and ATPase enzymatic activity.

### Drugs and Reagents

Analytical grade reagents were used in this investigation. Disodium ATP, calcium ionophore A23187, bovine

seroalbumin, MOPS (3-[n-morpholino]propanesulfonic acid), Tris (Tris[hydroximethyl]aminomethane), CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate), and reactive dye-agarose were from Sigma Chemical. $^{45}Ca$ (calcium chloride [$CaCl_2$]) was from New England Nuclear. Immunoglobulin G (IgG) anti-SERCA1, -2, and -3 antibodies were from Santa Cruz Biotechnology. Articaine chlorhydrate was kindly supplied by Septodont.

### Purification of SERCA on Reactive Dye-Agarose Columns

Purified SR membrane fragments rich in SERCA isoforms were later separated by using reactive dye-agarose affinity chromatography.[36] Columns were prepared by equilibration of 0.2 g reactive dye-agarose with 0.25 M sucrose, 100 mM potassium chloride (KCl), 2 mM sodium azide, 5 mM magnesium chloride ($MgCl_2$), and 100 mM imidazole hydrochloride (pH 6.8) containing 0.01% v/v CHAPS buffer. A 0.5-mL solution of solubilized native SR membranes (1 to 3 mg/mL) was applied to the column, which was then sealed and incubated at 4°C for 18 hours. The column was then eluted with CHAPS buffer, the first two 1-mL fractions were collected, and a further 5-mL volume was discarded. Elution was continued with CHAPS buffer containing 5 mM ATP, and 1-mL fractions were collected. Fractions were later assayed for Ca-ATPase activity as described below. Each purified SERCA isoform was checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)[37] assuming molecular weights of 110 KDa, 100 KDa, and 97 KDa for SERCA1, -2, and -3, respectively, and by enzyme-linked immunosorbent assay (ELISA) using anti-SERCA1, -2, and -3 antibodies. The protein concentration was estimated by the colorimetric procedure described by Lowry et al[38] by using bovine seroalbumin as standard.

### Identification of SERCA Isoforms

Sandwich ELISA was used for quantifying the SERCA1, -2, and -3 antigens in the native and purified SR membrane preparations as described by Leberer and Pette.[39] Samples of SR membrane fractions were incubated in buffer solution containing phosphate-buffered saline (PBS)–Tween added with 1% (w/v) milk powder, 10% (v/v) glycerol, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, and 1 mM phenylmethanesulfonylfluoride and Trasylol (100 kiu/mL). They were later introduced into wells of microtiter plates previously coated with anti-SERCA1, -2, and -3 IgG (10 mg/mL) and incubated for 2 hours at room temperature. The incubation was followed by three rinses with PBS-Tween and a further incubation for 3 hours at room temperature. After this period, samples were again rinsed three times with PBS-Tween and once with 0.01 M citrate buffer (pH 5.0). Blank solutions consisted of samples introduced into wells coated with control IgG and subjected to the procedure described above. Staining was carried out with a substrate solution containing 55 mg of phenylene-1,2-diamine dihydrochloride and 0.03 mL 30% hydrogen peroxide ($H_20_2$) in 100 mL of 0.1 M citrate buffer (pH 5.0) for 30 minutes at 37°C. The staining reaction was stopped by the addition of 0.05 mL of 2 M sulfuric acid ($H_2SO_4$). The optical density of each well was determined at 450 nm within 30 minutes by using a microplate reader (Metrolab 950). Samples and blanks were run simultaneously, and blanks were subtracted from the sample values.

### Determination of SERCA Activity

SR membranes (1 mg/mL) were subjected to incubation at 37°C in media containing 50 mM MOPS-TRIS buffer (pH 7.2), 3 mM ATP, 100 mM potassium chloride (KCl), 3 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.1 mM egtazic acid (EGTA), 10 µM calcimycin (calcium ionophore A 23187), and articaine in concentrations ranging from 0 to 90 mM. The addition of SR membranes to the described incubation medium started the reactions. After 2 minutes, the reactions were stopped by using cold trichloroacetic acid (at a final concentration of 5%). The media were then centrifuged at 3,600 rpm for 5 minutes to separate the denatured protein, and the concentration of inorganic phosphate (Pi) was determined in the supernatants as described by Baginski et al.[40] The Pi production during the reaction time was considered indicative of the SERCA activity. Blanks without SR membranes were run in parallel and subtracted from the experimental values. The results obtained with articaine were representative of six independent experiments performed in duplicate and simultaneously assayed for each SERCA isoform.

### Determination of SERCA Calcium Uptake

A radioisotopic method was used to determine SERCA calcium uptake. The procedure involved the incubation of SR membranes (0.1 mg/mL) at 37°C in media containing 0.1 mM $^{45}Ca$ ($CaCl_2$) (450 cpm/nmol), 0.1 mM EGTA, 3 mM ATP, 3 mM $MgCl_2$, 50 mM MOPS-TRIS buffer (pH 7.2), and articaine at concentrations ranging from 0 to 90 mM. The reactions were started by adding SR membranes to the described medium and were stopped after 30 seconds by filtration through filters of 0.45-µm pore size (Millipore filters) by the use of a vacuum pump. The filters were exhaustively rinsed with cold and freshly prepared 3 mM lanthanum(III) chloride ($LaCl_3$) solution, and the radioactivity retained on the filters was measured by liquid scintillation counting with a Beckman LS 6500 counter. ATP was not added to

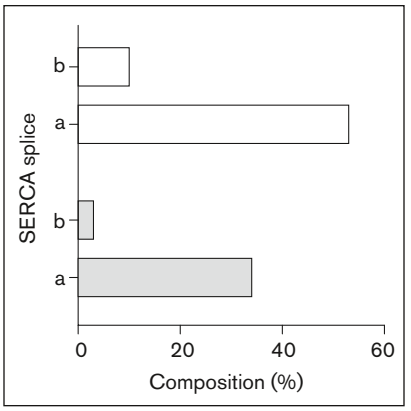


**Fig 1** Expression of SERCA1 and SERCA2 in medial pterygoid muscle: percent composition of the different SERCA splices identified in a typical SR membrane preparation by ultracentrifugation. SERCA isoforms were identified through ELISA by using anti-SERCA1 and -2 antibodies. Empty bars correspond to SERCA 2, and filled bars to SERCA1.

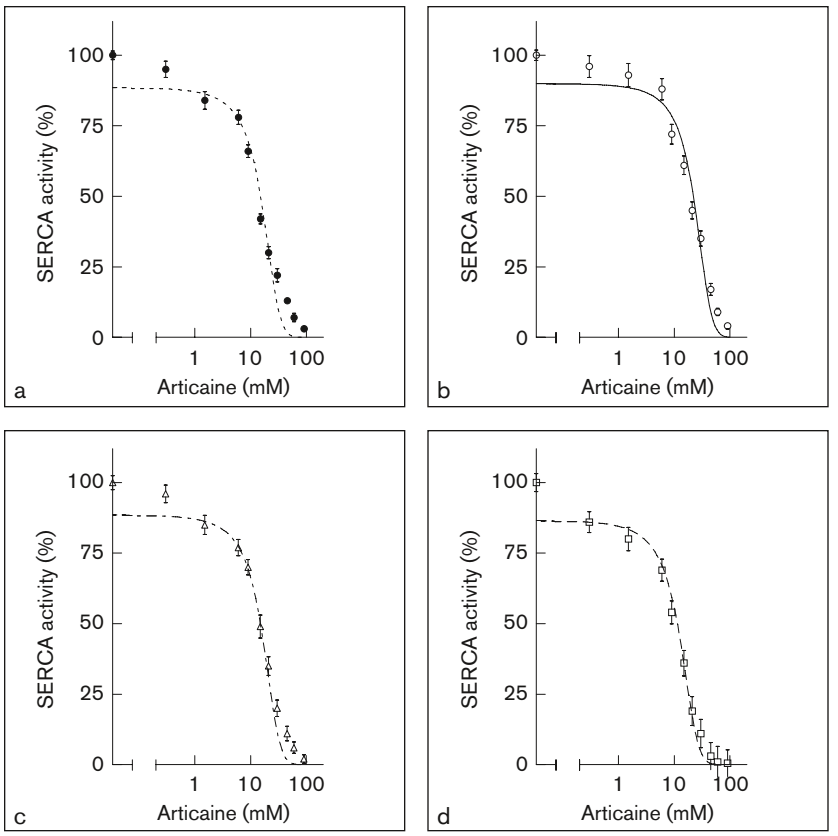


**Fig 2** Inhibitory effect of articaine on SERCA activity. SERCA membranes (0.1 mg/mL) were incubated with varying concentrations of articaine, and ATPase activity was measured spectrophotometrically. Results are expressed as mean ± standard deviation (SD) percent of maximal released µmol Pi mg protein$^{-1}$ h$^{-1}$ and taken as an index of enzymatic activity. Data were fitted with a single sigmoidal curve for **(a)** native SERCA, **(b)** SERCA1a, **(c)** SERCA2a, and **(d)** SERCA2b. Six tests were performed for each preparation.

the incubation medium for blanks. Samples and blanks were simultaneously run, and values of the blanks were subtracted from the experimental sample values. The assessment of the action of articaine was performed in six independent duplicated experiments performed at the same time for each SERCA isoform.

### Data Presentation and Statistical Analyses

Data analyses were comprised of the calculation of the mean and standard deviation (SD) values for activity and uptake. Differences among means were assessed with analysis of variance (ANOVA) and followed by Tukey's multiple comparisons test by using GRAPHPAD Prism 5.03 for Windows (GraphPad Software). The significance level used was $P < .05$.

## Results

Figure 1 shows the percent distribution of SERCA family expression assessed by ELISA in the medial pterygoid. SERCA1a was 34%, SERCA2a was 53%, SERCA2b was 10%, and other isoforms (SERCA1b together with SERCA3) amounted to 3%.

The maximum SERCA enzymatic activity under the experimental conditions without articaine was 250 µmol Pi mg protein$^{-1}$ h$^{-1}$. SERCA activity was inhibited by articaine in a dose-dependent manner. The plot showing SERCA enzymatic activity vs increasing articaine concentrations shown in Fig 2 reveals a sigmoidal dose-response profile with a negative slope. The fit of the data to that profile revealed an IC50 of 15.1 ± 2.1 mM for native SERCA preparation, 22.0 ± 2.3 mM for SERCA1a, 16.4 ± 2.4 mM for SERCA2a, and 11.3 ± 1.9 mM for SERCA2b. SERCA activity was fully inhibited at concentrations close to 90 mM.

The study of the action of articaine on calcium transport in SR membranes from the medial pterygoid was performed by determining the SERCA calcium uptake capability of the native SERCA preparation and the purified SERCA isoforms at increasing concentrations of the drug (Fig 3). The maximum value

of calcium uptake was recorded without articaine and corresponded to 28 nmol Ca mg protein$^{-1}$. Articaine inhibition of calcium uptake was enhanced with increases in the drug concentration, showing a sigmoidal dose-response curve with a negative slope and IC50 values of 25.2 ± 3.2 mM for native SERCA preparation, 31.1 ± 3.3 mM for SERCA1a, 24.8 ± 1.8 mM for SERCA2a, and 21.5 ± 1.5 for SERCA2b. In all cases, the articaine concentration required for the full inhibition of SERCA calcium uptake was around 90 mM.

Figure 4 shows the mean articaine IC50 values for inhibition of the enzymatic activity obtained for each SERCA isoform and the native SERCA preparation. Differences in mean values were statistically significant (ANOVA: $P < .0001$). The IC50 values followed the decreasing order: SERCA1a > SERCA2a > native SERCA > SERCA2b. Tukey test revealed significantly lower IC50 values for native SERCA compared to SERCA1a, for SERCA2a and SERCA 2b compared to SERCA1a, and for SERCA2b compared to SERCA2a. No significant differences in mean IC50 were observed between native SERCA and SERCA2a or SERCA2b. These findings indicate different articaine sensitivities for each SERCA isoform. SERCA2b was the most sensitive isoform, whereas SERCA1a was the least.

Figure 5 shows the differential inhibition of articaine on SERCA calcium transport. The articaine IC50 values obtained for the different SERCA isoforms and the native SERCA preparation are presented. The decreasing order of IC50 values was: SERCA1a > SERCA2a > native SERCA > SERCA2b. ANOVA revealed significant differences among mean IC50 values ($P = .0002$). Tukey test revealed significantly lower IC50 values for native SERCA,

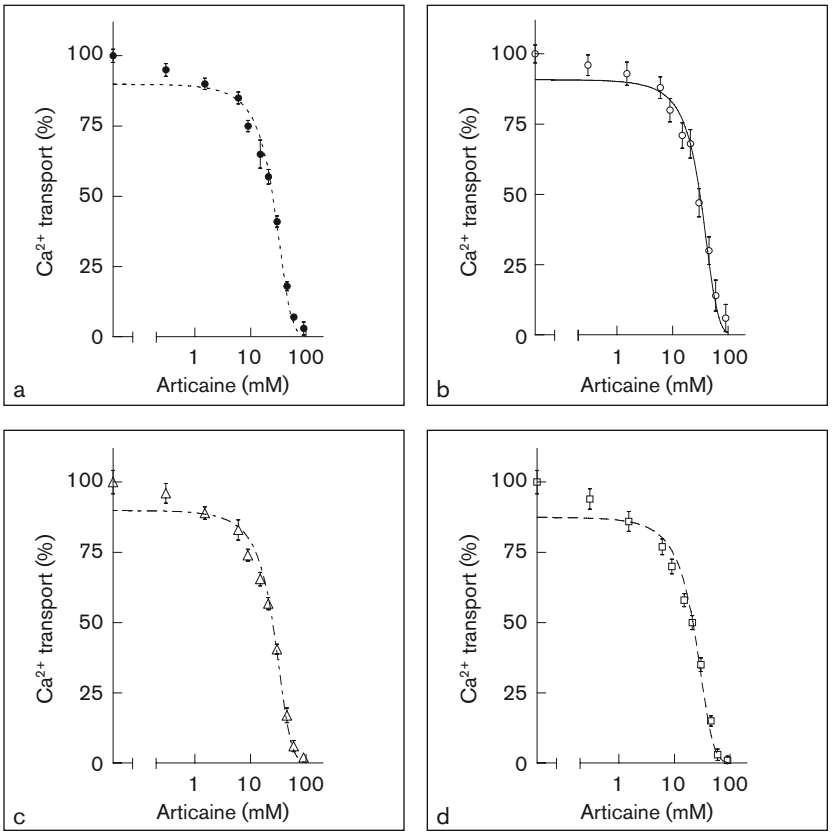


**Fig 3** Inhibitory effect of articaine on SERCA calcium transport. SR membranes (0.1 mg/mL) were incubated with varying concentrations of articaine, and calcium transport was determined as $^{45}$Ca uptaken by SR sealed vesicles. Results are expressed as percentage of maximal uptaken µmol Ca mg protein$^{-1}$. Data were fitted with a single sigmoidal curve for **(a)** native SERCA, **(b)** SERCA1a, **(c)** SERCA2a, and **(d)** SERCA2b. Six tests were performed for each preparation.

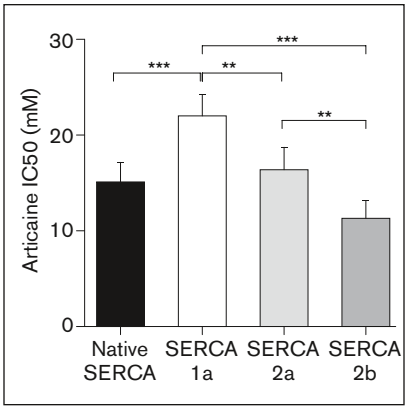


**Fig 4** Differential inhibition of articaine on SERCA activity. Mean half maximal inhibitory concentration (IC50) values obtained in the presence of articaine for the SERCA isoforms and native preparation of SR membranes isolated from the medial pterygoid muscle. Error bars show standard deviation. Capped lines show significantly different comparisons. $^{**}P < .001$, $^{***}P < .0001$.

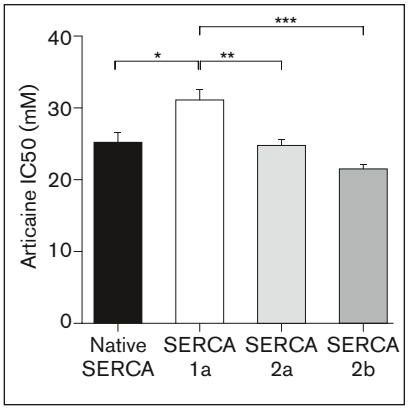


**Fig 5** Differential inhibition of articaine on SERCA calcium transport. Mean half maximal inhibitory concentration (IC50) values obtained in the presence of articaine for the SERCA isoforms and the native preparation of SR membranes isolated from the medial pterygoid muscle. Error bars show standard deviation. Capped lines show significantly different comparisons. $^{*}P < .01$, $^{**}P < .001$, $^{***}P < .0001$.

SERCA2a, and SERCA2b compared to SERCA1a. No significant differences were found between native SERCA and SERCA2a or SERCA2b. The comparison of mean IC50 values between SERCA2a and SERCA2b did not reach statistical significance. Therefore, the most sensitive isoform to articaine was SERCA2b, the least sensitive was SERCA1a, and the native SERCA preparation was as sensitive as SERCA2a and SERCA2b.

## Discussion

The inhibition of native SERCA by articaine in the muscles of mastication is in line with previous findings,[27,28,32] but the present investigation is the first to deal with the action of articaine on SERCA isoforms and used a previously unexplored approach to studying the differential effect of articaine on each SERCA isoform present in the medial pterygoid muscle. It is also noteworthy that the electrophoresis analysis (data not shown) revealed high SERCA protein content (> 90%) within the range of 100 to 110 KDa, similar to that previously reported by Sanchez et al.[15]

Three significant findings resulted from this study: *(1)* The extent of the inhibition caused by articaine on medial pterygoid SERCA depends on the relative presence of each known isoform; *(2)* the SERCA isoforms found in the medial pterygoid show different affinities for articaine; and *(3)* the articaine IC50 values obtained in nonpurified native SERCA membrane preparations are similar to those corresponding to the most abundant isoform.

Ester- and amide-type local anesthetics, including articaine, have been demonstrated to inhibit SERCA in both fast-twitch skeletal muscles[25,26,41–43] and masticatory muscles.[27,28,32] The IC50 values for lidocaine, bupivacaine, tetracaine, procaine, benzocaine, and articaine were significantly lower for SERCA isolated from the masticatory muscles compared to that isolated from fast-twitch skeletal muscles, indicating the local anesthetics had more effect on masticatory muscles. In previous reports,[7,27] it was hypothesized that the existence of an intrinsic SERCA isoform in masticatory muscles would account for this fact. Later, the report that SERCA expression in these muscles was a combined expression of SERCA isoforms rather than a ubiquitous isoform[15] allowed that hypothesis to be discarded, but the matter remained unclear. It was then alternatively hypothesized that the simultaneous expression of different isoforms in the masticatory muscles could explain the lower IC50 values compared to those reported for fast-twitch muscles. The present results confirm the combined presence of SERCA isoforms in the medial pterygoid muscle and provide evidence for the differential inhibition of articaine on the combination of the three present isoforms (SERCA1, -2, and -3), with different inhibitory potencies on each. The inhibition of SERCA transport activity by articaine implies the impediment of the active accumulation of calcium ions in the inner part of the SR. The resultant increase in the calcium concentration in the myoplasm triggers the contraction of muscle fibers and prevents their relaxation. Under these conditions, the most likely event is sustained contraction of the muscle. The present study provides evidence that SERCA inhibition could partly explain this event, which increases knowledge of the toxic effects of articaine on muscle tissues, a matter currently relevant to the clinical pharmacology of local anesthetics in general.[44–46] With regard to the clinical translation of the results of the present study, the critical reasoning proposed by Sánchez et al[27] could be useful. If the IC50 values for the inhibition of SERCA activity and calcium transport reported in the present study are compared to the usual concentration of articaine in cartridges for dental use (4%; 125 mM), it is clear that articaine inhibition of SERCA occurs at lower concentrations. As previously discussed by Sánchez et al,[32] it can be assumed that toxic effects on muscle fibers are likely to occur, for instance, following the unintended injection of articaine solution into the medial pterygoid during the supplementary buccal infiltration to enhance the effectiveness of lidocaine-produced inferior alveolar nerve block or during the infiltration of the temporal region with local anesthetics, a not-uncommon clinical treatment aimed at relieving the acute pain symptoms of trigeminal neuralgia.[47] In the latter case, the myotoxic effect would be on the temporalis muscle.

It is noteworthy that the articaine IC50 value for the combined expression of SERCA corresponds to that for the most abundant isoform (SERCA2). The present results show that in native SERCA preparations, articaine IC50 is similar to that for SERCA2, but the IC50 for SERCA1a is similar to that reported for fast-twitch muscle,[25,26] where SERCA1a is exclusively expressed. This implies that the affinity of a preparation of combined SERCA isoforms is determined by the relative content of the isoforms; eg, the higher the SERCA1 expression, the lower the affinity of the SERCA membrane preparation to articaine. This could be of interest in physiopathologic conditions where expression of SERCA is modified; for instance, in hyperthyroidism, there is a simultaneous increase of SERCA1 and decrease of SERCA2 expression, and in hypothyroidism, this situation is reversed.[48] Thus, it would be expected that SR membranes from hypothyroid animals would show higher articaine sensitivities because of higher SERCA2b expression.

The different affinities to articaine of SERCA isoforms must be addressed. Although structurally similar, amino acid sequences of SERCA isoforms are somewhat different. Perhaps differences in the positions of amino acids within the SERCA sequence could result in sites with higher or lower affinity for local anesthetics, but this should be further investigated. Preliminary experiments on this topic are being performed in the authors' laboratory.

With regard to the generalizability of the present results, it should be borne in mind that the results were obtained using male rabbits only. It is known that progesterone increases the expression of SERCA2a in female rodents[49]; therefore, depending on the blood level of progesterone, the proportions of SERCA isoforms in female rabbits are likely to vary from the ones reported here. Likewise, the enhancing effect of progesterone on SERCA2a activity is likely to imply a different inhibition pattern of articaine on the enzyme, which should be further studied.

## Conclusions

The differential inhibition of articaine on SERCA isoforms of the medial pterygoid muscle is evident at concentrations lower than those used in clinical dentistry. The inhibitory action accounts for the toxicity of articaine on muscle tissues, since the inhibition of SERCA likely induces impairment of muscle relaxation as a result of the intracellular calcium increase that occurs as a consequence of decreased activity and calcium transport of the enzyme. Inhibitory effects of a similar extent could be suspected for other local anesthetics in other masticatory muscles and could possibly be more significantly myotoxic in physiopathologic conditions of increased SERCA2b expression, such as hypothyroidism.

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