Adding Dexmedetomidine to Articaine Increases the Latency of Thermal Antinociception in Rats

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Articaine is a low-toxicity local anesthetic that is widely used in dentistry. Typically, epinephrine is added to prolong the duration of articaine local anesthesia; however, epinephrine exhibits adverse effects. Low-dose dexmedetomidine (DEX), an α_2-adrenoreceptor agonist, reportedly prolongs local anesthesia without notable adverse cardiovascular effects. The purpose of this study was to assess whether a combination of low-dose DEX and articaine would provide a low-toxicity local anesthetic option for dental procedures without adverse cardiovascular effects. Thus, this study investigated whether DEX could prolong the local anesthetic effect of articaine using a rat model of pain. Adult male Wistar rats ($N = 44$; 11 per group) received a 50-μL subcutaneous injection into the plantar surface of the hind paws; injections were composed of either normal saline, 4% articaine (2 mg articaine), combined 5 μg/kg DEX and 4% articaine (1.25 μg DEX + 2 mg articaine), or combined epinephrine (1:100,000) and 4% articaine (0.9 μg epinephrine + 2 mg articaine). Subsequent acute pain perception was determined by paw withdrawal movement in response to infrared radiant heat stimulation of the plantar region. Paw withdrawal latency was tested at 5-minute intervals. Paw withdrawal latency values at 35 and 40 minutes were 3.83 ± 1.76 and 3.29 ± 1.43 seconds for articaine alone, 7.89 ± 2.72 and 7.25 ± 3.37 seconds for DEX and articaine, and 8.95 ± 2.28 and 8.17 ± 3.01 seconds for epinephrine and articaine. DEX prolonged the paw withdrawal latency of articaine for up to 35 minutes ($p = .015$) but not 40 minutes after injection ($p = .052$) when compared to articaine alone. The combination of DEX and articaine can provide effective local anesthesia for up to 35 minutes after injection.

Key Words: Dexmedetomidine; Articaine; Epinephrine; Nociception.

For safe treatment of dental patients, a dental local anesthetic is required that exhibits low cardiac activity and low toxicity. Articaine is a widely used dental local anesthetic agent that differs from the other amide agents because of the presence of a thiophene ring and an ester group. It is hydrolyzed by plasma esterase and is biotransformed by hepatic microsomal enzymes, resulting in a short plasma half-life of approximately 20–30 minutes.[1] However, articaine has a plasma protein–binding capacity of approximately 95%, which is higher than that of lidocaine (65%).[1] Additionally, articaine has a dissociation constant of 7.8. Therefore, articaine constitutes an intermediate-potency, short-acting local anesthetic with a rapid onset of action. Its rapid metabolic characteristics and high plasma protein–binding capacity result in low toxicity; therefore, it can be used at a high dose (4%) as a dental local anesthetic.

In general, vasoconstrictors (eg, epinephrine) are used to prolong the duration and increase the efficacy of dental local anesthetics. Notably, articaine is commonly available in combination with either 1:100,000 or 1:200,000 epinephrine concentration. Because articaine exhibits lower cardio-depressant and vasodilator activity than lidocaine,[2] articaine can be combined with lower dosages of epinephrine than those used with lidocaine. However, the quantity of epinephrine used in most dental local anesthetics causes a transient minimal elevation in blood pressure as well as modest tachycardia.

Dexmedetomidine (DEX), a selective α_2-adrenoreceptor agonist, has been reported to increase the duration of analgesia in a dose-dependent manner when used with a local anesthetic.[3–6] In particular, DEX has been shown to enhance the potency of a local anesthetic (eg, lidocaine) without significant systemic effects following local injection into oral mucosa.[7] The exact mechanism by which DEX prolongs local anesthetic duration has

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not been fully clarified. However, DEX has a direct blockade effect on tetrodotoxin-resistant sodium (Na^+) channels.[8] Additionally, DEX inhibits the compound action potential.[9]

Consequently, it was theorized that a combination of DEX and articaine might comprise a low-toxicity dental local anesthetic without adverse cardiovascular effects when compared with a combination of epinephrine and articaine. Therefore, this study investigated whether a combination of DEX and articaine could prolong the duration of sensory blockade when directly compared to the effect of articaine alone and to the effect of a combination of epinephrine and articaine, using a rat model of pain.

MATERIALS AND METHODS

All experimental procedures and protocols used in the present study were approved by the Animal Care and Use Committee of the Nippon Dental University (approval number 17-21). All procedures conformed to the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experiments were performed using adult male Wistar rats ($N = 44$). The rats were acclimated in an animal room with water and food ad libitum for 1 week before experiments began. All rats weighed approximately 250 g (±20 g).

Articaine hydrochloride and DEX hydrochloride were purchased from Sigma-Aldrich (St Louis, Mo). Articaine was reconstituted using normal saline (NS) to prepare a 4% articaine solution (A). The final concentration of the A solution contained 2 mg articaine in each 50-μL aliquot. The DEX and articaine (DA) solution was prepared using a DEX concentration of 25 μg/mL that was added to the reconstituted A solution to facilitate a weight-based dosing of 5 μg/kg of DEX. Therefore, the final concentration of the DA solution contained 1.25 μg DEX and 2 mg articaine in each 50-μL aliquot. A solution of 4% articaine and 1:100,000 epinephrine (EA) was purchased from Septodont (Saint-Maur-des-Fossés, France). This commercially available solution contained 0.9 μg epinephrine and 2 mg articaine in each 50-μL aliquot. The pH of the A solution (4.54 ± 0.03) was used as a reference to which all other solutions were standardized.

In order to measure the local anesthetic effect, the thermal nociceptive block assessment protocol reported by Tsutsui et al[10] was implemented. First, a rat was placed in a wire net container, which was placed on a heated glass base. The hind paw plantar region was stimulated with intense heat by placing the plantar region of the hind paw on the infrared radiant stimulator (Ugo Basile, Collegeville, Pa). The perception of acute pain was measured by paw movement in response to stimulation; the initial measurement was regarded as baseline. At 5 minutes after baseline measurement, the plantar surface of the rat's hind paw was injected subcutaneously with 50 μL of one of the following solutions using a 31-gauge needle: NS, A, DA, or EA. One hind paw of each rat was injected with one solution, once per experiment. Then, the paw withdrawal latency was tested at 5-minute intervals (Figure 1). The cutoff time for withdrawal was designated as 10 seconds.

Statistical Analysis

All values of withdrawal latency are expressed as mean ± SD. A repeated-measures analysis of variance was used to evaluate the effects on paw withdrawal latency. A Dunnett post hoc test was used to determine the time dependency (baseline vs each time point after injection of NS, A, DA, or EA) of the effects; a Bonferroni post hoc test was used to compare paw withdrawal latency among the 4 study groups (NS, A, DA, and EA). Values of $p < .05$ were considered statistically significant. All statistical analyses were performed entirely using Microsoft Excel 2013 (Microsoft, Redmond, Wash).

Sample Size

An a priori power analysis was performed to establish the necessary sample size for this study. The subject sample size was calculated using G*Power software version 3.1.9.2 (E. Erdfelder, Psychologicsches Institute der Universitat Dusseldorf, Germany) with an α error probability of .05, a power of 0.8, and an effect size based on a mean difference in paw withdrawal latency of 3.0 seconds (SD 3.0 seconds) for each group. The estimate of the difference in paw withdrawal latency used for this analysis was obtained from a previous study by Tsutsui et al.[10] Based on these parameters, the power analysis demonstrated that 44 rats in total or 11 rats per group were required for this study.

RESULTS

The paw withdrawal latencies of rats were monitored after they received a subcutaneous injection of NS, A, DA, or EA solution in the hind paw plantar region (Figure 2 and Table 1). The paw withdrawal latency values for the A group were significantly longer than

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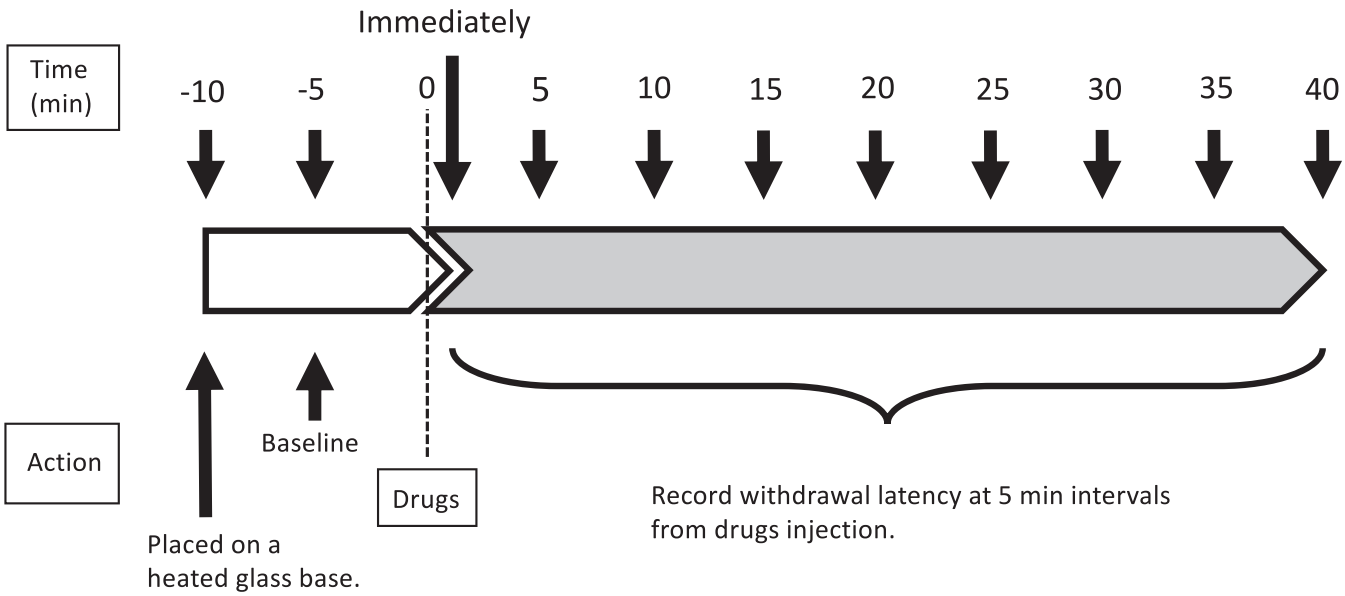

Figure 1. Schematic of the experimental protocol. Each rat was placed in a wire net container on a heated glass base 10 minutes prior to the start of the experiment. Baseline values were recorded 5 minutes prior to subcutaneous injection. Paw withdrawal latency was monitored immediately after the injection of the test solution; latency was then monitored every 5 minutes thereafter for 40 minutes.

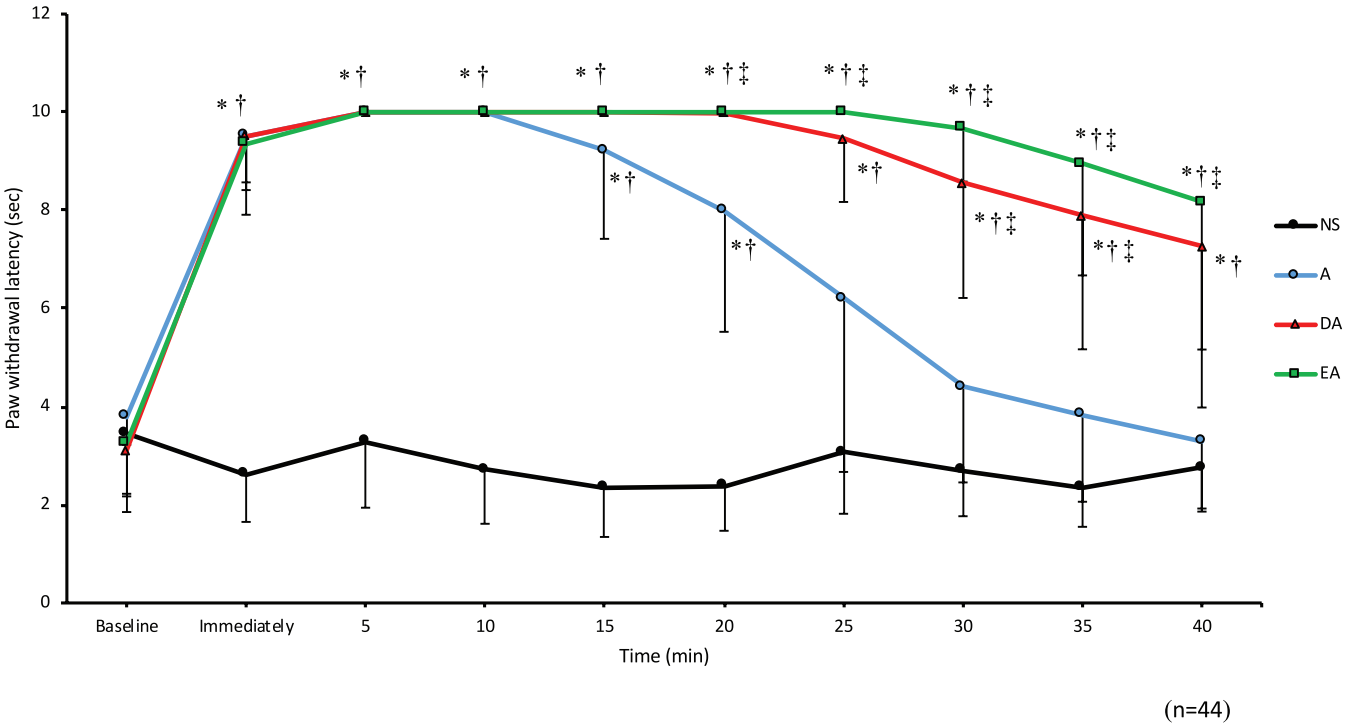

Figure 2. Paw withdrawal latency over time. NS indicates normal saline; A, 4% articaine (2 mg); DA, 5 μg/kg dexmedetomidine (1.25 μg) + 4% articaine (2 mg); and EA, 1:100,000 epinephrine (0.9 μg) + 4% articaine (2 mg). Paw withdrawal latency was monitored from 0 to 40 minutes after injection; $n = 11$ per study group. *Statistically significant difference compared to baseline. †Statistically significant difference compared to NS. ‡Statistically significant difference compared to A.

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Table 1. Paw Withdrawal Latency Values at Each Time Point From Immediately to 40 Minutes After Injection*

		Mean Paw Withdrawal Latency Value, s									
			Time After Injection, min								
	No.	*Baseline*	*0*	*5*	*10*	*15*	*20*	*25*	*30*	*35*	*40*
NS	11	3.45 ± 1.23	2.63 ± 0.98	3.29 ± 1.35	2.71 ± 1.10	2.35 ± 1.00	2.39 ± 0.92	3.07 ± 1.26	2.70 ± 0.93	2.35 ± 0.79	2.76 ± 0.84
A	11	3.79 ± 0.57	9.51 ± 0.94	10 ± 0	10 ± 0	9.23 ± 1.81	7.98 ± 2.46	6.20 ± 3.53	4.40 ± 1.94	3.83 ± 1.76	3.29 ± 1.43
DA	11	3.11 ± 0.94	9.51 ± 1.10	10 ± 0	10 ± 0	10 ± 0	9.98 ± 0.06	9.45 ± 1.28	8.56 ± 2.35	7.89 ± 2.72	7.25 ± 3.37
EA	11	3.25 ± 1.40	9.36 ± 1.45	10 ± 0	10 ± 0	10 ± 0	10 ± 0	10 ± 0	9.67 ± 1.09	8.95 ± 2.28	8.17 ± 3.01

* All values are expressed as mean ± SD. NS indicates normal saline; A, 4% articaine; DA, 5 μg/kg dexmedetomidine + 4% articaine; and EA, epinephrine + 4% articaine.

baseline values from 0 to 20 minutes after injection. The paw withdrawal latency values for the DA and EA groups were significantly longer than baseline values from 0 to 40 minutes after injection (Table 2).

In comparison to the NS group, the paw withdrawal latency values for the A group and the DA and EA groups were significantly longer than baseline from 0 to 20 minutes and 0 to 40 minutes after injection, respectively. In addition, the paw withdrawal latency values at 30 and 35 minutes after injection of the DA solution were significantly longer than those after injection of the A solution. Moreover, the paw withdrawal latency values from 20 to 40 minutes after injection of the EA solution were significantly longer than those after injection of the A solution (Table 3).

DISCUSSION

This study demonstrated that adding DEX to articaine prolongs the duration of sensory blockade to a thermal stimulus in rats; this has not been previously reported. First, this study investigated the paw withdrawal latency of rats treated with NS solution; the results showed that paw withdrawal latency was not significantly prolonged after injection of the NS solution. These results indicated that the subcutaneous injection of fluid did not skew the subjects' pain perception.

Next, this study compared paw withdrawal latency after injection of A, DA, and EA solutions from 0 to 40 minutes with baseline responses. Paw withdrawal latency after injection of the A solution was significantly longer compared to the baseline response until 20 minutes, which demonstrated that the duration of the local anesthetic effects of the A solution was approximately 20 minutes. By contrast, the paw withdrawal latency after injection of the DA and EA solutions was significantly longer compared to baseline until 40 minutes. These results suggested that the local anesthetic effect of DA and EA solutions lasted at least 40 minutes, which is consistent with the findings of a previous report.[1]

Third, this study investigated paw withdrawal latency in rats treated with either an A, DA, or EA solution; the results showed that paw withdrawal latency was significantly prolonged after injection of the A, DA, or EA solution, relative to injection of the NS solution. Moreover, the results showed that paw withdrawal latency was significantly prolonged after injection of the DA solution, compared with latency after injection of the A solution, at 30 and 35 minutes after injection. Thus, 5 μg/kg DEX (1.25 μg DEX for this study) prolonged the local anesthetic activity of articaine with similar effectiveness to epinephrine for up to 35 minutes

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Table 2. Differences (*P* Values) Between Baseline and Each Time Point From Immediately to 40 Minutes After Injection*

	Time After Injection, min								
Drug	*0*	*5*	*10*	*15*	*20*	*25*	*30*	*35*	*40*
NS	.375	1.000	.487	.215	.262	.982	.561	.231	.535
A	.000†	.000†	.000†	.000†	.005†	.863	.968	1.000	1.000
DA	.000†	.000†	.000†	.000†	.000†	.000†	.001†	.002†	.025†
EA	.000†	.000†	.000†	.000†	.000†	.000†	.000†	.001†	.006†

* NS indicates normal saline; A, 4% articaine; DA, 5 μg/kg dexmedetomidine + 4% articaine; and EA, epinephrine + 4% articaine

† Significant difference from baseline.

after injection. These results indicated that DEX prolonged the local anesthetic effect of articaine as well as epinephrine did up to 35 minutes after injection. These findings are consistent with those of previous reports in that the combination of DEX with a local anesthetic increases the duration of thermal antinociception.[3–6]

However, upon comparison of the paw withdrawal latency of DA and EA solutions, that of the DA solution (7.25 ± 3.37) was shorter than that of the EA solution (8.17 ± 3.01). However, the difference was not statistically significant. In this study, the paw withdrawal latency was only investigated until 40 minutes after injection of each solution. Further studies should investigate the latency until 50 or 60 minutes after injection of DA and EA solutions, as the results may then be statistically and clinically significant.

In this study, a low dose of DEX at 5 μg/kg (total dose of 1.25 μg DEX) was added into the 4% articaine solution. This low dose of DEX administered subcutaneously does not affect systolic blood pressure, diastolic blood pressure, or heart rate.[11] Moreover, this dosage was calculated as a human-equivalent dose that could be safely used in rats.[12] The 5 μg/kg DEX dosing for rats (1.25 μg DEX) would be equivalent to 0.82 μg/kg for humans. One microgram per kilogram DEX is often administered intravenously clinically for adult humans. Therefore, the dose used in this study was lower than the recommended clinical dosing for humans. Furthermore, 1 μg/kg DEX is often administered intravenously as a loading dose in adult humans to be given over 10 minutes to prevent the occurrence of cardiovascular complications. However, the cardiovascular impact of the DEX would be less abrupt following subcutaneous administration than that with intravenous administration because of the expected slower onset of the subcutaneous route.[13] Therefore, subcutaneous administration need not be performed over 10 minutes for the prevention of cardiovascular events. On the other hand, a high dose of DEX, such as 50 μg/kg (12.5 μg DEX for this study's subjects), would be expected to result in bradycardia and hypotension.[11] Therefore, the combination of a high dose of DEX and articaine would logically be considered potentially unsafe/unacceptable for use as a dental local anesthetic option, even though such a combination may exhibit a longer sensory blockade effect. Consequently, a dose of 5 μg/kg DEX (1.25 μg DEX) administered subcutaneously was selected for use in this study, as it was thought to be safe for use as a dental local anesthetic adjunct. However, this study did not investigate whether the combination of DEX and articaine induced any cardiovascular changes. In future studies, investigations of the cardiovascular effect of the combination of DEX and articaine are needed to confirm local anesthetic safety.

Table 3. Significant Differences Between Drugs (*P* Values) at Each Time Point From Immediately to 40 Minutes After Injection*

			Time After Injection, min								
Drug	*Compared vs*	*Baseline*	*0*	*5*	*10*	*15*	*20*	*25*	*30*	*35*	*40*
A	NS	.960	.000†	.000†	.000†	.000†	.000†	.252	.131	.225	.719
DA	NS	.843	.000†	.000†	.000†	.000†	.000†	.000†	.001†	.001†	.009†
	A	.349	.875	1.000	1.000	.470	.073	.127	.011‡	.015‡	.052
EA	NS	.901	.000†	.000†	.000†	.000†	.000†	.000†	.000†	.000†	.003†
	A	.546	.989	1.000	1.000	.470	.029‡	.012‡	.000‡	.003‡	.008‡
	DA	1.000	.978	1.000	1.000	1.000	.749	.266	.399	.817	.955

* NS indicates normal saline; A, 4% articaine; DA, 5 μg/kg dexmedetomidine + 4% articaine; and EA, epinephrine + 4% articaine

† Significant difference from normal saline.

‡ Significant difference from A solution.

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Articaine is widely known as a local anesthetic with low toxicity that is rapidly metabolized.[1] Articaine has been used clinically in a variety of nations (eg, Germany, Austria, Canada, and the United Kingdom) as a safe alternative to lidocaine for dental local anesthesia.[1,2] Typically, articaine is combined with 1:100,000 or 1:200,000 epinephrine to achieve prolonged dental local anesthesia; however, epinephrine has adverse effects. In this study, a low dose of DEX prolonged the duration of articaine in a manner similar to that of epinephrine (up to 35 minutes); additionally, a low dose of DEX reportedly has no effect on blood pressure or heart rate.[11]

This study had some limitations. First, this study did not investigate the sedative effects of DA, although DEX is known to have a dose-dependent sedative effect. Thus, it is possible that the rats may not have moved their hind paws because of the sedative effects of DEX. Second, this study did not investigate other forms of painful stimulus, such as mechanical stimulation. Heat stimulation causes cutaneous pain, whereas mechanical stimulation causes cutaneous and deep pain.[14] Thus, this study showed only the effects of DEX combined with articaine for inhibiting transmission of cutaneous pain. Third, we did not investigate mechanisms of prolongation in this study. Notably, mechanisms of prolongation have been reported to involve direct blocking of tetrodotoxin-resistant Na^+ channels by DEX[8]; this is similar to the mechanism by which anesthetics exert their effects by directly blocking Na^+ channels. However, mechanisms of prolongation may involve vasoconstriction around the injection site via the α_{2B}-adrenergic receptor.[15,16] This would affect the systemic absorption of articaine, which directly impacts safety. Therefore, an investigation of the cardiovascular effects of DEX is needed to show that a combination of DEX and articaine would be a safe local anesthetic alternative without adverse cardiovascular effects.

Moreover, it has been reported that DEX prolongs the local anesthetic effect via inhibition of inflammation and p38-MAPK phosphorylation.[17,18] In future studies, mechanisms underlying the prolongation effect should be elucidated by using the patch-clamp technique, Western blotting, and enzyme-linked immunosorbent assays.

CONCLUSION

In conclusion, a paw withdrawal latency test was used to investigate whether DEX prolonged the local anesthetic duration of articaine in this study. A combination of DEX at a dose of 5 μg/kg and 4% articaine (total dose 1.25 μg DEX + 2 mg articaine) was found to prolong paw withdrawal latency in a manner similar to that of an equivalent dose of articaine with epinephrine 1:100,000 for up to 35 minutes after injection. These findings suggest that a combination of low-dose DEX and articaine could potentially be a useful dental local anesthetic alternative to the common combination of epinephrine and articaine.

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