Altered Blood Flow in Terminal Vessels After Local Application of Ropivacaine and Prilocaine

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Background: Ropivacaine is primarily a local anesthetic, but it also acts as a vasoactive agent. Case reports have described a critical reduction in blood flow when higher concentrations of ropivacaine were used for peripheral-nerve blocks. One hypothesis is that local application of ropivacaine in tissues supplied by end arteries reduces tissue blood flow because of arterial vasoconstriction.

Methods: Rats were anesthetized by inhalation of isoflurane. The tail vessels were carefully dissected from the ventral side near the radix. Randomly, normal saline, prilocaine 0.5%, prilocaine 0.5% with epinephrine 1:200,000, ropivacaine 0.2%, ropivacaine 0.5%, or ropivacaine 0.75% was applied directly to the artery. Blood flow in the tail was continuously measured by use of laser Doppler flowmetry distal to the surgical site. Changes in temperature in the tail were detected by use of infrared thermography.

Results: Blood flow decreased after the application of ropivacaine at all concentrations in comparison with normal saline ($P < .01$ at t = 10 minutes, $P < .001$ at t = 20, 30, and 40 minutes). This effect was most pronounced at t = 30 minutes for ropivacaine 0.5% (with a 64.5% decrease in blood flow). Prilocaine 0.5% with epinephrine 1:200,000 reduced blood flow by up to 44.7% (t = 20 minutes, $P < .001$). In comparison with the placebo, the application of ropivacaine 0.5% and 0.75%, as well as prilocaine 0.5% with epinephrine 1:200,000, caused a significant reduction in tail temperature ($P < .001$ at t = 20, 30, and 40 minutes). No alteration in blood flow or temperature was seen after application of prilocaine 0.5%.

Conclusions: The application of ropivacaine directly to a rat's tail artery diminished blood flow and lowered regional skin temperature. These effects were dose related. The use of ropivacaine at higher concentrations can, therefore, not be recommended if tissues supplied by end arteries might be affected. *Reg Anesth Pain Med 2007; 32:233-239.*

Key Words: Ropivacaine, Prilocaine, Vasoconstriction, Blood flow, Laser Doppler flow, Thermography.

Local anesthetics also act as vasoactive agents. The administration of bupivacaine causes vasodilation in most tested vessels and tissues,[1] but depending on its chirality, it may also cause vasoconstriction.[2] By contrast, ropivacaine reduces blood flow when it is administered directly in vessels in the central nervous system or injected into the skin.[3] These findings are observed consistently in both animals and humans.[4]

A few case reports have described a critical reduction in blood flow when ropivacaine was used for peripheral-nerve block or epidural anesthesia.[5] The mechanism that causes vasoconstriction is as yet unknown. Also, the relation between the degree of vasoconstriction and the concentration of ropivacaine still needs to be clarified.

As far as we are aware, the effects of the application of ropivacaine on perfusion in terminal vessels have not previously been studied. Only case reports are available to document the vasoconstrictive properties of ropivacaine, which may even cause critical ischemia. In all of the cases concerned, immediate treatment was necessary to prevent permanent harm to the patient. The degree of vasoconstriction observed after application of ropivacaine in tissues supplied by end arteries is still unknown. This question is of clinical importance, as ropivacaine is often used for peripheral-nerve blocks.

In this study, the changes in tissue perfusion that occur after the application of ropivacaine at different concentrations were, therefore, investigated by

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use of laser Doppler flowmetry and infrared thermography. All of the measurements were carried out in rat tails. In addition, vasoactivity in the tail vascular system was tested to evaluate the reliability of this animal model.

An assumption was that ropivacaine in tissues supplied by end arteries causes arterial vasoconstriction and thus reduces blood perfusion. The degree of vasoconstriction was hypothesized to be directly proportional to the concentration of ropivacaine.

Methods

Anesthesia and Surgical Procedures

Institutional approval for the experiments was obtained from the Animal Care Committee of the district government of Muenster. The studies were conducted in 76 Sprague-Dawley (SD) rats weighing 250 to 275 g. The rats were allowed food and water ad libitum and were kept in an air-conditioned room in a 12-hour light-dark cycle. The rats were allowed to acclimatize to laboratory conditions for 1 week. Arterial and venous catheters were placed in the left carotid artery and right jugular vein, respectively.

All surgical interventions and measurements were carried out with the rats under inhalational anesthesia by use of 1.4 vol% isoflurane in 50% oxygen/50% room air via a face mask. Isoflurane concentration was only adjusted minimally within a range 1.1 to 1.4 vol%. Spontaneous breathing was maintained throughout the entire experiment. No apnea occurred. $PaCO_2$, as indicated by blood-gas analysis, was within normal range for all groups. The ventral aspect of the cervical region and the radix of the tail were shaved. The incision fields were disinfected.

The animals were placed on a medical water heating pad (T-Pump, Gaymar; AstraZeneca, Wedel, Germany) during the surgical procedures and observation period. Rectal temperatures were continuously measured. For hemodynamic control, a polyethylene catheter (PE 50, 0.96 mm) was inserted into the left carotid artery, and another catheter was placed in the superior vena cava via the right external jugular vein to administer fluids and medication. The carotid line was continuously flushed with 0.9% saline to maintain patency.

The tail was chosen for the experimental setup, as its arterial blood flow is supplied by a single artery, and changes in perfusion in the tail, therefore, depend only on changes in arterial blood flow. The tail's vascular system consists of 4 veins and 1 artery. Two veins and the artery course in the middle of the tail, in between the tail fibers, whereas the other 2 veins course superficially and laterally on each side of the tail.

The tail artery was prepared from the ventral side about 2 cm distal to the radix. The coat was carefully removed and the skin was disinfected. After mediolateral incision of the skin, fibers and tissue were spread to allow careful preparation of the vessels. The aim of the preparation was to identify the artery and ensure that the study medication definitely reached the vessels in the tail. The tail was carefully attached to the table by use of adhesive tape to prevent movement during spontaneous breathing.

Hemodynamic and Respiratory Control

Hemodynamic parameters were continuously recorded by use of a standard pressure transducer (PMSET 1DT; Becton Dickinson, Heidelberg, Germany) and a monitor (Sirecust 404; Siemens, Erlangen, Germany). An arterial blood sample was taken for blood gas analysis (ABL; Radiometer, Copenhagen, Denmark).

Study Protocol

The capacity of different local anesthetics to produce vasoconstriction was tested by applying them directly to the tail artery. Effects on perfusion were detected by laser Doppler flowmetry and thermography scans. Appropriate control groups were also studied. Medication was provided by AstraZeneca.

The animals were randomized into 6 groups ($n = 10$ in each group) in accordance with the study protocol. Ropivacaine was administered at 3 different concentrations (0.2%, 0.5%, and 0.75%). Prilocaine 0.5% was applied with and without the addition of epinephrine 1:200,000.

The control group received a saline solution (0.9%). In addition, vasoactive substances were applied locally, as well as systemically, to detect possible effects of surgical procedures on vasoreactivity in the tail artery. Vasopressin ($n = 3$) was infused to increase mean arterial blood pressure by 15 ± 2 mm Hg. Epinephrine (1:200,000, $n = 3$) was applied directly to the surgical site to induce vasoconstriction only at the tail artery. Sodium nitroprusside ($n = 3$) was infused until the mean arterial blood pressure was reduced by 15 ± 2 mm Hg. In addition, acetylcholine (1 mol, $n = 3$,) was administered to confirm the ability of the terminal tail arteriole to respond adequately to a locally applied vasodilating agent. Each animal in the control group was applied to only 1 of the test substances.

The influence of pH on vasoreactivity was also tested, as the pH of the test solutions used varied. Normal saline was titrated with HCl to pH values of

5.9 and 5.5 (n = 2 per group) and applied to the artery.

The investigator who performed the surgical procedures and the measurements was blinded to the treatment used. After preparation of the artery, 20 minutes of stabilization were allowed. At the end of the stabilization period, baseline laser Doppler flow and thermography readings were recorded. Then, 50 μL of the test substance were directly applied into the surgical site in the rat's tail. The test substance was not injected into the surrounding subcutaneous tissue because it was uncertain how to determine the amount of substance reaching the artery. All substances were applied at room temperature.

After the application of the test substance, blood flow and thermography were monitored continuously, and readings were recorded every 10 minutes. Measurements were completed after 40 minutes. At the end of the experiment, the anesthetized rats were euthanized by injecting thiopental.

Blood-Flow Measurement

Blood flow was measured by use of the laser Doppler flowmetry (LDF) technique. Laser Doppler signals were recorded in arbitrary blood perfusion units (BPU).

A laser Doppler probe (Standard Pencil Probe, ADI Instruments Blood Flow Meter, with PowerLab 4/20) was used to determine blood flow. The device was calibrated for each experiment. All other electrical devices (e.g., computers and the pump for the heating pad) were placed on a different table to minimize artefacts caused by vibration. Blood flow was allowed to stabilize for 20 minutes after the surgical procedures had been completed. Blood flow was measured 3 cm distal to the surgical site. Chart 4 for Windows was used for data analysis.

Thermography Scan

For thermography imaging, the PC-assisted high-resolution infrared thermography Varioscan 3021 system was used (InfraTec/Jenopitik, Dresden, Germany). The camera has a thermal resolution of 0.03°C. Liquid nitrogen is used for cooling. Thermographic images were taken at baseline and every 30 seconds for the entire experiment. A heating pad was placed under the rat's body to control body temperature. The tail was not placed on the heating pad, so that temperature contrasts with the environment could be better detected. Analysis of the thermographic images was carried out by use of IRBISplus (InfraTec, Dresden, Germany) to convert the images into numerical values.

The data are presented as means ± standard error of the mean (SEM). SigmaStat 3.1 software (SPSS, Chicago, IL) was used for statistical analysis. The data were evaluated using 2-way analysis for repeated measurement followed by the Student–Newman–Keuls method for pairwise multiple-comparison procedures. $P < .05$ was considered significant.

Results

Blood Flow

Blood-flow velocity index remained stable at baseline values in the control group throughout the entire experiment. The application of ropivacaine at all concentrations tested (Fig 1) caused a significant reduction in blood-flow velocity index in comparison with the control group at t = 10, 20, 30, and 40 minutes. The largest effect was noticed for ropivacaine 0.5%, with a blood-flow velocity index reduction of 64.5% after 30 minutes. For ropivacaine 0.75%, the maximum reduction in blood-flow velocity index was 53% after 30 minutes and 61% after 40 minutes.

Application of prilocaine 0.5% reduced blood-flow velocity index by 16.5% in comparison with the control group, but these changes were not significant ($P = .06$).

Prilocaine 0.5% with epinephrine 1:200,000 (Fig 2) reduced blood-flow velocity index by 44.7% at t = 20 minutes ($P \leq .001$), with a continuous increase toward the end of the observation period.

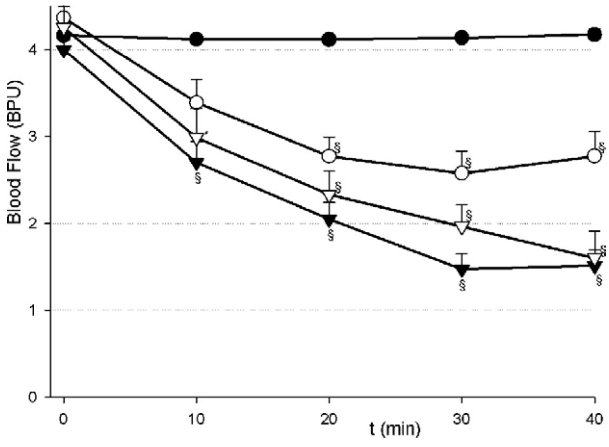

Fig 1. Blood flow (BPU) at baseline and at 10, 20, 30, and 40 minutes after application of ropivacaine in different concentrations in comparison with normal saline. Data are mean ± SEM. *$P < .01$; §$P < .001$. Filled circles = NaCl 0.9%; unfilled circles = ropivacaine 0.2%; filled triangles = ropivacaine 0.5%; unfilled triangles = ropivacaine 0.75%.

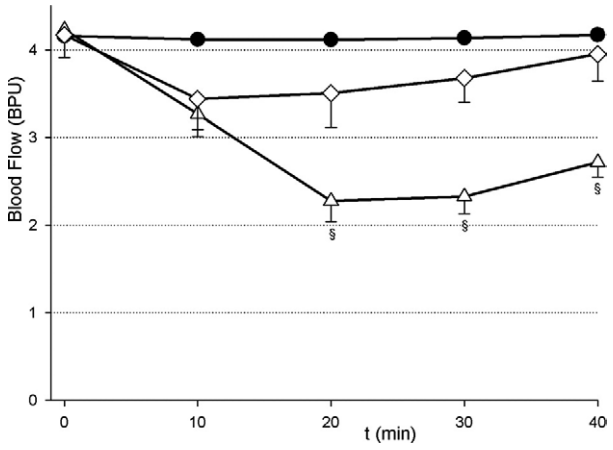

Fig 2. Blood flow (BPU) at baseline and at 10, 20, 30, and 40 minutes after application of prilocaine 0.5% plain (unfilled diamonds) and with epinephrine 1:200,000 (unfilled triangles) in comparison with normal saline (NaCl 0.9%, filled circles). Data are mean ± SEM. §$P <$ .001.

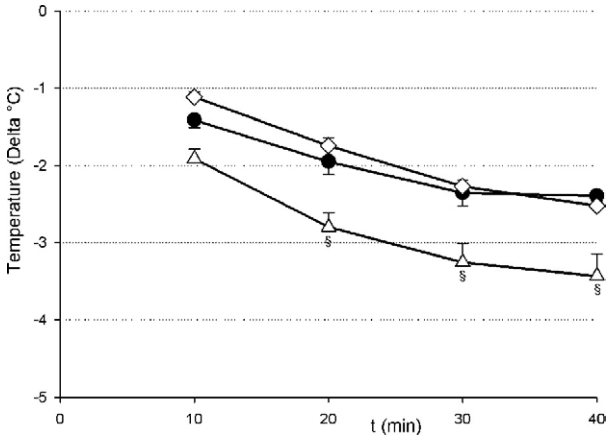

Fig 4. Change in temperature (°C) 10, 20, 30, and 40 minutes after application of prilocaine 0.5% plain (unfilled diamonds) and with epinephrine 1:200,000 (unfilled triangles) in comparison with normal saline (NaCl 0.9%, filled circles). Data are mean ± SEM. §$P <$ .001.

Thermography

Baseline values did not vary among the groups. All further calculations were based on differences in temperatures from the baseline (ΔT°). Skin temperature decreased in all groups during the course of the experiments.

In comparison with the control group, ropivacaine 0.75% and 0.5% (Fig 3) caused temperature to decrease at t = 20, 30, and 40 minutes (all $P \leq$ .001). Ropivacaine 0.2% had no influence on the tail temperature in comparison with the control group.

No decrease in the tail temperature occurred after the application of prilocaine in comparison with the control group (Fig 4). The addition of epinephrine 1:200,000 to prilocaine 0.5% decreased the temperature significantly in comparison with normal saline at t = 20, 30, and 40 minutes.

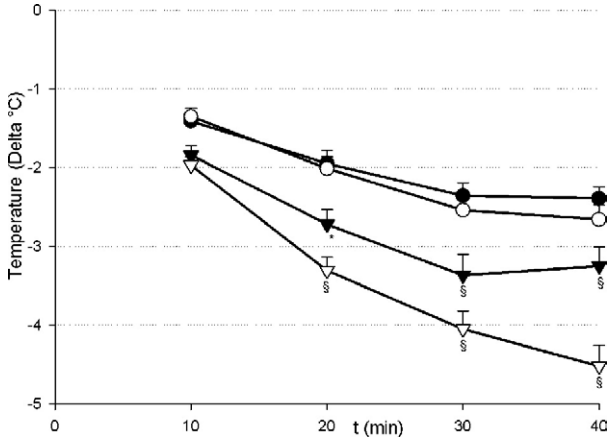

Fig 3. Change in temperature (°C) 10, 20, 30, and 40 minutes after application of ropivacaine in different concentrations in comparison with normal saline. Data are mean ± SEM. *$P <$ .01; §$P <$ .001. Filled circles = NaCl 0.9%; unfilled circles = ropivacaine 0.2%; filled triangles = ropivacaine 0.5%; unfilled triangles = ropivacaine 0.75%.

Local and Systemic Application of Vasoconstrictors and Vasodilators

Topical application of epinephrine (50 μL, 1:200,000) directly to the tail artery induced vasoconstriction and decreased blood-flow velocity index reversibly from 4.07 ± 0.02 BPU at baseline to minimum values of 1.00 ± 0.03 BPU ($P \leq$ .05). After local application of acetylcholine (50 μL, 1 mol) to the surgical site, an increase in blood-flow velocity index occurred, with BPU reaching maximum values of 6.25 ± 0.1 ($P \leq$ .05). The observed effects related to the application of acetylcholine were only of short duration, with blood flow returning to baseline within 5 to 8 minutes.

The effects of the systemic application of nitroprusside sodium and vasopressin are shown in Figure 5. Systemic infusion of sodium nitroprusside decreased mean arterial pressure (MAP) by 15 ± 2 mm Hg, with blood-flow velocity index increasing from 4.03 ± 0.04 BPU at baseline to 7.7 ± 0.04 BPU ($P \leq$ .05) after 30 minutes ($P \leq$ .001). Vasopressin was infused to increase MAP by 15 ± 2 mm Hg. Systemic vasoconstriction caused blood-flow velocity index in the tail to decrease to 0.47 ± 0.07 BPU after 40 minutes, corresponding to a blood-flow velocity index reduction of 88.7% in comparison

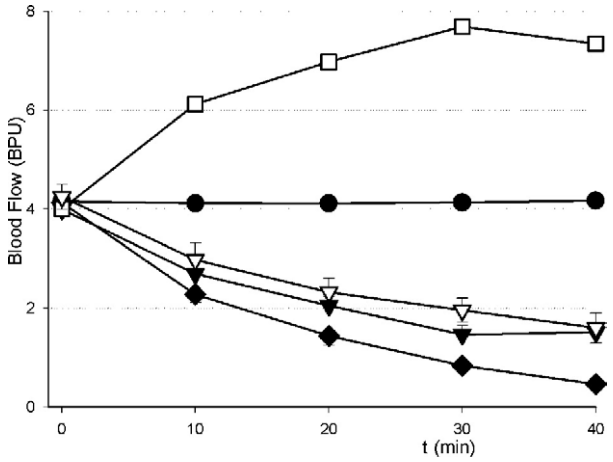

Fig 5. Blood flow (BPU) at baseline and at 10, 20, 30, and 40 minutes after application of ropivacaine in concentrations of 0.5% (filled triangles) and 0.75% (unfilled triangles), and systemic infusion of vasopressin (filled diamonds) and nitroprusside sodium (unfilled squares). Data are mean ± SEM. *$P < .01$; §$P < .001$. Filled circles = NaCl 0.9%.

with normal saline ($P \leq .001$). Application of vasopressin also lowered tail temperature in comparison with the control group at t = 30 and t = 40 minutes ($P \leq .001$). Data of ropivacaine 0.5% and 0.75% in Figure 1 and Figure 5 are the same.

Effects of Different pH Values

In comparison with normal saline (pH 7.0), solutions with acidic pH values (5.5 and 5.9) did not influence blood-flow velocity index as detected by laser Doppler flowmetry. BPU readings were stable at baseline values of 4 ± 0.05 and did not change during the observation period. Differences in pH between 5.5 and 7.0 had no influence on blood-flow velocity index.

Hemodynamic Monitoring and Blood-Gas Analysis

The mean arterial blood pressure and heart rate remained stable throughout the experiment. No significant differences were seen within or between the groups at any time.

Discussion

This study demonstrates that local application of ropivacaine at different concentrations reduces blood flow in the tail compared with a placebo. The vasoconstrictive response to the application of ropivacaine appears not to be directly dose dependent, with a maximum effect observed for ropivacaine 0.5%.

The temperature in the tail was also lower after the application of ropivacaine at concentrations higher than 0.2% in comparison with normal saline, which suggests that the vasoconstrictive effect of ropivacaine on the afferent arteriole of the tail induced hypoperfusion-related coolness of the tail skin. Comparable results were observed by adding epinephrine to prilocaine 0.5%, but prilocaine alone did not result in significant vasoconstriction or in a decrease in skin temperature in this experiment.

The surgical technique used in the experiment has not previously been described. The rat tail was chosen for the experimental setup because of its unique properties with regard to surgical access and measurement of blood flow and temperature. The blood supply to the tail tissue is provided by only a single artery. Changes in tissue blood flow are, therefore, highly dependent on blood-flow changes in the single end artery.

The study tested whether terminal arterial blood flow was altered by surgical manipulation at the radix of the tail. Vasoconstriction causing a significant decrease in blood flow was achieved by applying vasoconstrictors both locally and systemically. In contrast, vasodilative substances applied in the same way caused the blood flow to increase. Thus, the vascular reactivity of the tail artery was intact in the preparation, and the surgical procedures did not cause maximum vasoconstriction or vasodilation of the tail artery. Although vasoreactivity was carefully evaluated by applying substances with known effect on the vasotonus, we cannot completely exclude other physiologic effects simultaneously influencing the vasotonus of the tail's artery.

The laser Doppler flowmetry method has been described previously and is a well-established method of measuring surface-blood flow.[6] Laser Doppler flowmetry is considered to be a sensitive and reliable method of detecting immediate changes in perfusion. Both laser Doppler flowmetry and thermography have been applied simultaneously in several studies in humans. The authors are in agreement with previous studies reporting that the 2 methods show only a poor degree of correlation.[7,8]

Ropivacaine was introduced into clinical use more than 10 years ago.[9] Studies assessing the vascular response to the drug have produced inconsistent results. All of the published studies were carried out in relation to blood flow in human skin, in the central nervous system, or in isolated human or animal arteries. No experimental data are available on changes in tissue perfusion in end arteries after the administration of ropivacaine for peripheral-nerve blocks. However, evidence from case reports

indicates that ropivacaine at a concentration of 0.75% may cause ischemia in the area located distal to the injection site. After injection of ropivacaine 0.75% for a penile-nerve block, ischemia of the glans penis occurred and immediate infusion of iloprost (a prostaglandin I_2 analog) was necessary to prevent permanent harm.[5] Initial evaluations described vasoactive effects that ranged from "less vasodilative" to "slightly vasoconstrictive."[10,11]

Kopacz et al.[3] described a reduction in cutaneous blood flow in pigs after the injection of ropivacaine 0.25% and 0.75%. At both concentrations, ropivacaine decreased blood flow by 52% and 54%, respectively. In addition, all solutions that contained epinephrine reduced blood flow by 48% to 73%. Cederholm et al.[4] used laser Doppler flowmetry to examine the effects of intradermal application of ropivacaine on human skin blood flow. In contrast to previous studies, the degree of reduction in blood flow was found to be inversely related to the concentration of ropivacaine in this study. Further research confirmed these results.[12]

Both Iida et al.[13] and Ishiyama et al.[14] observed significant vasoconstrictive effects for ropivacaine when it was applied to canine spinal pial and parietal vessels. These results are consistent with the findings of the present study and are also supported by other data that show a reduction of epidural blood flow by up to 45%, depending on the concentration of ropivacaine.[13-15]

Dahl et al.[16] used the ^{133}Xe-clearance technique to evaluate changes in human epidural blood flow after applying different local anesthetics via an epidural catheter. Ropivacaine 0.5% lowered blood flow by 37%.

Nakamura et al.[17] compared the vascular effects of different local anesthetics when they were applied directly to femoral arteries and veins in dogs. Ropivacaine caused a maximum contraction of 51.5% in comparison with submaximal contraction caused by epinephrine.

Ropivacaine has been found to cause vasoconstriction in different types of tissue. Whether the reduction of blood flow by half after the application of ropivacaine is clinically relevant is still uncertain. Studies have shown that changes in evoked potentials and electroencephalographic activity are observed when perfusion diminishes by 50% to 66%.[18,19] In the present study, the maximum reduction in blood flow was 64.5%, capable of influencing neural activity. Whether this effect is harmful to the tissue supplied by the affected end artery is not clear.

Several studies have examined possible mechanisms that may cause the vasoconstriction observed after the application of ropivacaine, but the mode of action appears to be more complicated than was previously thought. Iida et al.[20] analyzed the influence of local anesthetics with different chiralities on cerebral pial arterioles in dogs. The ranking order of the vasoconstriction both in small and large arteries was (*S*)-ropivacaine > racemic ropivacaine > (*R*)-ropivacaine. A comparable enantioselective action is also present in coronary-resistance vessels.[2] Thus, the stereochemical structure of local anesthetics cannot explain their vascular effects.[20]

Data from different studies suggest that adrenergic receptors (α-receptors and β-receptors) and Na^+ or K^+ channels do not mediate the vascular effects of local anesthetics.[20,21] In addition, nitric oxide synthetase and prostaglandins do not affect the vascular responses to bupivacaine and ropivacaine.[22]

In addition, other postulated mechanisms include direct vascular interactions of ropivacaine.[15] Strong evidence suggests that ATP-sensitive K^+ channels and protein kinase C (PKC) are involved in vascular responses to local anesthetics.[21,23-25] The vascular effect of ropivacaine seems to be mediated by the endothelium and intracellular Ca^{2+}.[26,27]

In conclusion, this study demonstrated that ropivacaine causes a decrease in tissue blood flow when applied locally near an end artery. For prilocaine, no clear vasoactive response was observed, except when epinephrine was added. On the basis of the significant and long-lasting reduction in blood flow observed in this animal study, ropivacaine at higher concentrations cannot be recommended for peripheral-nerve block if an end artery is affected or preexisting vascular diseases are known that might alter the blood supply to the dependent tissue. The mechanism of vasoconstriction caused by ropivacaine remains to be clarified.

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