DRUG-INDUCED VASODILATION: IN VITRO AND IN VIVO STUDY ON THE EFFECTS OF LIDOCAINE AND PAPAVERINE ON RABBIT CAROTID ARTERY

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Flap ischemia is often encountered during pedicled and free tissue transfer. In this study, the vascular effects of varying doses of lidocaine, papaverine, and a combination of the two agents were evaluated and compared in an in vitro and in vivo model in the rabbit carotid artery. In the in vitro study, 14 rings from the rabbit carotid artery were bathed in Krebs-Ringers solution and stretched progressively to an optimal tension of 3.7–4.2 grams. Their isometric contractile activity was measured. The specimens were precontracted with norepinephrine (1 μM), and a dose response curve was established by adding cumulatively either lidocaine (to 7 arterial rings) or papaverine (to 7 arterial rings) at increasing concentrations. In the in vivo study, microvascular anastomoses were performed bilaterally in the rabbit carotid artery in 30 animals using 9–0 nylon suture and standard microsurgical techniques. In each animal, one side was treated with heparinized sodium chloride and served as the control. The other side was treated blindly, during and after the anastomoses, with a topical application of 1 ml of either lidocaine 2% (n = 5), lidocaine 20% (n = 5), papaverine (30 mg/ml, n = 5), lidocaine 2% combined with papaverine (30 mg/ml, n = 5), or lidocaine 20% combined with papaverine (30 mg/ml, n = 5). For 30–60 minutes after the procedure, blood flow changes in the vessels were continuously monitored with a transonic doppler applied to both carotid arteries. The 20% lidocaine group was flushed with saline at the end of the first hour and monitored for an additional 60 minutes. Papaverine elicited a concentration-dependent relaxation of norepinephrine precontracted carotid artery rings in vitro. Lidocaine elicited a biphasic response, with low concentrations (10^{-6}–10^{-4} M) increasing the norepinephrine-induced contraction and high concentrations (10^{-4}–10^{-2} M) relieving this contraction. Microsurgical anastomosis produced a significant decrease of blood flow through the rabbit carotid artery as measured by the transonic doppler. Drug application did not alter the systemic blood pressure of the animals. Topical application of lidocaine 2% did not significantly change the blood flow after microvascular anastomosis. Topical application of lidocaine 20%, papaverine (30 mg/ml), or lidocaine (2% or 20%) combined with papaverine significantly increased the blood flow in the rabbit carotid artery. In the lidocaine 20% group, the blood flow remained significantly increased after the drug was flushed with heparinized saline solution. These results demonstrate that topical lidocaine 20%, papaverine, and lidocaine 2% or 20% combined with papaverine significantly increase blood flow in the rabbit carotid artery after microvascular anastomosis. The data confirm the use of papaverine and lidocaine 20%, alone or in combination, as spasmolytics during clinical microsurgery. This suggests that lidocaine 2% alone is not the ideal drug to relieve vascular constriction, and further studies on the clinical use of low concentrations of topical lidocaine in microsurgery is warranted.

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Free tissue transfer is a common reconstructive technique used after cancer extirpation, burns, and trauma. Extreme vascular constriction is often encountered in microvascular surgery. Reduction of the blood vessel diameter causes a decrease in the blood flow. This can lead to prolonged ischemia, thrombosis, inflammation, and ultimately necrosis.[1,2] Several pharmacological and physical attempts have been made to relieve experimental and clinical ischemia and flap thrombosis, all with varying results.[3–8] Traditionally, topical lidocaine (2%) and papaverine (30 mg/ml) have been employed during microsurgery to prevent and counteract constriction in the vascular pedicle following the microvascular anastomosis. There are conflicting reports, however, on the efficacy of lidocaine 2%, with a biphasic response demonstrated with vasoconstriction at low concentrations and vasodilation at higher concentrations.[9–15] A long-lasting rebound contraction has also been described.[2,9] Pa-

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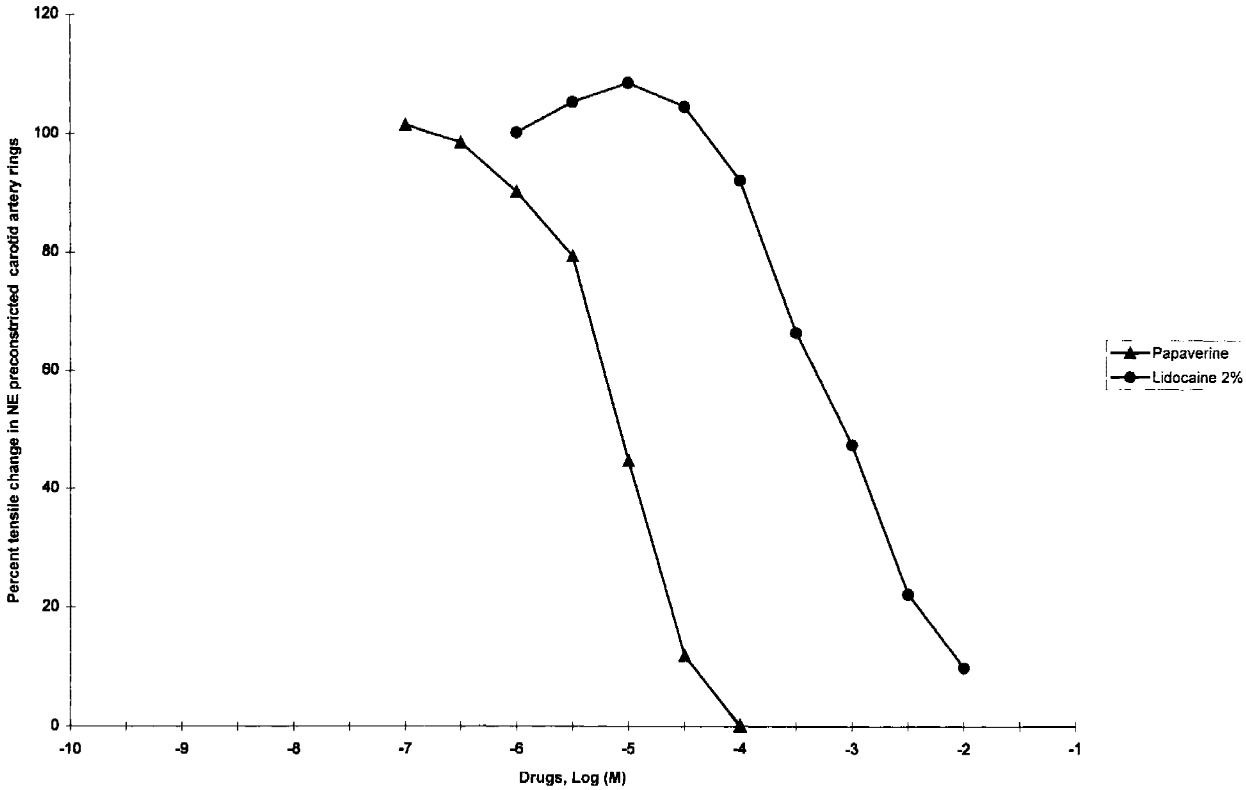

Figure 1. Effects of lidocaine (10^{-6}–10^{-2} M) and papaverine (10^{-7}–10^{-4} M) on norepinephrine (NE 5 × 10^{-3} M) preconstricted rabbit carotid artery rings in vitro. Papaverine elicits a dose-dependent relaxation of preconstricted arterial rings with no constriction seen at any concentration. Lidocaine exerts a biphasic action on the specimens, with low concentrations (10^{-6}–10^{-4} M) potentiating the contraction elicited by NE, while higher concentrations induce relaxation. Values are given as means, and concentrations are given in molar.

paverine has been shown to be a reliable tool in preventing and reversing arterial constriction.[16–26] In this work, an in vivo model is proposed to study blood flow changes after a microvascular anastomosis in the rabbit carotid artery, and the vascular effects of lidocaine alone and in combination with papaverine are compared. Furthermore, the effects of lidocaine and papaverine are tested in an in vitro system.

Animals used in this study are maintained in facilities approved by the American Association for Accreditation of Laboratory Animal Care, and in accordance with current United States Department of Agriculture, Department of Health and Human Services, and National Institutes for Health regulations and standards.

MATERIAL AND METHODS

In Vitro Study

Sections (2.5 cm) of the rabbit carotid arteries were harvested from 7 New Zealand male white rabbits (2.8–3.4 kg), after euthanasia (pentobarbital, 120 mg/kg IP). The specimens were cleaned of fat and connective tissue, cut into rings of 2 mm, and suspended in organ chambers containing 10 ml of modified Krebs-Ringers solution (118.3-mM NaCl, 4.7-mM KCl, 1.2-mM $MgSO_4$, 1.2-mM KH_2PO_4, 2.5-mM $CaCl_2$, 25.0-mM $NaHCO_3$, 0.016-mM CaEDTA, and 11.1-mM glucose) at 37°C, aerated with 95% O_2 and 5% CO_2. Care was taken not to injure the endothelium during the preparation. The rings (n = 14) were stretched progressively to an optimal tension (3.7–4.2 gm), and changes in the isometric tension were recorded via an isometric force transducer connected to a personal computer (IBM 386/30 MHZ). The presence of intact endothelium was verified by adding acetylcholine (1 μM), which resulted in relaxation of norepinephrine (NE, 1 μM) precontracted rings. The preparations were then rinsed three times with warm control solution and left to recovery for 30 minutes. Experiments were conducted by precontracting the specimens with NE (1 μM) and cumulatively adding lidocaine (10^{-6}–10^{-2} M, n = 7, Sigma Chemical Co., St. Louis, MO) or papaverine (10^{-7}–10^{-4} M, n = 7, Papaverine, Lilly) at increasing concentrations.

In Vivo Study

Thirty New Zealand male white rabbits (2.8–3.4 kg) were used. The animals were anesthetized by mask administration of 5% isoflurane (IsoFlo R Solvay Animal Health,

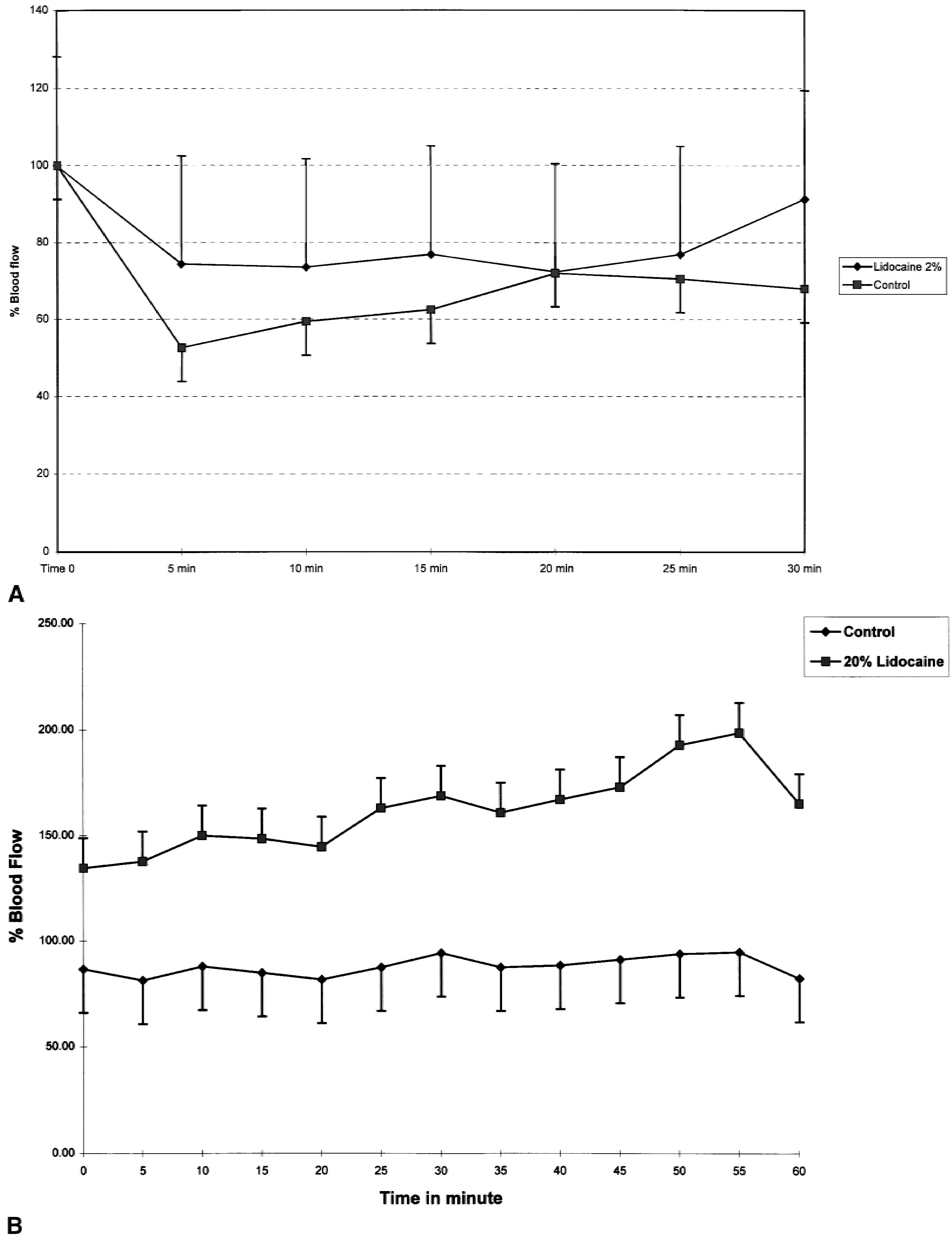

Figure 2. (Legend on following page.)

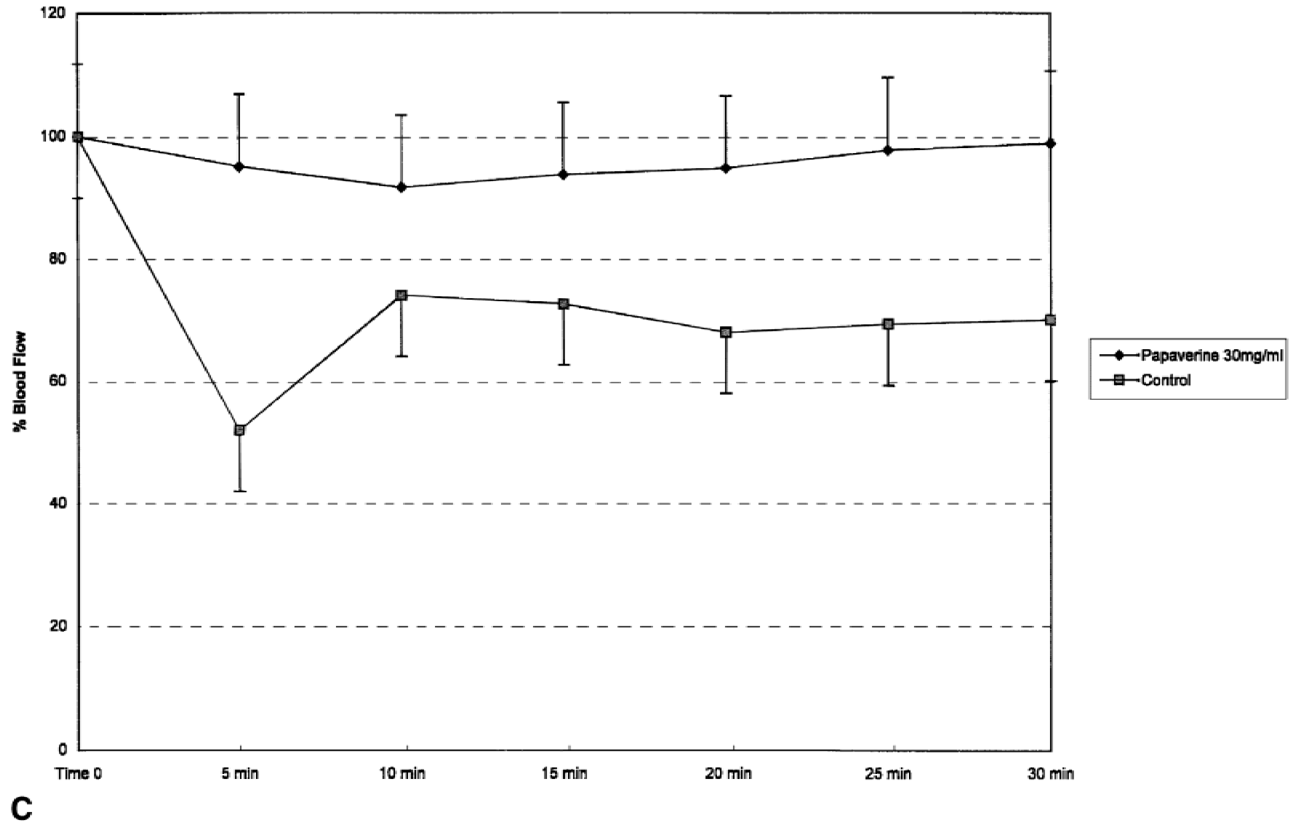

Figure 2. Effects of topical application of lidocaine and papaverine on the blood flow of the rabbit carotid artery after the microvascular anastomosis. The figure shows that topical lidocaine 2% (A) does not significantly affect the blood flow in the carotid artery when compared to the control. Lidocaine 20% (P = 0.0001) (B) and papaverine (30 mg/ml) (P = 0.0001) (C) induce a significant increase of the blood flow. Doppler values are given as means (SEM) and expressed as percentages of the preanastomotic blood flow readings.

Mendota Heights, MN) and a 95% O_2 mixture and intubated with a 3.0-mm orotracheal tube (Sheridan TM, Sheridan Catheter Corp., Argyle, NY). General anesthesia was maintained with 3.5% halothane (Halocarbon Laboratories, North Augusta, SC) and a 96.5% O_2 mixture. The rabbits were prepared for surgery in a routine fashion by shaving the neck hairs and applying antiseptic solution. Both common carotid arteries were exposed through a midline incision, and each carotid artery was fitted to a 1-mm 20-MHz doppler flow probe (Transonic Systems Inc., Ithaca, NY) for blood flow measurements. After a baseline flow was established (varied in each animal, but usually between 20–30 ml/min), the vessels underwent a microvascular anastomosis using 9-0 nylon suture. One side was treated with heparinized saline and served as the control. The opposite rabbit carotid artery served as the blinded experimental side. The side selected was not randomized prior to drug application. The animals were divided into five experimental groups. During and after the microvascular anastomosis, each group was treated with topical application of 1 ml of either lidocaine 2% (Sigma Chemical Co., n = 5), lidocaine 20% (Sigma Chemical Co., n = 5), papaverine (Papaverine, Lilly, 30 mg/ml, n = 5), lidocaine 2% and papaverine (Papaverine, Lilly, 30 mg/ml, n = 5), or lidocaine 20% and papaverine (Papaverine, Lilly, 30 mg/ml, n = 5). Five animals were further used as a control group for the microvascular anastomosis utilizing randomized sides without the application of drugs. This allowed for individual assessments of each anastomosis and the effects on blood flow of the opposite carotid artery. Blood pressure was measured in each animal by cannulating the femoral artery with a 22-gauge, 1-inch catheter (AbbocathT, Abbot Ireland, Sligo, Republic of Ireland) attached to a pressure transducer (Siemens Sirecrust R 404 pressure monitor, Siemens Medical Systems, Inc., South Iselin, NJ). Respiratory and cardiac functions were monitored throughout the procedure. The animals were systemically heparinized (600U) to decrease the thrombogenic potential of the microvascular anastomosis. This systemic heparin application did not appear to influence the effects of topical spasmolytics. Blood flow was recorded every 5 minutes for 1 hour after conclusion of the surgical procedure. All animals were euthanized at the conclusion of the blood flow studies.

Statistical Analysis

Percent change in doppler readings of the control side were compared to percent change on the experimental side using the two-tailed student's *t*-test (in vitro and in vivo).

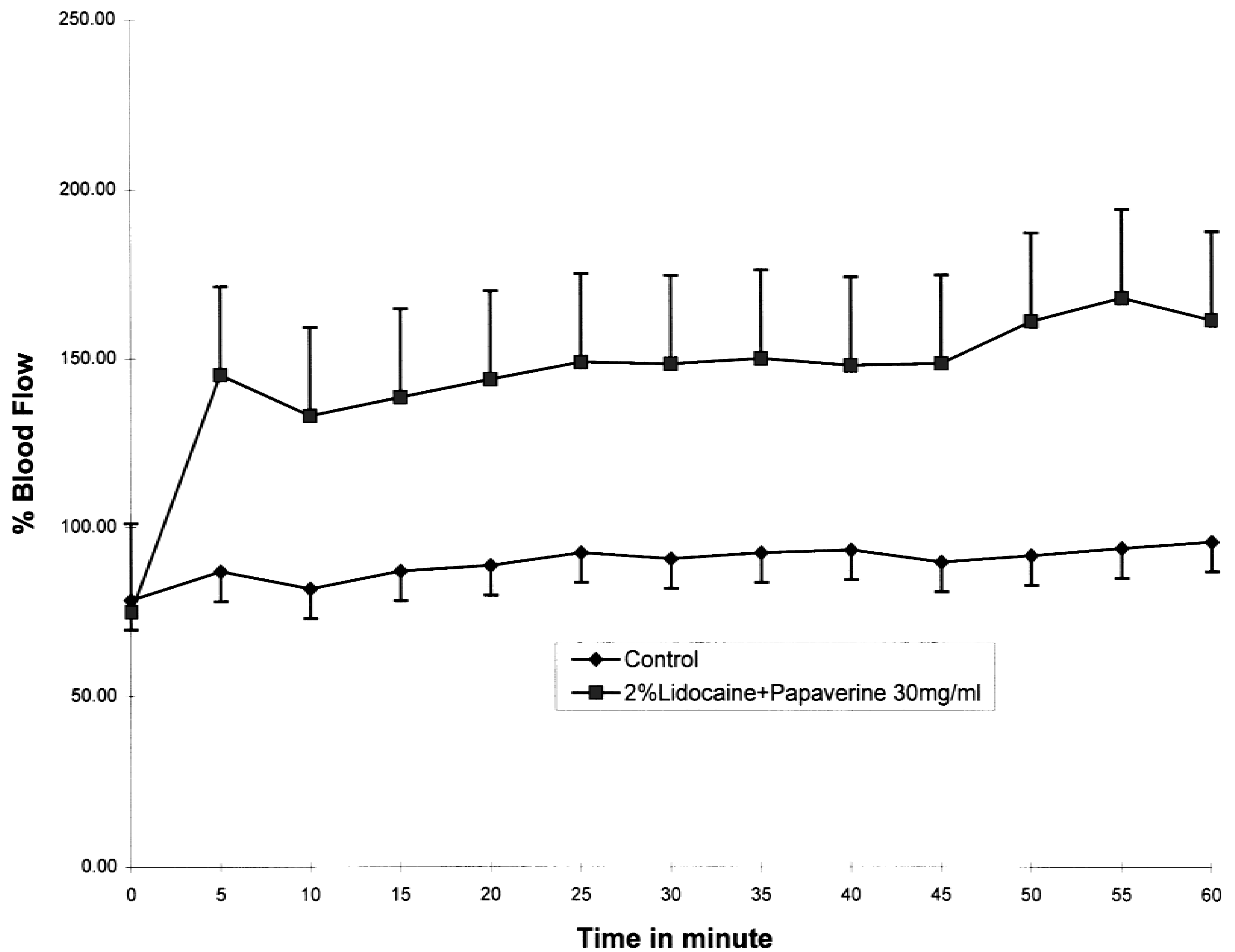

Figure 3. Effects of topical application of lidocaine (2%) in combination with papaverine (30 mg/ml) on the blood flow of the rabbit carotid artery after microvascular anastomosis. Application of the experimental drugs produces a significant increase of the blood flow in the carotid artery when compared to the control (P = 0.0007). Doppler values are given as means (SEM) and expressed as percentages of the preanastomotic blood flow readings.

Values are expressed as a percentage of the preanastomotic blood flow values. The level of statistical significance was set at $\alpha = 0.05$ and was adjusted with Bonferroni correction. The adjusted significance level was α/κ, where κ is the number of tests performed.

RESULTS

In Vitro Study

When applied to isolated rings of rabbit carotid arteries, NE (1 μM) caused a slowly developing contraction. The contraction reached a steady level of tension (after 20 minutes), and was maintained for several hours. When applied cumulatively during this sustained phase of tension, papaverine (10^{-7}–10^{-4} M) evoked a concentration-dependent relaxation (Fig. 1). Lidocaine at low concentrations (10^{-6}–10^{-4} M) increased the contraction elicited by NE while higher concentrations induced relaxation (Fig. 1).

In Vivo Study

Blood pressure was not significantly changed by the topical administration of experimental drugs. In all control animals a significant decrease in blood flow followed the microvascular anastomosis. Additional clamping of one carotid artery did not significantly alter blood flow in the opposite carotid vessel. Topical application of lidocaine 2% did not significantly alter blood flow through the anastomosis (Fig. 2A). Lidocaine (20%), papaverine (30 mg/ml), and lidocaine (2 or 20%) combined with papaverine (30 mg/ml) significantly increased blood flow ($P = 0.0001$, 0.0001, 0.0007, and 0.0001, respectively, Figs. 2B, 2C, 3, 4). Flushing 20% lidocaine with a heparinized solution did not elicit a significant change of blood flow.

DISCUSSION

In this study, papaverine elicited a concentration-dependent relaxation of carotid artery rings in vitro, while

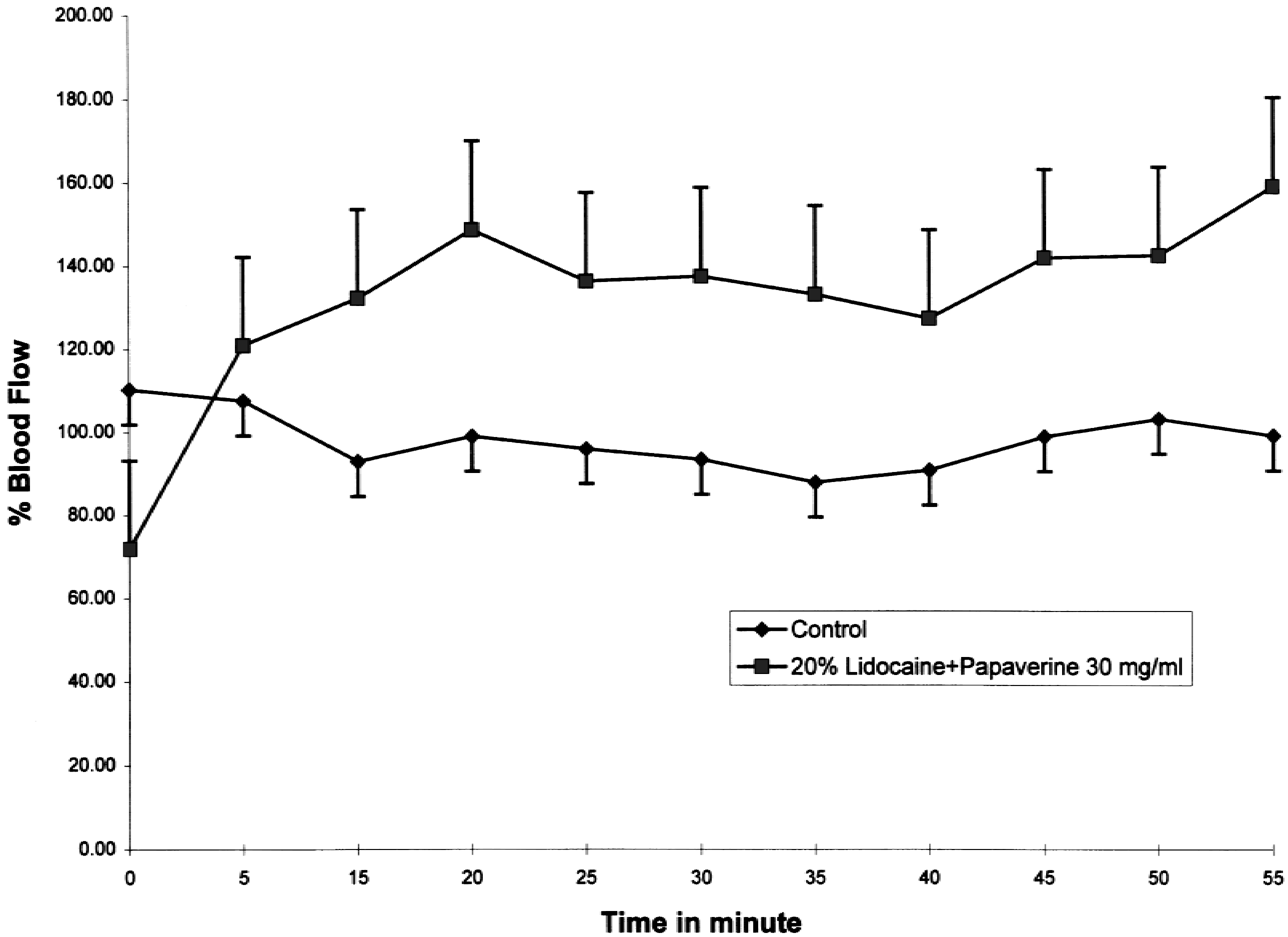

Figure 4. Effects of topical application of lidocaine (20%) in combination with papaverine (30 mg/ml) on the blood flow of the rabbit carotid artery after microvascular anastomosis. Application of the experimental drugs produces a significant increase in the blood flow in the carotid artery when compared to the control (P = 0.0001). Doppler values are given as means (SEM) and expressed as percentages of the preanastomotic blood flow readings.

lidocaine demonstrated a biphasic response; low concentrations (10^{-6}–10^{-4} M) increasing the norepinephrine-induced contraction, while higher concentrations relieved it. In the in vivo experiments, topical application of lidocaine 2% did not change blood flow through the microvascular anastomosis. Lidocaine 20%, papaverine, and lidocaine (2% or 20%) combined with papaverine all demonstrated beneficial effects in increasing blood flow in rabbit carotid arteries.

Lidocaine is a local anesthetic that acts as a stabilizer of the cell membrane. Its vasodilatory action has not been elucidated; however, it may work on nerves, smooth muscles, or both. The vasoconstrictive effects of lidocaine on in vitro human arteries and veins have been described[11,12,15] and lidocaine serum concentrations of 2×10^{-2} M have produced peripheral constriction in humans.[27] Other studies confirm a concentration-related ability of lidocaine to resolve ischemia.[28–30] These findings are in agreement with previous in vitro[10–12] and in vivo[5,6] studies demonstrating that the vasodilating properties of lidocaine are concentration-dependent. Low concentrations produce vasoconstriction and higher ones vasodilation.

Lidocaine 2% in this model did not alter the blood flow through anastomosed arteries; however, when combined with papaverine, vasodilation was observed. This suggests that the vasodilating effect of 2% lidocaine with papaverine may be due to the effects of papaverine and not lidocaine. Previous studies[2] demonstrated that the topical application of lidocaine after mechanically induced ischemia produced only partial relief of ischemia through vasodilation, followed by a long lasting vasoconstriction of the entire vessel. This same rebound effect was noted in human arteries and veins in vitro.[11,12] This vascular contraction observed after the lidocaine was flushed may account for the lack of blood flow increase in experimental random flaps.[5] It may be of importance in clinical microsurgery when initial vasodilation may be followed by a decrease of flap perfusion. This rebound effect was not observed in our model when lidocaine was flushed with the heparinized solution. This may be partially explained by the different model, the vessel chosen, the mechanism of vasospastic stimulation (a microvascular anastomosis vs. mechanical), the species specificity of lidocaine, or our small animal numbers.

Papaverine, a commonly used spasmolytic, is an opium alkaloid that exerts a direct action on vascular smooth muscle, probably by an inhibition of oxidative phosphorylation and calcium flux during muscle contraction.[17,20,31] It has been demonstrated that topical application of papaverine results in an increase of blood flow in the mammary artery in man and in the rabbit carotid artery after microvascular anastomosis.[16,19] Systemic administration has been shown to reverse cerebral[17,22,25,26] and internal mammary artery vasospasm[18] in man and experimental vasospasm in the basilar artery of dogs after a subarachnoid hemorrhage.[22] The failure of vasodilation with 2% lidocaine appears to be counteracted by the combination with papaverine. It is recommended that papaverine or lidocaine in high concentrations (20%) be used alone to relieve vasospasm.

CONCLUSIONS

Lidocaine at high concentration (20%) and papaverine (30 mg/ml) seem to be pharmacological tools capable of increasing the blood flow in anastomosed arteries. However, the biphasic effect of lidocaine, its concentration-dependent vascular action, and the described constrictive effects make 2% lidocaine a poor choice for a pharmacologic agent in the treatment of circulatory impairment during microsurgery.

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