Comparison of the Effect of Naloxone on Cerebral Versus Mesenteric Arterial Smooth Muscle in Feline and Primate Species

TOMIO SASAKI, M.D., NEAL F. KASSELL, M.D., DONN M. TURNER, M.D., AND HANS C. COESTER, B.S.

SUMMARY This study was conducted in order to investigate naloxone's in vitro action on both epinephrine-induced constriction of mesenteric artery and norepinephrine-induced constriction of cerebral arteries in different species (cat and monkey). Naloxone (3 × 10⁻⁵M) augmented the epinephrine-induced constriction of both feline and monkey mesenteric artery at epinephrine concentrations of 10⁻⁷ to 10⁻⁵M. Naloxone (3 × 10⁻⁵M) suppressed the constriction of feline basilar artery induced by high concentrations (10⁻⁴, 10⁻³ and 3 × 10⁻⁵M) of norepinephrine, while it failed to alter the constriction induced by lower concentrations (10⁻⁷ to 10⁻⁵M) of norepinephrine. The constrictor response of monkey basilar artery to norepinephrine (10⁻⁸ to 10⁻⁵M) was not altered by treatment with naloxone (3 × 10⁻⁴ and 3 × 10⁻⁵M). Such varying effects of naloxone in different tissues and species may have to be taken into account when evaluating the cerebral blood flow changes following naloxone administration.

Stroke Vol 15, No 6, 1984

THE OPIATE ANTAGONIST NALOXONE has recently been reported to inhibit the contractile response of canine cerebral artery to norepinephrine or epinephrine without altering the responses to KCL, serotonin or hemoglobin. This selective vasodilating effect of naloxone on norepinephrine-induced constriction of canine cerebral artery may, in part, participate in the improvement in cerebral blood flow (CBF) following naloxone administration. This hypothesis is supported by the observation that naloxone, in a high dose (10 mg/kg), causes an increase in CBF which is not accompanied by corresponding changes in cerebral metabolism. This phenomenon was also observed in dogs made acutely hypertensive by infusion of intravenous norepinephrine. However, there may be species differences in constrictor responses of cerebral arterial smooth muscle to naloxone, since varying effects of naloxone on CBF in different species have been demonstrated. Previous investigators have shown that naloxone increases CBF in dogs, decreases CBF in cats and fails to alter CBF in monkey or the human.

On the other hand, our recent study (unpublished, Sasaki, et. al.) has demonstrated that naloxone at concentrations of 3 × 10⁻⁵M to 3 × 10⁻⁴M selectively augments the constrictor responses of canine mesenteric artery to epinephrine. Such augmenting effects of naloxone observed in canine mesenteric artery have not yet been investigated in different species. The purpose of this study was to evaluate both the selective vasodilating effects of naloxone on norepinephrine-induced constriction of cerebral artery and the selective augmenting effects of naloxone on epinephrine-induced constriction of mesenteric artery in different species.

Materials and Methods

Adult cats and cynomolgus monkeys (macaca fascicularis) of either sex were anesthetized with sodium pentobarbitral (30 mg/kg) and sacrificed by exsanguination from the femoral artery. The basilar and mesenteric arteries from both species were rapidly removed. The arteries were dissected under magnification and placed immediately in oxygenated, nutrient Kreb's solution [(mM): NaCl, 120; KCl, 4.5; MgSO₄, 1.0; NaHCO₃, 27.0; KH₂PO₄, 1.0; CaCl₂, 2.5; and dextrose, 10.0] at 37°C, and gassed with 95% O₂ and 5% CO₂. The pH of the solution ranged from 7.40 to 7.50. Each artery was cut into 4 mm long ring segments which were suspended between L-shaped stainless steel holders in organ baths with a 10 ml working volume. Resting tension was adjusted to 2 g. The preparations were allowed to equilibrate at 37°C for 60 minutes before use. Dose-response curves for norepinephrine in basilar artery and for epinephrine in mesenteric artery were obtained by cumulative addition of the agonists. Contractile force was recorded isometrically using a Grass FT.03 force-displacement transducer. The transducer signal was then amplified and displayed on a Gould 260 multichannel recorder. Contractile activities of norepinephrine and epinephrine are expressed as a percentage of the contraction elicited by a standard dose of 40 mM KCl. This standard contraction by 40 mM KCl was obtained on each ring. In studies examining the effects of naloxone on the contraction induced by norepinephrine or epinephrine, preparations were exposed for five minutes to pure naloxone (3 × 10⁻⁵M) before addition of agonists. This particular concentration of naloxone was chosen since previous studies have demonstrated that naloxone at concentrations greater than 3 × 10⁻⁴M possesses nonspecific vasodilating effects, while naloxone at concentrations 3 × 10⁻⁵M to 3 × 10⁻⁴M selectively inhibits norepinephrine-induced constriction of canine basilar artery and selectively augments epinephrine-induced constriction of canine mesenteric artery. (Unpublished, Sasaki, et al).

Statistical analysis of the comparisons of control and treatment constrictor responses was done at each specific agonist concentration using a paired t-test, since the data were matched by preparation. For graphic
presentation, the control values were averaged together. When \( p \) was smaller than 0.05, values were considered to be significantly different. Computation of dose-response curves and \( ED_{50} \) values was done using probit analysis with the SAS (Statistical Analysis System) computer program. Drugs and solutions used in this study were (-)epinephrine (+) bitartrate (Sigma Chemical Co.), (±)-arterenol hydrochloride (Sigma Chemical Co.) and naloxone (Endo Laboratories, Inc. lot 82-037). Norepinephrine or epinephrine was dissolved using 50 mM phosphate buffer solution (pH 7.4). Naloxone was dissolved in saline.

**Results**

**Effects of Naloxone on Norepinephrine-induced Constriction of Basilar Artery**

The constrictor responses of feline basilar artery to low concentrations (<\( 10^{-5} \)M) of norepinephrine was poor, but it responded fairly well to higher concentrations (\( 10^{-4}, 10^{-3} \) and \( 3 \times 10^{-3} \)M) of norepinephrine. Naloxone at concentration of \( 3 \times 10^{-5} \)M suppressed the contraction of feline basilar artery induced by high concentrations (\( 10^{-4}, 10^{-3} \) and \( 3 \times 10^{-3} \)M) of norepinephrine while it failed to alter the contraction induced by lower concentrations (\( 10^{-7}, 10^{-6} \) and \( 10^{-5} \)M) of norepinephrine (fig. 1). Fifteen minutes after changing nutrient Kreb’s solution in the bath norepinephrine dose-response curve returned to original control values.

Monkey basilar artery responded well to norepinephrine. The \( ED_{50} \) value (95% confidence interval) of norepinephrine was 1.00 (0.7-1.6) \( \times 10^{-7} \)M. As shown in figure 2, naloxone at concentrations of \( 3 \times 10^{-6} \)M and \( 3 \times 10^{-5} \)M did not show any effect on the norepinephrine-induced constriction of monkey basilar artery at each norepinephrine concentration tested (\( 10^{-8} \) to \( 10^{-5} \)M).

**Effects of Naloxone on Epinephrine-Induced Constriction of Mesenteric Artery**

The constrictor responses of mesenteric artery to epinephrine were almost equal between cat and monkey. The \( ED_{50} \) values (95% confidence interval) of epinephrine in cat and monkey were 1.76 (0.85-3.67) \( \times 10^{-6} \)M and 2.32 (1.23-4.45) \( \times 10^{-6} \)M, respectively.

Naloxone at \( 3 \times 10^{-5} \)M augmented the epinephrine-induced constriction of feline mesenteric artery at epinephrine concentrations of \( 10^{-7}, 3 \times 10^{-7}, 10^{-6} \) and \( 3 \times 10^{-6} \)M (fig. 3). The epinephrine-induced constriction of monkey mesenteric artery was also augmented by \( 3 \times 10^{-5} \)M naloxone at epinephrine concentrations of \( 10^{-7} \) to \( 10^{-3} \)M (fig. 4). The constrictor responses of both feline and monkey mesenteric artery to high concentrations of epinephrine, however, were not altered by the treatment with \( 3 \times 10^{-5} \)M naloxone. The epinephrine dose-response curve returned to control values fifteen minutes after changing the nutrient Kreb's solution in the bath.

**Discussion**

The present results demonstrated that naloxone (\( 3 \times 10^{-5} \)M) suppressed the constrictor responses of feline basilar artery to high concentrations (\( 10^{-4}, 10^{-3} \) and \( 3 \times 10^{-3} \)M) of norepinephrine, while it failed to alter the responses to lower concentrations of norepinephrine. These findings may indicate the norepinephrine dose-response curve in the feline basilar artery could be resolved into two components: naloxone sen-
These findings indicate that naloxone exerts differing effects on norepinephrine-induced constriction of cerebral artery among species. Such varying effects of naloxone on the norepinephrine-induced constriction of cerebral artery may have to be taken into account in some species when evaluating the changes of CBF following naloxone administration. In dogs and monkeys, naloxone's action on norepinephrine-induced constriction of cerebral artery appears to be compatible with CBF changes following naloxone administration. In dogs, where naloxone showed a vasodilating effect on the norepinephrine-induced constriction of cerebral artery, it has been reported that CBF was increased by naloxone administration without corresponding changes in cerebral metabolism. In monkeys, where naloxone failed to alter the constrictor responses of cerebral artery to norepinephrine, CBF was not changed by naloxone administration.

In cats, however, naloxone's action on norepinephrine-induced constriction of the cerebral artery does not appear to be consistent with the CBF changes following naloxone administration. The present results demonstrated naloxone's vasodilating effect on norepinephrine-induced constriction of cerebral artery in vitro, while it has been reported by Grandison et al that CBF and brain metabolism were decreased by naloxone. Therefore, the vasodilating effect of naloxone on the norepinephrine-induced constriction in vitro seems to contradict the CBF changes in cats following naloxone administration. The reduction of CBF...
may be due to a reduction in brain metabolism which results in cerebral vasoconstriction through the metabolic component of autoregulation overriding the vasodilation caused by naloxone.

The effects of naloxone on epinephrine or norepinephrine-induced contraction were quite different when comparing the cerebral artery to the mesenteric artery. Naloxone ($3 \times 10^{-5}$) augmented the constrictor responses of either feline or monkey mesenteric artery to low concentrations of epinephrine. It has also been demonstrated that naloxone ($3 \times 10^{-7}$ to $3 \times 10^{-5}$) selectively augmented the epinephrine or norepinephrine-induced constriction of canine mesenteric artery (unpublished data, Sasaki, et al). Therefore, there seems to be no species differences in naloxone's augmenting effects on epinephrine-induced constriction of mesenteric artery. Those augmenting effects may, in part, participate in the improvement of systemic circulation following naloxone administration. Our recent work (unpublished, Sasaki, et al) has suggested that this augmenting effect of naloxone on the epinephrine-induced constriction results from inhibition of extrasynaptic uptake of catecholamines since the augmentation of norepinephrine-induced constriction of canine mesenteric artery by naloxone was inhibited by the pre-treatment of the specimens with normetanephrine.

In summary, the present results revealed the varying effects of naloxone on epinephrine or norepinephrine-induced constriction of arterial specimens in different tissues and species. Such varying effects of naloxone may have to be taken into account in some species when evaluating the changes of CBF following naloxone administration.

References
Comparison of the effect of naloxone on cerebral versus mesenteric arterial smooth muscle in feline and primate species.

T Sasaki, N F Kassell, D M Turner and H C Coester

Stroke. 1984;15:1025-1028
doi: 10.1161/01.STR.15.6.1025

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://stroke.ahajournals.org/content/15/6/1025