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

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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 x 10^-5M) augmented the epinephrine-induced constriction of both feline and monkey mesenteric artery at epinephrine concentrations of 10^-3 to 10^-5M. Naloxone (3 x 10^-5M) suppressed the constriction of feline basilar artery induced by high concentrations (10^-4, 10^-3 and 3 x 10^-3M) of norepinephrine, while it failed to alter the constriction induced by lower concentrations (10^-5 to 10^-4M) of norepinephrine. The constrictor response of monkey basilar artery to norepinephrine (10^-5 to 10^-4M) was not altered by treatment with naloxone (3 x 10^-4 and 3 x 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.

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. 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,2,4 decreases CBF in cats5 and fails to alter CBF in monkey6 or the human.7

On the other hand, our recent study (unpublished, Sasaki, et al.) has demonstrated that naloxone at concentrations of 3 x 10^-3M to 3 x 10^-5M 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 pentobarbital (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 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 x 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 x 10^-4M possesses nonspecific vasodilating effects, while naloxone at concentrations 3 x 10^-5M to 3 x 10^-4M selectively inhibits norepinephrine-induced constriction of canine basilar artery1 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 togeth-
er. When p was smaller than 0.05, values were consid-
ered to be significantly different. Computation of dose-
response curves and ED50 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 dis-
solved 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 concen-
trations (10^{-4}, 10^{-3} and 3 × 10^{-3}M) of norepineph-
rine. Naloxone at concentration of 3 × 10^{-5}M sup-
pressed the contraction of feline basilar artery induced
by high concentrations (10^{-4}, 10^{-3} and 3 × 10^{-5}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 nor-
epinephrine dose-response curve returned to original
control values.

Monkey basilar artery responded well to norepi-
nephrine. The ED50 value (95% confidence interval) of
norepinephrine was 1.00 (0.7-1.6) × 10^{-7}M. As
shown in figure 2, naloxone at concentrations of 3  ×
10^{-6}M and 3 × 10^{-5}M did not show any effect on the
norepinephrine-induced constriction of monkey basi-
lar 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 ED50 values (95% confidence interval)
of epinephrine in cat and monkey were 1.76 (0.85-
3.67) × 10^{-6}M and 2.32 (1.23-4.45) × 10^{-6}M,
respectively.

Naloxone at 3 × 10^{-5}M augmented the epineph-
rine-induced constriction of feline mesenteric artery at
epinephrine concentrations of 10^{-7}, 3 × 10^{-7}, 10^{-6}
and 3 × 10^{-6}M (fig. 3). The epinephrine-induced
constriction of monkey mesenteric artery was also aug-
menced by 3 × 10^{-5}M naloxone at epinephrine con-
centrations 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 × 10^{-5}M naloxone.
The epinephrine dose-response curve returned to con-
trol values fifteen minutes after changing the nutrient
Kreb’s solution in the bath.

Discussion

The present results demonstrated that naloxone (3
× 10^{-5}M) suppressed the constrictor responses of fe-
lime basilar artery to high concentrations (10^{-4}, 10^{-3}
and 3 × 10^{-5}M) of norepinephrine, while it failed to
alter the responses to lower concentrations of norepi-
nephrine. These findings may indicate the norepineph-
rine dose-response curve in the feline basilar artery
could be resolved into two components: naloxone sen-
These findings indicate that naloxone exerts contrasting 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 feline 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 mechanism of the vasodilating effects of naloxone on the constrictor responses to high concentrations of norepinephrine is unknown, though we have previously demonstrated that the vasodilating effect of naloxone on norepinephrine-induced constriction of canine basilar artery does not result from antagonistic action on opiate receptors, or from direct inhibition of α-adrenoceptors, or direct stimulation of β-adrenoceptors in cerebral arterial smooth muscle. Further studies will be necessary in order to investigate the precise mechanism of this vasodilating effect of naloxone.

The present results also demonstrated that the constrictor responses of monkey basilar artery to norepinephrine were not altered by the naloxone treatment.
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 extraneuronal 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

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