Responsiveness of Equine Basilar Artery to Transmural Nerve Stimulation Differs from That of Porcine and Bovine Basilar Arteries in Vitro
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(Received 3 October 1994/ Accepted 9 December 1994)

ABSTRACT. Transmural nerve stimulation (TNS) induced relaxations in porcine and bovine basilar arteries which were abolished by tetrodotoxin (TTX) and by L-nitro-arginine (LNAG). However, TNS induced contractions in equine basilar artery which were abolished by TTX and by guanethidine, but not by LNAG. These results suggest that the TNS-induced contractions of equine basilar arteries may be mediated by norepinephrine. KEY WORDS: equine basilar artery, norepinephrine, transmural nerve stimulation.


It has been reported that basilar arteries isolated from dogs [9, 10], pigs [1, 3, 4], oxen [8] and monkeys [9, 11] respond to transmural nerve stimulation (TNS) with relaxations and that the neurotransmitter responsible may be nitric oxide (NO).

The aim of the present study was to characterize TNS-induced responses of equine basilar artery and to compare them with those of porcine and bovine basilar arteries.

Basilar arteries of freshly slaughtered pigs, oxen and horses were obtained at an abattoir and transferred to our laboratory immersed in ice-cold physiological salt solution (mM: NaCl 119, KCl 4.7, CaCl₂ 1.6, MgCl₂ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2 and glucose 10) aerated with a mixture of 95% O₂ and 5% CO₂. Each basilar artery was dissected free, the adhering tissues were removed and rings (outer diameter: 0.5-0.8 mm in pigs, 1.0-1.4 mm in oxen and 1.0-1.5 mm in horses) about 4 mm long were cut off. Each ring was subjected to endothelial denudation by gently rubbing the intimal surface of a stainless steel rod with a diameter equivalent to that of the arterial lumen and then mounted vertically between two L-shaped stainless steel holders, the upper of which was fixed to an isometric force transducer (TB-611T, Nihon Kohden, Japan) and suspended in a 10-mL water-jacketed organ bath containing oxygenated physiological salt solution at 37°C (pH 7.4). The mounted rings were left to equilibrate for at least 120 min under a constant tension of 7.5 mN for porcine basilar artery or 10 mN for bovine and equine basilar arteries. Each resting tension was optimal for inducing maximal contractions to potassium chloride (KCl, 60 mM). Sixty mM KCl was applied every 30 min until the contraction amplitudes were constant. The change in the KCl concentration of the physiological salt solution was compensated for by an equimolar adjustment of the NaCl concentration. The isometric tension was displayed on an ink-writing recorder (WI-641G, Nihon Kohden).

A pair of parallel platinum stimulating electrodes was placed on either side of the artery. A cathodal wire coated with enamel except for the cut point was allowed to gently touch the surface of the artery, and an anodal plate was placed at a distance of approximately 2 mm from the arterial surface. Electrical stimulation with 0.2 ms square-wave pulses of 20 V and 20 Hz for 20 s, was applied to the periarterial nerves with these two platinum electrodes via a stimulator (SEN-2101, Nihon Kohden). These stimulus conditions were selected to elicit a maximal response.

The absence of endothelial cells was confirmed by testing no relaxant response to bradykinin (10⁻⁷ M) for the porcine artery or sodium fluoride (NaF, 2×10⁻² M) for the bovine and equine arteries [5, 6], and morphologically by scanning and transmission electron microscopy after the experiments. Some arterial rings from all three species were stored in physiological salt solution at 4°C for 7 days to achieve cold storage denervation [2, 7].

The following drugs were used: guanethidine sulfate, bradykinin acetate, tetrodotoxin, L-nitro-arginine (LNAG), D-arginine hydrochloride (Sigma, U.S.A.), (-)-phenylephrine hydrochloride (Wako, Japan), sodium fluoride, L-arginine, papaverine hydrochloride (Nacalai, Japan) and prostaglandin (PG) F₂α (Ono, Japan).

Figure 1 shows typical responses of porcine, bovine and equine basilar arterial rings without endothelium to TNS before and after contraction with PGF₂α (10⁻⁷ M for porcine, 10⁻⁶ M for bovine and equine basilar arteries). These concentrations of PGF₂α were chosen to produce stable contractions corresponding approximately 50% of the maximal responses elicited by 60 mM KCl. TNS induced porcine basilar arterial relaxations under resting and PGF₂α-precontracted conditions, whereas bovine arterial rings responded to TNS with weak relaxations under resting conditions, but clear relaxations when precontracted with PGF₂α. Equine basilar arterial rings, however, responded to TNS with contractions under both resting and PGF₂α-precontracted conditions. Similar results were obtained with arteries from five animals of each species. The TNS-induced relaxations and contractions were abolished by treatment with tetrodotoxin (10⁻⁶ M) and cold storage denervation (n=5 each, data not shown). These results indicate that the TNS-induced responses in the three species were of neurogenic origin. The porcine and bovine basilar arterial relaxations were abolished or inhibited markedly by LNAG (10⁻³ M), the inhibitory effects of LNAG were reversed by L-arginine (10⁻³ M), but not by D-arginine (10⁻³ M) (n=5 each, data not shown). These results suggest that NO may be involved in the nerve-mediated relaxations of porcine and bovine basilar arteries. The TNS-induced contractions of equine basilar arteries were not influenced by treatment with LNAG, suggesting that NO plays no significant...
Fig. 1. Typical responses of porcine (A), bovine (B) and equine (C, D) basilar arterial rings without endothelium to transmural nerve stimulation (+ S) before and after contraction with PGF2\textalpha\ (10^{-6} M for pig and 10^{-5} M for ox and horse). The concentrations of PGF2\textalpha\ were chosen to produce stable contractions corresponding to approximately 50% of the maximal responses elicited by 60 mM KCl. In D, the tension level before the addition of PGF2\textalpha\ is indicated by a horizontal line on the left of the recording trace. S; transmural nerve stimulation with square-wave pulses (0.2 ms duration, 20 V) at 20 Hz for a period of 20 s as indicated by bars. TTX; tetrodotoxin (10^{-4}M), PPV; papaverine (10^{-4}M), LNAG; L-nitro-arginine (10^{-5}M).

Fig. 2. A typical response of an equine basilar arterial ring without endothelium to transmural nerve stimulation (+ S) before and after treatment with guanethidine (10^{-6} M). Norepinephrine (●) at concentrations as marked induced a stepwise-increased contraction. Transmural nerve stimulation was made in the same way as in Fig. 1.

role in the contractile response.

Figure 2 shows a typical response of an equine basilar arterial ring without endothelium to TNS before and after treatment with guanethidine (10^{-6} M). The TNS-induced contraction was abolished by guanethidine treatment, whereas this allowed cumulative application of norepinephrine (10^{-7} - 10^{-3} M) to produce a stepwise-increased contraction. These results suggest that the TNS-induced contractions of equine basilar arteries may depend, to a large extent, on the release of norepinephrine. The TNS-induced relaxations of porcine and bovine basilar arteries were not affected significantly by guanethidine treatment (n=5 each, data not shown).

In conclusion, porcine and bovine basilar arteries responded to TNS with relaxations, whereas equine basilar arteries responded with contractions. The TNS-induced contraction of equine basilar arteries may be mediated by norepinephrine release.

REFERENCES


