EXPERIMENTAL STUDY

In vitro vasoactive effects of dexmedetomidine on isolated human umbilical arteries

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ABSTRACT

OBJECTIVE: We aimed to investigate the vasoactive effects of dexmedetomidine on isolated human umbilical arteries and possible mechanisms involved.

METHODS: Human umbilical artery strips were suspended in Krebs-Henseleit solution and dose-response curves were obtained for cumulative dexmedetomidine before and after incubation with different agents: propranolol, atropine, yohimbine, prazosin, indomethacin, verapamil. Effects of calcium on cumulative dexmedetomidine-induced contractions were also studied.

RESULTS: Cumulative dexmedetomidine resulted in dose dependent contraction responses. Incubation with propranolol (Emax: 93.3 ± 3.26 %), atropine (Emax: 92.0 ± 6.54 %), or indomethacin (Emax: 94.25 ± 2.62 %), did not attenuate dexmedetomidine-elicited contractions (p > 0.05). There were significant decreases in the contraction responses of cumulative dexmedetomidine with yohimbine (Emax: 12.1 ± 11.9 %), prazosin (Emax: 28.8 ± 4.6 %) and verapamil (Emax: 11.2 ± 13.6 %) (p < 0.05). In Ca+2 free medium contraction responses to cumulative dexmedetomidine was insignificant (Emax: 5.20 ± 3.42 %). Addition of cumulative calcium to the Ca+2 free medium resulted in concentration dependent increase in contractions (Emax: 64.83 ± 37.7 %) (p < 0.05).

CONCLUSION: Dexmedetomidine induces vasoconstriction in endothelial-free umbilical arteries via both, α1- and α2-adrenergic receptors and also extracellular Ca+2 concentrations play a major role. β-adrenergic receptors, muscarinic cholinergic receptors, and inhibition of cyclooxygenase enzyme are not involved in this vasconstriction (Fig. 3, Ref. 36).

KEY WORDS: alpha adrenergic receptors, dexmedetomidine, in vitro, umbilical cord, vascular smooth muscle.

Introduction

Dexmedetomidine, an α2-adrenergic receptor agonist, has been used for sedation and analgesia and as an adjunct to anesthesia because of its sedative, analgesic and sympatholytic properties (1). The α2:α1 adrenoreceptor specificity ratio of dexmedetomidine is ten times than clonidine (1,600 : 1) (2). This α2 specificity and a short half-life of six minutes make dexmedetomidine ideal for intravenous titration for sedation and anxiolysis. The Food and Drug Administration (FDA) has approved dexmedetomidine for limited use for sedation, mechanical ventilation and monitored anesthesia care in adults (3). There are current reports on the intraoperative use of dexmedetomidine for various indications including Cesarean sections (4–8). It is crucial to elucidate the possible effects of dexmedetomidine on the uterus and fetal circulation.

Dexmedetomidine increases uterine contractility (9) and is transported into the fetal circulation in small amounts (10). Except for the segment that is closest to the fetus, the umbilical cord and the placenta do not contain nerve fibers and are not innervated. The umbilical blood flow mainly depends on local vasoconstrictors such as endothelin-1 and thromboxane and also vasodilators such as prostacyclin (PGL) and nitric oxide (NO) (11, 12). The direct effects of vasoactive agents on umbilical vessels are essential due to lack of autonomic innervations (13).

There is no information on the direct effects of dexmedetomidine on human umbilical vessels. This in vitro study was designed to investigate the vasoactive effects of dexmedetomidine on isolated human umbilical arteries and possible mechanisms involved.

Materials and methods

The Institutional Human Ethics Committee approved this study. The umbilical cords were remnant tissues which would otherwise been discarded.

Collection of samples

After written maternal consent, human umbilical cords were collected from full-term healthy normal vaginal deliveries. After delivery, the umbilical cord was clamped at both placental and fetal ends. An untouched 10–15 cm long segment of the cord from the
placental end was removed within 10 min of delivery and placed in cold Krebs-Henseleit (KH) solution for immediate transport to the laboratory.

**Blood vessel preparation**

Umbilical arteries were separated from the surrounding tissue in KH solution. The isolated artery was cut spirally to form 2–3 mm wide and 15–20 mm long strips. The strips were transferred to organ baths containing 20 ml of KH solution maintained at 37 °C. Then the strips were suspended between two hooks; one anchored onto the organ bath and the other connected to a transducer. Smooth muscle contractions were recorded with a force-displacement transducer and digitized data acquisition system (MP35, BIOPAC, Goleta, CA, USA). Strips were aerated with a gas mixture of 95% O2: 5% CO2 throughout the experiment. Strips were initially placed under a resting tension of 1 g and were allowed to equilibrate for one hour. During this period the bath solution was changed every 15 minutes, and the resting tension was readjusted to the 1 g level.

**Experimental protocol**

Experimental procedures are summarized in Figure 1. Human umbilical artery strips without endothelium were used during the experiment. Endothelium removal was done by gently denuding the endothelium with cotton swabs. Following the washout period endothelium removal of the strips was examined by contracting the strips with phenylephrine (10⁻⁵ M) and testing with acetylcholine (10⁻⁶ M). Strips free of endothelium did not relax with acetylcholine. The strips were rewashed with the buffer solution and allowed to rest. Strips were randomly allocated to study groups.

To assess the possible mechanisms of dexmedetomidine’s vascular effects, first, the reactivity of the human umbilical artery to dexmedetomidine was examined. The strips at resting tension in each study group (a to g) (n = 6 or n = 5) were subjected to cumulative concentrations of dexmedetomidine (10⁻⁹ – 2x10⁻⁵ M), and dose-response curves were recorded. After washing and allowing the strips to rest for one hour, response curves of cumulative dexmedetomidine (10⁻⁹ – 2x10⁻⁵ M) were obtained in these groups after the strips were incubated for 20 min with different agents: a) propranolol (10⁻⁶ M) (n = 6) a non-selective β-adrenergic antagonist, b) atropine (10⁻⁶ M) (n = 6) a competitive antagonist of muscarinic cholinergic receptors, c) yohimbine (10⁻⁶ M) (n = 6) an α₂-adrenergic antagonist, d) prazosin (10⁻⁵ M) (n = 5) an α₁-adrenergic antagonist, e) indomethacin (10⁻⁶ M) (n = 5) a cyclooxygenase enzyme inhibitor, f) verapamil (10⁻⁶ M) (n = 5) a L-type voltage-sensitive Ca²⁺ channel blocker, and g) in Ca²⁺ free modified KH solution (n = 6). The effect of Ca²⁺ was assessed by first recording 2x10⁻⁵ M dexmedetomidine-induced control contraction in KH solution. The strips were washed and allowed to rest in Ca²⁺ free KH solution for one hour. At the end of the resting period, dose-response curves of cumulative dexametomidine (10⁻² – 2x10⁻¹ M) in Ca²⁺ free medium were recorded.
After recording the contraction to the maximum concentration of dexmedetomidine (2x10^{-5} M) in Ca^{2+} free medium, contraction responses were obtained by adding cumulative Ca^{2+} (10^{-4}–10^{-2} M) into the media. Each strip was used for only one experiment. Results are given as % of maximum contraction induced by 2x10^{-5} M dexmedetomidine in each group. Wilcoxon rank test was used for analysis, a p-value less than 0.05 was considered as significant.

Fig. 2. Effects of incubation with different agents on cumulative dexmedetomidine induced contractions in human umbilical arteries. Groups: propranolol (a), atropine (b), yohimbine (c), prazosin (d), indomethacin (e) or verapamil (f). Results are % of 2 x 10^{-5} M dexmedetomidine induced maximum contraction (mean ± SD), dex: dexmedetomidine, * p < 0.05 compared to same concentration of dexmedetomidine.

After recording the contraction to the maximum concentration of dexmedetomidine (2x10^{-4} M) in Ca^{2+} free medium, contraction responses were obtained by adding cumulative Ca^{2+} (10^{-4}–10^{-2} M) into the media.

Each strip was used for only one experiment. Results are given as % of maximum contraction induced by 2x10^{-5} M dexmedetomidine in each group. Wilcoxon rank test was used for analysis, a p-value less than 0.05 was considered as significant.
soconstriction. Our results also demonstrate that dexmedetomidine leads to vasoconstriction in endothelium-denuded umbilical artery strips via both the $\alpha_1$ and $\alpha_2$-adrenergic receptors at both low and high concentrations. These results are in part different from previous research which reported that through postsynaptic $\alpha_2$-receptor activation, dexmedetomidine causes vasoconstriction in various human and animal vessels, including coronary arteries, peripheral arterioles, cerebral arteries, gastroepiploic and brachial arteries (14–17). According to some of these studies, $\alpha_1$-adrenergic receptors contribute to vasoconstriction at high concentrations of dexmedetomidine (15, 17). In a study showing the vasoconstrictor effect of dexmedetomidine on human internal mammary artery (IMA), yohimbine attenuated the contraction resulting from lower doses of dexmedetomidine, whereas prazosin attenuated contraction resulting at higher doses of dexmedetomidine. Their data suggest that dexmedetomidine causes contraction by activating $\alpha_2$-adrenergic receptors at lower concentrations, but it may also activate $\alpha_1$-receptors at higher concentrations in IMA (15). It is suggested that the contractile adrenergic receptors in the human umbilical artery consist of both $\alpha_1$ and $\alpha_2$ subtypes (18).

In the present study $\alpha_1$-adrenergic receptor antagonist prazosin significantly inhibited the constriction at both low and high concentrations of dexmedetomidine; suggesting that both $\alpha_1$-receptors, as well as $\alpha_2$-receptors, are involved in the vasoconstrictor effect of dexmedetomidine on the umbilical artery.

The present study was conducted on endothelium denuded vascular rings, which provides us to study the direct effects of drugs on vascular smooth muscle by removing endothelial factors. In vivo, $\alpha_2$-adrenergic agonists can have different results. They may diminish norepinephrine release by prejunctional $\alpha_2$-adrenergic receptor stimulation, thereby decreasing local and circulating catecholamines (19, 20) or may activate $\alpha_1$-adrenergic receptors on endothelial cells, resulting in the release of endothelium-derived NO (14). Vasoconstriction is controlled by calcium-dependent and calcium sensitization mechanisms (21). Calcium influx from the ex-
tracellular space and release from the sarcoplasmic reticulum are associated with intracellular free Ca²⁺ concentrations. Dexametomidine-induced vasoconstriction involves calcium sensitization mediated by Rho kinase, protein kinase C, and phosphoinositide 3-kinase (22). In the present study, verapamil and Ca²⁺ free medium resulted in minor contractions. Incubation with verapamil and Ca²⁺ free medium significantly decreased but did not block these contractions suggesting significant involvement of extracellular Ca²⁺ rather than release from intracellular Ca²⁺ stores. Some of the effects of postsynaptic α₁-adrenergic receptors on vascular smooth muscle cells are mediated by molecular mechanisms that are common to α₁ and α₂ adrenergic receptors (23). Different G protein signal transduction pathways mediate the primary coupling of α₁ receptors signal through Gq-proteins (24), activate smooth muscle 3-kinase (22). In the present study, verapamil and Ca²⁺ free medium denuded vasculature aiming to study the direct effects of vasoactive substances of the endothelium such as NO. Dexmedetomidine-induced vasoconstriction is different from our in-vitro results. It was conducted on endothelium denuded vasculature aiming to study the direct effects of drugs on vascular smooth muscle. Our results were not affected by the vasoactive substances of the endothelium such as NO. Dexametomidine possesses an imidazole ring in its structure, and vascular Kᵥᵣ channel inhibition may be an underlying mechanism of the vasoconstriction (36). The present study did not assess the K⁺ channel activity.

Conclusion

Different from other vasculature, dexmedetomidine has a concentration-dependent vasoconstrictive effect on the endothelial-free umbilical artery via both α₁ and α₂ adrenergic receptors. Dexametomidine-induced vasoconstriction is dependent on extracellular Ca²⁺ concentrations and calcium influx via L-type voltage-sensitive Ca²⁺ channels. Also β-adrenergic receptors, muscarinic cholinergic receptors and inhibition of cyclooxygenase enzyme are not involved in direct mechanisms regulating the effects of dexmedetomidine on smooth muscle vascular tone of the umbilical artery.

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