

Locally Released Norepinephrine in the Oxygen-Dependent Regulation of Vascular Tone of Human Umbilical Vein

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ABSTRACT

In a previous study, human umbilical vein preparations constricted at P_{O_2} values above the physiologic intrauterine P_{O_2} range and dilated at hypoxia. Denudation of the endothelium reversed the hypoxic vasodilatation only, suggesting the release of a nonendothelial vasoconstrictor. We therefore hypothesized that norepinephrine from adrenergic nerve terminals could be responsible for the observed constricting effects. We measured intracellular membrane potential and isometric tension of human umbilical vein strips with and without functional adrenergic denervation by 6-OH-dopamine during variation of P_{O_2} . With P_{O_2} increasing from 5 to 104 mm Hg, intact preparations depolarized of from -58.6 ± 1.1 mV (SEM) to -53.3 ± 1.0 mV ($p < 0.001$) and isometric tension increased from 0.673 ± 0.037 g to 0.825 ± 0.044 g ($p < 0.02$). Intact preparations pretreated with 6-OH-dopamine depolarized from -58.0 ± 0.5 mV to

-55.8 ± 0.6 mV ($p < 0.01$), and isometric tension increased from 0.598 ± 0.040 g to 0.661 ± 0.018 g ($p < 0.02$). At P_{O_2} values above the physiologic intrauterine umbilical venous P_{O_2} range, membrane potential and isometric tension were significantly lower in preparations with 6-OH-dopamine pretreatment compared with matched controls ($p < 0.05$). Denudation of the endothelium reversed the hypoxic hyperpolarization and vasodilatation observed in intact preparations. However, membrane potential and isometric tension were not different between endothelium-denuded preparations with and without 6-OH-dopamine pretreatment. We conclude that locally released norepinephrine contributes to the depolarization and vasoconstriction of the human umbilical vein at hyperoxia but does not antagonize the endothelium-dependent vasodilatation at hypoxia. (*Pediatr Res* 55: 267–272, 2004)

The contribution of vascular tone of the umbilical vein to the regulation of blood flow from the placenta to the fetus had been regarded as negligible, because the umbilical vein was thought to be maximally dilated already at resting conditions (1, 2). However, in a recent study, we demonstrated that human umbilical vein was far from being a passive conduit but was able to adjust its vascular tone in response to changes in local P_{O_2} (3). Increasing P_{O_2} to values above the range present under physiologic intrauterine conditions resulted in constriction of human umbilical vein vascular strips, whereas hypoxia led to vasodilatation. In endothelium-denuded preparations, we saw not only a lack of hypoxic vasodilatation but also an increase in isometric tension at low P_{O_2} values. These findings were consistent with the action of a vasoconstricting factor, which resulted in human umbilical vein vasoconstriction at hyperoxia and which was more than antagonized by endothelial factors mediating hypoxic vasodilatation [e.g. prostacyclin (4), nitric

oxide (5), endothelium-derived hyperpolarizing factor (6, 7), the endothelin-B receptor-mediated actions of endothelin (8)].

We hypothesized that endogenous norepinephrine, released from adrenergic nerve terminals that remain in the vessel wall after dissection procedures (9), could account for the observed vasoconstricting effects. However, there is conflicting evidence from anatomical studies whether the human umbilical vein has any autonomic innervation in the extracorporeal part of the cord vessel (10–15). In this functional study, pretreatment of human umbilical vein preparations with 6-OH-dopamine was chosen to produce functional *in vitro* adrenergic denervation (9). We thus investigated a possible depolarizing and vasoconstricting contribution of locally released norepinephrine to the oxygen-dependent regulation of human umbilical vein vascular tone. On the one hand, we tested whether the depolarization and increase in isometric tension of intact preparations at high P_{O_2} were inhibited by pretreatment with 6-OH-dopamine. On the other hand, we tested whether the depolarization and increase in isometric tension of endothelium-denuded preparations at low P_{O_2} were diminished after pretreatment with 6-OH-dopamine.

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METHODS

Vessel dissection. Human umbilical cords were obtained from normal full-term deliveries after uneventful pregnancies with no evidence of hypertension in pregnancy or other pregnancy-related diseases. Additional characteristics of the newborns are summarized in Table 1. The study was approved by the local ethical committee. Within the first minutes of birth, cord segments were cut at least 10 cm from the infants, because this part of the human umbilical vein is thought definitely to be devoid of nerves (11, 15). Segments were placed into a modified Krebs solution (in mmol/L: 151 Na⁺, 4.69 K⁺, 2.52 Ca²⁺, 1.1 Mg²⁺, 145 Cl⁻, 16.3 HCO₃⁻, 1.38 H₂PO₄⁻, and 7.7 glucose) and stored at 4°C for less than 48 h until the experiments were done. Systematic studies had shown that up to 3 d of storage of blood vessels under these conditions does not affect vessel function (16). At the end of our experiments, we added 0.05 M of KCl to the bath solution to test the viability of the preparations. All preparations responded with vigorous contractions.

After carefully separating the vein from the Wharton's jelly, vascular rings, 0.5 cm in height, were allowed to equilibrate in Krebs solution gassed with 4% oxygen, 5% carbon dioxide, and balance nitrogen at 37°C for 2 h. Vascular rings were then cut lengthwise to create vascular strips.

Removal of the endothelium. For experiments with endothelium-denuded preparations, the endothelial layer was gently scraped off by scalpel just before the experiments. In a previous study, we had confirmed the effectiveness of our method to remove completely the endothelium as well as the integrity of the endothelium in the intact preparations (17).

Registration of isometric tension and of intracellular membrane potential. Vascular strips were attached to an isometric tension measuring device (KWS 522.C, K 52 C; Hottinger Baldwin, Darmstadt, Germany) recording the tension developed by the circularly oriented smooth muscle cells, which provide the main portion of the muscular layer of the umbilical vein vessel wall (18). Specimens were superfused in a bathing chamber by Krebs solution with a constant flow of 30 mL/min, applying a pretension of 1 g [equivalent to the wall stress of human umbilical vein under resting conditions according to the LaPlace relationship applied to a cylindrical vessel (19)]. This pretension was adjusted repeatedly during an initial period of 5 min.

Table 1. Characteristics of the newborns from whom the umbilical cords were obtained

	Sex	Gestational age (wk) median (range)*	Birth weight (g) median (range)*
Intact controls (n = 29)	16 girls, 13 boys	39 (37–42)	3570 (2650–4370)†
Endothelium-denuded controls (n = 23)	13 girls, 10 boys	39 (37–40)	3240 (2650–4650)‡

* Gestational age and birth weight were not different between intact and endothelium-denuded controls.

† All appropriate for gestational age, except five large-for-gestational-age newborns.

‡ All appropriate for gestational age, except three large-for-gestational-age newborns.

To record intracellular membrane potential simultaneously with mechanical tension, we inserted glass microelectrodes filled with 3 M of KCl into the venous smooth muscle cells from the intimal surface by means of a micromanipulator (19, 20). The electrodes were shielded to just below the tips. Their resistances ranged from 60 to 100 MΩ. Membrane potential was displayed at a large-screen oscilloscope (SGM 43 BN 901 S/H 293; Knott, Munich, Germany) and recorded by a personal computer. A sudden negative potential change was used as the criterion of a successful intracellular insertion, which reverted to zero upon withdrawal of the microelectrode.

To ensure that membrane potential recordings were derived from vascular smooth muscle cells, we excluded values > -45 mV from our final analysis (<5% of all recordings), because cultured human umbilical vein endothelial cells generate a resting potential of -25 mV (21, 22), whereas human venous smooth muscle cells generate a resting potential more negative than -50 mV (23).

Functional adrenergic denervation with 6-OH-dopamine. Specimens were pretreated with 6-OH-dopamine (6-hydroxydopamine hydrochloride; Sigma Chemical Co.-Aldrich Chemie, Taufkirchen, Germany) dissolved in Krebs solution at a concentration of 300 μg/mL according to the protocol of Aprigliano and Hermsmeyer (9), to produce functional adrenergic denervation.

Experimental protocol. Krebs solutions to superfuse the vessel preparations were bubbled by gas mixtures of 5% carbon dioxide, yielding a P_{CO2} of 40 mm Hg; by oxygen concentrations of 0, 2, 4, 8, or 14%; and by balance nitrogen in individual reservoirs. Gas flows to the reservoirs were adjusted to a constant rate by individual flow meters. A five-way stop cock allowed the observer to switch instantaneously from one equilibrated superfusate to another, maintaining a flow of 30 mL/min and thus to stepwise vary P_{O2} between 5, 22, 39, 68, and 104 mm Hg. Gas partial pressures were measured in the Krebs solution of the bathing chamber just above the specimens by a combined P_{O2}/P_{CO2} electrode (TCM 3; Radiometer, Copenhagen, Denmark). Temperature was kept at 37°C and pH between 7.20 and 7.40. In individual series of experiments with matched pairs of untreated control preparations and preparations pretreated with 6-OH-dopamine, variations of pH ranged from 0.04 to 0.11. Experiments were started at a P_{O2} of 39 mm Hg, i.e. a value close to the physiologic human umbilical venous P_{O2} range [22 to 35 mm Hg (24)]. Vessel strips were then exposed to first decreasing (39 to 5 mm Hg), then increasing (5 to 104 mm Hg), and finally again decreasing (104 to 39 mm Hg) P_{O2} for 15 min each, and isometric tension was recorded continuously. Isometric tension values at the end of a period of 15 min of exposure to the respective P_{O2} were averaged in each experiment. Intracellular membrane potential of multiple individual smooth muscle cells was recorded by individual impalements, lasting from seconds to minutes. Membrane potential values recorded during the second half of a period of 15 min of exposure of the preparations to the respective P_{O2} were averaged. The same protocol was applied in experiments with preparations pretreated with 6-OH-dopamine.

Membrane potential and isometric tension of 29 intact and 23 endothelium-denuded human umbilical vein preparations (untreated controls) were recorded. In addition, we studied five intact and five endothelium-denuded preparations with functional adrenergic denervation with 6-OH-dopamine. For experiments with and without 6-OH-dopamine pretreatment, we used vessel strips cut from the same umbilical cords (matched pairs), because the isometric tension generated by a vessel preparation depends on smooth muscle cell mass, and different umbilical veins have different muscle masses. Thus, isometric tension of the intact and endothelium-denuded preparations pretreated with 6-OH-dopamine were compared with that of their individual controls without 6-OH-dopamine treatment. We had difficulties obtaining a sufficient number of reliable recordings of membrane potential in the matched control preparations. However, the generation of membrane potential by an individual smooth muscle cell does not depend on the total muscle cell mass of a vessel preparation. We therefore thought it reasonable to compare membrane potential recordings of the five intact preparations pretreated with 6-OH-dopamine with those of a control group consisting of all 29 intact human umbilical vein preparations and to compare membrane potential recordings of the five endothelium-denuded preparations pretreated with 6-OH-dopamine with those of a control group consisting of all 23 endothelium-denuded preparations without pretreatment.

Statistical analysis. Results are expressed as mean \pm SEM. For statistical analysis, we used linear regression analysis, ANOVA, and the paired *t* test for comparison of treated preparations with their respective matched controls. Differences were considered statistically significant at *p* < 0.05.

RESULTS

Intact Human Umbilical Vein Preparations

Untreated intact preparations. With P_{O_2} increasing from 5 to 104 mm Hg, smooth muscle cell membranes of untreated intact human umbilical vein preparations (*n* = 29) depolarized from -58.6 ± 1.1 mV to -53.3 ± 1.0 mV (*p* < 0.001) and isometric tension increased from 0.673 ± 0.037 g to 0.825 ± 0.044 g (*p* < 0.02; Fig. 1). The changes in membrane potential and isometric tension were most pronounced between 22 and 39 mm Hg P_{O_2} , a range covering the physiologic intrauterine umbilical venous P_{O_2} range (24). There was a linear overall correlation between isometric tension and intracellular membrane potential at all P_{O_2} values investigated ($y = 0.030x + 2.41$, $r^2 = 0.99$, data not shown graphically).

Intact preparations pretreated with 6-OH-dopamine. With P_{O_2} increasing from 5 to 104 mm Hg, smooth muscle cell membranes of intact human umbilical vein preparations pretreated with 6-OH-dopamine (*n* = 5) depolarized from -58.0 ± 0.5 mV to -55.8 ± 0.6 mV (*p* < 0.01; Fig. 2). Between P_{O_2} values of 5 and 39 mm Hg, *i.e.* values below and within the P_{O_2} range present under physiologic conditions, vascular smooth muscle cell membrane potential of intact preparations with and without 6-OH-dopamine pretreatment was not statistically different. Between P_{O_2} 68 and 104 mm Hg, *i.e.* values above the physiologic intrauterine umbilical venous P_{O_2} range, mem-

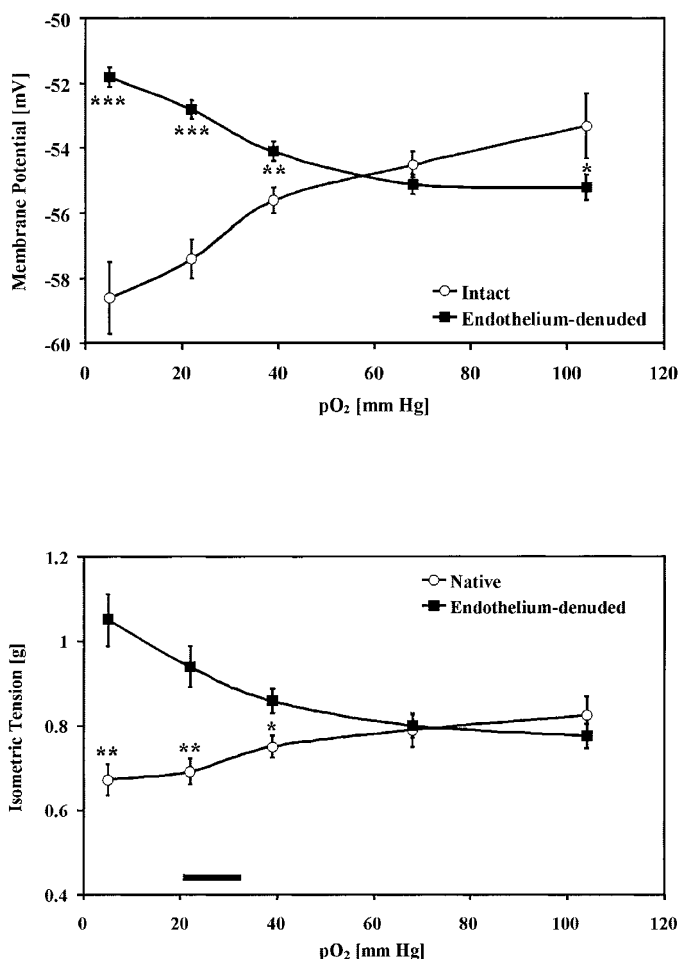


Figure 1. Oxygen-dependent regulation of membrane potential and vascular tone of untreated intact and endothelium-denuded human umbilical vein preparations. (Top) Membrane potential \pm SEM of intact (○; *n* = 29) and endothelium-denuded (■; *n* = 23) human umbilical vein preparations at P_{O_2} values between 5 and 104 mm Hg. ****p* < 0.0001, ***p* < 0.005, **p* < 0.05, intact vs endothelium-denuded preparations. (Bottom) Isometric tension \pm SEM of intact (○; *n* = 29) and endothelium-denuded (■; *n* = 23) human umbilical vein preparations at P_{O_2} values between 5 and 104 mm Hg. ***p* < 0.0001, **p* < 0.01, intact vs endothelium-denuded preparations. The horizontal bar represents the physiologic intrauterine umbilical venous P_{O_2} range (24).

brane potential was significantly less depolarized in preparations with 6-OH-dopamine pretreatment (*p* < 0.05).

With P_{O_2} increasing from 5 to 104 mm Hg, isometric tension of intact human umbilical vein preparations pretreated with 6-OH-dopamine (*n* = 5) increased from 0.598 ± 0.040 g to 0.661 ± 0.018 g (*p* < 0.02; Fig. 2). In contrast to untreated matched controls, isometric tension of intact human umbilical vein preparations pretreated with 6-OH-dopamine did not increase when P_{O_2} was increased to values above the physiologic intrauterine range. Between P_{O_2} 39 and 104 mm Hg, isometric tension remained unchanged: 0.660 ± 0.069 g versus 0.661 ± 0.018 g. At P_{O_2} 104 mm Hg, isometric tension was significantly lower in 6-OH-dopamine-pretreated preparations than in matched controls (*p* < 0.05). At P_{O_2} values below 104 mm Hg (68, 39, 22, and 5 mm Hg), isometric tension developed by intact preparations pretreated with 6-OH-dopamine was not different from that developed by intact controls. As in untreated

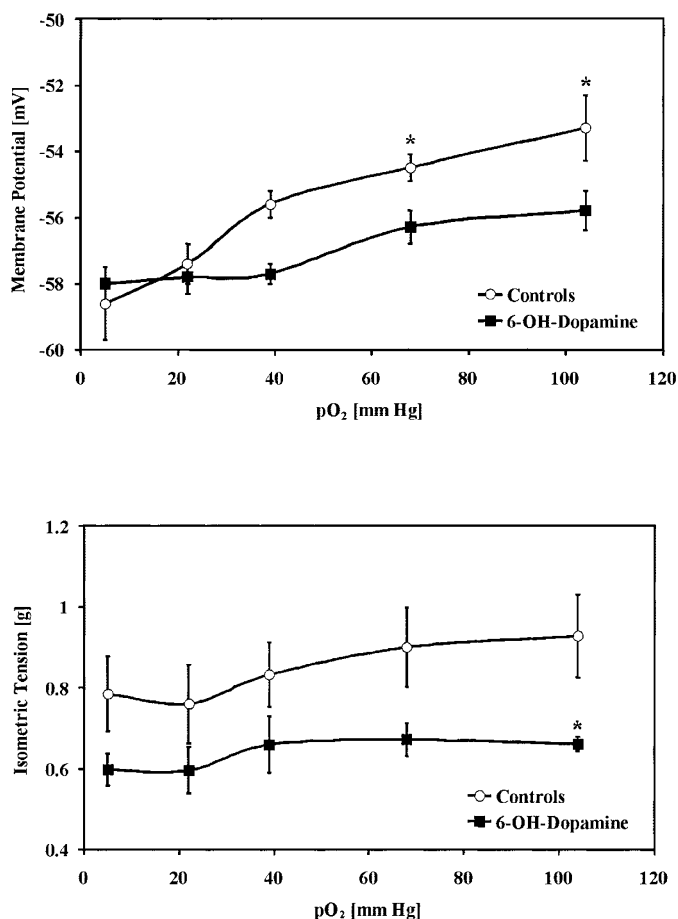


Figure 2. Effect of functional adrenergic denervation on oxygen-dependent membrane potential and vascular tone of intact human umbilical vein preparations. (Top) Membrane potential \pm SEM of intact human umbilical vein preparations with (■; $n = 5$) and without (○; $n = 29$, same as in Fig. 1, top) 6-OH-dopamine pretreatment at P_{O_2} values between 5 and 104 mm Hg. * $p < 0.05$, intact preparations pretreated with 6-OH-dopamine vs controls. (Bottom) Isometric tension \pm SEM developed by matched pairs of intact human umbilical vein preparations with (■; $n = 5$) and without (○; $n = 5$) 6-OH-dopamine pretreatment at P_{O_2} values between 5 and 104 mm Hg. * $p < 0.05$, intact preparations pretreated with 6-OH-dopamine vs controls.

intact preparations, there was a linear overall correlation between isometric tension and intracellular membrane potential at all P_{O_2} values investigated in intact preparations pretreated with 6-OH-dopamine ($y = 0.027x + 2.19$, $r^2 = 0.54$, data not shown graphically).

Endothelium-Denuded Human Umbilical Vein Preparations

Untreated endothelium-denuded preparations. With P_{O_2} increasing from 5 to 104 mm Hg, smooth muscle cell membranes of untreated endothelium-denuded human umbilical vein preparations ($n = 23$) hyperpolarized from -51.8 ± 0.3 mV to -55.2 ± 0.4 mV ($p < 0.0001$), most of the hyperpolarization occurring between 5 and 39 mm Hg (-51.8 ± 0.3 mV versus -54.1 ± 0.3 mV; $p < 0.0001$; Fig. 1). Correspondingly, isometric tension decreased from 1.051 ± 0.062 g to 0.776 ± 0.029 g ($p < 0.0005$). There was a linear overall correlation between isometric tension and intracellular mem-

brane potential of the endothelium-denuded preparations at all P_{O_2} values investigated ($y = 0.075x + 4.93$, $r^2 = 0.98$, data not shown graphically).

Endothelium-denuded preparations pretreated with 6-OH-dopamine. Endothelium-denuded human umbilical vein preparations pretreated with 6-OH-dopamine ($n = 5$) showed smooth muscle cell membrane hyperpolarization with increasing P_{O_2} similar to the hyperpolarization of untreated preparations (Fig. 3). With increasing P_{O_2} from 5 to 104 mm Hg, membrane potential hyperpolarized from -52.2 ± 0.6 mV to -55.4 ± 0.8 mV ($p < 0.005$). Correspondingly, isometric tension decreased from 1.122 ± 0.116 g to 0.881 ± 0.055 g. There was no statistically significant difference in isometric tension developed by matched pairs of endothelium-denuded human umbilical vein preparations with and without 6-OH-dopamine pretreatment at any P_{O_2} value investigated. Again, there was a linear overall correlation between isometric tension and intracellular membrane potential at all P_{O_2} values investi-

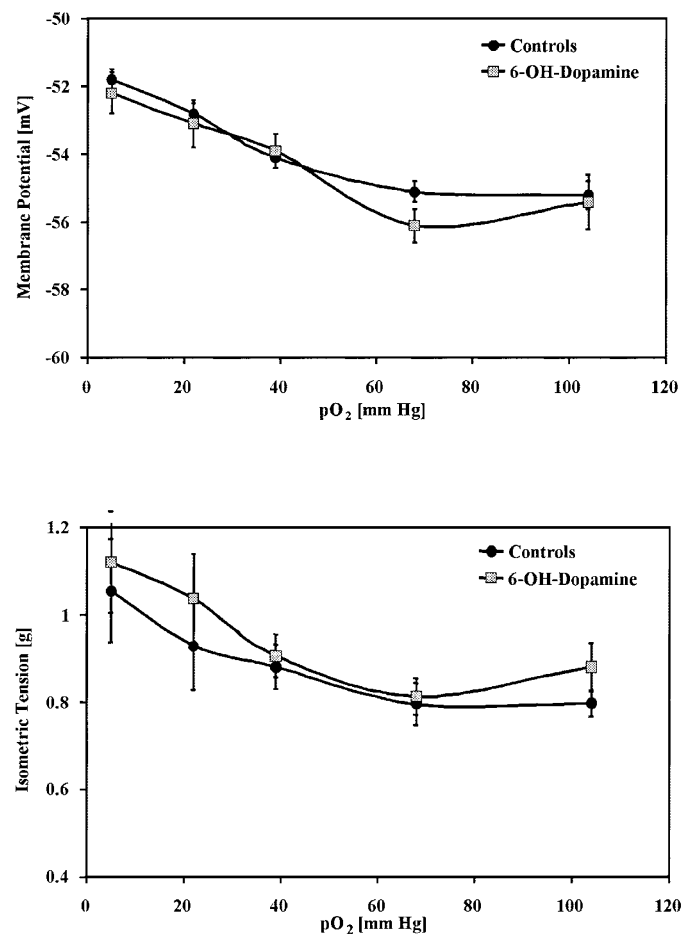


Figure 3. Effect of functional adrenergic denervation on oxygen-dependent membrane potential and vascular tone of endothelium-denuded human umbilical vein preparations. (Top) Membrane potential \pm SEM of endothelium-denuded human umbilical vein preparations with (■; $n = 5$) and without (●; $n = 23$, same as in Fig. 1, top) 6-OH-dopamine pretreatment at P_{O_2} values between 5 and 104 mm Hg. (Bottom) Isometric tension \pm SEM developed by matched pairs of endothelium-denuded human umbilical vein preparations with (■; $n = 5$) and without (●; $n = 5$) 6-OH-dopamine pretreatment at P_{O_2} values between 5 and 104 mm Hg.

gated in endothelium-denuded preparations with 6-OH-dopamine pretreatment ($y = 0.074x + 4.98$, $r^2 = 0.92$, data not shown graphically).

DISCUSSION

This study confirms previous results of an oxygen-dependent regulation of the human umbilical vein vascular tone that is directly related to the membrane potential of the vascular smooth muscle cells (3). The present study indicates that human umbilical vein membrane potential and vascular tone at P_{O_2} values present under physiologic conditions and decreasing to hypoxia are independent of the influence of locally released norepinephrine. In contrast, at P_{O_2} increasing to hyperoxia, a part of the observed membrane depolarization and increase of isometric tension can be attributed to the vasoconstricting action of endogenous norepinephrine released from the vessel wall of human umbilical vein.

Electromechanical coupling in human umbilical vein. At all P_{O_2} values investigated between 5 and 104 mm Hg, membrane potential and isometric tension were directly related, both in intact and in endothelium-denuded human umbilical vein preparations. This was the case both in untreated and in 6-OH-dopamine-pretreated preparations and suggests electromechanical coupling as a general principle in the human umbilical vein, as demonstrated in other vascular smooth muscle (25, 26). Graded membrane depolarization will lead to an increased open probability of voltage-dependent Ca^{2+} channels, resulting in an increased influx of Ca^{2+} ions into the vascular smooth muscle cells. The increased intracellular Ca^{2+} concentration in turn leads to myosin-actin interaction and so to force development (27, 28).

Human umbilical vein membrane potential and tone at high P_{O_2} . In accordance with our hypothesis, intact preparations pretreated with 6-OH-dopamine depolarized less and developed less isometric tension at increasing P_{O_2} above the physiologic range, when compared with untreated intact controls. We therefore conclude that locally released norepinephrine, possibly in concert with ATP or neuropeptide Y acting as co-transmitters (29), contributes to the vasoconstriction observed in the human umbilical vein when the local P_{O_2} increases.

However, in endothelium-denuded preparations, membrane potential and isometric tension did not differ between preparations with and without 6-OH-dopamine pretreatment. With respect to the results obtained from intact preparations, these findings are difficult to explain. Adrenergic nerve terminals are located in the adventitia and media of the blood vessel wall (10, 12), *i.e.* below the vascular endothelium. Given that pretreatment with 6-OH-dopamine diminished the membrane depolarization and constriction of intact human umbilical vein preparations in response to increasing P_{O_2} , one would also expect that pretreatment with 6-OH-dopamine resulted in a less depolarized membrane potential and in a diminished isometric tension of endothelium-denuded preparations with pretreatment. However, it has been described that catecholamines, apart from their predominant vasoconstricting effects through stimulation of α -adrenoreceptors on vascular smooth muscle

cells, are able to induce both release of vasoconstricting ATP (30) and release of vasodilating nitric oxide (31) through stimulation of adrenoreceptors located on vascular endothelial cells. Thus, our observation that oxygen-dependent changes in vascular tone did not differ between endothelium-denuded preparation with and without adrenergic denervation may be because removal of the endothelium removed both vasodilating and vasoconstricting adrenoreceptor-mediated functions of the endothelium.

Human umbilical vein membrane potential and tone at low P_{O_2} . Our results confirm that human umbilical vein has an endothelium-dependent vasodilator reserve (3) that may allow maintenance of an appropriate placental-fetal oxygen transport *via* a reduced resistance to flow at hypoxic conditions. However, because placental-fetal oxygen transport also depends on factors other than umbilical venous tone, we refrain from further speculations based on an *in vitro* model.

Locally released norepinephrine does not abolish the hypoxic vasodilator reserve of the human umbilical vein. Neither the hyperpolarization and vasodilatation of intact human umbilical vein preparations nor the depolarization and vasoconstriction of endothelium-denuded preparations at low P_{O_2} were altered by functional adrenergic denervation. The hypoxic vasoconstriction of endothelium-denuded preparations, if not from endogenous norepinephrine, may instead result from vascular smooth muscular factors [*e.g.* vasoconstricting prostanoids (32, 33)] and deserves further study. An alternative explanation is that an inhibition of voltage-dependent potassium channels (K_v channels) contribute to the hypoxic depolarization and increase in isometric tension observed in endothelium-denuded preparations with or without functional adrenergic denervation (34, 35).

Adrenergic innervation of human umbilical vein. The question of whether human umbilical cord vessels are provided with autonomic innervation has been investigated with conflicting results in histologic studies since the early 19th century (10). The current opinion that neural elements are absent in the umbilical cord, at least in the extracorporeal part of the umbilical vessels distant from the umbilical ring, is supported by anatomical studies (11, 13–15), although neural fibers have been demonstrated in human umbilical cord vessels by a modified methylene blue immersion technique (12). However, we found only two functional studies on the activity of putative autonomic nerves in the human umbilical vessels by electrical stimulation, with opposite results (13, 25). Functional adrenergic denervation of human umbilical vein—as in our approach—has not been studied. The results of our functional study provide indirect evidence for the presence of adrenergic nerve terminals in the extracorporeal part of the human umbilical vein.

CONCLUSION

In summary, our study confirms an oxygen-dependent regulation of vascular tone of the human umbilical vein on the basis of electromechanical coupling. Our study demonstrates the contribution of locally released norepinephrine to the hypoxic depolarization and vasoconstriction of human umbili-

cal vein and suggests the presence of adrenergic nerve terminals in the human umbilical vein vessel wall.

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