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Nitric Oxide Donors Mediate Vasodilation in Human Placental Arteries Partly Through a Direct Effect on Potassium Channels

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We have investigated the involvement of potassium channels in the NO-induced relaxation of small ET-1 precontracted arteries from placentas of normal pregnancies in the presence of the potassium channel modulating agents charybdotoxin, 4-AP, glibenclamide, TEA and the blocker of soluble guanylyl cyclase, ODQ, respectively. We have studied the effect of the NO-donor *S*-nitroso-*N*-acetylpenicillamine (SNAP) in vessels precontracted by different concentrations of potassium and we have also investigated the presence of BK_{Ca} channels in placental arteries by immunohistochemistry and immunoblotting.

Our results show that charybdotoxin, an inhibitor of large- and intermediate-conductance Ca^{2+}-activated potassium channels, inhibits relaxation in placental arteries. In presence of both charybdotoxin and ODQ, the inhibition of relaxation was significantly stronger, which indicates that NO-induced relaxation of human placental arteries is partly mediated through cGMP, and partly through a direct effect on potassium channels of the BK_{Ca} type.

The NO-donor SNAP preferentially relaxes contractions induced by 75 mM K^+ as compared to 100 mM K^+. This effect profile is a unique feature of drugs acting by K^+ channel opening. The immunohistochemistry shows that BK_{Ca} channels are located both in smooth muscle and in endothelium in placental arteries.
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INTRODUCTION

Nitric oxide (NO) is a multifunctional signalling molecule and an important modulator of cellular responses in a variety of tissues including those involved in human reproduction. NO is generated from L-arginine by the catalytic action of an enzyme, nitric oxide synthase (NOS). So far three different isoforms of NOS have been identified, cloned and characterized. While two of the three (type 1 or neuronal and 3 or endothelial) are constitutively expressed, the expression of the third isoform (type 2 or inducible) can be induced by cytokines and some other agents [1,2].

NO is known to have a powerful vasodilatory effect in resistance vessels throughout the body. Physiologically, this action is believed to be mediated by locally produced NO and in most instances by the subsequently generated second messenger molecule guanosine 3–5 cyclic monophosphate (cGMP) [3].

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The fetoplacental circulation is mainly regulated by locally produced and circulating factors since the vessels lack innervation. There is considerable evidence that local production of NO contributes to the maintenance of low vascular resistance in the fetoplacental circulation [4,5].

Endothelin-1 (ET-1) is a 21 amino acid, vasoconstrictor peptide released from the vascular endothelium [6]. It exerts its vascular effects through two receptor subtypes: ET_A and ET_B. ET_A receptors [7] are found on vascular smooth muscle (VSM), stimulation of these receptors induces constriction. ET_B receptors can be sub-divided into ET_{B1} and ET_{B2} receptors: ET_{B1} receptors are found on the endothelial layer and are involved in vasodilation [8]; ET_{B2} receptors are located on VSM and mediate vasoconstriction [9]. ET-1 generally induces VSM depolarisation associated with a biphasic elevation of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and cell contraction [10]. Initially there is a rapid rise in $[Ca^{2+}]_i$, due to Ca^{2+} release from the sarcoplasmic reticulum (SR), followed by a fall to plateau level, which is thought to be maintained by the persistent influx of extracellular Ca^{2+} through membrane ion channels [6,11–13]. The contribution of intracellular and extracellular Ca^{2+} sources to the ET-1-induced vasoconstrictor response varies between vessel types.

Our previous work has shown that placental arteries are able to contract in response to endothelin (ET-1) in a dose-dependent manner [14]. We have also shown that precontracted placental arteries relax in a dose-dependent manner in response to different nitric oxide donors [15]. However, we were not able to completely block the nitric oxide mediated relaxation with different inhibitors of the nitric oxide pathway (LY83583 (LY), 1*H*-[1,2,4]oxadiazole[4,3-*a*]quinoxalin-1-one (ODQ)), suggesting that other mechanisms also are involved in the nitric oxide mediated relaxation in placental arteries.

A number of in vitro studies on vascular smooth muscle have provided evidence for the involvement of potassium channels in the mechanism of NO-induced relaxation. In pulmonary artery smooth muscle cells, a selective activation of cGMP-dependent protein kinases was found to increase potassium currents [16]. Data from patch-clamp experiments suggest that large conductance calcium-activated K^+ channels (BK_{Ca}) may be activated via the NO-cGMP pathway [17] and that BK_{Ca} modulates the vasodilator response to both exogenous nitrovasodilators and endogenous receptor-mediated NO release in isolated arteries [18,19]. It has been suggested that drugs acting by K^+ channel opening relax smooth muscle more or less effectively depending on the external K^+ concentration [20].

However, other evidence suggests that NO may reduce vascular tone independent of cGMP and cGMP kinase I (cGKI) [21]. Consequently, current opinions on the nature of the mechanisms that are activated by NO and cGMP in smooth muscle are controversial.

BK_{Ca} has been identified to be present in both vascular smooth muscle and endothelial cells, and constitute a subgroup in the large family of potassium channels [22]. BK_{Ca} provides a negative feedback mechanism limiting the depolarising and Ca-increasing effects of vasoconstrictors. Opening of BK_{Ca} will allow K-flux out of the cell leading to a change in the membrane potential in a hyperpolarizing direction, and thus induce vasodilation [23].

BK_{Ca} has been described in a variety of electrically excitable and nonexcitable cells [24] and have been implicated in the regulation of neuroendocrine secretion, in the control of muscle contractility and in a number of other processes.

In our study, we have investigated the involvement of BK_{Ca} channels in the NO-induced relaxation of small, ET-1-precontracted chorion plate arteries from placentas of normal pregnancies. As an NO-donor we used a widely known and commonly used compound, *S*-nitroso-*N*-acetylpenicillamine (SNAP), which is known to release NO in a reproducible and stable manner [25]. We examined the placenta artery relaxation response in the presence or absence of the potassium channel modulating agents charybdotoxin, 4-AP, TEA and glibenclamide, as well as the blocker of soluble guanylate cyclase (sGC), ODQ. We also studied the effect of SNAP in vessels precontracted by different concentrations of potassium since potassium channel activity depends on the external potassium concentration. We have by immunohistochemistry studied the presence and subcellular distribution of the BK_{Ca} as well as the components involved in the nitric oxide induced relaxation in placenta, i.e. eNOS, cGMP, sGC and cGMP-dependent protein kinase type I (cGKI). We also examined the distribution of ET_A and ET_B receptors. Finally, we have studied the BK_{Ca} protein in placental arteries by immunoblotting.

MATERIALS AND METHODS

Patient

Human placentas were obtained from 79 women with normal pregnancy (Table 1). Delivery was either vaginally ($n = 67$) or by caesarean section ($n = 12$). None of the patients received any medication prior to delivery. Apgar scores for all the delivered infants were 9–10 after 5 min. Within 5–30 min of delivery of the placenta, tissue was excised for superfusion assay. The local ethics committee approved the procedures.

Superfusion assay

This was done essentially as described previously [14,15]. Tissue was excised and put into ice-cold Krebs–Ringer solution for dissection of placental arteries. Vessel strips (length 0.7–1 cm, inner diameter 0.3–0.7 mm) were cut longitudinally and mounted vertically in 2 mL organ baths containing Krebs–Ringer solution at 37 °C. From each patient, two or three strips were used. One strip from each patient was used as a temporal control and every strip was used in only one experiment. The Krebs–Ringer solution was of the following composition (in mM): NaCl 120, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.4, NaH_2PO_4 1.2 and glucose 5.7. The solution was bubbled with a gas mixture consisting of 95% O_2 and 5% CO_2 in order to keep a pH in the bath of around 7.35–7.45. The tension was recorded isometrically with a Grass FT03C force-displacement transducer and registered on a Grass model 7 polygraph. The vessels were given an initial passive load of about 50 mN and allowed to equilibrate for at least 30 min prior to the experiments. After the equilibrating period, vessels were stimulated with KCl (100 mM) in order to obtain a reference contraction. This contraction was defined as the maximal contraction to KCl. KCl and the concentration of 100 mM was chosen because we and others have previously found that it gives a robust and

Table 1. Clinical data of the patients ($n = 79$, mean ± SD)

Characteristic	Data
Age (years)	30.7 ± 5.1
First delivery (*n*)	36
Week of delivery (week)	39.5 ± 1.5
Birth weight (g)	3685 ± 504
Placenta weight (g)	591.2 ± 130

Patients were delivered by vaginal delivery (n = 67) or Caesarean section (n = 12). Reasons for Caesarean section were breech presentation (n = 4), imminent fetal asphyxia (n = 4) or psychosocial reasons (n = 4). Five-minute Apgar scores for all deliveries were 9–10.