Characterisation of kinin receptors on the human isolated umbilical artery

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Abstract

The aim of this study was to determine the kinin responses on human umbilical artery (HUA) and to characterise the kinin receptors present on this tissue. The HUA was found to constrict in response to both the B₂ receptor agonist, bradykinin (BK), and the B₁ receptor agonist, des-Arg⁹-BK. The presence of indomethacin (2.79 µM) was found to have no significant effect on the responses to BK and des-Arg⁹-BK. The presence of the B₂ receptor antagonist, HOE-140 (+2.79 µM indomethacin), resulted in a concentration-related rightward displacement of the concentration-effect curves to BK. The antagonism of the constrictor responses to BK by HOE-140 was found to be competitive (pA₂=7.5). The responses to BK were

not significantly affected by the B₁ receptor antagonist des-Arg⁹(leu⁸)BK. The presence of des-Arg⁹(leu⁸)BK (+2.79 µM indomethacin) resulted in a concentration-related rightward displacement of the concentration-effect curve to des-Arg⁹-BK. The antagonism of the response to des-Arg⁹-BK by des-Arg⁹(leu⁸)BK was found to be competitive (pA₂=5.9). The responses to des-Arg⁹-BK were not significantly affected by HOE-140. The results of the present study suggest that the products of the cyclooxygenase pathway are not involved in the kinin constrictor response on HUA and indicate the presence of B₁ and B₂ receptors on this tissue.

Journal of Endocrinology (1998) **156**, 389–394

Introduction

After delivery, constriction of the umbilical arteries occurs almost instantaneously and this *post partum* arterial closure is an irreversible process with the vessels remaining closed even if the cord is placed in anaerobic solutions (Bjoro & Stray-Pedersen 1986a). Several factors (mechanical, chemical and humoral) have been implicated in the control of umbilical blood flow in man and in spontaneous closure of the umbilical vessels at birth. Although the main events leading to constriction of the ductus arteriosus and umbilical arteries and veins at birth are a rise in the partial pressures of oxygen and mechanical alterations, these are not the only factors responsible for the circulatory changes at birth and kinins may also be capable of affecting/mediating some of the rapid circulatory changes required of the neonate (Altura *et al.* 1972, Soares De Moura & Cerqueira Lopes 1995). It has been speculated that the effects of oxygen on the ductus arteriosus and pulmonary vessels may even be mediated via bradykinin (BK) release (Assali *et al.* 1971).

BK receptors (B) were originally divided into B₁ and B₂ subtypes (Regoli *et al.* 1996) and kinins exert their vascular effects through either B₁ or B₂ receptors (Hall 1992, Gobeil *et al.* 1996). It has been proposed that BK receptor classification should be extended to include B₃ subtypes

(Farmer *et al.* 1989a,b, Hall 1997) as the presence of B₃ receptors has been demonstrated in guinea-pig trachea and taenia caeci (Field *et al.* 1992a,b). BK is a potent B₂ receptor agonist (Regoli & Barabé 1980, Hall 1992) with HOE-140 being a potent and selective B₂ receptor antagonist (Wirth *et al.* 1991a,b, Hock *et al.* 1991, Gobeil *et al.* 1996). Des-Arg⁹-BK is a potent B₁ receptor agonist (Regoli & Barabé 1980, Hall 1992, Gobeil *et al.* 1996) with des-Arg⁹(leu⁸)BK reported to be a selective B₁ receptor antagonist (Churchill & Ward 1986, Field *et al.* 1992a, Hall 1992, Campos & Calixto 1994, Drummond & Cocks 1995, Gobeil *et al.* 1996). In the present study the response of the human umbilical artery (HUA) to BK and des-Arg⁹-BK was investigated in terms of the kinin receptors involved.

BK has been shown to induce a potent contractile response on helically and longitudinally cut HUA (Altura *et al.* 1972, Félétou *et al.* 1995). Altura *et al.* (1972) suggested the possibility of the presence of specific receptors for BK on human umbilical arteries and veins, although they did not use any receptor selective antagonists. Félétou *et al.* (1995) reported the presence of B₂ receptors on HUA. BK and des-Arg⁹-BK have been reported to constrict also the human isolated umbilical vein (Gobeil *et al.* 1996) and the presence of B₁ and B₂ receptors has been demonstrated in this preparation. The

aim of this study was to characterise the response of HUA to BK and des-Arg⁹-BK, in terms of receptor involvement. To make this characterisation possible, HOE-140, a selective B₂ receptor antagonist, and des-Arg⁹(leu⁸)-BK, a selective B₁ receptor antagonist were used.

Materials and Methods

Tissue preparation

Umbilical cords were obtained from normal full term spontaneous deliveries, as soon as possible after delivery, usually within 60 min. All patients gave written consent and all procedures had ethical committee approval. Cords from mothers exhibiting eclampsia, hypertension, diabetes, a Rhesus problem or other overt diseases were not included in the study. Cords from mothers taking medication, such as antihistamines, morphine, adrenergic blockers or anticholinergic drugs during the last two months of pregnancy were also excluded. Lengths of umbilical cord (5–20 cm) were taken from the placental portion. This area of the cord is known to be nerve free (Davingdon *et al.* 1965, Altura *et al.* 1972, Bodelsson & Stjernquist 1995). The cords were immediately placed in Krebs' solution at room temperature and prepared for experimental procedures.

The samples of umbilical cord were first mounted onto a dissecting dish containing Krebs' solution, and umbilical arteries were dissected free of the Whartons jelly using micro-dissecting instruments. Care was required in the dissection, and handling was minimised in order to prevent tissue damage and prostaglandin release (Piper & Vane 1971, McGrath *et al.* 1985) which can cause constriction of the isolated HUA (Bjoro & Stray-Pedersen 1986a,c, DeMoraes *et al.* 1997). A magnifying lens was always used during the preparation procedure. The isolated arterial rings, with an intact endothelium (confirmed histologically), measuring 2–3 mm in diameter and 1–2 mm in length were suspended under 2 g tension in 10 ml organ baths. The organ baths contained Krebs' solution at 37 °C, aerated with 2.5% O₂/8% CO₂ and balance N₂ to mimic the gas tension and pH of the umbilical arterial blood *in utero*, i.e. pO₂ was 20 mm Hg, pCO₂ was 50 mm Hg and the pH was 7.28 (McGrath *et al.* 1985). The Krebs' solution also contained 2.79 µM indomethacin to inhibit cyclooxygenase (COX) activity, when required. A 2-h equilibration period was allowed before the administration of agonists, and during this time the Krebs' solution was changed every 15 min. When antagonists were used, they were added to the Krebs' solution and allowed to equilibrate for 30 min with the tissue before agonist concentration-effect curves were constructed. Responses were measured with isometric tension transducers (Dynamometer UFI; Astro-Med Inc., Slough, Berks, UK) coupled to a Grass Polygraph (Model 7D; Grass Instrument Co., Quincey, MA, USA)

4-channel pen recorder. Tissues which exhibited spontaneous activity were not utilised in the studies, as the spontaneous activity was superimposed on the agonist responses producing results which were difficult to interpret.

Agonist administration

Two cumulative concentration-effect curves were constructed using each preparation. The first concentration-effect curve was constructed with the agonists as a 'control'. The agonist was then washed out by changing the Krebs' solution every 15 min. A washout period of at least 90 min was allowed before construction of the subsequent concentration-effect curves in the presence of indomethacin alone or antagonist (+indomethacin). Agonist potency was expressed as the EC₅₀ value which was the effective concentration that produced a response equivalent to 50% of the maximal response elicited by the agonist, where the maximal response was taken as 100% (Baxter *et al.* 1995). EC₅₀ values were obtained for each experiment and data from separate experiments were grouped together and expressed as geometric means with 95% confidence limits (CL). On repetition, using the same preparation, concentration-effect curves for the agonists were reproducible.

Antagonist potency

Affinity estimates for antagonists were expressed as pA₂ values, calculated according to the method of Arunlakshana & Schild (1959). Tissues were incubated with antagonists for a period of at least 30 min. The pA₂, slope of the Schild plot and regression coefficient values were geometric means (95% CL) from five experiments.

Compounds used

BK, des-Arg⁹-BK, indomethacin and des-Arg⁹(leu⁸)BK were all obtained from the Sigma Chemical Company Ltd, Poole, Dorset, UK. HOE-140 was obtained from Hoechst AG (Frankfurt, Germany). BK, des-Arg⁹-BK, des-Arg⁹(leu⁸)BK and HOE-140 were dissolved in distilled water and final drug solutions were made up in 0.9% (w/v) NaCl. Indomethacin was dissolved in 1% (w/v) sodium bicarbonate solution. The vehicles used had no effect upon the tissue.

Statistical analysis

Student's *t*-test (for both paired and un-paired data) was used for analysis and differences were considered significant when *P* < 0.05. All tests for significance were carried out using the Statworks programme on the Apple Macintosh. Log-normal data such as EC₅₀ were expressed as geometric means. All other data in the text were grouped and expressed as arithmetic means ± S.E.M. of *n*

Figure 1 A typical sample trace showing the cumulative concentration-effect curve to BK on an HUA artery preparation. The arrows indicate point of BK (nM) administration.

determinations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicate significant differences between agonist alone and agonist in the presence of antagonist.

Results

Responses to BK $\pm$ 2.79 μ M indomethacin, HOE-140 or des-Arg⁹(leu⁸)BK

Cumulative addition of BK (1–1000 nM) resulted in concentration-dependent constriction ($EC_{50} = 38$ nM (range 31–46)) of HUA rings (Figs 1 and 2). The presence of indomethacin (2.79 μ M) had no significant effect on the responses to BK ($EC_{50} = 43$ nM (range 32–54)) (Fig. 2a). The presence of HOE-140 (0.1 μ M, 0.3 μ M and 0.6 μ M), in the presence of 2.79 μ M indomethacin, resulted in competitive antagonism of the responses to BK (Fig. 2b). The concentrations of HOE-140 used were in the same range as those used by Drummond & Cocks (1995) to antagonise the BK responses in the bovine isolated coronary artery.

The pA_2 value obtained for HOE-140, against the BK responses, was calculated to be 7.5 (range 7.45–7.62) with the slope of the Schild plot being 1.03 (range 0.73–1.18) and the regression coefficient being 0.99 (range 0.90–1.00). The slope of the Schild plot was not significantly different from one ($P > 0.05$), this indicates that in HUA preparations HOE-140 is a competitive antagonist against BK.

The presence of 10 μ M des-Arg⁹(leu⁸)BK had no significant effect on the responses to BK (data not shown). This concentration of des-Arg⁹(leu⁸)BK was chosen because 10 μ M des-Arg⁹(leu⁸)BK resulted in a significant ($P < 0.05$) reduction of the constrictor responses to des-Arg⁹-BK on HUA preparations.

Responses to des-Arg⁹-BK $\pm$ 2.79 μ M indomethacin, des-Arg⁹(leu⁸)BK or HOE-140

Cumulative addition of des-Arg⁹-BK (5–50 000 nM) resulted in concentration-dependent constriction ($EC_{50} = 320$ nM (range 151–605)) of HUA rings (Fig. 3a). The presence of indomethacin (2.79 μ M) had no significant effect on the responses to des-Arg⁹-BK ($EC_{50} = 418$ nM (range 112–1000)) (Fig. 3a). Des-Arg⁹(leu⁸)BK (3 μ M,

Figure 2 (a) Mean constrictor concentration-effect curves to BK alone (□) and in the presence of 2.79 μ M indomethacin (●), on human isolated umbilical artery. Data are expressed as arithmetic means of five determinations and vertical bars represent S.E.M. (b) Mean constrictor concentration-effect curves (+2.79 μ M indomethacin in Krebs' solution) to BK alone (□) and in the presence of 0.1 μ M HOE-140 (○), 0.3 μ M HOE-140 (●) and 0.6 μ M HOE-140 (Δ), on human isolated umbilical artery. Data are expressed as arithmetic means of five determinations and vertical bars represent S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significant difference between BK alone and in the presence of antagonist.

6 μ M and 10 μ M), in the presence of 2.79 μ M indomethacin, caused a progressive concentration-dependent rightward shift of the concentration-effect curves to des-Arg⁹-BK (Fig. 3b). The concentrations of des-Arg⁹(leu⁸)BK used were in the same range as those used by Drummond & Cocks (1995) to antagonise the des-Arg⁹-BK responses in the bovine isolated coronary artery. Note that 70% of the HUA preparations were found to respond to des-Arg⁹-BK, with no effect of des-Arg⁹-BK seen in 30% of the preparations.

Figure 3 (a) Mean constrictor concentration-effect curves to des-Arg⁹-BK alone (□) and in the presence of 2.79 μM indomethacin (●), on human isolated umbilical artery. Data are expressed as arithmetic means of five determinations and vertical bars represent S.E.M. (b) Mean constrictor concentration-effect curves (+2.79 μM indomethacin in Krebs' solution) to des-Arg⁹-BK alone (□) and in the presence of 3 μM des-Arg⁹(leu⁸)BK (○), 6 μM des-Arg⁹(leu⁸)BK (●) and 10 μM des-Arg⁹(leu⁸)BK (△), on human isolated umbilical artery. Data are expressed as arithmetic means of five determinations and vertical bars represent S.E.M. * $P < 0.05$, significant difference between des-Arg⁹-BK alone and in the presence of antagonist.

The pA_2 value obtained for des-Arg⁹(leu⁸)BK was calculated to be 5.90 (range 5.68–6.09) with the slope of the Schild plot being 1.06 (range 0.96–1.14) and the regression coefficient being 1.00 (range 0.97–1.00). The slope of the Schild plot indicates that in HUA preparations des-Arg⁹(leu⁸)BK is a competitive antagonist against des-Arg⁹-BK.

The presence of 1 μM HOE-140 had no significant effect on the responses to des-Arg⁹-BK (data not shown). The constrictor potency of des-Arg⁹-BK was significantly

($P < 0.001$) lower than that of BK and the maximum response was also lower than that of BK (see Figs 2 and 3).

Discussion

In vitro studies on the responses of the umbilical cord vasculature have been carried out mainly on isolated strips of the muscle layer or on short, isolated segments of the arteries. Human umbilical vessels are responsive to a number of vasoactive substances *in vitro* as well as *in vivo*, prostanoids and 5-hydroxytryptamine being two of the major substances to which these preparations are highly sensitive (Bjoro & Stray-Pedersen 1986a,b,c, Okatani *et al.* 1996). However, it has been demonstrated that human umbilical vessels are also sensitive to kinins (Gobeil *et al.* 1996). BK is a potent endogenous vasodilator in humans, but according to several studies results in constriction of HUA and human umbilical veins (Altura *et al.* 1972, Marceau *et al.* 1994, Félétou *et al.* 1995, Gobeil *et al.* 1996). BK causes dilatation of pulmonary arterioles and constriction of ductus arteriosus in the foetal lamb (Campbell *et al.* 1968). It also constricts calf and guinea-pig ductus (Kovalcik 1963, Eltherington *et al.* 1968). This is probably because BK can constrict and dilate vessels as required for the transitional circulatory changes that occur at birth (Assali *et al.* 1971).

In an attempt to obtain a pharmacological characterisation of kinin receptors on HUA preparation B₁ and B₂ agonists and antagonists were tested. The kinin receptor agonists were also tested in the presence of indomethacin in order to establish the role of the COX pathway products in the kinin response on HUA.

In the present study BK and des-Arg⁹-BK evoked constriction of the HUA, suggesting kinin receptors are present in this preparation. This view is supported by the findings in the presence of the B₁ and B₂ receptor antagonists. These results are in agreement with the findings of Gobeil *et al.* (1996) on human isolated umbilical vein and the responses seen to BK are in agreement with the BK responses reported by Altura *et al.* (1972) and Félétou *et al.* (1995) on HUA. Félétou *et al.* (1995) reported the presence of B₂ receptors on HUA but the presence of B₁ receptors on this preparation was not discussed by the authors.

The presence of indomethacin had no significant effect on the response to the kinins, thus indicating that the constrictor responses of HUA preparations to kinins do not involve COX products. This finding, in contrast to that of the myometrium where BK releases COX products (Abbas *et al.* 1996), suggests that in the HUA BK is acting directly on receptors to cause constrictor effects.

In this study competitive antagonism of the responses to BK by HOE-140 indicates the presence of B₂ receptors on HUA and this antagonism was competitive in nature. For most tissues studied HOE-140 acts as a mixed competitive and non-competitive or completely non-competitive

antagonist (Liebmann *et al.* 1993). In the present studies on HUA the pA_2 value of HOE-140, against the BK response, was calculated to be 7.5. The reported pA_2 values for HOE-140 range from 7.42 in the guinea-pig trachea to 9.19 in the rabbit jugular vein (Rhaleb *et al.* 1992). The results of the present study thus suggest that in the HUA BK mediates its responses through B_2 receptors and are in agreement with the results reported by Félétou *et al.* (1995) on HUA. Félétou *et al.* (1995) reported that the antagonism of the BK responses by HOE-140 on HUA was competitive and calculated the pA_2 value of HOE-140 to be 8.16 ± 0.16 . The possibility of B_3 receptor involvement cannot be ignored, since HOE-140 is also a B_3 receptor antagonist (Field *et al.* 1992a,b). However, so far guinea-pig trachea and taenia caeci have been the only B_3 receptor preparations reported (Field *et al.* 1992a,b, Hall 1997). The presence of $1 \mu M$ des-Arg⁹(leu⁸)BK did not significantly alter the responses to BK, thus implying that the BK responses are not mediated, to a significant extent, through B_1 receptors on this tissue.

The presence of des-Arg⁹(leu⁸)BK caused a progressive concentration-dependent rightward shift of the concentration-effect curve to des-Arg⁹-BK, thus suggesting the presence of B_1 receptors in this preparation. The pA_2 value for des-Arg⁹(leu⁸)BK, against des-Arg⁹-BK, was calculated to be 5.9 with a slope of 1.06, this pA_2 value was lower than that determined at B_1 receptors in the rabbit mesenteric artery (pA_2 of 6.5 reported by Churchill & Ward (1986)), and rabbit aorta (pA_2 of 6.8 reported by Regoli *et al.* (1977) and 7.3 reported by Drouin *et al.* (1979)). These results indicate that in HUA the responses to des-Arg⁹-BK are mediated through B_1 receptors and since HOE-140 ($1 \mu M$) had no effect on the contractile response to des-Arg⁹-BK the responses to this agonist do not involve B_2 receptors.

As far as the intracellular events that follow the stimulation of the membrane receptors by kinins are concerned kinin receptors behave in a similar manner to receptors for several other neurotransmitters and hormones. The majority of the effects of kinins are either contraction or relaxation of smooth muscles, and ions, especially Ca^{2+} , or cAMP or cGMP appear to be the second messengers for kinins (Hall 1992). The constriction of HUA in response to BK is reported to be accompanied by an increase of cGMP, dependent on extracellular Ca^{2+} (Clyman *et al.* 1975). This is possible as in rabbit saphenous vein BK elicits vasoconstriction by increasing Ca^{2+} influx from the extracellular space and the BK-induced increase in Ca^{2+} sensitivity is reported to be mediated by G-protein (Eguchi *et al.* 1997). Also in rat portal vein rings, BK-induced constrictions appear to rely largely on extracellular Ca^{2+} influx and activation of protein kinase C-dependent mechanisms (Campos & Calixto 1994). Arachidonic acid metabolites are considered to be of great importance in the regulation of umbilicoplacental blood flow (Tuvemo 1980, Mott & Walker 1984, Dadak *et al.* 1987), and they may

affect the vascular tone directly or may modulate responses to other vasoactive substances. In order to elucidate the role of endogenous prostanoids in the responses to the kinins on HUA the responses to BK will be evaluated in the presence of prostaglandins in further studies.

With respect to the findings of the present study, it may be speculated that the *in vitro* constrictor effects of kinins, primarily BK, may be representative of the *in vivo* situation as kinins are reported to be present in maternal plasma (Melmon *et al.* 1968, Soares De Moura & Cerqueira Lopes 1995), hence BK could produce constrictor responses at birth. Melmon *et al.* (1968) measured kinin concentrations by drawing blood from newborn infants and performing chemical assays and assays on the oestrous rat uterus. The authors reported the concentration range of BK in the cord blood of 56 newborn infants to be 12.8 ± 4.3 ng/ml, within the range (BK EC_{50} is 43 nM) found to cause HUA constriction in the present *in vitro* experiments.

In conclusion, the results of this study suggest that on the HUA BK is acting mainly through B_2 receptors and des-Arg⁹-BK is acting through B_1 receptors. The results also indicate that none of the constrictor responses to the kinins are mediated through COX products, since indomethacin had no effect on the response to the kinins. The B_3 subtype remains to be investigated. Finally, in view of the results of the present study the possibility must be entertained that BK and related kinins may play a role in the regulation of human umbilical cord blood flow *in vivo*, and may affect closure of human umbilical cord vessels at birth since previous studies (Melmon *et al.* 1968) have indicated that kinin umbilical cord plasma levels are markedly increased at birth.

Acknowledgements

The authors would like to acknowledge the Wellcome Trust for their support.

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Received 10 June 1997
Accepted 1 October 1997