Naunyn-Schmiedeberg's Arch Pharmacol (2001) 364 : 149–156
DOI 10.1007/s002100100434

ORIGINAL ARTICLE

**Andrea Emilse Errasti · Verónica Rey-Ares
Federico Manuel Daray · María Pía Rogines-Velo
Sergio Pablo Sardi · Cristina Paz
Ernesto Jorge Podestá · Rodolfo Pedro Rothlin**

Human umbilical vein: involvement of cyclooxygenase-2 pathway in bradykinin B_1 receptor-sensitized responses

Received: 30 October 2000 / Accepted: 23 March 2001 / Published online: 9 June 2001

Abstract In isolated human umbilical vein (HUV), the contractile response to des-Arg^9-bradykinin (des-Arg^9-BK), selective BK B_1 receptor agonist, increases as a function of the incubation time. Here, we evaluated whether cyclooxygenase (COX) pathway is involved in BK B_1-senzitized response obtained in 5-h incubated HUV rings. The effect of different concentrations of indomethacin, sodium salicylate, ibuprofen, meloxicam, lysine clonixinate or NS-398 administrated 30 min before concentration-response curves (CRC) was studied. All treatments produced a significant rightward shift of the CRC to des-Arg^9-BK in a concentration-dependent manner, which provides pharmacological evidence that COX pathway is involved in the BK B_1 responses. Moreover, in this tissue, the NS-398 pK_b (5.2) observed suggests that COX-2 pathway is the most relevant. The strong correlation between published pIC_{50} for COX-2 and the NSAIDs' pK_bs estimated further supports the hypothesis that COX-2 metabolites are involved in BK B_1 receptor-mediated responses. In other rings, indomethacin (30, 100 μmol/l) or NS-398 (10, 30 μmol/l) produced a significant rightward shift of the CRC to BK, selective BK B_2 agonist, and its pK_bs were similar to the values to inhibit BK B_1 receptor responses, suggesting that COX-2 pathway also is involved in BK B_2 receptor responses. Western blot analysis shows that COX-1 and COX-2 isoenzymes are present before and after 5-h in vitro incubation and apparently COX-2 does not suffer additional induction.

A.E. Errasti · V. Rey-Ares · F.M. Daray · M.P. Rogines-Velo
S.P. Sardi · R.P. Rothlin (✉)
Departamento de Farmacología, Facultad de Medicina,
Universidad de Buenos Aires,
Paraguay 2155, Piso 9, CP 1121 Buenos Aires, Argentina
e-mail: farmaco3@fmed.uba.ar,
Tel.: /Fax: +54-11-49620300

C. Paz · E.J. Podestá
Departamento de Bioquímica, Facultad de Medicina,
Universidad de Buenos Aires, Buenos Aires, Argentina

Keywords Human umbilical vein · Cyclooxygenase-1 · Cyclooxygenase-2 · Bradykinin B_1 and B_2 receptors · Non-steroidal antiinflammatory drugs

Introduction

In 1977, two mammalian bradykinin (BK) receptor subtypes were proposed, B_1 and B_2 (Regoli et al. 1977). BK and the decapeptide Lys-bradykinin (kallidin) are endogenous agonists for BK B_2 receptors. The cleavage of these peptides by arginine carboxipeptidases produces selective BK B_1 receptor agonists, des-Arg^9-BK and des-Arg^9-kallidin (Marceau et al. 1998). BK B_2 receptors are constitutively expressed in a variety of tissues and mediate most of the in vivo effects to kinins (Bathon and Proud 1991). On the other hand, the BK B_1 receptors are not present in any significant amount in normal tissues and their expression is often inducible rather than constitutive. BK B_1 receptor-mediated responses are up-regulated in a time- and protein synthesis-dependent process (Regoli et al. 1978; Bouthillier et al. 1987; Sardi et al. 2000).

Molecular cloning has revealed the primary structures of BK B_1 (Menke et al. 1994) and BK B_2 (McEachern et al. 1991) receptors, and has identified them as members of the G protein-coupled receptor family characterized by seven membrane-spanning α-helices. The identity of G protein subtypes linked to the BK B_1 receptor is similar to the ones coupled to BK B_2 receptor (de Weerd and Leeb-Lundberg 1997). Both BK B_1 and B_2 receptors are primarily linked to polyphosphoinositide phospholipase C activation. In rabbit vascular smooth muscle cells it has been described that BK B_1 receptors stimulate phosphatidylinositol hydrolysis leading to mobilization of intracellular calcium. In addition, rabbit BK B_1 receptors appear to be coupled to phospholipase A_2 pathway, which releases the prostaglandin precursor, arachidonic acid (Tropea et al. 1993; Schneck et al. 1994). On the other hand, BK B_2 stimulus has also been reported to cause arachidonic acid release via the rise in cytosolic free calcium and the activation of phospholipase A_2 that consequently

increases prostanoid production in porcine tracheal smooth-muscle cells (Tanaka et al. 1995). Furthermore, either BK B_1 or BK B_2 receptors stimulation induces activation of cyclooxygenase (COX) pathway, leading to the production of relaxing prostaglandins in mouse trachea (Li et al. 1998).

Two forms of cyclooxygenase exist and can generate prostaglandins from arachidonic acid: COX-1, which is constitutively expressed in many cells, and COX-2, which is inducible by a variety of stimuli. Both COX-1 and COX-2 are integral membrane proteins that have been localized to the endoplasmic reticulum and nuclear membranes (Otto et al. 1993; Morita et al. 1995).

In isolated human umbilical vein (HUV) the presence of both BK B_2 and BK B_1 receptors has been previously demonstrated using selective agonists and antagonists. In this tissue, BK promotes a potent and effective vasoconstrictor response and depends only on BK B_2 receptor stimulus (Sardi et al. 1997). On the other hand, the contractile effect of des-Arg^9-BK develops from an initial null level and increases in magnitude as a function of the in vitro incubation time and depends exclusively on BK B_1 receptor stimulation (Sardi et al. 1997). It has been proposed that BK B_1 receptor is induced under certain pathophysiological conditions such as tissue injury, inflammation or during trauma tissue isolation and incubation (Marceau et al. 1998).

The aim of this study was to investigate whether COX isoenzyme pathways are involved in contractile responses mediated by BK B_1 or BK B_2 receptor stimulation in HUV. Therefore, the effects of different non-steroidal antiinflammatory drugs (NSAIDs) on BK B_1 or BK B_2 receptor-mediated contractile responses were evaluated in this tissue.

Materials and methods

Preparation of tissues for tension measurements. Approximately 15–35 cm of human umbilical cords excised midway between the placenta and infant were obtained from normal full-term deliveries. Immediately, cords were placed in modified Krebs' solution at 4°C of the following composition (expressed in mmol/l): NaCl 119, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.0, EDTA 0.004, and D-glucose 11. The samples were placed onto dissecting dishes containing Krebs' solution and veins were carefully dissected free from Warthon's jelly using micro-dissecting instruments and cut into rings of approximately 3 mm width. The preparations were suspended in 10-ml organ baths and stretched with an initial tension of 3–5 g as described previously (Errasti et al. 1999). The time from delivery until the tissue was set up in the organ bath was approximately 3 h.

Changes in tension were measured with Grass isometric transducers (FT-03C; Grass Instruments, Quincy, Mass., USA) and displayed on a Grass polygraph (model 7D). During the incubation period, Krebs' solution was maintained at 37°C and at pH 7.4 by constant bubbling with 95% O_2/5% CO_2. Bath solution was replaced every 15 min. After 70 min of equilibration, each preparation was contracted with 40 mmol/l KCl to test its functional state. Optimal passive tension was adjusted throughout the equilibration period.

Des-Arg^9-BK and BK concentration-response curves in presence of COX inhibitors. After 5-h equilibration period cumulative concentration-response curves were obtained for des-Arg^9-BK or BK, BK B_1 or BK B_2 receptor-selective agonists, respectively. Only one agonist concentration-response curve was performed on a single ring. In all experiments, tissues were incubated with captopril (1 μmol/l) 30 min before BK receptor stimulation, to avoid peptide degradation by kininase II (angiotensin-converting enzyme). Some HUV rings were exposed to lysine clonixinate (100 μmol/l and 300 μmol/l), ibuprofen (100 μmol/l and 300 μmol/l), sodium salicylate (1 mmol/l), indomethacin (10, 30 and 100 μmol/l), meloxicam (3 μmol/l and 10 μmol/l), NS-398 (10 μmol/l and 30 μmol/l), 30 min before cumulative concentration-response curves were constructed. At the end of each experiment, the BK B_2 receptor agonist BK (0.1 μmol/l) or serotonin (10 μmol/l) was applied to determine the tissue maximal response.

Western-blot analysis of COX-1 and COX-2. HUVs were cut and the vein was carefully dissected free of surrounding tissues and mechanically denuded of endothelium. Tissues were homogenized in ice-cold buffer (50 mmol/l, Tris) with a Polytron homogenizer (Kinemateca, Switzerland). Microsomal fractions were obtained as previously described (Paz et al. 1999). Protein concentration was determined by the Bradford (1976) method using a Bio-Rad Kit. Microsomes were boiled in Laemmli sample buffer (Laemmli 1970) and microsomal protein (80 μg) was loaded to a 10% SDS-polyacrylamide gel electrophoresis. The resolved proteins were electrotransferred onto nitrocellulose membranes as described by Towbin et al. (1979), and stained briefly with Ponceau S to determine uniformity of electrophoretic transfer. Membranes were blocked in Tris-buffered saline (TBS) containing 0.5% Tween 20 and 1% BSA and then incubated overnight with 1:1000 dilution of either COX-1 or COX-2 rabbit polyclonal antibodies. Membranes were washed five times in TBS containing 0.5% Tween 20 prior to incubation with alkaline phosphatase-conjugated goat anti-rabbit IgG for 1 h. Immunoreactive bands were detected by enhanced chemiluminescence.

Chemicals and solutions. Ibuprofen, indomethacin and NS-398 (*N*-(2-cyclohexyloxy-4-nitrophenyl)-methanesulfonamide) were purchased from Biomol Research Laboratories (Plymouth Meeting, Pa., USA); BK, captopril and sodium salicylate were from Sigma Chemical (St. Louis, Mo., USA); des-Arg^9-BK was obtained from Bachem Bioscience (Torrance, Calif., USA); serotonin creatinine sulfate complex was purchased from Research Biochemicals (Natick, Mass., USA). COX-1 and COX-2 rabbit polyclonal antibodies were from Cayman Chemical (Ann Arbour, Mich., USA). Lysine clonixinate was a generous gift from Roemmers (Argentina) and meloxicam was donated by Boehringer Ingelheim (Argentina). All concentrations of drugs are expressed as final concentrations in the organ bath. Ibuprofen, indomethacin, meloxicam and sodium salicylate were made up in ethanol (99.5%) on the day of use. Stock solutions of peptides, serotonin and captopril were made up in distilled water, stored frozen in aliquots and thawed and diluted daily. NS-398 was dissolved in dimethylsulfoxide (DMSO) and stored at –20°C. The final concentrations of ethanol and DMSO in the bath solutions were always less than 1%. Lysine clonixinate was prepared daily in alkaline solution. Control trials were performed in the presence of the corresponding concentration of ethanol or DMSO.

Expression of results and statistical analysis. All data are presented as means ± SEM. Responses are expressed as grams of developed contraction. The pEC_{50} values, negative logarithms of the agonist concentration that produces 50% of the maximum, were determinated using ALLFIT, a nonlinear curve-fitting computer program (De Lean et al. 1978).

The NSAIDs' blocking potencies (pK_b) were estimated according to:

$$pK_b = \log\{(DR-1)/[NSAID]\} \quad (1)$$

where DR is the concentration ratio between the EC_{50} value in the presence and absence of NSAID. Only the lowest concentration of NSAID that shifts the curve to the right without affecting its max-

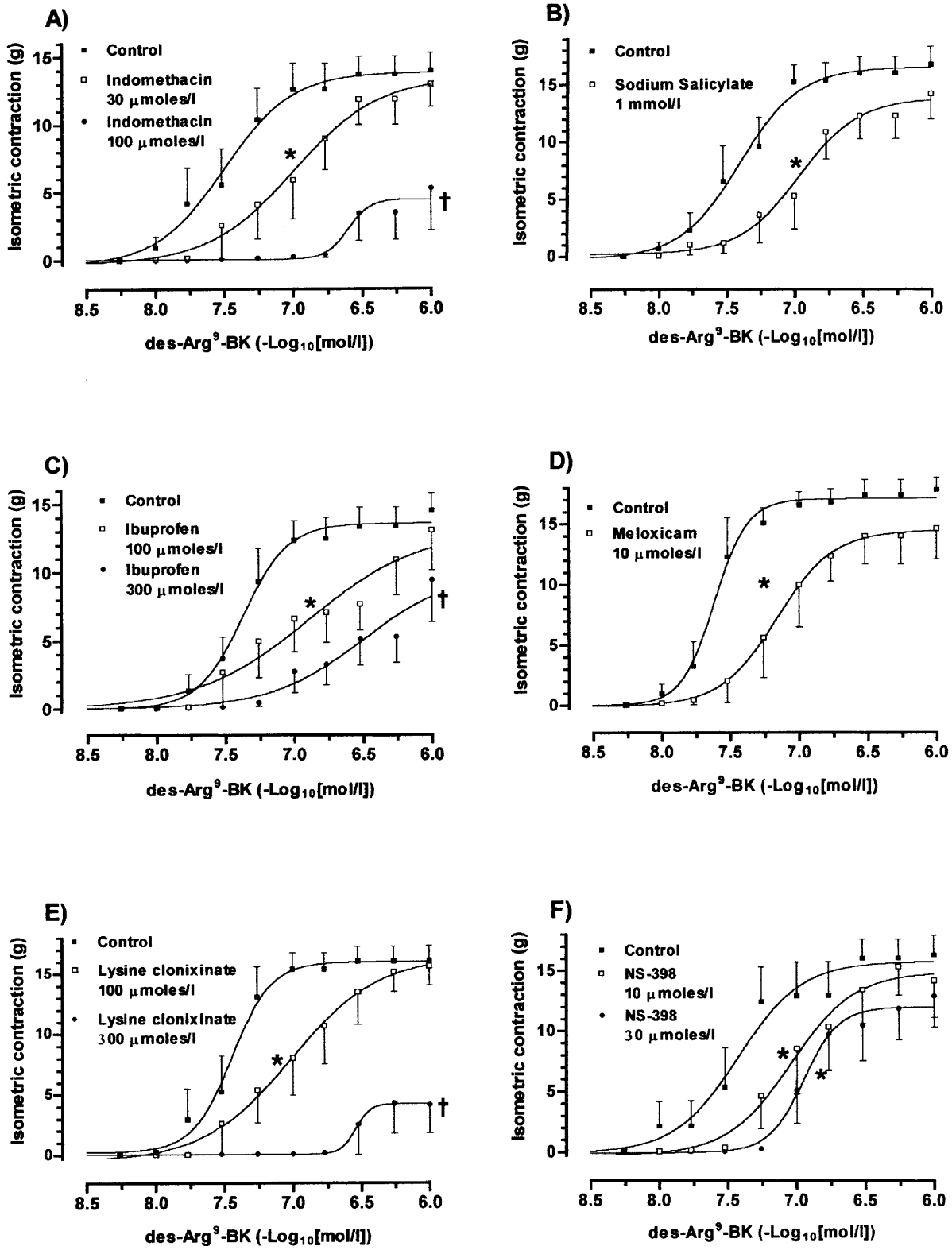

Fig. 1 Concentration-effect curves to des-Arg^9-BK on control HUV rings (■, **A** *n*=8, **B** *n*=7, **C** *n*=8, **D** *n*=5, **E** *n*=7, **F** *n*=6) and on tissues exposed to NSAIDs for 30 min. Tissues were exposed to: indomethacin (**A** 30 μmol/l, ❑, *n*=5; 100 μmol/l, ●, *n*=5); sodium salicylate (**B** 1 mmol/l, ❑, *n*=7); ibuprofen (**C** 100 μmol/l, ❑, *n*=5; 300 μmol/l, ●, *n*=6); meloxicam (**D** 10 μmol/l, ❑, *n*=5); lysine clonixinate (**E** 100 μmol/l, ❑, *n*=7; 300 μmol/l, ●, *n*=4) or NS-398 (**F** 10 μmol/l, ❑, *n*=6; 30 μmol/l, ●, *n*=4). *Points* represent the mean of *n* determinations made after the 5-h equilibration period; *vertical lines* show SEM. Responses are expressed in grams of developed contraction. *Abscissa scale:* $-\log_{10}$ of molar concentration. *,†Significant differences ($P<0.05$) between pEC_{50} and maximal response, respectively

Table 1 Effect of NSAIDs on the contractile responses to des-Arg^9-BK or BK in HUV (*NC* not calculated)

	pEC_{50}		Maximal response (g)		*n*
	Control	Treated	Control	Treated	
Des-Arg^9-bradykinin					
Indomethacin (10 μmol/l)	7.29±0.18	7.14±0.13	14.8±2.3	11.9±1.4	5
Indomethacin (30 μmol/l)	7.43±0.06	7.00±0.15*	14.7±1.1	13.2±2.0	5
Indomethacin (100 μmol/l)	7.45±0.20	NC	13.5±2.0	5.3±3.1*	5
Sodium salicylate (1 mmol/l)	7.40±0.08	6.97±0.10*	16.6±1.5	14.0±2.3	7
Ibuprofen (100 μmol/l)	7.43±0.06	6.90±0.11*	13.8±1.2	13.0±2.9	5
Ibuprofen (300 μmol/l)	7.36±0.07	NC	14.9±1.5	9.4±3.1*	6
Meloxicam (10 μmol/l)	7.62±0.04	7.16±0.10*	17.7±1.0	14.5±2.6	5
Lysine clonixinate (100 μmol/l)	7.44±0.06	7.01±0.15*	16.1±1.2	15.6±1.6	7
Lysine clonixinate (300 μmol/l)	7.31±0.06	NC	15.1±2.1	4.1±2.4*	4
NS-398 (10 μmol/l)	7.43±0.11	7.04±0.12*	16.2±1.6	14.0±3.0	6
NS-398 (30 μmol/l)	7.47±0.06	6.95±0.07*	16.9±2.4	12.8±2.6	4
Bradykinin					
Indomethacin (30 μmol/l)	9.59±0.07	9.30±0.10*	15.8±2.0	15.7±1.8	7
Indomethacin (100 μmol/l)	9.59±0.07	8.74±0.16*	15.8±2.0	15.6±2.6	7
NS-398 (10 μmol/l)	9.54±0.09	9.50±0.11	15.6±2.3	15.9±2.0	6
NS-398 (30 μmol/l)	9.61±0.06	9.08±0.10*	16.6±1.3	16.7±2.5	6

*Significant differences between treated and control paired tissues (P<0.05)

imal response was used for this calculation. The pK_b estimates obtained for the different NSAIDs in the HUV were plotted against previously published pIC_{50} of these NSAIDs for COX-1 or COX-2 (Futaki et al. 1994; Pallapies et al. 1995; Frölich 1997). Linear regression was used to correlate pK_b and IC_{50} values. Statistical analysis was performed by means of paired Student's *t*-test. *P*-values lower than 0.05 were taken to indicate significant difference between means.

Results

Effect of COX inhibition on BK B_1 receptor-mediated response in isolated HUV

The possible involvement of COX products on the des-Arg^9-BK contractile response has been assessed by testing the effect of NSAIDs with different relative COX-1 and COX-2 selectivity. All the concentration-response curves to the selective BK B_1 receptor agonist were performed after 5 h of incubation. HUV rings were exposed to NSAIDs 30 min before the construction of the curves. The effect of ibuprofen (100, 300 μmol/l), meloxicam (10 μmol/l), sodium salicylate (1 mmol/l) or lysine clonixinate (100, 300 μmol/l), which inhibit both COX-1 and COX-2 with similar potency, was evaluated. Moreover, HUV rings were treated with the COX-1-selective inhibitor, indomethacin (10, 30, 100 μmol/l) as well as the COX-2-selective inhibitor, NS-398 (10, 30 μmol/l). All treatments, except indomethacin 10 μmol/l, produced a significant rightward shift of the contractile response to des-Arg^9-BK (Fig. 1; Table 1). Furthermore, some NSAID concentrations diminished the maximal response to this selective BK B_1 receptor agonist (Fig. 1; Table 1), but did not modify the maximal response to the selective BK B_2 receptor agonist BK (0.1 μmol/l) at the end of each experiment (control: 15.8±2.1 g; indomethacin 100 μmol/l: 15.5±2.6 g, *n*=5; control: 18.5±1.4 g; ibuprofen 300 μmol/l: 17.2±2.2 g, *n*=6; control: 15.8±0.5 g; lysine clonixinate 300 μmol/l: 14.5±1.7 g, *n*=4).

The NSAIDs' potencies (pK_b) estimated for their antagonist effects on the BK B_1 receptor-mediated responses in HUV are shown in Table 2. Figure 2 shows the correlation between NSAIDs' pK_b in HUV and previously published pIC_{50} for COX-1 or COX-2. The scattergrams for COX-1 and COX-2 show a clear relationship between the present functional data (pK_b) and COX-2 affinities (COX-1: r^2= 0.276, P=0.28; COX-2: r^2=0.841, P<0.05; Fig. 2).

Effect of COX inhibition on BK B_2 receptor-mediated response in isolated HUV

In order to evaluate the possible involvement of prostanoids on BK B_2 receptor contractile responses, the effects of in-

Table 2 NSAIDs' potencies for their blocking actions on BK B_1 or BK B_2 responses in HUV and their previously published pIC_{50} for COX-1 and COX-2 (*ND* not determined)

	pK_b		pIC_{50}	
	BK B_1 receptor	BK B_2 receptor	COX-1	COX-2
Indomethacin	4.8	4.5	7.6[a]	5.8[a]
Sodium salicylate	3.2	ND	3.6[a]	3.1[a]
Ibuprofen	4.4	ND	5.3[a]	4.1[a]
Meloxicam	5.3	ND	6.7[a]	6.8[a]
Lysine clonixinate	4.2	ND	5.2[b]	4.2[b]
NS-398	5.2	5.0	<4.0[b]	5.4[b]

[a]Bovine aortic endothelial cells were used for determining COX-1 activity, J774.2 macrophages induced with lipopolysaccharide to express COX-2 (Frölich 1997)
[b]Purified enzyme preparations from sheep seminal vesicles (COX-1) and sheep placenta (COX-2; Futaki et al. 1994; Pallapies et al. 1995)

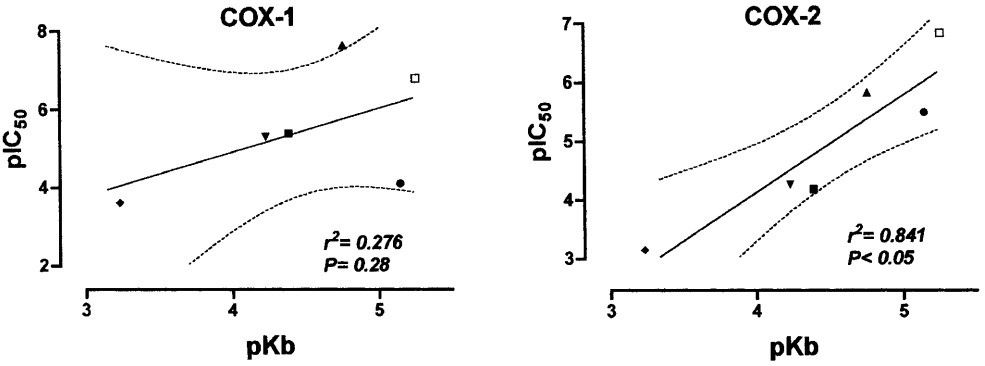

Fig. 2 Correlation plots comparing the NSAID potencies (pK_b) for their blocking effects on the BK B_1 receptor-mediated response and previously published pIC_{50} for COX-1 and COX-2. The following NSAIDs are shown: indomethacin (▲, 30 μmol/l); sodium salicylate (◆, 1 mmol/l); ibuprofen (■, 100 μmol/l); meloxicam (❑, 10 μmol/l); lysine clonixinate (▼, 100 μmol/l) and NS 398 (●, 10 μmol/l)

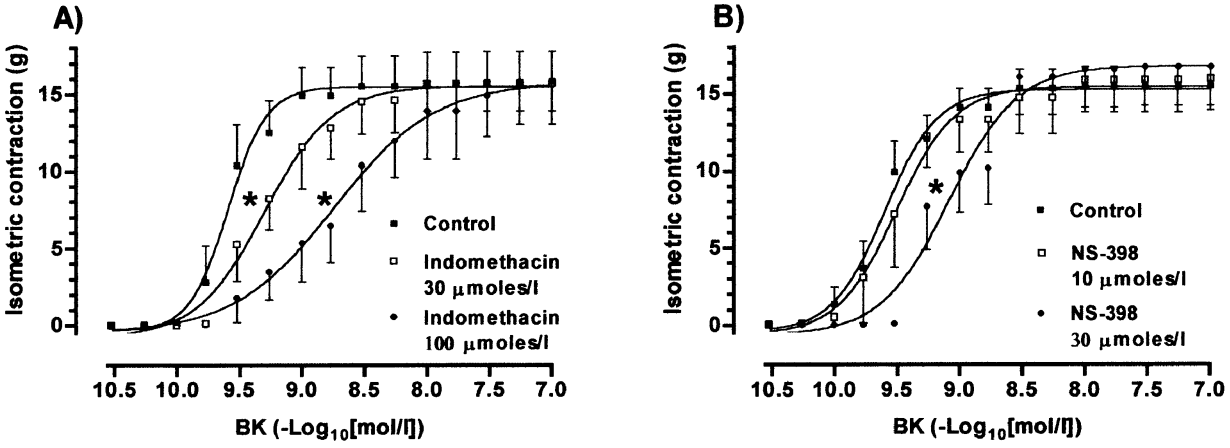

Fig. 3 Concentration-effect curves to BK on control HUV rings (■, **A** n=7, **B** n=12) and on tissues exposed to indomethacin or NS-398 for 30 min. Tissues were exposed to: indomethacin (**A** 30 μmol/l, ❑, n=7; 100 μmol/l, ●, n=7); NS-398 (**B** 10 μmol/l, ❑, n=6; 30 μmol/l, ●, n=6). *Points* represent the mean of n determinations made after the 5-h equilibration period; *vertical lines* show SEM. Responses are expressed in grams of developed contraction. *Abscissa scale:* $-\log_{10}$ of molar concentration. *Significant differences (P<0.05) between pEC_{50}

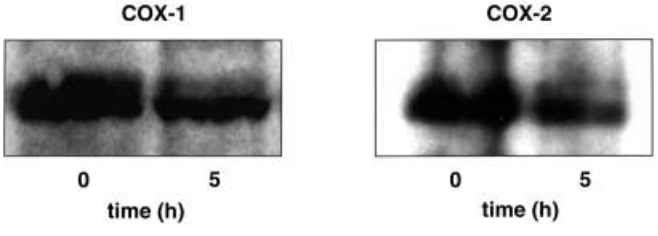

Fig. 4 COX-1 and COX-2 protein expression in HUV before and after in vitro incubation. HUV ring lysates were prepared immediately post-dissection (t=0 h) and after a prolonged in vitro incubation (t=5 h). Equal amounts of tissue protein (80 μg/lane) were separated on a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. Membranes were analysed by Western blot using human COX-1- and COX-2-specific antibodies. Both COX-1 and COX-2 bands migrated with an approximate molecular mass of 70 kDa. This figure is representative of three independent experiments

domethacin (30 μmol/l and 100 μmol/l) or NS-398 (10 μmol/l and 30 μmol/l) were studied. When HUV rings were exposed to indomethacin (30 μmol/l and 100 μmol/l) or NS-398 (30 μmol/l), a significant rightward shift of the concentration-response curves to BK was observed without affecting the maximal response (Fig. 3; Table 1). The NSAIDs' potencies (pK_b) estimated for their inhibitory effects on the BK B_2 receptor-mediated responses in HUV are shown in Table 2.

COX-1 and COX-2 expression in isolated HUV

HUV expresses both COX-1 and COX-2 proteins as shown by Western blot analysis (Fig. 4). COX-1 and COX-2 proteins were measured before (t=0 h) and after a prolonged in vitro incubation (t=5 h). Figure 4 suggests a decrease of COX-1 as well as COX-2 proteins at 5-h incubation period of the tissues. Antibodies against COX-1 or COX-2 identified bands of approximately 70 kDa in both experimental conditions.

Discussion

In the isolated HUV, we have previously demonstrated that contractile response to des-Arg^9-BK, selective BK B_1 receptor agonist, increases as a function of the in vitro incubation time (Sardi et al. 1997). The BK B_1 receptors of this tissue behave as their counterparts in rabbit vascular tissues since they are expressed de novo in a protein synthesis-, trafficking- and glycosilation-dependent process (Audet et al. 1994; Sardi et al. 1998, 1999). On the other

hand, BK B_2 receptors are present in a preformed and stable manner, and mediate a maximal vasoconstriction in HUV (Altura et al. 1972; Gobeil et al. 1996; Sardi et al. 1997).

The second messenger pathways activated by BK B_1 receptors are similar to those coupled to BK B_2 receptors. Both BK receptors are coupled to $G_{q/11}$ proteins to stimulate either phospholipase C or phospholipase A_2 (Marceau et al. 1998). In several tissues, it has been described that BK B_1 and B_2 receptors stimulate phosphatidylinositol hydrolysis leading to mobilization of intracellular calcium. In addition, both receptors, through phospholipase A_2 pathway, release arachidonic acid and consequently increase prostanoid production (Slivka and Insel 1988; Farmer et al. 1991; Tropea et al. 1993; Delamere et al. 1994; Schneck et al. 1994; Pang and Knox 1997).

In HUV, indomethacin (10 μmol/l) does not affect the concentration-response curve to the BK B_1 receptor agonist (see Table 1). This is in agreement with previously published data reported by our group (Sardi et al. 1997). However, when HUV rings were exposed to higher concentrations of indomethacin, a concentration-dependent rightward shift of the contractile response to des-Arg^9-BK was observed. The BK B_1 receptor-mediated responses were antagonized by indomethacin with a pK_b value of 4.8 (Table 2), that is a K_b value of 15.8 μmol/l. These results agree with previous reports showing that des-Arg^9-BK-induced effects in vitro could be mediated through the production of arachidonic metabolites that induce smooth muscle contraction (Meini et al. 1998; Bagate et al. 1999). Furthermore, in different tissues, the BK B_1 effects of des-Arg^9-BK are inhibited by indomethacin (Churchill and Ward 1986; Drapeau et al. 1991; Li et al. 1998; Bagate et al. 1999).

In the present study, a concentration-dependent rightward shift of the contractile response to des-Arg^9-BK was also obtained employing other NSAIDs with different COX-1 and COX-2 selectivity, such as sodium salicylate, ibuprofen, meloxicam, lysine clonixinate or NS-398. The inhibitory effects evoked by all the NSAIDs employed in this study on the responses mediated by the agonist des-arg^9-BK provide pharmacological evidence to support the view that COX pathway is involved in the signal transduction of the BK B_1 receptor in HUV.

Furthermore, the results obtained with the COX-2-selective inhibitor NS-398 suggest that COX-2 pathway is the most relevant. Previous studies employing different experimental conditions in various in vitro assay systems have shown variable IC_{50} values for inhibition of COX-2 activity by the highly selective inhibitor compound NS-398. These values range from 30 nmol/l to 3.8 μmol/l (Futaki et al. 1994; Masferrer et al. 1994; Panara et al. 1995; Miralpeix et al. 1997; Snyder et al. 1999). The results obtained with NS-398 under our experimental conditions allow us to estimate an inhibitory affinity value (pK_b: 5.2) on BK B_1 receptor-mediated responses. The NS-398 pK_b observed in HUV is similar to the inhibitory value on COX-2 activity (IC_{50}: 3.8 μmol/l, pIC_{50}: 5.4) obtained with this isoenzyme isolated from sheep placenta (Futaki et al. 1994). On the other hand, in two different preparations (Baculovirus-expressed recombinant murine COX-1 enzyme; Masferrer et al. 1994; and COX-1 purified enzyme from ram seminal vesicles; Futaki et al. 1994) NS-398 has not affected COX-1 activity at concentrations as high as 100 μmol/l.

On the other hand, these results allow us to estimate an inhibitory affinity value (pK_b) for each NSAID on BK B_1-mediated responses and speculate about which COX isoform is involved in these responses. Although correlation does not necessarily establish causality, our present results showing a strong correlation between the previously published pIC_{50} for COX-2 and the NSAIDs' pK_b estimates further support the hypothesis that COX-2 metabolites are involved in BK B_1-mediated responses in HUV.

In several tissues BK B_2 receptor-mediated contractions are associated with the production of metabolites of arachidonic acid generated through COX pathway. COX inhibitors reduce the contraction caused by BK in human foetal placental veins (Tulenko 1981), rat mesenteric arteries (Fasciolo et al. 1990; Weinberg et al. 1997), canine saphenous vein (Marsault et al. 1997) and guinea-pig trachea (Da Silva et al. 1995). In the present study, when HUV rings were exposed to indomethacin (30 μmol/l and 100 μmol/l) or NS-398 (30 μmol/l), a significant rightward shift of the concentration-response curves to BK was observed yielding NSAID potency values (pK_b) that are in agreement with previously published pIC_{50} for COX-2. Therefore, in isolated HUV, COX-2 metabolites seem to be involved in both BK B_1 and B_2 contractile responses.

In contrast to the constitutive expression of COX-1 in many organs and tissues, COX-2 is an isoenzyme that is characterized by absent or low expression under resting conditions whose induction is dependent on stimulation by cytokines, mitogens and a variety of tissue activators (Vane et al. 1998). On the other hand, both isolated arteries and veins of umbilical cords from full-term pregnancies produce great amounts of prostanoids (Bjoro et al. 1986). In endothelium-denuded HUV obtained from normal full-term deliveries, Western blot analysis shows that both COX-1 and COX-2 proteins are expressed. Considering that COX-1 is a constitutive protein and its expression should not be decreased during the incubation period, the apparently diminished expression of both isoenzymes at 5 h could be related to a protein loss throughout the in vitro incubation in addition with the washouts every 15 min. Moreover, the amount of COX-2 in HUV after 5-h incubation period suggests that this enzyme does not suffer an additional in vitro induction process. These results are in accord with previous studies in human foetal membranes and myometrium. In these tissues, COX-1 expression is not significantly changed with labour, whereas expression and activity of COX-2 are significantly increased before and during term and pre-term labour in myometrium, amnion, chorion and placenta (Slater et al. 1999; Sawdy et al. 2000).

In summary, our present results suggest that prostanoid pathway participates in the BK B_1-sensitized contractile

responses in HUV, preferentially through COX-2 isoenzyme. Moreover, in this tissue the COX-2 pathway also seems to be involved in BK B_2 receptor-mediated responses. Furthermore, we have shown that both COX-1 and COX-2 are present in the isolated HUV before incubation and, apparently, COX-2 does not suffer an additional up-regulation process with the 5 h of in vitro incubation. Further experiments with simultaneous measurements of endogenous eicosanoid synthesis in response to des-Arg^9-BK and BK, in the absence and presence of the NSAIDs, should be performed to determine which prostanoid(s) participate on the kinin responses in HUV.

Acknowledgements We wish to thank the Instituto Médico de Obstetricia (Buenos Aires) for their efforts in providing umbilical tissues. A.E. Errasti and S.P. Sardi are research fellows of the Universidad de Buenos Aires (UBA). This research was supported by grants from the UBA (Grant TM-049).

References

Altura BM, Malaviya D, Reich CF, Orkin LR (1972) Effects of vasoactive agents on isolated human umbilical arteries and veins. Am J Physiol 222:345–355

Audet R, Petitclerc E, Drapeau G, Rioux F, Marceau F (1994) Further analysis of the up-regulation of bradykinin B_1 receptors in isolated rabbit aorta by using metabolic inhibitors. Eur J Pharmacol 271:551–555

Bagate K, Develioglu L, Imbs JL, Michel B, Helwig JJ, Barthelmebs M (1999) Vascular kinin B(1) and B(2) receptor-mediated effects in the rat isolated perfused kidney – differential regulations. Br J Pharmacol 128:1643–1650

Bathon JM, Proud D (1991) Bradykinin antagonists. Annu Rev Pharmacol Toxicol 31:129–162

Bjoro K, Hovig T, Stokke KT, Stray-Pedersen S (1986) Formation of prostanoids in human umbilical vessels perfused in vitro. Prostaglandins 31:683–698

Bouthillier J, Deblois D, Marceau F (1987) Studies on the induction of pharmacological responses to des-Arg9-bradykinin in vitro and in vivo. Br J Pharmacol 92:257–264

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Churchill L, Ward PE (1986) Relaxation of isolated mesenteric arteries by des-Arg9-bradykinin stimulation of B1 receptors. Eur J Pharmacol 130:11–18

Da Silva A, Amrani Y, Trifilieff A, Landry Y (1995) Involvement of B2 receptors in the bradykinin-induced relaxation of guinea-pig isolated trachea. Br J Pharmacol 114:103–108

De Lean A, Munson PJ, Rodbard D (1978) Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. Am J Physiol 235:E97–E102

De Weerd WF, Leeb-Lundberg LM (1997) Bradykinin sequesters B2 bradykinin receptors and the receptor-coupled G-alpha subunits G-alphaq and G-alphai in caveolae in DDT1 MF-2 smooth muscle cells. J Biol Chem 272:17858–17866

Delamere F, Holland E, Patel S, Bennett J, Pavord I, Knox A (1994) Production of PGE2 by bovine cultured airway smooth muscle cells and its inhibition by cyclo-oxygenase inhibitors. Br J Pharmacol 111:983–988

Drapeau G, deBlois D, Marceau F (1991) Hypotensive effects of Lys-des-Arg9-bradykinin and metabolically protected agonists of B1 receptors for kinins. J Pharmacol Exp Ther 259:997–1003

Errasti AE, Rogines Velo MP, Torres RM, Sardi SP, Rothlin RP (1999) Characterization of alpha1-adrenoceptor subtypes mediating vasoconstriction in human umbilical vein. Br J Pharmacol 126:437–442

Farmer SG, Ensor JE, Burch RM (1991) Evidence that cultured airway smooth muscle cells contain bradykinin B2 and B3 receptors. Am J Respir Cell Mol Biol 4:273–277

Fasciolo JC, Vargas L, Lama MC, Nolly H (1990) Bradykinin-induced vasoconstriction of rat mesenteric arteries precontracted with noradrenaline. Br J Pharmacol 101:344–348

Frölich JC (1997) A classification of NSAIDs according to the relative inhibition of cyclooxygenase isoenzymes. Trends Pharmacol Sci 18:30–34

Futaki N, Takahashi S, Yokoyama M, Arai I, Higuchi S, Otomo S (1994) NS-398, a new anti-inflammatory agent, selectively inhibits prostaglandin G/H synthase/cyclooxygenase (COX-2) activity in vitro. Prostaglandins 47:55–59

Gobeil F, Pheng LH, Badini I, Nguyen-Le XK, Pizard A, Rizzi A, Blouin D, Regoli D (1996) Receptors for kinins in the human isolated umbilical vein. Br J Pharmacol 118:289–294

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Li L, Vaali K, Paakkari I, Vapaatalo H (1998) Involvement of bradykinin B1 and B2 receptors in relaxation of mouse isolated trachea. Br J Pharmacol 123:1337–1342

Marceau F, Hess JF, Bachvarov DR (1998) The B1 receptors for kinins. Pharmacol Rev 50:357–386

Marsault R, Illiano S, Vanhoutte PM (1997) Bradykinin-induced contractions of canine saphenous veins: mediation by B2 receptors and involvement of eicosanoids. Br J Pharmacol 120: 215–220

Masferrer JL, Zweifel BS, Manning PT, Hauser SD, Leahy KM, Smith WG, Isakson PC, Seibert K (1994) Selective inhibition of inducible cyclooxygenase 2 in vivo is anti-inflammatory and nonulcerogenic. Proc Natl Acad Sci USA 91:3228–3232

McEachern AE, Shelton ER, Bhakta S, Obernolte R, Bach C, Zuppan P, Fujisaki J, Aldrich RW, Jarnagin K (1991) Expression cloning of a rat B2 bradykinin receptor. Proc Natl Acad Sci USA 88 [Suppl 17]:7724–7728

Meini S, Lecci A, Cucchi P, Catalioto RM, Criscuoli M, Maggi CA (1998) Inflammation modifies the role of cyclooxygenases in the contractile responses of the rat detrusor smooth muscle to kinin agonists. J Pharmacol Exp Ther 287:137–143

Menke JG, Borkowski JA, Bierilo KK, MacNeil T, Derrick AW, Schneck KA, Ransom RW, Strader CD, Linemeyer DL, Hess JF (1994) Expression cloning of a human B1 bradykinin receptor. J Biol Chem 269:21583–21586

Miralpeix M, Camacho M, Lopez-Belmonte J, Canalias F, Beleta J, Palacios JM, Vila L (1997) Selective induction of cyclo-oxygenase-2 activity in the permanent human endothelial cell line HUV-EC-C: biochemical and pharmacological characterization. Br J Pharmacol 121:171–180

Morita I, Schindler M, Regier MK, Otto JC, Hori T, DeWitt DL, Smith WL (1995) Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. J Biol Chem 270: 10902–10908

Otto JC, DeWitt DL, Smith WL (1993) *N*-glycosylation of prostaglandin endoperoxide synthases-1 and -2 and their orientations in the endoplasmic reticulum. J Biol Chem 268:18234–18242

Pallapies D, Salinger A, Meyer zum Gottesberge A, Atkins DJ, Rohleder G, Nagyivanyi P, Peskar BA (1995) Effects of lysine clonixinate and ketorolac tromethamine on prostanoid release from various rat organs incubated ex vivo. Life Sci 57:83–89

Panara MR, Greco A, Santini G, Sciulli M, Rotondo M, Padovano R, Giamberardino M, Cipollone F, Cuccurullo F, Patrono C, Patrignani P (1995) Effects of the novel anti-inflammatory compounds, *N*-[2-(cyclohexyloxy)-4-nitrophenyl] methanesulphonamide (NS-398) and 5-methanesulphonamido-6-(2,4-difluorothio-phenyl)-1-inda none (L-745,337), on the cyclo-oxygenase activity of human blood prostaglandin endoperoxide synthases. Br J Pharmacol 116:2429–2434

Pang L, Knox AJ (1997) PGE2 release by bradykinin in human airway smooth muscle cells: involvement of cyclooxygenase-2 induction. Am J Physiol 273:L1132–L1140

Paz C, Cornejo MacIel F, Mendez C, Podesta EJ (1999) Corticotropin increases protein tyrosine phosphatase activity by a cAMP-dependent mechanism in rat adrenal gland. Eur J Biochem 265:911–918

Regoli D, Barabe J, Park WK (1977) Receptors for bradykinin in rabbit aorta. Can J Physiol Pharmacol 55:855–867

Regoli D, Marceau F, Barabe J (1978) De novo formation of vascular receptors for bradykinin. Can J Physiol Pharmacol 56:674–677

Sardi SP, Perez H, Antunez P, Rothlin RP (1997) Bradykinin B1 receptors in human umbilical vein. Eur J Pharmacol 321:33–38

Sardi SP, Rey Ares V, Errasti AE, Rothlin RP (1998) Bradykinin B1 receptors in human umbilical vein: pharmacological evidence of up-regulation, and induction by interleukin-1 beta. Eur J Pharmacol 358:221–227

Sardi SP, Daray FM, Errasti AE, Pelorosso FG, Pujol-Lereis VA, Rey-Ares V, Rogines-Velo MP, Rothlin RP (1999) Further pharmacological characterization of bradykinin B1 receptor up-regulation in human umbilical vein. J Pharmacol Exp Ther 290:1019–1025

Sardi SP, Rey Ares V, Errasti AE, Rogines Velo MP, Rothlin RP (2000) Bradykinin B_1 receptor in isolated human umbilical vein: an experimental model of the in vitro up-regulation process. Acta Pharmacol Sinica 21:105–110

Sawdy RJ, Slater DM, Dennes WJ, Sullivan MH, Bennett PR (2000) The roles of the cyclo-oxygenases types one and two in prostaglandin synthesis in human fetal membranes at term. Placenta 21:54–57

Schneck KA, Hess JF, Stonesifer GY, Ransom RW (1994) Bradykinin B1 receptors in rabbit aorta smooth muscle cells in culture. Eur J Pharmacol 266:277–282

Slater DM, Dennes WJ, Campa JS, Poston L, Bennett PR (1999) Expression of cyclo-oxygenase types-1 and -2 in human myometrium throughout pregnancy. Mol Hum Reprod 5:880–884

Slivka SR, Insel PA (1988) Phorbol ester and neomycin dissociate bradykinin receptor-mediated arachidonic acid release and polyphosphoinositide hydrolysis in Madin-Darby canine kidney cells. Evidence that bradykinin mediates noninterdependent activation of phospholipases A2 and C. J Biol Chem 263: 14640–14647

Snyder DW, Bach NJ, Dillard RD, Draheim SE, Carlson DG, Fox N, Roehm NW, Armstrong CT, Chang CH, Hartley LW, Johnson LM, Roman CR, Smith AC, Song M, Fleisch JH (1999) Pharmacology of LY315920/S-5920, [[3-(aminooxoacetyl)-2-ethyl-1-(phenylmethyl)-1*H*-indol-4-yl]oxy] acetate, a potent and selective secretory phospholipase A2 inhibitor: a new class of anti-inflammatory drugs, SPI. J Pharmacol Exp Ther 288: 1117–1124

Tanaka H, Watanabe K, Tamaru N, Yoshida M (1995) Arachidonic-acid metabolites and glucocorticoid regulatory mechanism in cultured porcine tracheal smooth-muscle cells. Lung 173:347–361

Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354

Tropea MM, Gummelt D, Herzig MS, Leeb-Lundberg LM (1993) B1 and B2 kinin receptors on cultured rabbit superior mesenteric artery smooth muscle cells: receptor-specific stimulation of inositol phosphate formation and arachidonic acid release by des-Arg9-bradykinin and bradykinin. J Pharmacol Exp Ther 264:930–937

Tulenko TN (1981) The actions of prostaglandins and cyclo-oxygenase inhibition on the resistance vessels supplying the human fetal placenta. Prostaglandins 21:1033–1043

Vane JR, Bakhle YS, Botting RM (1998) Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol 38:97–120

Weinberg ML, Moreira E, Weinberg J (1997) Arachidonic acid products-mediated contraction induced by bradykinin in relaxed mesenteric arterial rings from Holtzman rats. Eur J Pharmacol 320:145–150