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European Journal of Pharmacology 501 (2004) 127–135

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Arachidonic acid relaxes human pulmonary arteries through K^+ channels and nitric oxide pathways

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Received 16 June 2004; received in revised form 2 August 2004; accepted 4 August 2004
Available online 11 September 2004

Abstract

We aimed to investigate the role of K^+ channels and nitric oxide (NO) on the relaxant effects of arachidonic acid in the human intralobar pulmonary arteries. Arachidonic acid produced a concentration-dependent relaxation (E_{max}=93±3% of maximal relaxation induced by papaverine 0.1 mM; −log EC_{30}=7.03±0.09) that was antagonized by the cyclooxygenase inhibitor indomethacin (1 μM), by the combination of cyclooxygenase blockade and cytochrome P450 (CYP) blockade with 17-octadecynoic acid (17-ODYA, 10 μM), by the combination of cyclooxygenase inhibition and NO synthase (NOS) inhibition with N^{ω}-nitro-L-arginine (L-NOARG, 100 μM), by the simultaneous inhibition of CYP and NOS and by the simultaneous blockade of cyclooxygenase, CYP and NOS. Arachidonic acid-induced relaxation was significantly inhibited by glibenclamide (1 μM, ATP-dependent K^+ channel (K_{ATP}) blocker), apamin and charybdotoxin (0.3 μM small (SK_{Ca}) and 0.1 μM big (BK_{Ca}) conductance Ca^{2+}-sensitive K^+ channel blocker, respectively), and 4-aminopyridine (1 mM, voltage-dependent K^+ channel (K_V) blocker). Indomethacin and ketoconazole suppressed the antagonistic effects of glibenclamide and apamin and 17-ODYA those of all the K^+ channel blockers tested. L-NOARG suppressed only the antagonistic effect of glibenclamide. We suggest that K_{ATP}, SK_{Ca}, BK_{Ca} and K_V are involved in the arachidonic acid-induced relaxation of human pulmonary arteries. Cyclooxygenase metabolites are the main relaxing agents of arachidonic acid, involving K_{ATP} and SK_{Ca} channels. CYP-dependent metabolites modulate arachidonic acid-induced relaxation through a pathway involving K^+ channels. K_{ATP} channels are involved through a NOS-dependent pathway.

Keywords: Human pulmonary arteries; Arachidonic acid; Cyclooxygenase; Cytochrome P450; NO (Nitric oxide); K^+ channels

1. Introduction

Vascular smooth muscle cells of the lung have the ability to release arachidonic acid from membrane phospholipid stores through phospholipases, under various biological and physiological stimuli (Hyman et al., 1981). Once released, the free arachidonic acid may be oxidized along one of three major metabolic pathways: the cyclooxygenase pathway that produces prostaglandins, thromboxane and prostacyclin; the lipoxygenase pathway that synthesizes leukotrienes; and the cytochrome P450 (CYP)-dependent mono-oxygenase pathway that generates 19- and 20-hydroxyeicosatetraenoic acid (HETEs) by ω- and ω1-hydroxylase, respectively, and epoxyeicosatrienoic acid (EETs) by epoxygenase (Holtzman, 1991; Quilley and McGiff, 2000).

These metabolites have been described to possess various pharmalogical and physiological functions in the lung. Prostacyclin, a major metabolite of pulmonary artery endothelium, has vasodilator properties (Walch et al., 1999) whereas prostaglandin $F_{2\alpha}$ and thromboxane A_2 are

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doi:10.1016/j.ejphar.2004.08.007

both vasoconstrictors (Hyman et al., 1981). Lipoxygenase products are involved in airway inflammation and vascular permeability exhibited in asthma (Adelroth et al., 1986; Drazen, 1999). Recently, a large body of evidence has risen on the role of cytochrome P450 (CYP) pathway in the lung. For example, 20-HETE relaxes phenylephrine-constricted rabbit pulmonary artery rings and conversely blockade of its release enhances phenylephrine-induced contraction of pulmonary artery rings (Zhu et al., 2000a). Several studies support the view that EETs are also vasodilators in the pulmonary circulation (Stephenson et al., 1998; Stephenson et al., 1996; Tan et al., 1997). In addition, a relationship between cyclooxygenase and CYP pathways has been described. Small arteries from human lungs dilate upon exposure to 20-HETE in a cyclooxygenase-dependent manner (Birks et al., 1997) and EETs regioisomers contract pressurized rabbit pulmonary arteries in a cyclooxygenase- and endothelium-dependent manner (Zhu et al., 2000b). These observations suggest also that epoxygenase products may contribute to the tone of pulmonary vessels. To our knowledge, little is known about the role of K^+ channels and nitric oxide (NO) in the effects of arachidonic acid in the human pulmonary circulation. In others tissues, EETs are primary candidates for endothelium-derived hyperpolarizing factor (EDHF) (Fisslthaler et al., 1999) and arachidonic acid metabolites formed primarily by CYP produce potent endothelium-dependent dilation of human coronary arterioles through Ca^{2+}-activated K^+ channels (Miura and Gutterman, 1998).

The aim of this study was therefore to investigate in the human intralobar pulmonary arteries constricted with the thromboxane A_2 analogue, U46619, the involvement of K^+ channels and NO in the relaxant effects of arachidonic acid.

2. Material and methods

2.1. Pulmonary arteries preparation

Intralobar pulmonary arteries were removed from lung of 70 patients undergoing lobectomy for lung carcinoma. Just after resection, arteries were carefully dissected as far away as possible from the malignancy and were placed in Krebs–Henseleit solution (concentration mM): NaCl 116.3, KCl 5.4, $CaCl_2$ 1.8, NaH_2PO_4 1.04, $MgSO_4$ 0.83, $NaHCO_3$ 19, glucose 5.5; pH 7.4 and maintained at 4 °C. Arteries were dissected free of parenchyma and cut into small rings (internal diameter 1–3 mm, 5 mm in length). Each arterial segment was suspended in a 10 ml organ bath containing Krebs–Henseleit solution maintained at 37 °C and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide, by gently threading the rings onto a fixed, horizontal surgical steel wire. Once anchored a second wire of the same dimensions was connected to a force transducer. Arteries were subjected to an initial load of 1 g and were allowed to equilibrate over a 1 h time period during which Krebs–Henseleit solution was changed every 15 min. Changes in contraction force were measured isometrically (UF1-Pioden strain gauges, EMKA, Paris, France), amplified (Transbridge TBM4, WPI, Hertfordshire, UK) and recorded on a pen-writing oscillograph (LINSEIS, Munchen, Germany).

2.2. Functional procedures

In a preliminary set of experiments, following equilibration vessels were exposed to 80 mM potassium chloride (KCl) until a maximal contraction has been obtained. The Krebs solution was then changed four times at 5-min period intervals allowing vessels to relax back to baseline. Vessels where then re-exposed to 80 mM KCl and the average of these two contractions recorded. After washing by changing Krebs solution four times at 5-min period intervals the vessels were then allowed to equilibrate. The average of the two KCl-induced contractions was taken as 100% pre-constriction reference. This was used to determine the concentration of U46619, a stable analogue of the thromboxane A_2, responsible for a plateau of contraction at 80% of the 80 mM KCl-induced pre-constriction. This concentration of U46619 was therefore used in the following experiments.

In a first set of experiments, the involvement of cyclooxygenase, CYP and NO-synthase (NOS) in the vasodilator components of arachidonic acid response on the pulmonary arteries were determined by incubating artery rings, obtained from each individual patient in order to obtained pair wised experiments, with respective inhibitors before inducing contraction with U46619 (30 nM). Cyclooxygenase were blocked by indomethacin (1 μM), a concentration that is equal to 4.8 times and 2.7 times the concentration that has been shown to inhibit cyclooxygenase-1 and cyclooxygenase-2 activities, respectively, by 50% in assays of whole blood (Cryer and Feldman, 1998; FitzGerald and Patrono, 2001), ketoconazole, an imidazole derivative (10 μM), was used to inhibit epoxygenase activity (Capdevila et al., 1988), and 17-octadecynoic acid (17-ODYA, 10 μM) to inhibit the CYP4A-dependent hydroxylase that metabolizes arachidonic acid into 20-HETE and the formation of EETs by epoxygenases (Wang et al., 1998; Zou et al., 1994). NOS activity was inhibited by N^{ω}-nitro-L-arginine (L-NOARG, 100 μM).

After adding U46619, once a stable contraction was reached, cumulative concentration response-curves for arachidonic acid (1 nM to 10 μM) were constructed. Each additional concentration was added to the bath every 10–20 min until a plateau was reached. One additional paired ring was used as control for any time-related change in tension throughout the experimental procedure.

In a second set of experiments following the same experimental procedures, involvement of ATP-dependent

K^+ channel (K_{ATP}), small (SK_{Ca}) and big (BK_{Ca}) conductance Ca^{2+}-sensitive K^+ channel and voltage-dependent K^+ channel (K_V) were studied by incubating U46619-constricted arteries with K^+ channel inhibitors (glibenclamide, 1 μM; apamin, 0.3 μM; charybdotoxin, 0.1 μM and 4-aminopyridine, 1 mM to inhibit K_{ATP}, SK_{Ca}, BK_{Ca} and K_V channels, respectively). After adding inhibitors, once a stable contraction was reached again, cumulative concentration response-curves for arachidonic acid (1 nM to 10 μM) were constructed. These experiments were realized in the absence or in the presence of indomethacin, ketoconazole, 17-ODYA, L-NOARG and combination of 17-ODYA and L-NOARG added to the bath before U46619 contraction. One additional paired ring was used as control for any time-related change in tension throughout the experimental procedure.

The effects of agonists are expressed as a percentage of maximal relaxation induced by papaverine (0.1 mM) added to the bath at the end of the experiment as previously described (Bardou et al., 2002; Bardou et al., 2001; Walch et al., 1999). The formula for calculation is: $(A-x)/(A-B)$ where A is the absolute tension before beginning the cumulative concentration response curve, B is the absolute tension after adding papaverine and x is the tension level obtained after each addition of agonist. E_{max} indicates the maximal effect observed at the maximal concentration of arachidonic acid tested (10 μM). EC_{30} values indicate the concentration of each agonist producing 30% of the maximal response induced by papaverine (0.1 mM) and were calculated using the GraphPad Prism 4.01 computer program (GraphPad Software, San Diego, CA, USA). For analysis the EC_{30} values were log transformed and expressed as $-\log EC_{30}$ values.

Integrity of endothelium was assessed by relaxation obtained with acetylcholine (0.1 mM).

2.3. Statistical analysis of results

Relaxation was expressed as a percentage of maximal relaxation induced by papaverine 0.1 mM. Results are expressed as mean±S.E.M. Differences among groups were determined by analysis of variance (ANOVA) for repeated measures followed by the Bonferroni corrected t-test or by Student's t-test for paired or unpaired data as appropriate. All differences were considered significant when $P<0.05$.

2.4. Drugs

Drugs and chemicals used and their sources were: arachidonic acid (sodium salt), indomethacin, ketoconazole, 17-ODYA, glibenclamide, 4-aminopyridine, L-NOARG, acetylcholine chloride, U46619 (9,11-dideoxy-11α, 9α-epoxymethano-prostaglandin $F_{2\alpha}$), papaverine, Sigma-Aldrich Chemie (Saint Quentin Fallavier, France); apamin and charybdotoxin, Latoxan (Valence, France). Arachidonic acid was prepared in distilled water previously bubbled with nitrogen and divided into aliquots that were sealed under nitrogen and stored at −20 °C. Dilutions of arachidonic acid were made freshly for each experiment and kept on ice. Indomethacin was dissolved in ethanol and further dilutions were prepared in a mixture of ethanol–distilled water 1:1, ketoconazole in methanol and further dilutions in a mixture methanol–distilled water 0.6:0.4, 17-ODYA in ethanol and dilutions in a mixture of ethanol–distilled water 0.7:0.3, glibenclamide in dimethylsulfoxyde (DMSO) and dilutions in a mixture of DMSO–distilled water 0.3:0.7. 4-Aminopyridine, L-NOARG, acetylcholine, U46619, apamin and papaverine were prepared in distilled water, charybdotoxin in saline. The maximal concentration of ethanol (0.28%), methanol (0.24%) or DMSO (0.12%) in the bath did not by themselves exert any effect on U46619-induced vasoconstriction and did not modify the reactivity of the preparation. Drug concentrations are expressed as final bath concentrations.

3. Results

3.1. Effects of cyclooxygenase, CYP, NOS blockade and K^+ channels blockers on tone before and after U46619-induced-vasoconstriction

Treatment with cyclooxygenase, CYP or NOS inhibitors did not modify the pre-existing tone before the contraction induced by U46619. Indomethacin was associated with a significant increase of U46619-induced contraction compared to that obtained in control experiments (2.74±0.14 g versus 1.78±0.11 g; $P<0.001$). The other antagonists were devoid of any effects on the U46619-induced contraction. It is noticeable that none of the antagonists significantly modified the maximal relaxation induced by papaverine. Indeed, in each set of experiments, tone after papaverine went back to basal value before U46619-induced-contraction (Fig. 1).

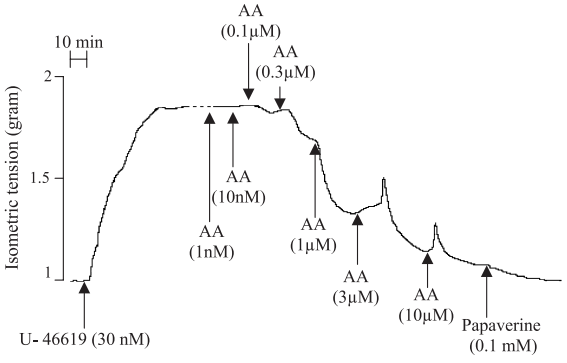

Fig. 1. Original shape of the effects of arachidonic acid (AA) in isolated human intralobar pulmonary arteries after U46619-induced contraction. Arachidonic acid (3 and 10 μM) caused early and transient vasoconstriction followed by a strong and sustained relaxation.

3.2. Effects of arachidonic acid on U46619-induced-vasoconstriction

Arachidonic acid (1 nM to 10 μM) produced a concentration-dependent relaxation of U46619-induced constriction of human intralobar pulmonary arteries. At the highest concentrations used (i.e. 3 μM and 10 μM), the responses were biphasic with an initial contraction followed by a sustained relaxation (Fig. 1). The concentration–response curve to arachidonic acid was significantly shifted to the right ($P<0.01$) by the blockade of cyclooxygenase by indomethacin but was unaffected by the blockade of CYP by ketoconazole or 17-ODYA, or by the blockade of NOS by L-NOARG (Fig. 2A). After the blockade of cyclooxygenase by indomethacin, the concentration–response curve to arachidonic acid was significantly shifted to the right ($P<0.05$) by either 17-ODYA, L-NOARG or 17-ODYA+L-NOARG (Fig. 2B). Whereas neither 17-ODYA nor L-NOARG displaced the concentration–response curve for arachidonic acid, or was significantly right-shifted ($P<0.05$) in the presence of a combination of both antagonists (Fig. 2C). The E_{max} and $-\log EC_{30}$ values are given in Table 1.

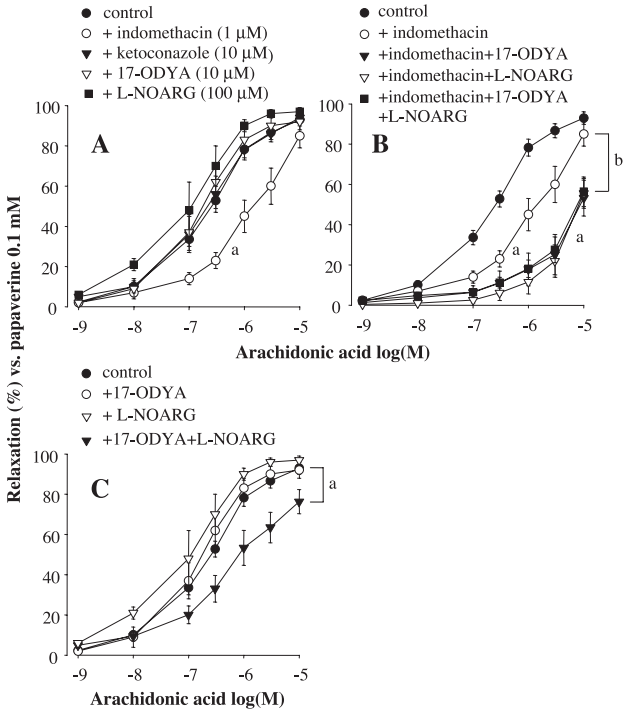

Fig. 2. Effects of arachidonic acid on U-46619-induced-vasoconstriction of human pulmonary arteries in the presence of inhibitors of cyclooxygenase (indomethacin 1μM, $n=7$), of CYP (ketoconazole 10 μM, $n=7$; 17-ODYA 10 μM, $n=6$) and of NOS (N^{ω}-nitro-L-arginine, L-NOARG, 100 μM, $n=7$) and combination of these inhibitors. Responses are expressed as a percentage of maximal relaxation induced with papaverine (0.1 mM). n is the number of patients. Data represent mean±S.E.M. [a] indicates curves that were significantly different from control using an ANOVA test ($P<0.001$). [b] indicates curves that were significantly different from curve "+indomethacin" using an ANOVA test ($P<0.01$).

Table 1
Effects of inhibitors of cyclooxygenase (indomethacin), CYP (ketoconazole and 17-octadecynoic acid, 17-ODYA) and NOS (N^{ω}-nitro-L-arginine, L-NOARG) on the vasodilator responses to arachidonic acid in the human pulmonary arteries

	E_{max} (%)	$-\log EC_{30}$	n
Control	93±3	7.03±0.09	7
Ketoconazole (10 μM)	94±2	7.16±0.21	7
17-ODYA (10 μM)	92±4	7.20±0.21	6
L-NOARG (100μM)	97±2	7.58±0.14	7
Indomethacin (1μM)	85±6	6.26±0.18[b]	7
17-ODYA+L-NOARG	79±5[a]	6.44±0.21[a]	8
Indomethacin+17-ODYA	58±10[a,b,c]	5.58±0.24[a,b,c]	8
Indomethacin+L-NOARG	56±7[a,b,c]	5.41±0.18[a,b,c]	8
Indomethacin+17-ODYA+L-NOARG	61±7[a,b,c]	5.70±0.20[a,b,c]	8

E_{max} indicates the maximal effect observed at the maximal concentration of arachidonic acid tested (10 μM). EC_{30} values indicate the concentration of each agonist producing 30% of the maximal response induced by papaverine (0.1 mM). Data are expressed as mean±S.E.M. n indicates the number of patients in each set of experiments. Statistical significance is given using an unpaired Student's t-test.

[a] $P<0.05$ vs. controls.

[b] $P<0.05$ vs. indomethacin.

[c] $P<0.05$ vs. 17-ODYA+ L-NOARG.

3.3. Influence of K^+ blockers and inhibitors of arachidonic acid metabolic pathways on the relaxation elicited by arachidonic acid

The blockade of K_{ATP}, SK_{Ca}, BK_{Ca}, and K_V channel with glibenclamide, apamin, charybdotoxin and 4-aminopyridine respectively, produced a significant ($P<0.001$) shift to the right of the concentration–response curve to arachidonic acid, with a significant reduction in both efficacy and potency of arachidonic acid. (Fig. 3, Table 2).

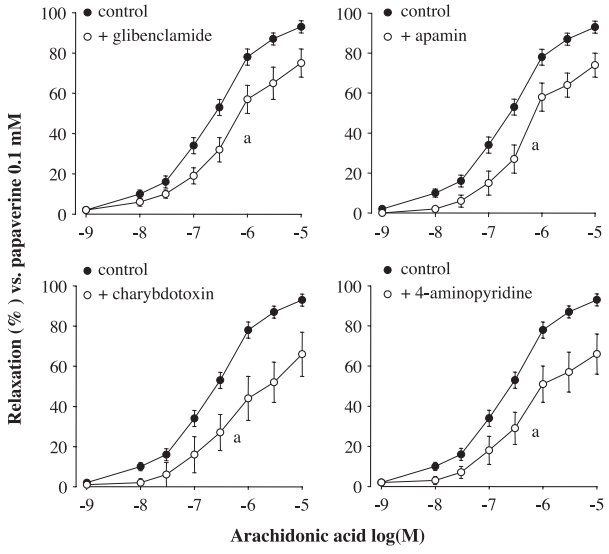

Fig. 3. Influence of K^+ channels blockers on the arachidonic acid-induced relaxation of human pulmonary arteries ($n=7$). Responses are expressed as a percentage of maximal relaxation induced with papaverine (0.1 mM). n is the number of patients. Data represent mean±S.E.M. [a] indicates curves that were significantly different from control using an ANOVA test ($P<0.001$).

Table 2
Effects of K^+ channel blockers on arachidonic acid concentration–relaxation response relationships in the human pulmonary artery (n=7), after the blockade of cyclooxygenase by indomethacin (1 μM, n=7) and NOS by N^{ω}-nitro-L-arginine (L-NOARG, 100 μM, n=7)

	Controls	Glibenclamide (1 μM)	Apamin (0.3 μM)	Charybdotoxin (0.1 μM)	4-Aminopyridine (1 mM)
E_{max} (%)	93±3	75±7[a]	74±6[a]	66±11[a]	66±10[a]
−log EC_{30}	7.09±0.1	6.29±0.39 [a]	6.49±0.16[a]	5.79±0.67[a]	6.30±0.37[a]
+indomethacin					
E_{max} (%)	85±6	84±7	87±5	74±12	82±6
−log EC_{30}	6.25±0.17	6.21±0.29	6.30±0.25	5.77±0.25	5.89±0.14
+L-NOARG					
E_{max} (%)	98±1	97±1	81±5[a]	90±3[a]	81±5[a]
−log EC_{30}	7.45±0.17	7.69±0.23	6.66±0.24[a]	6.54±0.14[a]	6.75±0.18[a]

E_{max} indicates the maximal effect observed at the maximal concentration of arachidonic acid tested (10 μM). EC_{30} values indicate the concentration of each agonist producing 30% of the maximal response induced by papaverine (0.1 mM). Data are expressed as mean±S.E.M. n indicates the number of patients in each set of experiments. Statistical significance is given using a paired Student's t test. In each set of experiments controls are performed in the absence of K^+ channel blockers.

[a] P<0.05 vs. controls.

After the blockade of cyclooxygenase by indomethacin, the shift of the concentration–response curve of arachidonic acid induced by the blockade of K_{ATP} and SK_{Ca} channels was abolished whereas that induced by the blockade of BK_{Ca} or K_V channels was reduced yet remaining significant (ANOVA, P=0.001) (Fig. 4, Table 2).

After the blockade of CYP by ketoconazole, the shift of the concentration–response curve of arachidonic acid induced by the blockade of K_{ATP} and SK_{Ca} channels was abolished whereas that induced by the blockade of BK_{Ca} or K_V channels was reduced yet remaining significant (ANOVA, P<0.05) (Fig. 5). Neither the E_{max} nor the −log EC_{30} values were significantly affected (data not shown). On the other hand, of the blockade of CYP by 17-ODYA suppressed the antagonistic effect of all the K^+ channels blockers tested on the relaxation induced by arachidonic acid (Fig. 6).

After the blockade of NOS by L-NOARG, the concentration–response curve to arachidonic acid was significantly shifted to the right by apamin, charybdotoxin and 4-aminopyridine (P=0.001), but not by glibenclamide, with

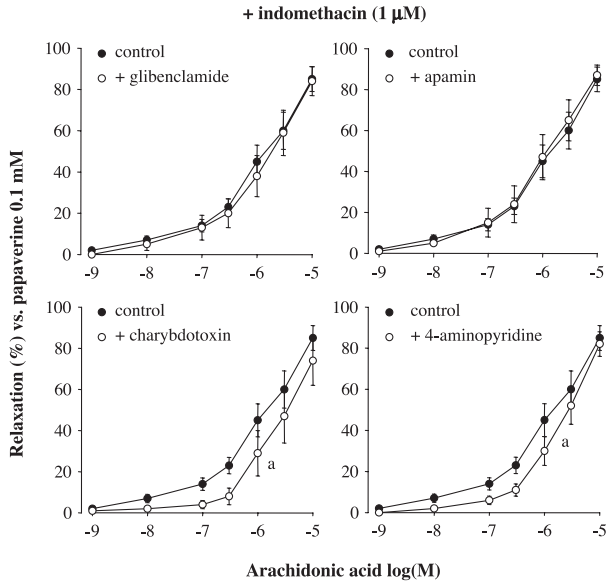

Fig. 4. Influence of K^+ channels blockers on the arachidonic acid-induced relaxation of human pulmonary arteries in the presence of the inhibitor of cyclooxygenase, indomethacin (n=7). Responses are expressed as a percentage of maximal relaxation induced with papaverine (0.1 mM). n is the number of patients. Data represent mean±S.E.M. [a] indicates curves that were significantly different from control using an ANOVA test (P<0.01).

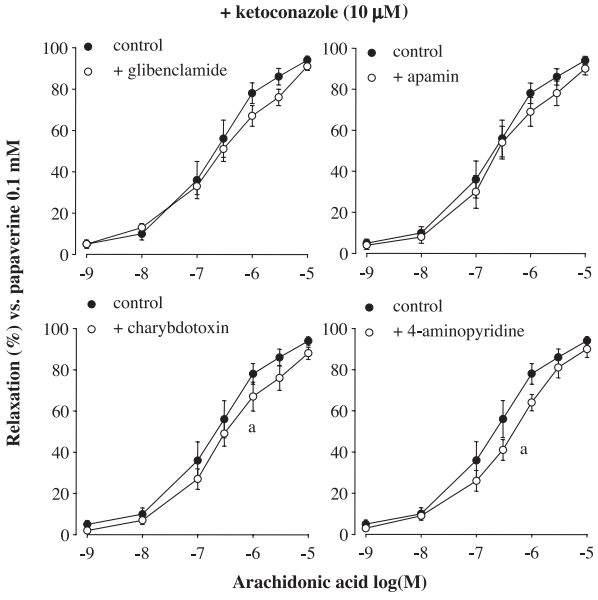

Fig. 5. Influence of K^+ channels blockers on the arachidonic acid-induced relaxation of human pulmonary arteries in the presence of the inhibitor of CYP, ketoconazole (n=7). Responses are expressed as a percentage of maximal relaxation induced with papaverine (0.1 mM). n is the number of patients. Data represent mean±S.E.M. [a] indicates curves that were significantly different from control using an ANOVA test (P<0.05).

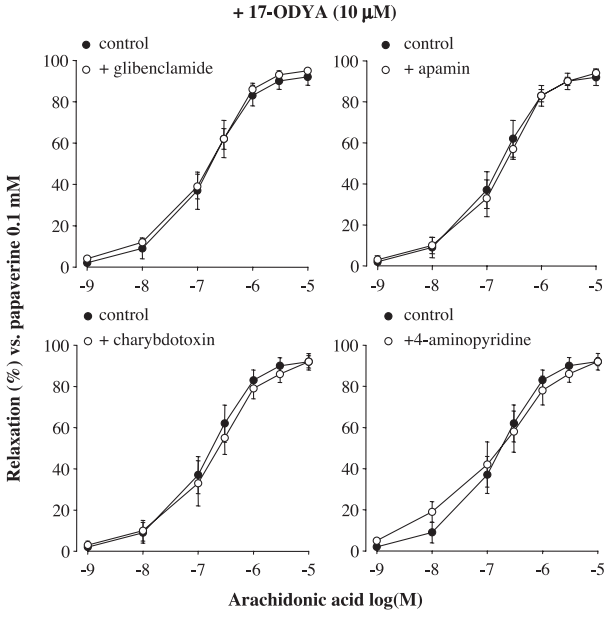

Fig. 6. Influence of K^+ channels blockers on the arachidonic acid-induced relaxation of human pulmonary arteries in the presence of the inhibitor of CYP, 17-ODYA (n=6). Responses are expressed as a percentage of maximal relaxation induced with papaverine (0.1 mM). n is the number of patients.

a significant diminution of the E_{max} and $-\log EC_{30}$ values (Fig. 7, Table 2).

In presence of a combined blockade of CYP by 17-ODYA and NOS by L-NOARG, the shift of the concentration–response curve of arachidonic acid induced by the

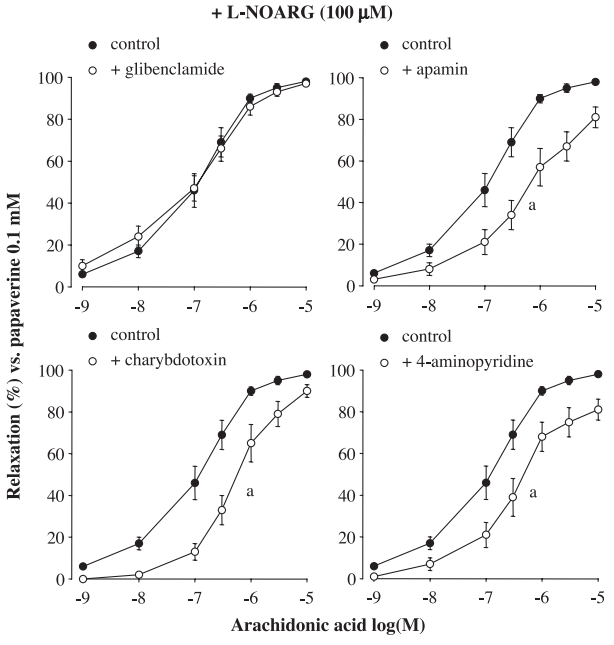

Fig. 7. Influence of K^+ channels blockers on the arachidonic acid-induced relaxation of human pulmonary arteries in the presence of the inhibitor of NOS, L-NOARG (n=7). Responses are expressed as a percentage of maximal relaxation induced with papaverine (0.1 mM). n is the number of patients. Data represent mean±S.E.M. [a] indicates curves that were significantly different from control using an ANOVA test (P<0.001).

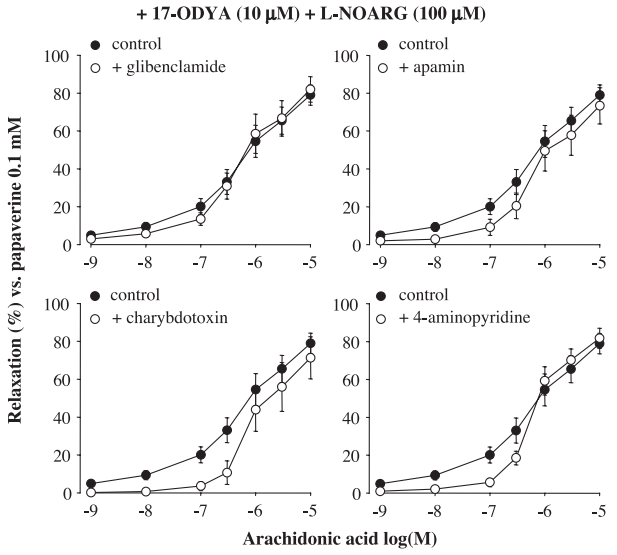

Fig. 8. Influence of K^+ channels blockers on the arachidonic acid-induced relaxation of human pulmonary arteries in the presence of the inhibitor of NOS, L-NOARG and inhibitor of CYP, 17-ODYA (n=7). Responses are expressed as a percentage of maximal relaxation induced with papaverine (0.1 mM). n is the number of patients. Data represent mean±S.E.M.

K_{ATP}, SK_{Ca}, BK_{Ca} and the K_V channel blockers was abolished (Fig. 8).

4. Discussion

To our knowledge the effects of arachidonic acid in human isolated pulmonary arteries have not been previously reported. In our study, we showed that arachidonic acid produced a concentration-dependent vasodilatation of human pulmonary arteries constricted with U46619. This finding is in keeping with previous results found in the rat perfused lung (Feddersen et al., 1990) and heart (Qiu and Quilley, 1999) as well as in isolated human coronary microvessels (Miura and Gutterman, 1998).

Although our study focused on the assessment of the relaxant effect of arachidonic acid in U46619-contracted pulmonary arteries, it has been shown that arachidonic acid metabolites can exert a vasocontractile activity in rat pulmonary artery (Daffonchio et al., 1984). U46619 is a stable analogue of thromboxane A_2, which is known to acts mainly trough TP-receptor stimulation (Lumley et al., 1989). It is therefore possible that thromboxane A_2 produced by arachidonic acid metabolism was no more able to contract over the U46619-generated tone thus resulting in a predominant relaxing effect of arachidonic acid, with minor transient contractions observed when existing tone had been reduced by the lowest concentrations of arachidonic acid used. This is in agreement with previous reports in the isolated perfused lung, particularly in lungs from chronically hypoxic rats (Russell et al., 1993). It should be noticed that arteries used in the present study were obtained from patients that were all but two, former

smokers, a condition that is associated with chronic hypoxia.

The arachidonic acid-induced relaxation was significantly antagonized by the blockade of cyclooxygenase by indomethacin. Since the maximal effect of arachidonic acid, expressed as a percentage of relaxation induced by papaverine (0.1 mM) added at the end of each experiment, was unaffected by indomethacine, the increase in U46619-induced contraction observed in presence of indomethacin is unlikely to explain this finding. This might be explained, at least partly, by a decrease in prostaglandin I_2 release (Birks et al., 1997) especially in a context of cyclooxygenase, and more specifically cyclooxygenase-2, overexpression. Indeed, it is widely accepted that these enzymes, are over-expressed in malignant and/or inflammatory tissues (Mitchell et al., 1995) leading to an increased sensitivity to cyclooxygenase inhibitors. Since we found that combination of cyclooxygenase and CYP blockade, by indomethacin and 17-ODYA respectively, was responsible for a more pronounced antagonistic effect on arachidonic acid-induced relaxation than does the blockade of cyclooxygenase alone, we might suggest that arachidonic acid metabolites of the CYP pathway contribute to a cyclooxygenase-dependent vasodilatation, as described previously (Birks et al., 1997).

In our study, the blockade of CYP by ketoconazole or 17-ODYA did not exert any antagonistic effect against arachidonic acid-induced relaxation. This is contrasting with other investigations that reported a negative influence of miconazole and 17-ODYA in the human coronary vessels (Miura and Gutterman, 1998) and of clotrimazole in the rat perfused heart (Qiu and Quilley, 1999). The role of CYP metabolites of arachidonic acid in the pulmonary vascular tone was also suggested by Kiss et al. (2000) who reported a release of EETs, leukotrienes, and HETEs in the vascular compartment from perfused and ventilated human lungs, isolated during surgery for bronchial carcinoma. In contrast, there was no evidence for a specific involvement of different pathways of arachidonic acid metabolism in the mechanism of hypoxic pulmonary vasoconstriction in rabbits (Weissmann et al., 1998) suggesting a minor role for the CYP-dependent mono-oxygenases pathway. This is in agreement with the work by Sobey et al. (1998) who described that the cerebral vasodilator response to arachidonate was abolished by indomethacin whereas clotrimazole and 17-ODYA had no effect. In agreement with previous work (Adeagbo and Malik, 1991; Miller et al., 2003), the blockade of NOS by L-NOARG did not affect arachidonic acid-induced relaxation whereas combination of CYP and NOS blockade was responsible of a significant antagonistic effect. This close relationship between CYP and NO pathways was suggested few years ago by Kerkhof et al. (1999) who described that inhibition of the CYP 4A pathway of arachidonic acid metabolism under normoxia induces NO production by the endothelium in rat cremaster arteries. This is likely to explain that we did not antagonise arachidonic acid-induced relaxation after CYP or NOS blockade whereas we did with a combination of both.

The involvement of K^+ channels in the relaxant properties of arachidonic acid in the human pulmonary arteries was suggested by the shift of the concentration–response curve to arachidonic acid observed after the blockade of K_{ATP}, SK_{Ca}, BK_{Ca}, or K_V channels by their respective antagonists, glibenclamide, apamin, charybdotoxin and 4-aminopyridine. Data on the involvement of K^+ channels in the vascular relaxant effects of arachidonic acid are controversial. The involvement of K_{Ca} and K_{ATP} channels in the relaxant properties of arachidonic acid has previously been described in the rat isolated mesenteric arteries, where their blockade with selective antagonists was responsible for a decrease in the relaxant properties of arachidonic acid (Adeagbo and Malik, 1991) as well as in piglet pulmonary resistance arteries (Fuloria et al., 2002), and that of K_V channels in the guinea-pig coronary arteries (Nishiyama et al., 1998). In non-vascular models arachidonic acid was shown recently to modulate both the peak amplitude and kinetics of the hippocampal A-current which is related to the Kv4 subfamily of voltage-gated K^+ channels (Holmqvist et al., 2001). In contrast, in the guinea-pig coronary arteries, a selective BK_{Ca}-channel blocker, iberiotoxin, significantly reduced responses to arachidonic acid whereas 4-aminopyridine did not (Eckman et al., 1998). It is admitted that epoxyeicosatrienoic acids, a metabolite of arachidonic acid can stimulate BK_{Ca} channel activity (Wu, 2003). No study apart from the present one has focused on the involvement of K^+ channels on the effect of arachidonic acid in human pulmonary arteries and further researches will be necessary to explain these discrepancies.

In the present study we found that the cyclooxygenase inhibitor, indomethacin, suppressed the inhibitory effects of glibenclamide and of apamin on the relaxant effects of arachidonic acid whereas those of charybdotoxin and 4-aminopyridine were reduced but remained significant. These results suggest that, in the human pulmonary arteries, the K^+ channels involved in the relaxant properties of cyclooxygenase-dependent metabolites of arachidonic acid belong mainly to the K_{ATP} and SK_{Ca} families. This is in agreement with the results of Schubert et al. (1996, 1997) and of Dumas et al. (1997) who demonstrated that, in the tail artery and in the isolated lung of rat, prostacyclin and iloprost, the stable analogue of prostacyclin, activate not only K_{ATP} channel but also K_{Ca} channels. This mechanism is likely to be responsible for our findings since prostacyclin is the main metabolite of arachidonic acid produced by cyclooxygenase in most blood vessels and particularly in pulmonary vessels. The inhibitory effects of glibenclamide and apamin on the relaxation induced by arachidonic acid were suppressed after blockade of CYP by ketoconazole whereas those of charybdotoxin and 4-aminopyridine were only reduced while remaining significant. Ketoconazole has been shown to be a selective inhibitor of the arachidonic acid-epoxygenase activities of phenobarbital-induced liver

microsomal fractions with IC_{50} value of 2 μM but not of ω, ω1-oxygenase activity of ciprofibrate-induced microsomal fractions (IC_{50}=50 μM) (Capdevila et al., 1988). This suggests that, in our study, the relaxant effect of arachidonic acid involving K_{ATP} and SK_{Ca} channels are dependent on epoxygenase. In contrast, Zou et al. (1994) showed that ketoconazole (10 μM) inhibited epoxygenase and ù-hydroxylase activity only by 50% and 20%, respectively. In consequence, we used an other CYP inhibitor, 17-ODYA, that has been described to inhibit both of ù-hydroxylation and epoxydation of arachidonic acid with IC_{50} values of 7 and 5 μM, respectively (Wang et al., 1998). After CYP blockade by 17-ODYA, the inhibitory effects of the four K^+ channels blockers used in this study were abolished. Taken together, these results suggest that CYP-dependent metabolites, as cyclooxygenase metabolites, shared some relaxing or some metabolic pathways in human pulmonary arteries both involving K^+ channels mainly K_{ATP} and SK_{Ca}, but also K_v and BK_{Ca} channels. This was previously suggested in the study by Birks et al. (1997). They demonstrated a 20-HETE-induced relaxation of isolated small human pulmonary arteries in a cyclooxygenase-dependent manner. It was shown that indomethacin blocked release of prostanoids without effects on the conversion of arachidonate into 20-HETE. This latter eicosanoid was converted by lung microsomes into prostanoids, raising the possibility that in vascular tissue cyclooxygenase may metabolize 20-HETE to a vasodilator compound. Such findings have also been suggested in another study where infusion of 5,6-EET in isolated dog lungs reduced the U46619-mediated increase in pulmonary vascular resistance by 23.6% and increased pulmonary prostaglandin I_2 synthesis from 70.5 to 675.9 ng/min (Stephenson et al., 1998). The involvement of BK_{Ca} channels in the effects of CYP-dependent metabolites was previously suggested by Li and Campbell (1997) in cell-attached patch-clamp experiments from small bovine coronary arteries. 11,12-EET produced a 0.5- to 10-fold increase in the activity of the K_{Ca} channels when added in concentrations of 1, 10, and 100 nM. The role od BK_{Ca} was recently reemphasised in the work by Archer et al. who suggested that 11,12-EET causes relaxation of human internal mammary arteries by activating BK_{Ca} channels (Archer et al., 2003). In the rat coronary arteries the activation of K_V channels was suggested to be dependent on the metabolism of arachidonic acid through 5-lipoxygenase and CYP-dependent mono-oxygenase pathways (Satake et al., 1997). In rat pulmonary arterial myocytes, the inhibition of CYP reduced voltage-gated K^+ currents (Yuan et al., 1995).

Finally, L-NOARG suppressed only the antagonistic effect of glibenclamide on the relaxation induced by arachidonic acid. This result suggests that arachidonic acid-induced relaxation of human pulmonary arteries is partly mediated through a NO release which in turns activates mainly K_{ATP} channels. We already reported the involvement of K_{ATP} channels in the relaxant properties of endogenous NO in rat main pulmonary arteries (Bardou et al., 2001). Our previous results suggest that, in the rat isolated lung preparation, iloprost, a stable analogue of prostacyclin, dilate pulmonary vessels partly through K_{ATP} channels and NO release (Dumas et al., 1997). Moreover, a synergistic interaction between endothelium-derived NO and prostaglandin I_2 in canine pulmonary artery has already been described. This interaction is mediated by activation of K_{ATP} channels, presumably by an endothelium-derived hyperpolarizing factor (EDHF) (Gambone et al., 1997). Therefore we can suggest that in human pulmonary arteries cyclooxygenase-dependent metabolites of arachidonic acid induce the release of NO which in turns dilate human pulmonary arteries partly through K_{ATP} channels activation. After CYP blockade by 17-ODYA and NOS blockade by L-NOARG, the inhibitory effects of the four K^+ channels blockers used in this study were abolished. This is not surprising since 17-ODYA by itself was responsible for a loss of inhibitory effect of all the K^+ channels on arachidonic acid-induced relaxation.

It appears from our data that arachidonic acid produced a relaxation of U46619-constricted human pulmonary arteries that is modulated by K_{ATP}, SK_{Ca}, BK_{Ca}, and K_V channels. Cyclooxygenase-dependent metabolites are the main products of arachidonic acid involved in these effects acting partly through K_{ATP} and SK_{Ca} channels. CYP-dependent metabolites modulate arachidonic acid-induced relaxation through a pathway involving K^+ channels mainly K_{ATP} and SK_{Ca}, but also BK_{Ca} and K_V channels. Involvement of K_{ATP} channels in the relaxant effects of arachidonic acid is likely to be attributable to a NOS-dependent pathway. These findings are reinforced by their consistency with previous studies in other models.

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