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Transcriptional Up-Regulation in Expression of 5-Hydroxytryptamine$_{2A}$ and Transcriptional Down-Regulation of Angiotensin II type 1 Receptors during Organ Culture of Rat Mesenteric Artery

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Abstract: The purpose of this study was to investigate in rat mesenteric artery if there is up-regulation of 5-hydroxytryptamine (5-HT) receptors and angiotensin II receptors and the potential role of protein kinase C activation in the smooth muscle cells during organ culture. Angiotensin II, 5-HT and potassium induced contraction of ring segments without endothelium, monitored by a sensitive *in vitro* pharmacology method. After the culture of the arterial ring segments for 24 hr, the concentration-contraction curves induced by 5-HT slightly shifted towards to the left with pEC_{50} from 6.64±0.11 to 6.84±0.11 and a significant increase in E_{max} from 147±11% to 246±15% ($P<0.05$), compared with that obtained in fresh segments. In contrast, the angiotensin II concentration-contraction curve only showed a significant decrease in E_{max} from 99±10% to 37±8%. Specific antagonists for the 5-HT type 2A receptors (5-HT_{2A}) and angiotensin II type 1 receptors (AT_1) demonstrated that the contractions occurred via 5-HT_{2A} and AT_1 receptors, respectively. Real-time PCR revealed that the 5-HT_{2A} receptor mRNA was up-regulated in parallel with the contractile response while there was a down-regulation of AT_1 receptor mRNA. Transcriptional inhibitor actinomycin D and specific protein kinase C inhibitor Ro31-8220 demonstrated that it was a transcriptional mechanism with involvement of protein kinase C that regulated the enhanced expression of 5-HT_{2A} receptors in the mesenteric artery.

5-Hydroxytryptamine (5-HT) and angiotensin II (AngII) are signaling molecules that have important roles in cardiovascular regulation (Turla & Webb 1989; Chester *et al*. 1993; Yildiz *et al*. 1998; Mizuno *et al*. 1989). Both have been seen to be synthesized in vascular endothelial cells (Lincoln *et al*. 1990). Their dominating receptors in smooth muscle cells of rat mesenteric artery are of the 5-HT type 2A (5-HT_{2A}) and Ang II type 1 (AT_1) receptors (Zemin *et al*. 1999). They are considered to be involved both in regulation of tone in the smooth muscle cells and in remodeling via mitogenesis (Florian & Watts 1998; Watts 1998; Ytterberg & Edvinsson 2001; Fang & Marwick 2002). Elevated levels of Ang II and 5-HT are seen in stroke (Nishimura & Suzuki 1995; Walther *et al*. 2002), and in congestive heart failure (Gschewend *et al*. 2003). As a feedback mechanism, there is local release of nitric oxide and prostacyclin, which overweighs the vascular constrictors but these may be particularly important in vascular disease with altered receptor expression. In situations of elevated levels of 5-HT or Ang II their impact on the smooth muscle cells might be pronounced, particularly, when the agonist concentrations in the concentration-response curves are logarithmic. We have previously shown enhanced expression of the endothelin type B receptor (ET_B) in experimental subarachnoid haemorrhage (Alafaci *et al*. 1990; Hansen-Schwartz *et al*. 2002), in stroke (Stenman *et al*. 2002) and in ischaemic heart disease (Wackenfors *et al*. 2004) which results in markedly enhanced responses to the agonists. The ET_B receptor may not only change its phenotype from a relaxant to a contractile ET_B receptor phenotype but also in increased receptor number as a result of organ culture (Adner *et al*. 1996). The mechanisms behind this phenomenon involve mitogen activated-protein kinase (Uddman *et al*. 2003), protein kinase C (Uddman *et al*. 2002) and transcription factors (Möller *et al*. 1997). However, we do not know if this phenomenon is unique to ET_B receptors or if alterations in other G-protein coupled receptors may occur during organ culture. There are data supporting since there is increased efficacy to Ang II in stroke (Mizuno *et al*. 1999; Stenman & Edvinsson 2004) and to 5-HT in SAH (Saida *et al*. 1997; Miranda *et al*. 1996) which imply a change in receptor expression (Zhu *et al*. 2000), while the elevated circulating level of Ang II in heart failure may results in the down-regulation of Ang II receptors (De Gasparo *et al*. 1994; Regitz-Zagrosek *et al*. 1994; Haywood *et al*. 1997).

The present study was designed to examine if organ culture of arterial segments change vascular smooth muscle cells receptor expression. The rat mesenteric artery has been used as a representative of a peripheral resistance vessel (Ralevic *et al*. 1991), and presently we have examined if there are alterations in the expression of Ang II and 5-HT

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receptors in rat mesenteric artery with functional and molecular methods. Actinomycin D (Strohm *et al*. 2002) and the specific protein kinase C inhibitor Ro 31-8220 (Trachsel & Keller 1995) were used to study transcription and involvement of protein kinase C, respectively.

Materials and Methods

Tissue preparation and organ culture procedure. Sprague-Dawley rats (body weight 250–300 g) were anaesthetized with CO_2 and exsanguinated. The superior mesenteric artery was removed gently, immersed into cold buffer solution (for composition, see below) and dissected free of adhering tissue under a microscope. The endothelium was denuded by perfusion of the vessel for 10 sec. with 0.1% Triton X-100 followed by another 10 sec. with a physiologic buffer solution (Hamel *et al*. 1987). The vessels were then cut into 1 mm long cylindrical segments, used directly (fresh group) or incubated for 6 hr–2 days at 37° in humified 5% CO_2 in O_2 (organ culture group). The segments for organ culture were placed in a 96-well plate, one segment in each well, containing 300 µl Dulbecco's modified Eagle's medium (DMEM) containing L-glutamine (584 mg/l) and supplemented with penicillin (100 U/ml) and streptomycin (100 µg/ml). The medium was changed to fresh daily. The experimental protocol was approved by Lund University Animal Ethic Committee (M217-03).

In vitro pharmacology. Fresh or incubated segments were immersed in temperature-controlled (37°) tissue baths containing a bicarbonate buffer solution. The solution was continuously aerated with 5% CO_2 in O_2 resulting in a pH of 7.4. Each segment was mounted on two L-shaped prongs, one of which was attached to a Grass FT-03 transducer (Grass Instr., Quincy, USA) connected to a PowerLab (ADInstruments, Hastings, UK) unit for continuous recording of isometric tension. A resting tone of about 2.5 mN was applied to each segment and the segments were allowed to stabilize at this tension for at least one hour before being exposed to a potassium-rich (60 mM) buffer solution with the same composition as the standard solution except that NaCl was replaced by an equimolar concentration of KCl. The potassium-induced contraction was used as a reference for the contractile capacity, and the segments was used only if potassium elicited reproducible responses over 1.0 mN. Concentration-response curves for vasoconstrictors were obtained by cumulative administration of the agents.

Buffer solutions and drugs. Standard buffer solution (mM): NaCl 119; $NaHCO_3$ 15; KCl 4.6; MgCl 1.2; NaH_2PO_4 1.2; $CaCl_2$ 1.5; glucose 5.5. Analytical grade chemicals and double distilled water were used for preparing all solutions. Dulbecco's modified Eagle's medium, penicillin and streptomycin were purchased from Gibco BRL, Paisley, Scotland, UK. Ang II, 5-HT, Ro 31-8220, ketanserin, candesartan, cyclohexamide and actinomycin D (Sigma, St. Louis, USA) were dissolved in sterile water with bovine serum albumin (0.1% w/v).

Real time RT-PCR. Smooth muscle isolated from fresh or the cultured segments was homogenated in 1 ml of the RNApro™ solution (Q-BIOgene, CA, USA) by using a FastPrep® instrument (Q-BIOgene, CA, USA). The total RNA was extracted following a protocol from the FastRNA® Pro kit supplier. Reverse transcription of total RNA to cDNA was carried out using the Gene Amp RT kit (PE Applied Biosystems) in a Perkin-Elmer 2400 PCR machine at 42° for 30 min. The real-time quantitative PCR was performed with the GeneAmp SYBR Green PCR kit (PE Applied Biosystems) in a Perkin-Elmer real-time PCR machine (PE, GeneAmp 5700 sequence detection system). The system automatically monitors the binding of a fluorescent dye to double-strand DNA by real-time detection of the fluorescence during each cycle of PCR amplification. Specific primers for rat AT_{1A} and 5-HT_{2A} receptors were designed based on gene bank data by using primer expression 2 software (PE Applied Biosystems).

5-HT_{2A} receptor primers;
Forward: 5′-CTCAGTCACCATGCGACAGG-3′
Reverse: 5′-TTGTCGCCAGGGTCCG-3′

AT_{1A} receptor primers;
Forward: 5′-3′ GGA TGG TTC GCA GAG AGA GTA CAT
Reverse: 5′-3′ CCT GCC CTC TTG TAC CTG TTG

The house keeping gene, GAPDH mRNA continuously expressed to a constant amount in the cells, was compared with the house keeping gene EF-1 in a pilot study by real-time PCR (Stenman *et al*. 2002). GAPDH was used as a reference in this study, but both were equally well constant in the tests.

GAPDH primers
Forward: 5′- GGCCTTCCGTGTTCCTACC-3′
Reverse: 5′-CGGCATGTCAGATCCACAAC-3′

The PCR reaction was performed in a 50 µl volume and started at a temperature of 50° for 2 min., 95° for 10 min. and the following 40 PCR cycles with 95° for 15 sec. and 60° for 1 min. Dissociation curves were run after the real time PCR to identify the specific PCR products. All primers were designed using the Primer Express 2.0 software (PE Applied Biosystems) and synthesized by TAG Copenhagen A/S (Denmark).

Data were analyzed with the comparative cycle threshold (CT) method. To evaluate the amount of 5-HT_{2A} and AT_{1A} mRNA in a sample, GAPDH mRNA was assessed in the same sample simultaneously. The CT values of GAPDH mRNA were used as a reference to quantity the relative amount of 5-HT_{2A} and AT_{1A} mRNA. The relative amount of mRNA was calculated with the CT values of 5-HT_{2A} and AT_{1A} receptor mRNA in relation to the CT values of GAPDH mRNA in the sample.

Calculation and statistics. Data are expressed as mean values ±S.E.M. Contractile responses in each segment are expressed as a percentage of the potassium-induced contraction. E_{max} represents the maximal contraction induced by an agonist. The pEC_{50} value was calculated from the line between the concentrations above and below the midpoint of the concentration-response curve. Statistical analysis was performed with unpaired Student's t-test or one-way ANOVA with Dunnet's post test. Differences were considered significant at $P<0.05$.

Results

5-Hydroxytryptamine receptors.

Contractile response. 5-HT, a general 5-HT receptor agonist, resulted in a concentration-dependent contraction. In fresh arterial segments the E_{max} and pEC_{50} values were 147±11% and 6.64±0.11, respectively. After 24 hr of incubation, the curves slightly shifted to the left with the pEC_{50} values from 6.64±0.11 to 6.84±0.11 and the maximal contraction increased significantly from 147±11% to 246±15% ($P<0.05$) (fig. 1A, table 1). The selective 5-HT_{2A} receptor antagonist ketanserin (10^{-9}–10^{-8} M) shifted the concentration-response in a concentration-dependent manner to the right without any change in maximum contraction (fig. 1B, table 1), indicating that the contraction induced by 5-HT was via a 5-HT_{2A} receptor subtype (Watts 2002). The pEC_{50} values of 5-HT with ketanserin 10^{-9} M and 10^{-8} M decreased

from 6.74±0.09 (without ketanserin) to 6.55±0.06 and 5.22±0.07, respectively. A Schild plot of the data yielded a pA_2 value of 9.43 (slope=1) (Watts 2002). 5-CT, a selective 5-HT_1 agonist, gave rise to a concentration-dependent contraction in the fresh arterial segments. The concentration-contraction curves of 5-CT did not shift obviously following organ culture for 24 or 48 hr and there was no significantly difference in the maximum contraction or the pEC_{50} values (data not shown).

Transcription and translation inhibition experiments. In order to examine the mechanisms of up-regulation of 5-HT_{2A} receptors, we used the transcription inhibitor actinomycin D (Strohm *et al.* 2002) and the translation inhibitor cyclohexamide (Möller *et al.* 1997). The results revealed that both inhibitors significantly decreased the enhanced 5-HT_{2A} receptor-mediated contraction. The enhanced contraction had returned to that seen in fresh arterial segments in actinomycin D (5×10^{-6} M) treated segments while cyclohexamide (5×10^{-5} M) only partially reduced the up-regulation (fig. 3, table 1). Furthermore, the involvement of protein kinase C was addressed by using its specific inhibitor Ro 31-8220 (10^{-6} M). The presence of Ro31-8220 during organ culture resulted in an attenuated 5-HT concentration-contraction curve with an E_{max} of 158±12% compared to that seen after organ culture in the absence of Ro31-8220 (246±15%) ($P<0.01$) (fig. 4, table 1).

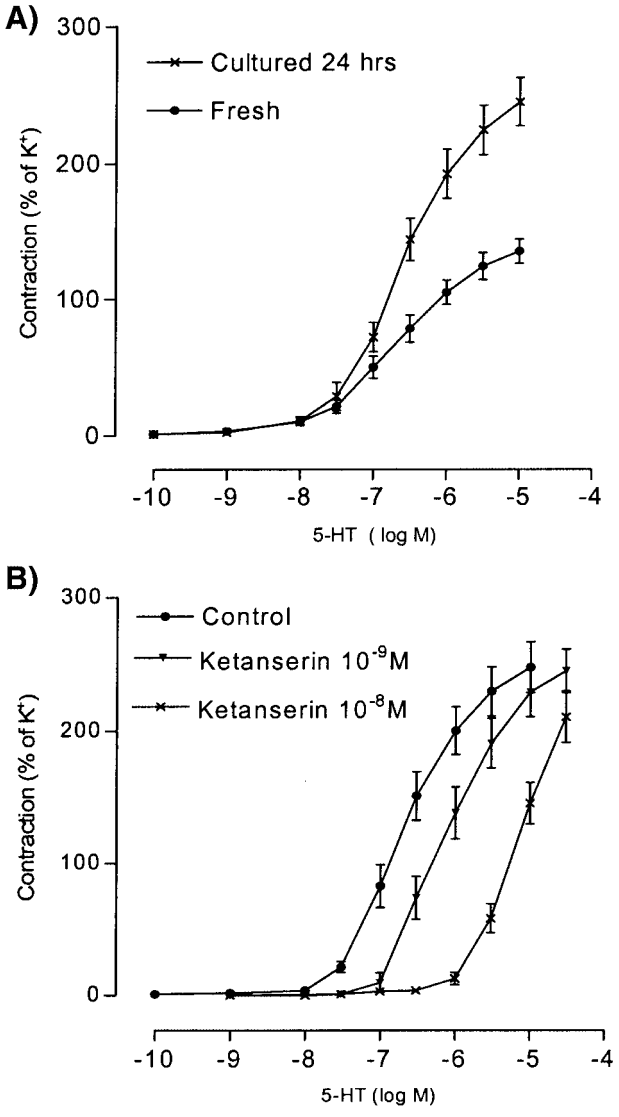

Fig. 1. Concentration response curves for 5-HT in fresh vessels and following 24 hr organ culture (A) or in presence of selective 5-HT_{2A} receptor antagonist ketanserin (10^{-8} M, 10^{-9} M) on rat mesenteric arteries after incubation 24 hr (B). Data are derived from 10–16 identical experiments and shown as mean±S.E.M.

Real-time PCR. To confirm the transcriptional mechanisms and the involvement of protein kinase C, changes in mRNA levels for 5-HT_{2A} receptors were analyzed before and after organ culture by real-time PCR. The mRNA level of 5-HT_{2A} receptors was significantly increased ($P<0.05$) following organ culture. The presence of the transcription inhibitor actinomycin D or the specific protein kinase C inhibitor Ro31-8220, but not the translation inhibitor cyclohexamide, significantly decreased the up-regulation of mRNA for the 5-HT_{2A} receptors (fig. 5A).

Table 1.

Overview of E_{max} and pEC_{50} values of 5-HT and Ang II induced contraction on rat mesenteric arteries.

Agonist	N	E_{max} (%)	pEC_{50}
5-HT			
Fresh	16	147±11	6.64±0.11
Organ culture 24 hr	16	246±15*	6.84±0.11
Antagonists			
Control	10	248±16	6.74±0.09
Ketanserin 10^{-9} M	10	245±14	6.11±0.09^{+}
Ketanserin 10^{-8} M	10	248±11	5.22±0.07*
Inhibitors			
Control	16	248±16	6.61±0.03
Actinomycin D 5×10^{-6} M	11	147±12*	6.50±0.02
Cyclohexamide 5×10^{-5} M	11	190±18^{+}	6.52±0.02
Ro 31-8220 10^{-6} M	16	158±12*	6.44±0.09
Angiotensin II			
Fresh	11	99±10	8.93±0.17
Organ culture 24 hr	8	37±8*	8.26±0.07
Organ culture 48 hr	10	33±12*	8.58±0.07
Antagonist			
Control	11	103±7	8.25±0.08
Candesartan 10^{-12} M	12	97±8	8.24±0.03
Candesartan 10^{-10} M	15	53±10^{+}	6.25±0.08*
Candesartan 10^{-8} M	15	17±8*	5.73±0.02*
Inhibitors			
Control	7	36±8	8.24±0.06
Actinomycin D 5×10^{-6} M	8	0	0
Cyclohexamide 5×10^{-5} M	8	0	0
Ro 31-8220 10^{-6} M	8	0	0

Maximum contractile effect E_{max} are expressed as percent of 60 mM K^{+}-induced contraction. pEC_{50} values mean negative logarithm of the molar concentration that produced half maximum contraction. Data are shown as mean±S.E.M. N denotes the number of vessel segments. *$P<0.01$ and $^{+}P<0.05$ versus fresh or control segments, respectively.

Angiotensin II receptors.

Contractile response. Ang II caused a concentration-dependent contractile response in fresh mesenteric artery segments. The maximum contraction was 99±10% and the pEC_{50} value 8.93±0.17. The contraction response curves were depressed markedly after organ culture for 24 hr or 48 hr (fig. 2A, table 1). The AT_1 receptor antagonist, candesartan, shifted the concentration-contractile curve to right and also reduced the maximum contraction (fig. 2B, table 1). The antagonism was similar to that previously seen in rabbit aorta (Morsing *et al.* 1999).

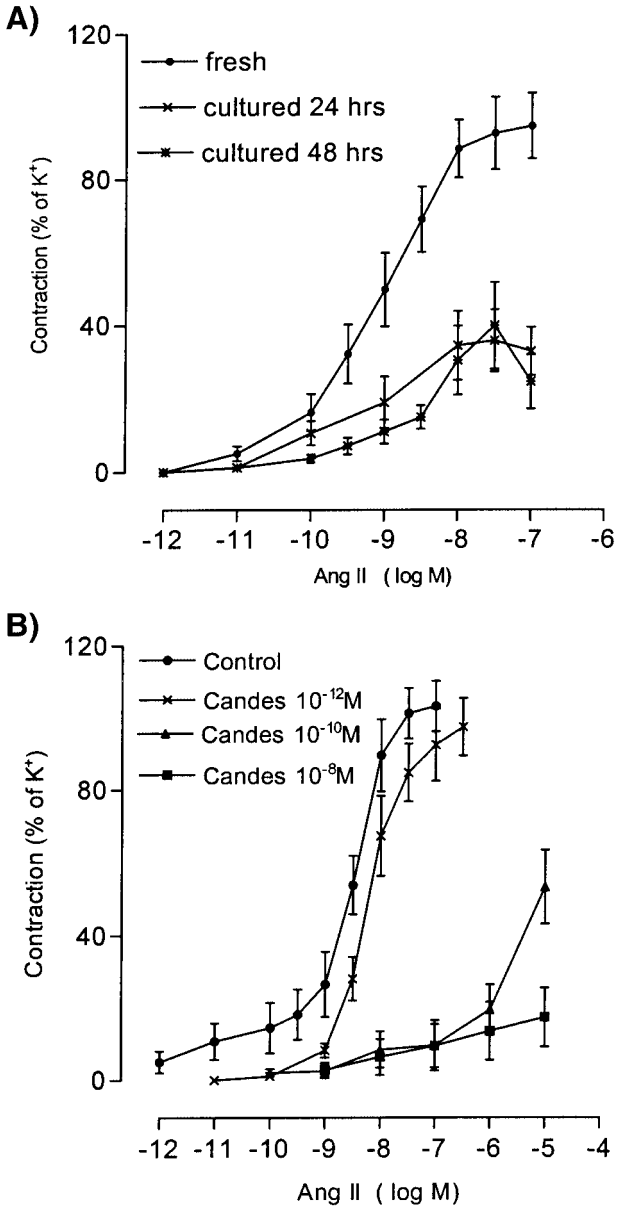

Fig. 2. Concentration response curves for Angiotension II in fresh and organ culture for 24, 48 hr (A) or in the presence of AT_1 receptor antagonist candesartan (10^{-8} M, 10^{-10} M, 10^{-12} M) on fresh mesenteric arteries of rat (B). Data are derived from 8–15 identical experiments and shown as mean±S.E.M.

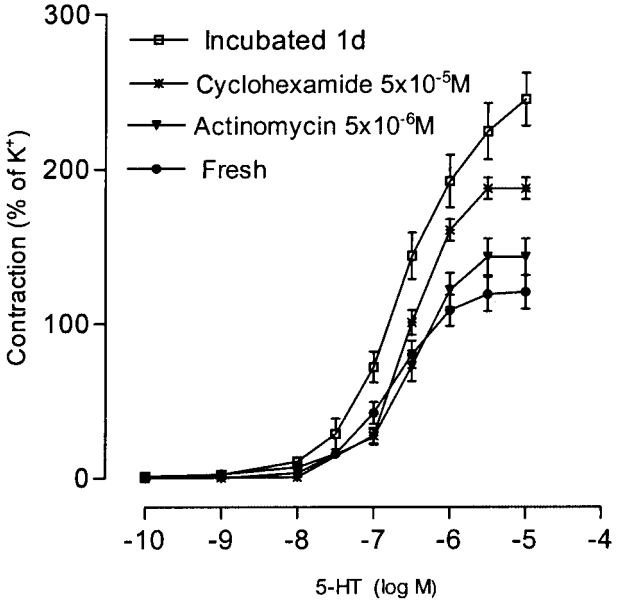

Fig. 3. The effects of transcriptional inhibitor actinomycin (5×10^{-6} M) and translation inhibitor cyclohexamide (5×10^{-5} M) exert on 5-HT induced concentration response curve which was co-cultured for 24 hr of rat mesenteric artery segments. Data are derived from 11–16 experiments and shown as mean±S.E.M.

*Real-time PCR.*The presences of mRNA for the AT_{1A} receptors was analyzed before and after organ culture by real-time PCR. In concert with the functional data above, the mRNA level of AT_{1A} was markedly decreased after organ culture of the mesenteric artery for 24 hr ($P<0.05$) (fig. 5B).

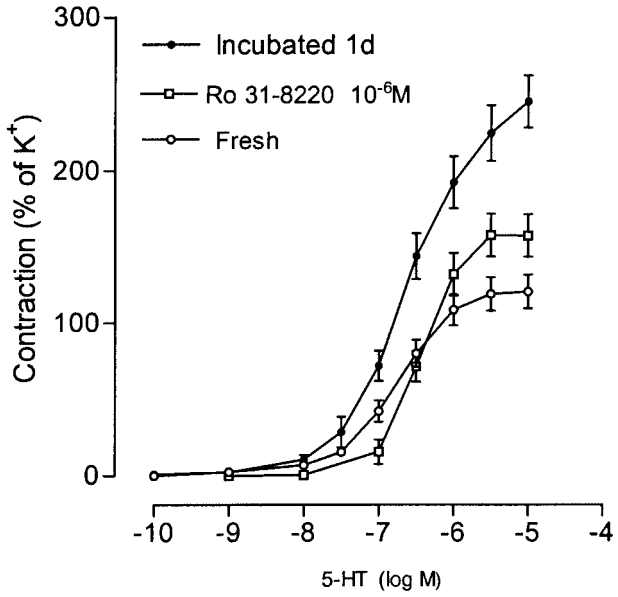

Fig. 4. The specific protein kinace C inhibitor Ro 31-8220 (10^{-6} M) altered the concentration-response curve of 5-HT which was co-cultured for 24 hr of rat mesenteric arteries. Data are derived from 16 identical experiments and shown as mean±S.E.M.

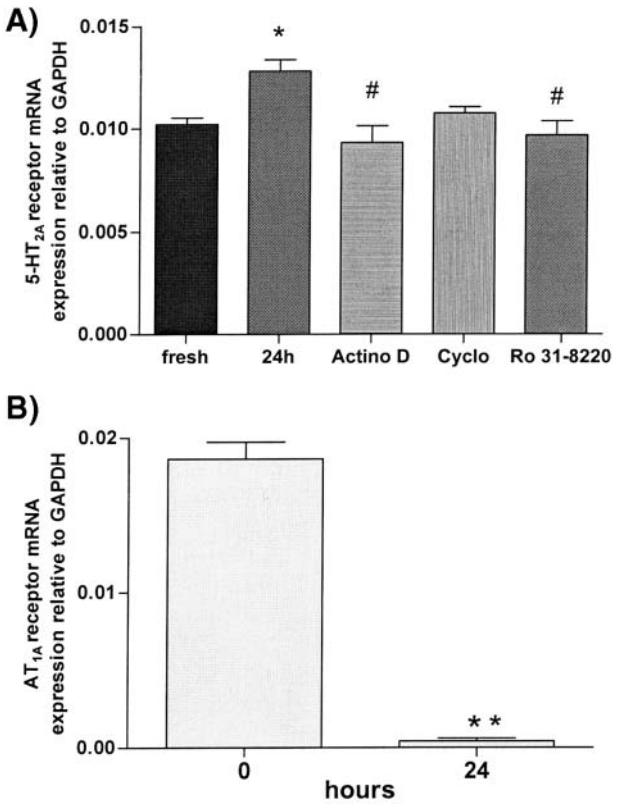

Fig. 5. The comparison of mRNA level on rat mesenteric arteries of fresh, organ culture for 24 hr and co-cultured with different inhibitors: translation inhibitor cyclohexamide, transcriptional inhibitor actinomycin and protein kinase C inhibitor Ro 31-8220 (A). The mRNA expression level of AT_1 receptor on fresh rat mesenteric segments or after organ culture for 24 hr (B). Data are derived from 3–5 identical experiments and shown as mean±S.E.M. Fresh versus organ culture *$P<0.01$, **$P<0.001$. Organ culture versus inhibitor $^{\#}P<0.05$.

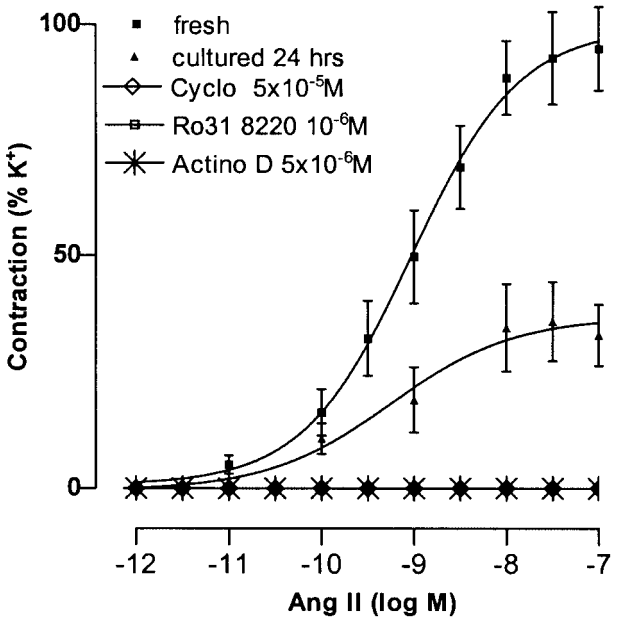

Fig. 6. The effects of protein kinase C inhibitor Ro 31-8220 (10^{-6} M), translation inhiobitor cyclohexamide 5×10^{-5} M and transcription inhibitor actinomycin D 5×10^{-6} M on angiotensin II induced contraction during organ culture of rat mesenteric artery for 24 hr compared with fresh. Data are derived from 7–8 identical experiments and shown as mean±S.E.M..

Protein kinace C inhibitor experiments. In order to study if protein kinase C activity was involved in the down-regulation of AT_1 receptors, the specific protein kinase C inhibitor Ro 31-8220 (10^{-6} M) was present during organ culture. Furthermore, inhibitors for transcription and translation, actinomycin D and cyclohexamide, were also studied. The presence of either of the three above inhibitors for 24 hr resulted in the total loss of the Ang II induced contractile responses; it decreased to zero in the organ cultured vessel segments (fig. 6).

Discussion

The present study has for the first time shown that organ culture of rat mesenteric artery results in altered expression of 5-HT_{2A} and AT_1 receptors in the smooth muscle cells. Alteration in the expression of these G-protein-coupled receptors may prove to be a key event in vascular disease resulting in vascular smooth muscle spasm, proliferation and vascular remodeling. We have previously demonstrated that the ET_B receptor shows plasticity by changing from a relaxant to a contractile phenotype during organ culture of cerebral and peripheral arteries, occurred not only *in vitro* but also *in vivo* e.g. in ischaemic stroke (Stenman *et al*. 2002) and subarachnoid haemorrhage (Hansen-Schwartz *et al*. 2003). The present study has revealed that there are alterations in the expression of 5-HT_{2A} and AT_1 receptors during organ culture of rat mesenteric arteries evident both at mRNA and functional levels. For ET_B receptors in cerebral arteries, this has been shown also at the protein level (Henriksson *et al*. 2004). The up-regulation of the 5-HT_{2A} receptor occurs via a transcriptional mechanism that involves protein kinase C. The results are well in line with our previous studies that have demonstrated that similar mechanisms are involved in the up-regulation of ET_B receptors *in vivo* and *in vitro* (Uddman *et al*. 2002). Protein kinase C activity seems to be a key factor in the transcription of G-protein-coupled receptors and thus blockage of protein kinase C activity during organ culture regulates this. We do not think that smooth muscle cell phenotype changes occurred in the present organ culture of the artery ring segments, since it is known that the contractile response to potassium did not change in the segments cultured for 5 days (Adner *et al*. 1996). In the present study, the segments only cultured for 1–2 days and the culture medium did not contain any serum and growth factors. Smooth muscle cell phenotype changes in primary culture requires longer than 6 days of culture period and also growth factors (Chamley-Campbell *et al*. 1981).

The postsynaptic 5-HT receptors in the peripheral circulation, e.g. the mesenteric artery of the rat, consist mainly

of the 5-HT$_{2A}$ type as based on responses to 5-HT and antagonism by ketanserin with a pA$_2$ 9.43; this is in concert with published data for 5-HT$_2$ receptors, pA$_2$=9.3 for 5-HT$_{2A}$ and 6.5 for 5-HT$_{2C}$ (Hoyer *et al*. 1994; Watts 2002), and the presence of mRNA of 5-HT$_{2A}$ receptors. In fresh rat mesenteric arteries, 5-HT produced a concentration-dependent contraction with an E_{max} of 147±11% which increased after organ culture for 24 hr. The selective 5-HT$_{2A}$ receptor antagonist, ketanserin shifted the concentration-contraction curves of 5-HT to the right in a parallel manner without any change of E_{max} which shows that it was a specific up-regulation of 5-HT$_{2A}$ receptors. The up-regulated receptor has the same pA$_2$ for ketanserin as that seen in fresh arteries. Real-time PCR is a method that allows the quantitative demonstration of receptor mRNA and here we have showed that the up-regulation is evident also at the mRNA level. This suggests a transcriptional mechanism responsible for the up-regulation. The transcription inhibitor actinomycin and the translation inhibitor cyclohexamide confirmed that the event occurs via a transcriptional mechanism. In agreement with the present study, Adner *et al*. (2002) have shown up-regulation of 5-HT$_{2A}$ receptors in smooth muscle cells during organ culture of mice tracheal segments.

Furthermore, analysis of the mechanisms triggering the up-regulation revealed that protein kinase C has a central role for ET$_B$ receptors (Uddman *et al*. 2002). In our study, the protein kinase C inhibitor Ro 31-8220 abolished both the increase in 5-HT$_{2A}$ receptor mRNA and reduced the contractile effects of 5-HT to that seen in fresh arteries. Thus, protein kinase C activity is involved in the up-regulation of 5-HT$_{2A}$ receptors. Protein kinase C is known to play an important role as an intracellular signal for activation of transcriptional factor nuclear factor-κB (NF-κB) and activation protein-1 (AP-1) (Sliva *et al*. 2002; Chuang *et al*. 2003). Since the protein kinase C inhibitor significantly inhibited the up-regulation of 5-HT$_{2A}$ receptors at both functional and mRNA levels, we have demonstrated that protein kinase C activity is required for the up-regulation. A similar phenomenon with protein kinase C has been demonstrated for ET$_B$ receptors and in addition the downstream extracellular signal-regulated kinase 1/2 (ERK1/2) is involved in the up-regulation. Thus, blockadge of protein kinase C activity might be a novel strategy of use in the regulation of the up-regulated G-protein-coupled receptors expression in vascular disease.

Ang II, the major effector molecule in the renin-angiotensin system, plays a central role in the control of sodium excretion and fluid volume as well as of vascular tone. The main actions of Ang II are mediated via a specific membrane-bound G-protein-coupled receptors AT$_1$ receptor, which plays an important role in the development and progression of many cardiovascular diseases. Available AT receptor blockers have been analysed in details and behave more or less as insurmountable antagonists (Ytterberg & Edvinsson 2001; Pantev *et al*. 2002). In heart failure there is down-regulation of AT receptors (Haywood *et al*. 1997; Touyz & Schiffrin 2000). This has been suggested as a feedback loop due to the elevated Ang II level in the circulation of subjects with heart failure (Makita *et al*. 1992; Lassegue *et al*. 1995). The intracellular mechanism behind the regulation of Ang II receptors is unclear, but has sometimes been considered due to enhanced receptor internalization. The contractile response to Ang II was in our study decreased in parallel with down-regulation of AT$_{1A}$ receptor mRNA during organ culture of rat mesenteric arteries for 24 hr. This demonstrates a transcriptional mechanism for the down-regulation of AT$_{1A}$ receptors. We do not know further intracellular mechanisms responsible for the down-regulation of AT$_1$ receptors, particularly since the experiments with actinomycin D or the protein kinase C inhibitor did not provide any clues. Production of Ang II, nitric oxide (Bucher *et al*. 2001), prostacyclin (Dusting *et al*. 1981; Oriji 1999) and protein kinase A activity (Xu & Murphy 2000) are putatively involved in the down-regulation since they have been shown to suppress G-protein-coupled receptor expression. Down-regulation of AT$_1$ receptors occur in human heart failure, failing human ventricular myocardium, hypertrophied human myocardium and atrial fibrillation (De Gasparo *et al*. 1994; Regitz-Zagrosek *et al*. 1994; Nozawa *et al*. 1996; Haywood *et al*. 1997; Tsutsumi *et al*. 1998) possibly via a mechanism similar to that seen during organ culture (Doan *et al*. 2004).

In conclusion, the present study demonstrated that 5-HT$_{2A}$ receptors were transcriptionally up-regulated whereas the AT$_1$ receptors were down-regulated at the transcriptional level in rat mesenteric artery following organ culture. Protein kinase C activity was involved in the up-regulation of 5-HT$_{2A}$ receptors during organ culture. Understanding of the G-protein-coupled receptor alterations that occur in organ culture may provide clues to their functions in cardiovascular disease.

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