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Analysis of the time course for organ culture-induced endothelin ET_B receptor upregulation in rat mesenteric arteries

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Abstract

In contrast to the constitutively expressed endothelin ET_A receptor, the distribution of endothelin ET_B receptors is more variable. The aim of the present study was to investigate the kinetics of organ culture-induced upregulation of contractile endothelin ET_B receptors in rat mesenteric arteries at both mRNA and functional levels. Assessment of mRNA expression revealed low levels of endothelin ET_B receptor mRNA relative to endothelin ET_A receptor mRNA after 3 h of culture, which gradually increased to reach a plateau level after 24 h. Correspondingly, vessels cultured for 3 h showed a negligible contractile response the selective endothelin ET_B receptor agonist sarafotoxin 6c. Subsequently, the contractile response to sarafotoxin 6c was successively increased during organ culture until 24 h and, thereafter, a further increase in potency was seen after 48 h. These results demonstrate a rapid induction of transcription within less than 7 h followed by an increase in the response to receptor stimulation.
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Keywords: Expression; Induction; Operational model; Upregulation

1. Introduction

The endothelin family of peptides are some of the most potent vasoactive substances known (Yanagisawa and Masaki, 1989). Their effect in mammals is mediated by two subtypes of receptors termed endothelin ET_A and ET_B (Arai et al., 1990; Sakurai et al., 1990). Both subtypes belong to the large family of G-protein-coupled seven-transmembrane domain receptors. The distribution and regulation of endothelin ET_A and ET_B receptors in the vascular bed have been the subject of many studies over the past decade. Endothelin ET_A receptors represent the predominant subtype mediating contraction in arterial smooth muscle (Maguire and Davenport, 1995). The endothelin ET_B receptor was found to be predominately expressed in the endothelium mediating vasodilatation via release of NO and prostaglandins (De Nucci et al., 1988). More recently, the existence of smooth muscle endothelin ET_B receptors has been demonstrated (Clozel et al., 1992; Haynes et al., 1995; Moreland et al., 1994), and variations in the expression of endothelin ET_B receptors were observed in pathological conditions such as atherosclerosis (Dagassan et al., 1996; Iwasa et al., 1999), congestive heart failure (Cannan et al., 1996) and in subarachnoid haemorrhage (Roux et al., 1995). In addition, recent studies have demonstrated changes in smooth muscle endothelin ET_B receptor expression after organ culture in a variety of vascular beds of both rat and man (Adner et al., 1998b; Möller et al., 1998), as well as in the rabbit carotid artery, where supraphysiologically high pressure induced a transcriptionally regulated increase of endothelin ET_B receptors (Lauth et al., 2000). In both models, the smooth muscle endothelin ET_A receptor expression remain unaffected. Little is known about the temporal kinetics of alterations in the endothelin-receptor balance in response to different stimuli. However, in other receptor systems, both fast and transient, as well as slower but sustained types of induced response to different stimuli have been reported (for review, see Donaldson et al., 1997).

The present work aims to compare the kinetics of organ culture-induced upregulation of smooth muscle endothelin ET_B receptors in the rat mesenteric artery at functional and transcriptional levels. This phenomenon has been shown to be regulated at the transcriptional level (Möller et al., 1997),

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independent of endothelial- or serum-derived factors (Adner et al., 1998a). The rat superior mesenteric artery has previously been shown to develop a significant endothelin ET_B receptor-mediated contractile response within 24 h of organ culture (Adner et al., 1998b) and this preparation was therefore chosen for the present study. For studies of receptor expression at the transcriptional level, we used a comparative reverse transcription-polymerase chain reaction (RT-PCR) assay that generates information about alterations in the ratio of endothelin ET_A and ET_B receptor mRNA expression (Möller et al., 1997). Functional responses were measured in vitro as changes in isometric force. Subsequently, we applied an operational model of agonism (Black and Leff, 1983) to the data to estimate relative changes in the number of functional receptors in the organ culture system.

2. Methods

2.1. Tissue preparation

Male Wistar–Kyoto rats (200–250 g; M&D, Denmark) were anaesthetised with CO_2 and killed by exsanguination. The superior mesenteric arteries with adherent branches were immediately removed and immersed in cold (4 °C) sterile Dulbecco's modified Eagle's medium (DMEM; Sigma, St. Louis, MO, USA). The distal branch of the artery was dissected free from adherent tissue under cold sterile conditions and the endothelium was removed by flushing the lumen with 0.01% Triton X-100 solution. The protocol was approved by the Ethical Committee of Lund University.

2.2. Organ culture procedure

Following removal of the endothelium, each vessel was divided into two parts: one for in vitro pharmacology and the other for RNA extraction and further RT-PCR. Before culturing, segments for in vitro pharmacology were cut into 1-mm-long circular segments whereas segments for RNA extraction remained intact. Fresh vessels were placed directly in buffer solution (for composition see below) and used for in vitro experiments as described below. The preparation time was standardised at 3 h, during which period the segments were kept in a gassed solution at 37 °C. It has previously been shown that this treatment does not alter the induction of contractile ET_B receptors (Adner et al., 1998a) and it was therefore considered to be equivalent to culture in DMEM. Segments for PCR analysis were incubated for the same duration in buffer solution. Results from these segments are therefore referred to as 3 h time point. Other segments were placed individually in a well plate with 1 ml DMEM containing 4500 mg/l D-glucose, sodium pyruvate (110 mg/l), L-glutamine (584 mg/l), supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). These vessel segments were incubated at 37 °C in humidified 5% CO_2 in air for 7, 12, 24 and 48 h. In addition, some segments were incubated for 7 h in the presence of the transcriptional inhibitor, actinomycin D (5 μg/ml). The absence of endothelium was verified functionally by the absence of acetylcholine-mediated (1 μM) relaxation following precontraction with noradrenaline (10 μM). As the endothelium was removed from the vessels before they were split, the absence of relaxation was also assumed to verify the absence of the endothelium in segments for RT-PCR.

2.3. RNA extraction

Immediately following organ culture, the vessels were snap-frozen in liquid nitrogen and stored at −80 °C until total cellular RNA was extracted using TRIzol reagent (Gibco BRL) following the supplier's instructions. The resulting RNA pellet was finally washed with 70% ice-cold ethanol, air-dried and re-dissolved in 10 μl diethyl-pyrocarbonate (DEPC)-treated water. The amount and purity of RNA was assessed by spectrophotometry using a microcuvette (Genquant, Pharmacia, Uppsala, Sweden).

2.4. Reverse transcriptase-polymerase chain reaction (RT-PCR)

RT-PCR was carried out using the GeneAmp RNA PCR kit (Perkin-Elmer, Foster City, CA, USA) on a DNA Thermal cycler (Perkin-Elmer). Specific primers for the rat endothelin ET_A (generating a 264-base pair product) and ET_B receptors (generating a 560-base pair product) were used as described previously (Uddman et al., 1999). First strand cDNA was synthesized from 1 μg total RNA in a 20-μl reaction volume using random hexamers as primers. The reaction mixture was incubated at 25 °C for 10 min, 42 °C for 15 min, heated to 99 °C for 5 min, and chilled to 5 °C for 5 min. For each primer pair, a 9-μl portion from the resultant cDNA was amplified by PCR in a final volume of 50 μl. The PCR was carried out with the following profile: 5 min at 95 °C for one cycle, followed by 30 cycles of 1 min at 95 °C, 1 min at 57 °C and 30 s at 72 °C; final extension was done for 7 min at 72 °C. Previous control experiments had shown that 30 cycles were within the exponential phase of the PCR. Samples extracted from vessels treated with actinomycin D were analysed after 40 cycles of amplification. A blank (water) was included in all experiments. The identity of the PCR products was verified by restriction analysis as described previously (Uddman et al., 1999).

2.5. Densitometric analysis of PCR products

Densitometric analysis of PCR products was essentially performed as previously described (Möller et al., 1997). Briefly, the PCR products were separated on a 1.5% agarose gel (Sambrook et al., 1989) and photographs of the gels were digitized and analysed densitometrically with NIH-Image software (v1.60) (Rasband and Bright, 1995). The values for the shorter endothelin ET_A receptor fragments

were corrected for their decreased ethidium bromide incorporation before the relative proportion of endothelin ET_A/ET_B band densities was calculated. Results are expressed as mean ± S.E.M. of the percentage of endothelin ET_B of the summed endothelin ET_A/ET_B band densities in each experiment.

2.6. In vitro pharmacology

Vasomotor reactivity was analysed in temperature-controlled (37 °C) tissue baths containing a buffer solution composed of 119 mM NaCl, 15 mM $NaHCO_3$, 4.6 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 1.5 mM $CaCl_2$ and 5.5 mM glucose. The solution was continuously gassed with 5% CO_2 in O_2 resulting in a pH of 7.4. The vessel segments were mounted on two L-shaped metal prongs. One prong was connected to a force-displacement transducer (FTO3C, Grass Instr., Quincy, USA) attached to a MacLab unit (ADInstruments, Hastings, UK) for continuous recording of isometric force by means of the Chart software (ADInstruments). The other prong was connected to a displacement device, allowing adjustment of the distance between the two parallel prongs. A passive force of 2 mN was applied to the segments before the specimens were allowed to stabilise at this level of tension for 60 min. The contractile capacity of each tissue segment was tested by exposure to a potassium-rich buffer solution (60 mM), which had the same composition as the standard solution except that NaCl was exchanged for an equimolar concentration of KCl. When two reproducible contractions had been obtained, the vessels were used for further studies. Concentration–response curves for sarafotoxin 6c were obtained by cumulative application of the peptide. At the end of each experiment, a single concentration of endothelin-1 (100 nM) was applied to the segments.

2.7. Analysis of functional data

All data were expressed as mean values ± S.E.M. Contractile responses in each segment were expressed as a percentage of the contraction induced by endothelin-1. Individual agonist concentration–effect curve data were fitted to the Hill equation using an iterative, least square method (GraphPad Prism, San Diego, CA, USA), to provide estimates of Hill coefficient (n_H), midpoint location (pEC_{50}) and upper asymptote (α). In the treatment groups with 3 and 7 h organ culture, not all the concentration–effect curves were fully defined and could not be fitted to the Hill equation. In the case of the 7 h organ culture, fitting parameters could be obtained from the mean experimental data.

2.8. Statistical analysis

Analysis of variance was followed by post hoc Bonferroni modified *t*-test (Graphpad Prism 3.0). Statistical significance was accepted when $P < 0.05$.

2.9. Operational model of agonism

Here we assume that the difference in responses seen following organ culture is due to differences in the level of receptor expression (see Discussion). If the classical assumption is made that equal receptor-occupancy produces equal tissue response, then the differences in receptor number following organ culture for different periods of time can be quantified. In this study, experimental data were directly fitted to the following derivation of an operational model of agonism (Black and Leff, 1983):

$$E = \frac{E_m[A]^n\tau^n}{(K_A + [A])^n + [A]^n\tau^n}$$

using the 'AR' programme (derivative-free, nonlinear regression analysis) within the BMDP statistical software package (BMDP New System 1.12, Core, Ireland). The model describes agonist concentration–effect data in terms of four parameters: E is pharmacological effect, [A] is agonist concentration, K_A is the agonist equilibrium dissociation constant, n is the slope of the transducer function and τ is the efficacy parameter or transducer ratio, which is given by the ratio of the total receptor concentration ($[R_0]$) and the midpoint location of the transducer function (K_E). E_m is the maximum pharmacological effect. In fact, τ and K_A were estimated as log values as these parameters have been shown to be log-normally distributed (Christopoulos, 1998). The goodness-of-fit of the model to the experimental data was assessed using a chi-squared (χ^2) analysis, where χ^2 was calculated by comparing the observed data points with the expected values obtained by model fitting using following equation:

$$c^2 = \sum \frac{(\text{observed} - \text{expected})^2}{m(\text{expected})^2 + c}$$

where the denominator is a correction factor applied to account for the linear relationship found between the variance (σ^2) and the square of the observed effect. The values

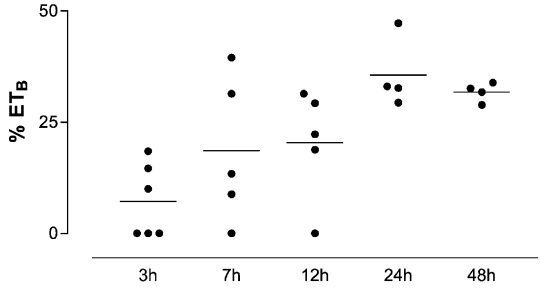

Fig. 1. Time course of alterations in endothelin ET receptor mRNA balance during organ culture. Each point represents the percentage of endothelin ET_B receptor mRNA of the total endothelin receptor mRNA population obtained from one animal. The horizontal bar in each column denotes mean.

of c and m were estimated from a linear regression analysis of these data, and the χ^2 values were corrected to account for any differences in the amplitude of the sarafotoxin 6c concentration–effect curves within the assay.

2.10. Drugs

Sarafotoxin 6c and endothelin-1 were dissolved in 0.1% (w/v) bovine serum albumin in sterile water (Auspep, Parkville, Australia) and further diluted in buffer solution. Actinomycin D, acetylcholine and all other chemicals were obtained from Sigma unless stated otherwise. Only high-grade chemicals and double-distilled water were used throughout.

3. Results

3.1. RT-PCR analysis

RT-PCR analysis of total RNA extracted from vessel segments incubated for 3–48 h demonstrated the presence of mRNA coding for ET_A receptors in all samples, whereas endothelin ET_B receptor mRNA was absent from some vessels of time points 3, 7 and 12 h. No signal was observed when the reverse transcription step was omitted.

In vessels cultured for 3 h, endothelin ET_B mRNA represented 7.18 ± 1.36% (n = 6) of the total mRNA coding for endothelin receptors. At this time point, three vessels showed no detectable signal for the endothelin ET_B receptor; however, we observed clear bands corresponding to endothelin ET_A mRNA, excluding the possibility of a PCR artefact (Fig. 1). After 7 h of incubation, the endothelin ET_B mRNA increased relative to endothelin ET_A representing 18.6 ± 7.3% (n = 5) of the total endothelin receptor mRNA. Even at this time point, we noticed the absence of endothelin ET_B bands in one sample. Longer incubation caused a significant (P < 0.05) increase of the endothelin ET_B proportion of the total endothelin receptor mRNA compared to 3 h culture. At 12 h of incubation, endothelin ET_B mRNA amounted 20.4 ± 5.6% (n = 5) with one sample showing no endothelin ET_B signal. Following 24 h of culture, endothelin ET_B mRNA comprised 35.6 ± 4.0%; (n = 4) relative to endothelin ET_A, and after 48 h of culture the change in mRNA ratio appeared to have reached a plateau (31.8 ± 1.1%; n = 4). These findings indicate an organ culture-induced time-dependent increase in endothelin ET_B mRNA relative to ET_A. Culture for 7 h in the presence of the transcriptional inhibitor actinomycin D reduced mRNA levels for both endothelin ET_A and ET_B receptor subtypes so that even 40 cycles of amplification revealed only faint bands corresponding to endothelin ET_A mRNA in two of four tested samples. No bands corresponding to the endothelin ET_B mRNA were detected under these conditions (not shown).

Table 1
Model fitting parameter estimates obtained for sarafotoxin 6c E/[A] curves on rat mesenteric artery following organ culture, fitted to the Hill equation (α, pEC_{50} and n_H) and an operational model of agonism (τ)

Time of organ culture (h)	n	α (% of ET-1)	pEC_{50}	Hill coefficient, n_H	log τ-value	τ-value
3	8	3 ± 1[a]	N.D.	N.D.	− 1.52[b]	0.030[b]
7	8	12[b]	8.93[b]	1.07[b]	− 0.64[b]	0.23[b]
12	8	55 ± 7	9.83 ± 0.24	1.16 ± 0.16	0.65 ± 0.28	4.50
24	7	82 ± 8	9.51 ± 0.09	1.29 ± 0.14	0.92 ± 0.10	8.26
48	5	97 ± 3	10.47 ± 0.36	1.33 ± 0.16	2.00 ± 0.39	99.5

ET-1: endothelin-1; N.D.: not determined.
[a] Calculated mean value.
[b] Estimated from the mean values.

3.2. In vitro pharmacology

Endothelium denuded segments of the rat mesenteric artery were tested for contractile responses towards endothelin-1 and sarafotoxin 6c. Prior to the experiment, the absence of the endothelium was verified in all segments by testing for acetylcholine-mediated dilation after precontraction with noradrenaline. None of the vessels tested responded to acetylcholine.

The contractile response to endothelin-1 did not change significantly between the different periods of culture when expressed as percentage of the contraction induced by 60 mM

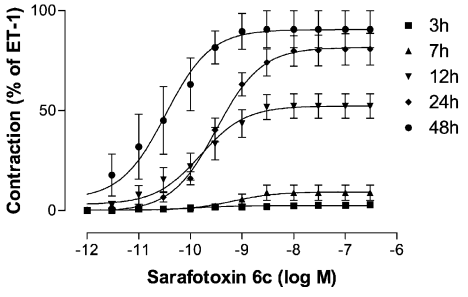

Fig. 2. Contractile responses elicited by cumulative application of the endothelin ET_B receptor selective agonist sarafotoxin 6c after 3, 7, 12, 24 and 48 h of organ culture. The contractions were normalised to the endothelin-1 response obtained after a single dose of 100 nM. Each point represents the mean ± S.E.M. of all segments tested. n = 5–8.

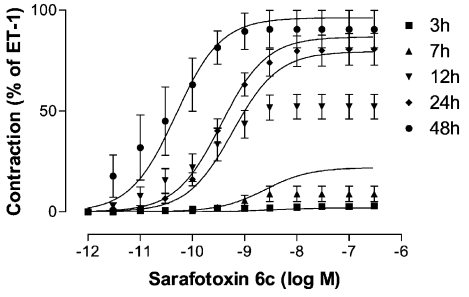

Fig. 3. Fitting of the operational model discussed in the text on the experimental data of Fig. 2.

K^+. However, earlier studies have shown that the contraction induced by KCl (60 mM) tends to be reduced following the organ culture procedure, whereas the responses to endothelin-1 are more reproducible (Adner et al., 1994). Therefore, responses to sarafotoxin 6c were normalised to the response to 100 nM endothelin-1 (Fig. 2). Following 3 h under culture conditions, there was virtually no response to sarafotoxin 6c; however, a gradual increase in maximum asymptote and pEC_{50} were observed with increasing incubation time (Fig. 1; Table 1). The maximum responses obtained after 24 and 48 h were indistinguishable although further leftward displacement of the concentration–response curve was observed, suggesting that the maximum tissue response had been achieved. After 7 h culture, treatment with the transcriptional inhibitor actinomycin D totally abolished the contractile responses to sarafotoxin 6c, while endothelin-1 still induced a clear contractile effect (99 ± 24% of the contraction without treatment of actinomycin D).

3.3. Application of the operational model to the data

The model was fitted simultaneously to the individual data (with the exception of the 3 and 7 h culture, were the mean values were used) with no constraint applied to any of the model parameters to be estimated. As judged by the goodness-of-fit ($\chi^2 = 192$; $\chi^2_{(0.05)} = 172$ at 143 degrees of freedom), the model did not provide a good description of the data. However, by inspection of the curves simulated from the model fit superimposed on the experimental data (Fig. 3), it seems that the model predicted the increase in the amplitude of the sarafotoxin 6c concentration–effect curve, but the increase in potency was not consistent with a simple increase in the τ-value with increased culture time in all cases. In particular, the sarafotoxin 6c concentration–effect curve following 12 h of culture was more potent and less efficacious than predicted by the model.

Notwithstanding this complexity, the best fit of the model generated an E_m estimate of 98 ± 2% of the endothelin-1-induced contraction (mean ± asymptotic standard deviation), a pK_A estimate of 8.48 ± 0.16 and transducer slope parameter of 1.01 ± 0.07. The model parameters R_0 and K_E are experimentally indistinguishable and are estimated as their ratio, τ. Thus, changes in the value of this parameter may reflect changes in receptor density or receptor–effector coupling efficiency. If it was assumed that the effect of organ culture is to increase the number of surface receptors, then the τ-values estimated would reflect a 3300-fold increase in receptor density between 3 and 48 h of culture (Table 1). A comparison of the mRNA and τ-values shows that the majority of the increase in receptor number took place after a plateau in relative increase in endothelin ET_B mRNA had been reached (Fig. 4).

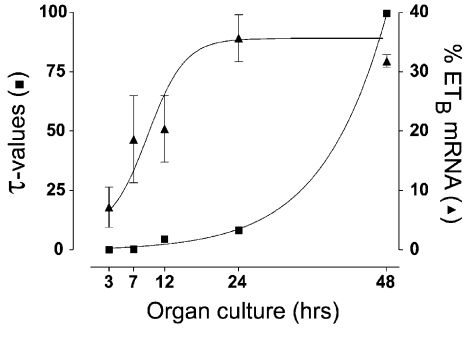

Fig. 4. Temporal relation between alterations in endothelin receptor density expressed as τ-values (left y-axis) and alterations of endothelin receptor expression on mRNA level (right y-axis).

4. Discussion

In the present study, we have examined the time course of organ culture-induced upregulation of smooth muscle endothelin ET_B receptors at both functional and transcriptional levels. Previous studies have shown this upregulation to occur in a wide range of vessels (Adner et al., 1998b) as a metabolically active process (Adner et al., 1998a), which is regulated at the transcriptional level (Möller et al., 1997). The results of this study demonstrate that the development of a contractile response to sarafotoxin 6c is preceded by a rapid transcriptional activation of the endothelin ET_B receptor gene. The weak response to sarafotoxin 6c seen after 7 h of culture could be blocked by treatment with the transcriptional inhibitor actinomycin D. This indicates that even this early response is dependent on transcription and does not reflect the activation of a pool of already present receptors. The observation in these experiments that the response to sarafotoxin 6c was abolished totally might indicate that the steady state pool of endothelin ET_B receptors present in fresh vessels is subject to a continuous turnover.

The role of the endothelium in the regulation of the endothelin ET_A and ET_B receptors is not clear. As recently described, the absence of the endothelium in fresh arteries potentiates the responses to endothelin-1, whereas no differences of the sarafotoxin 6c responses in denuded arteries cultured for 1 day was demonstrated (Adner et al., 1998a). This does not mean that the endothelium is without a role in the upregulation of endothelin ET_B receptors as it is possible that, for example, an effect of an unidentified inhibiting factor is abolished during culture. However, in this study, the endothelium was removed in order to minimize the role of dilatory endothelin ET_B receptors and/or nitric oxide that otherwise could alter the concentration–response curve for sarafotoxin 6c.

Using quantitative RT-PCR, we have previously observed that the absolute levels of endothelin ET_B mRNA in human omental arteries show large variability, whereas pharmacological responses and relative changes in mRNA expression do not (Möller et al., 1998). This observation confirms other studies in, for example, the kidney (Karet and Davenport, 1995), supporting the concept that the ratio

or balance of receptors rather than their absolute quantity might be of importance for the physiological state of a tissue (O'Reilly et al., 1992). For this reason and because of the limited amount of RNA extractable from the small vessels used, we applied a comparative RT-PCR assay for the estimation of changes in mRNA balance (Möller et al., 1997). In addition, the extent of the increase of contractile endothelin ET_B receptors was estimated using τ-values obtained from an operational model of agonism (Black and Leff, 1983). This approach is based on the idea that τ (the ratio of the model parameters R_0 and K_E) reflects the actual number of receptors if the effect of receptor stimulation is unaltered during culture. Although the model predicted the early and late time points well, it overestimated both pEC_{50} and α values at 7 and 12 h of organ culture. The failure to fit the experimental data at these time points might be explained by signal transduction pathways that are not adapted to handle stimulation from the rapid increase in the number of active surface receptors. At later stages, these pathways may have adapted, resulting in a better prediction of the experimental data by the model. If this interpretation is correct, the τ-values may provide a close approximation of the increase of active receptors between 3 and 48 h of organ culture.

The results of our study demonstrate a tight correlation between transcriptional activation of the endothelin ET_B receptor gene and the development of a contractile response towards the endothelin ET_B receptor selective agonist sarafotoxin 6c (Fig. 3). After 7 h of culture a twofold (although not significant) increase of endothelin ET_B receptor mRNA relative to endothelin ET_A receptor is seen, resulting in about 10 times higher receptor density as assessed by the model. This increase leads to a minor contractile response to sarafotoxin 6c. After 24 h of culture, the increase of endothelin ET_B receptor mRNA reaches a plateau with an approximately fivefold relative increase, while the number of receptors has increased by about 300 times compared to 3 h of incubation. Forty-eight hours of culture did not produce a further change of mRNA or α; however, we found a marked leftward shift of the concentration–response curve, indicating a continued increase in the number of receptors and consequently a larger τ-value, indicating an approximately 3000-fold increase in receptor density. Interestingly, almost identical kinetics of transcriptional and functional activation were recently found in the bradykinin B_2 receptor system in a rat model of acute myocardial infarction (Tschope et al., 2000). In that study, a twofold increase in mRNA was detected 6 h after induction of the infarction, that reached a steady state after 24 h with a fivefold increase compared to sham-operated rats. The bradykinin B_2 receptor protein levels as estimated by Western blot analysis increased gradually up to day 6 of the experiment.

Taken together, the present data indicate that in the rat mesenteric artery maximum transcriptional activation of the endothelin ET_B receptor gene is reached at about 24 h of culture, while the number of receptors increases continuously after a time delay of about 7 h. It appears that the increase in transcription reaches a plateau before the number of receptors. Although the physiological significance of spontaneously occurring endothelin ET_B receptor upregulation is not known, its occurrence in a wide range of vascular beds from different animals indicates a general mechanism. The nature of this mechanism as well as the stimuli involved are unknown, however, The possibility for the endothelin ET_B receptors mediate contraction and contribute to mitogenesis (Eguchi et al., 1994) indicate that a rapid induction of the receptors may be of importance for the regulation of both vascular tone and arterial remodelling (Iwasa et al., 1999). A more thorough investigation of the signal transduction pathways involved in the regulation of endothelin ET_B receptors in the rat mesenteric artery after organ culture is required. Recently, it has been demonstrated that the mechanosensitive transcription factor CCAAT/enhancer p-binding protein contributes to the upregulation of endothelin ET_B receptors in cyclic stretched rat aortic smooth muscle in culture (Cattaruzza et al., 2001). However, since dispersed cultured smooth muscle cells, in contrast to the smooth muscle cells in isolated whole tissues, rapidly change their phenotype from a contractile to a synthetic phenotype, data from these two different systems may not be comparable. While cyclic stretch may be an stimulus in cultured smooth muscle cells, the isolated cultured arteries in our organ culture model appear to respond with the upregulation of contractile ET_B receptors, regardless the presence or absence of tonus (Adner et al. 1998a). Thus, a link between these findings remains to be established.

In conclusion, the present study demonstrates that organ culture of the rat mesenteric artery induces the appearance of contractile endothelin ET_B receptors within 7 h of culture. Functional responses and mRNA changes were temporally associated and an operational model of agonism predicted the increase in response at later time points where the system had reached a stable state. This method may offer new opportunities for the quantitative analysis of receptor systems in cells showing plasticity and will assist future studies of the regulation and turn-over of receptors.

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