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# Cerebral vasoconstriction after subarachnoid hemorrhage - Role of changes in vascular receptor phenotype

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## 1. ABSTRACT

The pathological constriction of cerebral arteries known as cerebral vasospasm (CVS) is with a delay of 4 to 10 days linked to subarachnoid hemorrhage. Several agents have been suggested as being responsible; amongst these perhaps 5-hydroxytryptamine (5-HT) and endothelin-1 (ET-1) are the most prominent given their ability to elicit powerful constriction of cerebral arteries. Investigating both 5-HT and ET receptors we have observed that there are distinct changes in receptor phenotype after experimental SAH, namely upregulation of the $ET_B$ and $5\text{-}HT_{1B}$ receptors, and that this upregulation is linked to a higher sensitivity to the endogenous agonists. It has also been shown that reduction in regional cerebral blood flow (CBF) is associated with receptor upregulation and interventional animal experiments have shown a benefit from inhibiting the PKC and MAP kinase pathways on receptor upregulation, CBF and neurological outcome.

## 2. ASPECTS OF RECEPTOR UPREGULATION IN CEREBRAL ARTERIES

### 2.1. Changes in the vascular receptor phenotype

Some years ago it was discovered that segments of the rat and human arteries were able to change their receptor phenotype in the process of organ culture (1;2). Specifically the appearance of a contractile ETB receptor phenotype was noted (2). This was in stark contrast to the situation in normal fresh arteries where stimulation of the ETB receptor only produced a relaxant response (1). This discovery provided the impetus for the in-depth investigation of organ culture induced changes in the receptor phenotype of cerebral arteries and, given the focus on arterial narrowing as paramount in cerebral vasospasm, also for testing whether such alterations could play a role in this syndrome (3;4).

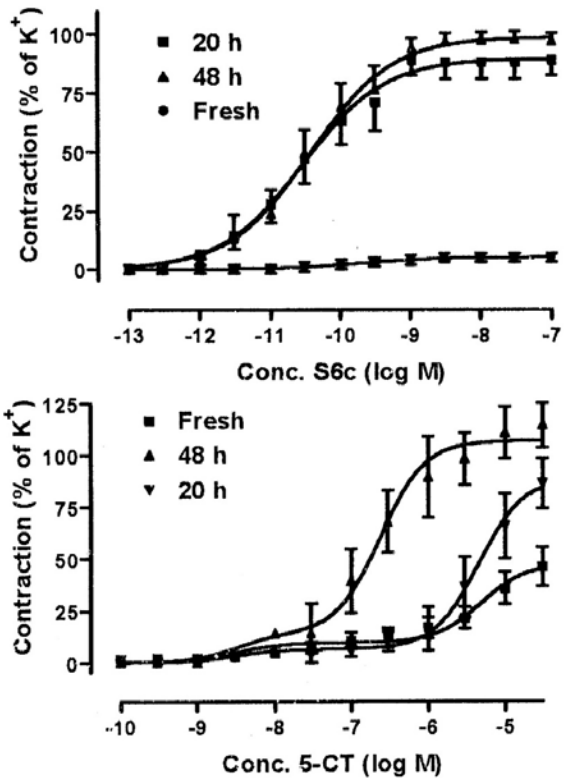


**Figure 1.** Concentration-contraction curves (rat cerebral artery) showing the time profile in the upregulation for the $ET_B$ receptor (left) and B the 5-$HT_{1B}$ receptor (right). The upregulation of the $ET_B$ receptor seems complete after 20 hours of organ culture, whereas in the case of the 5-$HT_{1B}$ receptor, much upregulation seems to be happening between 20 and 48 hours of organ culture.

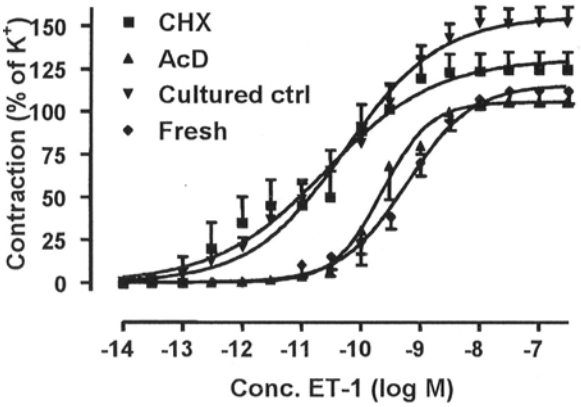


**Figure 2.** Concentration-contraction curves (human cerebral artery) showing the inhibitory effect of Actinomysin D (AcD) and Cyclohexamide (CHX) on the enhanced ET-1 response after organ culture. Clearly AcD inhibits the enhancement, the curve being similar to the fresh. CHX has a partial inhibiting effect; this was concluded not to be lack of effect, rather higher concentrations could not be employed for reasons of toxicity. The results indicate that gene expression is necessary to achieve the altered pharmacological phenotype.

**2.2. ET receptor upregulation in cerebral arteries**

The rat basilar artery was shown to exhibit a markedly altered receptor phenotype after organ culture (3). In fresh basilar artery ET-1 produced an $ET_A$ receptor dependent contraction with an $E_{max}$ of 122±15% and $pEC_{50}$ 8.7±0.1. The specific $ET_B$ receptor agonist S6c did not produce contraction in the fresh artery, but upon precontraction with U46619 a powerful endothelium dependent dilatation was observed. These observations were fully in line with those done on peripheral arteries in the rat (2) as well as on cerebral arteries from other species (5).

In situations of organ culture, the pattern of reactivity was modified dramatically; after 48 hours of organ culture the sensitivity to ET-1 had increased 1000-fold, $pEC_{50}$ now being 11.7±0.3, but without change in $E_{max}$ compared to fresh arteries. Also, S6c now gave rise to a powerful $ET_B$ receptor dependent contraction with an $E_{max}$ of 98±3% and $pEC_{50}$ of 10.6±0.3 (Figure 1).

Fresh human cortical arteries (removed in conjunction with neurosurgery) have been investigated by Nilsson *et al.* (6). They observed an $ET_A$ receptor mediated contraction in response to ET-1 with an $E_{max}$ of 112±8% and a $pEC_{50}$ of 9.2±0.2. In response to S6c an $ET_B$ receptor mediated dilatation was observed, though the effect was not as pronounced as the one observed in rat cerebral arteries. In cultured human cerebral arteries the picture was somewhat different compared to the observations done on rat basilar arteries (7). In similarity, the response to ET-1 increased both in terms of sensitivity and potency, $E_{max}$ 152±9% and $pEC_{50}$ 10.3±0.2 (Figure 2). The contraction was solely (in the pharmacological sense) $ET_A$ receptor dependent. However, and in contrast, S6c did not elicit any contraction.

In arteries from rats with induced SAH, an increased response to ET-1 was observed (8). In similarity with the results obtained with cultured human cerebral arteries, no contractile response toward S6c was observed, and the ET-1 induced contraction was shown to be dependent on the $ET_A$ receptor. The data were supplemented by quantitative RT-PCR, which showed the remarkable result that there was no increase in the number of $ET_A$ receptor mRNA copies; on the contrary, the number of $ET_B$ receptor copies was 4-fold increased.

It therefore seems reasonable to conclude that the appearance of the $ET_B$ receptor is instrumental in the process of increased pharmacological sensitivity to ET-1, although this receptor need not be functional in the sense of being able to produce contraction upon selective stimulation. The importance of the $ET_B$ receptor was furthermore confirmed in the SAH experiments since the ET-1 induced contraction in segments of cerebral arteries could be attenuated through either pre-treatment with the specific $ET_B$ receptor antagonist IRL 1038 or through desensitising any present $ET_B$ receptors by with S6c (used as pre-treatment).

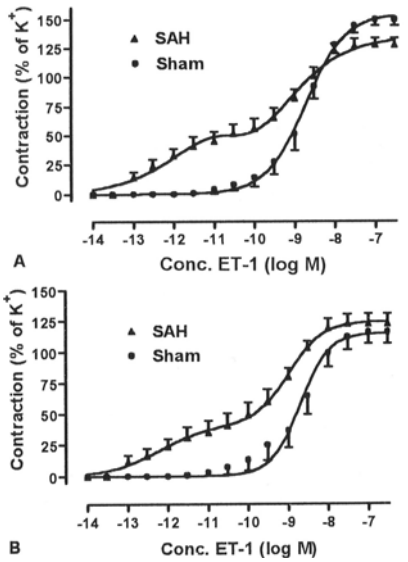


**Figure 3.** Concentration-contraction curves showing the enhanced ET-1 response in (A) the MCA and (B) the basilar artery from rats with induced SAH. A clearly enhanced response is observed. In the posterior communicating artery, no significant changes were observed (not shown). Molecular analysis (Figure 5) showed increased transcription of the $ET_B$ receptor. Selective $ET_B$ stimulation did not give rise to a functional response; thus, a complex interaction between the two ET receptors must be responsible for the enhanced response.

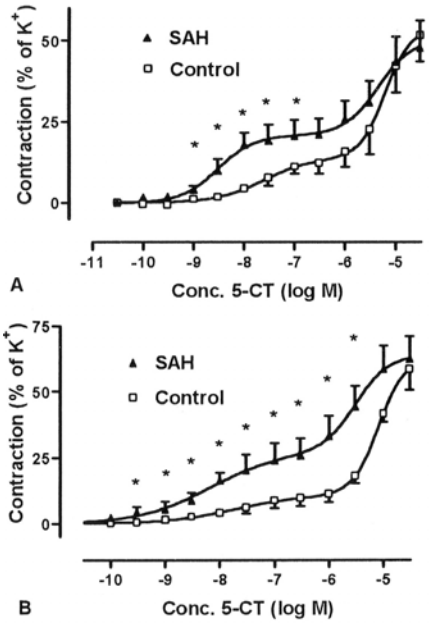


**Figure 4.** Concentration-contraction curves showing the enhanced 5-CT response in (A) the MCA and (B) the basilar artery from rats with induced SAH. Molecular analysis showed increased transcription of the $5\text{-}HT_{1B}$ receptor hence explaining the increased response to 5-CT (selective $5\text{-}HT_1$ receptor agonist).

At present the nature of interaction between the $ET_A$ and the $ET_B$ receptors may only be speculated at. It could range from possible dimerisation in the cell membrane over receptor synergism to interaction between the signal transduction pathways of the two receptors. In the ET-1 elicited contraction in small mesenteric arteries, Mickley *et al.* (9) found that $ET_B$ receptors contributed more to the contraction than could be predicted through the response to S6c alone, thus, also making allusion to collaboration between the two receptors. They termed this collaboration "cross-talk".

The role of the $ET_B$ receptor in arterial narrowing of the cerebral arteries after experimental SAH has also been studied by Vatter *et al.* (10;11;12). In agreement with the aforementioned observations they did not find any contractile of effect of selective $ET_B$ receptor stimulation, but did observe a time dependent decline in the dilatatory effect of $ET_B$ receptor stimulation. In contrast, however, quantitative protein analysis did not reveal any increase in $ET_B$ receptor protein in SMC.

### 2.3. 5-HT receptor upregulation in cerebral arteries

In fresh rat basilar artery 5-HT elicits a concentration-dependent contraction with an $E_{max}$ of 82±6% and a $pEC_{50}$ of 7.2±0.1, comparable to previously reported values (13). The contraction was found dependent on the $5\text{-}HT_{2A}$ receptor. 5-CT, a fairly specific agonist for the $5\text{-}HT_1$ receptor group, produced a biphasic response with an initial phase of low efficacy and a second with appreciable contraction. The first phase, but not the second, could be attenuated using the specific antagonist GR 55562 (specific $5\text{-}HT_{1B/1D}$ antagonist) indicating the vestigial presence of a $5\text{-}HT_{1B/1D}$ receptor in fresh arteries, and indicating a degenerate $5\text{-}HT_{2A}$ receptor response at high concentrations.

In cultured arteries, 5-CT gave rise to a powerful contraction with an $E_{max}$ of 113±11% and a $pEC_{50}$ of 6.8±0.4; a contraction that was competitively inhibited by GR 55562, thus indicating upregulation of a $5\text{-}HT_{1B/1D}$ type receptor. The response to 5-HT in cultured arteries was also increased compared to fresh arteries, $E_{max}$ being 117±4% and $pEC_{50}$ 7.3±0.4 (Figure 1). The functional results were confirmed using ordinary RT-PCR identifying $5\text{-}HT_{1B}$, $5\text{-}HT_{1D}$, and $5\text{-}HT_{2A}$ receptor mRNA.

In the MCA and basilar artery from rats with induced SAH, the $5\text{-}HT_{1B}$ receptor was also found upregulated, both functionally and in terms of number of mRNA copies (Figures 4 and 5)(14). Thus, 5-CT yielded an increased response in comparison to arteries from sham-operated animals, and the response could be attenuated using GR 55562. For the SAH studies quantitative RT-PCR was used. $5\text{-}HT_{1D}$ receptor mRNA was only found in negligible amounts. In contrast, the number of mRNA copies for the $5\text{-}HT_{1B}$ receptor was increased 5-fold (Figure 5). In conclusion, among the 5-HT receptors it appears to be the $5\text{-}HT_{1B/1D}$ receptor that is plastic, i.e. capable of being upregulated.

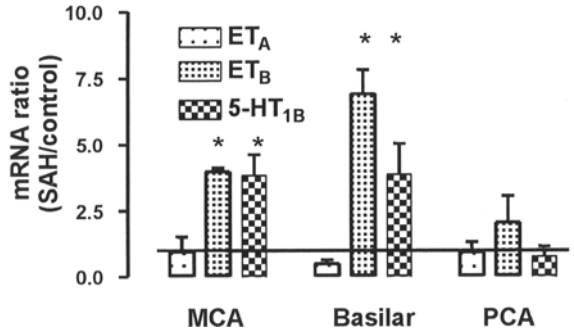


**Figure 5.** Bar chart showing the mRNA ratio (mRNA in experiment divided by control) in cerebral arteries of the various receptor types between arterial segments from rats with induced SAH and sham-operated animals. Transcription of the $ET_B$ and the $5\text{-}HT_{1B}$ receptors is increased in rats with induced SAH (figs. 3 and 4).

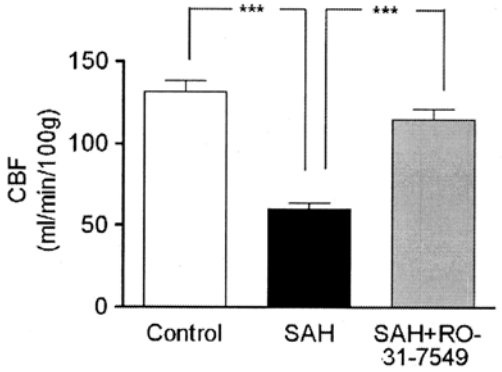


**Figure 6.** Effect of treatment with the PKC inhibitor RO-31-7549 on the global CBF after experimental SAH in rats. Data are expressed as mean±s.e.m. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

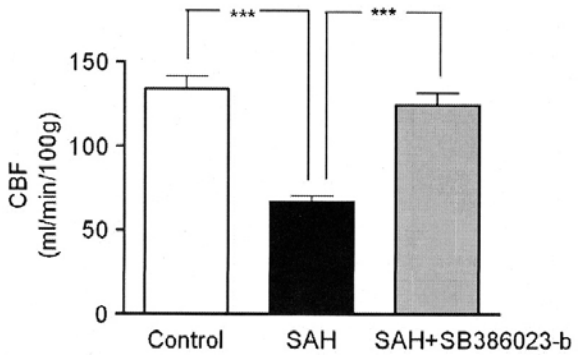


**Figure 7.** Effect of treatment with SB386023-b on the global CBF after experimental SAH in rats. Data are expressed as mean±s.e.m. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

### 2.4. The signal transduction pathway mediating receptor upregulation

It has been shown that the upregulation of receptors is an active process; co-culture with Actinomycin D inhibiting transcription prevented the phenotypical receptor changes described above (Figure 2) (10). Based on results gathered using *in vitro* organ culture of arterial segments where PKC and MAP kinases were found to be involved in the signal transduction process (15;16), a series of interventional experiments in a rat SAH model was conducted. PKC and MAPK inhibitors were administered in conjunction with the SAH as well as immediately after, observing whether this treatment blocked changes in receptor mRNA, receptor protein and vascular function, and most importantly, whether the SAH induced reduction in global and regional CBF and neurological score was prevented.

Experimental SAH in the rat gave rise to a significant drop in global CBF from 131±7 ml/l00g/min (as measured in the control group) to 60±4 ml/l00g/min after 48 hours of observation. Intrathecal administration of the PKC inhibitor RO-31-7549 (blocker of the classical and novel PKC's) did to some extent prevent the reduction in global CBF reaching a level of 115±6 ml/l00g/min (Figure 6)(4). As for regional CBF similar results were observed in 14 out of the 18 brain regions studied (Table 1 in (4)). Furthermore, the SAH induced enhancement of contractile responses to ET-1 and 5-CT in cerebral arteries was inhibited. As for protein levels, immunohistochemistry using specific receptor antibodies showed lower levels of vascular $ET_B$ and $5\text{-}HT_{1B}$ receptor protein in rats treated with RO-31-7549. In parallel, the increase in mRNA for these receptors was also prevented. For the study of the MAP kinase pathway the inhibitor SB386023-b was chosen (16). SB386023-b inhibits MAPKKK upstream of ERK1/2. Intrathecal treatment with SB386023-b efficiently prevented the aforementioned reduction in CBF after SAH to a level of 125±7 ml/l00g/min (Figure 7)(17). Furthermore, as observed before, the SAH induced enhancement of the contractile responses to ET-l and 5-CT in cerebral arteries was prevented. In addition, administration of SB386023-b prevented the upregulation of $ET_B$ and $5\text{-}HT_{1B}$ receptor mRNA and protein levels otherwise observed after SAH.

## 3. MAJOR CONCLUSIONS

- Cerebral arteries have an inherent capacity to increase their sensitivity to endogenous agonists such as ET-1 and 5-HT. It may be induced through organ culture of discrete arterial segments, but may also be induced *in vivo* in a rat model of SAH. Receptor upregulation and reduction in CBF (both globally and regionally) after experimental SAH have been associated.

- In the ET and 5-HT receptor systems, it is the $ET_B$ and $5\text{-}HT_{1B}$ receptors that are inducible. In terms of increased sensitivity to 5-HT, it seems governed by the appearance of the $5\text{-}HT_{1B}$ receptor, whereas the increased sensitivity to ET-1 involves the $ET_B$ receptor, but probably also involving a complex interplay between the $ET_A$ and the $ET_B$ receptors.

- The upregulation of receptors is an active process involving gene transcription and gene translation. Also, signal transduction pathways are involved. Both the

PKC and MAP kinase pathways have been shown to be involved both *in vitro* and experimental SAH. In the latter case inhibition of these pathways leads to normalization of CBF and prevents receptor upregulation.

## 4. ACKNOWLEDGEMENTS

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**Key Words**: Endothelin receptors, 5-HT receptors, Organ Culture, *In Vitro* Pharmacology, Reverse Transcriptase Polymerase Chain Reaction, Subarachnoid Haemorrhage, Review

**Abbreviations:** 5-HT: 5-Hydroxytryptamine, Ang II: Angiotensin II, CBF: Cerebral Blood Flow, CVS: Cerebral Vasospasm, DNA: Deoxyribonucleic Acid, $E_{max}$: The maximal attainable contraction of a vessel with a given agonist, ET: Endothelin, ET-1: Endothelin-1, MCA: Middle Cerebral Artery, mRNA: Messenger Ribonucleic Acid, $pEC_{50}$: The negative logarithm of the concentration of a given agonist needed to obtain 50% of the maximum contraction possible with that agonist, PKC: Protein Kinase C, RT-PCR: Reverse Transcriptase Polymerase Chain Reaction, S6c: Sarafotoxin 6c, SAH: Subarachnoid Haemorrhage, SMC: Smooth Muscle Cell

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