

Peer Tfelt-Hansen
Elisabeth Nilsson
Lars Edvinsson

Contractile responses to ergotamine and dihydroergotamine in the perfused middle cerebral artery of rat

Received: 2 November 2006
Accepted in revised form: 22 January 2007
Published online: 11 May 2007

P. Tfelt-Hansen
Department of Neurology,
Copenhagen University Hospital,
Glostrup, Denmark

E. Nilsson (✉) · L. Edvinsson
Department of Internal Medicine,
University Hospital of Lund,
S-22185 Lund, Sweden
e-mail: lars.edvinsson@med.lu.se
Tel.: +46-46-171484
Fax: +46-46-184792

L. Edvinsson
Department of Clinical Experimental
Research,
Copenhagen University Hospital,
Glostrup, Denmark

Abstract The vasomotor effects of ergotamine and dihydroergotamine (DHE) on the middle cerebral artery (MCA) of rats were studied using the pressurised arteriography method and *in vitro* myographs. MCAs from Sprague–Dawley rats were mounted on two glass micropipettes using the arteriograph, pressurised to 85 mmHg and luminally perfused. All vessels used attained spontaneous contractile tone ($34.9 \pm 1.8\%$ of resting tone) and responded to luminal adenosine triphosphate (ATP) with dilatation ($24.1 \pm 4.0\%$), which showed functioning endothelium. Luminally added ergotamine or DHE induced maximal contractions of $16.8 \pm 8\%$ and $22.4 \pm 0.9\%$, respectively, compared to the resting diameter, with a pEC_{50} of 8.7 ± 0.1 for ergotamine and 9.0 ± 0.1 for DHE. Abluminal application of ergotamine and DHE also caused concentration-dependent contractions of the perfused MCA by $21.4 \pm 2.1\%$ and $23.1 \pm 7.0\%$, respectively, with pEC_{50} values of

7.6 ± 0.2 for ergotamine and 8.4 ± 0.5 for DHE. The responses were blocked by the 5-HT_{2A} receptor antagonist ketanserin (concentration 10^{-12} to 10^{-5} M) and partially with the 5-HT_{1B} receptor antagonist BRL-11557PM-B. The 5-HT_{1D} receptor antagonist SB-224289-A had no significant effect. Using a myograph technique, isolated ring segments of the MCA with intact endothelium were mounted on two metal wires. Neither agonist caused relaxation of resting vessels, however, they both responded by weak contractile responses ($26 \pm 3\%$ of submaximal contractile capacity relative to 60 mM potassium). The contractions were typically slow in on and off set (about 30–60 min). The long duration of ergots should be investigated further in an attempt to design drugs with less recurrence.

Keywords Ergotamine · Migraine · Middle cerebral artery · Rats · Endothelium · Vascular smooth muscle

Introduction

Ergotamine and dihydroergotamine (DHE) are agents that have a broad spectrum of effects at amine receptors [1], including at the 5-HT_{1B/1D} receptors, and have for

decades been used as acute anti-migraine drugs [2, 3]. They have the ability to constrict human cranial arteries both *in vitro* and *in vivo* [3]. The constriction of intracranial arteries (cerebral and middle meningeal arteries) is considered an important target of anti-migraine therapy [3], but inhibition of the release of calcitonin gene-related

peptide (CGRP) from sensory nerves is considered a potential therapeutic target [4]. The vasoactive effects of the ergotamines and triptans in intracranial arteries correlate to the presence of 5-HT_{1B/1D} receptors in smooth muscle cells in man [8]. The 5-HT_{2A} receptor is expressed in extracranial and peripheral vessels [5]. We have shown that in man it is mainly the human temporal artery that has 5-HT₂ receptors [6] and this vessel is constricted by ergotamine [3]. Endothelial as well as smooth muscle cells of human intracranial vessels have been demonstrated to possess 5-HT_{1B} receptors using specific 5-HT_{1B/1D} receptor antibodies as well as analysis of receptor mRNA [7–11].

For many years the standard method for *in vitro* pharmacological examination of vascular pharmacology has been to hook up a vessel segment on two prongs, one fixed and the other connected to a strain gauge for recording of vasomotion, however, all components of the vessel walls are in these studies exposed to the drugs under examination or removal of the endothelium will involve trauma to the vessel wall. An alternative method has been developed, namely pressurised arteriography [12–14]. Apart from being more similar to the *in vivo* environment of blood vessels, the method offers the possibility of compartmentalised study of vascular responses (luminal vs. abluminal application).

The aim of the present study is to examine in detail the vasomotor effects of ergotamine and DHE on isolated perfused middle cerebral artery (MCA) of rats, particularly rats have often been used in migraine models [15–18]. The questions we address are: where do ergotamine and DHE act, can they pass the endothelium, and do they have similar pharmacodynamics? Here we reveal that the smooth muscle cells of the rat MCA contain primarily 5-HT_{2A} receptors, and only a minor population of 5-HT_{1B} receptors. Ergotamine and DHE can have effects despite an intact endothelium.

Materials and methods

Tissue preparation

The Animal Protocol Review committee at the University of Lund approved the experimental protocol.

Male Sprague–Dawley rats (250–300 g) were anaesthetised with CO₂ and decapitated ($n=20$). The brain was immediately removed and placed in cold (4°C) buffer solution of the following composition (mM); NaCl 119, NaHCO₃ 15, KCl 4.6, MgCl₂ 1.2, NaH₂PO₄ 1.2, CaCl₂ 1.5 and glucose 5.5. With the aid of a dissecting microscope, MCA segments were carefully harvested beginning at the circle of Willis and extending 5–8 mm distally.

Pressurised arteriograph

A section of the MCA (1–2 mm in length) was mounted in a pressurised arteriograph (Living Systems, Burlington, VT) as previously described [12, 13]. Micropipettes were inserted into both ends of the MCA and secured with 11-0 nylon ties. The MCA was superfused with the described buffer solution (37°C) equilibrated with a gas mixture consisting of 5% CO₂/95% O₂, resulting in a pH of 7.4. Via the glass micropipettes the MCA segment was perfused at a transmural pressure that was maintained at 85 mmHg by raising reservoirs connected to the micropipettes to the appropriate height above the MCA. Luminal perfusion was adjusted to 100 μ L/min by setting the two reservoirs at different heights. These pressure/flow characteristics are considered to approximate the *in vivo* situation well. Pressure transducers on either side of the MCA provided direct measurement of perfusion pressure across the MCA. The vessel was magnified 600-fold using a microscope coupled to a digital camera (Axis, Lund, Sweden) connected to a PC. The program Mary (Nihil KB, Lund, Sweden) saved the pictures at intervals of one second during the experiment as well as measuring the diameter of the vessels.

Any MCA that did not develop spontaneous tone of at least 20% compared to the initial diameter within 1 h was excluded from the experiment (approximately 15% of the total no. of vessels mounted). The presence of a functional endothelium was tested by luminal administration of adenosine triphosphate (ATP) (10⁻⁵ M). A dilatation of at least 10% of the resting diameter was considered indicative of a functional endothelium. Experimental protocols were not initiated until the MCA diameter was stable over a 15-min period. To test the vascular response to stimulation of endothelial 5-HT receptors, ergotamine and DHE were added to the luminal perfusate in the concentration range 10⁻¹² to 10⁻⁶ M. In separate experiments the agonists were added abluminally in the same concentration range to study the effect of administration route and avoiding interaction with the endothelium.

To characterise the responses to luminal or abluminal stimulation, ergotamine or DHE were used either alone or in the presence of antagonists for the 5-HT_{1B} (BRL-1557PM-B), 5-HT_{1D} (SB-224289) or 5-HT_{2A} receptor blockade with ketanserin. Inhibitors were added both to the abluminal bath or the luminal perfusate 20 min before commencing application of ergotamine

Table 1 The maximum relaxant responses and the pEC₅₀ values of ergotamine and dihydroergotamine in the perfused rat MCA

	MAX	pEC ₅₀
Abluminal		
Erg	21.4±2.1	7.6±0.2
DHE	23.1±7.0	8.4±0.5
Luminal		
Erg	16.8±0.8	8.7±0.1
DHE	22.4±0.9	9.0±0.1

or DHE, and were present during the experiments. Sumatriptan (10^{-10} M to 10^{-4} M) was added luminally and abluminally.

Tissue bath experiments

Using the same buffer as described above, MCA segments (1 mm in length) were mounted on two metal wires (Myograph, DMT, Aarhus, Denmark), one of which was connected to a force displacement device. The experiments were continuously recorded using PowerLab (ADInstruments, Oxford, UK) and the software program Chart (ADInstruments, Oxford, UK), as previously described. The segments were tensioned to 2 mN and allowed to rest at this tone for 1 h before commencing the experiments [19]. To test for viability the segments were exposed to a 60 mM K^+ buffer solution obtained by partial exchange of Na^+ with K^+ in the aforementioned buffer. The tension obtained hereby was also used as reference for tissue contractile capacity. Presence of a functional endothelium was tested through precontraction with U46619 (a thromboxane A2 receptor agonist) and subsequent relaxation with acetylcholine 10^{-5} M. A dilatation of over 70% of the precontracted value was considered indicative of a functional endothelium. The contractile response to ergotamine or DHE was studied through cumulative application in semilogarithmic steps, ending at 10^{-6} M. In additional experiments vessel rings from rat MCA were suspended and concentrations of 10^{-6} and 10^{-5} M were studied. When maximum effect was reached the bath was flushed repeatedly until baseline tension was reached and the time to maximum and time needed for washing out the effect was noted.

Statistical analysis

Data are expressed as mean values \pm SEM. For experiments performed on the pressurised arteriograph, changes in measured diameters of the vessel segments are expressed as a percentage of the resting diameter. For vessel bath experiments, contraction is expressed as a percentage of the contraction obtained exposing the vessel to 60 mM K^+ , and dilatation as a percentage of the tension obtained through precontraction. n refers to the number of blood vessels tested in each experiment. E_{max} denotes the maximum response elicited by an agonist whereas pEC_{50} denotes the negative logarithm of the concentration needed to elicit half the maximum response. Sigmoidal curve fitting was done using the computer program GraphPad Prism (GraphPad Software, San Diego, CA, USA). Based on the principal equation for a sigmoidal curve, the program makes iterated computations to derive a best fit based upon the actual experimental values. All concentrations expressed indicate the final concentration in the luminal or abluminal compartments of the pressurised arteriograph as well as in the vessel baths. Statistical analysis was performed using Student's t -test considering p values below 0.05 statistically significant.

Drugs used

ATP, acetylcholine, ergotamine, DHE, sumatriptan, ketanserin and U466169 were obtained from Sigma (USA). BRL-11557PM-B and SB-224289-A were generously provided by Smithkline Beecham Pharmaceuticals, UK.

Stock solutions of the drugs were made following manufacturers' instruction and stored frozen in small aliquots until use. For experiments, drugs were diluted in the described buffer solution immediately before use. All chemicals were obtained from Merck, Germany. Only double distilled water was used throughout the experiments.

Results

Pressurised arteriograph

The mean baseline diameter of the blood vessels examined was 187.3 ± 4.6 μ m after initial pressurisation and 121.4 ± 3.6 μ m after development of spontaneous tone ($n=20$, $p<0.001$). The spontaneous myogenic tone (i.e. contraction) developed was $34.9 \pm 1.8\%$ of the initial vessel diameter. ATP (10^{-5} M) applied luminally produced relaxation of the myogenic tone by $24.1 \pm 4.0\%$ ($p<0.001$). Of the agonists applied luminally, only sumatriptan ($n=7$, E_{max} $10 \pm 2\%$) induced relaxation of the MCA, with a pEC_{50} of 8.1 ± 0.5 ($n=7$). Thus, ergotamine and DHE did not produce any significant relaxant effect upon luminal application. The functional response to sumatriptan was independent of administration route. Thus, abluminal application also gave rise to relaxation with an E_{max} of $11 \pm 3\%$ and a pEC_{50} of 8.9 ± 0.5 . This was not statistically different from the values obtained applying the drug luminally ($p>0.05$). In separate tests this relaxant response has been characterised and shown to occur via an endothelial mechanism that involves 5-HT_{1B} receptors and endothelial relaxing factors [20].

Luminal and abluminal application of ergotamine or DHE caused concentration-dependent *contractions* of the perfused MCA (Fig. 1). The responses to *abluminal* application of ergotamine were $21.4 \pm 2.1\%$ while the luminal application resulted in a response of $16.8 \pm 0.8\%$, and the effects had pEC_{50} values of 7.6 ± 0.2 and 8.7 ± 0.1 , respectively (Fig. 1). The responses to DHE were similar, $23.1 \pm 7.0\%$ (abluminal) vs. $22.4 \pm 0.9\%$ (luminal) and a similar pEC_{50} of 8.4 ± 0.5 (abluminal) and 9.0 ± 0.1 (Fig. 1). The contractions elicited by ergotamine were mediated through the 5-HT_{2A} receptor as shown by the effect of ketanserin, which significantly ($p<0.05$) reduced the E_{max} from $21.4 \pm 2.1\%$ ($n=5$) to $4.7 \pm 0.2\%$ ($n=4$) (Fig. 2). For DHE the results were similar ($n=3$; data not shown). Pre-

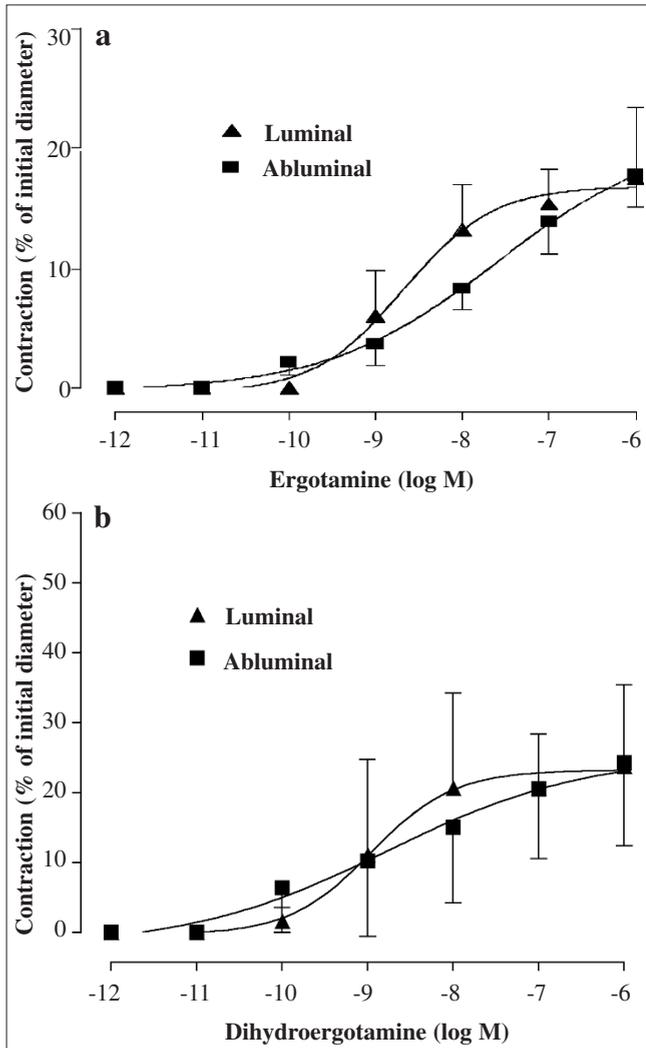


Fig. 1 Concentration–contraction curves for luminally and abluminally administered (a) ergotamine ($n=7$) and (b) dihydroergotamine ($n=6$) on rat MCA. Values are given as mean \pm SEM

treating the vessel segments with the 5-HT_{1B} antagonist BRL-119557PM-B ($n=5$) in part blocked the contractile responses (Fig. 3). The 5-HT_{1D} antagonist SB-224289-A had no effect (data not shown).

Tissue bath studies

The absolute contractile response of the MCA segments to 60 mM K⁺ was 3.0 \pm 0.2 mN. Contractile studies of ring segments of rat MCA using single-dose application of ergotamine or DHE (10⁻⁶ M) gave rise to contractile responses of up to 66 \pm 13% vs. 13 \pm 5%, respectively, as compared to the K⁺ response (set as 100%). The maximum effect of ergotamine was slowly developing and reached maximum at about 20 min and had returned to

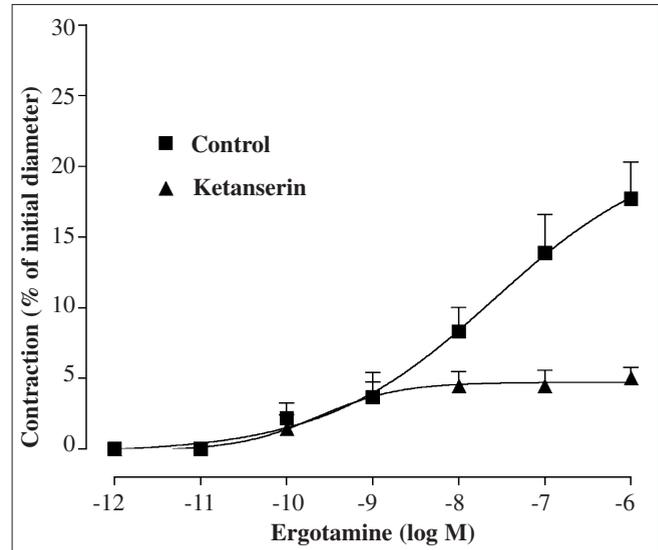


Fig. 2 The effect of the selective 5-HT_{2A} receptor antagonist ketanserin on the contractile response of rat MCA to abluminally administered ergotamine ($n=4$). Values are given as mean \pm SEM

baseline after 75 min after repeated washing. The effects of DHE were slightly quicker but qualitatively similar. The time to maximum contractile was 25–32 min with a return to baseline 13–48 min after initiating the washing. This illustrates the long on and off effects (Fig. 4).

Discussion

The present findings on the perfused MCA of the rat demonstrate for the first time the direct contractile effects of ergotamine while triptans act via an endothelial mechanism. The intraluminal or abluminal administration of ergotamine and DHE consistently produce contraction without any difference in magnitude abluminally and luminally. The relaxation elicited by sumatriptan was mediated through 5-HT_{1B} receptors on the endothelial cells (shown by the inhibitory action of GR 55562) and this involves the release of nitric oxide (dilatation was inhibited by L-NOARG) [20]. The observation agrees with immunocytochemical studies, which have shown that there are endothelial 5-HT_{1B} receptors both in rat [21] and in man [8]. It was notable that it is only in man that there is a rich supply of 5-HT_{1B} receptors in the medial layer [8]. The contractile effects of ergotamine and DHE were mainly mediated via 5-HT_{2A} receptors as the specific 5-HT_{2A} receptor antagonist ketanserin acted as a powerful antagonist. The 5-HT_{1B} blocker BRL-15572PM-B in part abolished the contractions while the 5-HT_{1D} antagonist SB-224289-A had no effect. This agrees well with a phar-

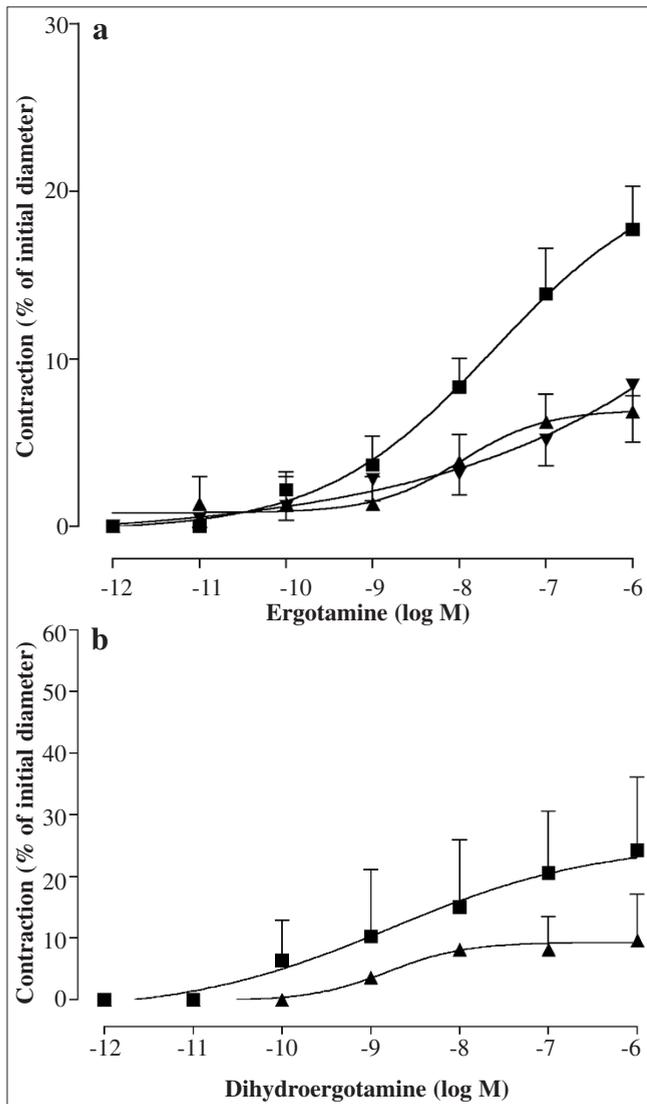


Fig. 3 The effect of the selective 5-HT_{1B} antagonist BRL-11557PM-B on contractile response of rat MCA to ablutally administered (a) ergotamine ($n=5$) and (b) DHE ($n=5$). Values are given as mean \pm SEM

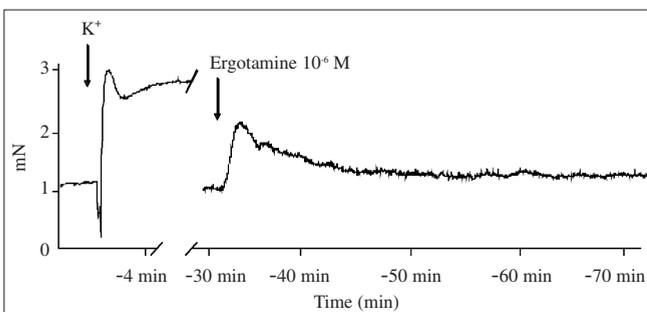


Fig. 4 Example of contractile response of rat MCA to first the administration of a high dose of potassium, followed by wash-out and the administration of ergotamine which resulted in a fairly strong contraction which levelled out at about 50% of maximum and persisted for the duration of study

macological study of the rat basilar artery showing powerful blockade by ketanserin of 5-HT-induced contractions [22]. A 5-HT₁ agonist 5-carboxytryptamine had, however, a weak contractive effect which could be blocked by a 5-HT_{1B/1D} antagonist, suggestive of a minor population of 5-HT_{1B} receptors in the rat basilar artery [22]. The effects of ergotamine on the isolated aorta have been studied extensively [23]. Ergotamine constricted the isolated rat aorta by acting on the 5-HT_{2A} receptor while DHE did not contract the rat aorta but acted as an insurmountable 5-HT_{2A} receptor antagonist [23]. In the present study both ergotamine and DHE acted as constrictors of the rat MCA primarily via 5-HT_{2A} receptors. The constrictive effect of ergotamine on human basilar arteries has also been found to be mediated by 5-HT receptors [24]. The vasoconstriction induced by ergotamine and DHE in the external carotid bed in dogs is mediated by 5-HT_{1B} receptors and $\alpha_{2A/2C}$ -adrenoceptors [25–27]. In the present study the vasoconstrictor effect was mediated by 5-HT_{2A} receptors. This illustrates that a vasoconstrictor may act on different receptors depending on what part of the vascular tree and species that is examined.

Ergotamine and DHE caused constriction of isolated bovine middle cerebral arteries with a pEC₅₀ of 8.0 [28]. Similarly, the pEC₅₀ of ergotamine and DHE for the rat MCA were 7.6–8.7 and 8.4–9.0, respectively, depending on route of administration. Ergotamine and DHE are thus potent vasoconstrictors that can selectively constrict the cranial bed [3], a possible reason for their efficacy in migraine. The two ergot alkaloids caused equally strong vasoconstriction when administered luminally and ablutally, indicating that they can cross the blood–brain barrier [29], however, the pEC₅₀ was slightly lower for the ablutal administration.

Whereas the effect of ergotamine on blood flow is short-lasting [30], the present study has demonstrated a slow onset and a longer-lasting effect on isolated MCA, lasting up to 45 min despite repeated flushing of the tissue bath. Similarly, the constrictor effect of ergotamine could not be washed out in human coronary arteries [31]. This is in agreement with the long duration of constrictor effect of ergotamine that can be seen both in man and in experimental studies. Thus, a single parenteral dose of ergotamine caused a gradually developing constriction of leg arteries lasting 30 h [30, 32]. In isolated veins the constrictor effect of DHE is slowly developing and persists despite repeated washings of the *in vitro* bath [33]. The slow onset and long duration of action of ergotamine is compatible with a slow dissociation from the receptor [30]. In contrast the effect of the new anti-migraine drugs, the triptans, occurs immediately and is quickly washed out. In rat sumatriptan dilates the MCA and basilar arteries [20, 22], but induces constriction in man [8] due to the

distribution difference in 5-HT receptor subtypes. The anti-migraine effects of sumatriptan, ergotamine and DHE have been compared in 5 randomised clinical trials; in each case the triptan has a quicker onset of action than the ergot alkaloids but at the same time a higher risk of relapse of migraine headache [3]. Clinically speaking, the triptans thus have one advantage over ergot alkaloids, quicker onset of effect (a feature highly valued by patients), but at the same time the high frequency of recurrence is a major problem often leading to repeated administrations of these drugs.

The ideal drug for treating a migraine attack would thus combine two features: the quick onset of a triptan and the long duration of effect of an ergot alkaloid. To design such a drug basic insight into the reason for the long duration of effect of the ergot alkaloids is needed. For the moment it is not possible to single this out from the disadvantage, the slow onset of effect. The effect of ergotamine and DHE differs markedly to the data we obtained with triptans; they consistently induced relaxation via an endothelial mechanism [20]. Obviously the dilatation of rat MCA is dependent on the presence of an intact

endothelium as evidenced by the dependency on NO. As sumatriptan is a specific agonist at the 5-HT_{1B/1D} receptors and an immunohistological study on human cerebral arteries has shown 5-HT_{1B/1D} receptors on the endothelium [8], it is therefore reasonable to assume that endothelial 5-HT_{1B/1D} receptors are present in the rat endothelial cells. In fact we have recently verified this using selective 5-HT_{1B} antibodies and confocal microscopy [21]. It is important to note that, as rat intracranial arteries have a minor population of contractile 5-HT_{1B} receptors in their medial smooth muscle cells, it is not a good species to examine the direct vasomotor effects of triptans. In conclusion, the present study, using the pressurised arteriograph, shows that ergotamine and DHE are potent constrictors of the rat MCA by acting at the 5-HT_{2A} receptor whereas sumatriptan acts as a vasodilator via a 5-HT_{1B} receptor. The effect of ergotamine is of long duration probably due to slow dissociation from the receptor.

Acknowledgements This work was supported by the Swedish Research Council (grant no. 5958), the Heart and Lung Foundation, the Danish Research Council and the Lundbeck Foundation.

References

- Müller-Schweinitzer E, Weidmann H (1978) Basic pharmacological properties. In: Berde B, Schild HO (eds) *Ergot alkaloids and related compounds*. Springer Verlag, Berlin, Heidelberg, New York, pp 87–232
- Olesen J, Tfelt-Hansen P, Welch KMA et al (eds) (2005) *The Headaches*, 3rd edn. Lippincott Williams & Wilkins, Philadelphia
- Tfelt-Hansen P, Saxena PR (2005) Ergot alkaloids in the acute treatment of migraine. In: Olesen J, Tfelt-Hansen P, Welch KMA (eds) *The Headaches*, 3rd edn. Lippincott Williams & Wilkins, Philadelphia, pp 459–467
- Edvinsson L (2004) Blockade of CGRP receptors in the intracranial vasculature: a new target in the treatment of headache. *Cephalalgia* 24:611–622
- Hoyer D, Clarke DE, Fozard JR et al (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev* 46:157–203
- Skärby T, Tfelt Hansen P, Gjerris F et al (1982) Characterization of the 5 hydroxytryptamine receptor in human temporal arteries; comparison between migraine sufferers and non sufferers. *Ann Neurol* 12:272–277
- Longmore J, Shaw D, Smith D et al (1997) Differential distribution of 5HT_{1D}- and 5HT_{1B}-immunoreactivity within the human trigemino-cerebrovascular system: implications for the discovery of new antimigraine drugs. *Cephalalgia* 17:833–842
- Nilsson T, Longmore J, Shaw D et al (1999) Contractile 5-HT_{1B} receptors in human cerebral arteries: pharmacological characterization and localization with immunocytochemistry. *Br J Pharmacol* 128:1133–1140
- Riad M, Tong X, el Mestikawy S et al (1998) Endothelial expression of the 5-hydroxytryptamine 1B antimigraine drug receptor in rat and human brain microvessels. *Neuroscience* 86:1031–1035
- Cohen Z, Bonvento G, Lacombe P, Hamel E (1996) Serotonin in the regulation of brain microcirculation. *Prog Neurobiol* 50:335–362
- Hamel E, Fan E, Linville D et al (1993) Expression of mRNA for the serotonin 5-hydroxytryptamine 1D beta receptor subtype in human and bovine cerebral arteries. *Mol Pharmacol* 44:242–246
- You J, Johnson TD, Childres WF, Bryan RM Jr (1997) Endothelial-mediated dilations of rat middle cerebral arteries by ATP and ADP. *Am J Physiol* 273:H1472–H1477
- Bryan RM Jr, Eichler MY, Swafford MW et al (1996) Stimulation of alpha 2 adrenoceptors dilates the rat middle cerebral artery. *Anesthesiology* 85:82–90
- Szok D, Hansen-Schwartz J, Edvinsson L (2001) In depth pharmacological characterization of endothelin B receptors in the rat middle cerebral artery. *Neurosci Lett* 314:69–72
- Akerman S, Williamson DJ, Kaube H, Goadsby PJ (2002) The effect of antimigraine compounds on nitric oxide-induced dilation of dural meningeal vessels. *Eur J Pharmacol* 452:223–228

16. Kowacs F, Williamson DJ, Goadsby PJ (2004) Neurogenic vasodilation of dural blood vessels is not mediated by cholinergic transmission in the anaesthetized rat. *Eur J Pharmacol* 493:133–137
17. Petersen KA, Dyrby L, Williamson D et al (2005) Effect of hypotension and carbon dioxide changes in an improved genuine closed cranial window rat model. *Cephalalgia* 25:23–29
18. Williamson DJ, Hargreaves RJ, Hill RG, Shephard SL (1997) Intravital microscope studies on the effects of neurokinin agonists and calcitonin gene-related peptide on dural vessel diameter in the anaesthetized rat. *Cephalalgia* 17:518–524
19. Högestätt ED, Andersson KE, Edvinsson L (1983) Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. *Acta Physiol Scand* 117:49–61
20. Hansen-Schwartz J, Lovland N, Nilsson E et al (2003) Endothelium-dependent relaxant responses to selective 5-HT_{1B/1D} receptor agonists in the isolated middle cerebral artery of the rat. *J Vasc Res* 40:561–566
21. Vikman P, Beg S, Khurana T et al (2006) Gene expression and molecular changes in cerebral arteries following subarachnoid haemorrhage in rat. *J Neurosurg* 105:438–444
22. Hoel NL, Hansen-Schwartz J, Edvinsson L (2001) Selective up-regulation of 5-HT_{1B/1D} receptors during organ culture of cerebral arteries. *Neuroreport* 12:1605–1608
23. Kalkman HO, Schneider F (1996) Effects of ergotamine and dihydroergotamine on 5-hydroxytryptamine-2A receptors in the isolated rat aorta. *Pharmacology* 53:351–355
24. Müller-Schweinitzer E (1983) Vascular effects of ergot alkaloids: a study on human basilar arteries. *Gen Pharmacol* 14:95–102
25. Villalón CM, De Vries P, Rabelo G et al (1999) Canine external carotid vasoconstriction to methysergide, ergotamine and dihydroergotamine: role of 5-HT_{1B/1D} receptors and alpha₂-adrenoceptors. *Br J Pharmacol* 126:585–594
26. Valdivia LF, Centurion D, Arulmani U et al (2004) 5-HT_{1B} receptors, alpha_{2A/2C}- and, to a lesser extent, alpha₁-adrenoceptors mediate the external carotid vasoconstriction of ergotamine in the vasosympathectomized dogs. *Naunyn Schmiedeberg Arch Pharmacol* 370:46–53
27. Villalón CM, Centurion D, Willems EW et al (2004) 5-HT_{1B} receptors and alpha_{2A/2C}-adrenoceptors mediate external carotid vasoconstriction to dihydroergotamine. *Eur J Pharmacol* 484:287–290
28. Roon KI, Maassen Van den Brink A, Ferrari MD, Saxena PR (1999) Bovine isolated middle cerebral artery contractions to antimigraine drugs. *Naunyn Schmiedeberg Arch Pharmacol* 360:591–596
29. Goadsby PJ, Gundlach AL (1991) Localization of 3H-dihydroergotamine-binding sites in the cat central nervous system: relevance to migraine. *Ann Neurol* 29:91–94
30. Tfelt-Hansen P (1986) The effect of ergotamine on the arterial system in man. *Acta Pharmacol Toxicol* 59[Suppl 3]:1–30
31. Maassen VanDenBrink A, Reekers M, Bax W et al (1998) Coronary side-effect potential of current and prospective antimigraine drugs. *Circulation* 98:25–30
32. Tfelt-Hansen P, Paalzow L (1985) Intramuscular ergotamine: plasma levels and dynamic activity. *Clin Pharmacol Ther* 37:29–35
33. Müller-Schweinitzer E (1980) In vitro studies on the duration of action of dihydroergotamine. *Int J Clin Pharmacol Ther Toxicol* 18:88–91