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European Journal of Pharmacology 382 (1999) 103–109

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Effects of alniditan on neurogenic oedema in the rat dura mater and on contraction of rat basilar artery

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Received 22 April 1999; received in revised form 13 August 1999; accepted 17 August 1999

Abstract

The non-indole 5-HT receptor agonist, alniditan (R 91274), was tested and compared to sumatriptan in an in vivo model of neurogenic inflammation within the meninges of rats and in rat basilar artery in a Mulvany–Halpern chamber in vitro. Alniditan dose dependently attenuated the neurogenic inflammation and was more potent than sumatriptan. The alniditan response was blocked by the 5-$HT_{1B/D}$ receptor antagonist, GR 127935 (2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide), but not by ketanserin, indicating that the effect is mediated through 5-$HT_{1B/D}$ receptors. Alniditan did not attenuate substance P-induced inflammation, suggesting that the mediating receptors are located prejunctionally. In vitro alniditan exhibited less vasoconstrictive effects on the rat basilar artery than did sumatriptan, although at a very high concentration (1 mM), alniditan caused intensive constriction, most likely through a mechanism independent from 5-HT receptor activation.

Keywords: Alniditan; 5-$HT_{1B/D}$ receptor agonist; Migraine; Inflammation neurogenic; Artery basilar

1. Introduction

Migraine is a form of headache which affects up to 15% of the general population (Rasmussen et al., 1991). While the detailed pathophysiology of migraine remains to be elucidated, a disturbance of cerebral vasomotor tone (Heyck, 1969) and an inflammatory process involving neurovascular regulation, possibly due to altered neuropeptide release (Moskowitz, 1992; Goadsby and Edvinsson, 1993), are being discussed as contributors for the manifestation of the disease. The investigation of migraine pathophysiology is hampered, however, by the lack of suitable animal models. Based on the above theories, isolated cerebral and other blood vessels as well as carotid arteriovenous anastomoses are being used to investigate antimigraine drug effects on vasomotor tone (Humphrey et al., 1988; Den Boer et al., 1991). On the other hand, consequences of neuropeptide release are frequently studied as nerve stimulation-mediated plasma extravasation in the meningeal arteries (Buzzi et al., 1991; Shepheard et al., 1995; Petty et al., 1997).

Agonists at 5-$HT_{1B/D}$ receptors, such as sumatriptan, generally produce effective contraction of isolated cerebral blood vessels (Connor et al., 1989) as well as of large cranial blood vessels in humans during migraine attacks (Friberg et al., 1991; Limmroth et al., 1996b). Moreover, this group of drugs causes inhibition of trigeminal ganglion stimulation-induced plasma extravasation (Buzzi and Moskowitz, 1990). Sumatriptan and several of its congeners have recently been introduced clinically for the treatment of migraine attacks, and are among the most effective treatment forms for this disease state (Diener et al., 1997). Side-effects such as coronary artery constriction, sedation, high rate of headache recurrence as well as poor pharmacokinetic properties such as short half-life and low oral bioavailability have stimulated the development of new 5-$HT_{1B/D}$ receptor agonists. Alniditan is the first non-indole, non-ergolide benzopyran derivative within the group of 5-$HT_{1B/D}$ receptor agonists (Van Lommen et al.,

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00602-0

Table 1
Binding profile of alniditan and sumatriptan on 5-HT_1 receptors: inhibition of radioligand binding expressed as pIC_{50} (adapted from Leysen et al., 1996)

Receptor	Tissue/cell-type (ligand)	Alniditan, $-\log M$	Sumatriptan, $-\log M$
5-HT_{1A}	Human HeLa cells ([^{3}H]8-OH-DPAT)	8.35	6.3
5-HT_{1B}	Rat striatum ([^{3}H]5-HT)	6.92	7.18
5-HT_{1D}	Calf substantia nigra ([^{3}H]5-HT)	8.69	7.48
5-$HT_{1D\alpha}$	Human C6 glioma cells ([^{3}H]5-HT)	9.03	8.05
5-$HT_{1D\beta}$	Human L929sA cells ([^{3}H]5-HT)	8.65	7.51
5-HT_{1E}	Human L929sA cells ([^{3}H]5-HT)	6.2	5.4
5-HT_{1F}	Human COS-7 cells ([^{3}H]5-HT)	6.2	7.7

1995) and might have an improved profile of side-effects since only slight effects on coronary, mesenteric and renal arterial blood flow have been observed (Van der Water et al., 1996). A pharmacological comparison of alniditan and sumatriptan is given in Table 1. In the present study, we investigated the ability of alniditan to inhibit nerve stimulation-induced plasma extravasation in rat dura mater in situ and to cause vasoconstriction in the isolated rat basilar artery in vitro. The prototypical compound, sumatriptan, was studied as a reference drug in both assays.

2. Methods

2.1. Studies on plasma extravasation in the rat dura mater

The experiments were performed as previously described (Limmroth et al., 1996a) following approval by the state animal welfare board at the Regierungspräsident Düsseldorf. Male Sprague–Dawley rats (200–250 g) were obtained from Charles River (Sulzfeld, Germany) and anaesthetized with phenobarbital (60 mg kg^{-1}). In some experiments, the rats received the indicated antagonist by intraperitoneal injection 45 min prior to stimulation. The 5-HT receptor agonists were injected intraperitoneally 30 min prior to stimulation. A bolus of [^{125}I]bovine serum albumin (50 μCi kg^{-1}) was injected in the left femoral vein 5 min prior to stimulation. Stimulation was performed either electrically, or chemically by systemic administration of substance P.

For electrical stimulation the rats were placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA) with the incisor bar at −2.5 mm. Skulls were exposed by a midline incision and symmetrical burr holes were drilled 3.2 mm lateral and 3.7 mm posterior from the bregma. Stainless steel bipolar electrodes (5-mm shaft; Rhodes Medical Instruments, Woodland Hills, CA, USA) were lowered into the trigeminal ganglia to a depth of 9.2 mm from the dura mater. Thereafter, the right trigeminal ganglion was stimulated for 5 min (0.6 mA, 5 ms duration, 5 Hz; Pulsemaster A300 and Stimulus Isolator A365, Word Precision Instruments, San Carlos, CA, USA). After the stimulation period, the animals were promptly perfused with 0.9% saline via the left cardiac ventricle for 2 min at a constant pressure of 100 mm Hg in order to remove intravasal [^{125}I]bovine serum albumin. The skull was then opened, the brain was removed and the cranial cavity was thoroughly rinsed with saline. The dura mater was dissected bilaterally. Radioactivity (cpm mg^{-1} wet weight) was compared between the stimulated and the unstimulated sides. The data are expressed as the ratio between the two sides (Limmroth et al., 1996a).

For chemical stimulation, substance P (1 nmol kg^{-1}) was infused intravenously. Ten minutes after substance P, the animals were perfused via the left cardiac ventricle with 0.9% saline. The dura mater on both sides was dissected, weighed and counted as described above, but the data were expressed as % of extravasation in drug-treated relative to vehicle-treated animals.

2.2. Studies on the basilar artery

Basilar arteries were carefully prepared under a stereoscopic microscope from the cranial vasculature of male Sprague–Dawley rats (250–300 g), which were obtained from the breeding facility at the University of Essen. The vessels were stored at 4°C for up to 24 h in Krebs–Henseleit buffer (see below). Artery segments of 2–3 mm length were then mounted in a Mulvany–Halpern myograph, and experiments were performed as previously described for mesenteric and intrarenal microvessels (Chen et al., 1996, 1997). Briefly, the vessels were mounted on 40 μm diameter stainless-steel wires in the myograph chamber for isometric recording of tension development. They were bathed in Krebs–Henseleit buffer of the following composition (mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $CaCl_2$ 2.5, ethylenediaminetetraacetic acid 0.026, glucose 11. The buffer temperature was maintained at 37°C, and the chamber was gassed continuously with 5% CO_2/95% O_2 to maintain the pH at 7.4. Following an equilibration period of 40 min the vessels were allowed to equilibrate for another 30 min. Thereafter, three cumulative concentration–response curves were obtained with extensive washing and 30 min resting in between. The first and the third curve were always obtained with 5-HT, while the second curve was generated with alniditan

or sumatriptan. Preparations were accepted only when the first and third curve yielded similar 5-HT effects. The force of contraction generated by alniditan and sumatriptan was expressed as % of the maximal force generated by 5-HT.

2.3. Data analysis

Data are given as means ± S.E.M. Statistical significance of differences was assessed by two-tailed, paired *t*-tests when two groups were compared. When more than two groups were compared, analysis of variance followed by a Bonferroni-post-hoc test was performed. ED_{50} values were determined by non-linear regression analysis using the Prism program (GraphPAD Software, San Diego, CA, USA). Values of $P < 0.05$ were considered significant.

2.4. Compounds

Alniditan, sumatriptan, GR 127935 and ketanserin were synthesized in house by the Janssen Research Foundation (Beerse, Belgium). Acetylsalicylic acid was kindly provided by Bayer (Leverkusen, Germany). [^{125}I]bovine serum albumin, substance P and 5-HT were obtained from New England Nuclear (Boston, MA, USA), and Sigmas (Deisenhofen, Germany), respectively.

3. Results

3.1. Studies on plasma extravasation in the rat dura mater

Trigeminal stimulation enhanced [^{125}I]bovine serum albumin extravasation (ratio: 1.72 ± 0.13), and this was reduced by alniditan in a dose-dependent manner (Fig. 1). The threshold dose for statistically significant reduction, estimated ED_{50} and maximally effective dose were 10 μg kg^{-1} (ratio: 1.24 ± 0.07), 9 μg kg^{-1} and 100 μg kg^{-1} (ratio: 1.06 ± 0.09), respectively. Similar effects were observed with sumatriptan (Fig. 1), and the corresponding values for estimated ED_{50} and maximally effective dose were 70 μg kg^{-1}, and 300 μg kg^{-1} (ratio: 1.06 ± 0.16), respectively. Thus, based on the ED_{50} values, alniditan was significantly more potent than sumatriptan under these experimental conditions ($P < 0.01$). The selective 5-$HT_{1B/D}$ receptor antagonist, GR 127935 (10 μg kg^{-1}, i.p.), shifted the dose–response curve for alniditan to the right by a factor of 20 (ED_{50} 190 μg kg^{-1}, $P < 0.01$ vs. data in the absence of GR 127935; Fig. 2). In contrast, a 10-fold higher dose of ketanserin (100 μg kg^{-1}, i.p.) did not attenuate the inhibitory effect of 100 μg kg^{-1} alniditan (ratio: 1.02 ± 0.08; Fig. 2). Neither antagonist alone inhibited extravasation (ratio GR 127935: 1.64 ± 0.06; ratio ketanserin: 1.61 ± 0.09, for each $n = 4$).

Infusion of substance P increased [^{125}I]bovine serum albumin extravasation to 160 ± 12% of the control values (Fig. 3). This stimulation by substance P was not significantly affected by 100 μg kg^{-1} alniditan (142 ± 6% of control, Fig. 3) but was abolished by 30 mg kg^{-1} acetylsalicylic acid (111 ± 5% of control, Fig. 3).

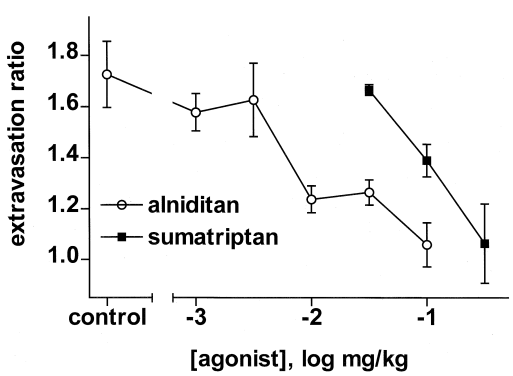

Fig 1. Dose–response curve for alniditan and sumatriptan on the inhibition of nerve stimulation-induced extravasation. The extravasation ratio is expressed as the ratio of the stimulated and the unstimulated side ($n = 4$–6 in each group). The intraperitoneal administration of alniditan ($ED_{50} = 9$ μg kg^{-1}) and sumatriptan ($ED_{50} = 70$ μg kg^{-1}) dose dependly reduced [^{125}I]-BSA extravasation in the rat meninges when done 30 min before stimulation.

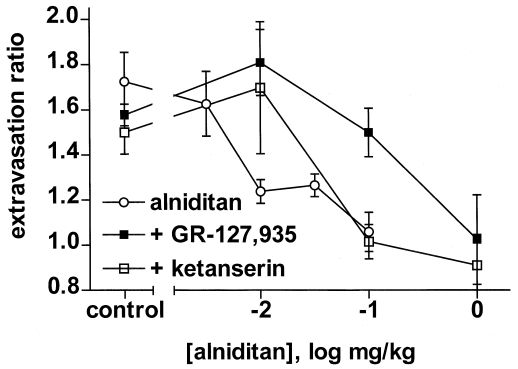

Fig 2. Dose–response curve for alniditan ($n = 4$–5 at each dose) in the absence and presence of GR 127935 (10 μg kg^{-1}) or ketanserin (100 μg kg^{-1}). The estimated ED_{50} values for alniditan were 9 μg kg^{-1} in the absence and 190 μg kg^{-1} in the presence of GR 127935.

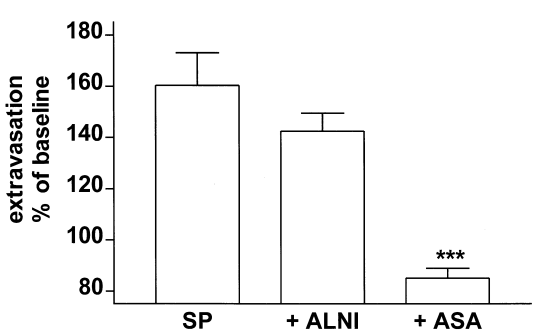

Fig 3. The effect of substance P (SP), SP + alniditan (ALNI; 100 μg kg^{-1}) and SP + acetylsalicylic acid (ASA; 30 mg kg^{-1}) on extravasation ($n = 4$–6 in each group). Data are expressed as percents of extravasation in vehicle-treated animals (''baseline'': 104 ± 7 cpm/mg wet weight). ***: $P < 0.001$ compared to SP.

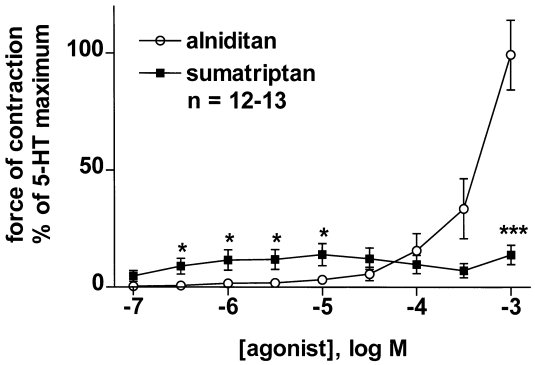

Fig 4. Concentration–response curve for alniditan and sumatriptan on the rat basilar artery in the Mulvany–Halpern chamber. The results are expressed as percents of maximum contraction force achieved with 5-HT alone. * and ***: $P < 0.05$ and < 0.001 vs. sumatriptan in a paired, two-tailed *t*-test.

3.2. Studies on the basilar artery

5-HT caused a concentration-dependent contraction of the rat basilar artery; the maximal effects of 5-HT were similar in the vessels used for the subsequent alniditan and sumatriptan evaluations (6.2 ± 1.0 vs. 6.4 ± 0.6 mN; $n = 13$ each). Alniditan caused only very weak contractions in concentrations of up to 10 μM ($3 \pm 2\%$ of maximum 5-HT values) but at very high concentrations (1 mM) was as efficacious as 5-HT (Fig. 4). In contrast, sumatriptan produced moderate contraction of rat basilar artery ($14 \pm 5\%$ of maximum 5-HT values); the contractions were already maximal at 1 μM sumatriptan and concentrations of up to 1 mM did not cause any additional contraction (Fig. 4). Accordingly, the contractile effects of sumatriptan were significantly greater than those of alniditan at concentrations of 0.3 to 10 μM but significantly smaller than those of alniditan at 1 mM (Fig. 4).

4. Discussion

4.1. The effect of alniditan

Previous studies on a vascular site of anti-migraine drug action have mainly relied on work with isolated blood vessels from rabbits (Cushing et al., 1994; Martin et al., 1997), dogs (Feniuk et al., 1985; Humphrey et al., 1988), primates (Connor et al., 1989), humans (Parsons et al., 1989) and on ateriovenous anastomoses of pigs (Willems et al., 1998). The pharmacological characterization of the 5-HT-induced contraction of these blood vessels has indicated that it occurs predominantly via 5-HT_{1B} receptors (Verheggen et al., 1998). This is supported by the finding that mRNA for 5-HT_{1B} receptors is found in these blood vessels (Hamel et al., 1993; Bouchelet et al., 1996). On the other hand, previous studies have demonstrated inhibition of plasma extravasation by 5-HT receptor agonists in rats, guinea pigs and mice (Markowitz et al., 1987; Buzzi and Moskowitz, 1990; Beattie and Connor, 1995; Yu et al., 1996). The present study investigated the effects of alniditan as an inhibitor of nerve stimulation-induced [^{125}I]bovine serum albumin extravasation and vasoconstricting agent in rats. While 5-$HT_{1B/D}$ receptor agonists such as sumatriptan are less potent in rats (present study) than in guinea pigs (Lee and Moskowitz, 1993; Beattie and Connor, 1995; Gupta et al., 1995) in extravasation assays but not on the isolated vessels, our approach allows the comparison of vascular and antiinflammatory properties within the same species. Moreover, the use of rats enables a comparison with anti-migraine drugs presumably acting independently of 5-HT receptors, since these drugs have mostly been studied in rats (Shepheard et al., 1995; Limmroth et al., 1996a).

Alniditan has a similarly high affinity for 5-HT_{1B} and 5-HT_{1D} receptors (0.9 and 1.2 nM, respectively) in radioligand binding studies (Lesage et al., 1998). Alniditan, however, differs from other sumatriptan congeners (Waeber and Moskowitz, 1995) in that it has very little affinity for 5-HT_{1F} receptors (Leysen et al., 1996). In studies with cloned human 5-HT_{1B} receptors heterologously expressed at high densities, alnditan and sumatriptan are full agonists for the inhibition of forskolin-stimulated cAMP accumulation (Lesage et al., 1998). In the present study, alniditan and sumatriptan fully suppressed nerve stimulation-induced [^{125}I]bovine serum albumin extravasation. Alniditan was approximately eight times as potent in this respect as was sumatriptan, a finding which is in good agreement with the 10–30-fold lower affinity of sumatriptan at cloned human 5-HT_{1B} and 5-HT_{1D} receptors (Lesage et al., 1998). The inhibitory effect of alniditan was antagonized by the 5-$HT_{1B/D}$ receptor antagonist, GR 127935, but not by the tenfold higher dose of the 5-HT_2 receptor antagonist, ketanserin. Since ketanserin has been shown to display a 100-fold higher affinity for 5-HT_{1D} receptors than for 5-HT_{1B} receptors (Bach et al., 1993; Zgombick et al., 1995) whereas GR 127935 was shown to have a higher affinity for 5-HT_{1B} than for 5-HT_{1D} receptors (Pauwels, 1995), these data indicate that alniditan-induced inhibition of extravasation occurs via 5-HT_{1B} receptors rather than 5-HT_{1D} or 5-HT_2 receptors.

GR 127935 has been suggested to act as a partial agonist at cloned human 5-$HT_{1D\alpha}$ receptor sites in C6-glial cells (Pauwels and Colpaert, 1995), based on neurochemical assays in the guinea pig nucleus raphe, on behavioural studies using guinea pigs (Hutson et al., 1995) and on an extravasation assay of the guinea pig dura mater (Yu et al., 1997). In our study, however, no effects as a partial agonist could be detected, confirming earlier results obtained with the rat dura mater (Yu et al., 1997). Similarly to that with other triptans (Buzzi and Moskowitz, 1990), substance P-induced [^{125}I]bovine serum albumin extravasation was inhibited by acetylsalicylic acid but not alniditan. These data suggest that alniditan and other sumatriptan

congeners inhibit [^{125}I]bovine serum albumin extravasation with a prejunctional site of action through 5-HT$_{1B}$ receptors only, presumably by reducing neuropeptide release from C-fibers (Buzzi et al., 1991).

In our in vitro studies sumatriptan was a weak partial agonist relative to 5-HT with an efficacy of only 14%. While this efficacy of sumatriptan is considerably lower than in human pial artery (Petty et al., 1997), rabbit saphenous vein or primate basilar artery (Martin et al., 1997), the potency of sumatriptan in the rat basilar artery in the present study was very similar to that in the other blood vessels, i.e., approximately 0.3 μM. On the other hand, alniditan produced only very little if any contraction of the rat basilar artery at concentrations compatible with 5-HT$_{1B/D}$ receptor activation; the marked contraction observed at a very high alniditan concentration is likely to have occurred independent of 5-HT receptors, possibly via α-adrenoceptors. Since effects on the basilar artery were seen only with high concentrations, experiments using specific 5-HT receptor antagonists to further characterize the receptors involved could not be performed. The weaker vasoconstricting effect of alniditan as compared to that of sumatriptan in the present study was in good agreement with its weaker effects on the tone of several vascular beds in vivo (Van der Water et al., 1996).

4.2. Implications for localisation and function of 5-HT$_{1B/D}$ receptors

It has previously been proposed that vasoconstriction of cerebral arteries occurs via 5-HT$_{1B}$ receptors because these receptors were found to be expressed on vascular smooth muscles (Hamel et al., 1993). Since the mRNA of 5-HT$_{1D}$ receptors had been discovered in the trigeminal ganglion (Rebeck et al., 1994), regulation of neuropeptide release as well as inhibition of nerve stimulation-induced [^{125}I]bovine serum albumin extravasation was thought to occur via 5-HT$_{1D}$ receptors. This concept was recently questioned when the mRNA of 5-HT$_{1B}$ receptors was discovered in the human trigeminal ganglion, using reverse transcription-polymerase chain reaction (RT-PCR) amlification (Bouchelet et al., 1996). The results from our study support these findings and provide further functional evidence that the 5-HT$_{1B}$ receptor has important functions at the prejunctional site. Thus, it is tempting to speculate that the 5-HT$_{1B}$ receptor in humans not only causes vasoconstriction of dilated cranial vessels but also modulates the neuropeptide release from trigeminal fibers.

At the functional level, our data demonstrating GR 127935 to be a more potent antagonist of alniditan than ketanserin argue against mediation of extravasation inhibition by 5-HT$_{1D}$ receptors in rats. Moreover, a comparison of the present data with results of published studies on 5-HT$_{1B/D}$ receptor agonists, including sumatriptan, zolmitriptan, MDL 74,721 ((*R*)-2-(*N*1,*N*1-dipropylamino)-8-methylaminosulfonylmethyl-1,2,3,4-tetrahydronaphthalene) and CP-122,288 (5-methylaminosulphonylmethyl-3-(*N*-methoxy-pyrrolidin-2R-yl-methyl)-1*H*-indole), (Martin et al., 1997; Petty et al., 1997), indicates that the ability to inhibit nerve stimulation-induced plasma extravasation does not correlate well with drug potencies at 5-HT$_{1D}$ receptors. Since alniditan, in contrast to other sumatriptan congeners, lacks relevant affinity for 5-HT$_{1F}$ receptors (Waeber and Moskowitz, 1995; Leysen et al., 1996), the 5-HT$_{1B}$ receptor remains the most likely candidate for mediation of alniditans effects on extravasation. However, it has recently been reported that selective 5-HT$_{1F}$ receptor agonists such as LY 334370 (4-fluoro-*N*-[3-(1-methyl-4-piperidiny)-1*H*-indol-5-yl]-benzamide) or LY 344864 (*N*-[3-(dimethylamino)-2,3,4,9-tetrahydro-1*H*-carbazol-6-yl]-4-fluorobenzamide), which lack vasoconstrictive activity, very effectively inhibit nerve stimulation-induced [^{125}I]bovine serum albumin extravasation as well as capsaicin-induced c-*fos* expression in the trigeminal nuclei (Phebus et al., 1997; Mitsikostas et al., 1999). Taken together, the available data on the agonist/antagonist profile for inhibition of nerve stimulation-induced extravasation do not match any known 5-HT receptor subtype. In this context it should be mentioned that, in studies using 5-HT$_{1B}$ knockout mice, 5-carboxamido-tryptamine and CP-122,288 but not sumatriptan reduced nerve stimulation-induced [^{125}I]bovine serum albumin extravasation (Yu et al., 1997). Thus, it is possible that inhibition of extravasation involves a complex mixture of known 5-HT receptor subtypes, which is difficult to dissect, or the existence of a still unknown additional receptor mediating extravasation. While the resolution of this question may require more selective tools, alniditan clearly is effective as an inhibitor of extravasation with few if any effects on vasomotor tone.

4.3. Conclusion

In summary, the 5-HT$_{1B/D}$ receptor agonist, alniditan, is fully effective as an inhibitor of nerve stimulation-induced [^{125}I]bovine serum albumin extravasation but has less effect than sumatriptan on rat basilar artery contraction. Meanwhile clinical dose-finding trials suggest that subcutaneous alniditan is at least as potent as subcutaneous sumatriptan (Goldstein et al., 1996). The results of our study further emphasize that the potential efficacy of a candidate for anti-migraine treatment cannot be predicted from models of vasoconstriction only, but require evaluation in different models, until a reliable ''anti-migraine-model'' has been developed.

Acknowledgements

The authors are indebted to Dr. F. Tegtmeier for his helpful remarks.

References

Bach, A.W.J., Unger, L., Sprengel, R., Mengod, G., Palacios, J., Seeburg, P.H., Voigt, M.M., 1993. Structure, functional expression and spatial distribution of a cloned cDNA, encoding a rat 5-$HT_{1D\text{-like}}$ receptor. J. Recept. Res. 13, 479–485.

Beattie, D.T., Connor, H.E., 1995. The pre- and post-junctional activity of CP-122,288, a conformationally restricted analogue of sumatriptan. Eur. J. Pharmacol. 276, 271–276.

Bouchelet, I., Cohen, Z., Case, B., Seguella, P., Hamel, E., 1996. Differential expression of sumatriptan-sensitive 5-hydroxytryptamine receptors in human trigeminal ganglia and cerebral blood vessels. Mol. Pharmacol. 50, 219–223.

Buzzi, M.G., Moskowitz, M.A., 1990. The antimigraine drug sumatriptan (GR43175) selectively blocks neurogenic plasma extravasation from blood vessels in dura mater. Br. J. Pharmacol. 99, 202–206.

Buzzi, M.G., Carter, W.B., Shimizu, T., Heath, H. III, Moskowitz, M.A., 1991. Dihydroergotamine and sumatriptan attenuate levels of CGRP in plasma in rat superior sagittal sinus during electrical stimulation of the trigeminal ganglion. Neuropharmacology 30, 1193–1200.

Chen, H., Fetscher, C., Schäfers, R.F., Wambach, G., Philipp, T., Michel, M.C., 1996. Effects of noradrenaline and neuropeptide Y on rat mesenteric microvessel contraction. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 314–323.

Chen, H., Bischoff, A., Schäfers, R.F., Wambach, G., Philipp, T., Michel, M.C., 1997. Vasoconstriction of rat renal interlobar arteries by noradrenaline and neuropeptide Y. J. Auton. Pharmacol. 17, 137–146.

Connor, H.E., Feniuk, W., Humphrey, P.P.A., 1989. Characterisation of 5-HT receptors mediating contraction of canine and primate basilar artery by use of GR 43175, a selective 5-$HT_{1\text{-like}}$ receptor agonist. Br. J. Pharmacol. 96, 379–387.

Cushing, D.J., Baez, M., Kusar, J.D., Schenck, K., Cohen, M.L., 1994. Serotonin-induced contraction in canine coronary artery and saphenous vein: role of a 5-$HT_{1D\text{-like}}$ receptor. Life Sci. 54, 1671–1677.

Den Boer, M.O., Villalón, C.M., Heiligers, J.P.C., Humphrey, P.P.A., Saxena, P.R., 1991. Role of 5-HT-1like receptors in the reduction of porcine cranial arteriovenous anastomotic shunting by sumatriptan. Br. J. Pharmacol. 102, 323–330.

Diener, H.C., Gendolla, A., Jüptner, M., Kaube, H., Limmroth, V., 1997. Emerging treatments in neurology: headache. Eur. Neurol. 38, 167–174.

Feniuk, W., Humphrey, P.P.A., Perren, M.J., Watts, A.D., 1985. A comparison of 5-hydroxytryptamine receptors mediating contraction in rabbit aorta and dog saphenous vein. Br. J. Pharmacol. 86, 697–704.

Friberg, L., Oleson, J., Iversen, H.K., Sperling, B., 1991. Migraine pain associated with middle cerebral artery dilatation: reversal by sumatriptan. Lancet 338, 13–17.

Goadsby, P.J., Edvinsson, L., 1993. The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. Ann. Neurol. 33, 48–56.

Goldstein, J., Dahlöf, C.G.H., Diener, H.C., Olesen, J., Schellens, R., Simard, D., Steiner, T.J., 1996. Alniditan in the acute treatment of migraine attacks: a subcutaneous dose-finding-study. Cephalalgia 16, 497–502, on behalf of the subcutaneous Alniditan study group.

Gupta, P., Brown, D., Butler, P., Ellis, P., Grayson, K.L., Land, G.C., Macor, J.E., Robson, S.F., Wythes, M.J., Shepperson, N.B., 1995. The in vivo pharmacological profile of a 5-HT1 receptor agonist, CP-122,288, a selective inhibitor of neurogenic inflammation. Br. J. Pharmacol. 116, 2385–2390.

Hamel, E., Grègoire, L., Lau, B., 1993. 5-HT_1 receptors mediating contraction in bovine cerebral arteries: a model for human cerebrovascular '5-$HT_{1D\beta}$' receptors. Eur. J. Pharmacol. 242, 75–82.

Heyck, H., 1969. Pathogenesis of migraine. Res. Clin. Stud. Headache 2, 1–26.

Humphrey, P.P.A., Feniuk, W., Perren, M.J., Connor, H.E., Oxford, A.W., Coates, I.H., Butina, D., 1988. GR 43175, a selective agonist for the 5-$HT_{1\text{-like}}$ receptor in dog isolated saphenous vein. Br. J. Pharmacol. 94, 1123–1132.

Hutson, P.H., Bristow, L.J., Cunningham, J.R., Hoog, J.E., Longmore, J., Murray, F., Pearce, D., Razzaque, Z., Saywell, K., Tricklebank, M.D., Young, L., 1995. The effects of GR 127935, a putative 5-HT_{1D} receptor antagonist, on brain 5-HT metabolism, extracellular 5-HT concentration and behaviour in the guinea pig. Neuropharmacology 34, 383–392.

Lee, W.S., Moskowitz, M.A., 1993. Conformationaly restricted sumatriptan analogues, CP 122,288 and CP 122,638 exhibit enhanced potency against neurogenic inflammation in the rat dura mater. Brain Res. 626, 303–305.

Lesage, A.S., Wouters, R., van Gompel, P., Heylen, L., Vanhoenacker, P., Haegeman, G., Luyten, W.H.M.L., Leysen, J.E., 1998. Agonistic properties of alniditan, sumatriptan and dihydroergotamine on human 5-HT_{1B} and 5-HT_{1D} receptors expressed in various mammalian cell lines. Br. J. Pharmacol. 123, 1655–1665.

Leysen, J.E., Gommeren, W., Heylen, L., Luyten, W.H.M.L., van de Weyer, I., Vanhoenacker, P., Haegeman, G., Schotte, A., van Gompel, P., Wouters, R., Lesage, A.S., 1996. Alniditan, a new 5-hydroxytryptamine$_{1D}$ agonist and migraine-abortive agent: ligand-binding properties of human 5-hydroxytryptamine$_{1Da}$, human 5-hydroxytryptamine$_{1Db}$, and calf 5-hydroxytryptamine$_{1D}$ receptors investigated with [^{3}H]5-hydroxytryptamine and [^{3}H]alniditan. Mol. Pharmacol. 50, 1567–1580.

Limmroth, V., Lee, W.S., Moskowitz, M.A., 1996a. $GABA_A$-receptor mediated effects of progesterone, its ringA-reduced metabolites and synthetic neuroactive steroids on neurogenic edema in the rat meninges. Br. J. Pharmacol. 117, 99–104.

Limmroth, V., May, A., Auerbach, P., Wosnitza, G., Eppe, T., Diener, H.C., 1996b. Changes of cerebral blood flow velocity after treatment with sumatriptan or placebo and its implication in the pathophysiology of migraine. J. Neurol. Sci. 138, 60–65.

Markowitz, S., Saito, K., Moskowitz, M.A., 1987. Neurogenically mediated leakage of plasma protein occurs from blood vessels in dura mater but not brain. J. Neurosci. 7, 4129–4136.

Martin, G.R., Robertson, A.D., MacLennan, S.J., Prentice, D.J., Barrett, V.J., Buckingham, J., Honey, A.C., Giles, H., Moncada, S., 1997. Receptor specificity and trigemino-vascular inhibitory actions of a novel 5-$HT_{1B/1D}$ receptor partial agonist, 311C90 (zolmitriptan). Br. J. Pharmacol. 121, 157–164.

Mitsikostas, D.D., Sanchez del Rio, M., Cutrer, F.M., Moskowitz, M.A., Waeber, C., 1999. Glutamate and 5-HT_{1F} receptors modulate trigeminal pain in rats. Cephalalgia 19, 314.

Moskowitz, M.A., 1992. Neurogenic versus vascular mechanisms of sumatriptan and ergot alkaloids in migraine. Trends Pharmacol. Sci. 13, 307–311.

Parsons, A.A., Whalley, E.T., Feniuk, W., Connor, H.E., Humphrey, P.P.A., 1989. 5-HT1-like receptors mediate 5-hydroxytryptamine-induced contraction of human isolated basilar artery. Br. J. Pharmacol. 96, 434–449.

Pauwels, P.J., 1995. Pharmacological properties of a putative 5-$HT_{1B/D}$ receptor antagonist GR 127935. CNS Drug Rev. 2, 415–428.

Pauwels, P.J., Colpaert, F.C., 1995. The 5-HT_{1D} receptor antagonist GR 127,935 is an agonist at cloned human 5-$HT_{1D\alpha}$ receptor sites. Neuropharmacology 34, 235–237.

Petty, M.A., Elands, J., Johnson, M.P., Linnik, M.D., Hamel, E., Moskowitz, M.A., Lee, W.S., McCarty, D.R., Hibert, M., Baron, B.M., 1997. The selectivity of MDL 74,721 in models of neurogenic versus vascular components of migraine. Eur. J. Pharmacol. 336, 127–136.

Phebus, L.A., Johnson, K.W., Audia, M.L., Cohen, B.A., Dressman, B.A., Fritz, J.E., Kaldor, S.W., Krushinski, J.H., Schenck, K.W., Zgombick, J.M., Branchek, T.A., Adham, N., Schaus, J.M., 1997. The serotonin 1F(5-HT_{1F}) receptor subtype: a rational target for migraine drug discovery. Cephalalgia 17, 245.

Rasmussen, B.K., Jensen, R., Schroll, M., Olesen, J., 1991. Epidemiology

of headache in a general population — a prevalence study. J. Clin. Epidemiology 44, 1147–1157.

Rebeck, G.W., Maynard, K.I., Hyman, B.T., Moskowitz, M.A., 1994. Selective 5-HT aserotonin receptor gene expression in trigeminal ganglia: implications for anti-migraine drug development. Proc. Natl. Acad. Sci. 91, 3666–3669.

Shepheard, S.L., Williamson, D.J., Williams, J., Hill, R.G., Hargreaves, R.J., 1995. Comparison of the effects of sumatriptan and the NK1 antagonist CP-99,994 on plasma extravasation in the dura mater and c-*fos* mRNA expression in the trigeminal nucleus caudalis of rats. Neuropharmacology 34, 255–261.

Van der Water, A., D'Aubioul, J., Van Gerven, W., Van Ammel, K., De Clerck, F., 1996. Selective vasoconstriction by alniditan in the carotid vascular bed of anaesthetized dogs. Eur. J. Pharmacol. 299, 127–137.

Van Lommen, G., De Bruyn, M., Schroven, M., Verschueren, W., Janssens, W., Verrelst, J., Leysen, J.E., 1995. The discovery of a series of new non-indole 5-HT1D agonists. Bioorg. Med. Chem. Lett. 5, 2649–2654.

Verheggen, R., Hundeshagen, A.G., Brown, A.M., Schindler, M., Kaumann, A.J., 1998. 5-HT_{1B} receptor-mediated contractions in human temporal artery: evidence from selective antagonists and 5-HT receptor mRNA expression. Br. J. Pharmacol. 124, 1345–1354.

Waeber, C., Moskowitz, M.A., 1995. Autoradiographic visualisaton of [^{3}H]5-carboxamido-tryptamine binding sites in the guinea pig and rat brain. Eur. J. Pharmacol. 283, 31–46.

Willems, E., De Vries, P., Heiligers, J.P.C., Saxena, P.R., 1998. Porcine carotid vascular effects of eletriptan (UK-116,044): a new 5-$HT_{1B/1D}$ receptor agonist with anti-migraine activity. Naunyn-Schmiedeberg's Arch. Pharmacol. 358, 212–219.

Yu, X.J., Waeber, C., Castanon, N., Scearce, K., Hen, R., Macor, J.E., Chaveau, J., Moskowitz, M.A., 1996. 5-Carboxamido-tryptamine, CP-122,288 and dihydroergotamine but not sumatriptan, CP-93,129, and serotonin-5-0-carboxymethyl-glycyl-tyrosinamide block dural plasma protein extravasation in knockout mice that lack 5-hydrotryptamine$_{1B}$ receptors. Mol. Pharmacol. 49, 761–765.

Yu, X.J., Cutrer, F.M., Moskowitz, M.A., Waeber, C., 1997. The 5-HT_{1D} receptor antagonist GR-127,935 prevents inhibitory effects of sumatriptan but not CP-122,288 and 5-CT on neurogenic plasma extravasation within guinea pig dura mater. Neuropharmacology 36, 83–91.

Zgombick, J.M., Schechter, L.E., Kucharewicz, S.A., Weinshank, R.L., Branchek, T.A., 1995. Ketanserin and ritanserin discriminate between recombinant human 5-HT1D$_{alpha}$ and 5-HT1D$_{beta}$ receptor subtypes. Eur. J. Pharmacol. 291, 9–15.