ELSEVIER

European Journal of Pharmacology 425 (2001) 203–210

ejp

www.elsevier.com/locate/ejphar

Serotonergic effects and extracellular brain levels of eletriptan, zolmitriptan and sumatriptan in rat brain

David E. Johnson [a], Hans Rollema [a,*], Anne W. Schmidt [a], Aileen D. McHarg [b]

[a] *Department of Neuroscience, Pfizer Global Research and Development, MS 8220-4159, Groton, CT 06340, USA*
[b] *Candidate Research Group, Sandwich, Kent, CT13 9NJ, UK*

Received 5 April 2001; received in revised form 8 June 2001; accepted 15 June 2001

Abstract

In vivo microdialysis was used to assess the central serotonergic effects and extracellular brain levels of the 5-$HT_{1B/1D}$ receptor agonists eletriptan, zolmitriptan and sumatriptan in rats after intravenous and intracerebral administration, while their binding affinities and functional potencies were determined at 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1A} receptors. In vitro studies showed that all three triptans are high affinity, full agonists at 5-$HT_{1B/1D}$ receptors, but that sumatriptan is functionally less potent as a 5-$HT_{1B/1D}$ agonist than zolmitriptan and eletriptan. Local intracortical perfusion with the compounds via the dialysis probe decreased cortical 5-HT (5-hydroxytryptamine, serotonin) release with ED_{50} values of approximately 0.1 μM for eletriptan and zolmitriptan and 0.5 μM for sumatriptan. At 3.2 mg/kg i.v., both eletriptan and zolmitriptan decreased 5-HT levels by about 35%, while sumatriptan had no effect, despite the fact that maximal sumatriptan concentrations in cortical dialysates were higher (8.8 nM at 20 min) than those of zolmitriptan (5.9 nM at 20 min) and eletriptan (2.6 nM at 40 min). The observation that eletriptan and zolmitriptan produce almost identical central serotonergic effects, after intracerebral as well as after systemic administration, is in agreement with their comparable functional 5-$HT_{1B/1D}$ receptor agonist potencies and their free levels in cortical dialysates after 3.2 mg/kg i.v. On the other hand, the lack of central serotonergic effects of 3.2 mg/kg i.v. sumatriptan is likely due to its weaker functional 5-$HT_{1B/1D}$ receptor agonist potency than eletriptan and zolmitriptan, rather than lower brain levels, consistent with sumatriptan's fivefold lower potency after intracerebral administration.

Keywords: 5-$HT_{1B/1D}$ receptor; 5-HT (5-hydroxytryptamine, serotonin) release; Brain level; Eletriptan; Zolmitriptan; Sumatriptan; Microdialysis

1. Introduction

The development of the 5-$HT_{1B/1D}$ receptor agonist sumatriptan represented a significant advance in the treatment of migraine headaches. Its mechanism of action has been ascribed to 5-$HT_{1B/1D}$ receptor activation on the innervating terminals of the cerebral vasculature, which results in vasoconstriction and a decreased release of neuropeptides such as calcitonin gene related peptide. Second generation compounds such as zolmitriptan, rizatriptan, naratriptan and eletriptan, have an improved bioavailability, are generally less hydrophilic than sumatriptan and are expected to have a better central nervous system (CNS) penetration (for reviews see Schoenen, 1997; Russel, 1999; Pauwels and John, 1999). This could be of importance since results of recent studies using several triptans, for example zolmitriptan (Goadsby and Hoskin, 1996; Goadsby and Knight, 1997a; Martin, 1997; Cumberbatch et al., 1998a), rizatriptan (Cumberbatch et al., 1997), naratriptan (Goadsby and Knight, 1997b; Cumberbatch et al., 1998b) and eletriptan (Goadsby and Hoskin 1999; Lambert et al., 2000) suggested a central component to their mechanism of action by acting at the trigeminal nucleus and higher pain centres of the brain. Sumatriptan, however, requires blood–brain barrier disruption before showing efficacy in this model (Kaube et al., 1993).

To examine the brain penetration of triptans in a functional model, we compared the central serotonergic effects of sumatriptan, zolmitriptan and eletriptan. Since it is known that 5-$HT_{1B/1D}$ receptors on serotonergic neurons in the CNS act as release modulating inhibitory autoreceptors (Millan et al., 2000), we applied in vivo microdialysis to demonstrate that local perfusion of 5-$HT_{1B/1D}$ agonists decreases cortical 5-HT levels in the rat prefrontal cortex. This response was then used as an in vivo marker to determine if systemic administration of eletriptan, zolmi-

* Corresponding author. Tel.: +1-860-441-6374; fax: +1-860-715-5378.
E-mail address: hans_rollema@groton.pfizer.com (H. Rollema).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01151-7

triptan or sumatriptan could produce a central effect. In addition, cortical dialysate concentrations of the triptans were determined following intravenous administration as an estimate of the extracellular brain concentrations of the compounds. Finally, binding affinities and functional agonist potencies at 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1A} receptors were determined to correlate the in vivo effects with in vitro binding and functional profiles.

2. Materials and methods

2.1. Receptor binding and effects on receptor-mediated cyclase activity

2.1.1. Receptor binding studies

Binding assays on membranes from rat and bovine brains were performed according to standard procedures (Seeger et al., 1995). Briefly, tissues were homogenised in 50 mM Tris–HCl buffer (pH 7.4) and resuspended in 50 mM Tris–HCl, 0.1% ascorbate 4 mM $CaCl_2$, 10 μM pargyline (pH 7.7). Incubations were initiated by the addition of tissue to tubes or 96-well plates containing test drugs and the appropriate tritiated ligand and masking reagents (1.5 nM [^{3}H]8-OH-DPAT (8-hydroxy-*N*,*N*-dipropylaminotetraline) for 5-HT_{1A} in rat hippocampus, 1 nM [^{3}H]CT (5-carboxytryptamine) + 3000 nM mianserin + 100 nM 8-OH-DPAT for 5-HT_{1B} in rat caudate and 2 nM [^{3}H]5-HT + 100 nM mesulergine + 100 nM 8-OH-DPAT for 5-HT_{1D} in bovine caudate). Nonspecific binding was determined by radioligand binding in the presence of a saturating excess of 5-HT (10 μM). After equilibration at room temperature, incubations were terminated by rapid filtration through Whatman GF/B filters, the membranes were washed with ice-cold 50 mM Tris buffer and membrane-bound ligand was determined by liquid scintillation counting of the filters in Ready-Safe scintillation cocktail or Betascint. The IC_{50} value (concentration at which 50% inhibition of specific binding occurs) was calculated by linear regression of the concentration–response data. K_i values were calculated according to the Cheng and Prusoff (1973) equation $K_i = IC_{50}/(1 + (L/K_d))$, where L is the concentration of the radioligand used in the experiment and the K_d value is the dissociation constant for the radioligand (determined previously by saturation analysis).

2.1.2. 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptor modulation of adenylate cyclase activity

Effects on adenylate cyclase activity were determined as described previously (Seeger et al., 1995). Briefly, membranes were prepared from freshly dissected rat substantia nigra (5-HT_{1B} receptors), rat hippocampus (5-HT_{1A} receptors) or guinea pig substantia nigra (5-HT_{1D} receptors) and incubated at 37 °C in a reaction medium containing 100 mM Hepes (pH 7.4), 3 μM forskolin, 2.0 mM $MgCl_2$, 0.5 mM ATP, 1.0 mM cAMP, 0.5 mM 3-isobutyl-1-methylxanthine, 10 mM phosphocreatine, 0.31 mg/ml creatine phosphokinase, 100 μM GTP, 1 μCi α-[^{32}P]ATP per tube. Incubations were terminated after 15 min by adding 2% sodium dodecyl sulfate. After separation of [^{32}P]cAMP from [^{32}P]ATP, the amount of [^{32}P]cAMP formed was determined by liquid scintillation counting, with results expressed as picomoles per minute per milligram of protein. EC_{50} values (the concentration at which the drug inhibits 50% of forskolin stimulated adenylyl cyclase activity) and efficacies relative to the full agonist ligand (5-HT for 5-HT_{1D}, 5-CT for 5-HT_{1B} and 8-OH-DPAT for 5-HT_{1A}) were calculated by linear regression analysis of the concentration–response curves.

2.2. Microdialysis

2.2.1. Surgery

In vivo microdialysis in prefrontal cortex was performed as previously described (Rollema et al., 2000) in awake, freely moving male Sprague–Dawley rats (280–320 g), which had free access to food and water and were maintained on a 12:12 h light/dark cycle. CMA/11 microdialysis probes (CMA Microdialysis, N. Chelmsford, MA) with a 4-mm cuprophane membrane (6 kDa cutoff) were stereotaxically implanted in the prefrontal cortex (AP +3.2 mm from bregma, −0.7 mm ML, DV −6.0 mm from dura; Paxinos and Watson, 1997) under ketamine/xylazine anaesthesia and fixed to the skull using bone screws and dental acrylic. Animals were allowed to recover for 24–48 h before experiments were started. The inlet and outlet of the probe were connected with flexible PEEK tubing (inside diameter 0.005″, Upchurch Scientific) and the probe was perfused at 1.5 μl/min with artificial cerebral spinal fluid (aCSF; NaCl 147 mM; $CaCl_2$ 1.3 mM; KCl 2.7 mM; $MgCl_2$ 1.0 mM) with a CMA/102 microperfusion pump.

2.2.2. Assays

For the 5-HT assay, microdialysate was collected at 18-min intervals directly into a 30-μl sample loop and analysed by on-line high performance liquid chromatography with electrochemical detection (HPLC-EC). Analytes were separated at 30 °C over a 150 × 3-mm C_{18} 3 μ Hypersil BDS column (Keystone Scientific, Bellefonte, PA) with a 20 × 3-mm Javelin guard column. The mobile phase consisted of 75 mM sodium acetate, 1.1 mM 1-heptanesulfonic acid, 0.1 mM EDTA, 9% methanol, pH 4.4 and was delivered at a flow rate of 0.5 ml/min (Shimadzu LC-10AD, Shimadzu Columbia, MD). Dialysate concentrations of 5-HT were measured amperometrically (ANTEC DECADE, Antec Leyden, The Netherlands), using a glassy carbon electrode set at 550 mV vs. Ag/AgCl.

Concentrations of the triptans in cortical dialysates were measured by a modification of an HPLC-EC assay for sumatriptan (Andrew et al., 1993). Dialysate was continuously collected on-line at 20-min intervals and the triptans

Table 1
Binding affinities (pK_i in M) of the triptans at native 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors

	Binding affinities $pK_i \pm$S.E.M. (n)		
	r 5-HT_{1B}	bv 5-HT_{1D}	r 5-HT_{1A}
Eletriptan	7.01 ± 0.10 (3)	8.28 ± 0.04 (4)	7.87 ± 0.08 (3)
Zolmitriptan	7.43 ± 0.08 (3)	7.92 ± 0.07 (2)	7.33 ± 0.10 (3)
Sumatriptan	7.05 ± 0.09 (3)	7.36 ± 0.18 (7)	6.64 ± 0.19 (3)

r = rat, bv = bovine, gp = guinea pig.

were separated over a 150 × 3-mm C_{18} 3 μ Hypersil BDS column at 35 °C with a mobile phase consisting of 24 mM Na_2HPO_4, 16 mM KH_2PO_4, 18% methanol (for sumatriptan and zolmitriptan) or 45% methanol (for eletriptan), pH 7.1, at 0.25 ml/min and detected amperometrically at 850 mV vs. AgAgCl (ANTEC DECADE).

2.2.3. Drug treatments

To study the effects on in vivo 5-HT release, drugs were locally perfused or administered i.v. after basal dialysate levels of 5-HT had stabilised. Eletriptan, zolmitriptan or sumatriptan were dissolved and diluted to the test concentration in aCSF and perfused through the dialysis probe at 0.1, 1 or 10 μM. For systemic administration, the drugs were dissolved in 0.9% NaCl and injected as an i.v. bolus at 0.1, 1.0 or 3.2 mg/kg via a jugular vein catheter.

To measure concentrations of eletriptan, sumatriptan or zolmitriptan in cortical dialysates, the compounds were dissolved in saline (1 ml/kg) and injected as a 3.2 mg/kg (~ 11 μmol/kg zolmitriptan and sumatriptan, ~ 9 μmol/kg eletriptan) i.v. bolus via the jugular vein cannula, after obtaining a stable baseline. Dialysate levels of the triptans were measured for at least 3 h and were expressed as nM ± S.E.M.

2.2.4. Probe recoveries / deliveries

The probe in vitro recovery for each triptan was determined by perfusing aCSF at 1.5 μl/min through a probe placed in a continuously stirred 20–100 nM triptan solution prepared in aCSF and measuring the concentration recovered in the perfusate. The recovery is calculated as $(C_{\text{perfusate}}/C_{\text{vial solution}}) \times 100\%$. The in vitro and in vivo deliveries of each triptan were determined via retrodialysis, by perfusing a 20–50 nM triptan solution in aCSF through the probe placed in a stirred aCSF solution, or through a probe implanted in rat cortex (at the end of an i.v. experiment), and measuring the remaining concentration in the perfusate. The delivery is calculated as $(C_{\text{perfusing solution}} - C_{\text{perfusate}})/C_{\text{perfusing solution}}) \times 100\%$.

2.3. Drugs and chemicals

Eletriptan hydrobromide, sumatriptan succinate, zolmitriptan mesylate and CP-94,253 [5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo[3,2-*b*] pyridine)] were synthesised by Pfizer (Sandwich, UK; Groton, USA). Ketamine/xylazine, 5-HT and agents used to define non-specific binding (mianserin, mesulergine, 8-OH-DPAT) were purchased from Research Biochemicals International (Natick, MA) or Sigma (St. Louis, MO). Radioligands were purchased from New England Nuclear (Boston, MA) or Amersham (Arlington Heights, IL). HPLC grade methanol was obtained from JT Baker (Philipsburg, NJ), all other analytical grade chemicals were purchased from Fluka-BioChemika (Ronkonkoma, NY).

2.4. Data analysis

All chromatographic data were acquired and analysed using EZChrom software (Scientific Software, San Ramon, CA) that quantified dialysate concentrations by comparing peak heights with those of standard solutions of 5-HT (0.2–2.0 nM) or the triptans (0.2–50 nM). Effects on extracellular 5-HT levels were monitored for at least 2 h and were expressed, for time–response curves, as the fraction of basal levels (i.e. mean of the last six samples before drug administration) ± S.E.M. or, for dose–response curves, as the area under the curve (AUC), calculated over a 2-h period after drug administration (AUC_{0-2h}) ± S.E.M. Statistical analyses were performed by two-way analysis of variance for repeated measures and Dunnett's post hoc multiple comparisons between baseline and post-treatment levels.

3. Results

3.1. Receptor binding and functional profiles

The in vitro binding affinities of eletriptan, zolmitriptan and eletriptan for rat 5-HT_{1B}, rat 5-HT_{1A} and bovine 5-HT_{1D} receptors are given in Table 1, and their in vitro

Table 2
Functional potencies and efficacies of the triptans to decrease 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1A} receptor mediated forskolin-stimulated adenylate cyclase activity

	Adenylate cyclase activities $pEC_{50} \pm$S.E.M. (n)		
	Relative efficacies vs. full agonist ligand (% ± S.E.M.)		
	r $5HT_{1B}$	gp $5HT_{1D}$	r $5HT_{1A}$
Eletriptan	6.95 ± 0.12 (3)	8.03 ± 0.25 (3)	6.85 ± 0.19 (3)
efficacy	*105 ± 4%*	*104 ± 3%*	*100 ± 9%*
Zolmitriptan	7.11 ± 0.12 (3)	7.55 ± 0.12 (3)	6.20 ± 0.09 (4)
efficacy	*106 ± 6%*	*104 ± 6%*	*104 ± 6%*
Sumatriptan	6.59 ± 0.05 (3)	7.03 ± 0.05 (8)	5.66 ± 0.19 (3)
efficacy	*103 ± 5%*	*97 ± 2%*	*96 ± 7%*

Potencies are given as $pEC_{50} \pm$S.E.M. (in M).
Efficacies relative to the full agonist ligand (5-CT for 5-HT_{1B}, 5-HT for 5-HT_{1D} and 8-OH-DPAT for 5-HT_{1A}) are given as % of maximal response ± S.E.M., in italics (r = rat, gp = guinea pig).

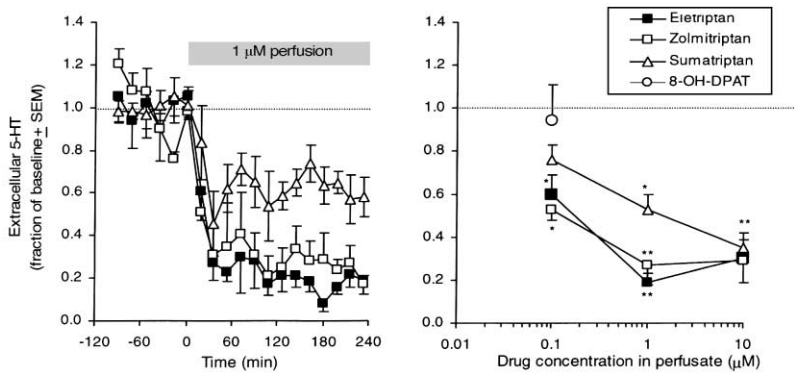

Fig. 1. Left panel: time courses for the effects of local intracortical perfusions (bar) with 1 μM eletriptan (■), zolmitriptan (□) and sumatriptan (△) on cortical 5-HT release. Data are expressed as average fraction of basal 5-HT levels ± S.E.M. ($n = 3–7$). Right panel: dose–response curves for the effects of locally perfused eletriptan, zolmitriptan, sumatriptan (0.1–10 μM) and 8-OH-DPAT (○, 0.1 μM) on cortical 5-HT release. Effects on 5-HT release were calculated as the average effect from 144 to 180 min after starting the perfusion and expressed as fraction of basal 5-HT levels ± S.E.M. ($n = 3–7$). $^{*}P < 0.05$, $^{**}P < 0.01$.

potencies and efficacies to decrease 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptor-mediated forskolin-stimulated adenyl cyclase activity in rat and guinea pig membranes, are listed in Table 2. The triptans bind with 10–100 nM affinities to the 5-HT_1 receptors tested, with the exception of eletriptan's higher 5-HT_{1D} receptor affinity (5 nM) and sumatriptan's lower 5-HT_{1A} receptor affinity (250 nM). The functional cyclase activity data demonstrated that the triptans are full agonists with 10–500 nM potencies at the three receptors, except for sumatriptan, which is a much less potent ($EC_{50} = 2$ μM), full 5-HT_{1A} receptor agonist.

3.2. Effects on 5-HT release

3.2.1. Intracortical perfusions

Determination of the in vitro and in vivo recovery and delivery for each triptan showed that the triptans diffuse with comparable efficiencies through the dialysis membrane of the CMA11/4 probe. In vitro recoveries (21.6–22.5%) were similar to in vitro deliveries (22.3–23.5%) and about four to five times more efficient than in vivo deliveries, measured by retrodialysis (5.4–7.5%).

Eletriptan, zolmitriptan and sumatriptan markedly decreased 5-HT release in rat prefrontal cortex during local perfusion of the compounds through the microdialysis probe into the cortex. The decrease in 5-HT release was sustained and dose-dependent with a maximal reduction of 70–80% after 10-μM perfusions. Fig. 1 (left panel) shows the time courses of the 5-HT response to inclusion of 1 μM of the 5-$HT_{1B/1D}$ receptor agonists in the perfusate. At this concentration, eletriptan and zolmitriptan produced near maximal inhibitions of 75% and 80%, respectively, while sumatriptan gave only a 45% decrease in 5-HT release. From the concentration–response curves (Fig. 1,

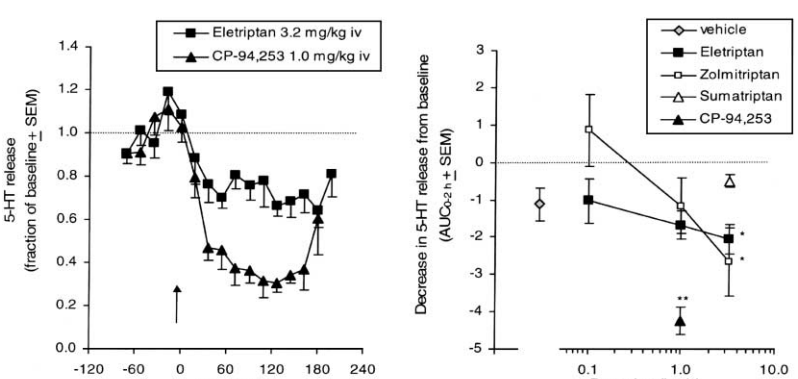

Fig. 2. Left panel: time courses for the effects of 3.2 mg/kg i.v. eletriptan (■) and 1.0 mg/kg i.v. CP-94,253 (▲) on cortical 5-HT release. Arrow indicates injection at $t = 0$. Data are expressed as average fraction of basal 5-HT levels ± S.E.M. ($n = 3–7$). Right panel: dose–response curves for the effects of i.v. injection with saline (♦), 0.1–3.2 mg/kg eletriptan (■), 0.1–3.2 mg/kg zolmitriptan (□), 3.2 mg/kg sumatriptan (△) and 1 mg/kg CP-94,253 (▲) on cortical 5-HT release. Effects on 5-HT release were calculated as the AUC over 0–2 h after drug injection and expressed as the decrease from baseline ± S.E.M. ($n = 3–7$). $^{*}P < 0.05$, $^{**}P < 0.01$.

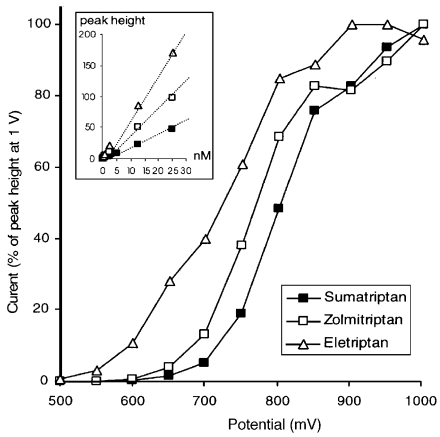

Fig. 3. Typical i.v. curves at pH 7.1 for eletriptan (■), zolmitriptan (□) and sumatriptan (△), determined by measuring the peak height of a 500 nM solution at increasing oxidation potentials. The response at each potential is expressed as the percentage of the maximal response at 1000 mV. Insert: calibration curves for the triptans (0.2–25 nM) determined at 850 mV. The amperometric response is given as percent of the maximal peak height at 1 nA/V. For chromatographic conditions, see Section 2.

right panel), EC_{50} values (the concentration that decreases 5-HT release to 50% of the maximal decrease) were estimated to be approximately 0.1 μM for eletriptan and zolmitriptan and 0.5 μM for sumatriptan, indicating that sumatriptan is approximately five times less potent than eletriptan or zolmitriptan when locally perfused. To examine the serotonergic effect of locally administered 5-HT_{1A} receptor agonists, 0.1 μM 8-OH-DPAT, a selective and potent 5-HT_{1A} agonist, was perfused through the dialysis probe. This treatment had no significant effect on cortical 5-HT release.

3.2.2. Intravenous administration of 5-HT$_{1B/1D}$ receptor agonists

Fig. 2 (left panel) shows the time courses for the effects of i.v. administration of 3.2 mg/kg eletriptan and 1 mg/kg CP-94,253, and the dose–response curves for eletriptan and zolmitriptan (right panel). The centrally acting, potent and selective 5-HT_{1B} receptor agonist, CP-94,253, (Koe et al., 1992) decreased extracellular cortical 5-HT levels by more than 60% at 1 mg/kg i.v., whereas 3.2 mg/kg i.v. eletriptan produced a much smaller, but still significant 35% decrease between 1 and 3 h after injection. Dose–response curves, constructed from the areas under the curve calculated over the 2-h period after drug administration (Fig. 2, right panel), show that at 3.2 mg/kg i.v., both eletriptan and zolmitriptan produced small but significant decreases in cortical 5-HT release of approximately 40%, while 3.2 mg/kg i.v. sumatriptan did not significantly inhibit 5-HT release. The vehicle (i.v. saline) produced a modest transient decrease in 5-HT release of about 18%.

3.2.3. Triptan levels in cortical microdialysate

Triptan microdialysate concentrations were determined by amperometric detection with a modified HPLC sumatriptan assay (Andrew et al., 1993). Based on current–voltage curves for the triptans at pH 7.1 (Fig. 3), we choose +850 mV vs. Ag/AgCl as the optimal oxidation potential. Under these assay conditions the limit of detection was about 0.2 nM for each triptan, while the amperometric response of all three triptans was at least linear between 0.2 and 125 nM (Fig. 3, insert showing 0.2–25 nM).

Time courses of triptan concentrations in rat cortical microdialysates (in nM, not corrected for recovery) after i.v. injection with 3.2 mg/kg of each of the triptans, are given in Fig. 4. Peak dialysate concentrations were reached in the first 0–20 min sample for sumatriptan (8.82 ± 1.15 nM) and zolmitriptan (5.86 ± 0.92 nM) and in the second 20–40 min sample for eletriptan (2.58 ± 0.23 nM).

4. Discussion

In the present study, we evaluated the in vivo central serotonergic effects and the brain dialysate levels of three antimigraine $5\text{-HT}_{1B/1D}$ receptor agonists, eletriptan, zolmitriptan and sumatriptan. The results demonstrate that eletriptan and zolmitriptan produce comparable central serotonergic effects, while sumatriptan, despite reaching

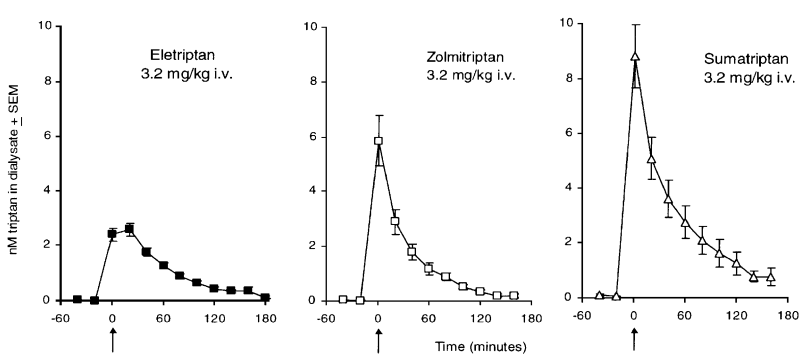

Fig. 4. Time courses of eletriptan (■), zolmitriptan (□) and sumatriptan (△) concentrations in microdialysates from rat prefrontal cortex following 3.2 mg/kg i.v. Data are dialysate concentrations (not corrected for recovery) in nM ± S.E.M. (n = 8–17). Arrows at t = 0 min indicate i.v. injection with 3.2 mg/kg of the triptan.

comparable dialysate concentrations, lacks central serotonergic activity, presumably because it is functionally less potent as a 5-$HT_{1B/1D}$ receptor agonist than eletriptan and zolmitriptan.

4.1. In vitro binding affinities and functional activities

In agreement with literature data, we found that eletriptan, zolmitriptan and sumatriptan bind with high affinity to native rat 5-HT_{1B} and bovine 5-HT_{1D} receptors, and that all three compounds act as full agonists at these receptors, but that the individual profiles differ substantially. Although the 5-$HT_{1B/1D}$ affinities of the three triptans vary among species, the present in vitro binding data are generally in good agreement with their binding profiles at human recombinant 5-HT_{1B} and 5-HT_{1D} receptors (Napier et al., 1999), both with regard to their relative rank order and their higher affinity for 5-HT_{1D} than for 5-HT_{1B} receptors. The functional cyclase data show that eletriptan and zolmitriptan are approximately 2.3–3.2-fold more potent as agonists at the 5-HT_{1B} receptor than sumatriptan, despite its comparable 5-HT_{1B} binding affinity. Eletriptan is the most potent 5-HT_{1D} receptor agonist, consistent with its high 5-HT_{1D} binding affinity and is 10-fold and 3-fold more potent than sumatriptan and zolmitriptan, respectively. The functional differences between zolmitriptan and sumatriptan are in line with reported data on the 5-$HT_{1B/1D}$ activities of these compounds in cyclase and GTPγS assays (Pauwels et al., 1997). The 5-HT_{1A} receptor agonist potencies of the triptans are an order of magnitude lower than their 5-HT_{1A} binding affinities, but show the same relative activities, eletriptan being about fourfold more potent than zolmitriptan and 16-fold more potent than sumatriptan.

4.2. Effects on 5-HT release

It has been well-documented that 5-$HT_{1B/1D}$ receptor agonists decrease terminal 5-HT release by activating presynaptic release regulating 5-HT autoreceptors, which are in serotonergic nerve terminals (for review see Millan et al., 2000). Several microdialysis studies have demonstrated a reduction in extracellular 5-HT levels in various species in vivo following systemic or intracerebral administration of 5-$HT_{1B/1D}$ receptor agonists, including sumatriptan (Sleight et al., 1990; Trillat et al., 1997; Roberts et al., 1997; Cremers et al., 2000), naratriptan (Moret and Briley, 1997), or selective 5-HT_{1B} receptor agonists such as CP-93,123 (Hjorth and Tao, 1991; Trillat et al., 1997; Hertel et al., 1999), and CP-94,325 (Tingley et al., 1992; Knobelman et al., 2000). The concentration-dependent decrease in extracellular 5-HT levels following local perfusions of eletriptan, zolmitriptan and sumatriptan confirms the 5-HT reducing effect of 5-$HT_{1B/1D}$ receptor agonists and demonstrates their ability to modulate terminal 5-HT release when present in the CNS. Since the amount of drug delivered through the dialysis probe during the local perfusions is practically identical for each triptan, the present data allow the first direct comparison of in vivo serotonergic effects of the three drugs at similar extracellular brain concentrations. Actual extracellular levels reached after a continuous local perfusion are not known, but can be roughly calculated with in vivo delivery efficiency by assuming that the compounds diffuse 1 mm from the probe (De Lange et al., 1997). After a 2-h perfusion at 1.5 μl/min with the triptans at their respective EC_{50} concentrations, the extracellular levels are then estimated to be 80 nM for eletriptan and zolmitriptan and 315 nM for sumatriptan. It is clear from these data that when locally perfused, sumatriptan is about four to fivefold less potent than eletriptan and zolmitriptan in decreasing cortical 5-HT release. The equal potency of eletriptan and zolmitriptan in this in vivo model, as well as the lower potency of sumatriptan, are consistent with their relative agonist potencies at the rat 5-HT_{1B} receptor as measured in the functional cyclase assay.

Intravenous dose–response studies showed that sumatriptan did not affect cortical 5-HT release at 3.2 mg/kg i.v., while at that dose both eletriptan and zolmitriptan produced a significant decrease in cortical 5-HT release. The 5-HT decrease following i.v. injection of eletriptan and zolmitriptan is most likely mediated via stimulation of inhibitory terminal 5-HT_{1B} autoreceptors, but systemically administered 5-$HT_{1B/1D}$ receptor agonists may in addition interact with somatodendritic 5-HT_{1D} autoreceptors. Activation of 5-HT_{1D} receptors could contribute to the decrease in terminal 5-HT release (Stamford et al., 2000), especially since eletriptan and zolmitriptan are more potent 5-HT_{1D} than 5-HT_{1B} receptor agonists. However, terminal 5-HT_{1B} receptor activation seems to be the more predominant mechanism, given the fact that a low dose of a centrally acting, selective 5-HT_{1B} receptor agonist, CP-94,253 ($K_{i\ 5\text{-}HT1B\ rat} = 2$ nM, $EC_{50\ cyclase} = 10$ nM; Tingley et al., 1992), markedly decreased cortical 5-HT release by more than 70%, in good agreement with the decrease in 5-HT release found in mouse striatum after 3.2 mg/kg i.p. CP-94,253 (Knobelman et al., 2000). Sumatriptan's lack of effect is again most likely due to its weaker 5-$HT_{1B/1D}$ functional potency, since it reaches somewhat higher dialysate levels than zolmitriptan and eletriptan (see below). Taken together, these data demonstrate that the triptans can reduce central 5-HT neurotransmission if their extracellular brain levels are sufficiently high, either following intracerebral local perfusions or after i.v. administration of relatively high doses.

The view that eletriptan decreases 5-HT release via 5-HT_{1B} receptors in rat frontal cortex is consistent with a recent in vitro autoradiography study on [^{3}H]-eletriptan binding in the rat brain (Clarke et al., 1999). It was reported that in vitro [^{3}H]-eletriptan binding occurs predominantly via 5-$HT_{1B/1D}$ and 5-HT_{1F} receptors in the substantia nigra, striatum/globus pallidus, cortex and hip-

pocampus. In addition, a large component of the binding in the cortex and hippocampus was found to be via 5-HT_{1A} receptors, consistent with the in vitro affinity of eletriptan for 5-HT_{1A} receptors. In view of these data it is conceivable that the reduction in extracellular 5-HT levels after the local perfusion with eletriptan and zolmitriptan, both moderately potent 5-HT_{1A} receptor agonists, is mediated via postsynaptic 5-HT_{1A} receptor-mediated feedback control, which may be operational in the cortex (Ceci et al., 1994; Casanovas et al., 1999). This seems unlikely, however, since intracortical perfusion with 0.1 μM of the potent 5-HT_{1A} receptor agonist, 8-OH-DPAT, did not change cortical 5-HT release. On the other hand, systemically administered 5-HT_{1A} receptor agonists are well known to produce decreases in cortical 5-HT release and it is thus possible that a small part of the 5-HT decrease after i.v. eletriptan and zolmitriptan is mediated via activation of presynaptic somatodendritic 5-HT_{1A} receptors in the raphe nuclei. We did not further investigate the relative contributions of 5-$HT_{1B/1D}$ and 5-HT_{1A} receptor activation in terminal or cell body areas, which are all central mechanisms and obviously require brain penetration of the triptans.

4.3. Extracellular brain triptan levels

The fact that the triptans were detected in cortical dialysates after an i.v. bolus injection of 3.2. mg/kg, confirms that these drugs can cross the blood–brain barrier into the CNS after systemic administration. Maximal extracellular levels of the triptans were reached very fast, within 20–40 min, indicating that the equilibrium between blood and brain concentrations is very rapid, consistent with the brain penetration of other hydrophilic compounds (De Lange et al., 1997). Dialysate levels declined to nondetectable levels after 3–4 h with $T_{1/2}$'s ranging from 31 to 45 min. Sumatriptan reached the highest dialysate concentrations with a C_{max} of 8.8 nM, while the C_{max} for zolmitriptan and eletriptan was 5.9 and 2.6 nM, respectively.

It is not possible to accurately calculate extracellular brain levels from the measured dialysate concentrations with in vivo recoveries determined by steady-state retrodialysis. However, since the in vitro and in vivo diffusion properties of the triptans through the membrane do not differ significantly, relative differences between extracellular levels of the triptan are reflected by the ratios of the areas under the curve over the 2-h period after drug administration. From these data it is estimated that free extracellular levels of sumatriptan are 2.2- and 2.5-fold higher than those of zolmitriptan and eletriptan, respectively.

The finding that peak extracellular levels of sumatriptan are actually higher than those of eletriptan and zolmitriptan after 3.2 mg/kg i.v., is consistent with reports presented in abstract form, that whole rat brain tissue levels after sumatriptan (13 nM) are comparable or higher than those of zolmitriptan (11 nM) and eletriptan (3 nM) after 3 mg/kg p.o. (O'Connor et al., 1997, 1999).

4.4. Conclusions and clinical significance

If inhibition of trigeminal neuronal firing is indeed an additional mechanism for antimigraine activity of second generation triptans (see Section 1), brain penetration of antimigraine drugs becomes an important issue. Very few studies, however, have measured actual brain levels or even more relevant for the drug action, have estimated free extracellular concentrations of the currently available triptans. Moreover, differences between functional agonist potencies of the novel triptans and sumatriptan are usually not discussed as a potential explanation for sumatriptan's lack of central activity. The results of the present study led us to conclude that sumatriptan lacks central serotonergic activity after systemic administration because of its weaker 5-$HT_{1B/1D}$ receptor agonist potency and not because of lower extracellular brain levels compared with eletriptan and zolmitriptan. Keeping in mind that the present studies used a rodent model, it appears that the assumption that the novel triptans have an additional central effect because of better brain penetration than sumatriptan could be incorrect. In view of the evidence presented in this paper, the purported central effects of novel triptans, such as eletriptan and zolmitriptan, might be adequately explained by their greater functional 5-$HT_{1B/1D}$ agonist efficacy than that of sumatriptan.

Finally, since eletriptan is equipotent to zolmitriptan as a 5-HT_{1D} receptor agonist and shows comparable brain penetration, its potential central serotonergic activity in man will be similar to that of zolmitriptan. Although it is still controversial whether central effects play an important role in the antimigraine efficacy of 5-$HT_{1B/1D}$ receptor agonists, these findings suggest that activation of central 5-$HT_{1B/1D}$ receptors may contribute to eletriptan's potent antimigraine activity (Goadsby et al., 2000).

References

Andrew, P.D., Birch, H.L., Phillpot, D.A., 1993. Determination of sumatriptan succinate in plasma and urine by high-performance liquid chromatography with electrochemical detection. J. Pharm. Sci. 82, 73–76.

Casanovas, J.M., Hervas, I., Artigas, F., 1999. Postsynaptic 5-HT_{1A} receptors control 5-HT release in the rat medial prefrontal cortex. NeuroReport 10, 1441–1445.

Ceci, A., Baschirotto, A., Borsini, F., 1994. The inhibitory effect of 8-OH-DPAT on the firing activity of dorsal raphe serotoninergic neurons in rats is attenuated by lesion of the frontal cortex. Neuropharmacology 33, 709–713.

Cheng, Y.-C., Prusoff, W.H., 1973. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I50) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Clarke, N.P., Johnson, D.E., Rollema, H., Leishman, D.J., Wallis, R.M.,

McHarg, A.D., 1999. Central binding sites and serotonergic effects of eletriptan. Soc. Neurosci. Abstr. 25, 175.

Cremers, T.I.F.H., De Boer, P., Liao, Y., Den Boer, J.A., Westerink, B.H.C., Wikström, H.V., 2000. Augmentation with a 5-HT_{1A}, but not a 5-HT_{1B} receptor antagonist critically depends on the dose of citalopram. Eur. J. Pharmacol. 397, 63–74.

Cumberbatch, M.J., Hill, R.G., Hargreaves, R.J., 1997. Rizatriptan has central antinociceptive effects against durally evoked responses. Eur. J. Pharmacol. 328, 37–40.

Cumberbatch, M.J., Hill, R.G., Hargreaves, R.J., 1998a. The effects of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptor agonists on trigeminal nociceptive neurotransmission in anaesthetized rats. Eur. J. Pharmacol. 362, 43–46.

Cumberbatch, M.J., Hill, R.G., Hargreaves, R.J., 1998b. Differential effects of the $5HT_{1B/1D}$ receptor agonist naratriptan on trigeminal versus spinal nociceptive responses. Cephalalgia 18, 659–663.

De Lange, E.C.M., Danhof, M., DeBoer, A.G., Breimer, D.D., 1997. Methodological considerations of intracerebral microdialysis in pharmacokinetic studies on drug transport across the blood–brain barrier. Brain Res. Rev. 25, 27–49.

Goadsby, P.J., Hoskin, K.L., 1996. Inhibition of trigeminal neurons by intravenous administration of the serotonin $(5HT)_{1B/D}$ receptor agonist zolmitriptan (311C90): are brain stem sites therapeutic target in migraine? Pain 67, 355–359.

Goadsby, P.J., Hoskin, K.L., 1999. Differential effects of low dose of CP122,288 and eletriptan on fos expression due to the stimulation of the superior sagittal sinus in the cat. Pain 82, 15–22.

Goadsby, P.J., Knight, Y.E., 1997a. Direct evidence for central sites of action of zolmitriptan (311C90): an autoradiographic study in cat. Cephalalgia 17, 153–158.

Goadsby, P.J., Knight, Y., 1997b. Inhibition of trigeminal neurones after intravenous administration of naratriptan through an action at 5-hydroxytryptamine (5-$HT_{1B/1D}$) receptors. Br. J. Pharmacol. 122, 918–922.

Goadsby, P.J., Ferrari, M.D., Olesen, J., Stovner, L.J., Senard, J.M., Jackson, N.C., Poole, P.H., 2000. Eletriptan in acute migraine: a double-blind, placebo-controlled comparison to sumatriptan. Neurology 54, 156–163.

Hertel, P., Nomikos, G.G., Svensson, T.H., 1999. The antipsychotic drug risperidone interacts with auto- and hetero-receptors regulating serotonin output in the rat prefrontal cortex. Neuropharmacology 38, 1175–1184.

Hjorth, S., Tao, R., 1991. The putative 5-HT_{1B} receptor agonist CP-93,129 suppresses rat hippocampal 5-HT release in vivo: comparison with RU 24969. Eur. J. Pharmacol. 209, 249–252.

Kaube, K., Hoskin, K.L., Goadsby, P.J., 1993. Inhibition by sumatriptan of central trigeminal neurones only after blood–brain barrier disruption. Br. J. Pharmacol. 109, 788–792.

Knobelman, D.A., Kung, H.F., Lucki, I., 2000. Regulation of extracellular concentrations of 5-hydroxytryptamine (5-HT) in mouse striatum by 5-HT_{1A} and 5-HT_{1B} receptors. J. Pharmacol. Exp. Ther. 292, 1111–1117.

Koe, B.K., Nielsen, J.A., Macor, J.E., Heym, J., 1992. Biochemical and behavioral studies of the 5-HT_{1B} receptor agonist, CP-94,253. Drug Dev. Res. 26, 241–250.

Lambert, G.A., Donaldson, C., Zagami, A.S., 2000. Eletriptan: selective effect on craniovascular second order sensory neurones when administered intravenously or iontophoretically. Cephalalgia 20, 397.

Martin, G.R., 1997. Pre-clinical pharmacology of zolmitriptan (Zomig™; formerly 311C90), a centrally and peripherally acting $5HT_{1B/1D}$ agonist for migraine. Cephalalgia 18, 4–14, Suppl.

Millan, M.J., Lejeune, F., Gobert, A., 2000. Reciprocal autoreceptor and heteroreceptor control of serotonergic, dopaminergic and noradrenergic transmission in the frontal cortex: relevance to the actions of antidepressant agents. J. Psychopharmacol. 12, 114–138.

Moret, C., Briley, M., 1997. 5-HT autoreceptors in the regulation of 5-HT release from guinea pig raphe nucleus and hypothalamus. Neuropharmacology 36, 1713–1723.

Napier, C., Stewart, M., Melrose, H., Hopkins, B., McHarg, A., Wallis, R., 1999. Characterisation of the 5-HT receptor binding profile of eletriptan and kinetics of [^{3}H]eletriptan binding at human 5-HT_{1B} and 5-HT_{1D} receptors. Eur. J. Pharmacol. 368, 259–268.

O'Connor, D., Capel, C., Rycroft, W., Tattersall, F.D., Locker, K., Sohal, B., Graham, M.I., Evans, D.C., 1997. Influence of physicochemistry on the brain penetration of the triptans in rat. Poster at XIV Helsinki Univ. Course on Drug Research, Helsinki, Finland.

O'Connor, D., Scott-Stevens, P., Beer, M.S., Tattersall, F.D., Rycroft, W., Cumberbatch, M.J., Hargreaves, R.J., Evans, D.C., 1999. Importance of central sites of action for the anti-migraine efficacy of 5-$HT_{1B/1D}$ agonists. Proceedings of the 9th North American ISSX Meeting, Nashville, TN, vol. 15, Abstr 339.

Pauwels, P.J., John, G.W., 1999. Present and future of 5-HT receptor agonists as antimigraine drugs. Clin. Neuropharmacol. 22, 123–136.

Pauwels, P.J., Tardiff, S., Palmier, C., Wurch, T., Colpaert, F.C., 1997. How efficacious are 5-$HT_{1B/1D}$ receptor ligands: an answer from GTPγS binding studies with stably transfected C6-glial cell lines. Neuropharmacology 36, 499–512.

Paxinos, G., Watson, C., 1997. The Rat Brain in Stereotaxic Coordinates. 3rd edn. Academic Press, San Diego.

Roberts, C., Price, G.W., Jones, B.J., 1997. The role of 5-$HT_{1B/1D}$ receptors in the modulation of 5-hydroxytryptamine levels in the frontal cortex of the conscious guinea pig. Eur. J. Pharmacol. 326, 23–30.

Rollema, H., Lu, Y., Schmidt, A.W., Sprouse, J.S., Zorn, S.H., 2000. 5-HT_{1A} receptor activation contributes to ziprasidone-induced dopamine release in rat prefrontal cortex. Biol. Psychiatry 48, 229–237.

Russel, M.G.N., 1999. Recent developments in 5-HT_{1D} agonists. IDrugs 2, 37–43.

Schoenen, J., 1997. Acute migraine therapy: the newer drugs. Curr. Opin. Neurol. 10, 237–243.

Seeger, T.F., Seymour, P.A., Schmidt, A.W., Zorn, S.H., Schulz, D.W., Lebel, L.A., McLean, S., Guanowsky, V., Howard, H.R., Lowe III, J.A., Heym, J., 1995. Ziprasidone (CP-88,059): a new antipsychotic with combined dopamine and serotonin receptor antagonist activity. J. Pharmacol. Exp. Ther. 275, 101–113.

Sleight, A.H., Cervenka, A., Peroutka, S.J., 1990. In vivo effects of sumatriptan (GR43175) on extracellular levels of 5-HT in the guinea pig. Neuropharmacology 29, 511–513.

Stamford, J.A., Davidson, C., McLaughlin, D.P., Hopwood, S.E., 2000. Control of dorsal raphé 5-HT function by multiple 5-HT_1 autoreceptors: parallel purposes or pointless plurality? Trends Neurosci. 23, 459–465.

Tingley, F.D., Schmidt, A.W., Macor, J.E., Schulz, D.W., 1992. In vivo activity of CP 94253, a selective serotonin 5-HT_{1B} receptor agonist. Soc. Neurosci. Abstr. 18, 1519.

Trillat, A.C., Malagie, I., Scearce, K., Pons, D., Jacquot, A.M.C., Hen, R., Gardier, A.M., 1997. Regulation of serotonin release in the frontal cortex and ventral hippocampus of homozygous mice lacking 5-HT_{1B} receptors—in vivo microdialysis studies. J. Neurochem. 69, 2019–2025.