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Sumatriptan elicits both constriction and dilation in human and bovine brain intracortical arterioles

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1 Little is known about serotonin (5-HT) receptors present on brain microvessels that are innervated by brainstem serotonergic neurons. Using 5-HT, sumatriptan and subtype selective 5-HT_1 receptor agonists and/or the 5-HT_1 receptor antagonist GR127935, we characterized the 5-HT receptors involved in regulating microvascular tone of pressurized intracortical arterioles ($\sim$40–50 μm) isolated from human and bovine cerebral cortex. The role of nitric oxide (NO) on these responses was assessed with the N^{ω}-nitro-L-arginine (L-NNA, 10^{-5} M), an inhibitor of NO synthesis. Bovine pial arteries were studied for comparative purposes.

2 At spontaneous tone, 5-HT induced a dose-dependent constriction of human and bovine microarteries (respective pD_2 values of 7.3 ± 0.2 and 6.9 ± 0.1); a response potently inhibited by GR127935 (pIC_{50} value of 8.5 ± 0.1) in bovine microvessels.

3 In both species, the 5-HT_1 receptor agonist sumatriptan induced a biphasic response consisting of a small but significant dilation at low concentrations (1 and/or 10 nM) followed by a constriction at higher doses (pD_2 for contraction of 6.9 ± 0.1 and 6.6 ± 0.2 in human and bovine vessels, respectively). Pre-incubation with L-NNA abolished the sumatriptan-induced dilation and significantly shifted the dose-response of the constriction curve to the left. In contrast, the selective 5-HT_{1D} (PNU-109291) and 5-HT_{1F} (LY344864) receptor agonists were devoid of any vasomotor effect.

4 In bovine pial vessels, 5-HT and sumatriptan elicited potent constrictions (respective pD_2 of 7.2 ± 0.1 and 6.6 ± 0.1), a weak dilation being occasionally observed at low sumatriptan concentrations.

5 A significant negative correlation was observed between pial and intracortical vessels diameter and the extent of the dilatory response to 10^{-9} M sumatriptan. Together, these results indicate that sumatriptan, most likely *via* activation of distinctly localized microvascular 5-HT_{1B} receptors, can induce a constriction and/or a dilation which is sensitive to inhibition of NO synthesis and dependent on the size and, possibly, the existing tone of the vessels.
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Abbreviations: CBF, cerebral blood flow; EDTA, ethylenediaminetetra acetic acid; GR127935, N-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol3-yl)[1,1-biphenyl]-carbozamide hydrochloride; 5-HT, serotonin, 5-hydroxytryptamine; L-NNA, N^{ω}-nitro-L-arginine; LY344864, (R)-(+)-N-(3-dimethylamino-1,2,3,4-tetrahydro-9H-carbazol-6-yl)-4-fluorobenzamide; MOPS, morpholinopropanesulphonic acid; NO, nitric oxide; NOS, nitric oxide synthase; PNU-109291, (S)-(−)-1[1-[4-(4-methoxyphenyl)-1-piperazinyl]ethyl]-N-methylisochroman-6-carboxamide

Introduction

Serotonergic fibres from peripheral and central neurons are known to respectively innervate blood vessels in the extra- and intracerebral circulation, and to exert direct vasomotor effects (for review see Lincoln, 1995; Cohen *et al*., 1996). In brain intracortical microvascular bed, the source of perivascular 5-HT nerves has been identified as the raphe nucleus (Reinhard *et al*., 1979) which upon stimulation can induce either an increase (vasodilation) or a decrease (vasoconstriction) in cerebral blood flow (CBF) (for review Cohen *et al*., 1996). This dual response has been attributed to several factors such as the initial tone of the blood vessels (Rosenblum & Nelson 1990), the region of the dorsal raphe nucleus stimulated (Underwood *et al*., 1992), and the method used to measure blood flow (see Cohen *et al*., 1996). However, the recent identification of numerous 5-HT receptor subtypes (some of which are known to mediate dilation or constriction) in human brain microvascular tissues and cells in culture (Cohen *et al*., 1999), raises the possibility that these different vasomotor responses can be due to activation of different receptor populations. The presence of specific 5-HT receptors in distinct endothelial and/or smooth muscle compartments of the microcirculation further points to the possibility that they both can regulate vasomotricity, as was shown previously in other vascular beds following removal of the

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endothelial cell layer (Faraci & Heistad, 1992; MacLean *et al*., 1994).

Of the various receptors identified in the brain microcirculation, the 5-HT_{1B} (formally identified in man as the 5-$HT_{1D\beta}$) subtype is of particular interest in light of its known vasomotor role in both the extracerebral and peripheral circulation. This receptor has been implicated in the contraction (Hamel & Bouchard, 1991; Hamel *et al*., 1993b; Kaumann *et al*., 1993; Maassenvandenbrink *et al*., 1998; De Vries *et al*., 1999; Bouchelet *et al*., 2000) and endothelium-dependent relaxation (Schoeffter & Hoyer, 1990; Gupta, 1992) of blood vessels. In brain cortical blood vessels, the 5-HT_{1B} receptor protein has been localized to both the smooth muscle and endothelial cells of large microvessels (Riad *et al*., 1998) but not in the endothelial cells of capillaries (Riad *et al*., 1998; Cohen *et al*., 1999) implying a possible role for the endothelial receptor in larger microvessels. To date the only recorded *in vitro* response to 5-HT application in intraparenchymal arterioles has been constriction (Dacey & Basset, 1987). This contrasts with early studies which documented, using the cranial window technique, that brain surface arterioles and small pial arteries (<200 μm) would dilate while larger arteries (>200 μm) would constrict, in response to 5-HT administration (Harper & Mackenzie, 1977; Edvinsson *et al*., 1978; Auer *et al*., 1985).

In the present study, we examined the response(s) of bovine and human brain intracortical arterioles to exogenously applied 5-HT. We further used a selective 5-HT_1 receptor antagonist and subtype selective 5-HT_1 receptor agonists to identify the receptor subtype(s) involved in the observed vasomotor responses. Since some of 5-HT_1 receptors are specifically targeted by acute antimigraine drugs (e.g. the triptans), the present data may have implications in the treatment of this disease.

Methods

Intracortical penetrating arterioles isolated from human (biopsies of temporal and/or frontal cortex from patients undergoing surgery for the treatment of epilepsy obtained with approval from the Institution research ethics committee) and bovine (frontoparietal cortex, Abattoir Ecolait, Terrebonne, QC, Canada) cerebral cortex were mounted and maintained ($37\pm1^{\circ}$C, pH 7.4 ± 0.1) in an arteriograph chamber system (Living Systems Instrumentation, Burlington, VT, U.S.A.) following a procedure previously described in detail (Elhusseiny & Hamel, 2000; Dacey & Duling, 1982). Briefly, penetrating arterioles (average intraluminal diameter ~47 μm) were dissected from cortical slices ($1-2$ mm thick) cut parallel to the brain surface with the overlying pia-arachnoid membrane intact. Slices were secured in a petri dish containing cold (4°C, pH 7.4 ± 0.1) MOPS solution (in mM: of NaCl, 144; KCl, 3.0; $CaCl_2$, 2.5; $MgSO_4$, 1.5; glucose, 5; pyruvate, 2.0; ethylenediaminetetraacetic acid (EDTA), 0.02; morpholinopropanesulphonic acid (MOPS), 2.0; and NaH_2PO_4, 1.21) and the pial membrane was gently pulled away from the brain surface to expose the attached penetrating arteries (for more details, Dacey & Basset, 1987; Elhusseiny & Hamel, 2000). These were isolated, suctioned with dissection fluid into a small glass pipette and kept in cold buffer until cannulation. For comparison, pial vessels (intraluminal diameters ~180 μm) were isolated from the surface of the bovine cerebral cortex and prepared in the exact same manner as the intracortical vessels.

Arterioles were cannulated on a glass micropipette ($20-30$ μm diameter) at one end and sealed to another glass micropipette (~40 μm diameter) on the other end of the arteriograph chamber, and filled with a de-bubbled MOPS/albumin (albumin 1%). Pial vessels were cannulated with the exact same procedure except that the glass micropipette diameters were larger (~80 μm). A pressure-servo micropump (Living Systems Instrumentation) was used to maintain intraluminal pressure at 60 mmHg (Dacey & Basset, 1987). Vessels were then superfused (6 ml m^{-1}) with MOPS solution and allowed to stabilize and acquire basal tone (45 min$-$1 h). On-line measurements of intraluminal vessel diameters were performed using a closed circuit video system (National Electronics, Taiwan) coupled to a video caliper (Imagen Instrumentation, Trenton, New Jersey, U.S.A.) (see Elhusseiny & Hamel, 2000). All compounds were added to the superfusing MOPS solution and thus were applied extraluminally for a period of 3 min. Maximal smooth muscle contractility was tested with 70 mM K^+ in MOPS. In some bovine vessels, the relaxation induced by 10^{-5} M acetylcholine at basal tone was used as a reference endothelial-dependent vasodilation. This response has been shown to correspond to about 65% of the maximal dilation with papaverine (Elhusseiny & Hamel, 2000). At the end of each experiment, the smooth muscle relaxant papaverine (10^{-4} M) was added to maximally dilate the vessels.

Graded concentrations of 5-HT were added to the superfusion solution bathing isolated intracortical arterioles (bovine and human) or pial arteries (bovine) at basal tone. In order to identify the receptor subtype(s) involved in the 5-HT-mediated vasocontractile response, bovine intracortical arterioles were exposed to 10^{-6} M 5-HT following pre-incubation (15 min) with different concentrations ($10^{-10}-10^{-7}$ M) of the selective $5HT_1$ receptor antagonist GR127935 (N-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol3-yl)[1,1-biphenyl]-carbozamide hydrochloride; Skingle *et al*., 1996; GlaxoWellcome, Greenford, U.K.). The reproducibility of the 5-HT contraction was verified by sequential application (four times) of 10^{-6} M 5-HT to the vessel segments and recording any change in the resulting response. In addition, dose-response curves (10^{-11} or $10^{-10}-10^{-5}$ M) of selective 5-$HT_{1B/1D/1F}$ (sumatriptan; GlaxoWellcome, Greenford, U.K.), 5-HT_{1D} (PNU-109291: (S)-(−)-1[1-[4-(4-methoxyphenyl)-1-piperazinyl]ethyl]-N-methylisochroman-6-carboxamide; Ennis *et al*., 1998; Pharmacia and Upjohn, Kalmazoo, MI, U.S.A.) and 5-HT_{1F} (LY344864: (R)-(+)-N-(3-dimethyl-amino-1,2,3,4-tetrahydro-9H-carbazol-6-yl)-4-fluorobenzamide, Phebus *et al*., 1997; Eli Lilly, Indianapolis, IN, U.S.A.) receptor agonists were generated in bovine and/or human intracortical arterioles. The role of nitric oxide (NO) in the sumatriptan-induced dilation of bovine and human arterioles was assessed by pre-incubation (30 min) of the vessels with the nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-L-arginine (L-NNA,10^{-5} M) before generation of the dose-response curves. As a comparison, the vasomotor responses of small bovine pial vessels to increasing concentrations of 5-HT and sumatriptan were also evaluated. All compounds used were readily dissolved in H_2O.

Calculations and statistical analysis

Agonists maximal response (expressed as per cent change in vessel diameter from spontaneous or basal tone) and potency (pD_2 values or $-\log$ of EC_{50}) were determined, and the efficacy of the 5-HT_1 receptor antagonist GR127935 expressed as a pIC_{50} value (negative logarithm of the molar antagonist concentration which induces 50% inhibition of maximal response) calculated from the antagonist concentration curve.

Values are expressed as the mean $\pm$ s.e.mean. The statistical differences between the means of two measurements were determined by either paired or unpaired Students *t*-test for independent observations as described in the legend to each figure. One way ANOVA followed by a Dunnett comparison test was used to analyse the inhibitory effects of the antagonist on the 5-HT-induced constriction, and a one way ANOVA followed by a Bonferroni comparison test was used to test the effect of L-NNA inhibition on the sumatriptan response. In all cases, $P<0.05$ was considered significant. All statistics were performed on the statistical software Prism (GraphPad Software Inc.)

Results

At basal tone, 5-HT (10^{-11} to 10^{-4} M) constricted human and bovine intracortical arterioles, in a dose-dependent manner (Figure 1A,B) with respective pD_2 values of 7.3 ± 0.2 and 6.9 ± 0.1, and maximal responses accounting for $10.5\pm1.5\%$ and $19.9\pm3.1\%$ from spontaneous tone. This constriction amounted to $49.4\pm9.9\%$ of that elicited by 70 mM K^+ in the same bovine vessels ($n=8$). Repeated (four times) application of 10^{-6} M 5-HT to bovine arterioles did not affect the constrictor response to 5-HT (Figure 2A). However, increasing concentrations of the 5-HT_1 receptor antagonist GR127935 significantly ($P<0.01$) inhibited the 5-HT-induced constriction (Figure 2B) with a pIC_{50} value of 8.5 ± 0.1 and a maximal inhibition ($94.9\pm2.2\%$) at 10^{-7} M GR127935 (Figure 2B, inset).

The 5-HT_1 receptor agonist sumatriptan elicited a biphasic response consisting of a dilation at low concentrations followed by a constriction. The dilation, while small ($25.5\pm3.7\%$ of the dilation obtained with 10^{-5} M acetylcholine in bovine vessels ($n=3$)), was shown to be significantly different from baseline diameters at 10^{-9} and/or 10^{-8} M sumatriptan in human and bovine arterioles (Figure 1A,B). In both human and bovine vessels, sumatriptan elicited constrictions with respective potencies (pD_2 values $=6.9\pm0.1$ and 6.6 ± 0.2) and maximal effects from spontaneous tone ($4.9\pm2.3\%$ and $12.7\pm1.7\%$) which were slightly but significantly lower ($P<0.05$) than those induced by 5-HT. In bovine arterioles, the sumatriptan-induced contractions corresponded to $44.2\pm10.7\%$ of those evoked by 70 mM K^+ ($n=3$). Application of the selective 5-HT_{1D} and 5-HT_{1F} receptor agonists PNU-109291 and LY344864, respectively, did not produce any significant changes in bovine vessel diameters (Figure 1B).

In both species, pre-incubation of the vessel segments with the NOS inhibitor L-NNA (10^{-5} M) abolished the vasodilation observed at low sumatriptan concentrations (Figure 3A, B). In these L-NNA-treated vessels, the sumatriptan dose-dependent contraction was significantly shifted to the left in human and bovine vessels (respective pD_2 of 7.7 ± 0.2, $P<0.001$ and 7.1 ± 0.1, $P<0.01$). In human vessels, blockade of NO synthesis by L-NNA (10^{-5} M) also resulted in a significantly higher maximal contractile response to sumatriptan ($21.2\pm6.7\%$ as compared to $4.9\pm2.3\%$, $P<0.05$) (Figure 3A).

Finally, in order to identify if the sumatriptan-induced dilation is also present in extracerebral blood vessels, the

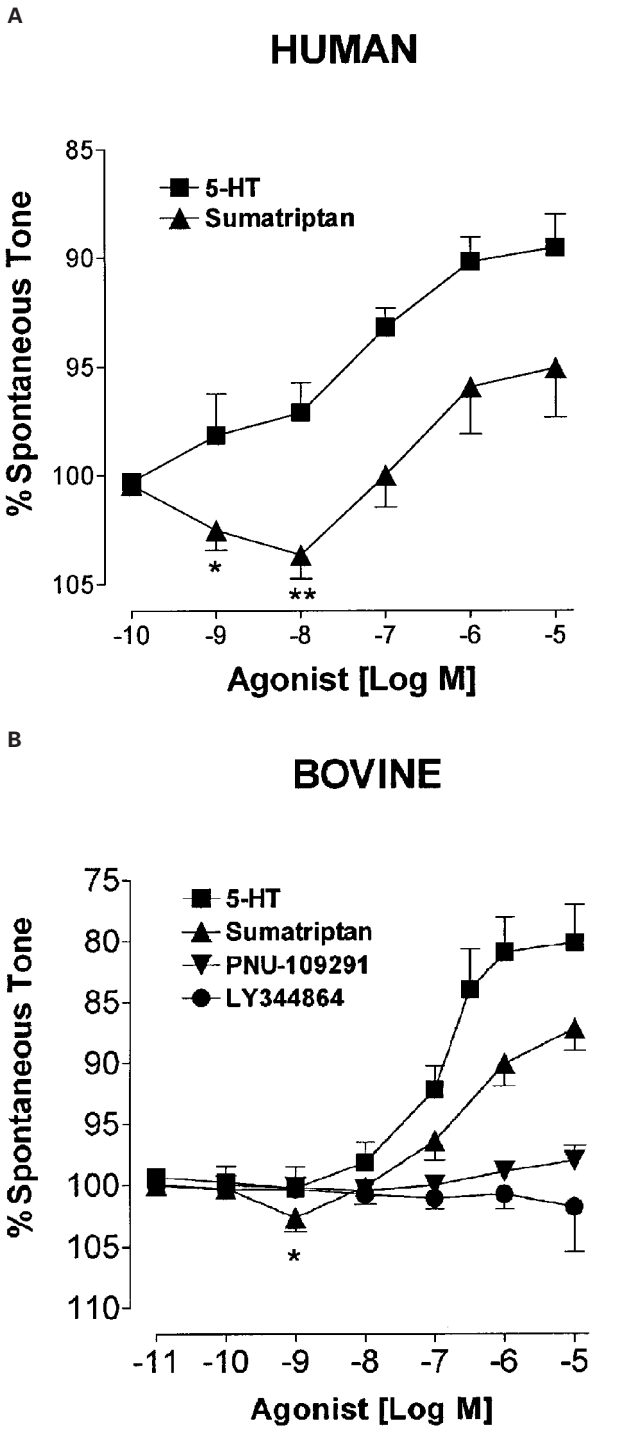

Figure 1 Concentration-dependent vasomotor responses to 5-HT, and selective 5-HT_1 receptor agonists in human (A: $n=3-8$) and/or bovine (B: $n=5-13$) intracortical arterioles. (*$P<0.05$, **$P<0.01$ by paired *t*-test from control).

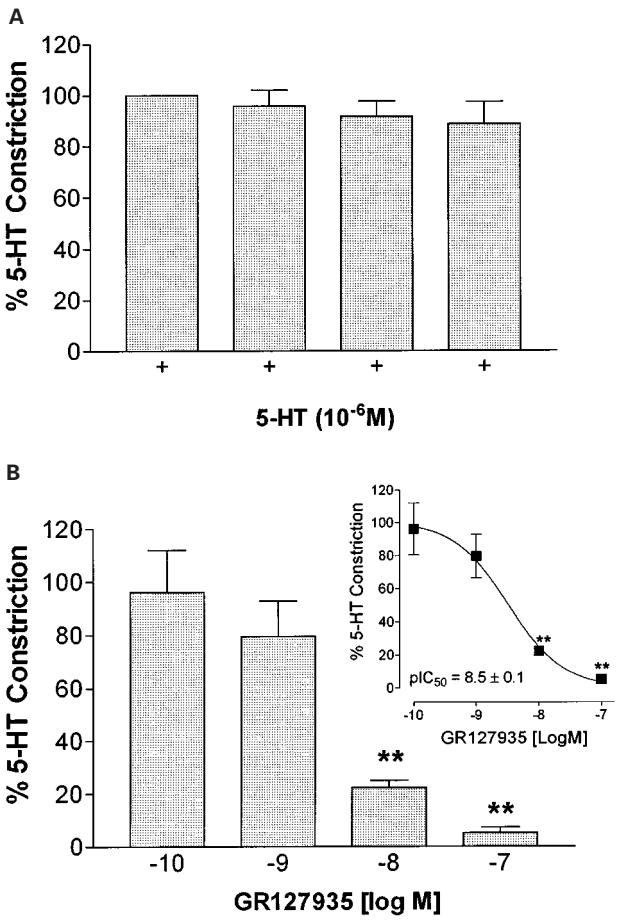

Figure 2 Repeated constriction upon application of 5-HT (10^{-6} M) to bovine intracortical arterioles in the absence (A), and presence (B) of graded concentrations of the 5-HT_1 receptor antagonist GR127935. Insert: inhibition concentration curve for GR127935 ($n=5$, $^{**}P<0.01$, by ANOVA).

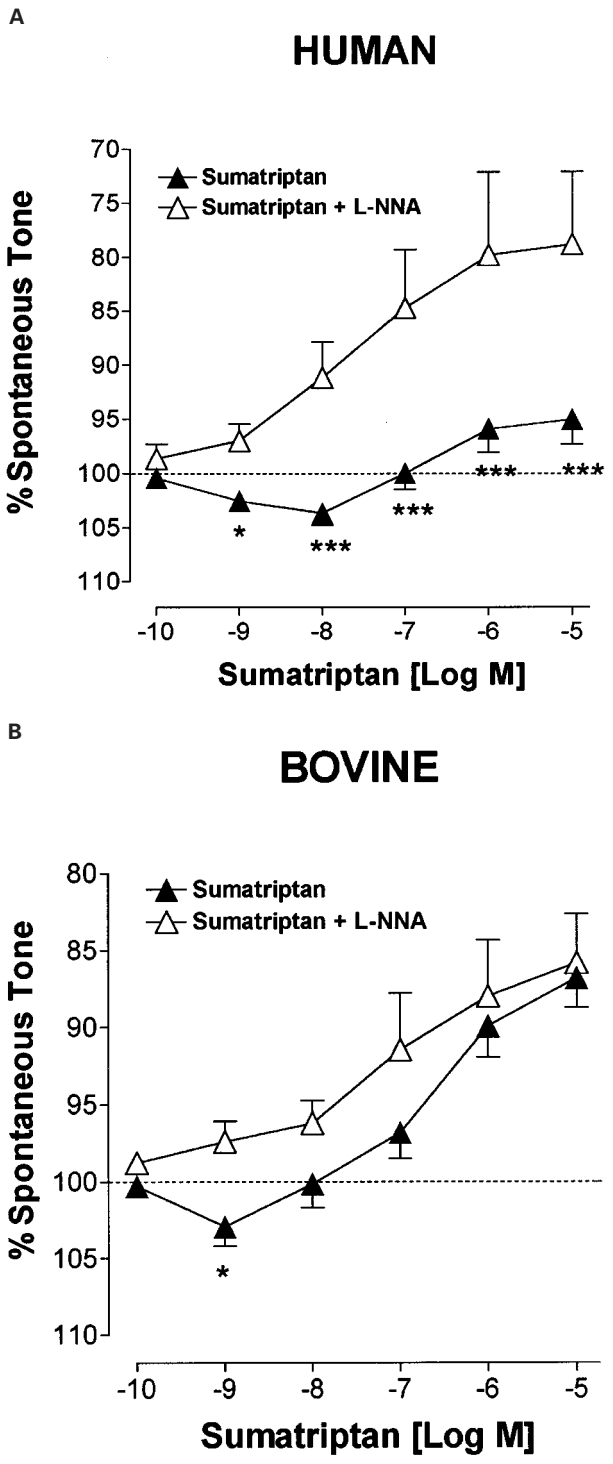

Figure 3 Effect of 10^{-5} M L-NNA on the sumatriptan-mediated vasomotor responses in (A), human ($n=5-8$, $^{*}P<0.05$, $^{***}P<0.001$, by ANOVA, B) and (B), bovine ($n=5-12$, $^{*}P<0.05$, by paired t-test) intracortical arterioles.

effect of 5-HT and sumatriptan was investigated in bovine small pial vessels (diameter $=179\pm34$, range $63-343$ μm; Figure 4). In these vessels, the predominant response to 5-HT and sumatriptan was a constriction of equipotent intensity ($25.9\pm2.5\%$ and $22.6\pm4.1\%$, respectively, from spontaneous tone) but sumatriptan was significantly less potent than 5-HT (respective pD_2 values of 6.6 ± 0.1 and 7.2 ± 0.02, $P<0.001$). A small but not significant dilation to low sumatriptan concentrations was also observed in about half (55.6%) of the pial vessels studied. The dilation was more pronounced in small size vessels than in large ones. In bovine, an overall significant negative correlation ($P<0.05$, $r=0.6$, slope $=-0.03\pm0.01$: Figure 5) was observed between vessel diameter at spontaneous tone (both intracortical and pial vessels) and increased diameter at 10^{-9} M sumatripan. This observation suggested that the dilation to sumatriptan was inversely related to vessel size with a break point at 201 μm (see Figure 5).

Discussion

The present *in vitro* findings demonstrate for the first time that 5-HT constricts human and bovine brain intracortical arterioles *via* a $5\text{-}HT_{1B}$ receptor as identified by the use of subtype selective 5-HT_1 receptor agonists and antagonist. Moreover, they show that low concentrations of the 5-HT_1 receptor agonist sumatriptan resulted in a small but significant dilation that was blocked by inhibition of NO synthesis, and overridden by a constriction at higher

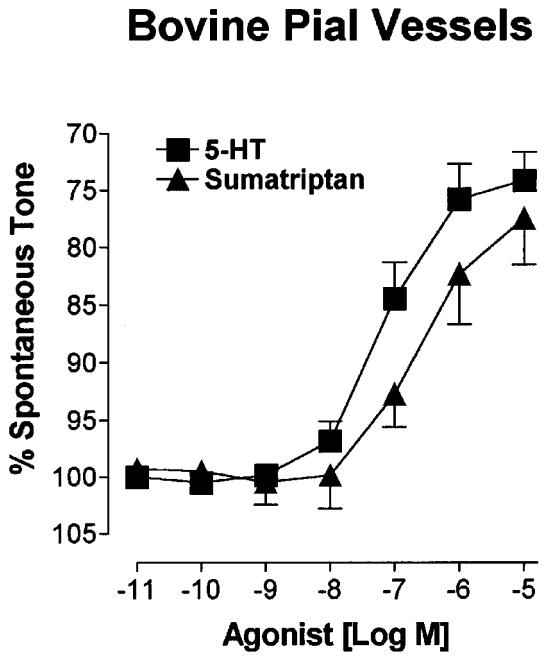

Figure 4 Dose-response curves to 5-HT ($n=9$) and sumatriptan ($n=6$) in bovine pial vessels.

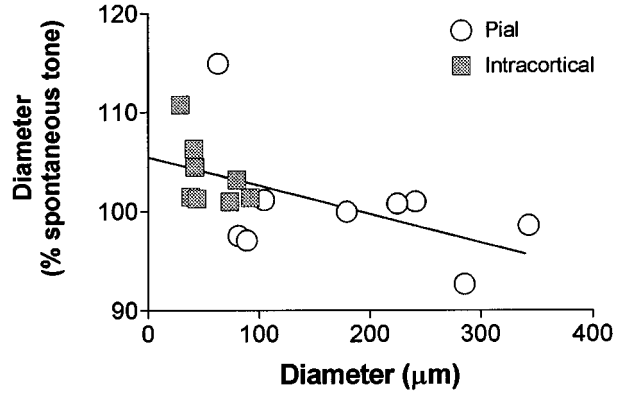

Figure 5 Correlation analysis between the vasomotor response to 10^{-9} M sumatriptan and the diameter of both pial ($n=7$) and intracortical ($n=9$) vessels ($r=0.6$, $P<0.05$).

concentrations or in large size vessels. Together with our previous localization of 5-HT_{1B} receptor in the endothelial and smooth muscle cells of intracortical arterioles (Riad *et al*., 1998), the present results suggest that, depending on its localization in the vessel wall, this receptor can mediate opposite vasomotor responses. We suggest that the NO-dependent dilation is mediated by an endothelial 5-HT_{1B} receptor and that its magnitude depends on the size of the vessel; small vessels will tend to dilate more than large ones, with vessels larger than 200 μm showing primarily, if not exclusively, a constriction.

The overall potency for 5-HT in constricting intracortical vessels compared very well with that reported in extracerebral blood vessels from both human and bovine brain (Edvinsson *et al*., 1978, Parsons *et al*., 1989; Hamel & Bouchard 1991; Hamel *et al*., 1993b; Bouchelet *et al*., 2000). No significant and reproducible dilation was observed with 5-HT, a finding that may appear at variance with previous studies which showed that 5-HT can act as a vasodilator under certain conditions (Cohen *et al*., 1983; Cocks & Angus, 1983; Potenza *et al*., 1998), especially in small size cerebral (Harper & MacKenzie, 1977; Edvinsson *et al*., 1978; Auer *et al*., 1985) and peripheral (Lamping, 1997) arteries and arterioles. However, the 5-HT_1 receptor agonist sumatriptan systematically and significantly dilated bovine and human intracortical arterioles at low concentrations while at higher doses, it elicited contraction, findings which suggest that a 5-HT_1 receptor is involved in both vasomotor responses.

Further, the participation of a 5-HT_{1B} receptor in the 5-HT_1-mediated constriction of bovine intracortical arterioles was suggested by the ability of the 5-HT_1 receptor antagonist GR127935 to potently antagonize this response, with an affinity ($pIC_{50}=8.5$) compatible with that reported for this compound at the 5-HT_{1B} receptors in large cerebral arteries (Skingle *et al*., 1996; Teng *et al*., 1998; Bouchelet *et al*., 2000). The failure of selective 5-HT_{1D} (PNU-109291) and 5-HT_{1F} (LY344864) receptor agonists – which exhibit affinities in the nM range for their respective receptor subtypes (Ennis *et al*., 1998; Phebus *et al*., 1997) – to mimic this response at concentrations as high as 10^{-5} M provided additional arguments that the arteriolar vasoconstriction is mediated by a 5-HT_{1B} receptor. The high level of expression of 5-HT_{1B}, and the virtual and complete absence of 5-HT_{1D} and 5-HT_{1F} receptor mRNAs in smooth muscle cells derived from human intracortical microvessels (Cohen *et al*., 1999), also argues for a role of 5-HT_{1B} receptors in mediating microvascular constriction, in agreement with their previously identified role in peripheral (Ennis *et al*., 1998; Johnson *et al*., 1998; Verheggen *et al*., 1998; Phebus *et al*., 1997; De Vries *et al*., 1999) and extracerebral (Bouchelet *et al*., 2000) blood vessels.

However, a novel finding from the present study was that low concentrations of sumatriptan exerted dilation of human and bovine brain intracortical arterioles, a response which was blocked by inhibition of NO synthesis by L-NNA. The possibility that the sumatriptan-induced relaxation was prevented by NOS inhibitors *via* increase in endogeneous tone (~20% in both species) (Elhusseiny & Hamel, 2000) cannot be totally ruled out. However, an endothelial, NO-dependent vasodilation appears more likely as such a response to 5-HT and/or sumatriptan has been documented before in cerebral (Faraci & Heistad, 1992) and peripheral (Cocks & Angus, 1983; Cohen *et al*., 1983; Schoeffter & Hoyer, 1990; Whiting & Cambridge 1995) blood vessels and suggested to be mediated by an endothelial 5-HT_1 (Molderings *et al*., 1989; Whiting & Cambridge, 1995; Lamping, 1997), most likely 5-HT_{1B} or 5-HT_{1D} (Schoefter & Hoyer 1990; Gupta *et al*., 1992), receptor subtype. Additionally, endothelial 5-HT_{1B} receptors albeit from coronary arteries have been identified (Ullmer *et al*., 1995; Ishida *et al*., 1998; Nilsson *et al*., 1999b), and recently found to promote NO synthesis (Ishida *et al*., 1998), an observation fully compatible with our present findings in brain microvessels. As neither the selective 5-HT_{1D} or 5-HT_{1F} receptor agonist, PNU109209 or LY344864, could elicit a vasodilatory response in our preparation, we conclude that the dilation of intracortical arterioles, like

the vasoconstriction, is mediated by 5-HT_{1B} receptors. Such statement also agrees with our ultrastructural localization of 5-HT_{1B} receptor protein in endothelial cells of large intraparenchymal microvessels (Riad *et al*., 1998).

The dilatory response was not restricted to intracortical vessels, as some pial vessels also dilated to low sumatriptan concentrations, a response which was inversely proportional to the diameter of the vessel at basal tone. Intracortical and/or pial vessels with diameters less than 200 μm were more likely to dilate, while larger vessels typically constricted, similar to earlier *in situ* reports in pial vessels (Edvinson *et al*., 1978; Harper & MacKenzie 1977; Auer *et al*., 1985). Differences in tone (Harper & MacKenzie, 1977), blood-brain barrier permeability (Auer *et al*., 1985) and 5-HT receptor subtypes (Lamping, 1997) between large and small vessels have been proposed as possible explanations for this effect. Alternatively, it could be that as vessels get larger, they contain more layers of smooth muscle cells endowed with contractile 5-HT_{1B} receptors (Hamel *et al*., 1993a; Bouchelet *et al*., 1996; Riad *et al*., 1998; Cohen *et al*., 1999; Nilsson *et al*., 1999a,b), which eventually pushes the equilibrium between endothelial and smooth muscle receptors (Riad *et al*., 1998; Nilsson *et al*., 1999a,b) towards constriction. This could explain why the majority of previous *in vitro* studies with large isolated cerebral vessels did not observe the vasodilatory effect. Moreover, 5-HT failed to induce a significant dilation of intracortical arterioles, possibly because it has the capacity, at least in bovine, to activate not only vasocontractile 5-HT_{1B} but also sumatriptan-insensitive 5-HT_{2A} receptors (Hamel *et al*., 1993b; Bouchelet *et al*., 2000; Kauman *et al*., 1993), a double interaction which could mask the 5-HT_{1B}-mediated vasodilator response. It could also be that, like recently reported in rat mesenteric small resistance vessels (Potenza *et al*., 1998), the relaxant potency and maximal response of sumatriptan are greater than those of 5-HT, thus allowing the vasodilatory response to be readily detected.

Incubation with L-NNA blocked the sumatriptan-induced dilation, shifted the contraction dose-response curve for sumatriptan to the left and, in human arterioles, significantly increased the maximal contractile response. Sumatriptan-(Whiting & Cambridge, 1995) and 5-HT-induced vasoconstrictions have previously been shown to increase upon endothelial denudation or application of NOS inhibitors (Cocks & Angus, 1983; Sercombe *et al*., 1985; Faraci & Heistad, 1992). We suspect that the potentiating effect of NOS inhibition of the sumatriptan-induced microarteriolar contractile response results from several factors including inhibition of NO production and release following activation of endothelial 5-HT_{1B} receptors (Whiting & Cambridge, 1995; Ishida *et al*., 1998), reduction in the constitutive endothelial NO synthesis by intracerebral arterioles (Kimura *et al*., 1994; Fergus *et al*., 1996; Elhusseiny & Hamel, 2000) and, possibly, as reported in pial vessels (Thorin *et al*., 1998) the release of other constrictor agents such as endothelin. The more pronounced potentiating effect of L-NNA in human than bovine arterioles could be explained by possible species differences in 5-HT_{1B} receptor density in smooth muscle and endothelial cells, or by the recently observed differences in NOS sensitivity to L-NNA between bovine and human intracortical arterioles (see Elhusseiny & Hamel, 2000).

Notably, the sumatriptan-induced vasoconstrictions occurred at slightly but significantly higher concentrations and were smaller in magnitude than those elicited by 5-HT in bovine and human microvessels. The greater response to 5-HT may be ascribed, at least in bovine vessels, to its possible interaction with 5-HT_{2A} receptors, a response that would be blocked by the highest concentration (10^{-7} M) of GR127935 used as this compound exhibits non-negligible affinity at these sites ($pK_i = 7.4$) (Skingle *et al*., 1996). However, it should be noted that the maximal constriction to sumatriptan in intracortical arterioles was calculated as the change from spontaneous tone, i.e. the baseline diameter of the vessel. If calculated as the change from the maximal dilation, then the maximal constrictions to sumatriptan (9.3 ± 2.2 and 15.0 ± 1.9% from dilation, respectively in human and bovine vessels) are not statistically different from those elicited by 5-HT (10.5 ± 1.5% and 9.9 ± 3.1 from basal tone), observations which suggest that the dilatory component of the response contributes to this apparent reduction in the magnitude of the sumatriptan-induced contraction. These observations may suggest that 5-HT_{1B} receptors are able to mediate a full contractile response in bovine intracortical arterioles, similar to what has been observed in ovine and/or bovine small cerebral arteries (Teng *et al*., 1998) as well as in vessels with vascular tone (MacLean *et al*., 1994), in which constriction to 5-HT tends to be preferentially mediated by the 5-HT_{1B} as opposed to the 5-HT_{2A} receptor.

In conclusion, this study provides the first functional evidence for a dual role of 5-HT_{1B} receptors in human and bovine brain microvessels. Identification of a dual response, which appears to depend on vessel size, could be an intrinsic feedback mechanism aimed at preventing excessive 5-HT-induced responses in smaller vessels. It also provides a basis for previous physiological findings which reported increases or decreases in local CBF upon stimulation of the raphe nucleus (see Cohen *et al*., 1996). It is thus possible that, in addition to the subregions of the raphe being stimulated and the intrinsic tone of the vessels, the equilibrium between endothelial (dilatory) and muscular (contractile) 5-HT_{1B} receptors might be an important contributing factor to the final adaptation of local blood flow to neuronal activity following raphe stimulation and 5-HT release. Further, since the present finding in microarterioles can be extrapolated to larger pial vessels which have recently been shown to be endowed with endothelial 5-HT_{1B} receptors (Nilsson *et al*., 1999a), the identification of a NO producing 5-HT_{1B} receptor becomes particularly interesting in light of the suggested role for endothelial NO-generating receptors in the aetiology of migraine headache (Schmuck *et al*., 1996, Fozard & Kalkman 1994).

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