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Sumatriptan (GR43175) inhibits cyclic-AMP accumulation in dog isolated saphenous vein

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Sumatriptan (GR43175) contracts rings of dog isolated saphenous vein by an action at 5-HT_1-like receptors. We have now examined the effects of sumatriptan on prostaglandin E_2 (PGE_2)-stimulated adenosine 3':5'-cyclic monophosphate (cyclic AMP) accumulation in this tissue. Sumatriptan and 5-hydroxytryptamine (5-HT) produced a concentration-dependent inhibition of PGE_2-stimulated cyclic AMP accumulation (EC_{50} values of 250 nM and 80 nM respectively), responses that were mimicked by 5-carboxamidotryptamine but not by U-46619 or methoxamine. The response to sumatriptan (1 μM) was antagonised by methiothepin (1 μM), but not by metergoline (0.1 μM), spiperone (1 μM) or ondansetron (GR38032, 1 μM). These results suggest that 5-HT_1-like receptors which mediate contraction of the dog isolated saphenous vein are negatively coupled to adenylate cyclase in this preparation.

Introduction Sumatriptan (GR43175) is a novel 5-HT_1-like receptor agonist which shows a high degree of selectivity for the 5-HT_1-like receptor mediating smooth muscle contraction in dog isolated saphenous vein (Humphrey *et al.*, 1988). It has been shown that sumatriptan has modest affinity for 5-HT_1 binding sites, with greatest affinity for the 5-HT_{1D} subtype which seems similar, but not identical to the 5-HT_1-like receptor in dog saphenous vein (Humphrey *et al.*, 1988; Peroutka & McCarthy, 1989; Sumner & Humphrey, 1989). Interestingly, 5-HT_{1D} binding sites appear to be negatively coupled to adenylate cyclase (Schoeffter *et al.*, 1988). We therefore sought to explore the receptor coupling mechanism for the 5-HT_1-like receptor in dog isolated saphenous vein. We now report that this receptor appears to be negatively coupled to adenylate cyclase in this preparation.

Methods Lengths of saphenous vein (4–7 cm long) were removed from beagle dogs (Glaxo, 7–10 kg of either sex) either during anaesthesia or after being killed by an overdose of sodium pentobarbitone. These tissues were used immediately or after storage overnight at 4°C in gassed (95% O_2, 5% CO_2), modified Krebs solution (Apperley *et al.*, 1976). Rings of 2–3 mm in length were pretreated for 30 min with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (100 μM) and used for measurements of adenosine 3':5'-cyclic monophosphate (cyclic AMP) formation and protein content as previously described (Sumner *et al.*, 1989). Results are expressed as arithmetic means ± s.e.mean of '*n*' observations, which is also the number of veins used. Student's unpaired *t* test was used to assess the significance of differences between mean values.

Results Sumatriptan (10 μM) reduced both basal and prostaglandin E_2 (PGE_2, 5 μM)-stimulated cyclic AMP accumulation in rings of dog isolated saphenous vein (basal: control 6.35 ± 1.23, sumatriptan 3.12 ± 0.61 pmol mg^{-1} protein, $n = 6$; PGE_2-stimulated: control 11.47 ± 1.48, sumatriptan 6.16 ± 1.34 pmol mg^{-1} protein, $n = 6$). The stimulation of cyclic AMP accumulation by PGE_2 (5 μM) and its inhibition by sumatriptan (10 μM) followed similar time courses, both effects being maximal after 1–2 min at 37°C (results not shown). Accordingly an incubation time of 2 min was used throughout the study. Sumatriptan and 5-hydroxytryptamine (5-HT) produced a concentration-dependent inhibition of PGE_2-stimulated cyclic AMP accumulation (Figure 1a), with both agonists producing a maximum of about 50% inhibition. Based upon this level of effect, the EC_{50} values for sumatriptan and 5-HT were 250 nM and 80 nM respectively ($n = 7$). The archetypal 5-HT_1-like receptor agonist, 5-carboxamidotryptamine (5-CT), also inhibited PGE_2-stimulated cyclic

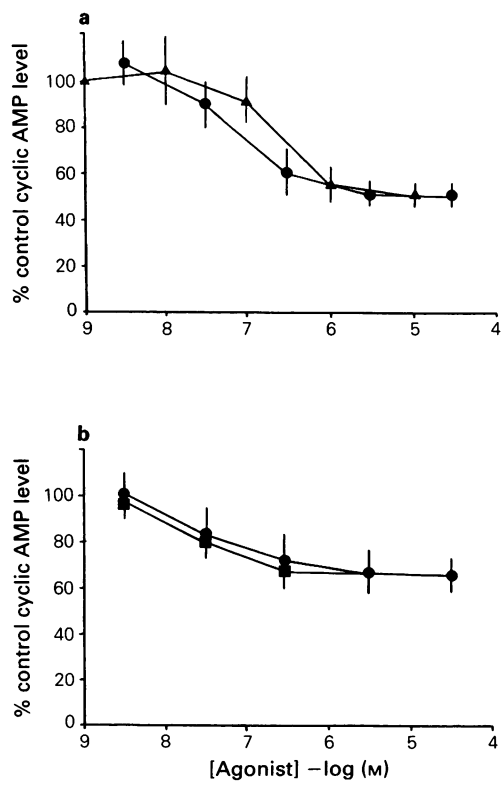

Figure 1 Inhibition of prostaglandin E_2 (PGE_2, 5 μM)-stimulated adenosine 3':5'-cyclic monophosphate (cyclic AMP) accumulation in rings of dog isolated saphenous vein by 5-hydroxytryptamine (5-HT, ●) and sumatriptan (▲), (a) or by 5-HT (●) and 5-carboxamidotryptamine (5-CT, ■) (b). Calculated pEC_{50} values were 7.10, 6.60, 7.47 and 7.85 respectively, control levels being 9.43 ± 1.07 (a) and 14.02 ± 1.99 (b) pmol cyclic AMP mg^{-1} protein. Results are means of 7 determinations (14 rings) with vertical lines showing s.e.mean.

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AMP accumulation (Figure 1b) with an EC_{50} value of 14 nM compared to 34 nM for 5-HT ($n = 7$). The contractile agonists U-46619 (11,9 epoxymethano-PGH_2) (1 μM) and methoxamine (10 μM) however, did not significantly inhibit PGE_2-stimulated cyclic AMP accumulation (levels of 16.54 ± 1.86 pmol mg^{-1} protein and 10.88 ± 1.54 pmol mg^{-1} protein, respectively, compared to a value for sumatriptan (10 μM) of 6.77 ± 0.43 pmol mg^{-1} protein, and a common control of 13.50 ± 0.64 pmol mg^{-1} protein, $n = 6$ for each). The inhibition of PGE_2-stimulated cyclic AMP accumulation (control 11.39 ± 0.92 pmol mg^{-1} protein ($n = 6$)) by a submaximal concentration (1 μM) of sumatriptan (7.96 ± 0.88 pmol mg^{-1} protein) was attenuated by methiothepin (1 μM; 12.29 ± 1.18 pmol mg^{-1} protein) but not by spiperone (1 μM), ondansetron (GR38032, 1 μM) or metergoline (0.1 μM) (levels of 8.21 ± 0.82, 7.18 ± 0.83 and 7.30 ± 0.88 pmol mg^{-1} protein respectively, $n = 6$ for each). None of these antagonists had any appreciable effect on PGE_2-stimulated cyclic AMP accumulation in their own right at these concentrations.

Discussion The inhibition of cyclic AMP accumulation (either basal or PGE_2-stimulated) by sumatriptan in rings of dog isolated saphenous vein provides the first indication that receptors for this agonist in smooth muscle may be negatively coupled to adenylate cyclase. Thus the receptors mediating both smooth muscle contraction (Humphrey *et al.*, 1988) and inhibition of cyclic AMP accumulation (this study) in this tissue appear identical and represent a sub-type of the 5-HT_1-like receptor class. In keeping with this, 5-HT was some 3–4 fold more potent than sumatriptan, with 5-CT being approximately twice as potent as 5-HT in this study. These relative potencies are almost the same as those for producing contraction in this preparation (Humphrey *et al.*, 1988). In addition, both the contraction of smooth muscle and the inhibition of cyclic AMP accumulation evoked by sumatriptan can be antagonized by methiothepin, but not by metergoline, spiperone, or ondansetron (Humphrey *et al.*, 1988; this study). Inhibition of adenylate cyclase has been implicated as the coupling mechanism for 5-HT_{1A} (Schoeffter & Hoyer, 1988), 5-HT_{1B} (Bouhelal *et al.*, 1988) and 5-HT_{1D} (Schoeffter *et al.*, 1988) binding sites in brain homogenates. Furthermore, sumatriptan shows greatest affinity for 5-HT_{1B} and 5-HT_{1D} binding sites (Peroutka & McCarthy, 1989; Hoyer & Middlemiss, 1989; Sumner & Humphrey, 1989). The 5-HT_1-like receptor in dog saphenous vein shows many similarities to 5-HT_{1D} binding sites (and 5-HT_{1B} sites in rats). There is, however, one obvious exception, namely the lack of effect of metergoline (0.1 μM) in the dog saphenous vein as already described, yet this antagonist shows a high affinity for 5-HT_{1D} binding sites in both radioligand binding and adenylate cyclase assays (Hoyer & Middlemiss, 1989). Despite this exception, there is a lot of data to suggest that the subtype of 5-HT_1-like receptor which subserves the responses to sumatriptan in the dog saphenous vein has its counterpart in 5-HT_{1D} (or 5-HT_{1B} in rats) binding sites, although the apparent heterogeneity of 5-HT_{1D} binding sites may confuse interpretation (Sumner & Humphrey 1989). Finally, although further experiments are required to reach definitive conclusions, it is interesting to note that smooth muscle contraction evoked by U-46619 and methoxamine in the dog saphenous vein was not accompanied by changes in cyclic AMP levels. Thus mechanistically, there does not appear to be an obligatory control of smooth muscle contraction by reduction of cyclic AMP levels in this vessel.

In summary, this study suggests that the subtype of 5-HT_1-like receptor which mediates sumatriptan-induced vascular smooth muscle contraction in dog saphenous vein is coupled to the inhibition of adenylate cyclase in this vessel. This further reinforces the similarity between this 5-HT_1-like receptor and the 5-HT_{1D} binding site.

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