Donitriptan, a Unique High-Efficacy 5-HT\textsubscript{1B/1D} Agonist: Key Features and Acute Antimigraine Potential

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ABSTRACT

We hypothesized that the limited acute therapeutic effectiveness of tryptamine derivatives in alleviating migraine headache could be explained by the relatively low intrinsic activity of these agents at 5-HT\textsubscript{1B/1D} receptors. Donitriptan is a novel arylpiperazide 5-hydroxytryptamine (5-HT) derivative which was designed to exploit the higher potency and efficacy properties of 5-HT compared to tryptamine at 5-HT\textsubscript{1B/1D} receptors.

In vitro, donitriptan has subnanomolar affinity for nonhuman and human 5-HT\textsubscript{1B/1D} receptors and micromolar affinity for the 5-HT\textsubscript{1P} subtype. Donitriptan potently inhibited forskolin-induced cAMP formation and enhanced specific GTP\textsuperscript{35S} specific binding to a greater extent than tryptamine derivatives and equivalent to 5-HT in C6 cells expressing human 5-HT\textsubscript{1B} or 5-HT\textsubscript{1D} receptors. Donitriptan produced more potent and larger amplitude increases in hyperpolarizing Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current than sumatriptan in guinea pig isolated trigeminal ganglion neurons, and was more potent than tryptamine derivatives in eliciting contractile responses in rabbit isolated saphenous vein rings.

In vivo, donitriptan evoked more potent, longer-lasting and greater amplitude carotid vasoconstrictor responses than tryptamine derivatives in anesthetized pigs; and in contrast to sumatriptan, naratriptan or zolmitriptan, produced long-lasting, dose-dependent decreases in unilateral carotid blood flow in conscious dogs at doses from 0.63 mg/kg p.o. without affecting heart rate or behavior. Oral donitriptan also evoked hypothermic responses in guinea pigs suggesting that the compound gains access to the brain.

Donitriptan is thus a selective, potent 5-HT\textsubscript{1B/1D} receptor agonist which can be distinguished from tryptamine derivatives in consistently exerting high intrinsic activity at these receptors in a series of vascular and neuronal models relevant to migraine. Advantages in terms of therapeutic effectiveness in the acute relief of migraine headache over currently
available triptans can be expected to include greater response rates and consistency of pain relief, a lower incidence of migraine recurrence and better tolerability. The acute anti-migraine potential of the first high efficacy $5\text{-HT}_{1B/1D}$ agonist of its kind, donitriptan, is currently being investigated in man.

**INTRODUCTION**

The first triptan (i.e., tryptamine derivative) to be marketed for the acute treatment of migraine, sumatriptan, has been so successful that a number of second generation triptans are now marketed or in the late stages of clinical development (3,27). The triptans represent a breakthrough in acute migraine treatment, being far more selective for $5\text{-HT}_{1B/1D}$ receptors than their relatively nonselective predecessors ergotamine and dihydroergotamine (DHE) (6,9). Three distinct pharmacological actions mediated by triptans have been identified as the most likely explanation for their antimigraine effectiveness: vasoconstriction of cranial blood vessels (11), inhibition of neurogenic inflammation in the dura mater (19), and inhibition of firing of trigeminal neurons (10).

Somewhat surprisingly, the second generation triptans which are already marketed (zolmitriptan, rizatriptan, and naratriptan) provide relatively little improvement, if any, over sumatriptan in terms of antimigraine effectiveness (6,27), despite improvements in pharmacokinetics, higher oral bioavailability, increased brain penetration, and longer plasma half-life. Less than half of the patients are pain free 2 h after treatment, and about a third of responders experience headache recurrence within 24 to 48 h (6,8,27). There appears to be room for substantial improvement over and above currently marketed triptans in terms of clinical antimigraine effectiveness and more so with respect to the “headache free at 2 h” criterion which is recommended by the International Headache Society Migraine Clinical Trials Committee as the primary end point in acute treatment evaluation (8). A recent comparative study between oral sumatriptan and eletriptan has clearly confirmed this perspective; only 29 and 37% of patients were completely pain-free at 2 h with 40- and 80-mg groups compared with 23% with sumatriptan 100-mg group and 6% in the placebo (7). Similarly, in another double-blind, placebo-controlled study comparing rizatriptan and sumatriptan, the percentage of completely headache-free patients at 2 h was 25 and 40% in the rizatriptan 5- and 10-mg groups, 33% in the sumatriptan 100-mg group, and 6% in the placebo group (16). These figures suggest that pain associated with migraine headache is not fully relieved 2 h after treatment in approximately 60% or more of migraine patients.
All of the triptans described to date, as well as ergotamine and DHE, are partial agonists at 5-HT1B receptors (12,18,20,21,24,32). We have hypothesized (12) that the relatively low intrinsic activity of currently available triptans at these receptors could explain their limited therapeutic response and/or ceiling effect in the acute treatment of migraine headache (6,9,20). Since the triptans, by definition, are tryptamine derivatives, it is noteworthy that the potency and intrinsic activity of tryptamine at 5-HT1B receptors is lower than that of serotonin (5-HT) (12). This provided us with the rationale for our chemical approach. In taking advantage of the potency and efficacy characteristics of 5-HT compared with tryptamine at 5-HT1B receptors, F 11356 was synthesized as a novel arylpiperazide 5-HT derivative (22,24) (Fig. 1), which distinguishes F 11356 from the tryptamine derivatives from a chemical point of view. F 11356 is a potent, selective, high-efficacy agonist at 5-HT1B receptors both in vitro and in vivo in vascular and neuronal models relevant to migraine. F 11356 displays unique craniovascular selectivity, is orally active, has a long duration of action, gains access to the brain, and is well tolerated in animals. Consequently, the drug is expected to provide superior acute therapeutic relief from migraine headache compared with the triptans.

CHEMISTRY

As mentioned above, our chemical approach focused on the use of 5-HT as the core structure instead of tryptamine, as with the triptans. Among the compounds synthesized...
(22–24), F 11356 (4-[4-2-(2-aminoethyl)-1H-indol-5-yloxyl]-acetyl-piperazin-1-yl] benzonitrile) (Fig. 1) was selected due to its exceptional pharmacological *in vitro* and *in vivo* profiles.

Two different salts of this compound were evaluated: F 11356 (hydrochloride) and F 12640 (mesylate), which shows improved water solubility and is stable under neutral or acidic conditions. Since both salts were shown to be pharmacologically equivalent (discussed later), F 12640 was selected for development.

**IN VITRO PHARMACOLOGY**

F 11356 has subnanomolar affinity for cloned human and nonhuman 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors with $K_i$ values ranging 0.1–4.3 nM. F 11356 has equivalent affinity at 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors (Fig. 2). The compound has approximately 200 times lower affinity for 5-HT<sub>1A</sub> receptors (Fig. 2) and, notably, over 500 times lower affinity for the 5-HT<sub>1F</sub> and other 5-HT receptors (Fig. 2). Similar affinity values were obtained with F 12640 (data not shown). F 11356 has little or no affinity at other neurotransmitter receptors, uptake sites or ion channel binding sites (Fig. 2; see ref. 12 for further details).

In cellular assays, F 11356 potently inhibits forskolin-induced cAMP accumulation mediated by recombinant human and nonhuman, stably transfected 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors with mean EC<sub>50</sub> values ranging 0.2–1.9 nM (12). In C6 glioma cells transfected...
with human 5-HT$_{1B}$ or 5-HT$_{1D}$ receptors, F 11356 enhanced specific GTP$_{35S}$ binding (21) to a greater extent than naratriptan, rizatriptan, sumatriptan, zolmitriptan, or DHE and to a level equivalent to that evoked by 5-HT at both 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (12), suggestive of greater intrinsic activity of F 11356 compared with the triptans and DHE.

In rabbit-isolated saphenous vein rings (30), F 11356 produced contractile responses in an equipotent manner to 5-HT (pD$_2$ = 7.1 and 7.2, respectively; Fig. 3). In comparison, naratriptan (pD$_2$ = 5.9), and sumatriptan (pD$_2$ = 5.8) were less potent in producing contractile responses, despite fairly similar response amplitudes (Fig. 3; 12). In addition, F 11356-induced contractions were sensitive to inhibition by GR 127935, a 5-HT$_{1B}$/5-HT$_{1D}$ receptor antagonist (1), (pIC$_{50}$ = 9.3 nM; 12), consistent with the involvement of 5-HT$_{1B}$ receptors and the high intrinsic activity of F 11356 compared with the triptans and DHE.

F 12640-evoked responses similar to F 11356 with a pD$_2$ value of 7.0. It may appear paradoxical that high efficacy (5-HT and F 11356) and partial (sumatriptan and naratriptan) 5-HT$_{1B}$/5-HT$_{1D}$ receptor agonists produced equivalent amplitude responses in some cases (i.e., saphenous vein contraction, inhibition of forskolin-evoked cAMP formation, stimulation of K$^+$ current in trigeminal ganglion cells) but not in others (enhancement of GTP$_{γ35S}$ specific binding [see above], or carotid vasoconstriction in vivo [12; discussed below]). However, as receptor theory points out (14), this is typical of agonist behavior. Indeed, high efficacy agonists will be expected to produce large amplitude responses irrespective of receptor density. Partial (i.e., low efficacy) agonists will produce smaller amplitude responses in cell/tissues where receptor density is lower, but may produce large amplitude responses elsewhere where receptor density is higher (14). Thus, the rabbit saphenous vein, for example, appears to be a useful model to detect agonist activity at recombinant 5-HT$_{1B}$ receptors, but it is not helpful in distinguishing between low- and high-efficacy agonists at this receptor. This ex-

![Fig. 4. Maximal increase in steady state (SS) outward hyperpolarizing K$^+$ current ($I_K$) evoked by F 11356 (1 μM) in guinea pig isolated trigeminal ganglion neurons in absence or presence of GR 127935 (0.1 μM) or EGTA (5 mM). Data are mean ± S.E.M. *P < 0.05 compared with vehicle controls, F 11356 + GR 127935 and F 11356 + EGTA. For further details see ref. 12.]
plains why no differences in intrinsic activity were observed between F 11356, 5 HT, sumatriptan, and naratriptan in this model (Fig. 3). In this respect, carotid vasoconstriction in anesthetized pigs (discussed later) is valuable to distinguish between different levels of intrinsic activity at vascular 5-HT\textsubscript{1B} receptors. Thus, relative agonist efficacy should ideally be assessed in a number of different models which are likely to present differences in receptor density. This has been our approach with F 11356, as this review will attempt to highlight.

F 11356 produced low amplitude contractile responses in canine isolated coronary artery rings (pD\textsubscript{2} = 6.7, \(E_{\text{max}} = 1.0 \text{ mN}\)), similar to those of sumatriptan (pD\textsubscript{2} = 4.8, \(E_{\text{max}} = 2.5 \text{ mN}\)) and naratriptan (pD\textsubscript{2} = 6.8, \(E_{\text{max}} = 1.7 \text{ mN}\); 13). These are in agreement with similar observations in human isolated coronary arteries (3,17).

In guinea pig-isolated trigeminal ganglion neurons, F 11356 produced increases in Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current (pD\textsubscript{2} = 7.3) which were sensitive to inhibition by GR 127935 (Fig. 4; 12; note potency value is similar to that obtained in the rabbit saphenous vein). The calcium chelator EGTA abolished these F 11356-induced increases in outward K\textsuperscript{+} current, indicating Ca\textsuperscript{2+}-dependency (Fig. 4). Outward Ca\textsuperscript{2+}-dependent K\textsuperscript{+} currents in neurons regulate excitability and can mediate hyperpolarization (25). Activation of such hyperpolarizing currents by F 11356 and triptans may provide a possible mechanism for the observed inhibitory effects of 5-HT\textsubscript{1B/1D} receptor agonists on trigeminal neuron firing (12,14,20).

Under similar experimental conditions, the 5-HT\textsubscript{1F} receptor agonist LY 334370 (31) failed to produce increases in Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current (0.01 to 10 \(\mu\text{M}\), \(n = 5–7\) per log unit concentration), indicating that 5-HT\textsubscript{1F} receptors are unlikely to be involved in mediating increases in hyperpolarizing currents by the triptans which have non-negligible affinity for these receptors (3,9), unlike F 11356 which does not recognize 5-HT\textsubscript{1F} sites (12). It remains to be determined, however, which specific channel protein mediates the Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current and whether this mechanism plays a role also in regulating trigeminal nociception in humans. These data nevertheless raise two interesting observations. First, in sensory neurons which express native 5-HT\textsubscript{1B/1D} receptors, positive coupling between the receptors and Ca\textsuperscript{2+} dependent K\textsuperscript{+} channels may occur, possibly via inositol phosphate calcium signaling as observed in studies in C6 glioma cells (15). In this respect, both native and recombinant 5 HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptors may concomitantly couple negatively to adenylate cyclase and positively to Ca\textsuperscript{2+} signaling (15,34,35). Second, the trigeminal ganglion neurons may represent an additional molecular target for F 11356 and the triptans in reducing neuronal firing involved in the nociceptive process during migraine headache. These two issues merit further investigation.

Similar findings were made in C6 glioma cells stably expressing cloned human 5-HT\textsubscript{1B} receptors, in which F 11356 produced concentration-related increases in Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current (pD\textsubscript{2} = 7.8 compared with 7.2 for sumatriptan), although no significant difference in maximal responses were noted between F 11356 and sumatriptan. Both F 11356 and sumatriptan-induced increases in outward K\textsuperscript{+} current were abolished by GR 127935 (0.1 \(\mu\text{M}\)), confirming the involvement of human 5-HT\textsubscript{1B} receptors. No effect was observed in wild-type C6 glioma cells with either F 11356 or sumatriptan.

Thus, F 11356 has high affinity, efficacy and selectivity at recombinant human and nonhuman 5-HT\textsubscript{1B} and 5-HT\textsubscript{1P} receptors and behaves as a potent, high-efficacy agonist in both vascular and sensory neuronal tissues that express these receptors. The mesylate salt, F 12640, behaves in quasi-identical fashion to F 11356 in these procedures.
Hypothermic responses in guinea pigs (26) were produced following oral F 11356 administration (ED<sub>50</sub> = 1.6 mg/kg compared with 8.3, 9.9, and 12.3 mg/kg for zolmitriptan, naratriptan and rizatriptan, respectively; 12), indicating superior potency of F 11356 and the likelihood that the compound gains access to the brain. F 12640 produced similar hypothermic responses to F 11356 with an ED<sub>50</sub> value of 0.9 mg/kg p.o. Access to the central nervous system is claimed to contribute to the therapeutic antimigraine effects of 5-HT<sub>1B/1D</sub> receptor agonists by reducing the excitability of central trigeminal neurons and hence nociception (9). Sumatriptan failed to produce hypothermia even at high doses (40 mg/kg p.o.) in agreement with its apparent poor brain penetration (13).

Some of the most significant observations on F 11356 were made during experiments carried out in anesthetized pigs and conscious dogs. In anesthetized pigs, F 11356 dose-dependently produced large-magnitude, GR 127935-sensitive increases in total carotid vascular resistance (TCVR) with only marginal increases in blood pressure (Fig. 4; 12,20,23). Vasoconstriction in the carotid vas-
circular bed is considered to be of importance in the migraine abortive effects of 5-HT\textsubscript{1B/1D} receptor agonists (4,5,11). F 11356 dose-dependently and concomitantly reduced carotid blood flow with an ED\textsubscript{50} value (and 95% confidence limits) of 0.53 (0.42–0.67) μg/kg i.v. (12). The corresponding potency value of F 12640 was 0.13 (0.07–0.28) μg/kg i.v. (n = 7). In comparison, increases in TCVR produced by the triptans investigated were considerably smaller and even presented bell-shaped, dose-response characteristics in the case of sumatriptan and naratriptan (12). F 11356, sumatriptan, naratriptan, and zolmitriptan produced similar marginal increases in arterial pressure (Fig. 5). Thus, F 11356 displays unique craniovascular selectivity over and above that observed for the triptans studied in this model. Similar to F 11356, dihydroergotamine (DHE) evoked large amplitude increases in total carotid vascular resistance, but unlike F 11356 the responses to DHE were accompanied by substantial increases (>40%) in mean arterial pressure (Fig. 5). 5-HT\textsubscript{1B/1D} receptors only partly mediate carotid vasoconstriction evoked by DHE, and other receptors appear to be involved (2). With respect to the large systemic pressor responses induced by DHE, it is well established that the drug is a vasoconstrictor agent on a wide variety of vessels, including veins, due to its poor receptor selectivity and interaction with a number of 5-HT and non-5-HT receptor subtypes, including α\textsubscript{1}- and α\textsubscript{2}-adrenoceptors (28).

Another observation of note was made in studies of F 11356 conducted in the anesthetized pig in which recovery of TCVR was followed for 60 min after drug infusion was stopped. Whereas TCVR had returned to near baseline values by 1 h after sumatriptan, rizatriptan, naratriptan, and zolmitriptan infusions were stopped, TCVR remained at near maximal values for F 11356 (Fig. 6), suggesting that F 11356 has a longer duration of action. Similar observations were made with DHE (Fig. 5, n = 7) and F 12640 (max. changes in TCVR being 92 ± 12% 1 h after stopping drug infusion; n = 7; P = NS compared with max. effect during drug infusion). Because carotid vascular response amplitudes evoked by F 11356 and DHE are large (and only slowly reversible) relative to that of
the triptans, a low incidence of headache recurrence in humans treated with F 11356 is suggested, as is the case with DHE (29,33).

Another significant observation was made in conscious dogs with a permanent flow probe placed around the left common carotid artery. F 11356, from 0.63 mg/kg p.o. produced long-lasting decreases in unilateral carotid blood flow, which were already maximal at 30 min at 2.5 mg/kg (Fig. 7) and remained decreased for over 12 h at 2.5 mg/kg. No effects were observed on heart rate or behavior (12,23) and the drug was well tolerated. The rapid onset of action F 11356 at 2.5 mg/kg is consistent with preliminary pharmacokinetic data in dogs and rats which indicate a $T_{\text{max}}$ of 30–45 min for the mesylate salt, F 12640. Such decreases in unilateral carotid blood flow, however, were not observed with sumatriptan (2.5 and 10 mg/kg), naratriptan (0.16 and 0.63 mg/kg), and zolmitriptan (0.16 and 0.63 mg/kg), since these triptans were not well tolerated and produced pronounced clinical signs possibly suggesting parasympatholytic-type activity (i.e., mydriasis, tachycardia, increased respiratory rate, vocalization; 12). Placebo had no significant effect on carotid blood flow, heart rate or behavior. These data clearly demonstrate that F 11356 is well-tolerated in conscious dogs (12).

Collectively, the in vivo data available on F 11356 and F 12640 confirm the high potency, selectivity, and efficacy of these compounds at 5-HT$_{1B/1D}$ receptors in vivo. F 12640 appears to be marginally more potent than F 11356 in the in vivo procedures investigated. F 11356 is active orally, has a rapid onset of action following oral administration, a long duration of action, gains access to the brain, and is well-tolerated.
Donitriptan is currently undergoing clinical evaluation for the acute treatment of migraine headache attacks. Based on the preclinical pharmacological characteristics of this compound, the following improvements over currently available triptans can reasonably be expected to include:

a.) A larger percentage (i.e., ≥40%) of migraineurs will be completely headache-free 2 h after treatment. In this respect, the hypothesis that the magnitude of intrinsic activity at 5-HT$_{1B/1D}$ receptors determines at least partly therapeutic antimigraine effectiveness will be assessed with donitriptan as the first high efficacy agonist of its kind. In comparison with donitriptan, currently available triptans behave as partial agonists at 5-HT$_{1B/1D}$ receptors, which could limit their therapeutic effectiveness (6,8,9,12,20).

b.) Consistency in headache response across the patient population studied can also be expected with donitriptan. This is reflected by the consistency of the drug to produce large amplitude responses in the various in vitro and in vivo experimental models relevant to migraine described earlier. In contrast, sumatriptan, naratriptan, zolmitriptan, or rizatriptan, fail to produce consistent, large amplitude responses in all of these models. Even bell-shaped, dose-response curves are observed in some cases (e.g., carotid vasoconstriction in pigs), typifying the partial agonist behavior of the latter drugs (12,20,23,24).

c.) A lower incidence of migraine recurrence. Until recently, it has been a widely held belief that headache recurrence is related to plasma half-life of the triptan in question (9); however, recurrence of headache occurs with all of the currently available triptans, and its incidence does not appear to correlate readily with plasma half-life for each drug (9,20). We tentatively suggest that the duration of pharmacological action of donitriptan, being significantly longer than that of representative triptans (see Fig. 6) and similar to that of DHE, might produce a lower incidence of headache recurrence, independently of its plasma half-life. Interestingly, the incidence of headache recurrence appears to be lower with DHE than with triptans (29,33).

In addition, donitriptan can be expected to have a relatively fast onset of action if the Tmax is around 1 h or less in man, as suggested by both rat and dog pharmacokinetic studies.

d.) It is also reasonable to expect that donitriptan will be well tolerated in migraineurs, as suggested by studies in conscious dogs. If this is the case, then a relatively large range of doses will be able to be evaluated for therapeutic antimigraine effectiveness in the absence of limitations due to poor tolerability. This would, in principle, permit the employment of well-tolerated, highly effective doses of donitriptan.

There are no expected disadvantages of donitriptan compared to the currently employed triptans, and systemic cardiovascular effects are expected to be similar.

CONCLUSION

Donitriptan is a potent and selective 5-HT$_{1B/1D}$ receptor agonist derived from 5-HT, which distinguishes itself from other triptans by exerting exceptionally high intrinsic activity (i.e., approaching that of the endogenous agonist 5-HT) at these receptors in vas-
cular and neuronal models relevant to migraine. The selective 5-HT$_{1B/1D}$ receptor agonists derived from tryptamine that have been described to date, including sumatriptan, naratriptan, zolmitriptan, and eletriptan, behave as partial agonists (i.e., exert lower intrinsic activity than 5-HT), which could limit their oral clinical antimigraine effectiveness (6,8,9,20). The room for improvement over the therapeutic effectiveness of currently available triptans is, therefore, substantial. Clinical evaluation of a high efficacy 5-HT$_{1B/1D}$ receptor agonist, of which donitriptan is the first, is warranted in order to verify the hypothesis that the magnitude of intrinsic activity at these receptors determines, at least in part, therapeutic antimigraine effectiveness. Thus, donitriptan may exert high efficacy agonist activity throughout the trigeminovascular system where 5-HT$_{1B/1D}$ receptors are located (i.e., at its peripheral sensory nerve-vessel interface, ganglion, and brainstem trigeminal nucleus caudalis). Consequently, donitriptan, by virtue of its unique pharmacological properties as a high efficacy agonist at 5-HT$_{1B/1D}$ receptors in combination with its excellent tolerability in animals, should provide a significant step forward in acute antimigraine therapy compared to currently available drugs.

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