Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 474 (2003) 117–120
www.elsevier.com/locate/ejphar

Short communication

SL65.0472 blocks 5-hydroxytryptamine-induced vasoconstriction in a dog hindlimb ischemia model

Fabrice Barbe, Etienne Gautier, Jean-Pierre Bidouard, Alain Grosset, Stephen E. O'Connor*, Philip Janiak

Sanofi-Synthélabo Research, Cardiovascular Thrombosis Research Department, 1 Avenue Pierre Brossolette, 91380 Chilly-Mazarin, France

Received 16 June 2003; accepted 27 June 2003

Abstract

We have studied the ability of SL65.0472 (7-fluoro-2-oxo-4-[2-[4-(thieno[3,2-*c*]pyridin-4-yl)piperazin-1-yl]ethyl]-1,2-dihydroquinoline-1-acetamide), a 5-hydroxytryptamine (5-HT) 5-HT_{1B}/5-HT_{2A} receptor antagonist, to antagonise the vasoconstrictor effects of 5-HT and sumatriptan in a canine model of hindlimb ischemia. Dogs underwent right external iliac artery ligation and right superficial femoral artery excision, resulting in decreased perfusion (− 31%, $P < 0.05$) in the right hindlimb. Following pretreatment with L-NAME, phentolamine and propranolol, intra-aortic injection of 5-HT markedly reduced blood flow to the right ischemic hindlimb (− 50 ± 2%, $P < 0,05$). 5-HT induced vasoconstriction was significantly inhibited (− 66%, $P < 0.05$) by SL65.0472 (300 μg/kg i.v.), but unaffected by ketanserin (300 μg/kg i.v.), a 5-HT_{2A} receptor antagonist. SL65.0472 also blocked sumatriptan-induced vasoconstriction in ischemic and normally perfused hindlimbs. Thus, SL65.0472 is an effective antagonist of 5-HT-receptor mediated hindlimb vasoconstriction.

Keywords: 5-HT receptor; Sumatriptan; Vasoconstriction; Hindlimb ischemia; SL65.0472; Ketanserin

1. Introduction

5-Hydroxytryptamine (5-HT) is a potent vasoconstrictor in many vascular beds. In addition to a well-characterised 5-HT_{2A}-receptor mediated component, recent evidence also demonstrates a contribution of 5-HT_{1B} receptors. For example, a ketanserin-resistant, 5-HT-induced constriction occurs in human coronary arteries in vivo and in vitro (McFadden et al., 1992; Kaumann et al., 1994), which appears to involve the 5-HT_{1B} subtype. Sumatriptan, a selective 5-$HT_{1B/1D}$ receptor agonist, is capable of causing coronary, pulmonary and systemic vasoconstriction in man (Macintyre et al., 1993). Molecular biological and functional studies confirm that constriction of human temporal artery (Verheggen et al., 1998), human coronary artery (Nilsson et al., 1999) and human small pulmonary arteries (Morecroft et al., 1999) are mediated by 5-HT_{1B} receptor stimulation. The participation of the different 5-HT receptor subtypes in the vasoconstrictor response to 5-HT has been shown to vary with the physiopathological condition. In pigs, intracoronary injection of 5-HT decreased coronary blood flow via the activation of 5-HT_2 receptors. However, after 4 weeks of chronic inhibition of nitric oxide synthesis, 5-HT-induced coronary constriction was exacerbated and resulted from combined stimulation of 5-HT_1 and 5-HT_2 receptors (Kadokami et al., 1996). Thus, endothelial dysfunction produced a shift in the relative contribution of 5-HT_1 and 5-HT_2 receptors to this response.

We have recently reported the profile of SL65.0472 (7-fluoro-2-oxo-4-[2-[4-(thieno[3,2-*c*]pyridin-4-yl) piperazin-1-yl]ethyl]-1,2-dihydroquinoline-1-acetamide), a potent new 5-HT receptor antagonist. SL65.0472 antagonises 5-HT_{2A} and 5-HT_{1B} receptor mediated vasoconstriction (Galzin et al., 2000; O'Connor et al., 2001) and demonstrates antithrombotic properties (Berry et al., 2001). The present study was designed to characterize the effects of SL65.0472, in comparison with ketanserin, on the vasoconstrictor responses mediated by 5-HT receptor stimulation in a canine model of chronic hindlimb ischemia. Although 5-HT typically increases blood flow in normal limbs, it can lead to a

* Corresponding author. Tel.: +33-1-69-79-7806; fax: +33-1-69-79-7810.
E-mail address: stephen-eric.o'connor@sanofi-synthelabo.com (S.E. O'Connor).

0014-2999/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0014-2999(03)02032-6

dramatic reduction in flow when administered to ischemic limbs. Indeed, collateral vessels show a hypersensitivity to vasoconstriction by 5-HT in animal models of hindlimb ischemia in several species (Orlandi et al., 1986; Bauters et al., 1995).

2. Materials and methods

2.1. Hindlimb ischemia in anesthetized dogs

This study was performed according to the European Community Standards on the Care and Use of Laboratory animals. Male Anglo-Poitevin dogs (ECDL, France, $n=35$) weighing 22–34 kg were used. Induction of hindlimb ischemia was performed under anesthesia with 30 mg/kg i.v. sodium pentobarbitone with supplements administered as required to sustain anesthesia. Animals were intubated for spontaneous respiration and body temperature maintained at 37 °C by a servo-controlled heating pad. After right lateral laparotomy, ligations of the right external iliac artery and the right deep femoral artery and excision of the right superficial femoral artery were performed. Then dogs were given penicillin (30 mg/kg i.m.) to prevent post-operative infection. After a 2-day recovery period, L-NAME (*N*-nitro-L-arginine methyl ester), an inhibitor of nitric oxide synthase was administered (10 mg/kg/day s.c.) to create endothelial dysfunction.

Two weeks after the induction of hindlimb ischemia, dogs were anesthetized by sodium pentobarbitone (42 mg/kg i.v.) followed by an i.v. infusion (6 mg/kg/h) throughout the study. Animals were intubated and ventilated with a respirator (SA1 Dräger, Germany). Both cephalic veins were catheterized for anesthetic infusion and drug administration. Mean arterial pressure was monitored by a MIKRO-TIP® catheter transducer (Millar Instruments, USA) inserted in the aorta and the hindlimb blood flows were measured by electromagnetic flow probes (Carolina Medical Electronics, USA) placed around the left internal and external iliac arteries and around the right internal iliac artery. In addition, both circumflex iliac arteries were catheterized for intra-aortic (i.a.) drug injections. All hemodynamic parameters were continuously monitored with HEM v3.1 software (Notocord, France).

Peripheral hemodynamic evaluation was performed under α- and β-adrenergic receptor blockade produced by phentolamine (0.2 mg/kg/h i.a.) and propranolol (1 mg/kg i.v.) in order to limit the involvement of catecholamines in the cardiovascular response to 5-HT. To optimize nitric oxide synthase inhibition, an additional dose of L-NAME (10 mg/kg i.v.) was administered at the beginning of the protocol. Following a stabilization period, 5-HT (1 and 3 μg/kg) was injected into the lower abdominal aorta via an infusion pump (10 ml/min, 20 s) in order to determine for each animal a dose giving at least a 25% decrease in right iliac blood flow without altering arterial pressure. This selected dose of 5-HT was then repeated before and 10 min after the administration of a 5-HT receptor antagonist, SL65.0472 (300 μg/kg i.v.) or ketanserin (300 μg/kg i.v.) or their vehicle ($n=8$ per group).

An additional group of similarly prepared animals received in the same fashion an injection of sumatriptan (3 or 10 μg/kg i.a.), which was repeated 10 min after administration of SL65.0472 (300 μg/kg i.v., $n=6$) or vehicle ($n=5$).

2.2. Drugs

5-HT and L-NAME were obtained from Sigma (St. Quentin Fallavier, France), sodium pentobarbitone from Sanofi (Toulouse, France), penicillin from Fort Dodge Santé (Paris, France), phentolamine from RBI (Illkirch, France), propranolol from Erypharm Italiana (Italy), nitroglycerin (Lenitral®) from Besins-Iscovesco Laboratories, Montrouge, France). SL65.0472 (7-fluoro-2-oxo-4-[2-[4-(thieno[3,2-*c*]pyridin-4-yl) piperazin-1-yl]ethyl]-1,2-dihydroquinoline-1-acetamide), sumatriptan and ketanserin were synthesized at Sanofi-Synthélabo (Chilly-Mazarin, France).

2.3. Data analysis

Data are expressed as mean ± S.E.M. Significance ($P<0.05$) was determined by *t*-test or two-way analysis of variance followed by a Dunnett's test (SAS Software®).

3. Results

Two weeks after the surgical induction of hindlimb ischemia, basal blood flow was significantly reduced (-31%, $P<0.05$, $n=24$) in the right ischemic leg as compared to the left control one. Mean arterial pressure, heart rate and limb blood flows were similar in the different experimental groups before the administration of the 5-HT receptor antagonists (Table 1). Injection of 5-HT above the iliac bifurcation reduced markedly blood flow in the right ischemic hindlimb ($-50\pm2\%$, $n=24$). In the contralateral normal leg, 5-HT produced a biphasic response characterized by a transient vasodilatation followed by vasoconstriction, which led to a flow reduction of $-25\pm4\%$ ($n=24$). Arterial blood pressure was not changed by the administration of 5-HT.

Vehicle administration had no effect on responses to 5-HT in normal or ischemic hindlimbs (Fig. 1). SL65.0472 (300 μg/kg i.v., $n=8$) substantially reduced the vasoconstriction to 5-HT in both the right hypoperfused leg (-66%, $P<0.05$) and the left control leg (-77%, $P<0.05$), without altering the 5-HT-mediated initial vasodilatation phase in the normal limb (Fig. 1). In contrast, vasoconstrictor responses to 5-HT in normal and hypoperfused hindlimbs were not blocked by administration of ketanserin (300 μg/kg i.v.)

Table 1
Hemodynamic effects of SL65.0472 (300 μg/kg i.v.), ketanserin (300 μg/kg i.v.) or vehicle in an anaesthetized dog model of hindlimb ischemia

Treatment	HR (bpm)		MAP (mm Hg)		LBF (ml/min)		RBF (ml/min)	
	Control	10 min	Control	10 min	Control	10 min	Control	10 min
Vehicle	140 ± 4	140 ± 4	129 ± 5	129 ± 5	135 ± 20	134 ± 21	71 ± 10	70 ± 9
Ketanserin	144 ± 2	137 ± 3[a]	138 ± 5	130 ± 5[a]	113 ± 13	143 ± 16[a]	90 ± 9	100 ± 9[a]
SL65.0472	135 ± 4	138 ± 3	134 ± 5	129 ± 6[a]	127 ± 13	146 ± 18[a]	93 ± 18	100 ± 19[a]

Heart rate (HR), mean arterial blood pressure (MAP), left iliac blood flow (LBF) and right iliac blood flow (RBF) were measured before (control) and 10 min after injection of treatment.
Values are means ± S.E.M., $n = 8$.
[a] $P < 0.05$ vs. control (Dunnett's test).

(Fig. 1). Table 1 shows the direct hemodynamic effects of SL65.0472 and ketanserin. Both compounds caused small falls in mean arterial pressure and modestly increased basal left and right iliac blood flows.

Sumatriptan caused monophasic falls in blood flow in ischemic and normal limbs of − 34 ± 4% and − 27 ± 5%, respectively ($n = 11$). These vasoconstrictor responses to sumatriptan were unchanged following vehicle but were significantly reduced by SL65.0472 (ischemic limb − 64%, $P < 0.05$ and normal limb − 68%, $P < 0.05$).

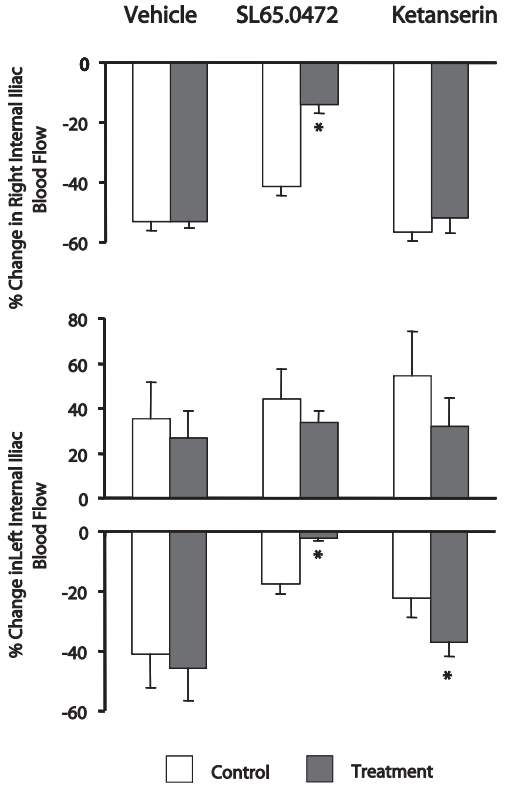

Fig. 1. Effects of 5-HT receptor antagonists on the vasoconstrictor response to 5-HT in the ischemic right hindlimb of the anesthetized dog (measured as the percentage decrease in right iliac blood flow) and on initial vasodilator and secondary vasoconstrictor responses to 5-HT in the normal hindlimb (measured as % increases and % decreases in left iliac blood flow, respectively). Three treatment groups were used comprising vehicle, ketanserin (300 μg/kg i.v.) and SL65.0472 (300 μg/kg i.v.). In each animal, the response to 5-HT (i.a.) was measured firstly under control conditions and secondly after administration of the treatment. Values are means ± S.E.M., $n = 8$ per group. *$P < 0.05$ versus control response.

4. Discussion

In this study, we describe a model of hindlimb ischemia induced by surgical excision of the right superficial femoral artery and ligation of the right external iliac artery and the right deep femoral artery, associated with L-NAME treatment. Two weeks after surgery, animals exhibited impaired perfusion of the right hindlimb accompanied by a strong vasoconstrictor response to 5-HT. Local 5-HT administration produced a marked vasoconstriction in the ischemic limb, which was antagonized by SL65.0472 but not by ketanserin. To our knowledge, this is the first demonstration of a ketanserin-resistant vasoconstrictor response to 5-HT in a model of hindlimb ischemia. Unlike ketanserin, SL65.0472 has a high affinity for the 5-HT_{1B} receptor subtype (IC_{50} 19 nM, Delahaye et al., 2001) and blocks 5-HT_{1B} receptor mediated vasoconstriction. We have demonstrated previously that SL65.0472 is capable of antagonizing ketanserin-resistant contractions of dog saphenous vein (O'Connor et al., 2001) and human coronary artery (Galzin et al., 2000). In the present study, the sensitivity of hindlimb vasoconstriction by 5-HT to antagonism by SL65.0472 (but not ketanserin) suggests an involvement of 5-HT_{1B} receptors. This was confirmed by the demonstration that the 5-$HT_{1B/1D}$ receptor agonist, sumatriptan, also produced vasoconstriction of normal and ischemic hindlimbs in this model, effects which were inhibited by SL65.0472.

The doses of antagonists chosen for this study were based on previous pharmacological studies and were intended to allow discrimination of a 5-HT_{1B}-mediated component in vivo. For example, ketanserin, a potent 5-HT_{2A}-receptor antagonist, does not modify 5-HT_{1B} mediated responses in the dog at a dose of 300 μg/kg i.v. (Drieu la Rochelle and O'Connor, 1995). Conversely, SL65.0472 300 μg/kg i.v. causes substantial antagonism of both 5-HT_{1B} and 5-HT_{2A} responses (O'Connor et al., 2001; Berry et al., 2001). These doses were also considered to be acceptable from the hemodynamic viewpoint, as confirmed by the relatively modest changes in blood pressure and limb blood flow observed in this study.

We have not attempted to characterize the transient initial vasodilatory response to 5-HT observed in the normal hindlimb in spite of treatment with L-NAME and which was unaffected by SL65.0472 or ketanserin. One candidate mechanism could be the 5-HT_7 receptor subtype, which mediates non-endothelial dependent vasodilation (Terron, 1996).

Platelet activation and aggregation at the site of atherosclerotic lesions and the release of platelet products (such as 5-HT) appear to play a key role in the pathogenesis of cardiovascular disease. High local levels of 5-HT in the vicinity of vascular lesions may cause vasospasm, thus aggravating ischemia. A recent study has highlighted the putative role of platelet-derived 5-HT as a risk factor for the development of vascular disease and subsequent cardiac events in man (Vikenes et al., 1999). Ketanserin is the only 5-HT receptor antagonist to have been extensively tested clinically for the treatment of cardiovascular indications; however, results of clinical studies in peripheral vascular disease have given contradictory results (Clement and Duprez, 1987).

It is possible that optimal antagonism of the vasoconstrictor effects of 5-HT in the setting of peripheral ischemia requires blockade of both 5-HT_{2A} and 5-HT_{1B} receptors. In this context, we have demonstrated recently that SL65.0472 improves distal perfusion after lower limb ischemia and inhibits 5-HT-induced limb vasoconstriction in fatty Zucker rats (Janiak et al., 2002), a model which exhibits overexpression of both 5-HT_{1B} and 5-HT_{2A} receptor subtypes in hypoperfused skeletal muscle 14 days after induction of ischemia. The present study reinforces the concept of a contribution of vascular 5-HT_{1B} receptors to hindlimb perfusion, including during chronic ischemia, and demonstrates anti-vasoconstrictor properties of SL65.0472 in a model where ketanserin is ineffective.

References

Bauters, C., Asahara, T., Zheng, L.P., Takeshita, S., Bunting, S., Ferrara, N., Symes, J.F., Isner, J.M., 1995. Recovery of disturbed endothelium-dependent flow in the collateral-perfused rabbit ischemic hindlimb after administration of vascular endothelial growth factor. Circulation 91, 2802–2809.

Berry, C.N., Lorrain, J., Lochot, S., Delahaye, M., Lale, A., Savi, P., Lechaire, I., Ferrari, P., Bernat, A., Schaeffer, P., Janiak, P., Duval, N., Grosset, A., Herbert, J.M., O'Connor, S.E., 2001. Antiplatelet and antithrombotic activity of SL65.0472, a mixed 5-HT_{1B}/5-HT_{2A} receptor antagonist. Thromb. Haemost. 85, 521–528.

Clement, D.L., Duprez, D., 1987. Effect of ketanserin in the treatment of patients with intermittent claudication: results from 13 placebo-controlled parallel group studies. J. Cardiovasc. Pharmacol. 10 (Suppl. 3), S89–S95.

Delahaye, M., Galzin, A.M., O'Connor, S.E., 2001. Antagonism of 5-HT induced contraction of porcine isolated coronary artery by SL 65.0472. Br. J. Pharmacol. 133, 103 p.

Drieu la Rochelle, C., O'Connor, S.E., 1995. Sumatriptan-induced saphenous venoconstriction in the anaesthetized dog through 5-$HT_{1\text{-like}}$ receptor activation. Br. J. Pharmacol. 116, 2207–2212.

Galzin, A.M., Delahaye, M., Hoornaert, C., McCort, G., O'Connor, S.E., 2000. Effects of SL 65.0472, a novel 5-HT receptor antagonist, on 5-HT receptor mediated vascular contraction. Eur. J. Pharmacol. 404, 361–368.

Janiak, P., Lainee, P., Grataloup, Y., Luyt, C.E., Bidouard, J.P., Michel, J.B., O'Connor, S.E., Herbert, J.M., 2002. Serotonin receptor blockade improves distal perfusion after lower limb ischemia in the fatty Zucker rat. Cardiovasc. Res. 56, 293–302.

Kadokami, T., Egashira, K., Kuwata, K., Fukumoto, Y., Kozai, T., Yasutake, H., Kuga, T., Shimokawa, H., Sueishi, K., Takeshita, A., 1996. Altered serotonin receptor subtypes mediate coronary microvascular hyperreactivity in pigs with chronic inhibition of nitric oxide synthesis. Circulation 94, 182–189.

Kaumann, A.J., Frenken, M., Posival, H., Brown, A.M., 1994. Variable participation of 5-$HT_{1\text{-like}}$ receptors and 5-HT_2 receptors in serotonin-induced contraction of human isolated coronary arteries. 5-$HT_{1\text{-like}}$ receptors resemble cloned 5-$HT_{1D\ beta}$ receptors. Circulation 90, 1141–1153.

Macintyre, P.D., Bhargava, B., Hogg, K.J., Gemmill, J.D., Hillis, W.S., 1993. Effect of subcutaneous sumatriptan, a selective 5HT_1 agonist, on the systemic, pulmonary, and coronary circulation. Circulation 87, 401–405.

McFadden, E.P., Bauters, C., Lablanche, J.M., Leroy, F., Clarke, J.G., Henry, M., Schandrin, C., Davies, G.J., Maseri, A., Bertrand, M.E., 1992. Effect of ketanserin on proximal and distal coronary constrictor responses to intracoronary infusion of serotonin in patients with stable angina, patients with variant angina, and control patients. Circulation 86, 187–195.

Morecroft, I., Heeley, R.P., Prentice, H.M., Kirk, A., MacLean, M.R., 1999. 5-hydroxytryptamine receptors mediating contraction in human small muscular pulmonary arteries: importance of the 5-HT_{1B} receptor. Br. J. Pharmacol. 128, 730–734.

Nilsson, T., Longmore, J., Shaw, D., Pantev, E., Bard, J.A., Branchek, T., Edvinsson, L., 1999. Characterisation of 5-HT receptors in human coronary arteries by molecular and pharmacological techniques. Eur. J. Pharmacol. 372, 49–56.

O'Connor, S.E., Grosset, A., La Rochelle, C.D., Gautier, E., Bidouard, J.P., Robineau, P., Caille, D., Janiak, P., 2001. Cardiovascular effects of SL65.0472, a 5-HT receptor antagonist. Eur. J. Pharmacol. 414, 259–269.

Orlandi, C., Blackshear, J.L., Hollenberg, N.K., 1986. Specific increase in sensitivity to serotonin of the canine hindlimb collateral arterial tree via the 5-hydroxytryptamine-2 receptor. Microvasc. Res. 32, 121–130.

Terron, J.A., 1996. The relaxant 5-HT receptor in the dog coronary artery smooth muscle: pharmacological resemblance to the cloned 5-ht_7 receptor subtype. Br. J. Pharmacol. 118, 1421–1428.

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

Vikenes, K., Farstad, M., Nordrehaug, J.E., 1999. Serotonin is associated with coronary artery disease and cardiac events. Circulation 100, 483–489.