0022-3565/92/2611-0202$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 261, No. 1
Printed in U.S.A.

LY215840, a Potent 5-Hydroxytryptamine (5-HT)$_2$ Receptor Antagonist, Blocks Vascular and Platelet 5-HT$_2$ Receptors and Delays Occlusion in a Rabbit Model of Thrombosis

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Accepted for publication January 3, 1992

ABSTRACT

Certain ergolines are potent and selective 5-hydroxytryptamine (5-HT)$_2$ receptor antagonists. Previous studies with two ergoline esters, LY53857 and sergolexole, documented their potency as 5-HT$_2$ receptor antagonists and their metabolism in rats to a less active metabolite, 1-isopropyl dihydrolysergic acid. LY215840, an ergoline amide, has been identified as a potent 5-HT$_2$ receptor antagonist that is not hydrolyzed to 1-isopropyl dihydrolysergic acid. In the rat jugular vein, LY215840 (3×10^{-10} to 10^{-8} M) blocked 5-HT$_2$ receptors mediating contraction to 5-HT *in vitro*. After i.v. and p.o. administration to rats, LY215840 was a potent 5-HT$_2$ receptor antagonist, documented by its ability to block the pressor response to 5-HT administered i.v. Furthermore, after i.v. and p.o. administration of LY215840, blockade of vascular 5-HT$_2$ receptors persisted in excess of 2 and 6 hr, respectively. LY215840 also blocked vascular 5-HT$_2$ receptors in doses that did not affect *alpha*-1, *beta*-1 receptors or angiotensin II pressor responses, documenting the selectivity of LY215840 as an inhibitor of 5-HT$_2$ and not other vascular receptors that modulate vasoconstriction. In addition to inhibiting vascular 5-HT$_2$ receptors, LY215840 also inhibited 5-HT-amplified, ADP-induced aggregation (another 5-HT$_2$ receptor-mediated response) in both rabbit and human platelets. Because of its ability to block both platelet and vascular 5-HT$_2$ receptors, we studied the effectiveness of LY215840 in the rabbit carotid artery model of vascular occlusion. Low i.v. doses of LY215840 markedly prolonged time to vascular occlusion. LY215840 had no marked effect on blood pressure, and only a transient elevation in carotid blood flow in rabbits, which returned to control values 15 to 30 min after its administration. Furthermore, LY215840 inhibited the *ex vivo* response of platelets to the combination of 5-HT and ADP. Thus, LY215840 is an ergoline amide that potently and selectively blocks 5-HT$_2$ receptors on both blood vessels and platelets, effects that likely account for its marked antiocclusive activity.

LY53857 (see fig. 1 for structure) is a prototypic ergoline 5-HT$_2$ receptor antagonist that has been studied extensively as a blocker of vascular (Cohen *et al.*, 1983 1985) and platelet (McBride, 1990) 5-HT$_2$ receptors. In addition, LY53857 inhibited cyclic flow variations in dogs (Ashton *et al.*, 1989; Golino *et al.*, 1987) and delayed vascular occlusion in rabbits (Wilson *et al.*, 1991). In an extensive exploration of the structure activity relationship of ergolines and their interaction with 5-HT$_2$ receptors (Marzoni *et al.*, 1987; Garbrecht *et al.*, 1988; Misner *et al.*, 1990), sergolexole (Cohen *et al.*, 1988, 1989) was identified (see fig. 1 for structure) as a more potent 5-HT$_2$ receptor antagonist than LY53857 after its p.o. administration to rats. However, sergolexole was metabolized rapidly in animals and humans to 1-isopropyl dihydrolysergic acid (Cohen *et al.*, 1989). Furthermore, sergolexole possessed only modest aqueous solubility, a factor limiting its maximum dosage by i.v. administration, the most desirable route for acute cardiovascular indications.

Received for publication August 22, 1991.

Recent findings documented the presence of 5-HT$_2$ receptors in human coronary arteries and the pronounced effect of 5-HT on coronary vessels in patients with atherosclerosis and angina (Golino *et al.*, 1991; McFadden *et al.*, 1991), rekindling interest in evaluation of cardiovascular indications for 5-HT$_2$ receptor antagonists. To obviate the rapid metabolism of the ergoline esters *in vivo*, studies on the structure activity relationships of ergoline amides were initiated (Misner *et al.*, 1990) and led to the development of LY215840 (see fig. 1 for structure), a water soluble ergoline amide that possesses high antagonist affinity at 5-HT$_2$ receptors. In the present study we document the ability of LY215840 to block 5-HT$_2$ receptors in a variety of *in vitro* and *in vivo* systems.

Methods

Effect on pressor responses to 5-HT in pithed rats. 5-HT receptor antagonism was evaluated in pithed Wistar normotensive rats (Charles River, Inc., Portage, MI, 240–374 g) because responses in the pithed preparation are primarily direct vascular effects. Rats were anesthetized with Metofane (methoxyflurane), pithed and ventilated

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); PRP, platelet-rich plasma.

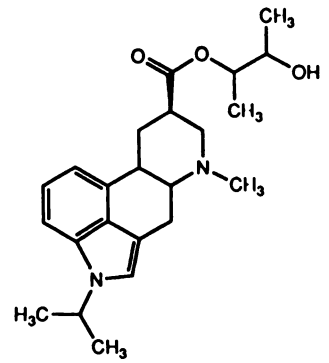
with room air (Cohen *et al.*, 1983, 1985). The carotid artery and femoral vein were cannulated and the trachea was intubated. Blood pressure was measured *via* a P23XL pressure transducer connected to the carotid arterial cannula, and drugs were injected into the right femoral vein. An equilibration period of 15 min was observed before control measurements or i.v. administration of drugs or vehicle. Increasing i.v. doses of 5-HT were injected 15 min after i.v. administration of 5-HT$_2$ receptor antagonist or vehicle.

For p.o. studies, LY215840 or vehicle (distilled water) was administered p.o. to conscious rats. The 5-HT dose-response was determined at approximately 60 min (or other times in studies on duration) after 5-HT$_2$ receptor antagonist or vehicle was administered p.o. Rats were pithed 15 to 30 min before 5-HT challenge. Blood pressure was allowed to recover to steady state before subsequent 5-HT doses were given.

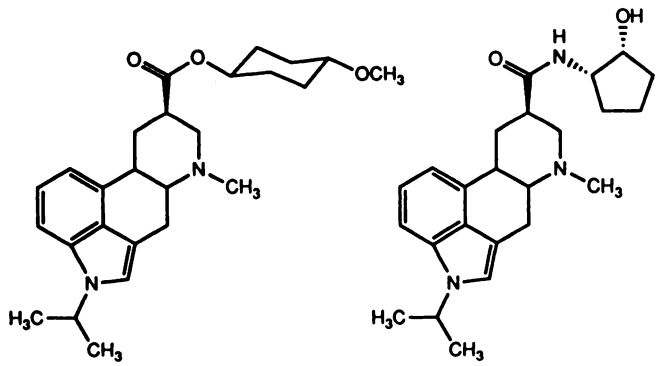
Effect on pressor responses to norepinephrine and angiotensin II and on heart rate responses to norepinephrine in pithed rats. Wistar normotensive rats were prepared as above. After a 15-min equilibration period, increasing i.v. doses of norepinephrine or angiotensin II were administered. The dose-response to norepinephrine or angiotensin II was repeated 15 min after i.v. administration of LY215840 or vehicle. Blood pressure was measured as above and heart rate was measured *via* a cardiotachometer triggered from the blood pressure signal.

Effect on 5-HT-induced contraction in the rat jugular vein *in vitro*. Male Wistar rats (240–400 g, Charles River, Portage, MI) were sacrificed by cervical dislocation. Ring preparations of external rat jugular veins were used as detailed previously (Cohen *et al.*, 1981).

Tissues were mounted in organ baths containing 10 ml of modified Krebs' solution of the following composition (millimolar concentrations): NaCl, 118.2; KCl, 4.6; $CaCl_2 \cdot 2H_2O$, 1.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; dextrose, 10.0; and $NaHCO_3$, 24.8. Tissue bath solutions were maintained at 37°C and aerated with 95% O_2-5% CO_2. Isometric contractions were recorded as changes in grams of force on a Beckman Dynograph with Statham UC-3 transducers and microscale accessory attachments. Tissues were allowed to equilibrate at an initial optimum resting force for 1 to 2 hr before exposure to drugs. After control cumulative contractile responses to 5-HT were obtained, tissues were incubated with appropriate concentrations of LY215840 for 1 hr. Responses to 5-HT were then repeated in the presence of antagonist.



LY53857



Sergolexole LY215840

Fig. 1. Chemical structures of LY53857, sergolexole and LY215840.

Effect on 5-HT-induced amplification of platelet aggregation. Blood (9 ml) was collected in a 10-ml syringe containing 1 ml of 3.8% Na citrate + 0.1% dextrose in saline (pH 7.4). Blood was centrifuged at 160 × *g* for 10 min at room temperature to provide PRP. Platelets were counted using a Cell-Dyn 900 hematology analyzer. For the aggregation studies, the PRP sample was adjusted using platelet-poor plasma to a platelet concentration of 300,000 × 10^6 cells per liter. Appropriate concentrations of LY215840 or vehicle (5.0 μl) and 5-HT (5.0 μl) were added to the PRP (225 μl), and aggregation was initiated by the addition of threshold concentrations of ADP (25 μl) (0.5–3.0 μM) to a final volume of 260 μl. Aggregation determinations were conducted at 37°C using the optical density method proposed by Born (1962). A platelet aggregation profiler (model PAP-4, Bio-Data Corporation, Harboro, PA) was used to measure optical density changes. Data are expressed as the area under the curve for the aggregation (change in optical density) occurring during 3 min.

Effect on vascular occlusion in a rabbit carotid artery. Male New Zealand White rabbits (2.5–3.5 kg) obtained from Langshaw Farms, (Augusta, MI) were anesthetized with ketamine HCl (50 mg/kg) and xylazine (16 mg/kg) s.c. In some experiments the left femoral artery was cannulated to obtain blood samples for platelet aggregation experiments. *Via* the right femoral artery, a Millar microtip pressure transducer (3 French size) was positioned in the descending aorta for blood pressure measurement.

The left carotid artery was isolated and a Carolina electromagnetic flow probe (4.5 mm circumference) was positioned proximally to monitor blood flow. A 1.5-cm section of the carotid artery distal to the flow probe was cross-clamped 10 times with a fine, serrated hemostat. A 26-gauge needle (0.5 cm in length) was inserted into the carotid artery distal to the flow probe at the site of endothelial cell damage. The needle was connected in series to a 9-V battery, a resistor and a potentiometer to produce 200 μamps d.c. current and was grounded to the s.c. neck tissues. A small vessel occluder was placed on the carotid artery over the site containing the indwelling needle, and mean carotid blood flow was reduced 25 to 35% of initial flow. Carotid blood flow was allowed to stabilize for 15 min to minimize production of spontaneous cyclic flow variations.

LY215840 or vehicle was administered *via* the marginal ear vein and 15 min later electrical stimulation of the carotid artery was initiated and maintained for 60 min. Time to carotid occlusion was measured from the beginning of electrical stimulation to the point at which carotid blood flow reached 0 ml/min. Experiments were terminated at 180 min after beginning stimulation.

Statistics. Data are presented as mean ± S.E.M. for the number of experiments indicated. Differences between means were determined with unpaired Student's *t* test and significant differences assumed when $P < .05$. Most Student's *t* tests were two-tailed except in figure 12 in which tests were one-tailed due to the unidirectional nature of treatment-induced changes.

Results

***In vivo* blockade of vascular 5-HT$_2$ receptors.** Given i.v., LY215840 (1–30 μg/kg) inhibited the pressor response to 5-HT in pithed rats (fig. 2), an effect known to be mediated by activation of 5-HT$_2$ receptors (Cohen *et al.*, 1983, 1985). Inhibition of the pressor response to 5-HT occurred with an ED_{50} of 2.8 μg/kg for LY215840, based on percentage of inhibition of the pressor response to 1 mg/kg i.v. of 5-HT.

When LY215840 (3–100 μg/kg) was administered p.o. (fig.

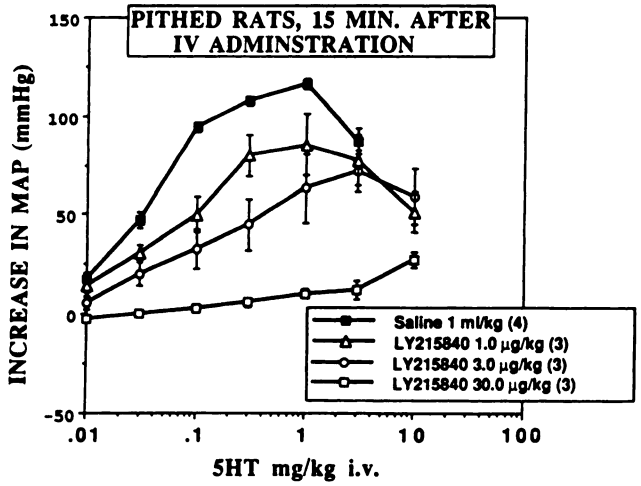


Fig. 2. Effect of i.v. administered LY215840 (1–30 µg/kg) to inhibit the pressor response to 5-HT administered 15 min after LY215840. Points are mean values and vertical bars represent S.E.M. for the number of animals indicated in parentheses. MAP, mean arterial pressure.

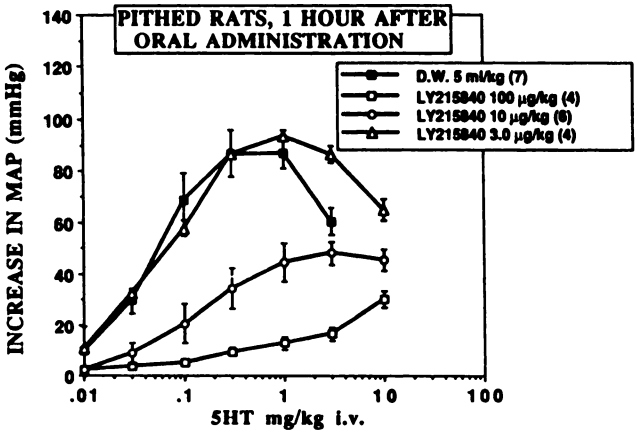


Fig. 3. Effect of p.o. administered LY215840 (3–100 µg/kg) on the pressor response to 5-HT administered 1 hr after LY215840. Points are mean values and vertical bars represent S.E.M. for the number of animals indicated in parentheses. MAP, mean arterial pressure; D.W., distilled water.

3), the pressor response to 5-HT was also inhibited markedly. The p.o. ED_{50} for LY215840 was approximately 10 µg/kg, based on percentage of inhibition of the pressor response to 5-HT (1 mg/kg i.v.). The p.o. to i.v. dose ratio approximated 4, suggesting excellent p.o. bioavailability in the rat.

To examine the duration of effective vascular 5-HT_2 receptor blockade after i.v. administration, LY215840 (3.0 µg/kg i.v.) was administered to pithed rats in a dose that produced approximately 70% inhibition of the pressor response to 5-HT (0.1 mg/kg i.v.). Inhibition of the pressor response to 5-HT persisted for > 2 hr after i.v. administration of LY215840 (fig. 4). Thus, persistent blockade of vascular 5-HT_2 receptors occurred after a single bolus i.v. dose of LY215840 to the rat.

LY215840 (100 µg/kg) was also evaluated for duration of vascular 5-HT_2 receptor blockade in pithed rats 1, 6 and 16 hr after its p.o. administration (fig. 5). Marked block of the pressor response to 5-HT occurred at 1 hr with responses returning, but not yet reaching control values, at 6 hr. By 16 hr after p.o. administration, responses to 5-HT returned to control values. LY215840 inhibited vascular 5-HT_2 receptors for longer than 6 hr after p.o. administration to rats.

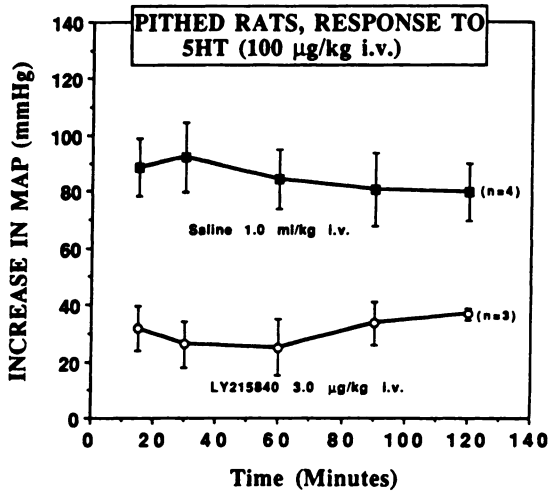


Fig. 4. Effect of LY215840 (3 µg/kg i.v.) to inhibit the repeated pressor response to 5-HT (100 µg/kg i.v.) for up to 2 hr after administration of LY215840. Points are mean values and vertical bars represent the S.E.M. for the number of animals indicated in parentheses. MAP, mean arterial pressure.

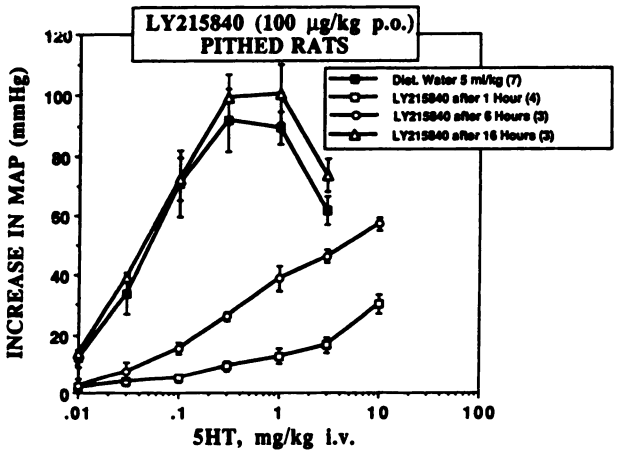


Fig. 5. Effect of LY215840 (100 µg/kg p.o.) to inhibit the pressor response to 5-HT at 1, 6 and 16 hr after its p.o. administration to pithed rats. Points are mean values and vertical bars represent S.E.M. for the number of animals indicated in parentheses. MAP, mean arterial pressure; Dist., distilled.

***In vivo* selectivity of LY215840.** LY215840 (100 µg/kg i.v.) was evaluated for its ability to inhibit norepinephrine-induced increases in heart rate (*beta*-1 adrenergic receptor-mediated), norepinephrine-induced increases in mean arterial pressure (*alpha*-1 adrenergic receptor-mediated) and angiotensin II-induced increases in arterial pressure (fig. 6). Although administered in doses in excess of those required to block pressor responses to 5-HT, LY215840 did not affect heart rate or pressor responses to norepinephrine or the pressor response to angiotensin II (fig. 6). Furthermore, LY215840 (up to 300 µg/kg i.v.) did not modify blood pressure or heart rate in pithed (data not shown) or urethane-anesthetized rats in doses well in excess of those required to block 5-HT_2 receptors (fig. 7). These data support the selectivity of LY215840 as an inhibitor of vascular 5-HT_2 receptors without affecting angiotensin-induced pressor responses, *alpha*-1 or *beta*-1 adrenergic receptors.

Effect of LY215840 on 5-HT-induced contraction in the rat jugular vein. 5-HT-induced contraction in the rat jugular vein is known to be mediated *via* activation of 5-HT_2

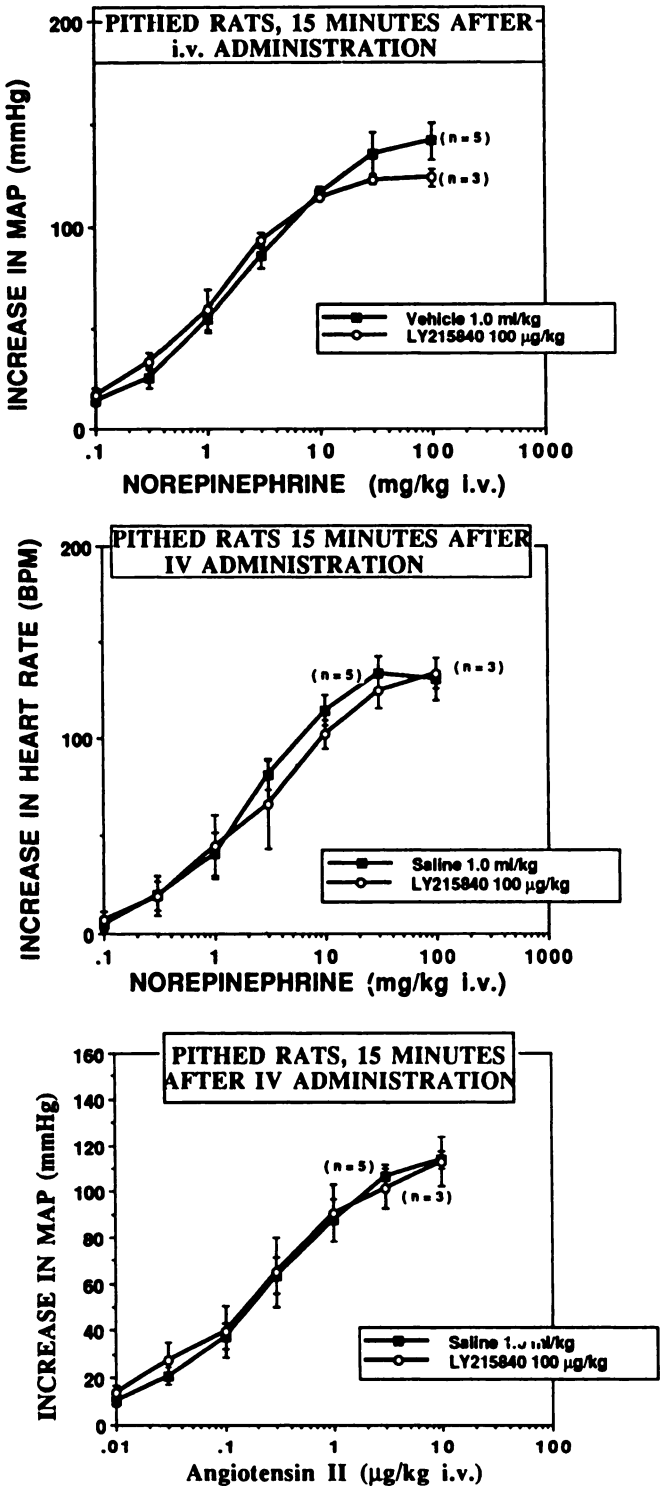


Fig. 6. Effect of LY215840 (100 µg/kg i.v.) on the pressor response to norepinephrine (top), heart rate increase to norepinephrine (middle) and pressor response to angiotensin II (bottom) when the rats were challenged 15 min after LY215840. Points are mean values and vertical bars represent S.E.M. for the number of animals indicated in parentheses. MAP, mean arterial pressure.

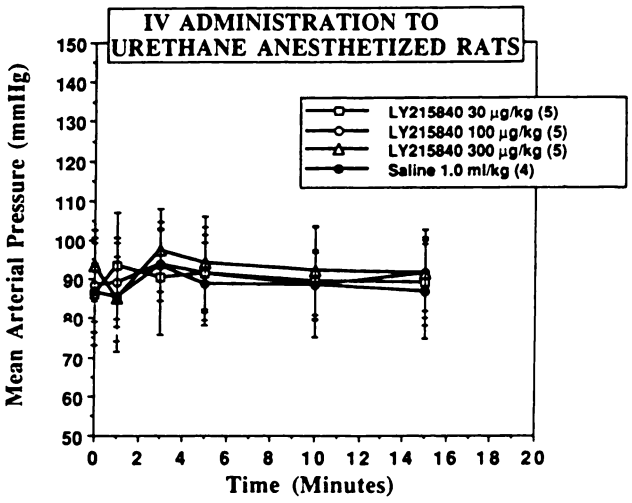


Fig. 7. Lack of effect of LY215840 (0.03–0.3 mg/kg i.v.)) on mean arterial pressure in urethane-anesthetized rats. Points are mean values and vertical bars represent S.E.M. for the number of animals indicated in parentheses. The point at 1 min was derived from peak changes occurring between 0 and 2 min after LY215840 administration.

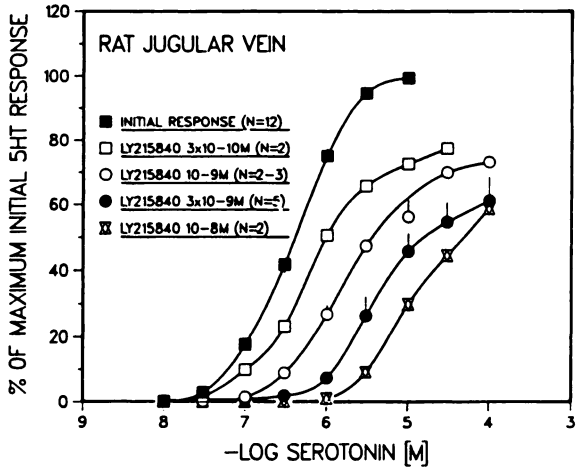


Fig. 8. Effect of LY215840 (3×10^{-10} to 10^{-8} M) to inhibit 5-HT-induced contraction in the rat jugular vein. Points are mean values and vertical bars represent S.E.M. for the number of tissues indicated in parentheses.

receptors (Cohen *et al.*, 1981). LY215840 (3×10^{-10} to 10^{-8} M) concentration dependently and potently inhibited the contractile response to 5-HT in the rat jugular vein (fig. 8). The inhibition was accompanied by a modest, but significant reduction in the maximal response to 5-HT. Thus, LY215840 possesses high affinity for the contractile 5-HT$_2$ receptor in the rat jugular vein.

Effect of LY215840 on 5-HT-amplified, ADP-induced platelet aggregation. 5-HT can amplify the platelet aggregation response produced by several aggregating agents. By using both rabbit and human platelets, 5-HT amplified aggregation produced by ADP (1 µM) (fig. 9). LY215840 markedly inhibited the amplified response produced by 5-HT in both human and rabbit platelets, similar to the LY53857-induced inhibition observed previously using rabbit platelets (Wilson *et al.*, 1991). Furthermore, LY215840 reduced the aggregation produced by the combination of 5-HT and ADP below aggregation seen with ADP alone in both rabbit and human platelets, suggesting that LY215840 also affected ADP-induced aggregation in rabbit and human platelets.

Effect of LY215840 to inhibit rabbit carotid artery occlusion induced by electrical stimulation. Endothelial cell injury of the rabbit carotid artery was induced by electrical stimulation and cross-clamping. Mechanical constriction, which reduced carotid blood flow by about 20 to 35%, increased

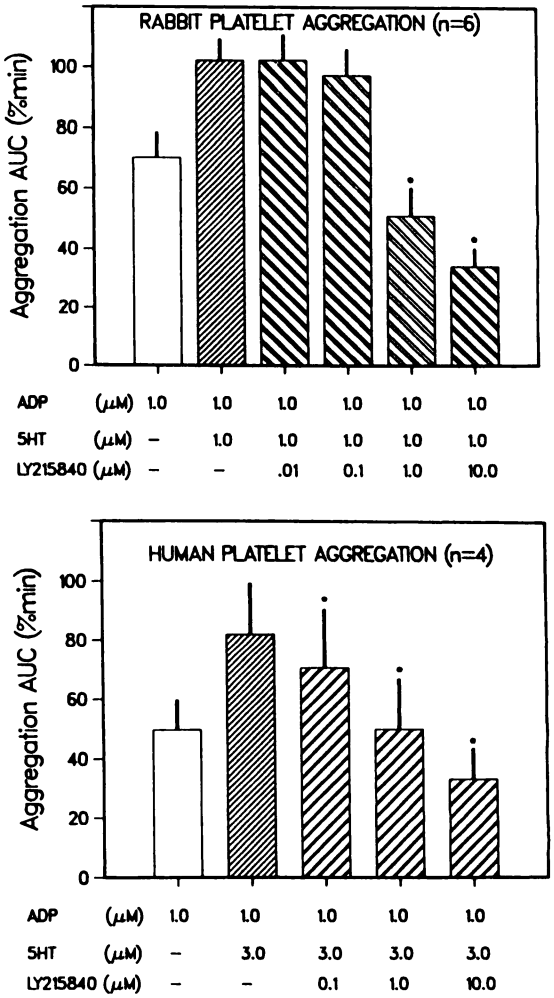


Fig. 9. Effect of increasing concentrations of LY215840 (0.01–10.0 μM) on platelet aggregation produced by the combination of ADP (1.0 μM) and 5-HT (1.0 μM) in rabbit (top) and human (bottom) platelets *in vitro*. Vertical bars represent mean values and vertical lines represent S.E.M. from four platelet samples derived from six rabbits and four humans. *Those values that differ significantly from the combination of ADP and 5-HT ($P < .05$). AUC, area under the curve.

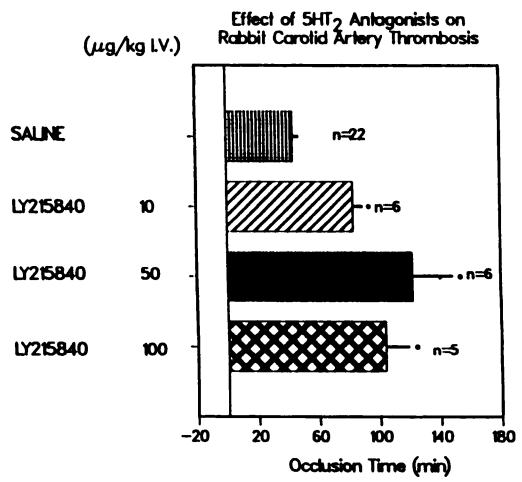


Fig. 10. Effect of increasing doses of LY215840 (10, 50 and 100 μg/kg i.v.) on occlusion times in the rabbit carotid artery thrombosis model. Bars represent mean values and horizontal lines indicate S.E.M. for the number of rabbits indicated in parentheses. *Those values that differ significantly from saline-treated rabbits ($P < .05$).

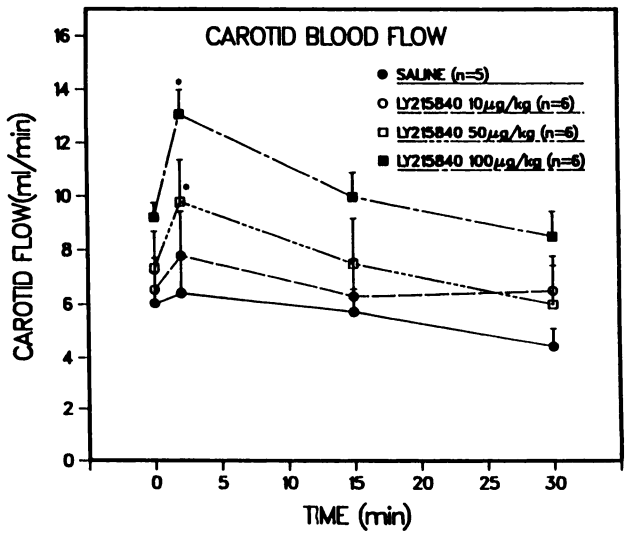


Fig. 11. Effect of LY215840 (10, 50 and 100 μg/kg i.v.) on carotid blood flow in anesthetized rabbits. Points are mean values and vertical lines represent the S.E.M. for the number of rabbits indicated. *Those values that differ significantly from saline-treated rabbits ($P < .05$).

shear force. Under these conditions, vascular occlusion can be measured by reduction in carotid blood flow to 0; the blood flow reduction occurred within 40 to 60 min after initiation of electrical stimulation of the artery. Under these conditions, LY215840 (10–100 μg/kg i.v.) prolonged the time to occlusion in the rabbit carotid artery (fig. 10). Furthermore, like LY53857 (Wilson *et al.*, 1991), LY215840 produced a transient increase in carotid blood flow in rabbits (fig. 11), an increase which returned toward control values 15 to 30 min after administration of LY215840. Because carotid blood flow was only transiently increased, it is unlikely that the increase in carotid flow could account for the prolongation to vascular occlusion which occurred for up to 2 hr after the bolus i.v. administration of LY215840. In the rabbit, LY215840 (50 and 100 μg/kg i.v.) produced a modest (8.6–13%) reduction in systolic blood pressure, respectively.

In some rabbits, the effect of LY215840 (100–300 μg/kg i.v.) on platelet aggregation in response to ADP and 5-HT was measured *ex vivo* (fig. 12). LY215840 inhibited the *ex vivo* ability of 5-HT to amplify platelet aggregation to ADP. Thus, prolongation of vascular occlusion time seen in the rabbit was associated with *ex vivo* inhibition of 5-HT-induced amplification of platelet aggregation by LY215840.

Discussion

LY215840 is an ergoline amide that is more potent p.o. (see fig. 13 for comparative data) as an antagonist of vascular 5-HT_2 receptors in the rat than either LY53857 (Cohen *et al.*, 1983, 1985) or sergolexole (Cohen *et al.*, 1988, 1989). The fact that this compound was also considerably more water soluble than sergolexole makes this ergoline amide 5-HT_2 receptor antagonist useful for studies where i.v. administration is important. Intravenous administration of 5-HT_2 receptor antagonists will permit evaluation of their therapeutic efficacy in cardiovascular indications. Furthermore, LY215840 possessed a long duration of action (in excess of 2 hr) after single bolus i.v. administration in rats. Likewise, p.o. administration of LY215840 also resulted in pronounced inhibition of vascular 5-

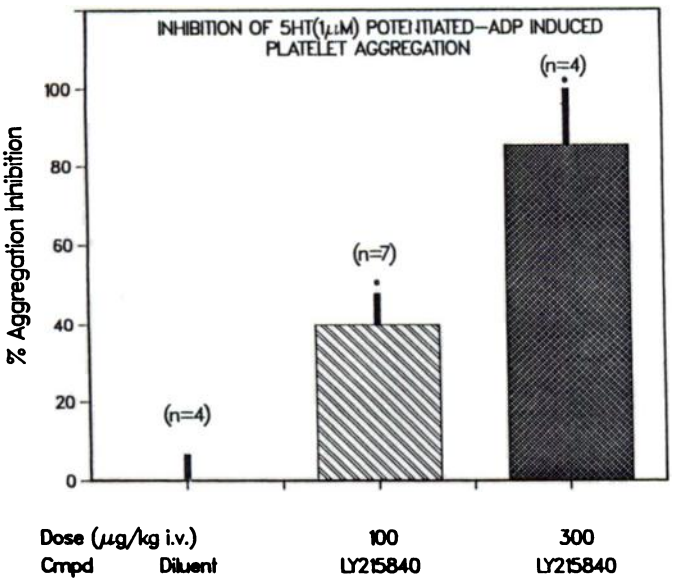


Fig. 12. Effect of LY215840 (100 and 300 μg/kg i.v.) on *ex vivo* platelet aggregation responses to 5-HT (1 mM) and ADP (1 mM) in rabbit platelets. Bars represent mean values and vertical lines indicate S.E.M. for the number of rabbits indicated in parentheses. *Those values that differ significantly from saline-treated rabbits ($P < .05$). Cmpd, compound.

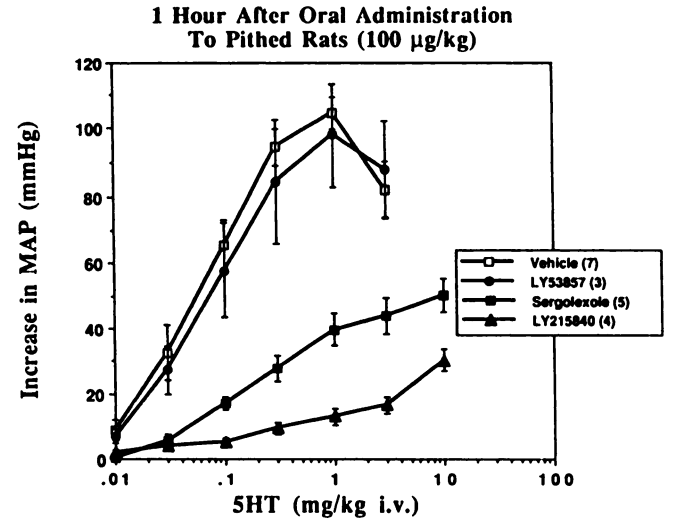


Fig. 13. Comparative effect of three potent 5-HT$_2$ receptor antagonists on pressor response to 5-HT after p.o. administration of 0.1-mg/kg 5-HT$_2$ receptor antagonists to pithed rats. Points are mean values and vertical lines represent the S.E.M. for the number of animals indicated in parentheses. MAP, mean arterial pressure.

HT$_2$ receptors 6 hr after its administration. The long duration of action may be due to its high affinity for the 5-HT$_2$ receptor as suggested by the dramatic inhibition of 5-HT-induced contractility in the rat jugular vein. Alternatively, formation of active metabolites may also contribute to its long action, *in vivo*. Finally, an evaluation of the i.v. to p.o. dose ratio suggests that LY215840, like sergolexole, has excellent p.o. bioavailability based on its p.o. to i.v. dose ratio.

With regard to cardiovascular indications, potential efficacy of 5-HT$_2$ receptor antagonists in vascular occlusive disorders is thought to arise from their simultaneous blockade of platelet 5-HT$_2$ aggregation and vascular 5-HT$_2$ constrictor responses. Studies with LY215840, using rabbit and human platelets, supported previous data documenting the ability of other ergoline 5-HT$_2$ receptor antagonists to block platelet aggregation responses to 5-HT (McBride *et al.*, 1990; Wilson *et al.*, 1991), effects mediated by activation of platelet 5-HT$_2$ receptors. In addition to the ability of LY215840 to inhibit 5-HT$_2$ receptors on rabbit and human platelets, LY215840 also appeared to inhibit ADP-induced platelet aggregation in both rabbit and human platelets, although the direct effect of LY215840 on ADP-induced platelet aggregation was not studied. A similar effect was documented for LY53857 which directly blocked ADP-induced aggregation in rabbit platelets only (Wilson *et al.*, 1991). These important observations suggest that ergoline 5-HT$_2$ receptor antagonists may be effective in inhibiting platelet aggregation not only in response to 5-HT, but in response to other aggregating agents as well. This may contribute to the potential effectiveness of LY215840 in treating vascular occlusive disorders.

As seen with both sergolexole and LY53857, this newest member of the ergoline family of 5-HT$_2$ receptor antagonists showed a high degree of selectivity in its ability to antagonize vascular 5-HT$_2$ receptors, without affecting pressor responses to either angiotensin II or to norepinephrine. Furthermore, *beta* adrenergic receptors were not affected by doses of LY215840 in excess of those required to block 5-HT$_2$ receptors. Based on the molecular similarities between 5-HT$_2$ and 5-HT$_{1C}$ receptors, LY215840, like LY53857 and sergolexole, showed similar affinity at both 5-HT$_2$ and 5-HT$_{1C}$ receptors with 50- to 1000-fold lower affinity at other 5-HT receptor subtypes (D. Nelson, personal communication). These data support the 5-HT$_2$/5-HT$_{1C}$ receptor selectivity of these ergoline derivatives. At the present time, the precise role of the 5-HT$_{1C}$ receptor affinity to the observed cardiovascular effects of LY215840 is unclear because 5-HT$_{1C}$ receptors have not been identified in blood vessels or platelets to date.

Most relevant is the observation that low doses of LY215840 were highly effective in delaying the occlusion time of endothelially damaged, electrically stimulated and mechanically constricted carotid arteries in the rabbit. In this regard, LY215840 (present study) was even more potent than LY53857 (Wilson *et al.*, 1991). LY215840 also caused a dose-dependent increase in carotid blood flow after its i.v. administration. The observation that two selective 5-HT$_2$ receptor antagonists, LY53857 (Wilson *et al.*, 1991) and LY215840 (present study) increased carotid blood flow in the rabbit may suggest that vasoconstriction in the carotid artery is tonically influenced by 5-HT. The possibility that 5-HT$_2$ receptor activation in local vascular beds can regulate flow has been proposed previously for ischemic myocardium (Simpson *et al.*, 1991), although this localized effect on vascular caliper does not translate to an important effect on blood pressure, which was not altered markedly by LY215840 or LY53857. Because carotid flow was restored to control values 15 to 30 min after administration of LY215840 in doses that markedly prolonged occlusion time through 2 hr, the increase in carotid blood flow was not required for prolongation of occlusion time in rabbits. The effectiveness of LY215840 in delaying occlusion time is likely related to its ability to block vascular 5-HT$_2$ receptors, coupled to its ability to antagonize platelet 5-HT$_2$ receptors. In fact, *ex vivo* examination of platelet aggregation indicated that LY215840 inhibited platelet aggregation to 5-HT in rabbits treated previously with LY215840 (fig. 10).

These data on LY215840 suggest that this rabbit model of carotid artery occlusion may be useful in probing the role of

vascular and platelet 5-HT_2 receptors in vascular occlusive disease. Furthermore, LY215840 is an ergoline amide 5-HT_2 receptor antagonist that is potent, selective, p.o. effective and has a long duration of activity in preclinical studies.

Acknowledgments

The authors acknowledge the skillful technical assistance of Mr. William Coffman, Mr. Dennis Thompson, Ms. Kathryn Schenck and the expert secretarial assistance of Ms. Sherry Pike and Ms. Virginia Santerre.

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