J. Pharm. Pharmacol. 1994, 46: 226–229
Received April 29, 1993

Comparative 5-HT_2-Receptor Antagonist Activity of Amesergide and its Active Metabolite 4-Hydroxyamesergide in Rats and Rabbits

MARLENE L. COHEN, KENNETH D. KURZ, RAY W. FULLER AND DAVID O. CALLIGARO

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285, USA

Abstract—Amesergide is an orally active ergoline amide, 5-HT_2-receptor antagonist with a long duration of action. Since a major metabolite of amesergide is 4-hydroxyamesergide, we questioned whether the formation of this metabolite might contribute to the pharmacological activity and long duration of action observed after oral administration of amesergide. 4-Hydroxyamesergide was a potent 5-HT_2-receptor antagonist with an affinity equal to or greater than amesergide under in-vitro conditions as measured by blockade of vascular 5-HT_2 receptors, and 5-hydroxytryptamine (5-HT)-amplified ADP-induced rabbit platelet aggregation. Furthermore, 4-hydroxyamesergide, like amesergide, inhibited the pressor response to 5-HT after its intravenous administration to rats and was about 3-fold more potent than amesergide in this regard. 4-Hydroxyamesergide was also a potent inhibitor of vascular 5-HT_2 receptors after oral administration to rats. After oral administration, 4-hydroxyamesergide had a similar or slightly greater duration of activity than the parent molecule. 4-Hydroxyamesergide, again like amesergide, also blocked central 5-HT_2 receptors after its in-vivo administration to rats as measured by its ability to inhibit quipazine-induced increases in serum corticosterone. Thus, the formation of 4-hydroxyamesergide after oral administration of amesergide to animals and man may contribute to the potency and long duration of action of amesergide as a 5-HT_2-receptor antagonist.

As part of a synthetic effort directed toward the discovery of potent and selective 5-HT_2/5-HT_{1C}-receptor antagonists, amesergide was identified as an orally active long-acting 5-HT_2/5-HT_{1C}-receptor antagonist (Misner et al 1990; Foreman et al 1992). The long duration of action of amesergide and the identification of 4-hydroxyamesergide as a primary metabolite in rats after oral administration of amesergide (Foreman et al 1992), have prompted the present comparative study of these two ergolines in an effort to understand whether 4-hydroxyamesergide might contribute to the pharmacological activity observed after administration of amesergide.

In the present study, we compared the in-vitro vascular and platelet 5-HT_2-receptor antagonist activity of amesergide and 4-hydroxyamesergide. Additionally, these two ergolines were also compared with regard to their in-vivo antagonism of both peripheral (blockade of a pressor response to 5-hydroxytryptamine (5-HT)) and central (blockade of quipazine-induced increases in serum corticosterone) 5-HT_2 receptors.

Materials and Methods

Effect on 5-HT-induced contraction in the rat jugular vein in-vitro

Male Wistar rats (240–400 g, Charles River, Portage, MI, USA) were killed 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 modified Krebs solution of the following composition (mM): 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.

Correspondence: M. L. Cohen, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285, USA.

Tissue bath solutions were maintained at 37°C and aerated with 95% O_2–5% CO_2. Isometric contractions were recorded as changes (g) on a Beckman Dynograph with Statham UC-3 transducers and microscale accessory attachments. Tissues were allowed to equilibrate at an initial optimum resting force of 1 g for 1–2 h before exposure to drugs. After control cumulative contractile responses to 5-HT were obtained, tissues were incubated with appropriate concentrations of antagonist for 1 h. Responses to 5-HT were then repeated in the presence of antagonist.

Effect on 5-HT-amplified ADP-induced platelet aggregation in-vitro

Male New Zealand White rabbits (Hazleton Research Animals, Kalamazoo, MI, USA) were anaesthetized with 50 mg kg^{-1} ketamine HCl and 16 mg kg^{-1} xylazine subcutaneously. Whole blood was withdrawn from the right carotid artery into 20-mL syringes containing 2·0 mL sodium citrate (3·8%). Portions (5 mL) of blood were dispensed into polystyrene tubes. Platelet-rich plasma (PRP) was obtained by centrifugation at 160 *g* for 12 min at 25°C. Supernatant PRP was removed and platelet-poor plasma (PPP) was obtained by centrifugation at 1500 *g* for 15 min. Platelets were counted on a Cell Dyne 1600 cell counter. PRP was diluted with autologous PPP to achieve $300\,000 \times 10^6$ cells L^{-1} for aggregation studies. Platelet aggregation was measured at 37°C on a Chrono-Log aggregometer (model 570 US) using the optical density method. Aggregation was followed for 5 min following the addition of agonists, and amplitude was measured as distance (mm) from base line.

Blockade of 5-HT_2 receptor-mediated pressor responses in-vivo

The 5-HT_2-receptor antagonist activity of amesergide and 4-

hydroxyamesergide was evaluated using the 5-HT-induced pressor response in pithed, spontaneously hypertensive rats (Taconic Farms, Inc., Germantown, NY, USA; 300–400 g). This model was selected because responses to 5-HT in the conscious animal are multiphasic and difficult to interpret due to chemo- and baroreceptor stimulation, whereas responses in the pithed animal are direct, vascular responses. Spontaneously hypertensive rats were anaesthetized with halothane (2% in nitrous oxide and oxygen), femoral venous and arterial catheters were implanted and the trachea was cannulated. Rats were pithed by passing a steel rod through the right orbit and down the entire length of the spinal column, where the rod remained for the duration of the experiment. Immediately after pithing, rats were ventilated with a rodent respirator using room air. The animals were allowed to stabilize for 15 min before any treatments or measurements. The maximal change in mean arterial blood pressure to intravenously administered 5-HT (0·001–10 mg kg^{-1}) was recorded before and 15 min after intravenously administered amesergide or 4-hydroxyamesergide. For oral activity, conscious rats were pretreated by gavage, 1, 6 or 24 h before determination of the pressor response to intravenously administered 5-HT. Blood pressure was allowed to return to control values between doses of 5-HT.

Determination of serum corticosterone concentrations in-vivo

Male Sprague-Dawley rats (Harlan Sprague-Dawley Inc., Indianapolis, IN, USA) were used in these studies. Quipazine maleate (2·5 mg kg^{-1}) was injected subcutaneously 1 h before rats were killed and 1 h after intraperitoneal administration of amesergide or its metabolite. Rats were decapitated and trunk blood was collected and allowed to clot. Serum samples were obtained by centrifugation of the clotted blood and were stored frozen (−15°C) before analysis. Serum corticosterone concentrations were determined by the spectrofluorometric method of Solem & Brinck-Johnsen (1965). Statistical evaluations were made using a Student's *t*-test and statistical significance was assumed when $P < 0{\cdot}05$.

Radioligand binding affinities at non-5-HT-ergic receptors

In-vitro binding affinities of 4-hydroxyamesergide at α_1-, α_2-, β-, D_1, D_2, H_1, benzodiazepine, $GABA_A$ and muscarinic receptors were determined in homogenates of frozen rat brains (PelFreez Biologicals Rogers, AR, USA) according to methods and conditions previously reported for amesergide (Foreman et al 1992). The apparent K_i for each receptor was determined from eleven concentrations in two separate experiments, analysing the data using nonlinear least-squares regression analysis (Munson & Rodbard 1980).

Results

In-vitro blockade of vascular 5-HT$_2$ receptors

Both amesergide and 4-hydroxyamesergide were potent inhibitors of 5-HT-induced contraction in the rat jugular vein (Fig. 1), an index of 5-HT$_2$-receptor blockade. Further, 4-hydroxyamesergide was approximately 30-fold more potent than amesergide as an inhibitor of vascular 5-HT$_2$ receptors in the rat jugular vein in-vitro.

In-vitro blockade of platelet 5-HT$_2$ receptors

Both amesergide and 4-hydroxyamesergide were potent inhibitors of the 5-HT-ergic component of 5-HT-amplified ADP-induced rabbit platelet aggregation (Fig. 2). In the concentration examined (10^{-6} M), both ergolines maximally inhibited the platelet aggregation response to 5-HT.

In-vivo blockade of the pressor response to 5-HT in pithed spontaneously hypertensive rats

Intravenous administration of amesergide (0·1 and 0·3 mg kg^{-1}, i.v.) and 4-hydroxyamesergide (0·01 mg kg^{-1}, i.v.) inhibited the pressor response to 5-HT in pithed spontaneously hypertensive rats, an effect known to be mediated by activation of 5-HT$_2$ receptors (Fig. 3). 4-Hydroxyamesergide was approximately 3-fold more potent than amesergide after intravenous administration as an antagonist at vascular 5-HT$_2$ receptors.

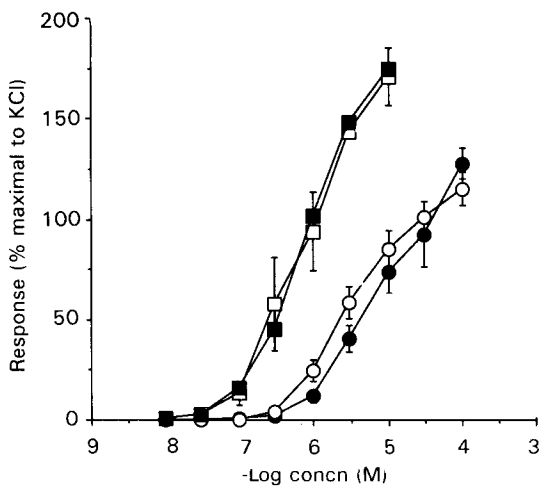

FIG. 1. In-vitro inhibition of 5-HT-induced contraction by amesergide and 4-hydroxyamesergide in the rat jugular vein. Points are mean values and vertical bars represent the standard error of the mean. ■ Initial response, n = 14; ● 4-hydroxyamesergide 3×10^{-9} M, n = 3–6; □ amesergide 3×10^{-9} M, n = 3; ○ amesergide 10^{-8} M, n = 5.

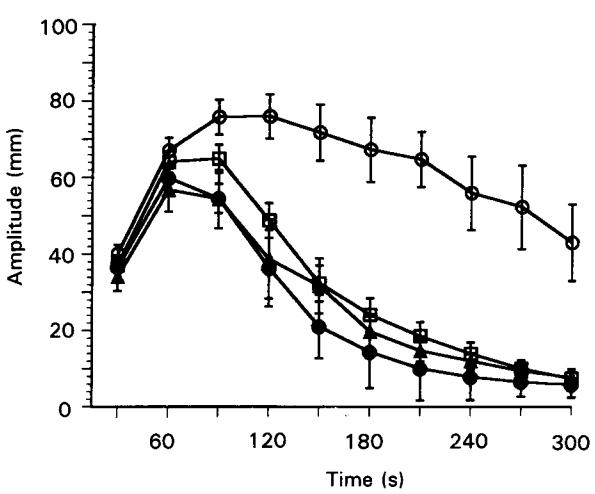

FIG. 2. In-vitro inhibition of 5-HT-amplified, ADP-induced rabbit platelet aggregation by amesergide and 4-hydroxyamesergide. Points are mean values and vertical bars represent the standard error of the mean. □ ADP 10^{-6} M, n = 16; ○ ADP 10^{-6} M + 5-HT 10^{-6} M + vehicle, n = 4; ▲ ADP 10^{-6} M + 5-HT 10^{-6} M + amesergide 10^{-6} M, n = 3; ● ADP 10^{-6} M + 5-HT 10^{-6} M + 4-hydroxyamesergide 10^{-6} M, n = 4.

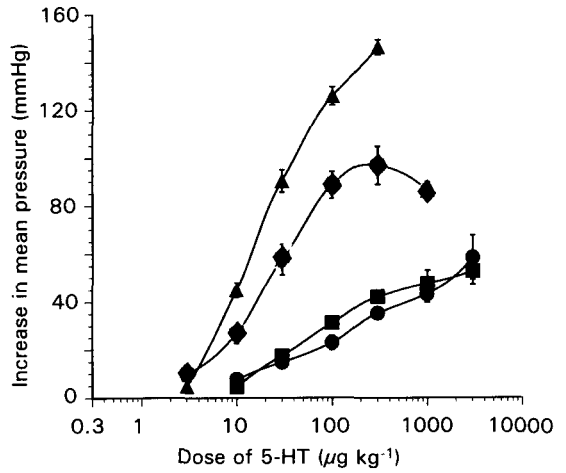

FIG. 3. Effect upon 5-HT pressor responses in pithed spontaneously hypertensive rats after 15-min intravenous pretreatment with amesergide and the metabolite 4-hydroxyamesergide. Baseline mean arterial blood pressure in the groups of pithed rats (n) before administration of 5-HT was: 58±2 mmHg in controls (▲ 4); 59±4 and 64±1 mmHg after 0·01 (◆ 7) and 0·03 mg kg^{-1} (■ 5) amesergide; and 61±1 mmHg after 0·01 mg kg^{-1} (● 4) 4-hydroxyamesergide.

Amesergide and 4-hydroxyamesergide were compared for their ability to inhibit the pressor response to 5-HT in the pithed preparation 1, 6 and 24 h after oral administration to conscious, spontaneously hypertensive rats. One hour after oral administration of these ergolines, amesergide at 0·01 and 0·1 mg kg^{-1} inhibited the pressor response to 5-HT slightly more than the equivalent doses of 4-hydroxyamesergide (Fig. 4, top). In contrast, by 6 h after oral administration, the inhibition of the pressor response to 5-HT was similar for both ergolines at each of the doses studied (Fig. 4, middle). Likewise, 24 h after oral administration, 0·01 mg kg^{-1} amesergide and 4-hydroxyamesergide no longer inhibited the pressor response to 5-HT whereas at the higher dose of 0·1 mg kg^{-1}, 4-hydroxyamesergide was slightly more potent than amesergide (Fig. 4, bottom). Thus, maximal inhibition of the pressor response to 5-HT occurred at 1 h with similar inhibition after 6 h for amesergide, whereas 4-hydroxyamesergide produced greater inhibition of the pressor response to 5-HT 6 h after its oral administration relative to its effectiveness after 1 h. Inhibition of 5-HT$_2$ receptors was long lasting for both agents after oral administration.

In-vivo inhibition of central 5-HT$_2$ receptors measured by blockade of quipazine-induced increases in serum corticosterone

Both amesergide and 4-hydroxyamesergide inhibited quipazine-induced increases in serum corticosterone (Table 1), an index of their efficacy in blocking brain 5-HT$_2$ receptors after in-vivo administration. The estimated ED50 values were 0·5 and 0·7 mg kg^{-1} respectively for 4-hydroxyamesergide and amesergide. Thus, both agents are capable of blocking central 5-HT$_2$ receptors.

Radioligand binding affinities at non-5-HT-ergic receptors

4-Hydroxyamesergide was also examined for its binding affinity at non-5-HT-ergic receptors (Table 2). Like other structurally related ergolines (Cohen et al 1985), 4-hydroxyamesergide had some modest affinity at α_2-adrenergic receptors (K_i=13 nM) with considerably lower to negligible affinity at α_1-, β-, D_1, D_2, H_1, benzodiazepine, GABA and muscarinic receptors.

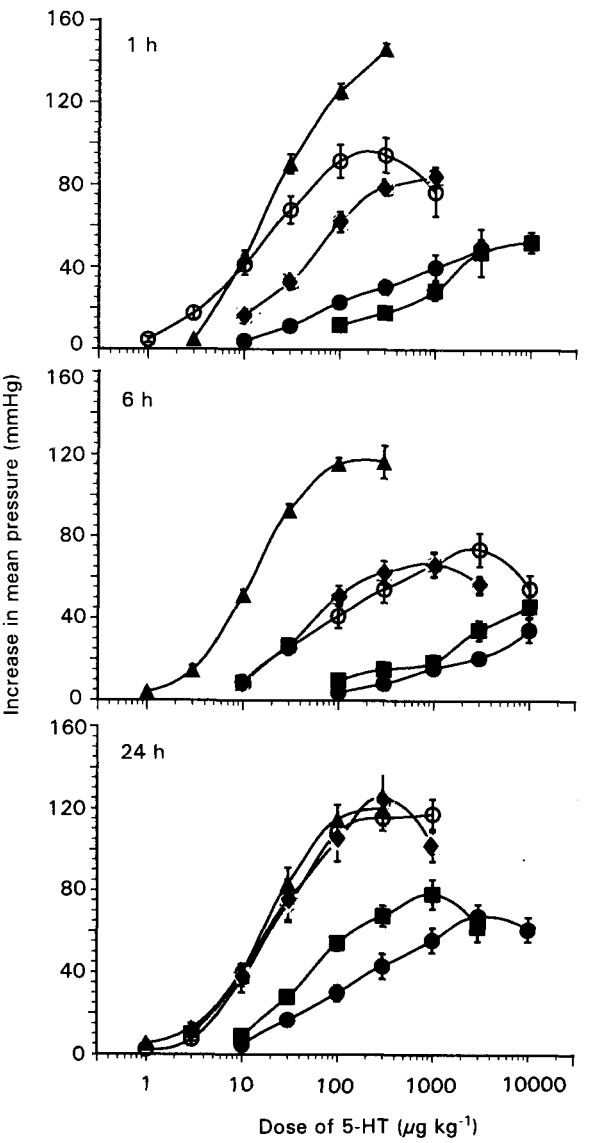

FIG. 4. Effect upon 5-HT pressor responses in pithed spontaneously hypertensive rats after oral pretreatment with amesergide and the metabolite 4-hydroxyamesergide. Conscious rats were treated by gavage at the times indicated and were anaesthetized 30 min before determination of pressor responsiveness. Baseline mean arterial blood pressure in the pithed rat before administration of 5-HT in the 1-, 6-, and 24-h groups (n) averaged respectively: 58±2(4), 60±2 (10) and 54±1(4) mmHg in the controls (▲); 66±2(11), 64±7(8) and 58±5(6) mmHg after 0·01 mg kg^{-1} amesergide (◆); 66±4(4), 54±2(8) and 66±4(4) mmHg after 0·1 mg kg^{-1} amesergide (■); 59±3(10), 67±3(6) and 59±5(4) mmHg after 0·01 mg kg^{-1} 4-hydroxyamesergide (○); and 55±3(5), 66±3(4) and 65±3(4) mmHg after 0·1 mg kg^{-1} 4-hydroxyamesergide (●).

Discussion

Amesergide is a potent, relatively selective 5-HT$_2$/5-HT$_{1C}$-receptor antagonist (Misner et al 1990) with a long duration of action (Foreman et al 1992). Amesergide is biotrans-

Table 1. Effect of intraperitoneal administration of 4-hydroxyamesergide and amesergide on quipazine-induced increases in serum corticosterone (μg/100 mL) in rats.

Dose (mg kg^{-1})	Vehicle	Quipazine
4-Hydroxyamesergide		
0	4·1 ± 0·3	40·7 ± 2·5[a]
0·3	4·1 ± 0·3	30·0 ± 3·9[a]
1	4·3 ± 0·3	12·0 ± 3·7[b]
3	4·3 ± 0·3	5·5 ± 1·1[b]
Amesergide		
0	4·1 ± 0·3	43 ± 2·3[a]
0·3	4·1 ± 0·3	43·2 ± 1·4[a]
1	6·0 ± 1·1	14·6 ± 6·4[a]
3	5·0 ± 0·2	11·8 ± 2·6[a,b]

All values are mean ± s.e.m. for five animals. [a] $P < 0·05$ compared with vehicle, [b] $P < 0·05$ compared with quipazine alone.

Table 2. Apparent affinity constants for 4-hydroxyamesergide at non-5-HT-ergic receptors.

Receptor	Apparent K_i (nM)
α_1	2300
α_2	13 ± 5
β	> 1000
D_1	155 ± 20
D_2	560 ± 14
H_1	2800
Benzodiazepine	> 10000
GABA	> 10000
Muscarinic	> 10000

formed in both rats and man to 4-hydroxyamesergide, a major metabolite. Previous studies on the structure-activity relationships of ergoline derivatives and their interaction with 5-HT$_2$ receptors had shown derivatives possessing an oxy or hydroxy moiety in the side chain at position 9 on the ergoline ring to be potent antagonists (Garbrecht et al 1988). The observation that amesergide was metabolized by hydroxylation on the cyclohexyl amide side chain of the ergoline structure, coupled with our previous information regarding the 5-HT$_2$-receptor antagonist structure-activity relationships and the observed long duration of pharmacological activity of amesergide following its oral administration, raised the possibility that 4-hydroxyamesergide was likely to be a potent 5-HT$_2$-receptor antagonist.

The in-vitro and in-vivo intravenous data are consistent with our previous information on synthetic ester analogs documenting the importance of an oxygen moiety on the cyclohexyl ring (Garbrecht et al 1988) to 5-HT$_2$-receptor blockade and extend those previous observations to amide derivatives.

Following oral administration, 4-hydroxyamesergide was slowly absorbed since its ability to inhibit vascular 5-HT$_2$ receptors at 1 h was less than that observed for amesergide at the same time. Nevertheless, at 6 h after oral administration, 4-hydroxyamesergide was equipotent to amesergide as an inhibitor of vascular 5-HT$_2$ receptors. At 24 h after oral administration, high doses of 4-hydroxyamesergide were more effective in blocking vascular 5-HT$_2$ receptors suggesting a slightly longer duration of 5-HT$_2$-receptor blockade with 4-hydroxyamesergide than observed with amesergide after oral administration to rats.

Of the non-5-HT-ergic receptors, 4-hydroxyamesergide had highest affinity at α_2-receptors analogous to previous observations with structurally related ergolines (Cohen et al 1985, 1988), including amesergide (Foreman et al 1992). Affinity of 4-hydroxyamesergide, like amesergide (Foreman et al 1992), was low to negligible at α_1-, β-, D_1, D_2, H_1, benzodiazepine, GABA and muscarinic receptors. The similar degree of receptor specificity of these ergolines reduces the probability that the in-vivo formation of 4-hydroxyamesergide following oral administration of amesergide would result in new or unpredicted side-effects.

The potent and long-lasting 5-HT$_2$-receptor blockade produced by 4-hydroxyamesergide both in-vitro and in-vivo, raises the likely possibility that formation of 4-hydroxyamesergide after oral administration of amesergide may contribute to the effectiveness of amesergide as a 5-HT$_2$-receptor antagonist, both in animals and in man (Goldberg et al 1992). In addition, these studies with 4-hydroxyamesergide reinforce the observation that oxygenation in the 4-position of the cyclohexyl amide side chain on the ergoline moiety results in potent 5-HT$_2$-receptor antagonists.

Acknowledgements

The authors thank Dr David Robertson and Mr Joseph Krushinski for supplying *trans*-4-hydroxyamesergide and acknowledge the expert technical assistance of Mr Tommy Smith, Mr William Bloomquist, Ms Kathryn Schenck and Mr Harold D. Snoddy.

References

Cohen, M. L., Fuller, R. W., Wiley, K. S. (1981) Evidence for 5-HT$_2$ receptors mediating contraction in vascular smooth muscle. J. Pharmacol. Exp. Ther. 218: 421–425

Cohen, M. L., Colbert, W., Wittenauer, L. A. (1985) Receptor specificity of the 5-HT$_2$ receptor antagonist, LY53857. Drug Dev. Res. 5: 313–321

Cohen, M. L., Fuller, R. W., Kurz, K. D., Parli, C. J., Mason, N. R., Meyers, D. B., Smallwood, J. K., Toomey, R. E. (1988) Preclinical pharmacology of a new serotonergic (5-HT$_2$) receptor antagonist, LY281067. J. Pharmacol. Exp. Ther. 244: 106–112

Foreman, M. A., Fuller, R. W., Nelson, D. L., Calligaro, D. O., Kurz, K. D., Misner, J. W., Garbrecht, W. L., Parli, C. J. (1992) Preclinical studies on LY237733, a potent and selective serotonergic antagonist. J. Pharmacol. Exp. Ther. 260: 51–57

Garbrecht, W. L., Marzoni, G., Whitten, K. R., Cohen, M. L. (1988) (8β)-Ergoline-8-carboxylic acid cycloalkyl esters as serotonin antagonists: structure-activity study. J. Med. Chem. 31: 444–448

Goldberg, M. J., Collins, J. F., Cerimele, B. J., Rowe, H. M. (1992) Effect of LY237733, a 5-HT$_2$ receptor antagonist, on serotonin hand vein constriction in humans. Meeting of the American Society for Clinical Pharmacology and Therapeutics, Orlando, Florida, March 18–20. Clin. Pharmacol. Ther. 51: 152

Misner, J. W., Garbrecht, W. L., Marzoni, G., Whitten, K. R., Cohen, M. L. (1990) (8β)-6-Methylergoline amide derivatives as serotonin antagonists: *N*-substituent effects on vascular 5-HT$_2$ receptor activity. J. Med. Chem. 33: 652–656

Munson, P. J., Rodbard, J. D. (1980) Ligand: a versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 107: 220–239

Solem, J. A., Brinck-Johnsen, T. (1965) An evaluation method for determination of free corticosteroids in minute quantitities of mouse plasma. Scand. J. Clin. Invest. 17 (Suppl. 80): 1–14