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# Serotonin$_{1A}$ Receptor Activation by Flesinoxan in Humans

## Body Temperature and Neuroendocrine Responses

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*The effects of the selective 5-HT$_{1A}$ receptor agonist flesinoxan on neuroendocrine function, temperature, and behavior were assessed in male healthy volunteers using a double-blind, placebo-controlled crossover design. Flexinoxan (7 and 14 μg/kg), administered intravenously in 11 healthy volunteers, elicited a dose-related decrease in body temperature and increases in growth hormone, adrenocorticotropic hormone (ACTH), cortisol, and prolactin plasma levels. In a second independent study, 12 healthy volunteers were pretreated sequentially, at one-week intervals, with either the 5-HT$_{1A}$ antagonist pindolol (30 mg, PO), the nonselective 5-HT$_{1,2}$ antagonist methysergide (4 mg, PO), or placebo, prior to being administered flesinoxan (1 mg, IV). The growth hormone response to flesinoxan was blocked by pindolol but not by methysergide, whereas the prolactin response was blocked by methysergide but not by pindolol. The ACTH and cortisol responses to flesinoxan were potentiated by methysergide. The flesinoxan-induced hypothermia was attenuated by both methysergide and pindolol, although the latter effects did not reach statistical significance. The present results suggest that the growth hormone response and the hypothermic response to the intravenous infusion of flesinoxan may serve as a valid index of 5-HT$_{1A}$ receptor function in humans.* ***[Neuropsychopharmacology 13:93–104, 1995]***

KEY WORDS: *Flesinoxan; Hypothermia; Growth hormone; Pindolol; Methysergide; 5-HT$_{1A}$ receptor*

Over the past decade, the development of nontoxic, selective serotonin (5-HT) receptor agonists has made it possible to probe 5-HT receptor function in humans in vivo by means of pharmacological challenges. More specifically, studies exploring the role of brain 5-HT$_{1A}$ receptor function in major affective and anxiety disorders have now been made possible using receptor probes such as buspirone (Meltzer and Maes 1994; Moeller et al. 1994), gepirone (Anderson et al. 1990), tandospirone (Miller et al. 1990), and ipsapirone (Lesch et al. 1989). Although buspirone has a high affinity for 5-HT$_{1A}$ receptors, its complex pharmacological effects on dopaminergic neurotransmission make us question its validity as an index of 5-HT$_{1A}$ function, particularly with respect to measurements of buspirone-induced prolactin release (Meltzer et al. 1983). Moreover, many of these agents share a common metabolite, 1-(2-pyrimidinyl) piperazine (1-PP), which displays $\alpha_2$-adrenoreceptor antagonist properties (Gobbi et al. 1990; Blier et al. 1991), which may constitute a confounding factor in the interpretation of these studies.

Flesinoxan is a newly developed substituted benzamide with high affinity and selectivity for the 5-HT$_{1A}$ receptor subtype (2 nM); (Olivier et al. 1991). In autoradiographic studies, flesinoxan showed a regional dis-

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tribution comparable to that of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT; Schipper et al. 1991). It decreased body temperature and reduced aggressivity scores in animal models (Schipper et al. 1991). Flesinoxan displays high potency at both somatodendritic and postsynaptic 5-$HT_{1A}$ receptors (Hadrava et al. 1995). For instance, in microiontophoretic studies, it was found 17 times more potent than gepirone in suppressing the firing rate of dorsal hippocampus pyramidal neurons (de Montigny et al. 1991). These characteristics, together with the fact that, contrary to the azapirones, it does not generate 1-PP, make it a potentially valuable probe for assessing 5-$HT_{1A}$ receptor function in humans.

The present study determined the effects of flesinoxan on body temperature, and neuroendocrine and behavioral measures in young male healthy volunteers. The selectivity of these responses for the 5-HT system, and more specifically for the 5-$HT_{1A}$ receptor, was further investigated in volunteers pretreated with the mixed 5-$HT_{1,2}$ antagonist methysergide, the 5-$HT_{1A}$ antagonist pindolol, and placebo

## MATERIALS AND METHODS

### Subjects

Twenty-eight healthy volunteers were recruited through local newspaper advertisements. All potential research subjects underwent physical and psychiatric examinations (using the Structured Clinical Interview for DSM-III-R, nonpatient version; Spitzer et al. 1992), had an electrocardiogram and routine laboratory tests, including serum sequential multiple analysis by computer, complete blood count, thyroid function tests, HIV test, urine analysis, and a urine toxicology-screen for drugs of abuse. Only physically healthy subjects without a personal history of psychiatric illness or a family history of mood disorders or alcoholism in first-degree relatives were invited to participate. Subjects scoring 2 or more on at least three symptoms on the Hopkins Symptom Checklist (HSCL-90) and more than 3 on the Beck Inventory for Depression were excluded. All subjects were medication free for a minimum of 2 weeks prior to testing, smoked less than 10 cigarettes per day, and ingested no more than the equivalent of five beers per week and three cups of coffee per day. This study was approved by the Research Ethics Board of the Department of Psychiatry of McGill University. Written informed consent was obtained from all subjects before enrollment in the study.

### Experimental Design

*Dose-Response Study.* In a randomized, double-blind, 3 × 3 Latin-square design, 11 men with a mean age of 29 years (SD = 4.8; range = 21 to 37 years; weight = 73.2 ± 10.5 kg; and height = 1.76 ± 0.15 m) each received two intravenous infusions of flesinoxan (7 μg/kg and 14 μg/kg) and one of saline, over a period of 10 minutes, on three separate occasions, a minimum of 1 week apart, between August and November 1992. Neuroendocrine, physiological, and psychological measures were collected over a 2-hour period during each session.

*Antagonist Study.* In a randomized, double-blind, 3 × 3 Latin-square design, 12 men (age = 28.3 ± 4.7 years; range = 20 to 40 years; weight 71.3 ± 5.6 kg; height = 1.78 ± 0.15 m) received orally, on three separate occasions each, a placebo, 30 mg of racemic pindolol, or 4 mg of methysergide, 90 minutes prior to the infusion of 1 mg flesinoxan over 10 minutes. Each subject was tested on three separate occasions, a minimum of 1 week apart, between November 1992 and February 1993. Five subjects participated in both the dose-response and the antagonist studies. Neuroendocrine, physiological, and psychological measures were collected over a 3.5-hour period during each session.

### Procedure

Subjects arrived at the Clinical Investigation Unit of the Royal Victoria Hospital in Montreal at 8:30 A.M., having fasted since midnight. They wore normal indoor attire and, after being weighed, reclined on a bed in a comfortable position with the head elevated. They were not allowed to eat, sleep, or watch television until the procedure was completed. Two indwelling venous catheters were inserted into the antecubital vein and kept opened with a slow infusion of 5% dextrose in a normal saline solution. After insertion of the intravenous catheters, baseline blood samples were obtained following a 45-min rest period and again, 15 minutes later, to determine baseline hormone concentrations. Immediately following the collection of the last baseline sample, around 10:00 A.M., subjects received an intravenous infusion of either flesinoxan or saline delivered over a 10-minute period. Additional blood samples were collected at 15, 30, 45, 60, and 120 minutes after the start of the infusion. In the antagonist study, the first blood sample was obtained 45 minutes after insertion of the intravenous catheters. Then, subjects were administered orally either a placebo, pindolol, or methysergide 90 minutes prior to the flesinoxan infusion. Blood samples were collected at −15 minutes, immediately prior to the flesinoxan infusion, and at 15, 30, 45, 60, and 120 minutes following the flexinoxan infusion.

### Neuroendocrine, Behavioral, and Physiological Measurements

***Neuroendocrine Measurements.*** Blood samples for measuring hormone plasma levels were collected in ice-cold plastic tubes containing 10 µl/ml of 0.5 M Na2 EDTA. They were centrifuged (20 minutes at 2,500 *g* at 4°C) within one hour of sampling. Plasma was aliquoted into plastic tubes and stored at −70°C until time of assay. Plasma prolactin (PRL), growth hormone (GH), and adrenocorticotropic hormone (ACTH) levels were determined by standard immunoradiometric assays using commercially available kits (Immunocorp, Montreal, QC; ICN Biomedicals, Montreal, QC; Nichols Institute Diagnostics, San Juan Capistrano, CA). Plasma cortisol was determined by radioimmunoassay using a commercially available kit (Kallestad, Austin, TX). The inter- and intra-assay coefficients of variation were as follows: PRL, 3.9% and 2.4%; GH, 12.1% and 12.3%; ACTH, 7.8% and 3.2%; cortisol, 7.6% and 8.4%. At the end of each study, all measurements were carried out in one assay by a technician blind to the order and nature of drug treatments.

***Assessment of Hypothermic and Cardiovascular Responses.*** Sublingual body temperature was measured using a thermistor probe (Electro-therm, model TM99A), and digital readings were obtained at the end of a 2-minute recording period. Blood pressure and pulse were measured and recorded using an automated monitor (Criticare 508, Criticare Systems, Inc., Waukesha, WI). Sublingual body temperature, blood pressure, and heart rate were recorded every 15 minutes throughout the procedure.

***Assessment of Mood and Other Psychological Responses.*** Three questionnaires were used to assess the psychological and somatic changes associated with flesinoxan infusions: the Bipolar Profile of Mood States (McNair et al. 1988), the NIMH Self-Rating Scale (Murphy et al. 1989), and a somatic symptom checklist. The Bipolar Profile is highly sensitive to subclinical changes in mood states and consists of 72 adjectives commonly used to describe transitory mood states. Subjects are required to rate each adjective on a four-point scale ranging from "not at all" (0) to "extremely" (3). Adjectives are grouped together in six clusters representing specific mood states (Agreeable-Hostile, Composed-Anxious, Elated-Depressed, Confident-Unsure, Energetic-Tired, and Clearheaded-Confused). The NIMH Self-Rating Scale consists of 20 items used to describe changes in psychological states. Subjects rated each item on a seven-point scale ranging from "absent" (0) to "very marked" (6). Items were then grouped into six specific factors: Anxiety, Activation-Euphoria, Depressive Affect, Dysphoria, Altered Self-Reality, and Functional-Deficit. The somatic symptom checklist is a compendium of the most frequent somatic complaints commonly encountered during treatment with serotonergic agents, in particular flesinoxan (flesinoxan investigator brochure, Solvay-Duphar, Weesp, Holland); it consists of 29 physical symptoms rated by subjects on a five-point scale (none, slight, moderate, much, severe). In the dose-response study, the psychological questionnaires were administered 15 minutes prior to, and 30 and 120 minutes following the flesinoxan infusion; in the antagonist study, the psychological questionnaires were administered 90 and 15 minutes prior to, and 30 and 120 minutes following, the flesinoxan infusion.

### Drugs

Flesinoxan was provided by Solvay-Duphar in lyophilized powder contained in a closed sterile container. Flesinoxan and saline infusion vials were prepared in identical forms, by the pharmacy department of the Royal Victoria Hospital. Flesinoxan was dissolved in saline 0.9%, following Solvay-Duphar instructions, and stored in individual vials of 13 ml each (0.05 mg/ml; 7 µg/kg; 0.1 mg/ml: 14 µg/kg). The concentration, identity, pH, purity, lack of pyrogenicity, and stability of flesinoxan were verified by Société d'Analyses Biopharmaceutiques (Laval, QC). Flesinoxan was diluted in 20 ml of saline 0.9% and administered via an antecubital vein over 10 minutes. The use of flesinoxan as an intravenous infusion was approved by the Health Protection Branch of Health and Welfare, Canada.

Pindolol (Visken®) and methysergide (Sansert®) are both commercially available in Canada. Placebo (lactose), pindolol, and methysergide pills were provided by the pharmacy department of the Royal Victoria Hospital. Pindolol and methysergide were administered blindly in random order 90 minutes prior to flesinoxan infusion at a dose of 30 mg and 4 mg, respectively.

### Statistical Analysis

Because some samples were accidentally destroyed during storage and centrifugation, neuroendocrine results are reported for 10 subjects only, except for levels of ACTH, which are reported for 9 subjects in the dose-response study and 7 subjects in the antagonist study. Analysis of variance (ANOVA) was used to confirm that there were no significant ($p > .05$) differences between the treatment groups at baseline. The results of the studies were submitted to analysis of covariance for repeated measures, appropriate to the Latin-square design, with the baseline value as covariate (Fleiss 1986). To avoid assumptions about the structure of the variance-covariance matrix for repeated measures on the

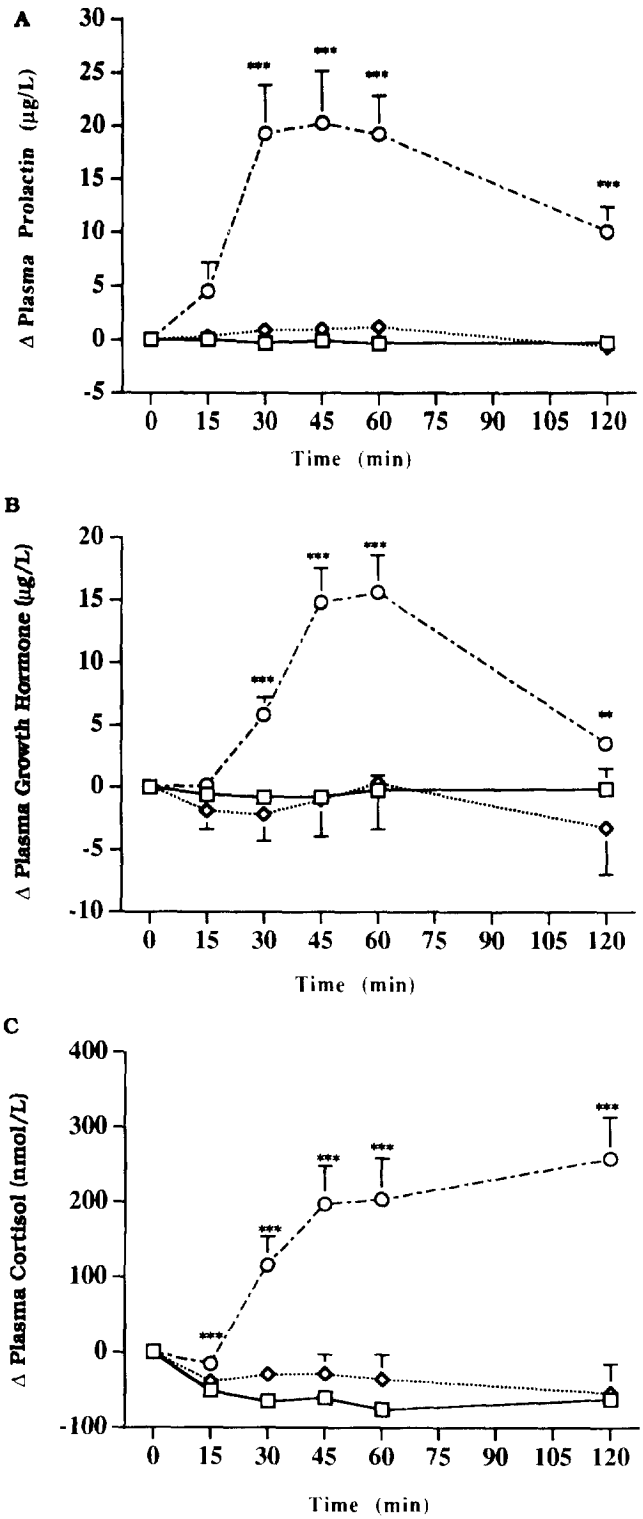


**Figure 1.** Time-dependent effect of flesinoxan (7 μg/kg, diamonds; 14 μg/kg, circles) or placebo (squares) on plasma prolactin **(A)**, growth hormone **(B)**, and cortisol **(C)** concentrations. Each point represents mean ± SEM obtained from 10 healthy volunteers. $^{**}p < .01$; $^{***}p < .001$, flesinoxan 14 μg/kg vs. placebo, ANCOVA.

same subjects, the hypothesis of no treatment × time interaction was tested by multivariate analysis, by computing Wilks's lambda statistic transformed to an F variate (Morrison 1976). When the treatment × time interaction was significant ($p < .05$), indicating differences between the treatments with respect to the profile of response over time, the equality of treatment effects was tested at each time point, by separate univariate analyses of covariance. In the absence of a significant treatment × time interaction, the treatments were compared with respect to the average response over time by a univariate analyses of covariance. The homogeneity of the regression slopes of the response variable on the covariate (baseline value) for each treatment group (parallelism of regression lines) was tested by the treatment × covariate interaction. No significant departures from homogeneity occurred ($p > .05$). Thus, the magnitude of the differences between treatments can be assumed to be independent of the baseline values of the covariate. Because of missing data for the ACTH response to flesinoxan, in the dose-response study, only maximum change (independent of time) relative to baseline (Δ peak) could be analyzed. The hypothesis of no difference between the treatments with respect to the incidence of side effects was tested by the chi-square test for contingency tables. All statistical analyses were performed with the Systat software package (MacIntosh version, 1989). All data are expressed as means ± SD, unless specified otherwise.

## RESULTS

### Dose-Response Study

#### *Comparison of Baseline Measurements*

Prior to placebo or flesinoxan administration (whether 7 or 14 μg/kg), baseline neuroendocrine, temperature, blood pressure, and pulse were within normal limits for all subjects: plasma PRL (μg/L), 5.8 ± 3.2, 6.7 ± 2.9, and 6.1 ± 2.5; plasma GH (μg/L), 3.5 ± 4.1, 6.0 ± 11.6, and 2.1 ± 0.9; plasma cortisol (nmol/L), 248.1 ± 59.8, 279.8 ± 112.1, and 280.1 ± 77.9; plasma ACTH (pmol/L), 4.7 ± 2.1, 5.1 ± 2.7, and 4.7 ± 2.2; temperature (°C), 36.5 ± 0.2, 36.5 ± 0.3, and 36.6 ± 0.2. For these measurements, subjects showed negligible variations between each baseline session, with the exception of one subject, who showed a high baseline plasma GH on one occasion.

#### *Neuroendocrine Measurements*

PROLACTIN. The 7 μg/kg dose of flesinoxan produced no alteration of PRL levels (Figure 1A). However, at the dosage 14 μg/kg, flesinoxan significantly increased PRL levels over baseline (placebo: Δ peak = −2%; flesinoxan [7 μg/kg]: Δ peak = 15%; flesinoxan [14 μg/kg]:

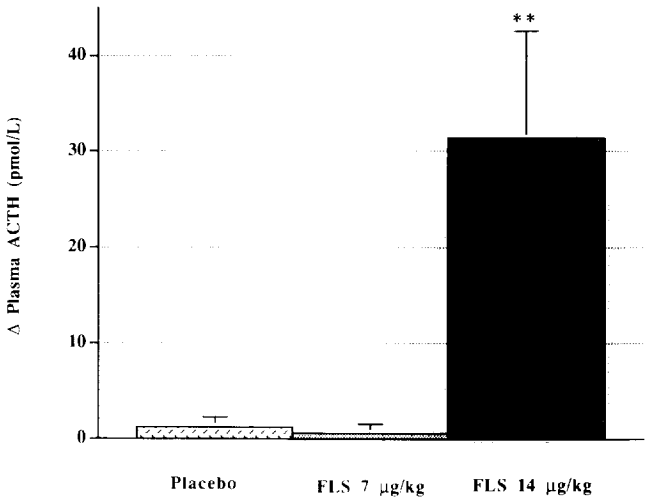


**Figure 2.** Effect of flesinoxan or placebo on plasma ACTH levels (maximum response). Data obtained from 9 healthy volunteers; mean ± SEM. $^{**}p < .005$, flesinoxan 14 μg/kg vs. placebo, ANCOVA.

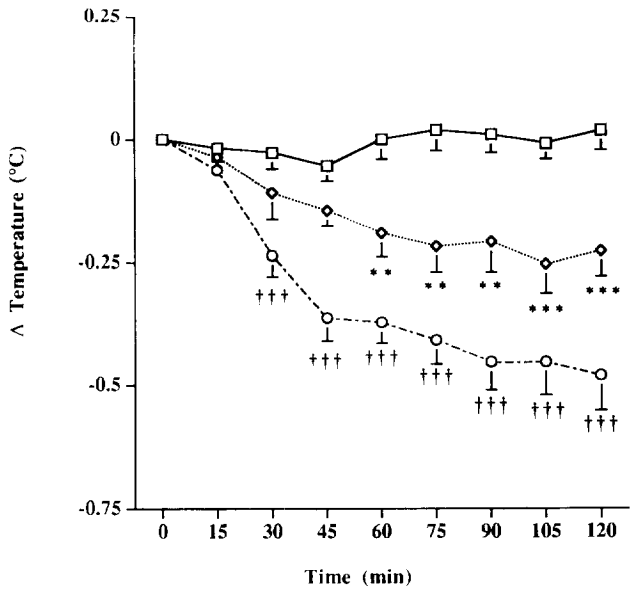


**Figure 3.** Time-dependent effect of flesinoxan (7 μg/kg, diamonds; 14 μg/kg, circles) or placebo (squares) on oral temperature. Each point represents mean ± SEM (obtained from 11 healthy volunteers). Flesinoxan 7 μg/kg vs. placebo, $^{**}p < .01$, $^{***}p < .001$; flesinoxan 14 μg/kg vs. placebo, $^{†††}p < .001$, ANCOVA.

Δ peak = 366%). Multivariate analysis indicates a significant interaction [treatment × time] for PRL (Wilks's $F_{8,24} = 6.93$; $p < .001$). The maximal PRL response occurred 45 minutes after administration of the high dose of flesinoxan (14 μg/kg) ($F_{1,15} = 44.80$; $p < .001$) and plasma PRL levels were still significantly increased at 120 minutes (Figure 1A).

GROWTH HORMONE. Flesinoxan, at the dosage 14 μg/kg, increased significantly plasma GH levels over baseline (placebo: Δ peak = −7%; flesinoxan [7 μg/kg]: Δ peak = 5% flesinoxan [14 μg/kg]: Δ peak = 743%). Multivariate analysis revealed a significant interaction [treatment × time] for GH (Wilks's $F_{8,24} = 5.44$; $p <$ .001). With the high dose of flesinoxan the maximal GH response occurred at 60 minutes ($F_{1,15} = 54.12$; $p <$ .001), then declined rapidly over the next hour in all subjects (Figure 1B). Plasma GH levels remained significantly increased over baseline at 120 minutes.

CORTISOL. Significant long-lasting increases in plasma cortisol were produced by flesinoxan at the high dosage (placebo: Δ peak = −25%; flesinoxan [7 μg/kg]: Δ peak = −19%; flesinoxan [14 μg/kg]: Δ peak = 92%). Multivariate analysis revealed a significant interaction [treatment × time] for cortisol (Wilks's $F_{8,24} = 7.00$; $p <$ .001). The maximal cortisol response occurred 120 minutes after administration of the high dose of flesinoxan ($F_{1,15} = 75.34$; $p < 0.001$) (Figure 1C). Consistent with circadian rhythm, a decrease over time was noted for plasma cortisol levels in the placebo group. The peak PRL and cortisol responses elicited by the high dose of flesinoxan correlated significantly ($r_8 = .68$, $p < .05$).

ACTH. Flesinoxan at the dosage 14 μg/kg increased significantly plasma ACTH levels over baseline (placebo: Δ peak = −21%; flesinoxan [7 μg/kg]: Δ peak = −6%; flesinoxan [14 μg/kg]: Δ peak = 585%) (Figure 2). The ANCOVA showed a significant treatment effect ($F_{2,13} = 8.18$; $p < .005$) due to a greater ACTH response associated with administration of the high dose of flesinoxan.

### *Body Temperature, Blood Pressure, and Heart Rate*

Flesinoxan decreased oral temperature in a dose-dependent manner (Figure 3). The maximum effect was recorded at 120 minutes after administration of either dose of flesinoxan (placebo: Δ peak = 0°C; flesinoxan [7 μg/kg]: Δ peak = −0.25°C; flesinoxan [14 μg/kg]: Δ peak = −0.50°C) (Figure 3). Multivariate analysis revealed a significant interaction [treatment × time] for oral temperature (Wilks's $F_{14,22}$; $p < .001$). At the time of maximum response, there was a greater hypothermic response to flesinoxan (7 μg/kg) ($F_{1,15} = 18.55$; $p <$ .001) and to flesinoxan (14 μg/kg) ($F_{1,15} = 74.93$; $p <$ .001) than to placebo. There was no significant effect of flesinoxan at either dosage level or placebo on both blood pressure and heart rate.

### *Psychological Effects*

PROFILE OF MOOD STATES. The high dose of flesinoxan was associated with a moderate increase in feelings of tiredness and fatigue. Multivariate analysis revealed no significant interaction [treatment × time], but a significant treatment effect for the factors energetic/tired ($F_{2,17} = 11.05$; $p < .001$), confident/unsure ($F_{2,17} =$

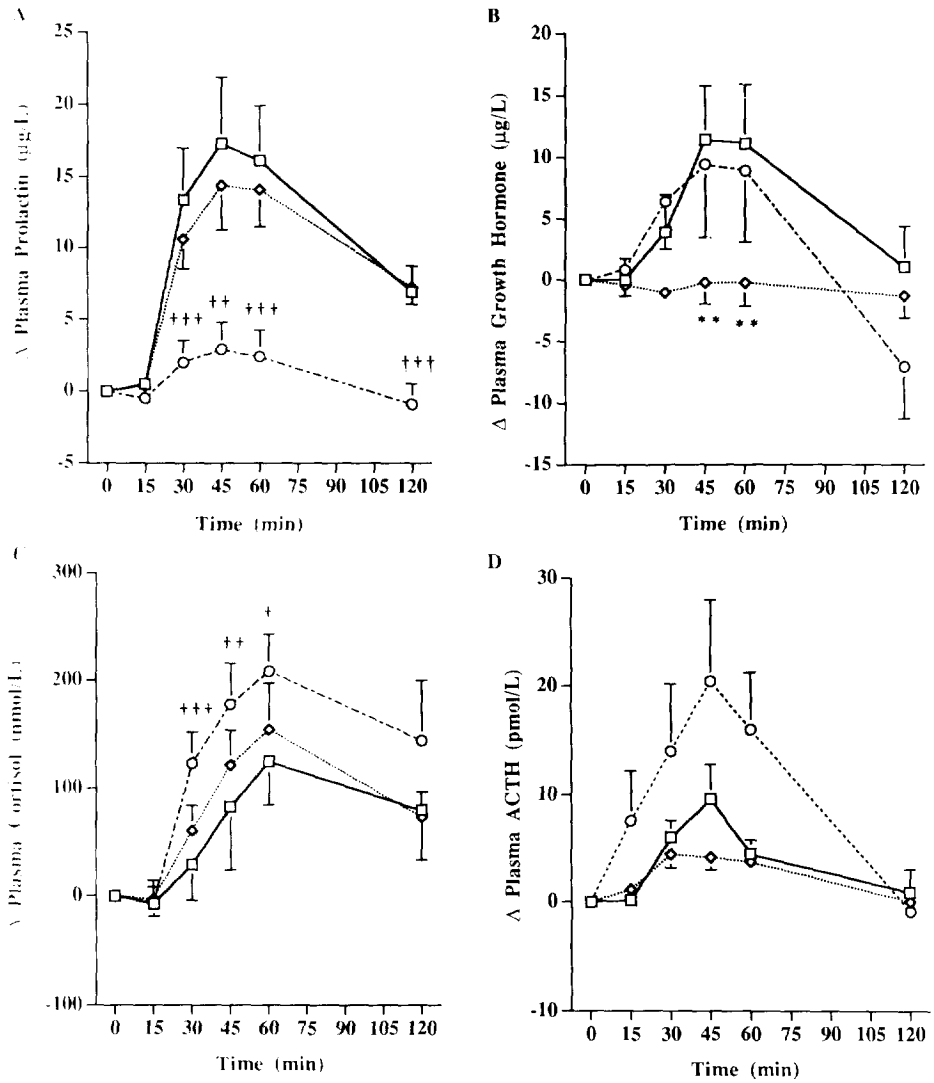


**Figure 4.** Effect of pindolol (30 mg, diamonds), methysergide (4 mg, circles), and placebo (squares) administered orally, 90 minutes prior to flesinoxan intravenous infusion (1 mg) on plasma concentrations of prolactin **(A)**, growth hormone **(B)**, cortisol **(C)**, and ACTH **(D)**. Each point represents the mean ± SEM obtained from 10 healthy volunteers, with the exception of ACTH responses, where each point represents mean ± SEM from 7 subjects. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$, [pindolol + flesinoxan] vs. [placebo + flesinoxan]; and $^{+}p < .05$; $^{++}p < .01$; $^{+++}p < .001$, [methysergide + flesinoxan] vs. [placebo + flesinoxan], ANCOVA.

12.14; $p < .001$), and clearheaded/confused ($F_{2,17} = 6.8$; $p < .01$). Maximal responses occurred at 30 minutes: energetic/tired ($F_{1,17} = 21.57$; $p < .001$); confident/unsure ($F_{1,17} = 17.64$; $p < .001$); and clearheaded/confused ($F_{1,17} = 14.10$; $p < .002$).

NIMH SELF-RATING SCALE. Subjects noticed significant but moderate feelings of fatigue and lack of energy after the high dose of flesinoxan but not after the low dose. Multivariate analysis revealed a significant interaction [treatment × time] *n* ($F_{2,17} = 4.76$, $p < .05$) for altered self-awareness and a significant treatment effect ($F_{2,17} = 4.49$, $p < .05$) for functional deficit. Maximum responses occurred at 30 minutes, and altered self-awareness ($F_{1,17} = 4.35$, $p < .05$) and functional deficit ($F_{1,17} = 9.21$, $p < .01$). The effect decreased slightly over the following hour.

***Side Effects.*** Six subjects reported transient side effects following the low dose of flesinoxan, and 10 reported them following the high dose of flesinoxan. Contingency table analyses showed significant treatment effects for the following side effects: drowsiness, lightheadedness, nausea, numbness, headache, faintness, asthenia, dizziness, and sweating. Two subjects complained of headache and difficulty concentrating after placebo. Ratings of nausea ($r_9 = .63$, $p < .05$) and lightheadedness ($r_9 = .71$, $p < .05$) were correlated significantly with the peak flesinoxan-induced cortisol response.

## Antagonist Study

***Comparison of Baseline Measurements.*** All baseline measurements collected prior to the administration of placebo, pindolol, and methysergide were within normal limits: PRL (μg/L), 8.9 ± 7.2, 7.1 ± 5.7, and 7.2 ± 6.3; GH (μg/L), 5.1 ± 8.1, 4.3 ± 4.9, and 11.9 ± 12.9; cortisol (nmol/L), 246.9 ± 71.8, 246.8 ± 98.7, and 356.1 ± 156.4; ACTH (pmol/L), 4.0 ± 1.3, 4.6 ± 1.9, and 9.7 ± 9.8; temperature (°C), 36.4 ± 0.2, 36.3 ± 0.2, and 36.4 ± 0.2.

***Effects of Pindolol and Methysergide.*** Administration of either pindolol or methysergide did not significantly ($p > .05$) alter baseline measurements of hormone levels, body temperature, systolic blood pressure, pulse, and psychological subscores prior to flesinoxan infusion. However, methysergide showed a trend toward increasing GH (interaction [treatment × time]: $F_{2,15} = 2.96$; $p = .083$) and cortisol (treatment effect: $F_{2,15} = 3.52$; $p = .056$). Moreover, methysergide increased ACTH levels slightly but not significantly. Diastolic blood pressure was significantly affected by both pindolol and methysergide (treatment effect; $F_{2,19} = 25.78$; $p < .001$): the former drug decreased it by 4 mm of Hg ($F_{1,19} = 12.81$; $p < .01$), whereas the latter increased it by 2 mm of Hg ($F_{1,19} = 5.59$; $p < 0.05$).

### *Effects of Pindolol and Methysergide on Flesinoxan-Induced Neuroendocrine Responses*

PROLACTIN. Methysergide, but not pindolol, significantly blocked the PRL response to flesinoxan (interaction [treatment × time]; Wilks's $F_{8,24} = 3.78$; $p < .005$) (placebo, Δ peak = 194%; pindolol, Δ peak = 203%; methysergide, Δ peak = 40%) (Figure 4A).

GROWTH HORMONE. Pindolol, but not methysergide, completely abolished the GH response to flesinoxan (interaction [treatment × time]; Wilks's $F_{8,24} = 3.52$; $p < .008$) (placebo, Δ peak = 224%; pindolol, Δ peak = 6%; methysergide, Δ peak = 79%) (Figure 4B).

CORTISOL. Both methysergide and pindolol failed to block the cortisol response to flesinoxan. It was even potentiated by methysergide (interaction [treatment × time]; Wilks's $F_{8,24} = 3.59$; $p < .05$) (placebo, Δ peak = 51%; pindolol, Δ peak = 63%; methysergide, Δ peak = 59%) (Figure 4C).

ACTH. As for the cortisol response, methysergide showed a trend toward potentiating the ACTH response to flesinoxan (treatment effect; $F_{2,9} = 3.04$; $p = .098$). On the other hand, pretreatment with pindolol resulted in a slight attenuation of peak ACTH levels (placebo, Δ peak = 148%; pindolol, Δ peak = 113%; methysergide, Δ peak = 206%) (Figure 4D).

***Effects of Pindolol and Methysergide on Flesinoxan-Induced Hypothermia.*** Both methysergide and pindolol showed a trend toward attenuating the maximal hypothermic response to flesinoxan (interaction [treatment × time]; Wilks's $F_{14,26} = 1.83$; $p = .09$). Strikingly, the hypothermic effect of flesinoxan was present in the first hour irrespective of the nature of the pretreatment administered (Figure 5). When temperature data were analyzed separately for each time point, statistically significant treatment differences emerged indicating an attenuation by methysergide of the flesinoxan-induced hypothermia at 90, 105, and 120 minutes following flesinoxan administration (at 105 min. $F_{1,19} = 9.19$; $p < .05$) (placebo Δ peak = −0.32°C; pindolol Δ peak = −0.21°C; methysergide Δ peak = −0.17°C) (Figure 5).

***Effects of Pindolol and Methysergide on Cardiovascular Parameters.*** Multivariate analysis revealed no significant interaction [treatment × time] for either blood pressure or pulse values following flesinoxan infusion.

***Effects of Pindolol and Methysergide on Flesinoxan-Induced Psychological Effects.*** As in the dose-response study, flesinoxan infusion produced a mild to moderate increase in the subjects' feelings of tiredness and fatigue, although of a lesser magnitude. This effect was potentiated by methysergide but was unaffected by pindolol (interaction [treatment × time]; energetic/tired: $F_{2,19} = 7.86$; $p < .01$; confident/unsure: $F_{2,19} = 5.72$; $p < .05$). In addition, some subjects reported a subclinical increase in the subjective perception of altered self-awareness, dysphoria, and functional deficit as measured by the NIMH Self-Rating Scale. None of these responses were affected by pretreatment with either methysergide or pindolol.

***Side Effects.*** Overall, this procedure was well tolerated, with subjects reporting even fewer or less intense side effects after flesinoxan than they reported in the

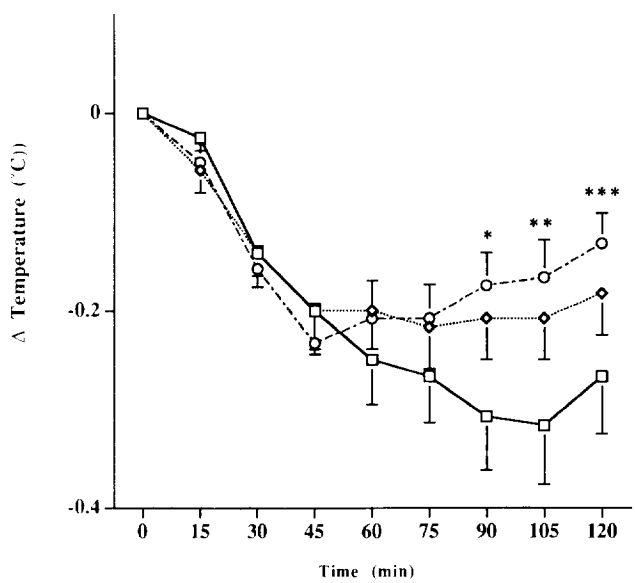


**Figure 5.** Effect of pindolol (30 mg, diamonds), methysergide (4 mg, circles), and placebo (squares) administered orally, 90 minutes prior to flesinoxan intravenous infusion (1 mg) on oral body temperature. Each point represents the mean ± SEM obtained from 12 healthy volunteers. $*p < .05$; $**p < .01$; $***p < .005$, [methysergide + flesinoxan] vs. [placebo + flesinoxan], ANCOVA. There is no significant effect of pindolol pretreatment.

dose-response study. Drowsiness, lightheadedness, and difficulty concentrating were reported by the same proportion of subjects. Pindolol showed a trend toward attenuating and methysergide a trend toward potentiating the side effects induced by flesinoxan. However, the chi-square test demonstrated no significant ($p > .05$) differences between the two treatment conditions. Two subjects dropped out of the study following the administration of methysergide administered in combination with flesinoxan (vomiting, $n = 1$; vertigo, $n = 1$).

### Test-Retest

Five subjects participated in both studies, 40 days apart, and hence were suitable for a test-retest comparison of their responsiveness to flesinoxan. The subjects' PRL and GH responses in both studies were of comparable magnitude (plasma PRL [μg/L]: Δ peak = 16.4 ± 11.1 and 11.8 ± 11.7; plasma GH [μg/L]: Δ peak = 12.8 ± 12.2 and 7.4 ± 16.3), whereas their ACTH and cortisol responses to flesinoxan were lower during retest (plasma ACTH [pmol/L]: Δ max = 16.5 ± 13.4 and 8.8 ± 11.6; plasma cortisol [nmol/L]: Δ peak = 83.2 ± 162.9 and 26.0 ± 80.2). There was a significant correlation in the peak GH ($r_4 = 0.881$, $p < .05$) but not in the peak PRL or cortisol responsiveness across the two studies.

The hypothermic response to flesinoxan was of the same intensity in both studies (Δ peak = −0.5 ± 0.2 and −0.4 ± 0.2°C).

## DISCUSSION

Flesinoxan administered intravenously to male healthy volunteers elicited a dose- and time-dependent hypothermic effect as well as increases in plasma GH, ACTH/cortisol, and PRL levels. The rise in plasma levels of GH was blocked by pindolol but was not affected by methysergide. Conversely, the PRL response to flesinoxan was blocked by methysergide but not by pindolol. The hypothermic response to flesinoxan was attenuated by both pindolol and methysergide but only significantly so by methysergide. Methysergide potentiated the ACTH and cortisol responses to flesinoxan.

### Flesinoxan-Induced Growth Hormone Release

In general, studies with selective 5-HT agonists or antagonists tend to support the stimulatory action of 5-HT on GH secretion (Tuomisto and Männistö 1985). This 5-HT effect seems due to its postsynaptic action in the rodent hypothalamus, since the 5-HT synthesis inhibitor parachlorophenylalanine was reported to decrease basal GH levels (Tuomisto and Männistö 1985). In support of this contention, the stimulatory effect of the 5-$HT_{1A}$ agonist buspirone on GH release is unaffected in humans with experimentally lowered serotonergic neurotransmission, induced by a dietary depletion of tryptophan (Blier et al. 1994b).

In the present study, flesinoxan infusion resulted in a time-dependent increase in plasma GH levels, consistent with results obtained with other selective 5-$HT_{1A}$ agonists in humans (Meltzer et al. 1983; Lesch et al. 1989; Anderson et al. 1990; Miller et al. 1990). Blockade of the GH response to flesinoxan by pindolol but not by methysergide is consistent with a 5-$HT_{1A}$-mediated phenomenon. This finding concurs with previous reports of pindolol blocking the GH response to ipsapirone (Lesch 1991) and buspirone (Anderson and Cowen 1992). The failure of the 5-$HT_{1/2}$ receptor antagonist methysergide to antagonize the GH response to flesinoxan was not unexpected, given the limited effectiveness of methysergide and metergoline (another similar 5-$HT_{1/2}$ antagonist) in attenuating 5-$HT_{1A}$ receptor-mediated functional responses (Koenig et al. 1987; McCance et al. 1987; Neuhauser et al. 1988; Sharp et al. 1989; Fuller and Snoddy, 1990; Gartside et al. 1992). This might be related to the weak capacity of methysergide to displace [$^{125}$I] LSD from 5-$HT_{1A}$ binding sites (Lovenberg et al. 1993).

### Flesinoxan-Induced Hypothermia

There is now good evidence that 5-$HT_{1A}$ agonists produce hypothermia in rodents via postsynaptic 5-$HT_{1A}$ receptors (for review see Millan et al. 1993). Recent experimental evidence, using the hypothermic response to buspirone as an index of 5-$HT_{1A}$ receptor function in healthy volunteers undergoing a tryptophan depletion paradigm, suggests that humans respond similarly (Blier et al. 1994a). The 5-$HT_{1A}$ agonists ipsapirone, gepirone, and buspirone induce hypothermia in both rats (Koenig et al. 1988) and humans (Anderson et al. 1990; Lesch et al. 1990a; Anderson and Cowen, 1992), which is blocked by pindolol (Lesch et al. 1990a; Anderson and Cowen, 1992). Consistent with 5-HT mechanisms, methysergide significantly attenuated the hypothermic response to flesinoxan. A similar effect was observed with pindolol but did not reach statistical significance. Although these data could indicate that flesinoxan-induced hypothermia is a 5-$HT_2$ receptor-mediated event, such an interpretation is unlikely for the following reasons: (1) the affinity of flesinoxan for 5-$HT_2$ receptors in binding studies is very low (>4,500 nM) (Olivier et al. 1992); (2) in rodents and humans, 5-$HT_2$ agonists cause hyper- rather than hypothermia (Strassman and Qualls 1994); (3) in data recently obtained from our laboratory, the hypothermic response elicited in rats by flesinoxan was prevented by pindolol (Hadrava et al. 1995).

A more conservative interpretation of this relative

failure of pindolol to significantly block the flesinoxan-induced hypothermia may be that pharmacokinetic (dose, duration of treatment, brain penetration) or, perhaps to a lesser extent, pharmacodynamic factors (relative selectivity/affinity of pindolol for 5-HT$_{1A}$ versus other 5-HT receptors and/or adrenoceptors in humans) may have prevented the achievement of sufficient blockade of 5-HT$_{1A}$ receptors. In fact, it is noteworthy that, for both 5-HT antagonists, the rate at which hypothermia manifested itself was unaffected in the first hour following flesinoxan injection and that an antagonism was apparent only in the second hour.

### Flesinoxan-Induced Release of ACTH and Cortisol

In this study, the high but not the low dose of flesinoxan produced significant increases in plasma ACTH and cortisol levels. Hypothalamic-pituitary-adrenal activation was not likely due to anxiety since all subjects reported feeling free of anxiety throughout the study; nor was it due to a reflexive cardiovascular effect, as neither blood pressure nor heart rate was affected by the procedure. The finding of a positive correlation between the nausea and lightheadedness induced by flesinoxan and cortisol plasma levels suggests, however, that part of the flesinoxan-induced release of cortisol might be related to somatic side effects.

In contrast to results obtained with selective 5-HT$_{1A}$ agonists in rats (Koenig et al. 1987; Gilbert et al. 1988; Haleem et al. 1989; Fuller 1990; Fuller and Snoddy 1990; Di Sciullo et al. 1990; Gartside et al. 1992; Pan and Gilbert 1992, 1993) and in humans (Lesch et al. 1990b), the effect of flesinoxan on ACTH or cortisol release was not prevented by pindolol (Figure 4C,D). The failure of pindolol to block the flesinoxan-induced rise in ACTH and cortisol plasma levels suggests that this effect was not 5-HT$_{1A}$ receptor-mediated. This conclusion is consistent with a recent report that 8-OH-DPAT, administered centrally in rodents, exerts a biphasic effect on the hypothalamic-pituitary-adrenal axis, with the stimulatory effect being non-5-HT$_{1A}$ receptor-mediated (Welch et al. 1993). However, given the breadth of the literature suggesting otherwise (Gilbert et al. 1988; Pan and Gilbert 1992, 1993), these conclusions are tentative and await replication. An alternative interpretation may be that, as in the case of flesinoxan-induced hypothermia, other pharmacological factors may have limited the ability of pindolol to block potently 5-HT$_{1A}$ receptor-mediated responses. The potentiation of the cortisol response by methysergide is most likely due to its partial 5-HT agonistic properties (Apperley et al. 1980; Dumuis et al. 1988; Hoyer 1988; Schoeffter and Hoyer 1988).

### Flesinoxan-Induced Release of Prolactin

The demonstration of enhanced PRL secretion in response to flesinoxan is consistent with earlier reports that 8-OH-DPAT elicits PRL release in rats (Simonovic et al. 1984; Willoughby et al. 1988; Aulakh et al. 1988; Di Sciullo et al. 1990; Kellar et al. 1992; Flores et al. 1992), which is blocked by methysergide (Flores et al. 1992) or metergoline (Willoughby et al. 1988; Aulakh et al. 1988; Kellar et al. 1992). In humans, increased PRL secretion was reported with buspirone (Meltzer et al. 1983; Coccaro et al. 1990; Anderson and Cowen, 1992) and gepirone (Anderson et al. 1990), but not with ipsapirone (Lesch et al. 1989) or tandospirone (Miller et al. 1990). Differences in the magnitude of the PRL response to various 5-HT$_{1A}$ receptor probes in humans might be explained by differences in their respective pharmacological profiles, in particular with respect to their relative affinity for 5-HT$_{1A}$ versus D$_2$ receptors (Olivier et al. 1992; Nash and Meltzer 1989). Interpretation of the PRL data is further complicated by the report that nonselective 5-HT$_{1-2}$ antagonists, such as metergoline, block the PRL release produced by haloperidol, thus suggesting dopamine-mediated mechanisms (Ellis et al. 1991). The pattern observed in the present study—that the PRL response is blocked by methysergide but not by pindolol—suggests that the flesinoxan-induced rise in PRL might not be 5-HT$_{1A}$ receptor-mediated. Thus the blockade by methysergide of the PRL response to flesinoxan could indicate either a 5-HT$_7$ or D$_2$ receptor-mediated event (Krulich et al. 1981; Hoyer 1991). Given the high affinity of methysergide for 5-HT$_7$ receptors (32 nM; Lovenberg et al., 1993), the lack of affinity of pindolol for this recently cloned receptor, and the moderate affinity of flesinoxan for D$_2$ receptors (140 nM; Olivier et al. 1992), it is possible that the PRL response to flesinoxan may involve both 5-HT and dopaminergic activation. However, presently it is not known whether flesinoxan, like 8-OH-DPAT, has significant affinity for 5-HT$_7$ receptors, which are present in high density in the hypothalamus (Lovenberg et al. 1993).

The differential effectiveness of pindolol to antagonize 5-HT$_{1A}$ responses in the present study might be due to the involvement of different populations of 5-HT$_{1A}$ receptors with distinct pharmacological properties. Indeed, in rats, a low dose of racemic pindolol (4 mg/kg) blocks the 8-OH-DPAT-induced hypothermia but leaves unaffected the PRL increase produced by this 5-HT$_{1A}$ receptor agonist (Aulakh et al. 1988). Yet, (−) pindolol, which is the enantiomer with the 5-HT$_{1A}$ affinity, at a dose of 15 mg/kg, blocks the PRL response to 8-OH-DPAT (Blier et al. 1994b), thus indicating that the PRL-response in rats to 8-OH-DPAT is mediated by a 5-HT$_{1A}$ receptor. Interestingly, the suppressant effect of microiontophoretically applied 8-OH-DPAT on the firing activity of rat dorsal hippocampus pyramidal neurons is not blocked by (−) pindolol. Several other lines of evidence accumulated in recent years also indicate that pre- and postsynaptic 5-HT$_{1A}$ recep-

tors have different pharmacological properties. For instance, the buspirone analogue BMY 7378 readily blocks 5-$HT_{1A}$ receptors in the rat dorsal hippocampus while ineffectively blocking 5-$HT_{1A}$ autoreceptors in the dorsal raphe (Chaput and de Montigny 1988). In contrast, acute spiperone injection blocks the effect of microiontophoretic applications of 5-HT onto 5-HT neurons but not onto hippocampus neurons (Blier et al. 1993). Consequently, the failure of pindolol in the present study to block the effect of a rather selective 5-$HT_{1A}$ receptor agonist, such as flesinoxan, by no means rules out the possibility that the observed physiological response is not mediated by a 5-$HT_{1A}$ receptor.

## CONCLUSION

The present results provide novel evidence that the intravenous infusion of flesinoxan is a safe and valid probe of serotonergic function. More specifically, the attenuation or blockade by pindolol of the flesinoxan-induced hypothermic and GH responses suggests that these measures qualify as reliable indices of 5-$HT_{1A}$ receptor function in man.

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