Central CRF inhibits gastric emptying of a nutrient solid meal in rats: the role of CRF\(_2\) receptors

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Martinez, V., E. Barquist, J. Rivier, and Y. Taché. Central CRF inhibits gastric emptying of a nutrient solid meal in rats: the role of CRF\(_2\) receptors. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G965–G970, 1998.—Corticotropin-releasing factor (CRF)-related peptides exhibit different affinity for the receptor subtypes 1 and 2 cloned in the rat brain. We investigated, in conscious rats, the effects of intracisternal (IC) injection of CRF (rathuman) on the 5-h rate of gastric emptying of a solid nutrient meal (Purina chow and water ad libitum for 3 h) and the CRF receptor subtype involved. CRF, urotensin I (suckerfish), and sauvagine (frog) injected IC inhibited gastric emptying in a dose-dependent manner, with \(ED_{50}\) values of 0.31, 0.13, and 0.08 \(\mu g\) rat, respectively. Rat CRF-(6—33) (0.1–10 \(\mu g\) IC) had no effect. The nonselective CRF\(_1\) and CRF\(_2\) receptor antagonist, astressin, injected IC completely blocked the inhibitory effect of IC CRF, urotensin I, and sauvagine (frog) injected IC by 10 to 20 times. These data show that central CRF and CRF-related peptides are potent inhibitors of gastric emptying of a solid meal with a rank order of potency characteristic of the CRF\(_2\) receptor subtype affinity (sauvagine > urotensin I > CRF). In addition, the reversal by astressin but not by the CRF\(_1\)-selective receptor antagonist further supports the view that the CRF\(_2\) receptor subtype is primarily involved in central CRF-induced delayed gastric emptying.
increases in ACTH plasma levels (12). Astressin injected intracisternally (IC) is also more potent to antagonize central CRF-induced delayed gastric emptying of a nonnutrient solution in rats (21). Several lines of evidence indicate that CRF-induced pituitary ACTH secretion and anxiogenic behavior are mediated by the activation of the CRF$_1$ receptor (6, 30, 38). However, the CRF receptor subtype that underlies the autonomic nervous system-mediated changes in gastric emptying is not known.

In the present study, we investigated 1) the effect of central injection of CRF on the gastric emptying of a physiological meal (ingestion of solid Purina chow) in conscious rats and 2) the CRF receptor subtype subserving IC CRF-induced inhibition of gastric emptying of a solid meal. To determine the pharmacological characteristics of the CRF receptors involved, we compared the potency profiles of r/hCRF with the nonmammalian CRF-related peptides sauvagine and urotensin I. We also tested the specificity of the response by using the middle fragment r/hCRF-(6–33), which is devoid of intrinsic activity at both CRF receptor subtypes (11, 33). In addition, we examined the antagonist action of astressin, the potent CRF$_1$/CRF$_2$ receptor antagonist (12), and NBI-27914, a nonpeptide CRF$_1$-selective receptor antagonist (6), against inhibition of gastric emptying induced by CRF-related peptides.

**MATERIALS AND METHODS**

Animals. Adult male Sprague-Dawley rats (Harlan, San Diego, CA) weighing 280–320 g were maintained on a 12:12-h light-dark cycle with controlled temperature (21–23°C). Animals were housed in group cages with free access to food (Purina rat chow) and tap water. All experiments were performed in rats fasted 18–20 h, with free access to water.

Drugs and treatments. The following peptides were synthesized and purified as previously described (12): r/hCRF$_1$, amphibian sauvagine, suckerfish urotensin I, r/hCRF$_1$-(6–33)-OH, and cyclo(30–33)-[d-Phe$_{12}$,Nle$_{21}$,38,Glu$_{30}$,Lys$_{33}$]r/hCRF$_1$-(12–41) (astressin) (Salk Institute, Clayton Foundation Laboratories for Peptide Biology, La Jolla, CA). Peptides were kept in powder form at −70°C, and, immediately before use, CRF and its related peptides were dissolved in sterile saline, and astressin was dissolved in double-distilled water (adjusted to pH 7.0, warmed to 37°C). The nonpeptide CRF$_1$-selective receptor antagonist NBI-27914 (Neurocrine Biosciences, San Diego, CA) was synthesized as a tosylate salt as previously described (6). Before use, NBI-27914 was dissolved in 100% DMSO served as the control vehicle.

Substances were injected IC under short enflurane anesthesia (2–3 min; 5.5% vapor concentration in O$_2$; Ethrane-DMSO, and 100% DMSO served as the control vehicle. Drugs and treatments.

**Experimental protocols.** All experiments were started between 7:30 AM and 8:00 AM in rats fasted for 18–20 h. Rats were given preweighed Purina chow and water ad libitum for a 3-h period. Then food and water were removed, and under short enflurane anesthesia, rats were injected IC with either saline (10 µl), CRF (0.1, 0.3, and 1 µg/rat in 10 µl), sauvagine (0.03, 0.1, 0.3, or 1 µg/rat in 10 µl), urotensin I (0.03, 0.1, 0.3, or 1 µg/rat in 10 µl), or CRF-(6–33) (0.3, 1, or 10 µg/rat in 10 µl). In a second experiment, rats were injected IC with either vehicle (water or DMSO, 5 µl/rat), astressin (1, 1.5, 3, or 5 µg/rat in 5 µl), or NBI-27914 (50 µg/rat in 5 µl). Immediately afterward, the rats were injected with either vehicle (5 µl saline), CRF (0.3 or 1 µg/rat in 5 µl), sauvagine (0.3 or 1 µg/rat in 5 µl), or urotensin I (0.3 µg/rat in 5 µl).

In each daily experiment, a control and several doses of peptides were included and repeated on multiple days. The doses of astressin were based on the previous antagonistic action of the peptide injected IC against CRF- or stress-induced inhibition of gastric emptying by a nonnutrient solution (21). The dose of NBI-27914 used corresponds to the highest effective dose tested using other peptide CRF antagonists, to prevent CRF- or abdominal surgery-induced delay of gastric emptying (35).

After the IC injections, rats were returned to their home cages without food and water, and after a 5-h period, rats were euthanized to measure the rate of gastric emptying of the ingested meal. A group of rats in which no IC injection was performed was also studied.

**Statistical Analysis**

Results are expressed as means ± SE. Comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls multiple-comparisons test. P values < 0.05 were considered statistically significant. ED$_{50}$, defined as the dose of peptide that induced 50% inhibition of gastric emptying compared with the rate of emptying in vehicle-treated rats (taken as 0% inhibition), was determined by nonlinear regression to a sigmoidal equation with variable slope (Prism, version 2.0; GraphPad, San Diego, CA).

**RESULTS**

During the 3-h feeding period after an 18-h fast, rats ate 6.46 ± 0.10 g of Purina chow. The rate of gastric emptying of the food ingested was 61.8 ± 3.8% (n = 8) as measured at 5 h after the end of the feeding period in control rats (nontreated group). Saline, injected IC under short enflurane anesthesia at the end of the feeding period, did not significantly modify gastric emptying of food ingested (53.1 ± 4.6%, n = 12, P > 0.05).
Effect of Intracisternal CRF and CRF-Related Peptides on Gastric Emptying of a Solid Nutrient Meal

r/hCRF injected IC (0.1, 0.3, and 1 µg) dose-dependently inhibited gastric emptying of the solid nutrient meal to 49.8 ± 6.1%, (n = 5, P > 0.05), 26.2 ± 8.8% (n = 6, P < 0.05), and 6.7 ± 5.5% (n = 6, P < 0.05), respectively (F4,32 = 14.686, P < 0.05; Fig. 1).

The CRF-related peptides, suckerfish urotensin I and amphibian sauvagine, also inhibited gastric emptying of the solid nutrient meal in a dose-dependent manner. Urotensin I injected IC had no significant effect at 0.03 µg (49.5 ± 5.5%), whereas at 0.1 and 0.3 µg urotensin I decreased gastric emptying to 33.1 ± 7.5% and 8.5 ± 4.2%, respectively (F5,36 = 15.44, P < 0.05, n = 5 for each dose; Fig. 1). There was no additional inhibitory effect at a higher dose (1 µg) of urotensin I (Fig. 1).

Sauvagine, injected IC at 0.03, 0.1, and 0.3 µg/rat, decreased the 5-h rate of gastric emptying to 42.1 ± 8.3% (P > 0.05), 20.4 ± 7.7% (P < 0.05), and 11.6 ± 5.3% (P < 0.05), respectively (n = 4–5 for each dose, Fig. 1). At a dose of 1 µg, sauvagine completely suppressed gastric emptying for the 5-h experimental period (Fig. 1). Based on ED50 values calculated from nonlinear regression analysis of the dose-response curves, the rank order of potency to inhibit gastric emptying of the solid meal was sauvagine > urotensin I > CRF (Table 1).

The midsequence CRF analog, r/hCRF-(6–33) (0.3–10 µg ic), did not significantly influence the 5-h percent-age of gastric emptying (0.3 µg, 44.1 ± 3.0%, n = 4; 1 µg, 59.9 ± 4.1%, n = 5; 10 µg, 66.3 ± 4.0%, n = 5) compared with the vehicle-treated group (53.1 ± 4.6%, n = 12; F4,22 = 2.94, P = 0.05681).

Effect of Intracisternal A stressing on Inhibition of Gastric Emptying of a Solid Nutrient Meal Induced by Intracisternal CRF and CRF-Related Peptide

In animals injected with vehicle (5 µl ic distilled water + 5 µl ic saline), 53.5 ± 4.0% (n = 8) of the meal had emptied from the stomach after 5 h. The basal rate of emptying was not significantly modified by astressin (3 or 5 µg) followed by the injection of saline (44.5 ± 3.5%, n = 6; and 53.3 ± 4.3%, n = 5, respectively). The antagonist-to-agonist ratios required for IC injection of astressin to completely block IC CRF-, urotensin I-, and sauvagine-induced inhibition of gastric emptying of a solid meal were 3:1, 10:1, and 16:1, respectively (Fig. 2). The inhibition of emptying of a solid meal induced by 0.3 µg CRF injected IC (23.5 ± 7.3%, n = 4) was completely prevented by astressin at 1 and 3 µg as values reached 40.2 ± 5.7% and 47.7 ± 4.7%, respectively (n = 4–5 per group, P > 0.05 compared with astressin alone or vehicle) (Fig. 2).

CRF
Urotensin-I
Sauvagine

Fig. 1. Effect of rat/human corticotropin-releasing factor (r/hCRF) and its related peptides, amphibian sauvagine and suckerfish urotensin I, injected intracisternally (IC), on 5-h rate of gastric emptying of a solid nutrient meal in rats. Fasted rats were exposed to Purina chow ad libitum for a 3-h period. Vehicle or peptides were then injected IC under short enflurane anesthesia, and gastric emptying was monitored 5 h later. Data are means ± SE of 4–12 animals per group. *P < 0.05 vs. control or vehicle-treated animals (ANOVA).

Fig. 2. Effect of the CRF1/CRF2 antagonist, astressin, in IC r/hCRF-, urotensin I-, and sauvagine-induced inhibition of gastric emptying of a nutrient solid meal in conscious rats. Protocols similar to those in Fig. 1 were followed, except that rats received 2 consecutive IC injections (5 µl each) of either water or astressin followed by vehicle or peptides. Data are means ± SE of 4–8 animals per group; data were pooled from different doses of agonists and antagonists at the same ratio of antagonist to agonist. Dashed line represents 5-h rate of emptying in control conditions (animals injected with vehicle or astressin + vehicle).

Table 1. Potency of r/hCRF and the related peptides amphibian sauvagine and suckerfish urotensin I to inhibit gastric emptying of a solid nutrient meal

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ED50 (µg/rat) (95% confidence interval)</th>
<th>r²</th>
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<tbody>
<tr>
<td>r/hCRF</td>
<td>0.31 (0.261–0.350)</td>
<td>0.99</td>
</tr>
<tr>
<td>Urotensin I</td>
<td>0.13 (0.096–0.172)</td>
<td>0.99</td>
</tr>
<tr>
<td>Sauvagine</td>
<td>0.08 (0.041–0.151)</td>
<td>0.98</td>
</tr>
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</table>

Rats, under short enflurane anesthesia, were injected intracisternally with each peptide at the end of the 3-h feeding period, and gastric emptying of the solid meal was monitored 5 h later. ED50 values correspond to the dose of peptide (µg/rat) inhibiting gastric emptying by 50% compared with vehicle-treated animals (taken as 0% inhibition), according to a nonlinear regression model. r/hCRF, rat/human corticotropin-releasing factor.
completely prevented the CRF (1 µg) inhibitory effect when injected at a dose of 3 µg (gastric emptying, 51.5 ± 10.5%, n = 5) but had no effect when injected at a dose of 1 µg (ratio 1:1) (17.4 ± 6.0%, n = 4) (Fig. 2).

Inhibition of gastric emptying induced by urotensin I injected IC at 0.3 µg (14.2 ± 5.7%, n = 6) was partially blocked by 1.5 µg astressin (ratio 5:1; 23.4 ± 7.4%, n = 5) and completely prevented by 3 µg astressin (ratio 10:1; 52.0 ± 5.5%, n = 4, Fig. 2). Sauvagine (0.3 µg ic) inhibited gastric emptying to 18.6 ± 6.1% (n = 5). Astressin at 3 µg (ratio 10:1) partly blocked the sauvagine effect (30.7 ± 15.9%, n = 4) and completely prevented it when injected at 5 µg (ratio 16:1) (49.4 ± 4.3%, n = 5, Fig. 2).

Effect of Intracisternal NBI-27914 on Inhibition of Gastric Emptying of a Solid Nutrient Meal Induced by Intracisternal CRF and CRF-Related Peptide

In animals injected with vehicle (5 µl DMSO + 5 µl saline, ic), the 5-h rate of gastric emptying was 52.0 ± 3.8% (n = 6). The basal rate of emptying was not significantly modified by NBI-27914 (50 µg), followed by the injection of vehicle (Table 2). Injection of NBI-27914 (50 µg/rat ic) immediately before peptide administration at a low dose (0.3 µg/rat) did not modify CRF-, sauvagine-, or urotensin I-induced inhibition of gastric emptying of a solid meal (Table 2).

DISCUSSION

Rats fasted for 18–20 h and given access to Purina chow and water ad libitum ingested 6.46 ± 0.10 g of food within the 3-h period, of which 62% was emptied from the stomach after 5 h. r/hCRF and the CRF-related peptides, amphibian sauvagine and suckerfish urotensin I, injected into the cisterna magna in picomolar amounts, dose dependently inhibited the gastric emptying of the solid nutrient meal. By contrast, the CRF analogs, r/hCRF-(6—33) or cyclo(30—33)-[D-Phe12,Nle21,38,Glu30,Lys33]r/hCRF-(12—41) (astressin) injected IC at similar or higher doses, as well as IC injection of vehicle, did not influence the rate of gastric emptying compared with the nontreated control group. These results show the specificity of the inhibitory action induced by r/hCRF and the nonmammalian CRF-related peptides, urotensin I and sauvagine. Previous studies showed that r/hCRF injected into the cerebrospinal fluid inhibited gastric emptying of an intragastrically delivered noncaloric (2, 15, 18, 21, 36, 37, 39) or caloric solution (7, 32) in rats. Broccardo and Improta (2) and Improta (15) also reported that sauvagine and urotensin I injected into the lateral brain ventricle delayed gastric emptying of a nonnutrient solution. These data, together with the present results, establish CRF and nonmammalian CRF-related peptides as potent inhibitors of gastric emptying in rats, irrespective of the nature of the meal (caloric liquid or solid, or noncaloric liquid or viscous). Likewise, in the dog, intracerebroventricular injection of CRF delayed the total gastric emptying time of a solid meal (17).

A recent study indicates that there is an active carrier-mediated brain-to-blood transport of CRF (22). However, several control experiments established that the inhibition of gastric emptying of a liquid meal induced by IC injection of CRF reflects a central nervous system-mediated action (37). Therefore it is likely that the long-lasting inhibition of gastric emptying of a solid meal induced by CRF, sauvagine, and urotensin I injected into the cisterna magna at picomolar amounts reflects an action initiated in the central nervous system. The site of action of CRF injected into the cisterna magna may involve the dorsal vagal complex. Microinjection of CRF at this site, as opposed to nearby nuclei, mimicked the effect of IC injection by suppressing central vagal stimulation of gastric motility in anesthetized rats (10, 14). In addition, the inhibitory effect of central CRF and sauvagine on gastric emptying is dependent on the vagus (2, 36).

CRF mediates its effects in the brain through interaction with high-affinity CRF1 and CRF2 receptor subtypes. Convergent sets of evidence are consistent with the involvement of the CRF2 receptor subtype in IC CRF-induced delay in gastric emptying of a solid meal. The potency order of IC CRF and the nonmammalian CRF-related peptides to inhibit gastric emptying of a solid meal exhibits a characteristic profile similar to that defined for the CRF2 subtype (sauvagine > urotensin I > r/hCRF), unlike that expected for CRF1 receptor (r/hCRF ≈ urotensin I > sauvagine) (5). The ED50 values, defined as the molar dose necessary to inhibit the 5-h rate of gastric emptying by 50%, were ~17 pmol for sauvagine and 26 pmol for urotensin I, values ~3.8-fold and ~2.5-fold lower, respectively, than the r/hCRF ED50 (65 pmol). Consistent with these observations, the 20-min rate of gastric emptying of a nonnutrient liquid meal was inhibited with a similar rank order of potency (sauvagine > urotensin I > CRF) when peptides were injected into the lateral brain ventricle in rats (2, 15).

Recently, several novel molecules with antagonist activity to CRF receptors have been described (6, 12, 30). A stressin, a CRF-derived antagonist, exhibits equally high affinity at both the CRF1 and CRF2

<table>
<thead>
<tr>
<th>Treatment, µg/rat</th>
<th>Gastric Emptying, %</th>
<th>Vehicle (DMSO)</th>
<th>n</th>
<th>NBI-27914</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>52.0 ± 3.8</td>
<td>6</td>
<td>47.2 ± 5.2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>r/hCRF</td>
<td>0.3</td>
<td>31.4 ± 2.3*</td>
<td>5</td>
<td>20.9 ± 3.5*</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>21.1 ± 1.4*</td>
<td>5</td>
<td>12.8 ± 7.4*</td>
<td>6</td>
</tr>
<tr>
<td>Sauvagine</td>
<td>0.3</td>
<td>23.4 ± 6.4*</td>
<td>5</td>
<td>25.9 ± 2.6*</td>
<td>5</td>
</tr>
<tr>
<td>Urotensin I</td>
<td>0.3</td>
<td>24.9 ± 6.3*</td>
<td>5</td>
<td>26.2 ± 3.4*</td>
<td>5</td>
</tr>
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</table>

Data are means ± SE and represent % of gastric emptying in 5 h. Rats fasted for 18–20 h were given ad libitum Purina chow and water for 3 h, and then food and water were removed and under short enflurane anesthesia rats were injected intracisternally with DMSO (5 µl/rat) or NBI-27914 (50 µg/rat) followed by vehicle (saline) or peptides; 5 h later gastric emptying was measured. *P < 0.05 vs. vehicle (DMSO) + vehicle (saline) or NBI-27914 + vehicle (saline).
receptor subtypes and greater in vitro and in vivo potency than the previously developed antagonists, α-helical CRF-(9—41) and [d-Phe12,Nle21,38]r/hCRF-(12—41) (12). In addition, nonpeptide CRF receptor antagonists have also been developed (6, 30); among them, NBI-27914 exhibits high CRF1-selective antagonist action (6). IC injection of astressin at doses of 3–5 µg/rat, which by themselves had no effect on the basal rate of gastric emptying, completely blocked CRF, sauvagine, and urotensin I inhibitory action at antagonist-to-agonist ratios (µg) of 3:1, 10:1, and 16:1 respectively. The higher IC antagonist-to-agonist ratio needed to block sauvagine compared with CRF is in line with the higher affinity of sauvagine on CRF2 receptors compared with CRF (5). We previously reported that similar doses of astressin injected IC antagonized IC CRF-induced delay of gastric emptying of a noncaloric viscous solution at an antagonist-to-agonist ratio of 5:1 in rats (21). By contrast, NBI-27914 injected IC at 50 µg, the higher effective dose determined for other CRF antagonists (35), did not modify CRF- or CRF-related peptide-induced inhibition of gastric emptying. It is unlikely that the lack of action of the NBI-27914 is related to the use of a subeffective treatment. In cells stably transfected with the CRF1 receptor, astressin and NBI-27914 shared similar affinity (K_i in the 2 nM range) to inhibit CRF binding (6, 12). In the present study, NBI-27914 was injected IC at an antagonist-to-agonist ratio (µg) of 167:1, which is 300-fold higher on a molar basis than the effective ratio for astressin and CRF. Because NBI-27914 is devoid of activity in cells transfected with the CRF2 receptor subtype, whereas astressin displays a similar affinity for both receptor subtypes (6, 12), these results further support the view that peptide interaction with the CRF2 receptor subtype is likely to mediate the central CRF action to inhibit gastric motor function.

The pituitary response to CRF involved an interaction with the CRF1 receptor subtype, as shown by the equal potency of CRF, sauvagine, and urotensin I to stimulate in vitro and in vivo pituitary ACTH release, which are blocked by both NBI-27914 and astressin (6, 28, 38). The lack of influence of the specific CRF1 receptor antagonist on gastric stasis is consistent with previous reports indicating that the inhibition of gastric emptying induced by central CRF administration is mediated through autonomic vagal pathways and is independent from its pituitary action in rats and dogs (2, 7, 17, 37). Interestingly, sauvagine injected into the lateral ventricle was reported to be 5- to 10-fold more potent than CRF to induce an autonomic nervous system-mediated increase in plasma catecholamine and glucose levels, elevation of mean arterial pressure and thermogenesis from brown adipose tissue, and a decrease in gastric vagal efferent discharges in rats (3, 16, Kosoyan and Taché, unpublished observations). These data suggest that autonomic-dependent gastrointestinal, cardiovascular, and thermogenic responses to central CRF and CRF-related peptides may be primarily mediated by the activation of brain CRF2 receptors. This is also supported by the presence of CRF2 receptors in the hypothalamus, amygdala, lateral septum, and brain stem (4, 20), which contain autonomic regulatory centers (i.e., paraventricular nucleus of the hypothalamus and dorsal vagal complex) that are target sites of action of CRF-induced inhibition of gastric motor function (24, 25, 37).

Central injection of the CRF receptor antagonists α-helical CRF-(9—41), [d-Phe12,Nle21,38]r/hCRF-(12—41), or astressin (12, 13) prevented gastric stasis induced by various stressors (7, 19, 21, 34, 37). By contrast, the CRF receptor antagonists did not influence the basal rate of gastric emptying of a liquid meal (37). Likewise, astressin injected IC at doses sufficient to antagonize central CRF did not alter gastric emptying of a solid nutrient meal. These data suggest that brain stem CRF receptors are not involved in postprandial regulation of gastric emptying, at least under nonstressed conditions.

In conclusion, IC CRF and its related nonmammalian peptides, amphibian sauvagine anduckerfish urotensin I, dose dependently inhibit gastric emptying of a physiological meal in rats. The rank order of potency of the peptides (sauvagine > urotensin I > r/hCRF) to inhibit gastric emptying of a solid nutrient meal is consistent with the profile characterized in vitro for activation of the CRF2 receptor subtype, rather than the CRF1 receptor subtype. This assumption is further supported by the complete blockade of IC CRF-, sauvagine-, and urotensin I-induced delay of gastric emptying by the CRF2 receptor antagonist, astressin, whereas the CRF2-selective receptor antagonist, NBI-27914, had no effect.

We thank Dr. E. B. De Souza and Dr. J. R. McCarthy (Neurocrine Biosciences, San Diego, CA) for the generous donation of NBI-27914. P. Kirsch is acknowledged for help in the preparation of the manuscript.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-33061 (Y. Taché) and DK-26741 (J. Rivier) and by National Institute of Mental Health Grant MH-00063 (Y. Taché).

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Received 20 August 1997; accepted in final form 29 January 1998.

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