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Effects of CI-943, a Potential Antipsychotic Drug, and Haloperidol on Regional Brain Neurotensin Concentrations

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ABSTRACT Treatment with efficacious antipsychotic drugs such as haloperidol increases the concentrations of neurotensin (NT) in the nucleus accumbens and caudate nucleus of the rat. These increases in NT concentrations may be associated with the therapeutic and/or side effects of these drugs. CI-943, a novel compound without appreciable affinity for dopamine-binding sites, produces behavioral effects in animals, which suggest that it may possess antipsychotic activity. This study evaluated the effects of subchronic treatment (3 weeks) with CI-943 or haloperidol on regional brain NT concentrations in rats. Haloperidol treatment (1 mg/kg) produced significant increases in the concentrations of NT in the nucleus accumbens and caudate nucleus but not in the other brain regions studied. Like haloperidol, CI-943 (40 mg/kg) increased NT concentrations in the nucleus accumbens and caudate but differed in that CI-943 produced significantly greater increases in NT concentration in the caudate than haloperidol and also increased NT content in the substantia nigra/ventral tegmental area and hypothalamus. The regional specificity of the NT alterations produced by chronic treatment with CI-943, a nondopamine receptor ligand, was similar to that previously reported after treatment with multiple doses of methamphetamine.

INTRODUCTION

Considerable evidence supports a role for the endogenous neuropeptide neurotensin (NT) in the pathophysiology of schizophrenia and in the mechanism of action of antipsychotic drugs (for review see Levant and Nemeroff, 1988). The anatomical localization of NT-containing neurons and NT receptors suggests interactions between NT and dopamine (DA) neural circuits, particularly the mesolimbocortical DA pathway. Centrally administered NT has been shown to produce pharmacological effects that are remarkably similar to those produced by antipsychotic drugs, including increased DA turnover, decreased locomotor activity, antagonism of amphetamine-induced hyperactivity, potentiation of barbiturate-induced sedation, induction of hypothermia, and decreased responding in a conditioned avoidance paradigm. Furthermore, the concentration of NT in the CSF of schizophrenic patients has been repeatedly shown to be decreased when compared to age- and sex-matched controls (Garver et al., 1990; Lindstrom et al., 1988; Widerlov et al., 1982). In two populations of schizophrenic patients with subnormal CSF NT concentrations, treatment with antipsychotic drugs effected an increase in NT concentrations to normal levels (Widerlov et al., 1982; Breslin, Nemeroff, Bissette, Weinberger, unpublished observations). These findings strongly implicate the NT system in the pathophysiology of schizophrenia and/or the mechanism of action of antipsychotic drugs.

Preclinical studies also provide evidence for the involvement of NT systems in antipsychotic drug action. Studies in rats have shown that a variety of typical, clinically efficacious antipsychotic drugs, including haloperidol, chlorpromazine, and pimozide, produce increases in NT concentrations in specific brain regions, most notably the nucleus accumbens and the caudate nucleus, whereas phenothiazines without antipsychotic efficacy, such as promazine and promethazine, do not (Govoni et al., 1980). Moreover, although tolerance develops to many of the effects of typical antipsychotic drugs on DA neurons and receptors, increases in NT concentrations have been observed after 8 months of

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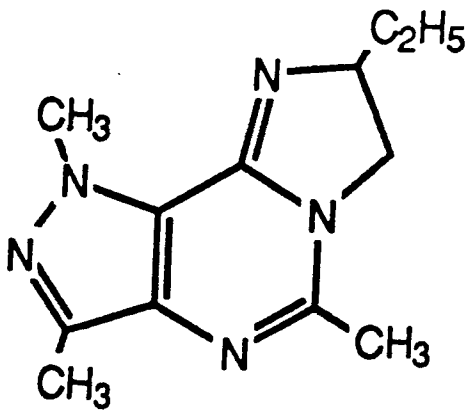

CI-493 (8-ethyl-7,8-dihydro-1,3,5-trimethyl-1H-imidazo[1,2-c]pyrazolo[3,4-e]-pyrimidine)

Fig. 1. Structure of CI-943.

continuous haloperidol administration (Radke et al., 1988), further suggesting that the observed increases in NT concentrations may underlie some of the therapeutic effects of these drugs—effects to which tolerance does not develop. Several atypical and potential antipsychotic drugs, such as clozapine, S(+)-N-n-propylnorapomorphine ((+)NPA), BMY 14802, and rimcazole, have also been reported to increase the concentrations of NT in either the nucleus accumbens, caudate, or both (Kilts et al., 1988; Levant et al., 1990; Levant and Nemeroff, 1990; Nemeroff et al., 1990). These data provide further support for the hypothesis that increases in the concentrations of NT in the nucleus accumbens, and perhaps other brain regions, may be associated with antipsychotic efficacy.

The pyrazolo-pyrimidine CI-943 (Fig. 1) produces behavioral effects in animals, such as decreased spontaneous locomotor activity and decreased avoidance (but not escape) responding in conditioned avoidance paradigms, which are shared with clincally efficacious antipsychotic drugs (Heffner et al., 1989). Like certain atypical antipsychotics, CI-943 fails to produce either supersensitivity to dopamine agonists, extrapyramidal dysfunction at behaviorally active doses in monkeys, or an up-regulation of dopamine receptors, all suggesting that it may not produce the neurological side effects seen with many typical antipsychotic drugs (Heffner et al., 1989; Pugsley et al., 1989). Interestingly, unlike classical and atypical antipsychotic drugs, which antagonize amphetamine-induced locomotor activity, CI-943 potentiates this effect (Heffner et al., 1989). Behavioral, neurochemical, and receptor binding data indicate that this drug does not produce its effects through interactions with dopamine receptors (Heffner et al., 1989; Meltzer et al., 1989; Pugsley et al., 1989).

In light of the hypothesized role for NT in the mechanism of action of antipsychotic drugs and the behavioral evidence suggesting that CI-943 may possess antipsychotic activity, this study was designed to compare the effects of subchronic treatment with CI-943 or haloperidol on regional brain NT concentrations.

MATERIALS AND METHODS

Animals

Adult, male, Sprague-Dawley rats (225–250 g) were housed two per cage with free access to laboratory chow and water. The temperature and humidity controlled animal facility had a 12-hour dark-light cycle. Rats were obtained from Charles River Laboratories (Raleigh, NC and Harlan, IN).

Experimental procedure

For the haloperidol dose-response experiment, rats (n = 10 per group) were injected intraperitoneally (ip) with haloperidol or vehicle at the same time each day for 21 days. For the comparison of the effects of haloperidol and CI-943, rats (n = 10–12 per group) were injected (ip) with haloperidol (1 mg/kg), CI-943 (40 mg/kg), or vehicle for 23 days. Rats were killed by decapitation 18 hours after the last injection. Brains were rapidly removed, frozen on dry ice, and stored at −70°C until dissection. Seven discrete brain regions (frontal cortex, nucleus accumbens, anterior caudate, posterior caudate, hypothalamus, amygdala, and substantia nigra/ventral tegmental area) were isolated by freehand dissection on ice by a modification of the method of Glowinski and Iversen (1966).

Neurotensin radioimmunoassay

Brain tissues were extracted by sonic dismembranation in ice cold 1N HCl in polypropylene microcentrifuge tubes. Homogenates were centrifuged at 8,000 × g for 15 min at 4°C; supernatants were transferred to separate polypropylene microcentrifuge tubes and vortexed. Duplicate aliquots of supernatant were transferred to borosilicate glass tubes on ice. Aliquots were lyophilized, reconstituted in assay buffer, and assayed by a single equilibrium radioimmunoassay according to methods previously described (Bissette et al., 1984). The antiserum is directed toward the midportion of the NT molecule, amino acids 6,7, and 8 (Lys-Pro-Arg) and was used at a final dilution of 1:14,000. Synthetic NT_{1-13} (Bachem, Inc., Torrance, CA) was used as standard and was iodinated for use as trace. Goat-antirabbit antiserum (Arnel Products, New York City) was used as second antibody. The assay has a sensitivity of 1.25 pg/tube and an IC_{50} of 80 pg/tube. Pellets from NT extraction were dissolved in 1N NaOH and assayed for protein concentration by the method of Lowry et al. (1951) with bovine serum albumin used as standard. NT concentrations are expressed as pg NT/mg protein.

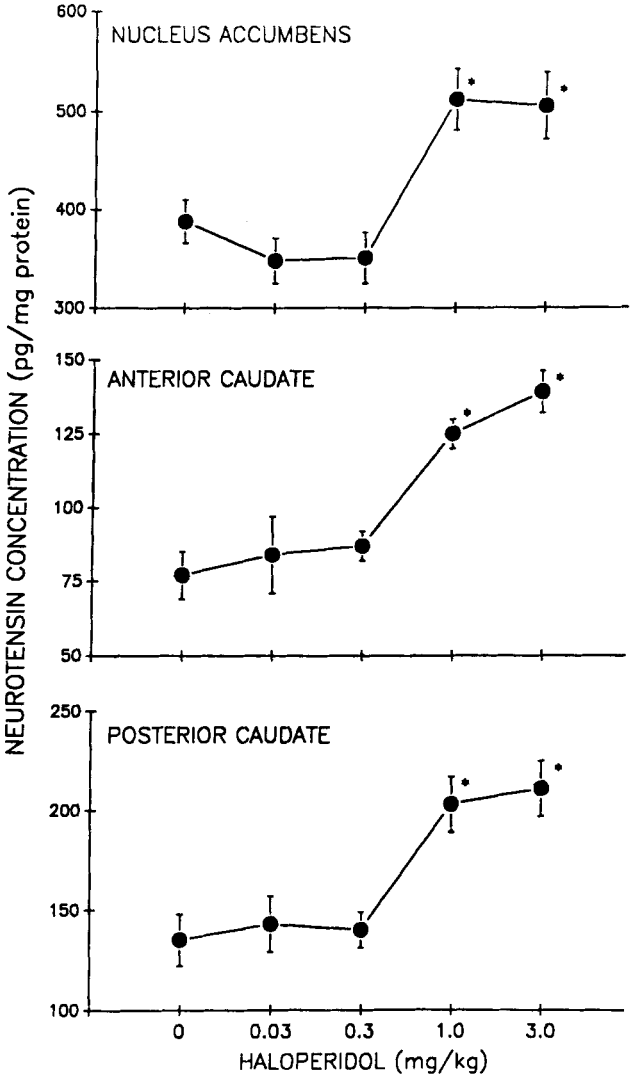

Fig. 2. Dose-response effects of haloperidol on neurotensin concentrations in the nucleus accumbens, anterior caudate, and posterior caudate. Rats were injected (ip) with haloperidol for 21 days. *$P < 0.01$ by ANOVA and the Student-Newman-Keuls multiple comparisons test.

Drugs

Haloperidol (Research Biochemical, Natick, MA) was administered in a vehicle of 0.3% tartaric acid. CI-943 (synthesized at Parke-Davis by Drs. H. Dewald and L. Wise) was administered in 0.9% saline. All drugs were administered in a volume of 1 ml/kg. In the haloperidol dose-response study, control animals were treated with 0.3% tartaric acid; in the CI-943 study, 0.9% saline.

Statistical analysis

Data are expressed as the mean (x) ± the standard error of the mean (S.E.). Data were analyzed for statistical significance by analysis of variance (ANOVA) and the Student-Newman-Keuls multiple comparisons test. Significant differences between means was assumed at $P < 0.05$.

RESULTS

Rats were treated subchronically with haloperidol at doses of 0.03 mg/kg, 0.3 mg/kg, 1 mg/kg, and 3 mg/kg. Statisticaly significant increases in NT concentrations of the nucleus accumbens, anterior caudate, and posterior caudate were observed after treatment with doses of 1 mg/kg and 3 mg/kg (Fig. 2). NT concentrations were unaltered by any dose tested in the other brain regions studied (data not shown). The effects of haloperidol at 1 mg/kg and 3 mg/kg were not significantly different.

To compare the effects of CI-943 and haloperidol, rats were treated subchronically with CI-943 (40 mg/kg) or haloperidol (1 mg/kg). Both CI-943 and haloperidol produced statistically significant increases in the concentrations of NT in the nucleus accumbens and anterior and posterior caudate. Whereas increases of similar magnitude were produced by both drugs in the nucleus accumbens, CI-943 produced significantly larger increases in NT concentration in the anterior and posterior caudate than haloperidol. In both regions of the caudate, CI-943 produced a fivefold increase in the concentration of NT compared to a twofold increase produced by haloperidol. CI-943 also produced significant increases in the concentrations of NT in the substantia nigra/ventral tegmental area (264% of control) and hypothalamus (125% of control), which were not observed after treatment with haloperidol. A small but significant decrease in NT concentration in the hypothalamus was observed after treatment with haloperidol. NT concentrations in the frontal cortex and amygdala were not altered by either drug. These data are summarized in Figure 3.

DISCUSSION

As previously described, haloperidol produced significant increases in the concentrations of NT in the nucleus accumbens and caudate nucleus (Govoni et al., 1980; Kilts et al., 1988; Levant and Nemeroff, 1990; Levant et al., 1990). Alterations in NT content in other brain regions, as well as in the nucleus accumbens and caudate, have been reported in certain studies, such as those of Kilts et al. (1988) and Nemeroff et al. (1990). However, these studies employed a micropunch dissection technique, which allows the isolation of discrete nuclei and subregions. Certain NT alterations reported in these and similar studies were most likely facilitated by the high degree of anatomical resolution of the dissection method. The haloperidol dose-response profile observed in this study is similar to that reported by Govoni et al. (1980). Because the effects of haloperidol at 1 mg/kg and 3 mg/kg were not significantly different,

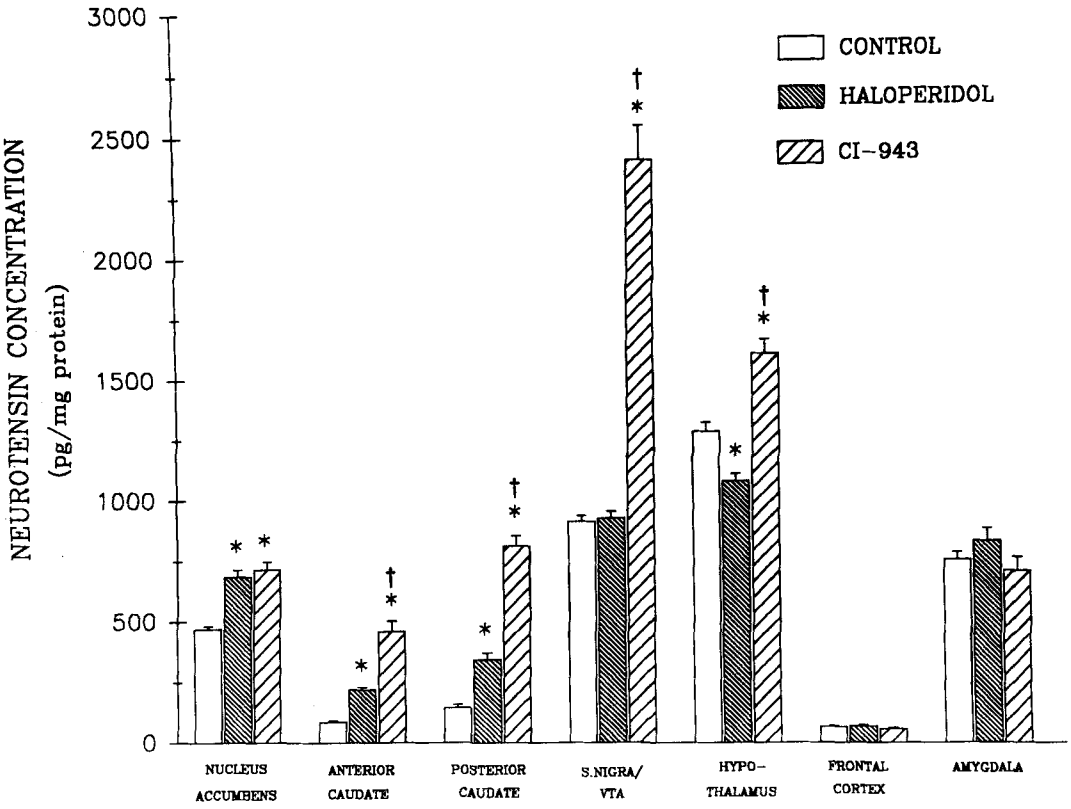

Fig. 3. Effects of CI-943 and haloperidol on regional brain neurotensin concentrations. Rats were injected (ip) with CI-943 (40 mg/kg) or haloperidol (1 mg/kg) for 23 days. *$P < 0.01$ compared to control; †$P < 0.01$ compared to haloperidol by ANOVA and the Student-Newman-Keuls multiple comparisons test.

the dose of 1 mg/kg was used for the subsequent experiment. A small but significant decrease in the concentration of NT was observed in the hypothalamus after treatment with haloperidol (Fig. 3). A decrease in NT concentration in this brain region has not been reported in previous studies and should be explored further.

Among typical, atypical, and other potential antipsychotic drugs tested thus far, subchronic treatment with CI-943 produces a unique pattern of NT alterations. As described above, haloperidol and other typical antipsychotic drugs, such as chlorpromazine, thioridazine, and pimozide, have been shown to produce increases in the concentrations of NT in the nucleus accumbens and caudate in the range of 150%–200% of control (Frey et al., 1986; Govoni et al., 1980). Likewise, a variety of atypical and potential antipsychotic drugs like clozapine, sulpiride, BMY 14802, rimcazole, and (+)NPA, have been found to produce increases in NT concentrations of similar magnitude in one or both of these brain regions (Kilts et al., 1988; Levant and Nemeroff, 1990; Nemeroff et al., 1991; Levant et al., 1991). Administered at a dose that produces maximal, or near maximal, response in a variety of behavioral paradigms (as does haloperidol at 1 mg/kg), CI-943, like haloperidol, produced significant increases in the concentrations of NT in the nucleus accumbens and anterior and posterior caudate. Unlike haloperidol, CI-943 produced significantly greater increases in NT concentration in the caudate (fivefold vs. twofold after treatment with haloperidol) and also increased the concentrations of NT in the substantia nigra/ventral tegmental area and hypothalamus.

The regional specificity of NT alterations produced by chronic treatment with CI-943 is similar to that reported after treatment with large, multiple doses of methamphetamine or cocaine, which also increased NT concentrations in the nucleus accumbens, caudate, substantia nigra, and hypothalamus (Hanson et al., 1989; Letter et al., 1987; Merchant et al., 1987). Certain amphetamine analogs such as methylenedioxyamphetamine (MDA) produced increases in NT concentrations of a magnitude more similar to those observed after treatment with CI-943 than those produced by methamphetamine; however, no alteration in hypothalamic NT concentration was reported after treatment with MDA (Merchant et al., 1987). Although certain similarities exist between the present findings and those of Hanson et al. (1989), Letter et al. (1987), and Merchant et al. (1987), it is important to note that the experimental designs in the above mentioned studies differ substan-

tially with respect to doses, schedules of administration, and time of sacrifice from the present study. These methodological factors may affect both the magnitude and regional specificity of the NT alterations detected.

The mechanism of action of CI-943 is currently unknown. Extensive receptor binding studies have failed to detect any receptors to which the compound binds with high affinity (Pugsley et al., 1989). It is therefore possible that CI-943 produces its CNS effects through actions that do not involve classical neurotransmitter receptors. It is clear however, that neither CI-943 nor its metabolites act as direct or indirect dopamine agonists or antagonists. In addition to decreasing spontaneous locomotor activity and producing no alteration in dopamine receptor number after chronic administration, CI-943 does not alter prolactin secretion (Heffner et al., 1987; Pugsley et al., 1987) and does not affect the ability of dopamine agonists to decrease the firing of brain dopamine neurons in vivo (Meltzer et al., 1989). CI-943 does increase dopamine turnover in the striatum and mesolimbic brain regions but to a lesser extent than haloperidol (Pugsley et al., 1987). A small increase in dopamine outflow has been observed with intracerebral microdialysis after treatment with CI-943, but this drug is much less potent than methamphetamine and other psychostimulants (Davis and Heffner, unpublished observation). Furthermore, CI-943 does not produce stimulant effects in a variety of behavioral tests and is devoid of amphetamine-like effects in mice, rats, and primates. In fact, the behavioral effects of CI-943 resemble those of antipsychotic drugs in all species tested (Heffner et al., 1989).

The NT alterations produced by methamphetamine and cocaine have been hypothesized to result from the stimulation of dopamine receptors, particularly D_1 receptors, because these increases were blocked by concomitant treatment with D_1 antagonist SCH 23390 (Hanson et al., 1989; Merchant et al., 1988). D_1 agonist SKF 38393 was also observed to produce modest increases in NT concentrations in the nucleus accumbens and striatum; however, combined stimulation of D_1 and D_2 receptors with SKF 38393 and LY 171555, respectively, was shown to produce no alteration in the concentrations of NT in these brain regions (Merchant et al., 1989). Clearly, treatment with methamphetamine or cocaine would result in increased stimulation of both D_1 and D_2 receptors. Thus it appears that whereas some stimulation of D_1 receptors may be permissive for these increases to occur, the increases in NT concentrations are not principally the result of D_1 receptor stimulation. The similarity of the NT alterations produced by CI-943 and methamphetamine also suggests that both drugs may modulate NT systems through a nondopaminergic mechanism.

The clinical efficacy of CI-943 has not yet been determined. The fact that CI-943 can increase NT concentrations in the nucleus accumbens and caudate suggests that it would possess clinical antipsychotic activity. However, the ability of CI-943 to potentiate amphetamine-induced behavior and the similarity of the NT increases after CI-943 in certain other brain regions to the regional NT alterations seen after high doses of methamphetamine indicate that CI-943 may possibly exacerbate psychotic symptoms. Further work will be required to elucidate the extradopaminergic mechanisms responsible for the observed effects of CI-943 on NT concentrations.

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