Dopamine Antagonist Haloperidol Decreases Substance P, Substance K, and Preprotachykinin mRNAs in Rat Striatonigral Neurons*

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Rat genomic clones were used to quantitate preprotachykinin mRNAs in the rat basal ganglia, while the tachykinin peptide products substance P and substance K were measured by radioimmunoassay. Administration of the dopamine antagonist (antipsychotic) drug haloperidol significantly decreased substance P, substance K, and both α (substance P encoding) and β (substance P/substance K encoding) preprotachykinin mRNAs, suggesting a drug-induced decrease in striatonigral tachykinin biosynthesis. The time course for decreased preprotachykinin mRNAs and tachykinins apparently parallels the period of maximum risk for the development of certain antipsychotic drug-induced extrapyramidal side effects seen clinically. Tachykinin interaction with dopamine neurons may play an important role in the modulation of basal ganglia function.

The tachykinin neuropeptide substance P is present throughout the brain and peripheral tissues. The prominent substance P projection from the striatum to the substantia nigra is thought to play an important role in the regulation of basal ganglia function and the response to dopaminergic and other pharmacological agents (1). However, the physiological and pharmacological significance of this striatonigral tachykinin system has been difficult to assess, since no useful measures of substance P turnover have been available. Previous studies have shown that the repeated administration of dopamine antagonist (antipsychotic) drugs decreases the concentration of substance P in the substantia nigra (2–6). Although the relationship between substance P concentration and substance P turnover remains obscure, the antipsychotic drug-induced decrease in this tachykinin has generally been thought to result from an imbalance between increased substance P biosynthesis and an even greater acceleration of substance P release (2, 3).

It has recently been demonstrated (7, 8) that two mRNAs derived from one preprotachykinin gene by alternate RNA splicing encode for either substance P alone (α preprotachykinin mRNA) or for both substance P and the newly discovered tachykinin (9, 10) substance K (β preprotachykinin mRNA). Thus, the biosynthesis of these two tachykinins could be differentially regulated in response to dopamine antagonists or other stimuli. We have quantitated the acute and chronic effects of the antipsychotic drug haloperidol on the levels of striatonigral preprotachykinin mRNAs (as well as substance P and substance K peptide levels) in single rat brain striata as a dynamic index of tachykinin turnover. Tachykinin-encoding mRNAs rapidly decreased after haloperidol, followed by decreased tachykinin peptides, suggesting that antipsychotic drugs may, in fact, decrease striatonigral tachykinin biosynthesis.

EXPERIMENTAL PROCEDURES

Animals—Adult male Sprague-Dawley rats (Charles River, 225–250 g body weight) were killed by decapitation either 2–4 h after haloperidol or 24 h after 1–10 days of haloperidol treatment (1 mg/kg, intraperitoneally; McNeil). Brains were blocked in a chilled aluminum brain mold (Activational Systems, Warren, MI), dissected using previously described procedures for the striatum and substantia nigra (11), quickly frozen on dry ice, then stored at −70 °C until further use.

Radioimmunoassays—Tissues were extracted and substance P-like immunoreactivity and substance K-like immunoreactivity were assayed by radioimmunoassay as previously described (12–14). Substance K showed approximately 1% cross-reactivity compared to substance P in the substance P radioimmunoassay; conversely, substance P cross-reacted to approximately 1% in the substance K radioimmunoassay. High performance liquid chromatographic analysis coupled to the tachykinin radioimmunoassays established that all detected striatal and nigral substance P immunoreactivity was authentic substance P, while authentic substance K accounted for at least 86% of striatal and 94% of nigral substance K immunoreactivity (15). Therefore, substance P- and substance K-immunoreactivity are hereafter referred to simply as substance P and substance K, respectively. Protein concentrations were determined by the Lowry method (16).

Preparation of Total Nucleic Acids—One or two striata (30 mg of tissue each) previously stored at −70 °C were rapidly Dounce homogenized in 3 ml of SET buffer (1% sodium dodecyl sulfate, 5 mM EDTA, 10 mM Tris, pH 8) containing 100 μg/ml proteinase K (17). After a 1.5-h incubation at 42 °C, 100 μg of tRNA was added as a carrier, and the samples were extracted 2–3 times with 2 volumes of 1:1 Tris-saturated phenol/chloroform. Following a further extraction with 2 volumes of 1:1 chloroform/butanol, total nucleic acids in the aqueous phase were precipitated overnight at −20 °C with 0.4 M sodium acetate, 2.5 volumes of ethanol.

Isolation and Preparation of 32P-Labeled Probes—The construction, isolation, and characterization of plasmids containing rat preprotachykinin genomic sequences is described elsewhere (18). Plasmids RSP p23 and RSP p18 were purified from HB101 lysates using a DEAE high performance liquid chromatography column (Alltech). Restriction endonuclease digestion yielded DNA probes containing exon 7 (~460 base pairs) or exon 6 (~300 base pairs), respectively. Restriction fragments were isolated by gel electrophoresis, purified on Nucleobond columns (New England Nuclear), and labeled by nick-translation (19) using [α-32P]dATP (~3000 Ci/mmol, Amersham) to a specific activity of ~2 × 104–5 × 104 cpm/μg DNA.

Nucleic Acid Electrophoresis, Blot Transfer, and Hybridization—Nucleic acids were denatured in 50% formamide, 6% formaldehyde at 65 °C for 5–10 min and electrophoresed in 1% agarose (formaldehyde-containing) gels (20). Gels were stained with ethidium bromide, 6640
RESULTS AND DISCUSSION

As previously reported (2, 5, 23, 24), neither nigral nor striatal substance P concentrations were altered within 2–4 h after the acute administration of the antipsychotic drug haloperidol (Fig. 1, A and B). After 3–10 days of haloperidol treatment, substance P levels were decreased in the substantia nigra, the terminal field of these striatonigral tachykinin neurons (Fig. 1A), as previously shown by others (2–6). A haloperidol-induced decrease in substance P of similar magnitude but earlier onset was seen in the striatum, the brain region containing the striatonigral tachykinin cell bodies (Fig. 1B). At all times assessed, changes in the concentration of substance P were exactly paralleled by changes in the concentration of substance K, its newly discovered tachykinin co-transmitter (Fig. 1, A and B).

Total (α and β) preprotachykinin mRNA from single rat strata was quantitated as described under "Experimental Procedures" and in Fig. 2 using a clone derived from the preprotachykinin gene exon 7, common to both mRNA species. Following hybridization, the prominent band visualized corresponded in migrational position (~1 kilobase) to the previously reported size of the bovine preprotachykinin mRNAs (7, 8). Since α and β preprotachykinin mRNAs differ by only ~50 nucleotides (7, 8), these two bands were unresolved under the conditions employed. Upon longer exposure of blots, a higher molecular weight (>6 kilobase) band, which conceivably corresponds to an unprocessed or partially processed precursor of mature preprotachykinin mRNA, was also visualized (see Fig. 2B). Further experiments are necessary to clarify the nature of this higher molecular weight band.

Four h after a single dose of haloperidol, a time when striatonigral tachykinin peptide levels were unaltered, total preprotachykinin mRNA levels were dramatically reduced (Fig. 1C). The reduction of preprotachykinin mRNA persisted with continued haloperidol treatment (Fig. 1C). Similar results were obtained by dot blot hybridization quantitated by either densitometry or liquid scintillation counting (data not shown). When β preprotachykinin mRNA was specifically quantitated using an exon 6-containing clone, parallel changes of a similar magnitude were seen (data not shown). Thus, in response to haloperidol administration, both α (substance P encoding) and β (substance P/substance K encoding) preprotachykinin mRNAs rapidly decrease in parallel. The reduction in tachykinin mRNAs prior to decreased tachykinin peptides (Fig. 1, A–C) strongly suggests that haloperidol decreases striatonigral substance P and substance K biosynthesis, presumably through decreased preprotachykinin gene transcription (although an effect on mRNA stability cannot be ruled out at present).

The present findings may offer some insight into the effects of acute and chronic antipsychotic drug administration on basal ganglia function. Dopamine is thought to normally provide a tonic inhibitory influence in the striatum (25). Antipsychotic drugs like haloperidol, by blocking striatal dopamine receptors, may cause a disinhibition of many striatal cells, including γ-aminobutyric acid- and/or enkephalin-containing inhibitory neurons. For example, striatal enkephalin mRNA and peptide levels increase after haloperidol (26–29).
Haloperidol Decreases Preprotachykinin mRNA

Thus, disinhibition of striatal inhibitory cells could account for the subsequent inhibition of tachykinin-containing neurons. Striatonigral tachykinins decrease after haloperidol with a time course that apparently parallels the period of maximal risk for the development of certain antipsychotic drug-induced extrapyramidal (Parkinsonian) side effects in man (30). Thus, it is conceivable that decreased striatonigral tachykinin output is somehow related to the production of these adverse neurological symptoms. In support of this suggestion, the loss of nigrostriatal dopamine cells in Parkinson’s disease is also accompanied by a decrease in basal ganglia substance P content (31). The data presented suggest that, despite brain tissue heterogeneity, neuropeptide-encoding mRNAs from discrete brain regions of individual animals can be quantitated as a rapid, dynamic index of peptide biosynthesis.

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REFERENCES
