SYNAPSE 28:302–312 (1998)

# Effects of Risperidone and Haloperidol on Tachykinin and Opioid Precursor Peptide mRNA Levels in the Caudate-Putamen and Nucleus Accumbens of the Rat

**M. JANNEKE MIJNSTER,[1] ALAIN SCHOTTE,[2] GERRIT J. DOCTER,[1] AND PIETER VOORN***
[1]*Graduate School Neurosciences Amsterdam, Research Institute Neurosciences, Department of Anatomy, Vrije Universiteit, 1081 BT Amsterdam, The Netherlands*
[2]*Department of Biochemical Pharmacology, Janssen Research Foundation, Beerse, B-2340 Belgium*

*KEY WORDS:* antipsychotic drugs; in situ hybridization; enkephalin; dynorphin; substance P; striatum

*ABSTRACT* We investigated whether the two output pathways of the striatum are differently affected by the novel atypical drug risperidone and the conventional typical antipsychotic drug haloperidol. To this end, changes in mRNA levels of preproenkephalin-A, preproenkephalin-B, and preprotachykinin were determined in the rat striatum following chronic drug treatment for 14 days, using quantitative in situ hybridization. Furthermore, we studied the contribution of the dopamine $D_2$ and serotonin 5-$HT_{2A}$ antagonist components of risperidone in establishing its effects on neuropeptide mRNA levels in the striatum. The results showed that both risperidone and haloperidol had major effects on the preproenkephalin-A mRNA and thus on the indirect striatal output route, whereas they had minor effects on preproenkephalin-B and preprotachykinin mRNA, contained by the direct output route. When both drugs were administered in the same dose, preproenkephalin-A mRNA was much more elevated by haloperidol than by risperidone. However, when doses of risperidone and haloperidol were modified to attain comparable dopamine $D_2$ receptor occupancy, the drugs had comparable effects on preproenkephalin-A mRNA levels. It was further found that 5-$HT_{2A/C}$ receptor blockade with ritanserin had only modest effects on preproenkephalin-B and preprotachykinin mRNA levels and did not affect preproenkephalin-A mRNA levels. We conclude that risperidone and haloperidol, administered in the same dose, differently affect the striatal output routes. Furthermore, the results suggest that the effects of risperidone on neuropeptide mRNA levels are fully accounted for by its $D_2$ antagonism and that no indication exists for a role of 5-$HT_{2A}$ receptor blockade in this action. **Synapse 28:302–312, 1998.** 

## INTRODUCTION

Antipsychotic drugs share a common feature in that they all block dopamine $D_2$ receptors to some extent. These drugs may have either a high (typical drugs) or a low (atypical drugs) tendency to induce extrapyramidal side effects (EPS). The EPS observed after typical antipsychotic drug treatment seem to be attenuated by serotonin 5-$HT_{2A/2C}$ antagonist treatment (Reyntjens et al., 1986; Bersani et al., 1990). This observation has led to the development of antipsychotic drugs, like risperidone (Risperdal®), that show predominant affinity for serotonin 5-$HT_{2A}$ receptors and lower affinity for dopamine $D_2$ receptors. Risperidone seems to act like an atypical drug, since its use results in a low incidence and severity of EPS (Borison et al., 1992; Claus et al., 1992; Chouinard et al., 1993).

In the striatum, treatment with typical antipsychotic drugs results in dramatically increased levels of preproenkephalin-A mRNA which encodes the neuropeptide enkephalin. On the other hand, mRNA levels of preproenkephalin-B and tachykinin, encoding, among others, the neuropeptides dynorphin and substance P, are decreased or remain largely unchanged after such treatments (for review see Augood et al., 1993). Dynorphin and substance P are colocalized in the majority of striatal neurons that project to the substantia nigra

*Correspondence to: Dr. P. Voorn, Department of Anatomy, Faculty of Medicine, Vrije Universiteit, v.d. Boechorststraat 7, 1081 BT Amsterdam, The Netherlands. Email: P.Voorn.Anat@med.vu.nl

Received 29 October 1996; accepted in revised form 16 September 1997.

(the so-called "direct" striatal output route), while enkephalin is present in the majority of striatal neurons projecting to the globus pallidus (the so-called "indirect" striatal output pathway) (Gerfen and Young, 1988; Anderson and Reiner, 1990). Possibly EPS arise as a result of a disturbed balance between the activity of the two output routes of the striatum (DeLong, 1990). If indeed the balance between the two striatal output pathways is essential for normal motor behavior, it may be hypothesized that the atypical drug risperidone affects the striatal output routes in a different manner than a typical drug, such as haloperidol, on the basis of their different EPS-inducing profile. This was investigated in the present study by determining changes in levels of mRNA encoding preproenkephalin-A, preproenkephalin-B, and tachykinin in the striatum with quantitative in situ hybridization. In order to shed light on the role of the 5-$HT_{2A}$ receptors in the effects of risperidone, we took a dual approach. First, we induced comparable dopamine $D_2$ receptor occupancy in vivo with risperidone and haloperidol in order to cancel out $D_2$-induced effects and focus on the 5-$HT_{2A}$ antagonist component of risperidone. Second, we investigated the effects of the 5-$HT_{2A/2C}$ antagonist ritanserin on neuropeptide mRNA levels. In view of the fact that the nucleus accumbens, in particular the shell, is regarded as a target site for antipsychotics (Deutch, 1993) and the caudate-putamen may be involved in EPS (Ellenbroek et al., 1985; Hauber and Schmidt, 1993; Merchant and Dorsa, 1993; Prinssen et al., 1995), we quantified changes in mRNA levels in several striatal (sub)regions.

## MATERIALS AND METHODS

### Animal treatment

Male Wistar rats (200 g) (Harlan, Zeist, The Netherlands) were maintained on a 12:12 h light/dark cycle with food and water ad libitum. In all experiments, drugs were administered orally through a canula that was brought into the esophagus for each administration. Drugs were administered for 14 days, twice daily, at 9 AM and 6 PM, in order to induce a chronic receptor blockade. On the fifteenth day, the animals were decapitated, and their brains were removed, rapidly frozen in isopentane (−40°C), and stored at −80°C. The experimental procedures have been approved by the animal care and use committee of the Medical Faculty of the Vrije Universiteit.

Haloperidol, risperidone, and ritanserin (all 1 mg/kg) were dissolved in acidified 2.5% ethanol (vehicle 1) and administered in a volume of approximately 0.2 ml. Animals were decapitated 16 h after the last administration. In a separate experiment, haloperidol (0.5 mg/kg) and risperidone (2.5 mg/kg) were dissolved in acidified aqua dest (vehicle 2) and administered in a volume of approximately 1 ml. Animals were decapitated 2 h after the last drug administration. Control groups received the appropriate vehicle (i.e., acidified 2.5% ethanol (vehicle 1) or acidified aqua dest [vehicle 2]). For all groups n = 6, except for aqua dest (n = 5).

For the generation of ex vivo dopamine $D_2$ and serotonin 5-$HT_{2A}$ receptor occupancy curves, male Wistar rats (200 g) (Janssen Research Foundation, Beerse, Belgium) received a single treatment (p.o.) with risperidone or haloperidol at seven dosages ranging from 0.01–40 mg/kg body weight (n = 6 per dosage). Saline-treated animals were used as controls (n = 6). Animals were sacrificed by decapitation 2 h after drug administration, and brains were treated as described above.

### In situ hybridization

Transverse cryostat sections of 14 µm were cut through the striatum and thaw-mounted on gelatin-coated slides. Sections were fixed, treated with acetic anhydride, dehydrated, defatted, and hybridized as described by Young (1990). $^{35}$S-UTP–labeled cRNA probes for preproenkephalin B (ppEnk-B), preproenkephalin-A (ppEnk-A), and preprotachykinin (ppT) were hybridized to the sections ($1.10^6$ cpm per section) in hybridization buffer (50% formamide, 4× SSC, pH 7.2, 500 µg/ml sheared single-stranded DNA, 250 µg/ml yeast tRNA, 1× Denhardt's (0.02% ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 10% dextran sulfate (MW = 500.000) and 100 mM dithiothreitol) overnight at 50°C under coverslips in a humid chamber. After removing the coverslips in 1× SSC, stringency washes were carried out as follows: 2 × 15 min in 2× SSC/50% formamide at 45°C, 1 × 25 min in RNAse A/2× SSC (Boehringer, Mannheim, Germany) at 37°C, and 3 × 15 min in 2× SSC/50% formamide at 45°C. Finally sections were rinsed with 2× SSC at room temperature.

PpEnk-B cDNA (Civelli et al., 1985) was subcloned into pBluescript KS+. PpEnk-A cDNA (Yoshikawa et al., 1984), cloned into pSP64 (pYSEA1), was kindly provided by Dr. S.L. Sabol (NIH, Bethesda, MD). β-ppT cDNA, subcloned into pGEM2 (Krause et al., 1987), was kindly provided by Dr. J.E. Krause (Washington University, St. Louis, MO). Polymerase chain reaction (PCR) fragments of full-length cDNA were generated using primers for the RNA polymerase promotor sites flanking the cDNA sequences or for plasmid sequences flanking the RNA polymerase promotor regions. The PCR fragments were used as templates in the in vitro transcription procedure to synthesize cRNA. In vitro transcription was carried out according to the protocol provided by Promega (Madison, WI) the manufacturer of the RNA polymerases used. In short, the particular cDNA fragment was incubated with RNA polymerase and $^{35}$S-UTP, ATP, CTP, GTP in a reaction mixture consisting of transcription buffer, dithiothreitol, and RNAsin for 2 h at 37°C. After in vitro transcription, the DNA template was eliminated with RNAse-free DNAse

(10 min at 37°C, 2U per 20 µl assay), and the RNA probe was hydrolyzed in order to increase penetration of RNA fragments into the tissue (0.09 M NaCl, 0.24 N NaOH, and 0.01 M dithiothreitol in ETS buffer for 30 min (ppEnk-A) or 45 min (ppEnk-B) at 0°C).

Method specificity was controlled for by performing hybridization with sense cRNA probe and hybridization with antisense probe to RNAse-treated tissue sections. No hybridization signal was detected in these experiments.

After hybridization, sections were exposed to X-ray–sensitive film (Amersham Hyperfilm MP, Amersham, Buckinghamshire, UK) (exposure time 1 day for ppEnk-A, 2 days for ppT and 3 days for ppEnk-B), developed in Kodak (Rochester, NY) D-19 and fixed in Kodak Rapidfix.

### Method of analysis and quantification of in situ hybridization results

An IBAS image analysis system (Kontron, Germany) was used for densitometrical quantification of the results. Eight to ten sections were examined per animal, taken with an interspace of approximately 150 µm and spanning the entire rostrocaudal extent of the nucleus accumbens (from 2.20 mm to 0.70 mm anterior to bregma) (Paxinos and Watson, 1986). In each section, the caudate-putamen and the core and shell of the nucleus accumbens were interactively delineated as illustrated in Figure 1, and pixel gray levels were measured in the outlined areas. For each pixel, these values were converted to radioactivity/milligram of tissue using calibration data from coexposed, $^{35}$S-cross-calibrated, $^{14}$C standards (ARC, St. Louis, MO) and subsequently averaged over the measured area. Specific labeling was calculated by subtracting background radioactivity levels (measured per section over the corpus callosum) from striatal radioactivity values.

For all treatment groups, mean values of bound radioactivity/milligram of tissue were calculated for each section (i.e. each rostrocaudal level) per subregion. A covariance test was used to test for drug effects, with rostrocaudal level as a covariate. In addition, independent *t*-tests were performed at each individual rostrocaudal level in order to specify the exact rostrocaudal levels where the drug effects occurred. The required assumptions for parametric tests were tested in the following way. A Kolmogorov-Smirnov goodness of fit test was used to test whether the data of each treatment group followed a normal distribution, and a Levene's test for equality of variances was performed to test the equality of variances between the different treatment groups. The requirements for parametric tests were fulfilled in all cases except for the ppEnk-A–risperidone groups (both 1 mg/kg and 2.5 mg/kg) and the ppEnk-A–haloperidol groups (both 1 mg/kg and 0.5 mg/kg). In the latter cases, instead of a covariance test and *t*-tests, nonparametric Mann-Whitney U tests were performed on all rostrocaudal levels grouped together as well as on individual rostrocaudal levels.

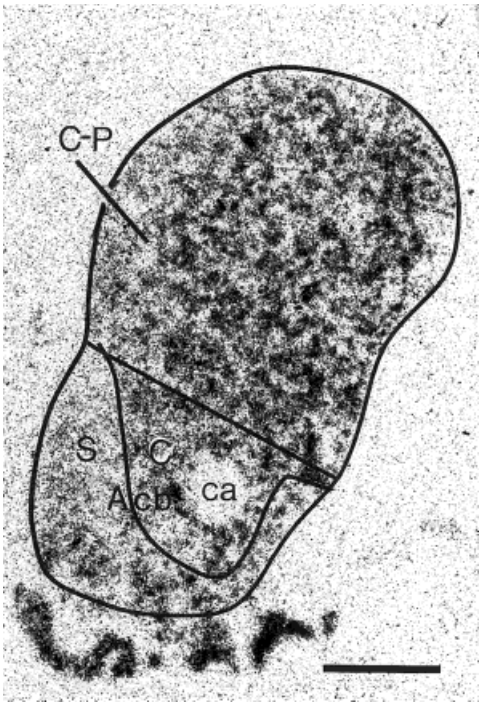


Fig. 1. Subdivisions of the striatum. Transversal section of the striatum, hybridized with ppEnk-A cRNA, illustrating the definition of the borders of caudate-putamen (C-P) and shell (S) and core (C) of the nucleus accumbens (Acb). Anterior commissure (ca). Scale bar is 1 mm.

Finally, in order to test whether the drug effects were dependent on the rostrocaudal level, the drug effects in each rostrocaudal level were calculated as percentage of control values, and subsequently a Spearman's rank correlation test was applied.

### Ex vivo autoradiography

For the generation of $D_2$ and 5-$HT_{2A}$ occupancy curves, ex vivo autoradiography techniques were applied as previously reported (Schotte et al., 1996). Briefly, transverse 20 mm thick frontal sections were cut through the striatum (1.20 µm anterior to bregma) (Paxinos and Watson, 1986) and frontal cortex (3.70 mm anterior to bregma) and thaw-mounted on adhesive microscope slides (Star Frost, Knittel Gläser, Germany). The sections were kept at −20°C until use. For the ex vivo receptor binding after chronic antipsychotic treatment, transverse cryostat sections of 14 mm were cut through the striatum (level 1.20 mm anterior to bregma) (Paxinos and Watson, 1986) and thaw-mounted on gelatin-coated slides. Sections were kept at −80°C until use.

On the day of the experiment, sections were thawed and dried at room temperature for about 30 min, and radioligand solution (200 µl) was applied on each section. Incubation was restricted to 10 min at room

temperature in order to minimize dissociation of the drug from the receptor. Brain sections of drug-treated and saline-treated animals were incubated in parallel. After the incubation, the brain sections were rinsed for $2 \times 2$ min in TRIS-HCl (50 mM, pH 7.4) at 4°C followed by a quick dip in $H_2O$). The sections were then dried under a cold air stream and apposed to an Ektascan GRL film (Kodak) in a light-tight cassette. After an exposure time of 1 week, the films were developed in a Kodak X-Omat processor. Radioligands include [$^{125}$I]-7-amino-8-iodo-ketanserin at 0.1 mM for the 5-$HT_{2A}$ receptor and [$^{125}$I] iodosulpiride at 0.2 mM for the $D_2$ receptor; specific activity for both ligands was 2,000 Ci/mmol, and both ligands were obtained from Amersham.

Autoradiograms from receptor-binding experiments were quantified using an M1-MCID image analyzer (Imaging Research, St-Catharine's, Ontario, Canada). Optical densities were transformed to levels of bound radioactivity after calibration of the image analyzer using optical densities generated by coexposed commercially available polymer standards ([$^{125}$I]Micro-scales; Amersham). Specific binding was given as the difference between total binding and nonspecific binding measured in adjacent sections. Nonspecific binding to 5-$HT_{2A}$ and $D_2$ receptors was determined with BW501 (1 μM) and domperidone (1 μM), respectively. Receptor labeling in drug-treated animals was expressed as the percentage of labeling measured in corresponding sections of saline-treated animals. Percent drug-induced receptor occupancies (=100% minus the percent receptor labeling) were plotted vs. dosage. Each individual value was the mean of measurements in three sections of the same rat. The sigmoid log dose-effect curve of best fit was calculated by nonlinear regression analysis, using a computer program modified from Oestreicher and Pinto (1987). Differences in percentage occupancy between risperidone and haloperidol were tested with independent *t*-test, and differences between caudate-putamen and nucleus accumbens were tested with paired *t*-test. Data were log-transformed before statistical testing.

## RESULTS

Ex vivo receptor binding was used for risperidone and haloperidol to select doses for chronic treatment at which similar or dissimilar dopamine $D_2$ receptor occupancy would be attained.

### $D_2$ and 5-$HT_{2A}$ receptor binding

$D_2$ receptor occupancy was determined in the caudate-putamen and nucleus accumbens after single oral administration of the drugs. Figure 2 shows the curve for $D_2$ occupancy by risperidone and haloperidol in the caudate-putamen. A similar curve was found for the nucleus accumbens (data not shown). On the basis of these curves, we selected for a first experiment doses of 1 mg/kg for both drugs, at which $D_2$ receptor binding for haloperidol was 66%, whereas risperidone resulted in 18% $D_2$ occupancy (Fig. 2). In a separate experiment, we used doses of 0.5 mg/kg haloperidol and 2.5 mg/kg risperidone which, according to the curves shown in Figure 2, should result in a similar, moderate degree of approximately 40% $D_2$ occupancy. Higher doses of risperidone that would allow higher $D_2$ occupancy were avoided because other receptor types might become involved and because 5-$HT_{2A}$ receptor occupancy at 2.5 mg/kg is already maximal (Fig. 2). In contrast, 5-$HT_{2A}$ occupancy is low after haloperidol at 0.5 and 1 mg/kg (i.e., 17% and 22%, respectively) (Fig. 2). Finally, ritanserin at 1 mg/kg can be expected to induce supramaximal 5-$HT_2$ occupancy (Leysen et al., 1985).

Animals received chronic oral treatment with haloperidol (0.5 and 1 mg/kg), risperidone (1 and 2.5 mg/kg), and ritanserin (1 mg/kg) and probe hybridization levels were determined (see below). In addition, post hoc assessment of $D_2$ and 5-$HT_{2A}$ receptor occupancy was carried out after chronic treatment of animals with 0.5 mg/kg haloperidol and 2.5 mg/kg risperidone. After chronic treatment, $D_2$ occupancy appeared to be somewhat different compared to treatment with a single dose. In the caudate-putamen (C-P), on average 47% of the $D_2$ receptors were occupied by haloperidol, while risperidone gave rise to a significantly lower $D_2$ occupancy of 31% (Table I). In the nucleus accumbens, 48% of the $D_2$ receptors were occupied by haloperidol and 41% by risperidone (Table I). With regard to the 5-$HT_{2A}$ receptors, chronic treatment with risperidone (2.5 mg/kg) resulted in 89% occupancy, whereas haloperidol (0.5 mg/kg) induced 18% occupancy (Table I).

### Effects of haloperidol, risperidone and ritanserin on neuropeptide hybridization signal

Both risperidone (1 mg/kg) and haloperidol (1 mg/kg) induced an increase in striatal ppEnk-A hybridization signal, as illustrated in Figures 3 and 4. However, the magnitude of the effect was more dramatic after haloperidol (e.g., 71% average increase in C-P) than after risperidone treatment (e.g., 14% average increase in the C-P) (Table II). The PpEnk-B and ppT hybridization signal was also differently affected by the two antipsychotic drugs. Whereas the hybridization signal was unchanged after risperidone, treatment with haloperidol resulted in increased ppEnk-B levels (10% average in C-P) and minor changes in ppT hybridization signal (Table II). There was a tendency for a decrease of ppT hybridization signal in the caudate-putamen and for an increase in the nucleus accumbens core and shell (see Table II). The 5-$HT_{2A/2C}$ antagonist ritanserin (1 mg/kg) did not affect ppEnk-A hybridization signal (Figs. 3, 4)

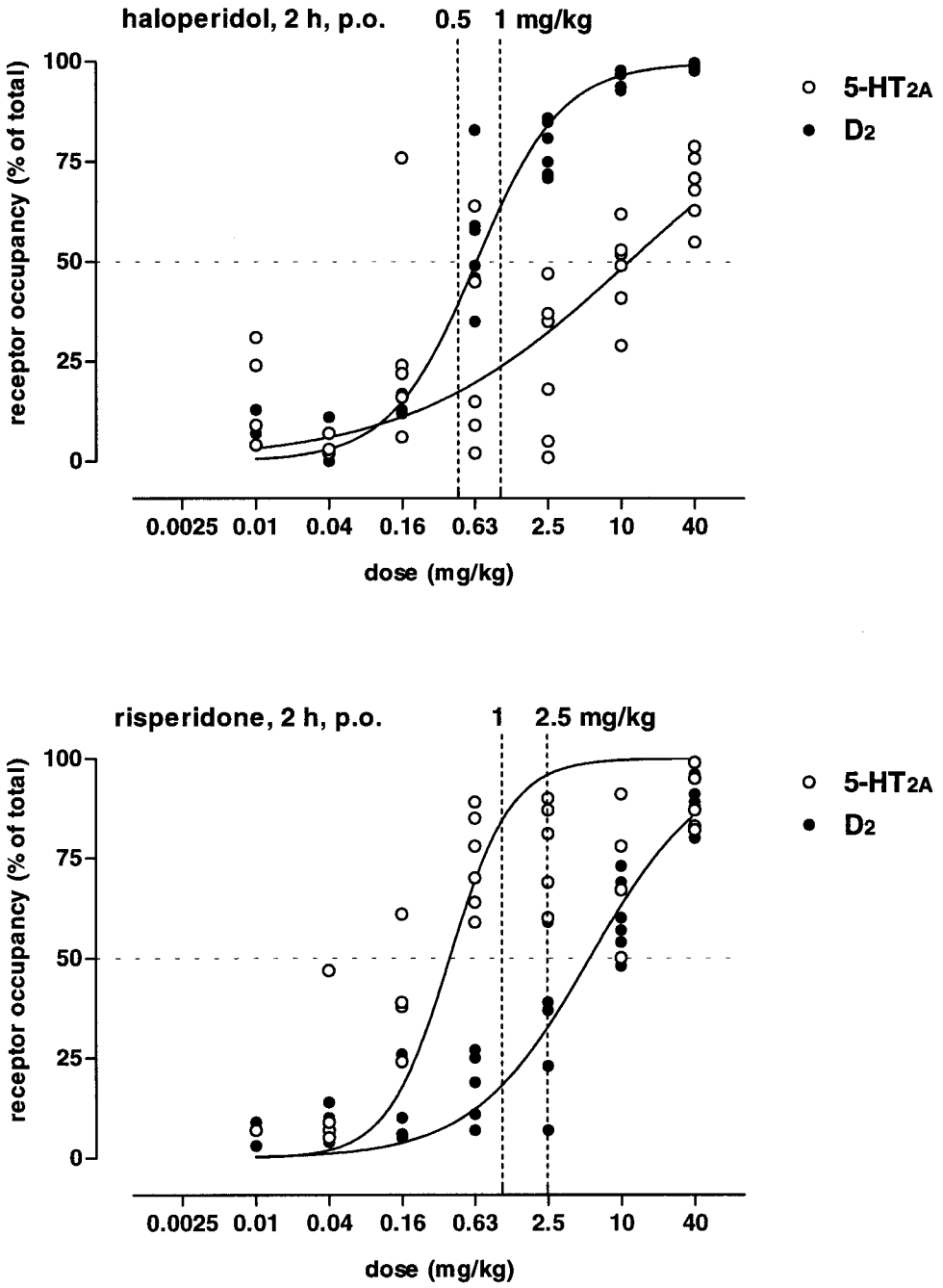


Fig. 2. $D_2$ and 5-$HT_{2A}$ receptor occupancy after a single administration of risperidone and haloperidol. Receptor occupancy was measured ex vivo in the rat caudate-putamen ($D_2$) and frontal cortex (5-$HT_2$) by autoradiography. Animals received a single oral administration with haloperidol or risperidone and were decapitated 2 h later. Values from individual animals are shown as well as the derived curves calculated by computerized analysis of the data as described in Materials and Methods. Dashed lines indicate $D_2$ and 5-$HT_2$ occupancies at doses of risperidone and haloperidol selected for chronic treatment.

but did increase the ppEnk-B hybridization signal (on average 10% in C-P) and had a tendency to increase the ppT hybridization signal (Table II).

Similar to the equal-dosage experiment described above, 0.5 mg/kg of haloperidol and 2.5 mg/kg of risperidone appeared to have major effects on ppEnk-A and minor effects on ppEnk-B and ppT mRNA (Table III). Both antipsychotic drugs increased the ppEnk-A hybridization signal. However, the changes were of similar magnitude (e.g., on average 57% for risperidone and 67% for haloperidol in C-P) (Table III; Figs. 3, 4). Levels of the ppT hybridization signal were slightly decreased by both drugs, similar to the 1 mg/kg experiment. Finally, the ppEnk-B hybridization signal was

*TABLE I. $D_2$ and 5-$HT_{2A}$ receptor occupancy after chronic treatment with haloperidol and risperidone*[1]

| | Haloperidol (0.5 mg/kg) | | | Risperidone (2.5 mg/kg) | | |
|---|---|---|---|---|---|---|
| | C-P | Accumbens | Cortex | C-P | Accumbens | Cortex |
| $D_2$ | 47% ± 4.1 | 48% ± 3.7 | | 31% ± 2.4* | 41% ± 3.7** | |
| 5-$HT_{2A}$ | | | 18% ± 5.3 | | | 89% ± 4.5* |

[1]Animals (n = 6 per group) received oral drug treatment for 14 days and were decapitated 2 h after the last treatment. Values are expressed as percentages of drug-induced receptor occupancies (=100% minus the percent receptor labeling) measured in saline-treated animals ± S.E.M. Data were log-transformed before applying *t*-tests. The data in this table can be directly related to the in situ hybridization results in Table III. *A significant difference in binding between the two drugs, as tested with an independent *t*-test on the means per animal ($P < 0.05$). **A significant difference between nucleus accumbens and caudate-putamen, as tested with a paired *t*-test on the means per animal ($P < 0.05$).

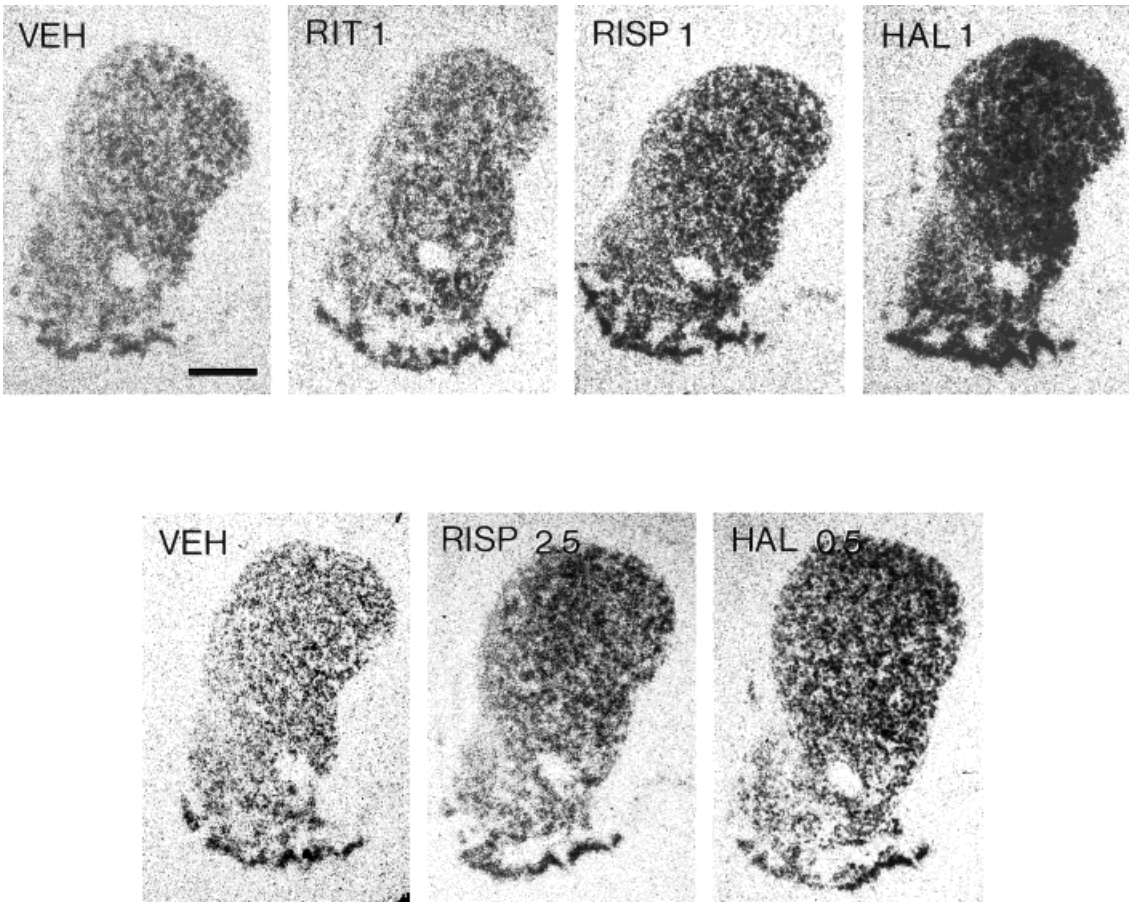


Fig. 3. PpEnk-A hybridization pattern after oral treatment with haloperidol, risperidone, and ritanserin. Photographs are shown of transverse sections of the striatum hybridized with ppEnk-A cRNA. Photographs in the first row represent striata from animals chronically treated with vehicle 1 (VEH), ritanserin 1 mg/kg (RIT 1), risperidone 1 mg/kg (RISP 1) and haloperidol 1 mg/kg (HAL 1). The pictures show that ppEnk-A hybridization signal is not affected by ritanserin, increased by risperidone and more increased by haloperidol. Photographs in the second row represent striata from animals treated chronically with vehicle 2 (VEH), risperidone 2.5 mg/kg (RISP 2.5), and haloperidol 0.5 mg/kg (HAL 0.5), illustrating that risperidone and haloperidol increase ppEnk-A hybridization signal in a similar fashion. NB: The first row cannot be directly compared to the second row since the data are derived from different hybridization experiments with different survival times after last drug administration. Magnification bar is 1 mm and is valid for all pictures.

slightly increased after risperidone treatment, whereas it was slightly decreased in the haloperidol-treated group, accounting for a significant difference between haloperidol and risperidone (Table III).

It appeared that the effects of risperidone and haloperidol on the ppEnk-A hybridization signal were most pronounced in the caudate-putamen, less prominent in the nucleus accumbens core, and least remarkable in the nucleus accumbens shell in all experiments (Tables II, III; Fig. 4). In the nucleus accumbens, the effect on ppEnk-A mRNA was differentiated along the rostrocaudal axis, with proportionally larger effects rostrally in the core and shell than caudally (see Fig. 4). As indicated by Spearman's rank correlation tests, the effects of both doses of haloperidol and the highest dose of risperidone were significantly dependent on the

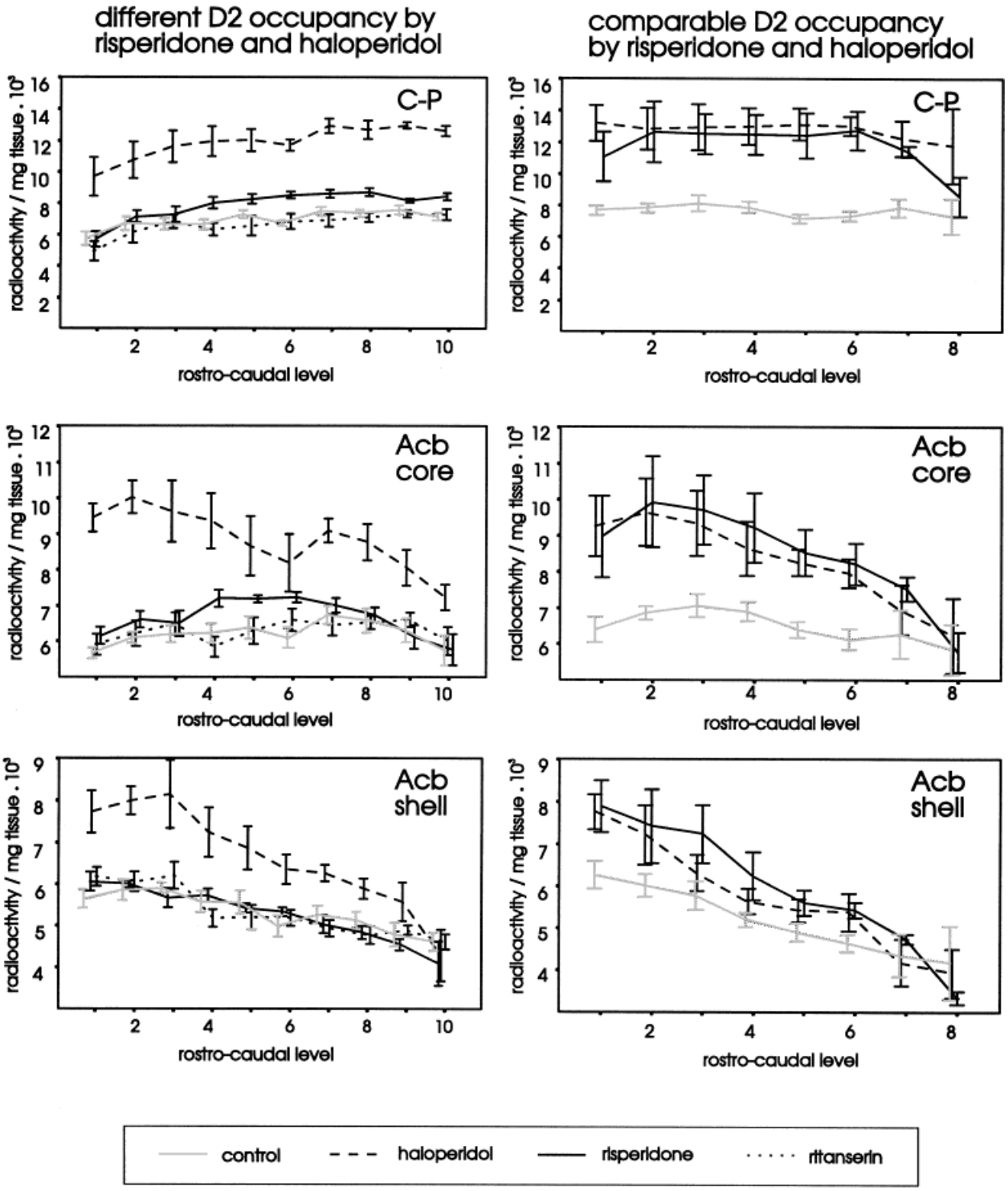


Fig. 4. Rostrocaudal differences in ppEnk-A hybridization signal levels in striatal regions after oral treatment with haloperidol, risperidone, and ritanserin. The amount of hybridized ppEnk-A cRNA probe is represented as mean radioactivity/milligram of tissue $\times 10^3$ per rostrocaudal level $\pm$ S.E.M. Each line represents the mean value of six animals (n = 5 for the control group in graphs in first column). Different treatments are indicated by different lines. Eight to ten sections were examined per animal, spanning the entire rostrocaudal extent of the nucleus accumbens (from 2.20 mm to 0.70 mm anterior to bregma). Graphs in the first column show the results of 1 mg/kg doses of haloperidol, risperidone, and ritanserin in the caudate-putamen (C-P), core (Acb core), and shell (Acb shell) of the nucleus accumbens, whereas graphs in the second column show results of 0.5 mg/kg haloperidol and 2.5 mg/kg risperidone in similar brain areas. A significant difference in bound radioactivity levels was demonstrated between haloperidol and risperidone in the first column over the entire rostrocaudal extent, whereas no significant difference was shown between the two antipsychotic drugs in the second column, as tested with Mann-Whitney U tests on the means of individual rostrocaudal levels. NB: Absolute values of radioactivity can be directly compared only within experiments (i.e. within columns and not between experiments (i.e., between columns) since the data are derived from different hybridization experiments with different survival times after last drug administration. Changes in bound radioactivity averaged over all rostrocaudal levels are indicated in Tables II and III. To test whether a rostrocaudal high-to-low gradient in the increase of ppEnk-A mRNA was present in the nucleus accumbens after both doses of haloperidol and the highest dose of risperidone, we calculated the drug effects as percentage of controls (not shown) and performed Spearman's rank correlation tests. The tests indicated that the effects of both doses of haloperidol and the highest dose of risperidone were significantly dependent on the rostrocaudal level ($P < 0.05$) in the nucleus accumbens core and shell.

*TABLE II. Bound radioactivity of neuropeptide cRNA probes in striatal regions after oral treatment with haloperidol (1 mg/kg), risperidone (1 mg/kg), and ritanserin (1 mg/kg)*[1]

| | ppEnk-A | ppEnk-B | ppT |
|---|---|---|---|
| Vehicle 1 | | | |
| C-P | 69.4 ± 3.7 | 12.1 ± 1.0 | 18.4 ± 0.8 |
| Core | 62.0 ± 2.9 | 18.9 ± 1.3 | 15.5 ± 1.3 |
| Shell | 53.2 ± 2.8 | 19.4 ± 1.5 | 19.9 ± 1.1 |
| Haloperido *l (1 mg/kg)* | | | |
| C-P | 118.8 ± 7.9***,**** all levels +71% | 13.3 ± 1.0*,** caudal +10% | 17.7 ± 0.3*,** caudal −4% |
| Core | 88.7 ± 6.3***,**** all levels +43% | 2.2 ± 1.1*,** caudal +12% | 16.2 ± 1.1* caudal +5% |
| Shell | 66.7 ± 6.2***,**** all levels +25% | 21.0 ± 1.1*,** caudal +8% | 21 ± 0.8*,** caudal +5% |
| Risperidone (1 mg/kg) | | | |
| C-P | 79.4 ± 4.6***,**** intermediate and caudal +14% | 12.0 ± 0.8 −1% | 18.5 ± 0.6 +1% |
| Core | 66.8 ± 3.1***,*** intermediate +8% | 19.0 ± 0.9 +1% | 15.0 ± 1.3 −3% |
| Shell | 52.7 ± 3.1 −1% | 18.7 ± 1.1 −4% | 19.7 ± 1.2 −1% |
| Ritanserin (1 mg/kg) | | | |
| C-P | 66.1 ± 4.9 −5% | 13.4 ± 1.1*,** caudal +10% | 19.2 ± 0.8* +5% |
| Core | 62.9 ± 2.8 +1% | 19.9 ± 1.3* +5% | 15.5 ± 1.2 +1% |
| Shell | 53.4 ± 3.1 +0% | 19.9 ± 1.3 +3% | 19.8 ± 1.1 −1% |

[1]Animals received drug treatment for 14 days and were decapitated 16 h after the last treatment. Values represent the mean bound radioactivity/milligram of tissue × $10^3$ ± S.E.M. of six animals (n = 5 in the control group), averaged over ten rostrocaudal levels per animal. In addition the % change compared to control values is indicated. Levels 1–3 were considered as rostral, 4–6 as intermediate, and 7 and 8 as caudal regions. The changes in ppEnk-A along the entire rostrocaudal axis are indicated in Fig. 3A–C. *Significant difference from control levels ($P < 0.05$), as tested with covariance analysis. **Significant difference from control levels ($P < 0.05$), tested with *t*-test per individual rostrocaudal level. ***Significant difference from controls, ($P < 0.05$), tested with Mann-Whitney U test on all rostrocaudal levels grouped together. ****Significant difference from controls, as tested with Mann-Whitney U per rostrocaudal level ($P < 0.05$).

*TABLE III. Bound radioactivity of neuropeptide cRNA probes in striatal regions after oral treatment with haloperidol (0.5 mg/kg) and risperidone (2.5 mg/kg)*[1]

| | ppEnk-A | ppEnk-B | ppT |
|---|---|---|---|
| Vehicle 2 | | | |
| C-P | 77.0 ± 3.8 | 13.8 ± 0.9 | 22.7 ± 0.6 |
| Core | 65.4 ± 3.6 | 20.3 ± 1.2 | 18.7 ± 1.8 |
| Shell | 52.5 ± 4.0 | 20.0 ± 1.3 | 22.9 ± 1.6 |
| Haloperidol (0.5 mg/kg) | | | |
| C-P | 128.5 ± 10.7***,*** all levels +67% | 12.8 ± 0.5*,** rostral −7% | 21.4 ± 0.6*,** rostral −5% |
| Core | 84.6 ± 7.8***,*** rostral and intermediate +29% | 20.6 ± 0.9 +1% | 17.7 ± 1.8*,** causal −6% |
| Shell | 58.9 ± 6.4*** +12% | 20.4 ± 1.0 +2% | 22.5 ± 1.7 −2% |
| Risperidone (2.5 mg/kg) | | | |
| C-P | 120.0 ± 12.6***,**** all levels +57% | 14.6 ± 0.6[2]* all levels +6% | 21.7 ± 0.7* −3% |
| Core | 87.3 ± 8.8***,**** rostral and intermediate +34% | 22.1 ± 1.1*,** intermediate +9% | 18.8 ± 1.7 +1% |
| Shell | 62.3 ± 6.9*** +19% | 21.7 ± 1.1* +9% | 23.2 ± 1.3 +1% |

[1]Animals received drug treatment for 14 days and were decapitated 2 h after the last treatment. Values represent the mean bound radioactivity/milligram of tissue × $10^3$ ± S.E.M. of six animals, averaged over ten rostrocaudal levels per animal. Levels 1, 2, 3 were considered as rostral, 4, 5, 6 as intermediate and 7, 8 as caudal regions. The changes in ppEnk-A along the entire rostrocaudal axis are indicated in Fig. 4D–F. [2]Significant difference between haloperidol and risperidone (indepedent *t*-test per (level). *Significant difference from control levels ($P < 0.05$), as tested with covariance analysis. **Significant difference from control levels ($P < 0.05$), tested with *t*-test per individual rostrocaudal level. ***Significant difference from controls, ($P < 0.05$) tested with Mann-Whitney U test on all rostrocaudal levels grouped together. ****Significant difference from controls, as tested with Mann-Whitney U per rostrocaudal level ($P < 0.05$).

rostrocaudal level in the nucleus accumbens core and shell.

Unlike ppEnk-A, changes in the ppEnk-B and ppT hybridization signal did not show a differentiated distribution over the striatal regions. Alterations in the ppEnk-B and ppT hybridization signal in the nucleus accumbens were in a number of cases most pronounced at caudal or intermediate levels (Tables II, III).

## DISCUSSION

### Comparison of the actions of risperidone and haloperidol on the striatal output pathways

In the present study, the receptor occupancy measured after chronic drug administration was different in the caudate-putamen for both drugs, viz. 31% after risperidone and 47% after haloperidol. This difference notwithstanding, the present data indicate a $D_2$ receptor occupancy that lies within the same range on a scale from weak to moderate to strong. The comparable, moderate $D_2$ receptor blockade by risperidone and haloperidol allows comparison of the effects of the two drugs on striatal mRNA levels.

The results of this study show that both the typical neuroleptic haloperidol and the atypical neuroleptic risperidone have major effects on preproenkephalin-A mRNA and thus on the indirect striatal output pathway, whereas they have minor effects on preproenkephalin-B and preprotachykinin mRNA, contained in the direct striatal output pathway. When the two drugs were administered in the same dose of 1 mg/kg, they affected the two striatal output routes in a different manner, since haloperidol caused a much higher elevation of ppEnk-A mRNA than did risperidone. However, when the dose of risperidone was increased and that of haloperidol was decreased to attain comparable $D_2$ receptor occupancy, the drugs had comparable effects on ppEnk-A mRNA levels and ppEnk-B/ppT mRNA levels. It was further demonstrated that 5-$HT_{2A/2C}$ blockade with ritanserin had modest effects on ppEnk-B and ppT mRNA levels but did not affect ppEnk-A mRNA levels.

Our finding that both antipsychotic drugs affect striatal preproenkephalin-A mRNA is in line with previous studies demonstrating that haloperidol and a wide range of other antipsychotics induce increases of ppEnk-A mRNA and peptide levels of varying magnitudes (Hong et al., 1980; Tang et al., 1983; Angulo et al., 1990b; Houdi and Van Loon, 1990; Herman et al., 1991).

In contrast to the major effects of risperidone and haloperidol on ppEnk-A mRNA, only minor changes in ppEnk-B (increase) and ppT mRNA levels (decrease) were observed, which is consistent with earlier reports on haloperidol (Quirion et al., 1985, Bannon et al., 1987; Li et al., 1987; Angulo et al., 1990a; Humpel et al., 1990; Shibata et al., 1990; however, see also Li et al., 1986; Morris et al., 1988a; Trujillo et al., 1990). The changes previously reported for ppT mRNA levels were more pronounced than those we observed. Possibly the present treatment schedule with oral administration accounts for the difference in magnitude of the response.

### Regional differentiation of risperidone and haloperidol effects

We observed that the effects of risperidone and haloperidol on ppEnk-A mRNA levels were always most prominent in the caudate-putamen and least pronounced in the shell of nucleus accumbens. A similar difference between the nucleus accumbens and caudate-putamen in the magnitude of the adaptive response of the enkephalinergic system to antipsychotics has previously been reported by Hong et al. (1978) and Angulo et al. (1990b). The observed smaller effect in nucleus accumbens is not necessarily due to lower occupation of dopamine $D_2$ receptors since $D_2$ occupancy was found to be equal, or even higher in the case of risperidone, compared to the caudate-putamen. However, $D_2$ receptor density in the nucleus accumbens is lower than in the caudate-putamen (Bardo and Hammer, 1991). Furthermore, the pharmacological properties of $D_2$ receptors in the nucleus accumbens are different from those in the caudate-putamen (Stoof et al., 1987).

In the present experiments, a rostrocaudal high-to-low gradient in the increase of ppEnk-A mRNA was observed in the nucleus accumbens after both doses of haloperidol and the highest dose of risperidone. Voorn et al. (1994) demonstrated a similar rostrocaudal gradient in the increase of ppEnk-A mRNA after a midbrain 6-hydroxydopamine lesion. The similarity of the effects of a dopamine denervation and $D_2$ receptor blockade by antipsychotics is probably explained by the fact that changes in ppEnk-A synthesis are mainly mediated by the $D_2$ receptor and that $D_2$ receptor binding follows a similar high-to-low rostrocaudal gradient in the nucleus accumbens (Voorn et al., 1994). Our findings are in contrast with those of Caboche et al. (1993) who observed a low-to-high gradient in the effect of haloperidol on ppEnk-A mRNA levels in the nucleus accumbens. However, it is not clear from the latter study which rostrocaudal levels of the nucleus accumbens were measured.

### Receptors involved in effects of risperidone on striatal mRNA levels

A major finding of the present study is that risperidone and haloperidol, in dosages inducing different serotonin 5-$HT_{2A}$ but comparable dopamine $D_2$ receptor occupancy in vivo, have similar effects on ppEnk-A mRNA levels. This by itself suggests that the effects of risperidone on ppEnk-A are fully accounted for by its $D_2$ antagonism and that there is no clear indication for a role of the 5-$HT_{2A}$ receptor in modulating this action. Our results on the 5-$HT_{2A/2C}$ receptor antagonist ritanserin support this viewpoint since no changes in ppEnk-A mRNA levels in caudate-putamen or nucleus accumbens were observed after ritanserin. The latter finding is in agreement with results on cinanserin, another 5-$HT_2$ antagonist (Angulo et al., 1990b). In addition, the serotonin reuptake blocker fenfluramine has been shown not to affect striatal ppEnk-A mRNA levels (Mocchetti et al., 1985). The possibility arises that striatal ppEnk-A mRNA levels are not regulated by the 5-$HT_{2A/2C}$ receptor or maybe not by serotonin at all. Furthermore, our results with risperidone do not indicate the presence of complex interactions between 5-$HT_{2A}$ and $D_2$ receptors during simultaneous blockade, such as potentiation or counteraction of the effect on ppEnk-A mRNA transcription.

With respect to ppEnk-B, a slight increase in mRNA levels was observed after ritanserin treatment, but changes in ppT mRNA were rather insignificant. After a lesion of the serotonergic system with 5,7-DHT, Morris et al. (1988b) found a decrease of ppEnk-B mRNA levels. The present data suggest that 5-HT receptors different from the 5-$HT_{2A/2C}$ subtype account for the lesion-induced effect. Similar to ppEnk-A, our experiments with risperidone give no evidence for any complex interactions at the level of 5-$HT_{2A}$ and $D_2$ receptor blockade. In conclusion, 5-$HT_{2A}$ receptor blockade does not appear to counteract the $D_2$-induced disturbance of the balance between striatal output pathways by either attenuating the increase in ppEnk-A mRNA or by increasing ppEnk-B and ppT mRNA levels.

### Disturbed balance in striatal output routes and extrapyramidal side effects of antipsychotic drugs

The present results suggest that haloperidol treatment leads to a profound disturbance of the balance between the direct and the indirect striatal output pathways that has been proposed to be essential for normal motor function (DeLong, 1990). Treatment with risperidone at the same dose leads to a smaller imbalance between the two pathways since the activation of the indirect pathway is minor compared to that after haloperidol. The suggestion of a milder balance disturbance by atypical vs. typical antipsychotics is supported by data on the atypical drug clozapine. This drug reportedly does not affect or increases the peptides and their mRNA levels in the direct striatal output pathway (Nylander and Terenius, 1986; Angulo et al., 1990a; Humpel et al., 1990) and increases ppEnk-A mRNA levels in the indirect pathway. However, like risperi-

done in the present experiments, it appears less potent in elevating ppEnk-A mRNA than typical antipsychotics (Hong et al., 1980; Angulo et al., 1990b). The data on clozapine and the present data on risperidone suggest that a lesser degree of balance disturbance is due mainly to relatively small effects on ppEnk-A. Thus, these data suggest a possible role for ppEnk-A in the extrapyramidal side effects (EPS) of antipsychotic drugs. The observation that antipsychotic-induced ppEnk-A mRNA elevations are more pronounced in the caudate-putamen than in nucleus accumbens is congruent with this idea (Hong et al., 1978; Angulo et al., 1990b; present study).

Extrapyramidal side effects after treatment with antipsychotic drugs can occur acutely, such as parkinsonism, or be of late onset, such as dyskinesias. Several studies on primate and rat models of Parkinson's disease have found an increase of preproenkephalin-A mRNA or peptide in the striatum and are thus supportive of a role of enkephalin in parkinsonian symptoms (Young et al., 1986; Voorn et al., 1987; Dacko and Schneider, 1991; Gerfen et al., 1991; Roeling et al., 1995). In addition, a role for enkephalin in dyskinesias can also be suggested from the results of several studies (Lindenmayer et al., 1988; Stoessl et al., 1993; Egan et al., 1994; however, see also Egan et al., 1995).

Substantial evidence suggests that blockade of 5-$HT_2$ receptors protects to some degree against EPS in humans and $D_2$-induced catalepsy in laboratory animals (Reyntjens et al., 1986; Meltzer et al., 1989; Bersani et al., 1990; Leysen et al., 1993; Schotte et al., 1996). If enkephalin plays a role in EPS, then the present results, which do not provide evidence for a role of 5-$HT_{2A/2C}$ blockade in ppEnk-A regulation, suggest that the 5-$HT_{2A}$ antagonistic component of risperidone is establishing risperidone's low EPS profile by a mechanism different from modulating striatal ppEnk-A mRNA levels. However, a role for enkephalin cannot be excluded since 5-$HT_2$ blockade may exert its influence at the level of peptide metabolism and not at the level of gene transcription (see Mocchetti et al., 1985).

## ACKNOWLEDGMENTS

We thank P.F.M. Janssen, P. te Riele, and C. Wintmolders for performing the receptor-binding experiments and analyses and D. de Jong for photographic assistance. We are grateful to Profs. J.E. Leysen and H.J. Groenewegen for valuable discussions and comments.

## REFERENCES

Anderson, K.D., and Reiner, A. (1990) Extensive co-occurrence of substance P and dynorphin in striatal projection neurons: An evolutionary conserved feature of basal ganglia organization. J. Comp. Neurol. 295:339–369.

Angulo, J.A., Cadet, J.L., and McEwen, B.S. (1990a) Effect of typical and atypical neuroleptic treatment on protachykinin mRNA levels in the striatum of the rat. Neurosci. Lett., 113:217–221.

Angulo, J.A., Cadet, J.L., Woolley, C.S., Suber, F., and McEwen, B.S. (1990b) Effect of chronic typical and atypical neuroleptic treatment on proenkephalin mRNA levels in the striatum and nucleus accumbens of the rat. J. Neurochem. 54:1889–1894.

Augood, S.J., Westmore, K., Faull, R.L., and Emson, P.C. (1993) Neuroleptics and striatal neuropeptide gene expression. Prog. Brain Res. 99:181–199.

Bannon, M.J., Elliott, P.J., and Bunney, E.B. (1987) Striatal tachykinin biosynthesis: Regulation of mRNA and peptide levels by dopamine agonists and antagonists. Brain Res. 427:31–37.

Bardo, M.T., and Hammer, R.P., Jr. (1991) Autoradiographic localization of dopamine D1 and D2 receptors in rat nucleus accumbens: Resistance to differential rearing conditions. Neuroscience, 45:281–290.

Bersani, G., Grispini, A., Marini, S., Pasini, A., Valducci, M., and Ciani, N. (1990) 5-HT2 antagonist ritanserin in neuroleptic-induced parkinsonism: A double-blind comparison with orphenadrine and placebo. Clin. Neuropharmacol., 13:500–506.

Borison, R.L., Pathiraja, A.P., Diamond, B.L., and Meibach, R.C. (1992) Risperidone: Clinical safety and efficacy in schizophrenia. Psychopharmacol. Bull. 28:213–218.

Caboche, J., Vernier, P., Rogard, M., and Besson, M.J. (1993) Haloperidol increases PPE mRNA levels in the caudal part of the nucleus accumbens in the rat. Neuroreport, 4:551–554.

Chouinard, G., Jones, B., Remington, G., Bloom, D., Addington, D., MacEwan, G.W., Labelle, A., Beauclair, L., and Arnott, W. (1993) A Canadian multicenter placebo-controlled study of fixed doses of risperidone and haloperidol in the treatment of chronic schizophrenic patients. J. Clin. Psychopharmacol. 13:25–40.

Civelli, O., Douglass, J., Goldstein, A., and Herbert, E. (1985) Sequence and expression of the rat prodynorphin gene. Proc. Natl. Acad. Sci. U.S.A., 82:4291–4295.

Claus, A., Bollen, J., De Cuyper, H., Eneman, M., Malfroid, M., Peuskens, J., and Heylen, S. (1992) Risperidone versus haloperidol in the treatment of chronic schizophrenic inpatients: A multicentre double-blind comparative study. Acta Psychiatr. Scand. 85:295–305.

Dacko, S., and Schneider, J.S. (1991) Met-enkephalin immunoreactivity in the basal ganglia in symptomatic and asymptomatic MPTP-exposed monkeys: Correlation with degree of parkinsonian symptoms. Neurosci. Lett., 127:49–52.

DeLong, M.R. (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci., 13:281–285.

Deutch, A.Y. (1993) Prefrontal cortical dopamine systems and the elaboration of functional corticostriatal circuits: Implications for schizophrenia and Parkinson's disease. J. Neural Transm. Gen. Sect., 91:197–221.

Egan, M.F., Hurd, Y., Hyde, T.M., Weinberger, D.R., Wyatt, R.J., and Kleinman, J.E. (1994) Alterations in mRNA levels of D2 receptors and neuropeptides in striatonigral and striatopallidal neurons of rats with neuroleptic-induced dyskinesias. Synapse, 18:178–189.

Egan, M.F., Ferguson, J.N., and Hyde, T.M. (1995) Effects of chronic naloxone administration on vacuous chewing movements and catalepsy in rats treated with long-term haloperidol decanoate. Brain Res. Bull., 38:355–363.

Ellenbroek, B., Schwartz, M., Sontag, K.-H., Jaspers, R., and Cools, A. (1985) Muscular rigidity and delineation of a dopamine-specific neostriatal subregion: Tonic EMG activity in rats. Brain Res., 345:132–140.

Gerfen, C.R., and Young, W.S., III (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: An in situ hybridization histochemistry and fluorescent retrograde tracing study. Brain Res. 460:161–167.

Gerfen, C.R., McGinty, J.F., and Young, W.S. (1991) Dopamine differentially regulates dynorphin, substance P, and enkephalin expression in striatal neurons: In situ hybridization histochemical analysis. J. Neurosci., 11:1016–1031.

Hauber, W., and Schmidt, W.J. (1993) Discrete quinolinic acid lesions of the lateral but not of the medial caudate-putamen reversed haloperidol-induced catalepsy in rats. J. Neural Transm. Gen. Sect., 94:103–114.

Herman, Z.S., Huzarska, M., Kmieciak-Kolada, K., and Kowalski, J. (1991) Chronic treatment with chlorpromazine, thioridazine or haloperidol increases striatal enkephalins and their release from rat brain. Psychopharmacology 104:106–112.

Hong, J.S., Yang, H.Y., Fratta, W., and Costa, E. (1978) Rat striatal methionine-enkephalin content after chronic treatment with cataleptogenic and noncataleptogenic antischizophrenic drugs. J. Pharmacol. Exp. Ther., 205:141–147.

Hong, J.S., Yang, H.Y., Gillin, J.C., and Costa, E. (1980) Effects of long-term administration of antipsychotic drugs on enkephalinergic neurons. Adv. Biochem. Psychopharmacol., 24:223–232.

Houdi, A.A., and Van Loon, G.R. (1990) Haloperidol-induced increase

in striatal concentration of the tripeptide, Tyr-Gly-Gly, provides an index of increased enkephalin release in vivo. J. Neurochem., 54:1360–1366.

Humpel, C., Knaus, G.A., Auer, B., Knaus, H.G., Haring, C., Theodorsson, E., and Saria, A. (1990) Effects of haloperidol and clozapine on preprotachykinin-A messenger RNA, tachykinin tissue levels, release and neurokinin-1 receptors in the striato-nigral system. Synapse, 6:1–9.

Krause, J.E., Chirgwin, J.M., Carter, M.S., Xu, Z.S., and Hershey, A.D. (1987) Three rat preprotachykinin mRNAs encode the neuropeptides substance P and neurokinin A. Proc. Natl. Acad. Sci. U.S.A., 84:881–885.

Leysen, J.E., Gommeren, W., Van Gompel, P., Wynants, J., Janssen, P.F.M., and Laduron, P.M. (1985) Receptor-binding properties in vitro and in vivo of ritanserin, a very potent and long acting serotonin-$S_2$ antagonist. Mol. Pharmacol., 27:600–611.

Leysen, J.E., Janssen, P.M., Schotte, A., Luyten, W.H., and Megens, A.A. (1993) Interaction of antipsychotic drugs with neurotransmitter receptor sites in vitro and in vivo in relation to pharmacological and clinical effects: Role of 5HT2 receptors. Psychopharmacology 112:S40–S54.

Li, S., Sivam, S.P., and Hong, J.S. (1986) Regulation of the concentration of dynorphin A1-8 in the striatonigral pathway by the dopaminergic system. Brain Res., 398:390–392.

Li, S.J., Sivam, S.P., McGinty, J.F., Huang, Y.S., and Hong, J.S. (1987) Dopaminergic regulation of tachykinin metabolism in the striatonigral pathway. J. Pharmacol. Exp. Ther., 243:792–798.

Lindenmayer, J.P., Gardner, E., Goldberg, E., Opler, L.A., Kay, S.R., van Praag, H.M., Weiner, M., and Zukin, S. (1988) High-dose naloxone in tardive dyskinesia. Psychiatry Res., 26:19–28.

Meltzer, H.Y., Matsubara, S., and Lee, J.C. (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2, and serotonin2 pKi values. J. Pharm. Exp. Ther., 251:238–246.

Merchant, K.M., and Dorsa, D.M. (1993) Differential induction of neurotensin and c-fos gene expression by typical versus atypical antipsychotics. Proc. Natl. Acad. Sci. U.S.A., 90:3447–3451.

Mocchetti, I., Schwartz, J.P., and Costa, E. (1985) Use of mRNA hybridization and radioimmunoassay to study mechanisms of drug-induced accumulation of enkephalins in rat brain structures. Mol. Pharmacol., 28:86–91.

Morris, B.J., Hollt, V. and Herz, A. (1988a) Dopaminergic regulation of striatal proenkephalin mRNA and prodynorphin mRNA: Contrasting effects of D1 and D2 antagonists. Neuroscience, 25:525–532.

Morris, B.J., Reimer, S., Hollt, V., and Herz, A. (1988b) Regulation of striatal prodynorphin mRNA levels by the raphe-striatal pathway. Brain Res., 464:15–22.

Nylander, I. and Terenius, L. (1986) Chronic haloperidol and clozapine differentially affect dynorphin peptides and substance P in basal ganglia of the rat. Brain Res., 380:34–41.

Oestreicher, E.G., and Pinto, G.F. (1987) A microcomputer program for fitting enzyme inhibition equations. Comput. Biol. Med., 17:317–321.

Paxinos, G., and Watson, C. (1986) The rat brain in stereotaxic coordinates. Sydney: Academic Press.

Prinssen, E.P., Ellenbroek, B.A., Stamatovic, B., and Cools, A.R. (1995) Role of striatal dopamine D2 receptors in the paw test, an animal model for the therapeutic efficacy and extrapyramidal side effects of neuroleptic drugs. Brain Res., 673:283–289.

Quirion, R., Gaudreau, P., Martel, J.C., St-Pierre, S. and Zamir, N. (1985) Possible interactions between dynorphin and dopaminergic systems in rat basal ganglia and substantia nigra. Brain Res., 331:358–362.

Reyntjens, A., Gelders, Y.G., Hoppenbrouwers, M.J.A., and Vanden Bussche, G. (1986) Thymostenic effects of ritanserin (R 55667), a centrally acting serotonin-S2 receptor blocker. Drug Dev. Res., 8:205–211.

Roeling, T.A.P., Docter, G.J., Voorn, P., Melchers, B.P.C., Wolters, E.C., and Groenewegen, H.J. (1995) Effects of unilateral 6-hydroxydopamine lesions on neuropeptide immunoreactivity in the basal ganglia of the common marmoset, callithrix jacchus, a quantitative immunohistochemical analysis. J. Chem. Neuroanat., 9:155–164.

Schotte, A., Janssen, P.F.M., Gommeren, W., Luyten, W.H.M.L., van Gompel, P., Lesage, A.S., de Loore, K., and Leysen, J.E. (1996) Risperidone compared with new and reference antipsychotic drugs: In vitro and in vivo receptor binding. Psychopharmacology 124: 57–73.

Shibata, K., Haverstick, D.M., and Bannon, M.J. (1990) Tachykinin gene expression in rat limbic nuclei: Modulation by dopamine antagonists. J. Pharmacol. Exp. Ther., 255:388–392.

Stoessl, A.J., Polanski, E. and Frydryszak, H. (1993) The opiate antagonist naloxone suppresses a rodent model of tardive dyskinesia. Mov. Disord., 8:445–452.

Stoof, J.C., Verheijden, P.F., and Leysen, J.E. (1987) Stimulation of D2-receptors in rat nucleus accumbens slices inhibits dopamine and acetylcholine release but not cyclic AMP formation. Brain Res., 423:364–368.

Tang, F., Costa, E., and Schwartz, J.P. (1983) Increase of proenkephalin mRNA and enkephalin content of rat striatum after daily injection of haloperidol for 2 to 3 weeks. Proc. Natl. Acad. Sci. U.S.A., 80:3841–3844.

Trujillo, K.A., Day, R., and Akil, H. (1990) Regulation of striatonigral prodynorphin peptides by dopaminergic agents. Brain Res., 518:244–256.

Voorn, P., Roest, G., and Groenewegen, H.J. (1987) Increase of enkephalin and decrease of substance P immunoreactivity in the dorsal and ventral striatum of the rat after midbrain 6-hydroxydopamine lesions. Brain Res., 412:391–396.

Voorn, P., Docter, G.J., Jongen-Rêlo, A.L., and Jonker, A.J. (1994) Rostrocaudal subregional differences in the response of enkephalin, dynorphin and substance P synthesis in rat nucleus accumbens to dopamine depletion. Eur. J. Neurosci., 6:486–496.

Yoshikawa, K., Williams, C., and Sabol, S.L. (1984) Rat brain preproenkephalin mRNA. cDNA cloning, primary structure, and distribution in the central nervous system. J. Biol. Chem., 259:14301–14308.

Young, W.S. (1990) In situ hybridization histochemistry. In: Methods for the Study of Neuronal Microcircuits and Synaptic Interactions: Handbook of Chemical Neuroanatomy, Vol. 8. F.G. Wouterlood, and A.N. Van den Pol, eds. Elsevier, Amsterdam, pp. 481–512.

Young, W.S., Bonner, T.I., and Brann, M.R. (1986) Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. Proc. Natl. Acad. Sci. U.S.A., 83:9827–9831.