REGULATION OF NEUROPEPTIDE Y-CONTAINING NEURONS

IN THE BASAL GANGLIA AND LIMBIC SYSTEM

OF THE RAT

by

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SUPERVISORY COMMITTEE APPROVAL

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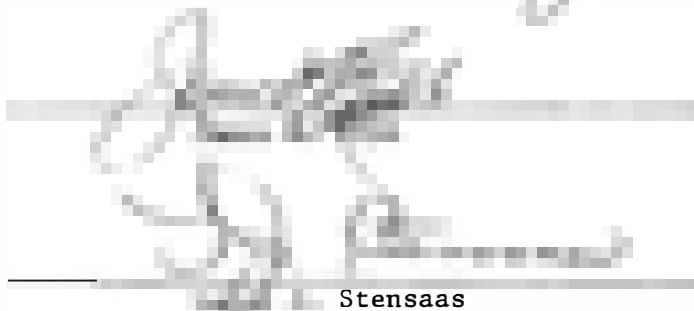
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ABSTRACT

Single or multiple administrations of the psychotomimetic drug, phencyclidine-HCl (PCP), decreased striatal, cortical and accumbens, but not nigral, neuropeptide Y-like immunoreactivity (NPYLI) levels at some time point(s) after administration. The effect of the noncompetitive NMDA receptor antagonist, MK-801, on striatal, cortical and accumbens NPYLI content was similar to PCP, suggesting that extrapyramidal and limbic NPY systems are modulated by glutamatergic activity through NMDA receptor mechanisms. Administration of the GABA-T inhibitors, amino-oxyacetic acid (AOAA) or gamma-vinyl-GABA (GVG) alone had no effect on striatal or cortical NPYLI levels; however, AOAA administration alone decreased accumbens NPYLI levels. Administration of AOAA or GVG prior to or concurrently with PCP treatment completely blocked PCP-induced changes in striatal and cortical NPYLI levels while accumbens NPYLI changes were attenuated. These data suggest that striatal, cortical and accumbens NPY systems are modulated by glutamatergic activity and that the interaction between these two transmitter systems is mediated, at least in part, by GABAergic mechanisms. The role of dopamine (DA) and several other transmitter or receptor systems in PCP-induced extrapyramidal and limbic NPYLI changes was also evaluated. Neither the dopamine D-1 receptor, SCH 23390, nor the dopamine D-2 antagonist, sulpiride, by themselves altered cortical NPY systems but in combination they totally blocked the PCP-induced changes. In contrast, sulpiride alone significantly reduced striatal and accumbens NPYLI

content and enhanced the PCP-induced decreases, while SCH 23390 alone had no effect on striatal and accumbens NPYLI levels but did attenuate PCP-induced effects. These data suggest that basal ganglia and limbic NPY systems are differentially modulated by NMDA and dopaminergic activity. Examination of the effects of multiple administrations of selective D-1 and D-2 agonists and antagonists on striatal, nigral, accumbens, pallidal and cortical NPY systems indicated that within structures such as the caudate and nucleus accumbens there exists multiple distinct NPY systems which are uniquely influenced by DA receptors. Initial studies suggest that both GABA-A and -B receptors are involved in mediating the PCP-induced NPYLI changes, and that PCP causes both release of NPY from nerve terminals and increased peptide biosynthesis.

To Gary

-for making it all possible

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CHAPTER 1

OVERVIEW

Introductory Remarks

Neuropeptide Y (NPY) is a 36-amino acid peptide first isolated from porcine brain in 1982 (Tatemoto, 1982). The peptide shares sequence homology with members of the pancreatic polypeptide family and contains both N- and C-terminal tyrosine residues, hence the name neuropeptide Y (Tatemoto, 1982; Tatemoto et al., 1982). NPY, NPY message, and NPY binding sites are widely distributed in the central nervous system of various mammalian species (Allen et al., 1983; Gehlert et al., 1987; Martel et al., 1987). NPY meets many of the criteria for an interneuronal neurotransmitter and has important effects in regulating neuroendocrine function, circadian rhythms, consummatory behavior, central autonomic control, memory processing (Heilig and Widerlov, 1990; Aakerlund et al., 1990; Sheikh et al., 1989; Hinson et al., 1988;) and in regulating contraction and relaxation of cerebral vessels (Abel and Han, 1989; Suzuki et al., 1989). NPY colocalizes in specific brain areas with several transmitters including noradrenaline, GABA and somatostatin, and interacts with neurotransmitter systems such as noradrenaline, dopamine (DA) and GABA (Beal et al., 1987; Kerkarian et al., 1989; Salin et al., 1990; Massari et al., 1988; Aoki and Pickel, 1989; Everitt et al, 1984; Kubota et al., 1988).

Altered tissue or cerebrospinal fluid (CSF) levels of neuropeptide Y-like immunoreactivity (NPYLI) have been implicated in several clinically important psychiatric disturbances, including depression, anxiety and schizophrenia (Heilig and Widerlov, 1990; Peters et al., 1990; Wahlestedt et al., 1989; Widerlov et al., 1987). In addition, several of the transmitter systems that are altered in these psychiatric illnesses are known to interact with NPY- containing neurons.

Considerable interest exists about the role of NPY in several neurodegenerative diseases. In Huntington's chorea, the medium-sized aspiny striatal neurons containing NPY, somatostatin and NADPH-diaphorase are selectively spared (Dawbarn et al., 1985; Ferrante et al., 1985; Chan-Paly, 1988; Beal et al., 1988). Observations that NPY- and somatostatin-sparing lesions can be produced by quinolinic acid but not other excitotoxins were reported but replication of these results has been equivocal (Beal et al., 1986; Boegman et al., 1987; Davies and Roberts, 1987). In Alzheimer's and Parkinson's diseases there appears to be a dissociation between the colocalized neuropeptides, NPY and somatostatin (Wahlestedt et al., 1989). Reports of dissociation between NPY and somatostatin and of NPYLI tissue degeneration indicate significant regional specificity, but studies are often conflicting (Heilig and Widerlov, 1990). However, the NPY-containing cortical and hippocampal neurons in the Alzheimer's disease patient do undergo serious degenerative changes (Heilig and Widerlov, 1990; Wahlestedt et al., 1989). In Parkinson's patients, no change in neocortical and hippocampal NPY concentration have been found despite significant reduction in somatostatin content (Allen et al., 1985). In addition, levels of NPY in frontal cortex, hippocampus and amygdala-

/pyriform cortex are significantly elevated after acute kainic acid-induced seizures (Marksteiner et al., 1989) and in rats with decreased seizure thresholds due to pentylenetetrazole kindling (Marksteiner and Sperk, 1988).

We previously reported that dopamine-releasing drugs such as methamphetamine (METH), MDMA and cocaine significantly alter neurotensin-, dynorphin- and substance P-like immunoreactivity (LI) levels in a number of brain structures (Hanson et al., 1987 a and b; Letter et al., 1987; Merchant et al., 1987). Subsequent studies indicated that NPYLI content is also affected by these dopamine-releasing drugs and is significantly altered in striatum, nucleus accumbens and frontal cortex (Midgley et al., 1989). These data suggest that NPYLI levels may be altered by dopaminergic action on specific postsynaptic or presynaptic receptors. More recently, we identified that phencyclidine-HCl (PCP) alters striatal NPYLI levels (Midgley et al., 1990) in a manner similar to, but more profound than, that of MDMA and METH. The fact that PCP is a less potent DA releaser than METH suggested that some of PCP's actions on NPY-containing neurons may be mediated by systems other than dopamine (Contreras et al., 1987). Thus, Chapter 2 examines the effects of PCP administration on extrapyramidal NPY systems and identifies neurotransmitter systems that are important in mediating these PCP-induced NPYLI changes; Chapter 3 similarly examines the effects of PCP administration on limbic NPY systems; Chapter 4 evaluates the effects of administration of specific dopaminergic agonists and antagonists on NPY systems in discrete structures in the basal ganglia and limbic system; Chapter 5 examines the mechanisms by which these PCP-induced NPYLI changes are occurring.

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CHAPTER 2

CHARACTERIZATION OF PHENCYCLIDINE-INDUCED
EFFECTS ON NEUROPEPTIDE Y SYSTEMS IN
THE RAT CAUDATE-PUTAMEN

Introduction

NPY-like immunoreactive structures are found in high concentrations in many

brain structures, including the basal ganglia (Beal et al., 1987; Martel et al., 1987).

NPY-containing cells in the caudate-putamen, nucleus accumbens and frontal cortex are

primarily aspiny interneurons that form symmetrical junctions suggestive of inhibitory

action (Aoki and Pickel, 1990; Massari et al.,1988; Vuillet et al., 1989). Afferent

synapses with NPY perikarya and dendrites in the striatum are of both the symmetrical

and asymmetrical type suggesting differential regulation of these neurons by other

transmitter systems (Vuillet et al., 1989). In addition, axonal processes containing

neuropeptide Y-like immunoreactivity are often found in proximity to cerebral vessels

(Aoki and Pickel, 1990; Vuillet et al., 1989) and NPY alters contraction and relaxation

of cerebral vessels in rat striatum (Suzuki et al., 1989) and in isolated rabbit cerebral

arteries (Abel and Han, 1989). Furthermore, striatal NPY-containing neurons interact

with several different neurotransmitter systems including dopamine and GABA (Aoki and

Pickel, 1990; Beal et al., 1986; Kerkarian et al., 1988; Massari et al., 1988).

As mentioned in Chapter 1, alterations in tissue and cerebrospinal fluid levels of NPYLI have been implicated in several psychiatric disturbances (Wahlestedt et al., 1989). Cerebrospinal fluid levels of NPY are significantly lower in depressed patients (Widerlov et al., 1987) and administration of antidepressants such as imipramine (a noradrenaline (NA) uptake blocker) and zimeldine (a 5-hydroxytryptamine (serotonin; 5-HT) uptake blocker) increase the concentration of NPYLI in rat brain (Heilig and Widerlov, 1990). In addition, centrally administered NPY suppresses open-field and home cage activity in rat and produces anxiolytic-like effects in animal anxiety models (Heilig and Widerlov, 1990). Biochemical (Heilig and Widerlov, 1990; Tatsuoka et al., 1987) and immunohistochemical data (Kerkarian et al., 1988; Lindefors et al., 1990) support the hypothesis that NPY and dopaminergic systems are interrelated and reciprocal regulation exists. This interaction with dopaminergic systems suggests that brain NPY parameters may be changed by conditions in which normal dopaminergic transmission is thought to be altered, such as schizophrenia. In support of this suggestion, Peters et al. (1990) reported increased NPYLI content in the CSF of drug-free schizophrenics; furthermore, withdrawal from haloperidol maintenance therapy was associated with significantly increased CSF levels of NPYLI. Reduced NPY concentrations have also been observed in the temporal cortex of a population of schizophrenic patients (Heilig and Widerlov, 1990).

Phencyclidine HCl (PCP) is a common drug of abuse that can induce schizophrenia-like psychotic behavior in users. PCP is known to interact with noradrenergic, cholinergic, serotonergic, GABAergic, and dopaminergic transmitter systems (Contreras

et al., 1987; Johnson and Jones, 1990; Junien and Leonard, 1989). We recently assessed

levels of the rate-limiting enzyme in dopamine synthesis, tyrosine hydroxylase (TH), in

striatal tissues 15 h after multiple PCP administrations and found a 20% decrease in TH

activity (data not shown). In addition, PCP is a noncompetitive NMDA antagonist and

binds with high affinity at the sigma-opioid receptor (Contreras et al., 1987; Johnson and

Jones, 1990; Junien and Leonard, 1989). Other drugs of abuse that can cause symptoms

of schizophrenia in abusers, such as amphetamine and cocaine, alter NPY parameters in

the rat brain (Midgley et al., 1989; Tatsuoka et al., 1987; Tessel et al., 1985; Wahlestedt

et al., 1991). In this study, we observed that PCP also profoundly alters NPY systems

in the basal ganglia. We elucidated the nature of these PCP-induced changes and discuss

their relevance to NPY regulation by other transmitter systems.

Materials and Methods

Animals and treatments

Male Sprague-Dawley rats (180-270 g, Simonsen Laboratories, Gilroy, Ca) were

maintained in a controlled environment with 12-h light/dark cycles and allowed free

access to food and water. Drugs dissolved in 0.9 % saline included: phencyclidine HCl

(PCP, National Institute on Drug Abuse, Rockville, MD); the serotonin toxin, DL-p-

chloroamphetamine HCl (PCA, Sigma Chemical Co., St. Louis, MO); the alpha$_1$-

adrenergic antagonist, prazosin HCl (Pfizer Inc., Groton, CN); the alpha$_2$-adrenergic

antagonist, idazoxan HCl (Research Biochemicals Inc., Natick, MA); the dopamine D-1

antagonist, SCH 23390 HCl (Research Biochemicals Inc., Natick, MA); the sigma

antagonist, rimcazole HCl (Burroughs Wellcome, Research Triangle Park, NC); the muscarinic cholinergic antagonist, atropine sulfate (Sigma Chemical Co., St. Louis, MO); the opioid antagonist, naloxone HCl (Research Biochemicals Inc., Natick, MA); the GABA-T (GABA transaminase) suicide inhibitor, gamma-vinyl-GABA (GVG, vigabatrin, MDL 71,754; Marion Merrell Dow Inc., Cincinnati, OH; Jung et al., 1977); the NMDA (N-methyl-D-aspartate) antagonist, MK-801 hydrogen maleate (dizocilpine, Research Biochemicals Inc., Natick, MA). Vehicles used for dissolving the following drugs were as follows: the dopamine D-2 antagonist, ($\pm$) sulpiride (Sigma Chemical Co., St. Louis, MO), in 2% lactate + 25% propylene glycol-saline; the indirect GABA-T inhibitor, AOAA (Churchich, 1982) (aminooxyacetic acid hemi HCl, Sigma Chemical Co., St. Louis, MO), in saline neutralized with 20% of 1 N NaOH; the GABA antagonist, bicuculline (Sigma Chemical Co., St. Louis, MO), in saline acidified with 10% of 0.1 N HCl and the sigma antagonist, BMY 14802 (Bristol-Meyers Squibb Co., Wallingford, CT), in 1% lactate-saline. All drugs were administered intraperitoneally with the exceptions of PCP and the GABA antagonist, bicuculline, which were administered subcutaneously. Rats administered PCA received two doses (10 mg/kg/dose) 24 h apart, 1 week prior to challenge. This dosing regimen depletes striatal serotonin levels by 85%(Smiley et al., 1990). Those administered the GABA-T suicide inhibitor, GVG, received 2 doses (1200 mg/kg/dose) 48 and 1 h prior to challenge. Drug doses, except for MK-801, were calculated for their free forms. Control animals received identical treatment regimens using vehicles only.

In acute experiments, animals received a single dose of PCP (15 mg/kg) and were sacrificed 12 h after treatment; or 6, 12 and 24 h after drug injection for recovery studies. For multiple dose experiments, animals received five doses of PCP (15 mg/kg/dose), MK-801 (1 mg/kg/dose) or antagonist/agonist (unless otherwise specified above) 6 h apart and were sacrificed 18 h; 10, 18 or 34 h; or 12-15 h after the final dose for dose-effect, recovery and combination drug studies, respectfully. All antagonists were given 20 min prior to PCP administration.

Dissections

All animals were sacrificed by decapitation at the indicated times following treatment. Brains were rapidly removed and placed on ice, striata (caudate-putamen) were excised, and the remaining brain tissues immediately frozen on dry ice and stored at -80°C until assayed for NPYLI. The substantia nigrae were identified according to the atlas of Konig and Klippel (1963), bilaterally dissected out from 1.0-mm thick frozen coronal slices and stored at -80°C until assayed for NPYLI content.

Neuropeptide Y antiserum

The antiserum to NPY was prepared in our laboratory as described by Letter et al. (1987) using a modification of standard techniques (Hurn and Chantler, 1970; Suess et al., 1986). Porcine NPY (1-36) was conjugated to thyroglobulin or hemocyanin with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide as described by Skowsky and Fischer (1972). The mixture was incubated at room temperature for 24 h, after which an equal

volume of 1 M hydroxylamine was added to the reaction mixture. Following 4 or more hours of incubation, the conjugate was emulsified with complete or incomplete Freund's adjuvant and immediately injected intradermally into multiple sites on the backs of New Zealand White rabbits. Injections were administered once a week for 4 weeks and subsequently at 6-week intervals. The antiserum was harvested by heart puncture 10 days after the last booster.

The resultant NPY antiserum reliably detects 8 pg of NPY when assayed at an antiserum dilution of 1:15,000. In the presence of 1000-fold excess concentrations of related peptides, the NPY antiserum demonstrated less than 1% cross-reactivity with peptide PPP and NPY fragment 18-36, less than 5% cross-reactivity with peptide PYY and less than 10% cross-reactivity with [Leu^{31}, Pro^{34}]-NPY. Results are expressed as pg/mg protein of NPYLI. No significant cross-reactivity was observed between the NPY antiserum and unrelated peptides such as substance P and neurotensin.

NPY radioimmunoassay

The radioimmunoassay for NPYLI was performed according to a modification of the methods described by Hanson and Lovenberg (1980). Briefly, selected portions of brain were homogenized in 0.01 N HCl from which an aliquot was removed for protein analyses as described by Bradford (1976). The remaining homogenate was boiled for 10 min, then centrifuged for 1 h at 3000 g. The supernatant was removed, lyophilized and stored at -80^0 C until assayed for NPYLI content. The samples were reconstituted in phosphate-buffered saline with gelatin plus 0.1% Triton X-100. Duplicate aliquots

(approximately 0.04% of total sample per aliquot) were mixed with NPY antiserum (1:15,000 final dilution) and incubated at 4°C for 4 days. ^{125}I-labelled NPY (Amersham Co., Arlington Heights, IL) was added to the sample tubes and the reaction mixture was incubated at 4°C for another 2 days. Free and antibody bound ^{125}I-labelled NPY were separated by mixing the reactants with a dextran-coated charcoal slurry (Herbert et al., 1965). Quantitation of NPYLI content was achieved by comparing bound to free ^{125}I-labelled NPY in each sample to a standard curve.

Statistical analysis

To facilitate comparisons between groups, results in all figures are shown as percentages of respective controls. Control and experimental groups of animals from each study were run in a single assay. The NPYLI control values are included in the figure legends. Bars represent the mean of treatment groups ± S.E.M. Data were analyzed using a one factor ANOVA; if the F ratio was significant, a Fischer-PLSD (Protected Least Significant Difference) test was used to compare differences between the means of individual groups. Unless otherwise specified, differences were considered significant when the probability that they were zero was less than 5%.

Results

Dose-effect response of striatal NPY systems to multiple

PCP administrations

To evaluate the effects of PCP on striatal NPYLI content, rats were administered multiple doses (five doses, 6 h apart) of PCP at 5, 10 or 15 mg/kg/dose and sacrificed approximately 18 h after the final dose. PCP decreased striatal NPYLI levels in a dose-dependent manner to 79, 55 and 35% of control, respectively (Figure 2.1).

Recovery of striatal NPY systems after acute and multiple

doses of PCP

To assess the recovery of striatal NPY systems after acute PCP administration, a single injection of PCP (15 mg/kg) was given and rats were sacrificed 6, 12 or 24 h later. After acute treatment, striatal NPYLI levels were unchanged at 6 h, significantly decreased to 70% of control at 12 h, and recovered by 24 h (Figure 2.2 A).

To assess the recovery of striatal NPY systems after multiple doses of PCP, rats received five injections of PCP (15 mg/kg/dose) at 6-h intervals, and were sacrificed 10, 18, 34 or 58 h after the final dose. Striatal NPYLI levels were decreased to 55, 48, and 56% after 10,18 and 34 h, and had returned to control by 58 h (Figure 2.2 B).

Effects of single and multiple doses of PCP on NPYLI content

in substantia nigra

Because the substantia nigra is functionally related to the striatum, we assessed
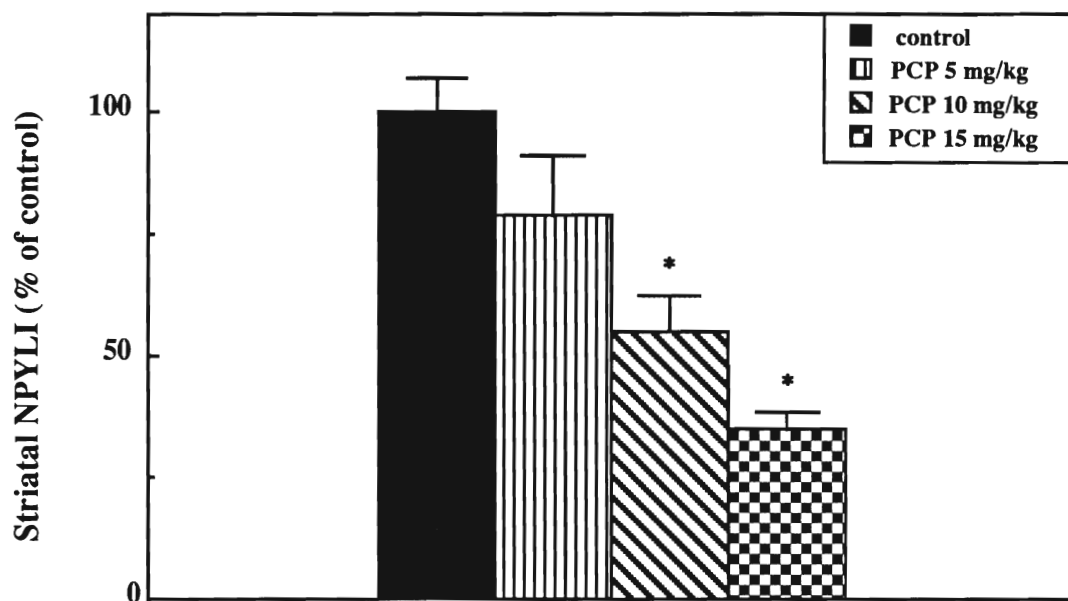
Figure 2.1. Effects of multiple administrations of various doses of PCP on striatal NPYLI content. Rats were given five doses of PCP (5, 10 or 15 mg/kg/dose) at 6-h intervals and sacrificed 18 h after the final dose. Results are expressed as percent of control ± S.E.M. (n=6-8). The average value for control NPYLI levels was 328 ± 28 pg/mg protein. * p < 0.01 vs. control.
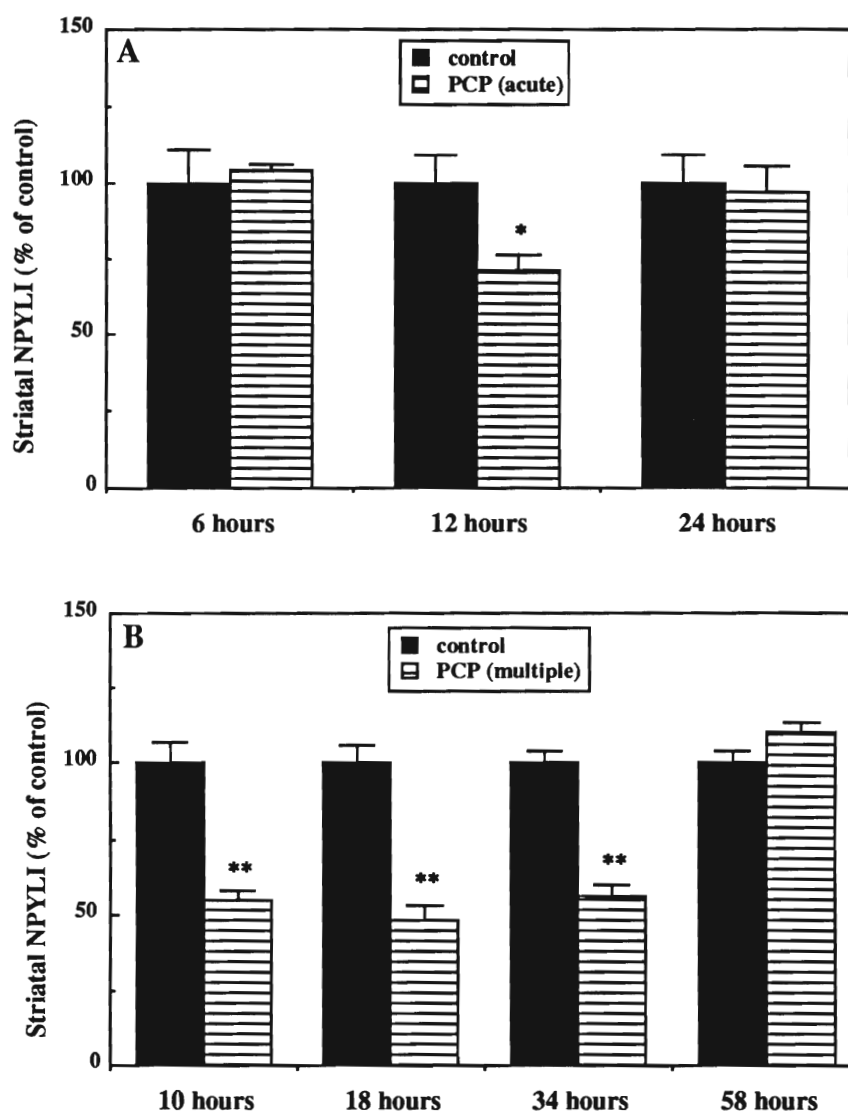
Figure 2.2. Effects of single and multiple PCP administrations on striatal NPYLI content. A. Rats were given a single dose of PCP (15mg/kg/dose) and sacrificed 6, 12 or 24 h after injection. The average value for control NPYLI levels was 610 ± 65 pg/mg protein. * p < 0.05 vs. respective control. B. Rats were administered five doses of PCP (15 mg/kg/dose, s.c.) 6 h apart and sacrificed 10, 18, 34 or 58 h after the final dose. Average control value for NPYLI level was 555 ± 41 pg/mg protein. ** p < 0.01 vs. respective control. Results shown in both panels are expressed as percent of respective control ± S.E.M. (n=6).

the effects of single and multiple PCP administrations on nigral NPYLI content. Rats received a single (dose) or multiple doses (5 injections, 6 h apart) of PCP (15 mg/kg/dose) and were sacrificed approximately 12-15 h after the single or final dose, to coincide with time points of maximal striatal effects. We observed no significant changes in nigral NPY levels with either acute or multiple administrations (data not shown).

Role of monoamines in PCP-induced changes in striatal

NPYLI content

To investigate the relationship between monoamine systems and striatal NPY systems, 5-HT was depleted or selective monoaminergic antagonists were given alone or with PCP and NPYLI content was determined. 5-hydroxytryptamine was depleted with PCA (10 mg/kg/dose) as described in Materials and Methods. The dosing protocol for the antagonists and PCP was five doses administered with a 6-h interval between doses; animals were sacrificed approximately 10-15 h after the last dose. We observed that depletion of 5-HT alone with PCA did not significantly alter striatal NPYLI levels nor did it affect PCP-induced effects (Figure 2.3). Administration of idazoxan (0.125 mg/kg/dose), a selective alpha$_2$-adrenergic receptor antagonist, produced no effects alone or in combination with PCP; however, prazosin (0.5 mg/kg/dose), a selective alpha$_1$-adrenergic receptor antagonist, decreased striatal NPYLI levels alone (79%) and attenuated PCP's effect when given in combination (66 % vs. 44 %). Administration of the selective D-1 receptor antagonist, SCH 23390 (0.5 mg/kg/dose) had no effect by itself but significantly attenuated PCP's effects on striatal NPYLI content to 60% of
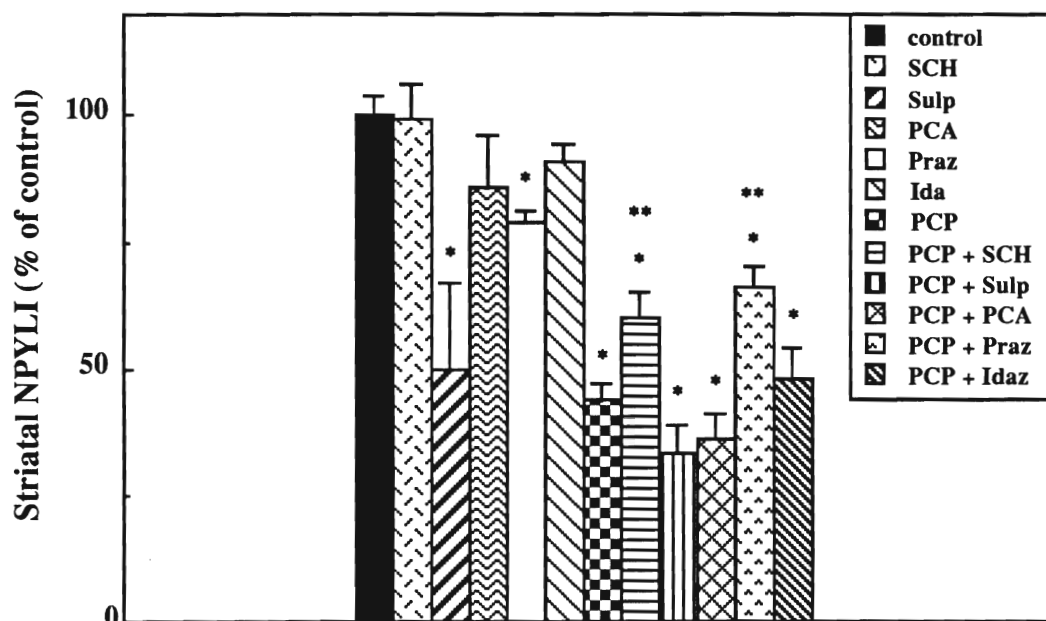
Figure 2.3. Effects of 5-HT depletion or selective monoamine receptor blockade alone and in combination with PCP on striatal NPYLI content. 5-HT was depleted with PCA, or animals were given multiple doses of SCH 23390 (0.5 mg/kg/dose), sulpiride (80 mg/kg/dose), prazosin (0.5 mg/kg/dose) or idazoxan (0.125 mg/kg/dose) alone or in combination with PCP (15 mg/kg/dose) as described in Materials and Methods. Rats were sacrificed approximately 10-15 h after the final dose. Results are expressed as percent of control ± S.E.M. (n=6). Average control value for striatal NPYLI content was 463 ± 50 pg/mg protein. * p < 0.05 vs. control, ** p < 0.05 vs. PCP.

control (Figure 2.3). The selective D-2 antagonist, sulpiride (80 mg/kg/dose), significantly decreased striatal NPYLI levels to 50% of control by itself but did not alter PCP-induced striatal NPYLI changes.

Effects of a cholinergic antagonist alone and in combination
with PCP on striatal NPYLI content

To investigate the relationship between the cholinergic system and striatal NPY systems, we administered the cholinergic antagonist, atropine (1 mg/kg/dose), alone and in combination with PCP. Animals received five doses of atropine, PCP or a combination as previously described and were sacrificed approximately 15 h after the final dose. Atropine alone had no effect (107 $\pm$ 13 of control); coadministration of atropine did not significantly alter PCP-induced NPY changes (data not shown).

Effects of sigma and nonselective opioid antagonists alone and
in combination with PCP on striatal NPYLI content

To evaluate the possible role of sigma and opioid receptors in regulating striatal NPY systems and in PCP-induced NPYLI changes, we administered selective antagonists in combination with PCP. Animals received multiple doses of PCP, antagonist or a combination as previously described and were sacrificed approximately 10-15 h after the final dose (Table 2.1). The putative sigma antagonist, rimcazole (12.5 mg/kg/dose), had no effect alone on striatal NPY levels and did not significantly alter PCP-induced changes, although a trend was suggested (two tailed p=.08 for combination vs. PCP

Table 2.1

Effects of sigma and nonselective opioid receptor antagonists, alone and in combination with PCP, on striatal NPYLI content.

Rats were administered five doses (6 h apart) of rimcazole (12.5 mg/kg/dose), BMY 14802 (35.0 mg/kg/dose) or naloxone (0.5 mg/kg/dose) alone and in combination with PCP (15 mg/kg/dose) and sacrificed 10-15 h after the final dose. Results represent combined data from three experiments expressed as percent of control $\pm$ S.E.M. (n= 6-18). Average control values for striatal NPYLI content were 331 $\pm$ 48 pg/mg protein. * p <0.05 vs. vehicle without PCP.

	NPYLI (percent of control)	
	Without PCP	With PCP
Vehicle	100 $\pm$ 5	* 47 $\pm$ 4
Rimcazole	94 $\pm$ 12	* 62 $\pm$ 7
BMY 14802	* 61 $\pm$ 3	* 42 $\pm$ 12
Naloxone	111 $\pm$ 4	* 49 $\pm$ 7

alone). In contrast, the putative sigma antagonist, BMY 14802 (35 mg/kg/dose), significantly decreased NPYLI content to 61% of control by itself and had little effect on PCP-induced NPYLI changes. The opioid antagonist, naloxone (0.5 mg/kg/dose), had no effect on striatal NPYLI content by itself or when coadministered with PCP.

Effects of GABA agonists or antagonists alone and in
combination with PCP on striatal NPYLI content

To investigate the role of GABA in the response of the striatal NPY system to PCP, a GABA antagonist and GABA-T inhibitors were administered alone and in combination with PCP (Figure 2.4 A and B). All animals were sacrificed approximately 10-15 h after multiple dosing. The GABA antagonist, bicuculline (1 mg/kg/dose) produced a small but significant decrease in NPYLI levels when administered alone (79%) and had no effect on PCP-induced NPYLI alterations (Figure 2.4 A). The indirect GABA agonists, AOAA (15 mg/kg/dose) and GVG, had no effects by themselves, but both completely blocked PCP-induced changes in striatal NPYLI content (Figure 2.4 B).

Effects of MK-801 and GABA agonists alone and in
combination on striatal NPYLI content

To determine if the PCP effect on striatal NPY systems was the result of NMDA receptor blockade, MK-801 (1 mg/kg/dose), a similar noncompetitive NMDA antagonist was administered in a manner like that described for PCP. Levels of striatal NPYLI declined to 71% following MK-801 treatment. Coadministration of the GABA-T
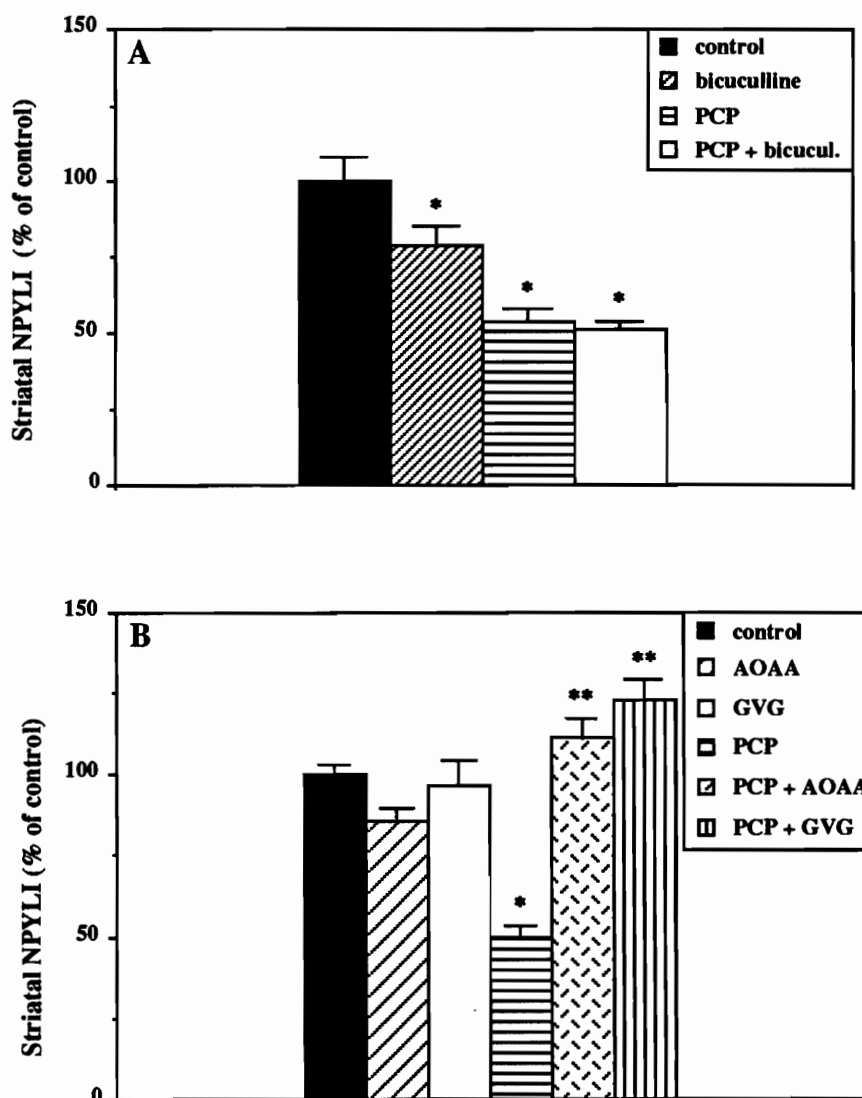
Figure 2.4. Effects of GABA agonists and antagonist alone and with PCP on striatal NPYLI content. Rats were treated with multiple doses of bicuculline (1 mg/kg/dose; A), GVG (1200 mg/kg/dose; B) or AOAA (15 mg/kg/dose; B) alone and in combination with PCP (15 mg/kg/dose). Rats were sacrificed approximately 15 h after the last dose. Results are expressed as percent of control ± S.E.M. (n=6). Average control values for panels A and B NPYLI content were 220 ± 17 and 496 ± 37 pg/mg protein, respectively. * $p < 0.05$ vs. respective controls, ** $p < 0.05$ vs. PCP.

inhibitors totally blocked the MK-801-induced effects in a manner similar to that observed with PCP (Figure 2.5).

Discussion

These studies demonstrate that PCP administration has profound effects on striatal NPY systems. Multiple doses of PCP reduced striatal NPYLI content in a dose-dependent manner to as much as 35% of control (Figure 2.1). Significant declines occurred after both single and multiple PCP administrations; however, the effects of multiple administrations were more profound and of longer duration (Figure 2.2, A and B). These data demonstrate that PCP affects striatal NPY systems like other schizophrenia-inducing drugs of abuse, such as amphetamines and cocaine (Midgley et al., 1989; Tatsuoka et al., 1987; Tessel et al., 1985; Wahlestedt et al., 1991). Furthermore, the fact that acute administration of PCP produced significant decreases in striatal NPYLI content as soon as 12 h after administration suggests that the acute effects may be due in part to increased release and turnover of the peptide, although the possibility of altered synthesis cannot be excluded. No effects on nigral NPYLI content were observed after either acute or multiple PCP treatments at the identified time points. The lack of a nigral NPY response suggests regional differences in regulation and function of basal ganglia NPY systems. Because monoamines participate in the function of the basal ganglia and PCP affects the activity of these pathways (Bowyer et al., 1984; Smith et al., 1977), the role of dopamine, norepinephrine and serotonin systems in the NPY response to PCP was examined. In this study, the selective D-1 antagonist, SCH 23390, alone lacked an effect
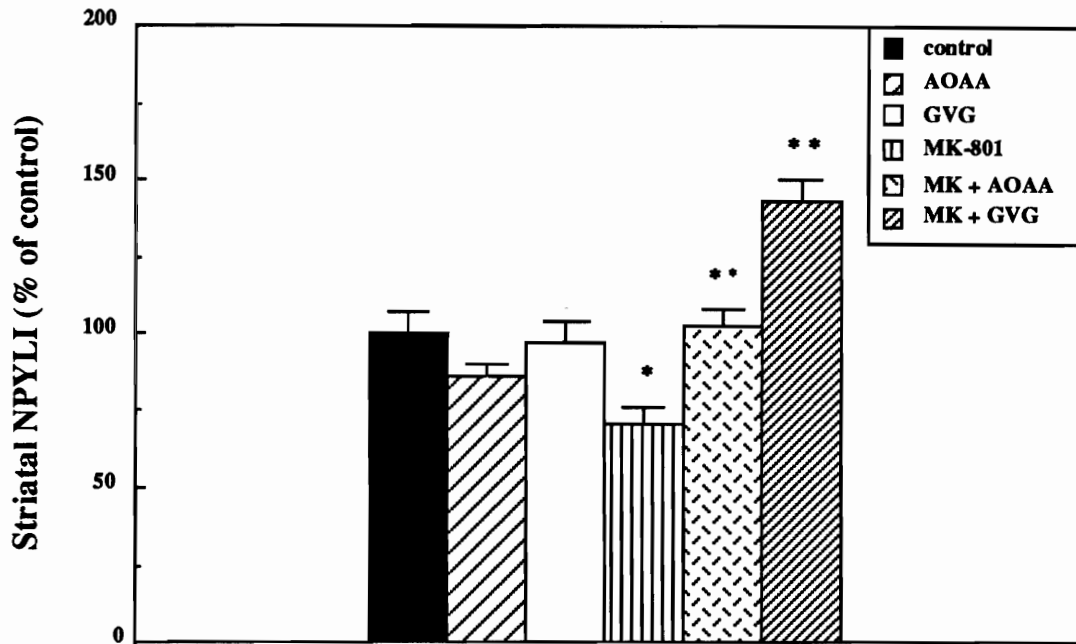
Figure 2.5. Effects of MK-801 administrations with and without GABA agonists on striatal NPYLI content. Rats were treated with GVG (see Materials and Methods) or AOAA (15 mg/kg/dose, as previously described) alone and in combination with MK-801 (1 mg/kg/dose). Rats were sacrificed approximately 15 h after the final dose. Results are expressed as percent of control ± S.E.M. (n=6). Average control value for NPYLI content was 596 ± 61 pg/mg protein. * p < 0.05 vs. control, ** p < 0.05 vs. MK-801.

on striatal NPYLI but significantly attenuated the effects of PCP on striatal NPYLI levels (Figure 2.3). These data suggest that some of PCP's actions on NPY systems are mediated through the D-1 receptor. Other studies conducted in our laboratories have shown that methamphetamine produces a similar though less dramatic effect as PCP on striatal NPY levels, and its effects also are attenuated by D-1 receptor blockade (Midgley et al., 1989). The selective dopamine D-2 antagonist, sulpiride, significantly decreased striatal NPYLI content by itself, suggesting that D-2 receptors exert tonic activity over striatal NPY-containing neurons. These observations support the findings of Kerkarian et al. (1988) that D-2 receptor blockade decreases the number of striatal NPY immunoreactive cells. However, D-2 receptor blockade had no effect on the PCP-induced changes in striatal NPYLI levels (Figure 2.3).

Depletion of striatal 5-HT by PCA pretreatment had no significant effect on striatal NPYLI content either alone or in combination with PCP (Fig. 2.3). Furthermore, multiple administrations of the 5-HT uptake blocker, fluoxetine, failed to change striatal NPY levels alone and did not alter PCP-induced NPY alterations (data not shown). These data suggest that 5-HT receptor activation is not important in maintenance of basal NPYLI levels nor do serotonin systems contribute significantly to PCP-induced effects on NPYLI levels in the striatum.

Interestingly, the alpha$_1$-adrenergic antagonist, prazosin, produced a small but significant decrease in striatal NPYLI content by itself and significantly attenuated PCP's effects, whereas the alpha$_2$-adrenergic antagonist, idazoxan, lacked an effect either alone or when coadministered with PCP (Figure 2.3). These results suggest that alpha$_1$ but not

alpha$_2$ receptors play a small role in basal NPY regulation and contribute to the PCP-induced striatal NPY response. However, the mechanism by which this occurs can not be determined from these data.

Because of the reputed interaction of PCP (Contreras et al., 1987; Johnson and Jones, 1990; Junien and Leonard, 1989) and NPY (Roman et al., 1989) with the sigma receptor, and in view of data suggesting the involvement of sigma and other opioid receptors in regulation of nigrostriatal dopaminergic neurons (Iyengar et al., 1990; Manzanares et al., 1991), we evaluated the effects of two putative sigma antagonists, rimcazole and BMY 14802 (Ferris et al., 1986; Taylor and Dekleva, 1987), and the nonspecific opioid antagonist, naloxone. Interestingly, neither rimcazole or naloxone alone altered striatal NPY levels, whereas BMY 14802 significantly decreased striatal NPY content by itself (Table 2.1). The affinity of the BMY compound is over 20 times that of rimcazole for the the D-2 receptor (Largent et al., 1988). In view of these data and other reports that dopamine D-2 antagonism significantly reduces striatal NPY content (Kerkarian et al., 1988; Miyake et al., 1990; Midgley et al., 1989), it is possible that alteration of D-2 receptor activity is the mechanism by which BMY 14802 affects striatal NPY systems. However, since BMY 14802 has a relatively low affinity for D-2 receptors and some affinity for receptors, such as the 5-HT$_2$ and alpha$_1$-adrenergic, other mechanisms may be involved as well (Largent et al., 1988). Neither of the two sigma antagonists, nor naloxone, altered the effects of PCP administration, although rimcazole showed a trend toward attenuation (Table 2.1). These data suggest that sigma and opioid systems are not the major components in PCP-induced alterations in striatal NPY content.

PCP has also been suggested to have cholinergic activity (Contreras et al., 1987; Johnson and Jones, 1990; Junien and Leonard, 1989). We therefore administered the cholinergic antagonist, atropine, alone and in combination with PCP. Atropine alone did not significantly alter striatal NPY levels and had no effect on PCP-induced alterations in striatal NPY, although there was considerable variability in the atropine alone group. These findings suggest that the cholinergic system is not a principal regulator of basal striatal NPY content and that PCP's effects on striatal NPY systems are not mediated by cholinergic activity.

Vuillet et al. (1990) have shown that GABAergic neurons make synaptic contacts with NPY-containing neurons in the rat striatum. We therefore investigated the possibility that PCP-induced effects on striatal NPY content involve GABAergic systems. To test this hypothesis, we increased striatal GABA levels by administering inhibitors of the primary degradative enzyme for GABA, GABA-transaminase (GABA-T), or administered a selective GABA antagonist, bicuculline, at subconvulsive doses. As shown in Figure 2.4, administration of the indirect GABA agonists alone did not affect striatal NPY content, while administration of the GABA antagonist, bicuculline, significantly reduced striatal NPYLI levels. The failure of bicuculline to completely mimick the effects of PCP was likely due to the dose utilized or its GABA-A receptor selectivity (i.e., perhaps the effect of PCP is both GABA-A- and -B-mediated). These data suggest that tonic GABAergic activity is necessary for maintenance of baseline striatal NPY levels. Furthermore, both indirect GABA agonists, AOAA and GVG, completely blocked PCP-induced changes in striatal NPY content, while bicuculline had little

additional effect on the large PCP-induced depletions. These findings suggest that the PCP effects on striatal NPY systems are mediated by GABA pathways.

PCP has been identified as a potent noncompetitive antagonist at the NMDA receptor (Contreras et al., 1987; Johnson and Jones, 1990; Junien and Leonard, 1989). Stimulation of NMDA receptors releases GABA both from striatal neurons in primary culture (Weiss, 1990) and from striatal tissues in vivo (Young and Bradford, 1990). Furthermore, striatal NPY-containing neurons are influenced by corticostriatal and thalamostriatal projections (presumably glutamatergic)(Kerkarian et al., 1989; Vuillet et al., 1989). Because of these reports and our findings, we evaluated the effects of the selective NMDA antagonist, MK-801, alone and in combination with the GABA-T antagonists, AOAA and GVG (Figure 2.5) to compare with the PCP-induced effects. Treatment with MK-801 decreased striatal NPY content in a PCP-like manner, and the effect was similarly blocked by administration of the indirect GABA agonists, AOAA and GVG. These data confirm that striatal NPY-containing neurons are modulated by glutamatergic activity through NMDA receptors. In addition, the glutamatergic modulation of these striatal NPY neurons is mediated by GABAergic systems.

In summary, the results of this study suggest that striatal NPY pathways are regulated by NMDA receptors through GABAergic mechanisms. In addition, striatal NPY neurons also might be regulated by noradrenergic (through the $alpha_1$-receptor) and dopaminergic systems (through D-2 and D-1 receptors). PCP has a profound, reversible effect on striatal NPY systems that likely reflects the activity of this drug on NMDA and GABA systems; however, noradrenergic and dopaminergic factors may be involved as

well. The exact significance of the NPY changes in response to PCP is presently unclear, but these changes may contribute to locomotor, psychiatric or vascular responses to this drug.

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CHAPTER 3

DIFFERENTIAL REGULATION OF NEUROPEPTIDE Y

SYSTEMS IN LIMBIC STRUCTURES OF THE RAT

Introduction

NPY-containing cells in limbic structures such as the frontal cortex and nucleus

accumbens are primarily aspiny interneurons that form symmetrical (inhibitory) junctions

with other neurons; afferent synapses with cortical and accumbens NPY perikarya and

dendrites are of both the symmetrical and asymmetrical type (Aoki and Pickel, 1989;

Massari et al., 1988). NPY colocalizes with somatostatin in both the frontal cortex and

nucleus accumbens (Beal et al., 1987); these neurons are also NADPH-diaphorase

positive (Heilig and Widerlov, 1990). Most cortical NPYLI neurons are also positive

for the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD) (Aoki and

Pickel, 1988). NPYLI neurons in the nucleus accumbens receive direct GABAergic

input (Massari et al., 1988), while direct contact between NPY-terminals and GABAergic

somata or dendrites in the cortex has been observed (Aoki and Pickel, 1988). In

addition, NPY neurons are associated with dopaminergic systems in several brain struc-

tures and reciprocal regulation may occur (Heilig and Widerlov, 1990; Kerkarian et al.,

1988). Specifically, dopamine stimulates NPY gene expression in the fronto-parietal

cortex (Lindefors et al., 1990) and 6-hydroxydopamine (6-OHDA) lesion of nigral dopaminergic neurons produces decreases in accumbens NPYLI (Salin et al., 1990).

As mentioned in the previous chapter, PCP is a potent drug of abuse that produces agitation and a schizophrenia-like psychosis in abusers. PCP is a noncompetitive NMDA antagonist and interacts with a number of neurotransmitters, including dopamine, GABA and serotonin systems (Contreras et al., 1987; Johnson and Jones, 1990). In addition, PCP binds with high affinity to the sigma-opioid receptor (Contreras et al., 1987; Johnson and Jones, 1990). In Chapter 2, we reported that PCP administration significantly affects extrapyramidal NPY systems (Midgley et al., 1992). In this chapter, the profound effects of PCP on limbic NPY systems is described. These effects appear to be differentially regulated in the frontal cortex and nucleus accumbens.

Materials and Methods

Animals and treatments

Male Sprague-Dawley rats (180-270 g, Simonsen Laboratories, Gilroy, Ca) were maintained in a controlled environment as described in Chapter 2. Drugs dissolved in 0.9% saline included: PCP (National Institute on Drug Abuse, Rockville, MD); the serotonin toxin, DL-p-chloroamphetamine HCl (PCA, Sigma Chemical Co., St. Louis, MO); the dopamine D-1 antagonist, SCH 23390 HCl (Research Biochemicals Inc., Natick, MA); the sigma antagonist, rimcazole HCl (Burroughs Wellcome, Research Triangle Park, NC); the GABA-T suicide inhibitor, gamma-vinyl-GABA (Jung et al., 1977) (GVG, vigabatrin, MDL 71,754; Marion Merrell Dow Inc., Cincinnati, OH) and

the NMDA antagonist, MK-801 hydrogen maleate (dizocilpine, Research Biochemicals Inc., Natick, MA). Vehicles used for dissolving the following drugs were as follows: the dopamine D-2 antagonist, ($\pm$) sulpiride (Sigma Chemical Co., St. Louis, MO), in 2% lactate + 25% propylene glycol-saline; the indirect GABA-T inhibitor, aminooxyacetic acid hemi HCL (Churchich, 1982) (AOAA; Sigma Chemical Co., St. Louis, MO), in saline neutralized with 20% 1 N NaOH. All drugs were administered intraperitoneally with the exception of PCP which was administered subcutaneously. PCA-treated rats received two doses (10 mg/kg/dose) 24 h apart, 1 week prior to PCP challenge. We have found this dosing regimen depletes striatal serotonin levels by 85% (Smiley et al., 1990). Animals administered GVG received two doses (1200 mg/kg/dose) 48 and 1 h prior to PCP challenge. Drug doses, except for MK-801, were calculated for their free forms. Control animals received identical treatment regimens using vehicles only.

In acute recovery studies, animals received a single dose of PCP (15 mg/kg) and were sacrificed 6, 12 and 24 h after drug injection. For multiple dose experiments, animals received five doses of PCP (15 mg/kg/dose), MK-801 (1 mg/kg/dose) or antagonist/agonist (unless otherwise specified above) 6 h apart and were sacrificed at 10, 18, 34 or 58 h after the final dose for recovery studies and 12-15 h after the final dose for combination drug studies. All antagonists and agonists were given 20 min prior to PCP administration, unless otherwise specified.

All animals were sacrificed by decapitation at the indicated times following treatment. Brains were rapidly removed and placed on ice, frontal cortices were excised, and the remaining brain tissues immediately frozen on dry ice and stored at -80°C until

assayed for NPYLI. The nucleus accumbens was identified according to the atlas of Konig and Klippel (1963), bilaterally dissected out from 1.0-mm thick frozen coronal slices and stored at -80°C until assayed for NPYLI content.

Neuropeptide Y radioimmunoassay

The antiserum to NPY was prepared in our laboratory as described in Chapter 2. The resultant NPY antiserum reliably detects 8 pg of NPY when assayed at an antiserum dilution of 1:15,000. Results of cross reactivity assays and a description of the radioimmunoassay for NPYLI were presented in the previous chapter. Results are expressed as pg/mg protein of NPYLI.

To facilitate comparisons between groups, results in all figures are shown as percentages of respective controls. The NPYLI control values are included in the figure legends. Bars represent the mean of treatment groups $\pm$ S.E.M. Data were analyzed using a one factor ANOVA; if the F ratio was significant, a Fischer-PLSD (Protected Least Significant Difference) test was used to compare differences between the means of individual groups. Unless otherwise specified, differences were considered significant when the probability that they were zero was less than 5%.

Results

Effects of acute and multiple doses of PCP on cortical and

accumbens NPY systems after varying times

The time course for effects of acute and multiple doses of PCP on cortical and accumbens NPY systems is described in Figures 3.1 and 3.2. To evaluate the response of cortical and accumbens NPY systems to acute PCP administration, a single dose of PCP (15 mg/kg) was administered and rats were sacrificed 6, 12 or 24 h after injection. After acute treatment, cortical NPYLI levels were unchanged at 6 h, decreased to 74% of control at 12 h, and returned to control by 24 h (Figure 3.1 A). In contrast, accumbens NPYLI levels were significantly decreased to 76% of control at 6 h, and recovered by 12 h (Figure 3.2 A).

To evaluate the response of cortical and accumbens NPY systems to multiple doses of PCP, animals received five injections of PCP (15 mg/kg/dose) at 6-h intervals, and were sacrificed 10, 18, 34 or 58 h after the last dose. Cortical NPYLI levels were decreased to 52% and 62% after 10 and 18 h, respectively, and returned to control by 34 h (Figure 3.1 B). NPYLI levels in the nucleus accumbens were decreased to 49, 44 and 68% after 10, 18 and 34 h, respectively, and recovered by 58 h (Figure 3.2 B).
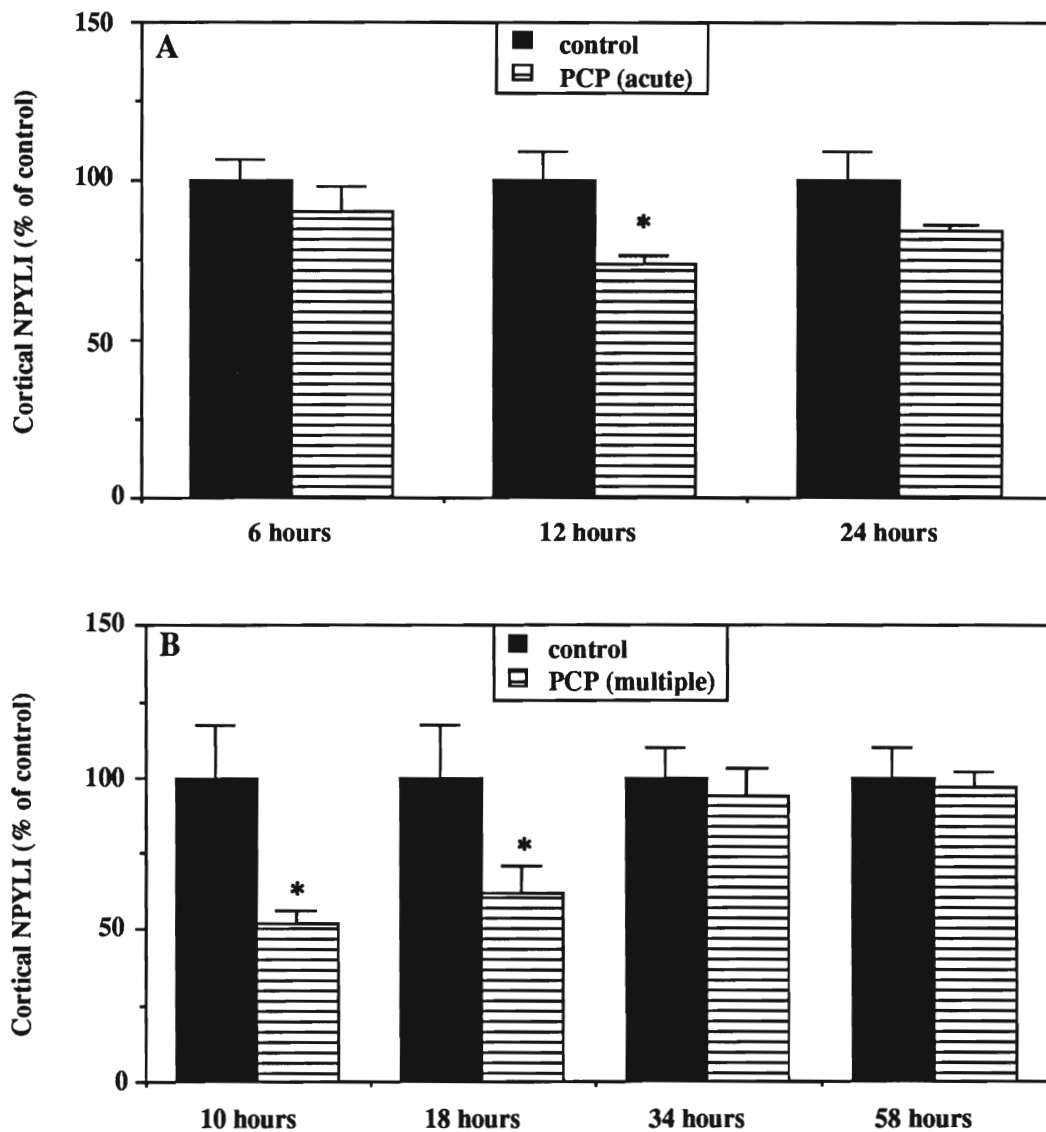
Figure 3.1. Effects of acute and multiple administrations of PCP on cortical NPYLI content. A. Rats received a single dose of PCP (15 mg/kg) and were sacrificed 6, 12 or 24 h after injection. B. Rats were administered five doses of PCP (15 mg/kg/ dose) 6 h apart and sacrificed 10, 18, 34 or 58 h after the final dose. Results shown in both panels are expressed as percent of respective control ± S.E.M. (n=5-6). The average value for control NPYLI levels was 598 ± 49 pg/mg protein. * p < 0.05 vs. respective control.
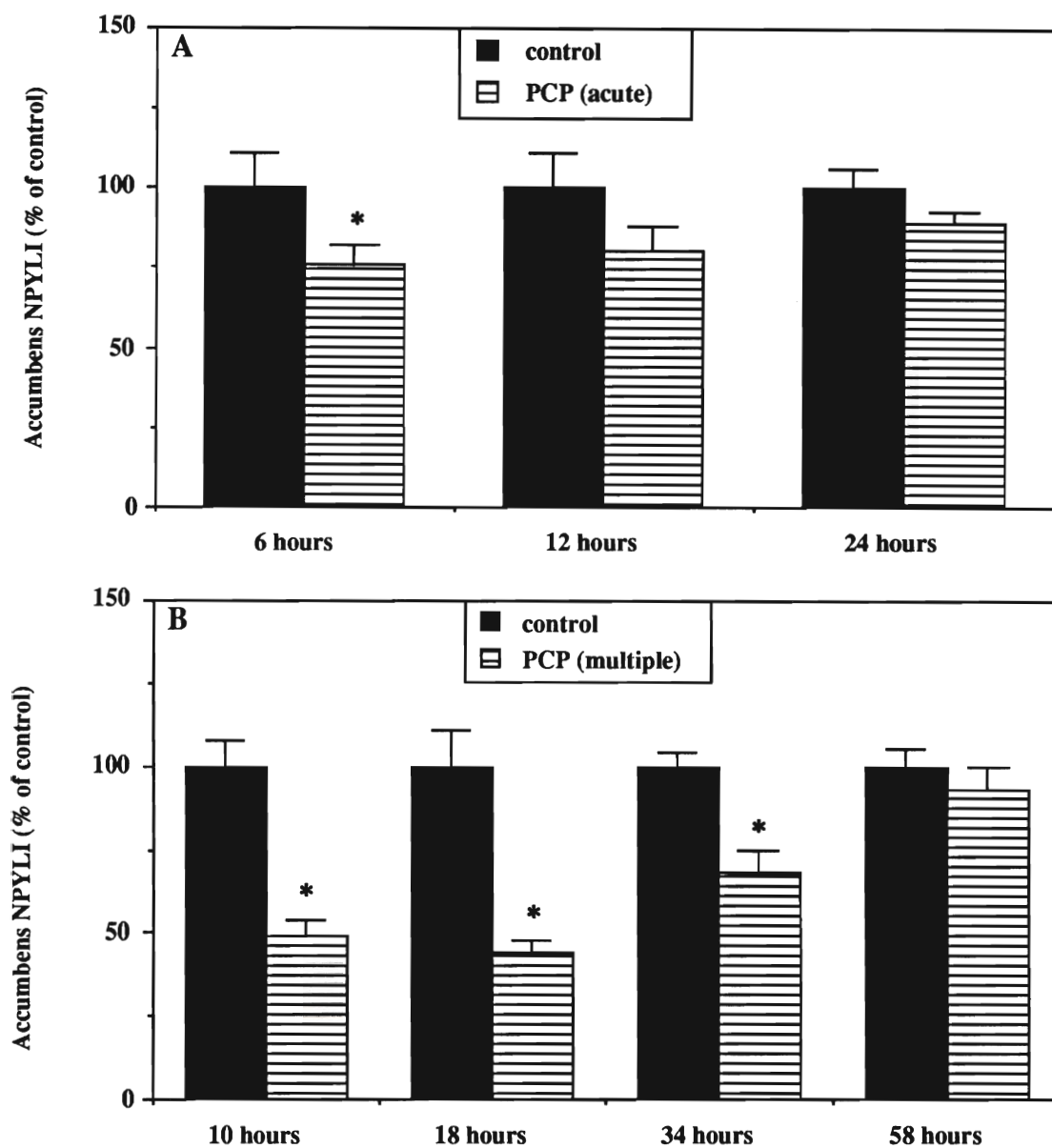
Figure 3.2. Effects of acute and multiple administrations of PCP on NPYLI content in the nucleus accumbens. Rats were treated with one (A) or multiple (B) doses of PCP as described for Figure 3.1. Results in both panels are expressed as percent of respective control ± S.E.M. (n=6). The average value for control NPYLI levels was 6842 ± 273 pg/mg protein. * p < 0.05 vs. respective control.

Effects of 5-hydroxytryptamine depletion and sigma, dopamine

D-1 and D-2 receptor blockade alone and in combination

with PCP on cortical and accumbens NPY systems

To assess the role of selected monoamine pathways in regulating cortical and accumbens NPY systems and PCP-induced changes in NPYLI content, 5-hydroxytrypta-mine was depleted or selective dopaminergic antagonists were administered alone and in combination with PCP (Figures 3.3 and 3.4). In addition, the possible role of sigma receptors in the regulation of these systems and in the PCP-induced changes was evaluated by cotreatment with the selective sigma antagonist, rimcazole. 5-hydroxytryp-tamine content was depleted with PCA (10 mg/kg/dose) pretreatment as described in Materials and Methods. Animals received five doses of PCP (15 mg/kg/dose), antagonists or combinations at 6-h intervals and were sacrificed approximately 10-15 h after the final dose. All antagonists were administered 20 min before each injection of PCP. Neither the selective D-1 antagonist, SCH 23390 (0.5 mg/kg/dose), the selective D-2 antagonist, sulpiride (80 mg/kg/dose), 5-HT depletion or the putative sigma antagonist, rimcazole (12.5 mg/kg/dose) alone had any effect on cortical NPYLI content (Figure 3.3 A). In contrast, accumbens NPYLI content was significantly decreased to 73% and 85% of control by sulpiride and PCA, respectively (Figure 3.4 A). The selective D-1 antagonist, SCH 23390, attenuated PCP-induced NPYLI changes in the cortex and accumbens (from 73% and 54% to 92% and 71%, respectively) (Figures 3.3 A and 3.4 A). The effects of the selective D-2 antagonist, sulpiride, appeared to be additive with PCP-induced NPYLI decreases in the accumbens (Figure 3.4 A).
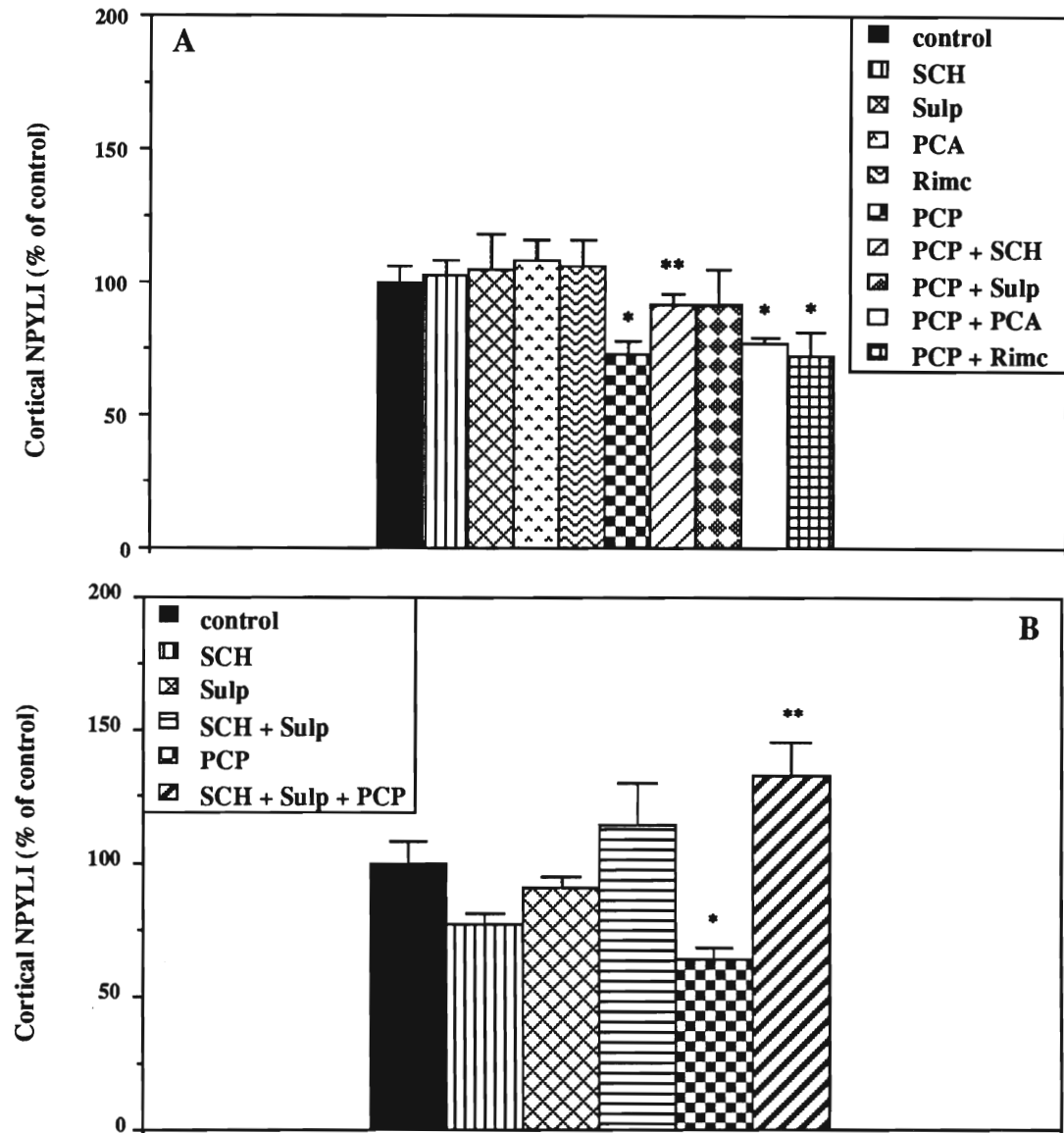
Figure 3.3. Effects of 5-HT depletion or selective sigma, dopamine D-1 or D-2 receptor blockade alone and in combination with PCP on cortical NPYLI content. A. 5-HT was depleted with PCA, or animals received multiple doses of SCH 23390 (SCH; 0.5 mg/kg/dose), sulpiride (Sulp; 80 mg/kg/dose) or rimcazole (Rimc; 12.5 mg/kg/dose) alone and in combination with PCP (15 mg/kg/dose) as described in Materials and Methods. Average control value for NPYLI levels was 948 ± 61 pg/mg protein. B. Rats were administered multiple doses of SCH 23390 (0.5 mg/kg/dose), sulpiride (80 mg/kg/dose) or a combination of the two with or without PCP (15 mg/kg/dose). Average control value for NPYLI levels was 666 ± 52 pg/mg protein. Rats in both experiments were sacrificed approximately 10-15 h after the final dose. Results are expressed as percent of control $\pm$ S.E.M. (n=6). *p < 0.05 vs. control; **p < 0.05 vs. PCP.
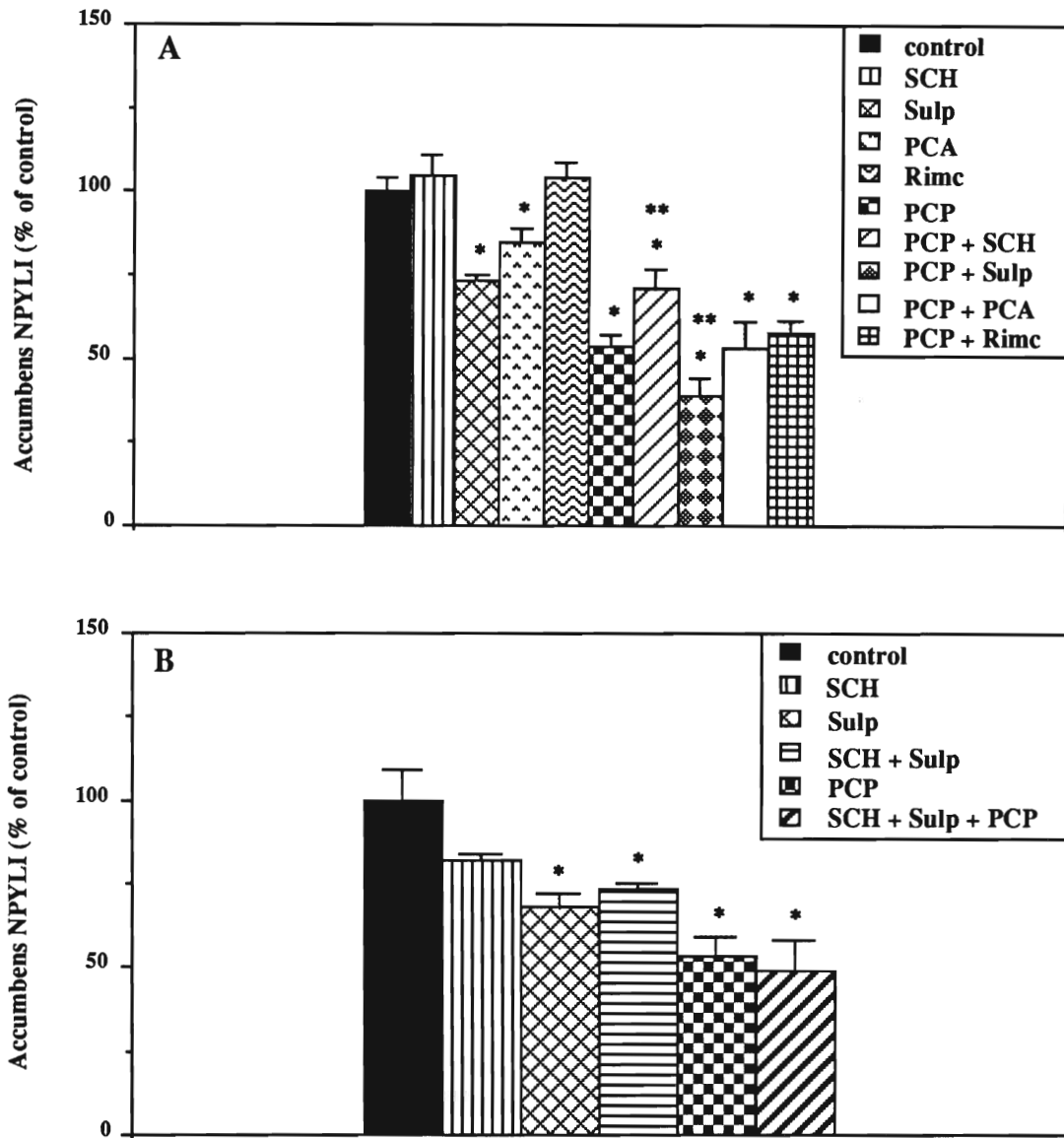
Figure 3.4 Effects of 5-HT depletion or selective sigma, dopamine D-1 or D-2 receptor blockade alone and in combination with PCP on NPYLI content in the nucleus accumbens. Rats were treated as described for Figure 3.3. Average control value for NPYLI levels in Figure 3.4 A was 2695 ± 107 pg/mg protein. Average control value for NPYLI levels in Figure 3.4 B was 1916 ± 171 pg/mg protein. Results are expressed as percent of control $\pm$ S.E.M. (n=6). * $p < 0.05$ vs. control; ** $p < 0.05$ vs. PCP.

However, sulpiride did not enhance PCP-induced changes in the cortex (Figure 3.3 A). Neither PCA or rimcazole altered PCP-induced changes in either structure (Figures 3.3 A and 3.4 A).

To evaluate further the differences in dopaminergic regulation of cortical and accumbens NPY systems and the role of DA pathways in PCP-induced changes in NPYLI content, we administered SCH 23390, sulpiride, and a combination of the two drugs alone and with PCP (Figures 3.3 B and 3.4 B). Treatment paradigms were similar to those used for corresponding A panels. Coadministration of SCH 23390 plus sulpiride had no effect on NPYLI content in the frontal cortex but completely blocked PCP-induced decreases (from 64% to 125% of control) in cortical NPYLI levels (Figure 3.3 B). In contrast, coadministration of SCH 23390 and sulpiride decreased accumbens NPYLI content (to 73% of control) in a manner similar to sulpiride alone (68% of control) and did not significantly alter PCP-induced NPYLI decreases (53% of control) (Figure 3.4 B).

Effects of multiple doses of MK-801 and PCP with and without

GABA-enhancing drugs on NPYLI content in the frontal

cortex and nucleus accumbens

To investigate the role of GABA in regulating cortical and accumbens NPY and the response to PCP treatment, we administered selective GABA-T inhibitors alone and in combination with PCP (15 mg/kg/dose)(Figures 3.5 and 3.6). In addition, to determine if the effects of PCP on NPYLI content in these structures were the result of

Figure 3.5 Effects of multiple MK-801 (MK) or PCP administrations with and without GABA agonists on cortical NPYLI content. Rats were treated with GVG (see Materials and Methods) or AOAA (15 mg/kg/dose) alone and in combination with MK-801 (1 mg/kg/dose) or PCP (15 mg/kg/dose). Rats were sacrificed approximately 15 h after the final dose. Results are expressed as percent of control $\pm$ S.E.M. (n=4-9). Average control value for NPYLI content was 492 ± 30 pg/mg protein. * $p < 0.05$ vs. control; ** $p < 0.05$ vs. PCP; § $p < 0.05$ vs. MK-801.
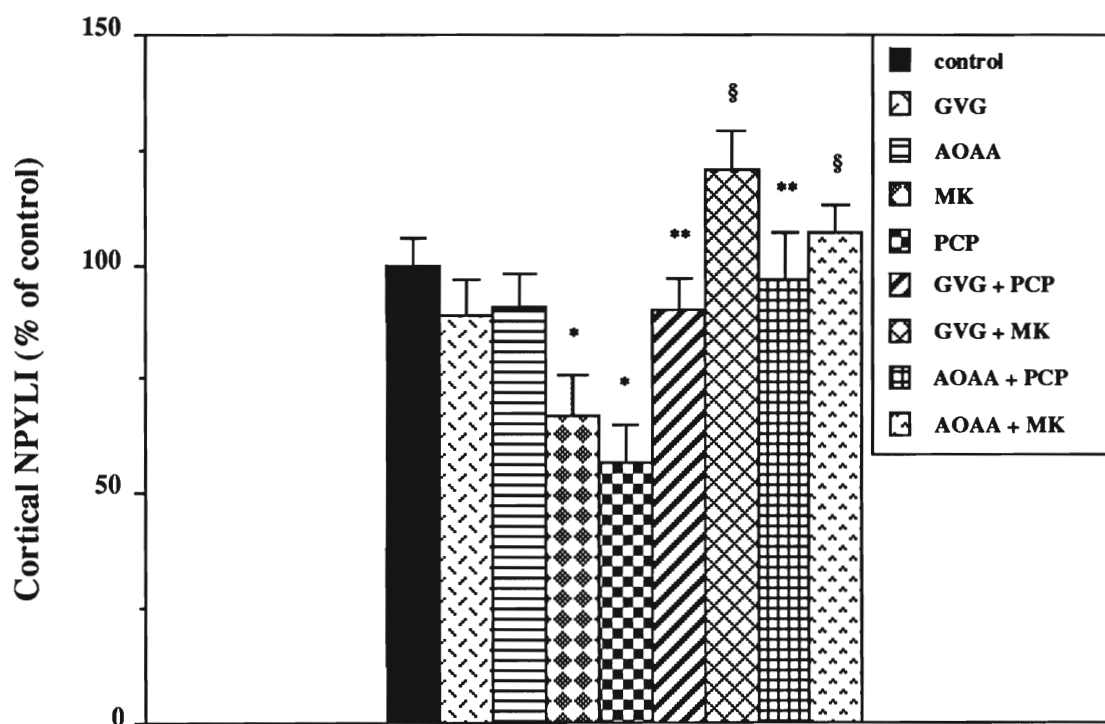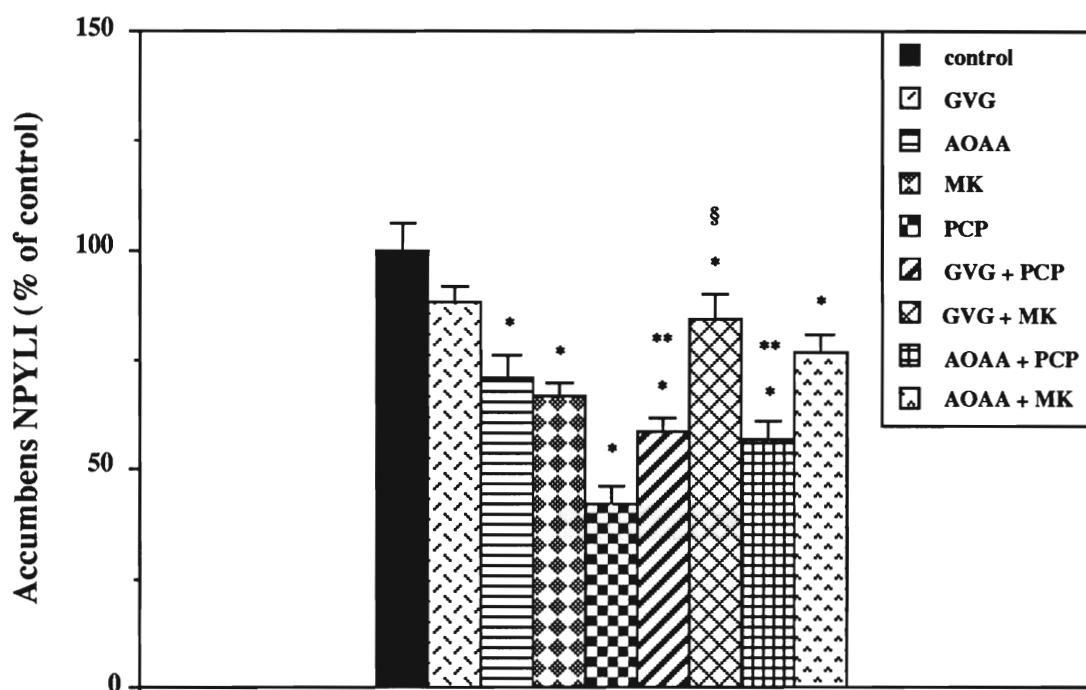
Figure 3.6. Effects of multiple MK-801 (MK) or PCP administrations with and without GABA agonists on NPYLI content in the nucleus accumbens. Rats were treated as described for Figure 3.5. Results are expressed as percent of control ± S.E.M. (n=5-9). Average control value for NPYLI content was 2937 ± 255 pg/mg protein. * p < 0.05 vs. control; ** p < 0.05 vs. PCP; § p < 0.05 vs. MK-801.

NMDA receptor blockade, MK-801 (1 mg/kg/dose), a PCP-like noncompetitive NMDA antagonist, was administered in the same manner as PCP (Figures 3.5 and 3.6). Animals received five doses of PCP, MK-801 or AOAA (15 mg/kg/dose) at 6-h intervals and were sacrificed approximately 10-15 h after the final dose. GVG was administered as described in Materials and Methods. PCP alone decreased cortical and accumbens NPYLI content to 57% and 42% of control, respectively; MK-801 alone decreased cortical and accumbens NPYLI levels in a PCP-like manner to 67% of control in both structures (Figures 3.5 and 3.6). Higher doses of MK-801 produced quantitatively similar changes to this dose of PCP (data not shown), but were not used due to potential lack of selectivity. The indirect GABA agonists, GVG and AOAA, had no effect by themselves on cortical NPYLI levels; AOAA alone decreased (to 71% of control) accumbens NPYLI content. Coadministration of either GABA-T inhibitor completely blocked both the PCP and MK-801-induced cortical decrease in NPYLI content. However, the effect of enhancing GABA activity on PCP or MK-801-induced changes in accumbens NPY systems is more difficult to interpret since at least one of the GABA-T inhibitors (AOAA) alone appeared to have an effect on accumbens NPYLI levels. GVG and AOAA attenuated accumbens PCP-induced decreases from 42% to 60% and 58%, respectively. GVG significantly attenuated MK-801-induced accumbens effects from 67% to 86%; the trend towards attenuation by AOAA from 67% to 78% did not reach statistical significance. It is not clear if enhancing GABA activity totally blocked the PCP and MK-801 effects on accumbens NPYLI levels because AOAA plus PCP or MK-801 was not different from AOAA alone nor was GVG plus MK-801 different from

GVG alone. GVG plus PCP was somewhat different in that the combination effect was intermediate between either drug alone. Even so, from these data it is clear that GABA systems are involved in the response of limbic NPY pathways to the effects of PCP and MK-801.

Discussion

Acute and multiple dose experiments revealed that PCP administration has profound effects on NPY systems in the frontal cortex and nucleus accumbens. The present data also suggest that cortical and accumbens NPY systems are differentially regulated. The disparate time courses for PCP-induced NPYLI decreases in these two structures could reflect differences in drug distribution, regulation by other transmitter systems, release and turnover of the peptide, or peptide synthesis. Additional experiments must be conducted to determine which of these possibilities are responsible for the differences.

PCP alters brain dopaminergic and serotonergic parameters and binds to sigma opioid receptors (Contreras et al., 1987; Johnson and Jones, 1990). Central NPY systems interact with dopaminergic and serotonergic systems as well as with sigma receptors (Heilig et al., 1990; Roman et al., 1989; Schlicker et al., 1991). Thus, in order to determine if any of these systems are involved in mediating the PCP effects on limbic NPY pathways, we depleted 5-HT with PCA or administered selective dopaminergic or sigma antagonists alone and in combination with PCP (Figures 3.3 and 3.4). Interestingly, there were significant differences in the responses of cortical and

accumbens NPY systems to the selective dopaminergic antagonists alone and in combination with PCP, supporting the hypothesis of differential regulation. The selective D-1 antagonist, SCH 23390, had no significant effect on either cortical or accumbens NPYLI levels. However, in both structures coadministration of SCH 23390 with PCP attenuated PCP-induced effects (Figures 3.3 A and 3.4 A), suggesting that the PCP-induced decreases in both structures are mediated in part through D-1 receptors. The selective D-2 antagonist, sulpiride, alone significantly decreased accumbens NPYLI content but had no effect on cortical NPYLI levels, indicating that accumbens, but not cortical, D-2 receptors may be important in maintaining basal NPY levels. Coadministration of sulpiride with PCP had no significant effect on cortical PCP-induced changes, yet enhanced PCP-induced decreases in accumbens NPYLI content (Figures 3.3 A and 3.4 A). This enhancement of PCP's effects in the accumbens could be due to the summation of separate or similar mechanisms: this study cannot distinguish between these two possibilities. In additional experiments, we administered SCH 23390 and sulpiride together with PCP. Coadministration of SCH 23390 and sulpiride had no significant effect on cortical NPYLI levels and decreased accumbens NPYLI levels like sulpiride alone. Significantly, administration of the D-1 and D-2 antagonists together completely blocked PCP-induced cortical NPYLI changes but had no effect on PCP-induced NPYLI decreases in the nucleus accumbens. These data suggest that the dopaminergic system through both D-1 and D-2 receptors plays an important role in mediating PCP-induced changes in the cortex. In contrast, only the D-1 receptor has a partial role in mediating PCP-induced decreases in accumbens NPYLI levels.

Collectively, these dopamine antagonist data indicate that there are important differences in the regulation of cortical and accumbens NPY systems by the dopaminergic system.

Depletion of 5-HT with PCA produced no change in cortical NPYLI content and a small but significant decrease (15%) in accumbens NPYLI level. This decrease could reflect direct or indirect interactions between accumbens NPY and serotonin systems. However, in neither structure did PCA pretreatment alter PCP-induced changes, suggesting that 5-HT systems do not play an important role in mediating PCP-induced NPYLI decreases in the cortex and accumbens (Figure 3.3 and 3.4).

Administration of the selective sigma antagonist, rimcazole, had no effect on NPYLI content alone nor did it alter the PCP-induced NPYLI changes in either structure (Figures 3.3 A and 3.4 A). These data suggest that sigma receptor activity has little effect on cortical and accumbens NPY systems nor is it involved in mediating PCP-induced changes in limbic NPY pathways.

In 1988, Pin et al. demonstrated that NMDA-evoked GABA release from primary cultures of striatal neurons could be blocked in a competitive or noncompetitive manner by DL-2-amino-5-phosphovalerate (APV) or PCP, respectively. In addition, Young and Bradford (1990) reported that NMDA releases GABA from rat striatum in vivo. We recently observed that administration of both PCP and the more selective noncompetitive NMDA antagonist, MK-801, significantly decreased striatal NPYLI levels; moreover, these changes were completely blocked by coadminstration of inhibitors of the primary degradative enzyme for GABA, GABA-transaminase (GABA-T) (Midgley et al., 1992). Because of the NMDA effects of PCP and the fact that both cortical and accumbens NPY

systems interact with GABAergic systems (Massari et al., 1988; Aoki and Pickel, 1989),

we administered the GABA-T inhibitors, AOAA and GVG, alone or in combination with

PCP and MK-801 (Figure 3.5 and 3.6). Administration of GVG or AOAA alone had

no effect on cortical NPYLI content. In the nucleus accumbens, GVG had no significant

effect on NPYLI levels whereas AOAA decreased NPYLI content to 71% of control.

This difference is possibly due to the distinct mechanisms of the two drugs. GVG is a

selective suicide inhibitor of GABA-T whereas AOAA inhibits binding of the cofactor

(pyridoxal phosphate) to GABA-T, and is thus less selective (Jung et al., 1977;

Churchich, 1982). Multiple administrations of MK-801 decreased NPYLI content in both

limbic structures in a manner similar to PCP, confirming that cortical and accumbens

NPY-containing neurons are modulated by glutamatergic activity through NMDA

receptors. Coadministration of GVG and AOAA completely blocked both PCP- and MK-

801-induced cortical NPYLI changes, suggesting that glutamatergic modulation of

cortical NPY-containing neurons is mediated primarily by GABAergic systems (Figure

3.5). The results in the nucleus accumbens are more difficult to explain. In this tissue,

GVG appeared to attenuate only PCP- and MK-801-induced changes. AOAA

significantly attenuated the PCP-induced decrease in accumbens NPYLI levels but not

MK-801 induced changes; this lack of attenuation of MK-801-induced effects was likely

due to the decrease in NPYLI content produced by AOAA alone and to the less dramatic

decreases in NPYLI levels produced by this dose of MK-801. These data suggest that

glutamatergic modulation of accumbens NPY-containing neurons is only partially

mediated by GABAergic systems, and that differential regulation of cortical and accumbens NPY systems by GABAergic systems exists.

In summary, the results of this study indicate that PCP has a profound, reversible effect on cortical NPY systems which is likely due to the effects of this drug on NMDA, GABAergic and dopaminergic systems. Specifically, these data demonstrate that regulation of cortical NPY pathways by NMDA receptors occurs through GABAergic mechanisms. The complete blockade of PCP-induced cortical NPYLI decreases by coadministration of dopamine D-1 and D-2 receptor antagonists suggests that dopaminergic receptor activation is also important in mediating these PCP-induced changes. These studies also suggest that accumbens NPY systems are profoundly and reversibly affected by PCP administration, likely due to the effects of this drug on NMDA systems. In contrast to the cortex, GABAergic mechanisms appear to mediate only partially NMDA receptor regulation of accumbens NPY systems. In addition, accumbens NPY systems seem to be tonically regulated by D-2 receptor activity, and PCP-induced accumbens NPYLI changes partially mediated through the D-1 receptor. Further studies must be performed to clarify the relationship between NMDA, dopaminergic and GABAergic systems in the regulation of NPY-containing neurons in the limbic system.

The significance of limbic NPY alterations in response to a psychotomimetic drug such as PCP cannot presently be ascertained. Decreases in NPYLI levels can be caused by (1) an increase in the neuronal release of NPY, resulting in increased metabolism by degrading peptidases, (2) a decrease in synthesis or, (3) a combination of both (1) and (2). Additional research is necessary to identify the mechanisms involved. Because of

the possible association of NPY with psychiatric disorders (Widerlov et al., 1989), changes in NPY systems by PCP may contribute to the psychiatric manifestations of this drug. Furthermore, the differential regulation and modulation of these limbic NPY systems could suggest problems or solutions in treatment strategies for psychiatric disorders in which NPY systems are thought to be involved.

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CHAPTER 4

DYNAMIC DOPAMINERGIC REGULATION OF

NEUROPEPTIDE Y SYSTEMS IN DISCRETE

STRIATAL AND ACCUMBENS REGIONS

Introduction

Numerous studies support the hypothesis that neuropeptide Y and dopaminergic

(DA) systems are interrelated and that reciprocal regulation occurs. Centrally adminis-

tered NPY produces a dose-related increase in the levels of DA and its metabolite,

DOPAC, in striatum, frontal and parietal cortex, hypothalamus and brainstem in the rat

(Heilig and Widerlov, 1990). Immunohistochemical data indicate that NPY-immunoreac-

tive (NPYIR) neurons receive synaptic input from tyrosine hydroxylase-immunoreactive

axon terminals in the rat neostriatum (Kubota et al., 1988; Vuillet et al, 1989). A

unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal dopamine pathway

markedly increases NPYLI in striatal perikarya in the ipsilateral deafferented side, which

can be reversed by administration of the direct-acting dopamine agonist, apomorphine.

Interestingly, administration of the nonselective dopamine receptor antagonist,

haloperidol, or the dopamine synthesis inhibitor, alpha-methyl-p-tyrosine, produces a

decrease in the number and labeling intensity of striatal NPY like-immunoreactive cells

(Kerkarian et al., 1988). Salin et al. (1990) showed that unilateral 6-OHDA lesions of

the nigrostriatal dopaminergic pathway result in bilateral decreases in accumbens NPY neuron density which are more marked on the contralateral than ipsilateral side. This effect is completely reversed ipsilaterally and partially reversed on the contralateral side by apomorphine administration. Lindefors et al. (1990) showed that unilateral 6-OHDA lesions increase NPY and somatostatin mRNA in the caudate-putamen and decrease NPY and somatostatin mRNA in the fronto-parietal cortex, suggesting tonic inhibition and stimulation, respectively, of NPY-containing neurons by DA in these structures. These data indicate an important, albeit complex, role for dopamine in the regulation of telencephalic NPY-containing neurons.

To understand further the role of DA in the regulation of striatal NPY-containing neurons, several investigators have utilized selective D-1 and D-2 receptor antagonists and agonists. Immunohistochemical data indicate that the selective D-2 antagonist, sulpiride, decreases, while the selective D-1 antagonist, SCH 23390, produces slight increases in the number of striatal NPYLI cells (Kerkerian et al., 1988). These data suggest an opposite role for D-1 and D-2 receptor subtypes in dopaminergic control of rat striatal NPY neurons. We previously reported that multiple dosing with either the selective D-2 antagonist, sulpiride, or the D-2 agonist, quinpirole, significantly decreases rat striatal NPYLI content (Midgley et al, 1989). We observed no significant differences in striatal NPYLI content after similar administrations of either the selective D-1 antagonist, SCH 23390, or the D-1 agonist, SKF 38393. Engber et al. (1992) reported that intermittent quinpirole administration decreases, while continuous quinpirole administration increases rat striatal NPYLI levels. In addition, they observed that both

intermittent and continuous administration of the D-1 agonist, SKF 38393, significantly reduces striatal NPYLI content. These findings suggest that striatal NPY systems are regulated by selective dopaminergic activity at specific postsynaptic or presynaptic receptors. In this chapter we elucidate further the role of DA in modulating NPY systems in the rat basal ganglia and limbic system, and the response of these NPY systems to stimulation or blockade of D-1 or D-2 receptors. We observed significant differences in dopaminergic regulation of NPY systems in discrete structures within extrapyramidal and limbic systems.

Materials and Methods

Animals and treatments

Male Sprague-Dawley rats (180-270 g, Simonsen Laboratories, Gilroy, CA) were maintained in a temperature-controlled environment as previously described. The dopamine D-1 antagonist, SCH 23390 (Research Biochemicals Inc., Natick, MA), was dissolved in 0.9% saline. The dopamine D-1 agonist, SKF 38393 (Research Bio-chemicals, Inc.), and the dopamine D-2 antagonist, ($\pm$) sulpiride (Sigma Chemical Co., St. Louis, MO), were dissolved in 2% lactate + 25% propylene glycol-saline; the dopamine D-2 agonist, quinpirole (Lilly 171555; Research Biochemicals Inc.), was dissolved in 1% lactate-saline. Animals received five doses of SKF 38393 (20 mg/kg/dose, i.p.), quinpirole (5 mg/kg/dose, i.p.), SCH 23390 (0.5 mg/kg/dose, i.p.) or sulpiride (80 mg/kg/dose, i.p.) at 6-h intervals and were sacrificed 18 h (single time point study) or 3, 12 and 48 h (recovery study) after the final dose.

Dissections

All animals were sacrificed by decapitation at the indicated times following treatment. For whole structure analyses, brains were rapidly removed and placed on ice, frontal cortex and caudate-putamen excised, and the remaining brain tissues immediately frozen on dry ice and stored at -80⁰C until assayed for NPYLI. Nucleus accumbens and substantia nigrae were identified according to the atlas of Konig and Klippel (1963), bilaterally dissected out from 1.0-mm thick frozen coronal slices and stored at -80⁰ C until assayed for NPYLI content. For regional studies, brains were rapidly removed, frozen on dry ice and stored at -80⁰ C until dissection. The caudate-putamen was dissected into very anterior caudate (VAC), medial anterior caudate (MAC), lateral anterior caudate (LAC), medial posterior caudate (MPC), lateral posterior caudate (LPC) and very posterior caudate (VPC) as represented in Figure 4.1 A-D. Nucleus accumbens was dissected into anterior (ANA) and posterior (PNA) regions as identified in Figure 4.1 A and B. Globus pallidus was dissected according to Figure 4.1 D. Tissue slices were 1-mm thick and dissected posteriorly from the following locations: 2.5 mm anterior to bregma to remove the VAC and ANA, 1.5 mm anterior to bregma for removal of MAC, LAC and PNA, 0.5 mm anterior to bregma to remove MPC and LPC, and 0.5 mm posterior to bregma for removal of VPC and GP. All tissues were dissected on ice and stored at -80⁰ C until assayed for NPYLI content.

Determination of regional NPYLI content

Preparation of the antiserum to NPY and the radioimmunoassay for NPYLI are
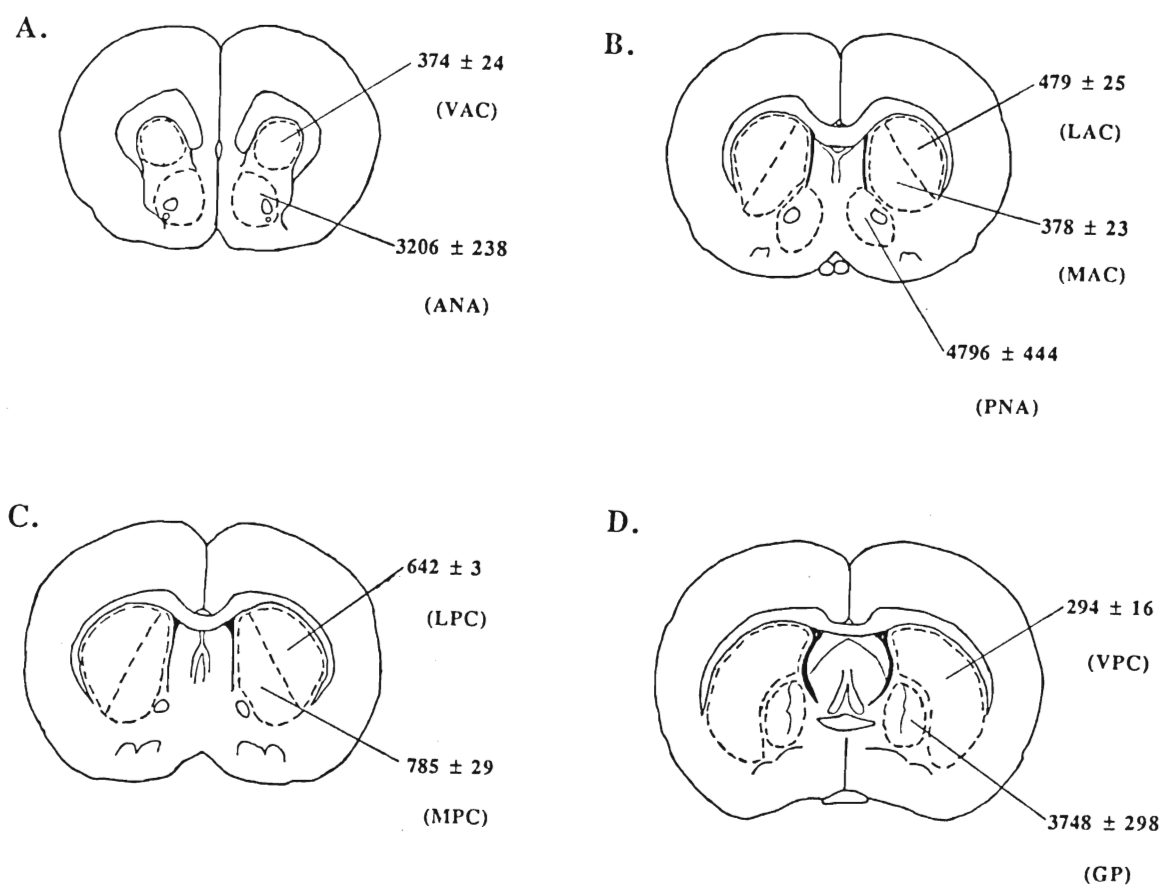
Figure 4.1. Description of regional dissections of caudate, nucleus accumbens and globus pallidus and their respective control levels of NPYLI (pg/mg protein). A. Very anterior caudate (VAC) and anterior nucleus accumbens (ANA), 2.5 mm anterior to bregma. B. Medial anterior caudate (MAC), lateral anterior caudate (LAC) and posterior nucleus accumbens (PNA), 1.5 mm anterior to bregma. C. Medial posterior caudate (MPC) and lateral posterior caudate (LPC), 0.5 mm anterior to bregma. D. Very posterior caudate (VPC) and globus pallidus (GP), 0.5 mm posterior to bregma.

described in Chapter 2. Results are expressed as pg/mg protein of NPYLI.

To facilitate comparisons between groups, results in all figures are shown as percentages of respective controls. The NPYLI control values are included in the figure legends. Bars represent the mean of treatment groups ± S.E.M. Data were analyzed using a one-factor ANOVA; if the F ratio was significant, a Fischer-PLSD (Protected Least Significant Difference) test was used to compare differences between the means of individual groups. Unless otherwise specified, differences were considered significant when the probability that they were zero was less than 5%.

Results

Effects of multiple dose DA agonist and antagonist treatments

on NPYLI content in striatum, substantia nigra, nucleus

accumbens, and frontal cortex.

To investigate the importance of dopaminergic activity in regulating extrapyramidal and limbic NPY systems, we administered multiple doses of the selective D-1 agonist, SKF 38393, the D-2 agonist, quinpirole, the D-1 antagonist, SCH 23390, or the D-2 antagonist, sulpiride, at 6-h intervals and sacrificed the rats 18 h after the final dose (Table 4.1). Neither the dopamine D-1 agonist (SKF 38393) nor the D-1 antagonist (SCH 23390) produced any significant effects on striatal NPYLI content. However, both quinpirole and sulpiride decreased striatal NPYLI levels (69 and 59% of control, respectively). There were no significant effects on nigral NPYLI systems with any of

Table 4.1

Effect of D-1 and D-2 stimulation and blockade on NPYLI content in the striatum, substantia nigra, nucleus accumbens and frontal cortex.

Animals were administered five doses of SKF 38393 (20 mg/kg/dose), quinpirole (5 mg/kg/dose), SCH 23390 (0.5 mg/kg/dose) or sulpiride (80 mg/kg/dose) at 6-h intervals and sacrificed 18 h after the final dose. Results are expressed as percent of control $\pm$ S.E.M. (n= 6-8). Average control values for striatal, nigral, accumbens and cortical NPYLI content were 704 $\pm$ 81, 552 $\pm$ 69, 1618 $\pm$ 67 and 898 $\pm$ 70 pg/mg protein, respectively. * $p < 0.05$ vs. control.

NPYLI (Percent of Control)

	Striatum	Nigra	Accumbens	Cortex
Vehicle	100 $\pm$ 12	100 $\pm$ 13	100 $\pm$ 4	100 $\pm$ 12
SKF 38393	97 $\pm$ 8	123 $\pm$ 7	* 79 $\pm$ 4	84 $\pm$ 7
Quinpirole	* 69 $\pm$ 6	110 $\pm$ 15	* 69 $\pm$ 7	78 $\pm$ 3
SCH 23390	102 $\pm$ 8	124 $\pm$ 6	103 $\pm$ 3	89 $\pm$ 6
Sulpiride	* 59 $\pm$ 8	109 $\pm$ 7	* 66 $\pm$ 5	89 $\pm$ 7

the treatments. The response in the nucleus accumbens was similar to that in the striatum. The D-1 antagonist (SCH 23390) had no significant effect, while quinpirole and sulpiride decreased accumbens NPYLI content to 69 and 66% of control, respectively. However, in contrast to the striatum, the D-1 agonist, SKF 38393, produced a small but significant decrease (to 79% of control) in accumbens NPYLI content. There were no significant effects on cortical NPYLI levels with any of these drug administrations. In addition, acute DA agonist and antagonist treatments produced no effects on NPYLI content in any of the identified structures (data not shown).

Response of NPY systems in anterior caudate regions to D-1 and
D-2 receptor stimulation and blockade after various times

To characterize further the effects of dopaminergic activity on extrapyramidal NPY systems, we administered SKF 38393, quinpirole, SCH 23390 or sulpiride as described in the previous section and sacrificed the animals 3, 12 or 48 h after the final dose (Figure 4.2). The anterior caudate was dissected into three portions (VAC, MAC and LAC) from frozen tissue as identified in Figure 4.1 A and B. At the 3-h time point, significant alterations in NPY content were found only in the quinpirole group (to 76% of control) in the VAC and the sulpiride group (to 65% of control) in the MAC; in the LAC, both quinpirole and sulpiride significantly decreased 3-h NPYLI levels to 59% and 55% of control, respectively. Twelve hours after treatment, quinpirole had comparably decreased NPYLI levels in the VAC, MAC and LAC to 47, 65 and 59% of control, respectively. Interestingly, sulpiride also decreased NPYLI content in the VAC, MAC

Figure 4.2. Effects of multiple doses of dopamine agonists and antagonists on NPY systems in discrete regions of the anterior caudate after various times. Rats were given five doses of SKF 38393 (20 mg/kg/dose), quinpirole (5 mg/kg/dose), SCH 23390 (0.5 mg/kg/dose), or sulpiride (80 mg/kg/dose) and sacrificed 3, 12 or 48 h after the final dose. Average values for control NPYLI levels are shown in Figure 4.1. Results are expressed as percent of respective control ± S.E.M. (n=6).
* p < 0.05 vs. respective controls.

and LAC to 71, 35 and 55% of control, respectively, at this time point. However, at 12 h, regional differences occurred in response to alterations in dopamine D-1 receptor activity. The D-1 agonist, SKF 38393, decreased NPYLI levels in the VAC to 59% of control, while the D-1 antagonist, SCH 23390, significantly increased NPYLI content in the LAC to 124% of control. No effects in the MAC occurred with either D-1 agonist or antagonist treatment at 12 h. After 48 h, NPYLI content in all treatment groups in the MAC had returned to control levels. Conversely, NPYLI levels in the LAC in SKF 38393-, SCH 23390-, quinpirole- and sulpiride-treated groups were decreased to 74, 63, 68 and 61% of control, respectively. NPYLI content in the VAC remained decreased to 57 and 59% of control in animals receiving SKF 39393 and quinpirole treatments, respectively; NPYLI levels in the SCH 23390 treatment group remained unchanged while the sulpiride treatment group had returned to control (Figure 4.2 A).

Response of NPY systems in posterior caudate regions to

D-1 and D-2 receptor stimulation and blockade

after various times

To evaluate the effects of dopaminergic activity on NPY systems in the posterior caudate, SKF 38393, SCH 23390, quinpirole and sulpiride were administered as previously described and the posterior caudate dissected from frozen tissues (MPC, LPC and VPC; Figure 4.3 A, B and C, respectively) as identified in Figure 4.1 C and D. Three hours after the final dose, quinpirole had decreased NPYLI content in the MPC,
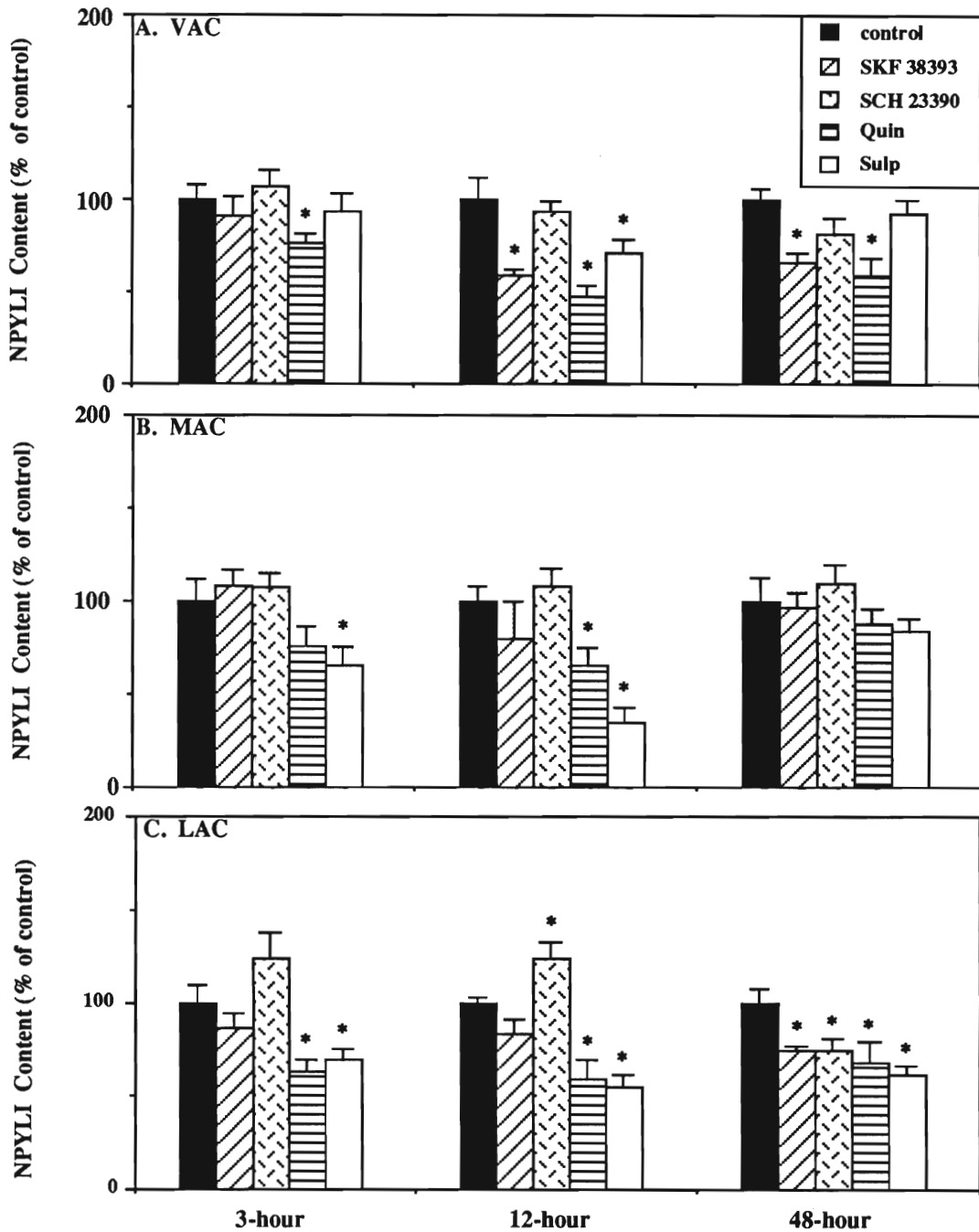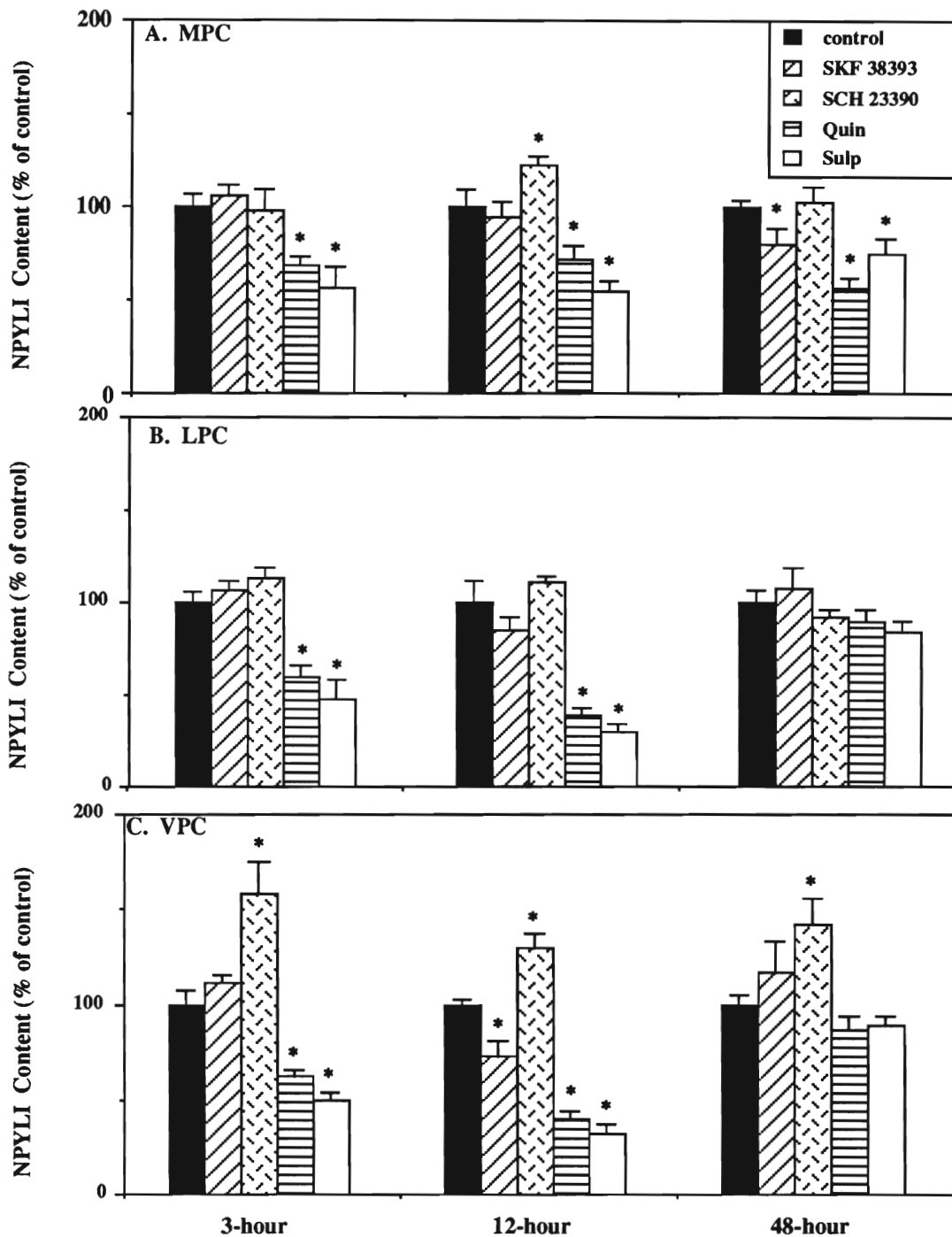
Figure 4.3. Effects of multiple doses of dopamine agonists and antagonists on NPY systems in discrete regions of the posterior caudate after various times. Animals were treated as for Figure 4.2. Average values for control NPYLI levels are shown in Figure 4.1. Results are expressed as percent of respective control ± S.E.M. (n=6). * p < 0.05 vs. respective control.

LPC and VPC to 69, 60 and 63% of control, respectively, while sulpiride had decreased NPYLI levels in these structures to 57, 48 and 50%, respectively. NPYLI content in the VPC was increased by SCH 23390 administration to 158% of control, while SKF 39393 had no effect on any of the three structures at this time point. At 12 h after treatment, quinpirole had decreased NPYLI levels in the MPC, LPC and VPC to 72, 39, and 40% control, respectively; sulpiride had decreased these levels to 55, 30 and 32% of control, respectively. NPYLI content in the VPC remained elevated 12 h after SCH 23390 administration to 130% of control; in the MPC the SCH 23390 treatment group was increased to 122% of control. In addition, SKF 38393 administration significantly reduced NPYLI levels in the VPC to 73% of control at this time point. After 48 h recovery, NPLI levels in all treatment groups in the LPC had returned to control. In the MPC, NPYLI levels in the SCH 23390 group had returned to control, but had decreased in the SKF 38393 group to 75% of control. NPYLI content in the MPC remained decreased to 57 and 75% of control, respectively, 48 h after quinpirole and sulpiride administration. In the VPC, NPYLI content in all treatment groups had returned to control except for the SCH 23390 group which remained increased to 142% of control (Figure 4.3 C).

Response of NPY systems in anterior and posterior nucleus

accumbens to D-1 and D-2 receptor stimulation and

blockade after various times

To assess the involvement of dopaminergic receptor activity in the regulation of

limbic NPY systems, we administered SKF 38393, SCH 23390, quinpirole and sulpiride

as previously described and dissected the nucleus accumbens into anterior (Figure 4.4 A,

ANA) and posterior (Figure 4.4 B, PNA) portions as identified in Figure 4.1 A and B.

Three hours after treatment, only SCH 23390 administration significantly altered NPYLI

content in the ANA (to 140% of control), while SKF 38393, quinpirole and sulpiride all

decreased NPYLI levels in the PNA to 67, 69 and 63% of control, respectively. Twelve

hours after quinpirole treatment, NPYLI content decreased in the ANA and PNA to 56

and 59% of control, respectively; NPYLI levels decreased in the ANA and PNA to 46

and 59% of control, respectively, 12 h after sulpiride treatment. No significant

differences were seen after either D-1 agonist or antagonist treatment in either region at

this time point. Unlike most regions of the caudate, by 48 h NPYLI levels in all

treatment groups in both regions of the nucleus accumbens had returned to control

(Figure 4.4).

Response of NPYLI systems in globus pallidus to D-1 and D-2

receptor stimulation and blockade after various times

To assess the effect of dopaminergic systems on NPY-containing neurons in the

globus pallidus, we administered SKF 38393, SCH 23390, quinpirole and sulpiride as
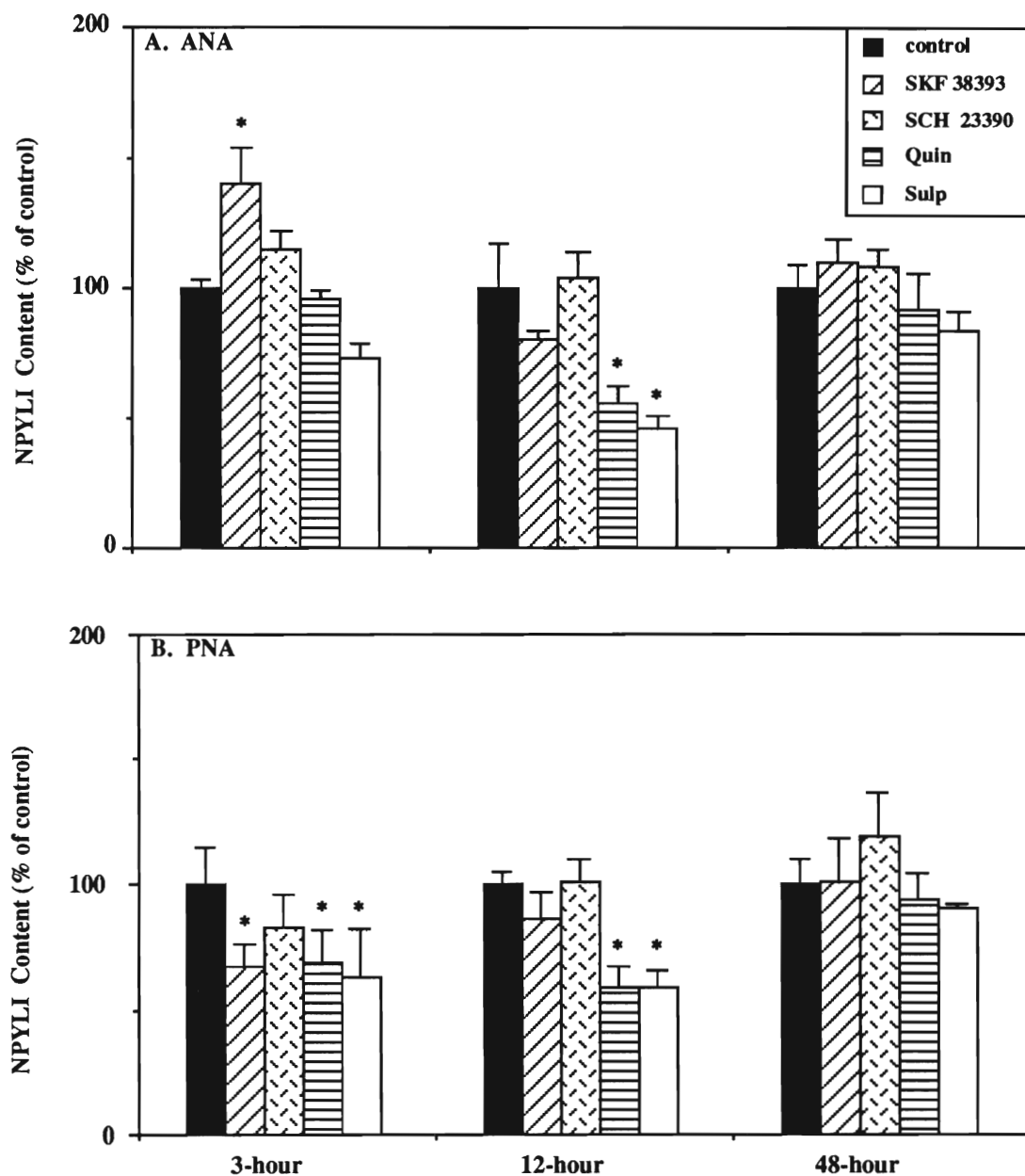
Figure 4.4. Effects of multiple doses of dopamine agonists and antagonists on NPY systems in discrete regions of the nucleus accumbens after various times. Rats were treated as for Figure 4.2. Average values for control NPYLI levels are shown in Figure 4.1. Results are expressed as percent of respective control ± S.E.M. (n=6). * p < 0.05 vs. respective control.

previously described and dissected the structure from frozen tissue as identified in Fig. 4-1 D (Figure 4.5) . Only quinpirole administration significantly altered globus pallidus NPYLI content (51% of control) 3 h after treatment. Twelve hours after drug administration, NPYLI levels in the SKF 38393-, quinpirole- and sulpiride-treatment groups were decreased to 57, 59 and 47% of control, respectively, while the SCH 23390 treatment group remained unchanged. Recovery occurred in all treatment groups by 48 h except for sulpiride-treated animals. It is of interest to note that control NPYLI levels in the globus pallidus are quantitatively more similar to those in the nucleus accumbens than in the striatum (Figure 4.1).

Discussion

These findings demonstrate the existence of complex interactions between dopaminergic and NPY systems in extrapyramidal and limbic structures which are time- and region-dependent. It is likely that the distinct regional patterns of changes in NPYLI content to selective stimulation and blockade of D-1 and D-2 receptors reflect changes in basal release and synthesis activities and rebound responses. Without additional information it is impossible to explain the nature of specific changes in each region examined. However, these findings do support general conclusions and have important implications for future research in this area.

The results of this study suggest that regional differences in the regulation of neuropeptide Y systems exist in structures frequently analyzed in their entirety (Table 4.1). These data may explain the lack of significant peptidergic changes seen with our
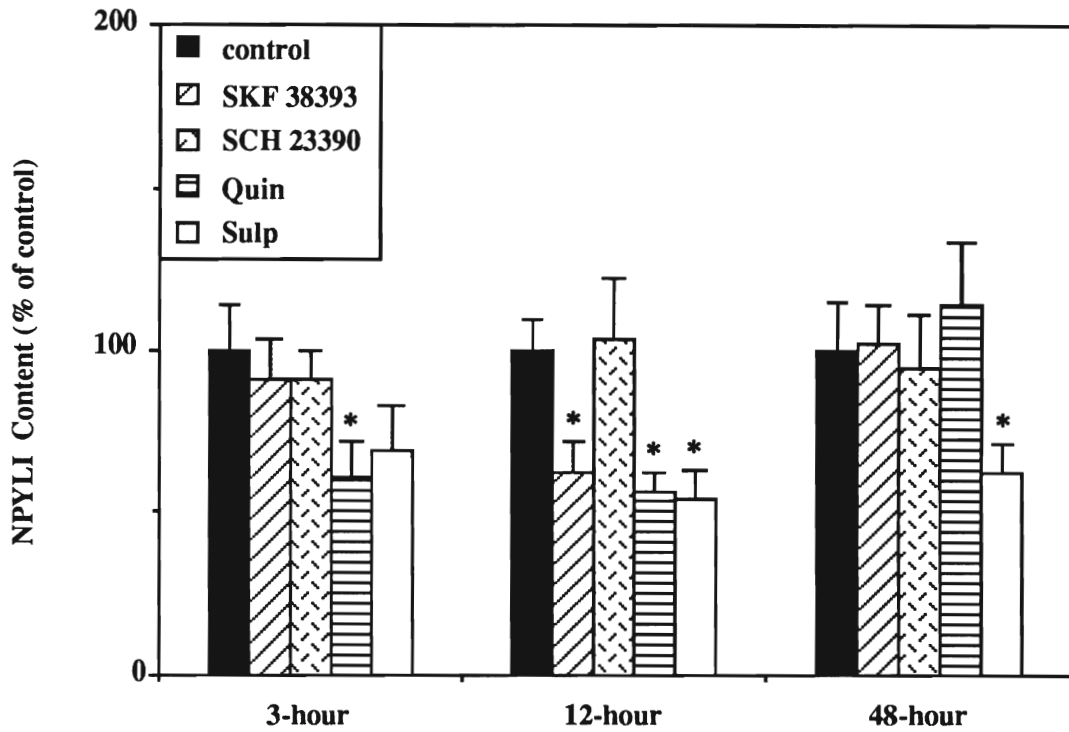
Figure 4.5. Effects of multiple doses of dopamine agonists and antagonists on NPY systems in the globus pallidus after various time points. Rats were treated as described for Figure 4.2. Average value for control NPYLI level is shown in Figure 4.1. Results are expressed as percent of respective control $\pm$ S.E.M. (n=6). * p < 0.05 vs. respective control.

previous administrations of selective D-1 agonists and antagonists, and support our earlier findings (Midgley et al., 1989) that striatal NPY systems respond similarly to intermittent administration of both D-2 agonists and antagonists.

Kerkarian et al. (1988) have reported that treatment with the DA D-1 antagonist, SCH 23390, produces a small but constant increase in the number of NPY-immunoreactive rat striatal neurons. Engber et al. (1992) found that both intermittent and continuous administration of the D-1 agonist, SKF 38393, produce decreases in rat striatal NPYLI content. The present study confirms the work of both investigators, and demonstrates that regions within the striatum have distinct responses to D-1 stimulation and blockade. Since striatal NPY neurons receive synaptic input from tyrosine hydroxylase-positive axons (Kubota et al., 1988; Vuillet et al., 1989), and D-1 receptor mRNA is expressed in a small number of somatostatin-containing neurons (NPY colocalizes with somatostatin in the striatum; Le Moine et al., 1991), these changes could be mediated either directly, indirectly or both. In addition, our observation of localized striatal responses to D-1 stimulation and blockade suggests that whole structure analyses may result in a washout effect in which regions that remain unchanged in response to drug treatments mask the change in responsive areas. This diluting effect may account for our inability to see significant changes in response to these drugs in previous studies at the whole structure level (Midgley et al., 1989). Regional differences in response to these drugs might reflect differences in basal dopaminergic tone; those with high tone (such as the LAC and VPC) would be more likely to respond to the antagonist, whereas those with low tone (such as the VAC) would more likely respond to the agonist.

Interestingly, the D-1 agonist, SKF 38393, elevated NPYLI levels after three hours in the ANA and decreased it in the PNA (Figure 4.4 A and B). However, the variability in responses to the D-1 agonist is not surprising as Salin et al. (1990) found that accumbens NPY systems responded to unilateral 6-OHDA lesions in a manner opposite that of striatal NPY systems. Furthermore, they reported that apomorphine treatment of animals after a 6-OHDA lesion completely reversed ipsilateral anterior but not posterior NPYLI decreases.

In contrast to the opposing effects on striatal NPY systems produced by D-1 receptor agonist and antagonist treatments (e.g., Figure 4.3 A and C), intermittent administrations of the D-2 agonist, quinpirole, or the D-2 antagonist, sulpiride, both decreased caudate, pallidal and accumbens NPY levels, although in some regions the time course for these effects varied. The apparent contradiction of these results might be explained by several mechanisms: 1) The effects of quinpirole and sulpiride could be mediated through different populations of neurons, regulated by differential basal dopaminergic activity. These effects could be either direct or indirect, but are more likely the latter since Le Moine et al. (1991) reported that no DA D-2 receptor mRNA exists in striatal NPY-containing neurons. We previously reported that PCP- or MK-801-induced decreases in striatal NPYLI content are blocked by administration of indirect GABA agonists (Midgley et al., 1992). In addition, Maura et al. (1988) have shown that stimulation of D-2 receptors blocks glutamate release in striatal synaptosomes. Therefore, it is possible that the D-2 agonist acts indirectly through glutamate and GABA systems to produce its effects on NPYLI levels. Kerkarian-Le Goff et al. (1991) recently

reported that striatal choline acetyltransferase-positive neurons receive some nigral dopaminergic input and that these neurons have reciprocal synaptic interactions with NPY-containing neurons. Perhaps this system, or a similar one, could be responsible for the decreases induced by the D-2 antagonist. 2) The effects of the dopamine D-2 agonist, quinpirole and the D-2 antagonist, sulpiride, on striatal NPYLI systems could actually be mediated principally by two different dopamine receptor subtypes. Recently, Schwartz et al. (1992) reported that quinpirole has considerably higher affinity for the D-3 receptor, while sulpiride has higher affinity for the D-2 receptor; possibly other DA receptor subtypes will yet be identified for which the two drugs have differing affinities. 3) A complex interrelationship between DA postsynaptic receptors and presynaptic autoreceptors may account for these complex results. For example, stimulation of D-1 receptors is necessary for the full expression of postsynaptic effects of dopamine D-2 agonists (Carlson et al., 1987; Watchel et al., 1989). Thus, the action of quinpirole at presynaptic dopamine D-2 autoreceptors might inhibit dopamine release and thereby remove the D-1 stimulation necessary for the expression of postsynaptic effects which might be expected from D-2 receptor stimulation. In addition, the similar responses by striatal NPY systems to D-2 agonists and antagonists could reflect such mechanisms as differences in second messenger systems or in synthesis and release of the peptide induced by the two drugs. Further studies are necessary to identify the specific mechanism(s) involved.

In addition to quantitative differences in regional NPYLI content observed in response to administrations of D-1 and D-2 agonists and antagonists, differences in basal

NPYLI levels and the time course of NPYLI changes in response to drug treatment were also identified. As described in Figure 4.1, accumbens NPYLI levels are much higher than striatal NPYLI levels and posterior striatal levels higher than anterior or very posterior striatal levels. In addition, NPYLI levels in the globus pallidus approximated those in the nucleus accumbens. These regional differences in basal NPYLI content suggest differences in the role or regulation of these regional NPY systems. The responses of these regional NPYLI systems to the dopaminergic agonists and antagonists also differed in their time courses. For example, SCH 23390 increased NPYLI levels early in the VPC but later in the MPC and LAC (Figure 4.3 C and A, Figure 4.2 C); SKF 38393 decreased NPYLI levels early in the PNA, but later in the VPC and LAC (Figure 4.4 B, Figure 4.3 C and Figure 4.2 C); quinpirole, but not sulpiride, decreased NPYLI content in the VAC by 3 h and quinpirole but not sulpiride remained decreased at 48 h (Figure 4.2 A) whereas sulpiride, but not quinpirole, decreased NPYLI content in the MAC at 3 hr (Figure 4.2 B); and quinpirole but not sulpiride decreased pallidal NPYLI content by 3 h while only sulpiride remained decreased by 48 h (Figure 4.5). The regional differences in time courses for these drugs could reflect differences in kinetics, regional regulation of NPY-containing neurons, or synthesis and release of the peptide.

In summary, the results of this study suggest that intermittent administration of a D-1 agonist or antagonist decreased or increased, respectively, NPYLI content in selected regions of the rat caudate and globus pallidus at the indicated time points. The D-1 agonist increased NPYLI levels in the anterior and decreased them in the posterior

nucleus accumbens. The D-1 antagonist had no significant effect in either region of the accumbens; however, it did increase NPYLI levels in LAC, MPC and VPC at identified time points after treatment. Intermittent administration of either D-2 agonist and antagonist reduced NPYLI content in all identified regions of each structure at some time point, although the time course and magnitude of effects varied. While the explanation for these varied fluctuations is not yet known, they confirm our earlier work (Midgley et al., 1989) and that of Kerkarian et al. (1988) and Enger et al. (1992) concerning the role of DA receptor subtypes in the regulation of striatal NPY. These data further suggest that studies must be carefully designed not to overlook potential regional differences which may confound or be diluted out in whole structure analyses.

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CHAPTER 5

MECHANISMS OF PCP-INDUCED CHANGES IN

CENTRAL NEUROPEPTIDE Y SYSTEMS

Introduction

Data from the previous chapters suggest that extrapyramidal and limbic NPY

systems are modulated by glutamatergic activity and that the interaction between these

two systems is mediated, at least in part, by GABAergic mechanisms (Midgley et al.,

1992). However, the previous data do not indicate which GABAergic receptor subtype(s)

(A or B) may be involved, nor the specific mechanism(s) of action by which PCP is

affecting central NPY systems. For example, alterations in NPYLI tissue content may

result from changes in NPY synthesis, release or both.

Assessment of mechanisms is made difficult by the presence of multiple pop-

ulations of NPY-containing neurons with differential response patterns in discrete brain

structures (Kubota et al., 1991; Salin et al., 1990; Kuljis and Rakic, 1989; Kerkarian et

al., 1989). In addition, we have recently completed studies which suggest that the effects

of PCP and MK-801 on NPY neurons in the anterior caudate and nucleus accumbens,

and the globus pallidus are very similar; however, in the posterior caudate and nucleus

accumbens they are dissimilar (data not shown). In this chapter we evaluate the

mechanisms by which PCP may change NPYLI content by: 1) assessing the effects of

selective GABAergic agonists on PCP-induced striatal NPYLI changes, 2) evaluating the effects of PCP on striatal NPY release and 3) examining the effects of PCP, MK-801, quinpirole and sulpiride on striatal, accumbens and cortical NPY messenger ribonucleic acid (mRNA).

Materials and Methods

Animals and treatments

Male Sprague-Dawley rats (180-270 g, Simonsen Laboratories, Gilroy, CA) were used in all the following experiments and maintained as described in Chapter 2. For GABA agonist and mRNA studies, animals received five doses each of PCP (15 mg/kg/dose), MK-801 (2 or 2.5 mg/kg/dose for agonist and mRNA studies, respectfully), quinpirole (5 mg/kg/dose), sulpiride (80 mg/kg/dose), muscimol (2 mg/kg/dose), baclofen (25 mg/kg/dose) or drug combinations at 6-h intervals and were sacrificed 15 h after the final dose. Agonists were given 20-30 min prior to administration of PCP or MK-801. Drug doses, except for MK-801, were calculated for their free forms. All drugs were administered intraperitoneally except PCP which was administered subcutaneously. All drugs were dissolved in 0.9% saline except for baclofen and quinpirole, which were dissolved in 1% lactate-saline, and sulpiride which was dissolved in 2% lactate + 25% propylene glycol-saline.

For NPY release studies, animals were anesthetized with chloral hydrate (375 mg/kg) and placed in a stereotaxic apparatus. A push-pull cannula was placed 0.5 mm anterior to bregma, (-) 2.6 mm lateral to bregma and 5.5 mm ventral from the skull. Rats

were allowed to stabilize for 2.5 to 3 h before baselines of NPY release were obtained. Samples were collected for 30 min at a flow rate of 13 μl/min into 60 μl of 0.1 N HCl. Artificial cerebral spinal fluid (CSF) (Tatsuoka et al., 1987) consisted of 140 mM NaCl, 3.5 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.0 mM HEPES and 0.1% bovine serum albumin (BSA). Striata were perfused with artificial CSF during the stabilization period and baseline collections. Subsequently, animals received one dose of PCP (15 mg/kg, s.c.) and samples were collected for the remainder of the experiment (usually two more collections).

Dissections

Animals used for GABA agonist and mRNA studies were sacrificed by decapitation 15 h following treatment. Brains were rapidly removed and those used in mRNA studies were immediately frozen on dry ice and stored at -80^0C until processed. Brains from the GABA agonist study had striata excised on ice and brains stored as identified in Chapter 2. Animals used in release studies were sacrificed at the end of each experiment, their brains removed and cannula placement verified.

NPY radioimmunoassay

The antiserum to NPY was prepared as described in Chapter 2. Assays for NPYLI were conducted as previously described except in release studies 200 μl of artificial CSF was substituted for buffer in the standards and samples were run individually due to limited NPY concentrations in perfusate.

In situ hybridization histochemistry

A modification of methods previously described by Merchant et al. (1992) was used. Briefly, brains were cut into 16-μM-thick slices, thaw-mounted onto gelatin-coated slides and stored at -80^{0}C until processed. Slides were warmed to room temperature for 10 min, fixed in 4% w/v paraformaldehyde, acetylated with 0.25% v/v acetic anhydride in 0.1 M triethanolamine (pH 8.0), dehydrated through a graded series of ethanol, delipidated in chloroform, rehydrated to 95% v/v ethanol and air dried. A 36 base-pair oligonucleotide probe (5'-GGA-GTA-GTA-TCT-GGC-CATGTC-CTC-TGC-TGG-CGC-GCT; Baskin et al., 1990; Higuchi et al., 1988) was terminal deoxyribo-nucleotidyl transferase (TDT) labelled (Lewis et al., 1986) with ^{35}S dATP to a specific activity of 10.44 μCi/pmol. The labelled probe was applied at saturating concentrations (2 pmol/ml) in a hybridization solution (10 mM Tris-HCl buffer, pH 8.0, containing 50% v/v deionized formamide, 0.3 M NaCl, 1mM EDTA, 10% w/v dextran sulfate, 1 x Denhardt's solution, 10 mM dithiothreitol, and 0.5 mg/ml yeast tRNA). Sections were covered with siliconized coverslips and the slides incubated overnight in a humid chamber at 27^{0}C (20^{0}C below the calculated melting point). The coverslips were removed in 1 x SSC (0.15 M NACl + 0.015 M sodium citrate, pH 7.0), and the slides washed 3 x 20 min at 60^{0}C in 1 x SSC. Slides were then washed in 1 x SSC at room temperature for 2 x 60 min., dehydrated through graded alcohols containing 0.6 M ammonium acetate, and air dried.

Autoradiography

Following hybridization, slides were apposed to Hyperfilm-max (Amersham) for 7 days and films developed in Kodak D-19 solution. Slides were then dipped in Kodak NTB2 nuclear tract emulsion diluted 1:1 with 0.6 M ammonium acetate, air dried in the dark for 2 h, and exposed for 3 weeks. The emulsion was developed in D-19 diluted 1:1 with water. Slides were counter-stained in 0.1% w/v cresyl violet acetate, dehydrated and coverslipped with Permount. Brain sections from different treatment groups were anatomically matched according to the atlas of Konig and Klippel (1963) under bright field prior to evaluation of films and distribution of autoradiographic grains in dark field.

Statistical analysis

Data for Figure 5.1 and 5.2 were analyzed as described in Chapter 2. NPYLI control value is included in the figure legend.

Results

Regulation of striatal NPY systems by GABA A and B receptors

To examine the influence of selective GABAergic receptors in the regulation of striatal NPY systems, we administered the GABA A agonist, muscimol (2 mg/kg/dose, i.p.), or the GABA B agonist, baclofen (25 mg/kg/dose, i.p.) alone and in combination with PCP (15 mg/kg/dose, s.c.) or MK-801 (2 mg/kg/dose, i.p.). Animals received five doses, 6 h apart and were sacrificed 15 h after the final dose. Agonists were adminis-tered 20-30 min prior to PCP or MK-801 treatments. As shown in Figure 5.1, both
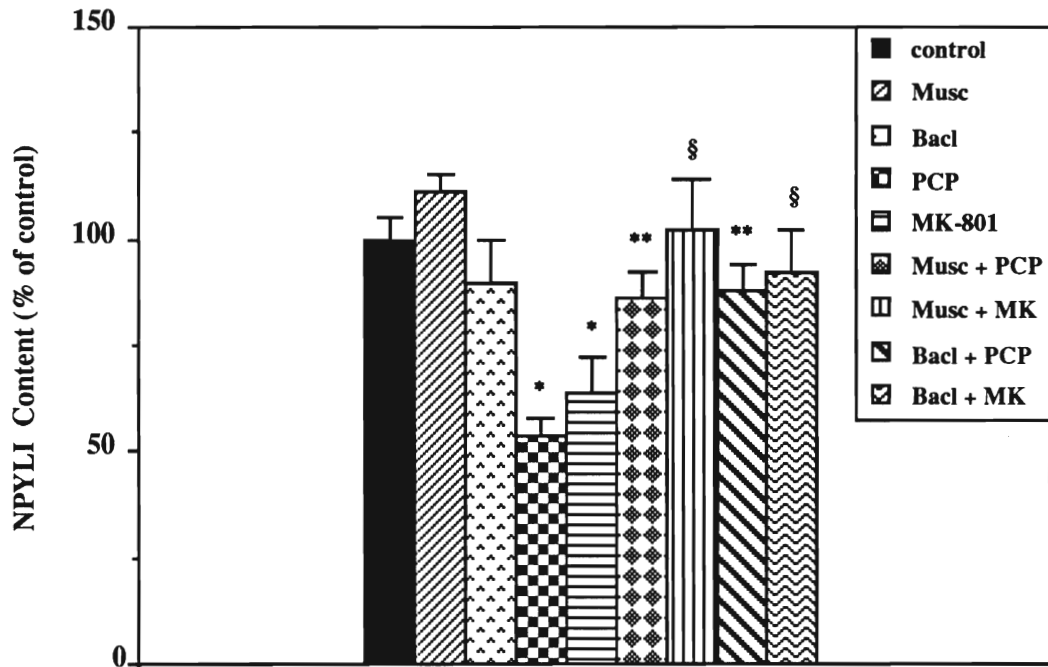
Figure 5.1. Effects of multiple doses of selective GABA A and B agonists on striatal NPYLI content alone and in combination with PCP or MK-801. Rats were treated as described in Materials and Methods. Average value for control NPYLI level was 750 ± 39. Results are expressed as percent of control $\pm$ S.E.M. (n=4-6). * p< 0.05 vs. control; ** p < 0.05 vs. PCP; § p < 0.05 vs. MK-801.

PCP and MK-801 decreased striatal NPYLI content, and both the GABA A and B agonists completely blocked the PCP- and MK-801-induced changes. We were unable to determine clearly the effects of these selective GABAergic drugs on accumbens and cortical PCP and MK-801-induced NPYLI decreases because both the GABAergic drugs increased NPYLI levels in both tissues in separate experiments (data not shown)

The role of release in PCP-induced striatal NPYLI changes

To evaluate the effects of systemic PCP administration on NPY release, we administered a single dose of PCP (15 mg/kg, s.c.) to chloral hydrate-anesthetized animals implanted with a push-pull cannula as described in Materials and Methods. As shown in Figure 5.2, acute PCP administration produced a small (10%) but significant increase in the release of striatal NPYLI.

The role of peptide synthesis in PCP-, MK-801-, quinpirole- and
sulpiride-induced NPYLI changes

To evaluate whether multiple PCP, MK-801, quinpirole or sulpiride administrations exert their effects on striatal, accumbens and cortical NPY systems through altering peptide synthesis, we performed *in situ* hybridization histochemistry on tissue slices from animals treated with these drugs as described in Material and Methods. As shown in Figure 5.3 A and B, striatal cell bodies were specifically labelled by the oligonucleotide probe for NPY mRNA. Cells positive for NPY message were generally distributed in
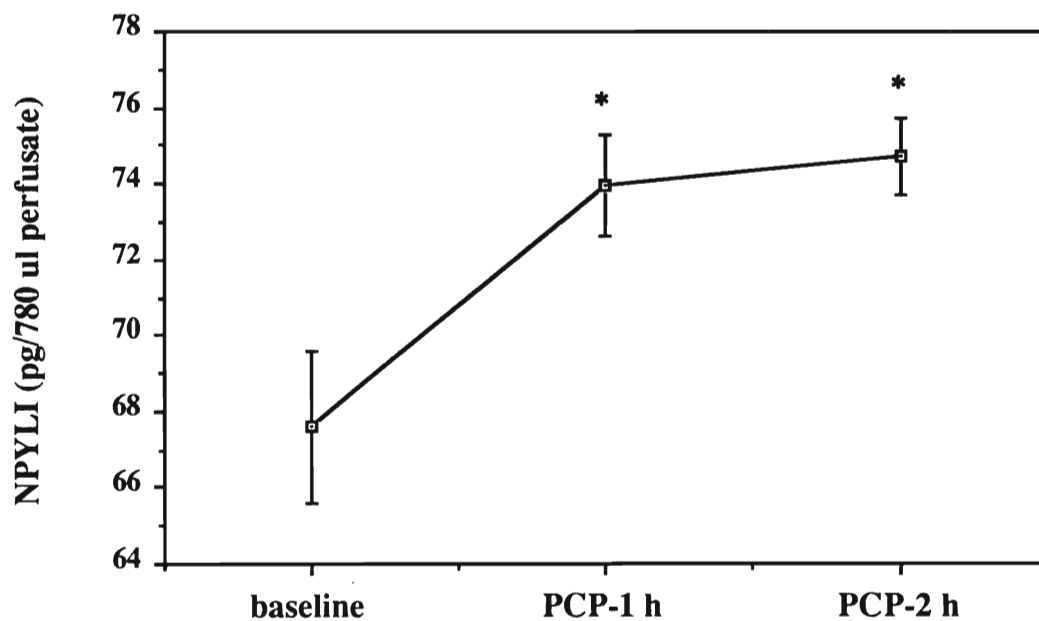
Figure 5.2. Effect of a single systemic PCP administration (15 mg/kg) on striatal NPYLI release. Animals were treated as described in Materials and Methods. Each data point represents the total of two 30-min collections (n = 5). Average control value was 67.6 ± 2 pg/780 ul perfusate. * $p < 0.05$ vs. control.

a dispersed pattern throughout the striatum, accumbens and cortex. Using both film autoradiography and counting NPY-positive cells on slides we were unable to detect any significant overall change in striatal, accumbens or frontal cortex NPY mRNA induced by any of the drugs at this time point (data not shown). However, we found significant regional induction of NPY mRNA by PCP treatment in two cortical areas at 15 h after treatment. The cortical region from anterior caudate to bregma (more posterior structures were not examined), which borders the corpus callosum, appeared particularly sensitive to the NMDA antagonists. As shown in Figure 5.4 A-C, PCP and MK-801 treatments significantly increased the presence of NPY-positive neurons in this region to 577% and 416% of control, respectively. In addition, NPY-positive cells in the cortex lateral to the mid-ventral striatum were increased by PCP administration to 257% of control (Figure 5.5 A and B).

Figure 5.3. Localization of striatal NPY mRNA to cell body with an oligonucleotide probe. Animals were treated and tissues processed as described in Materials and Methods. Panel A is a light field and panel B a dark field representation. Section was taken approximately 1.37μ anterior to bregma in the dorso-medial striatum.
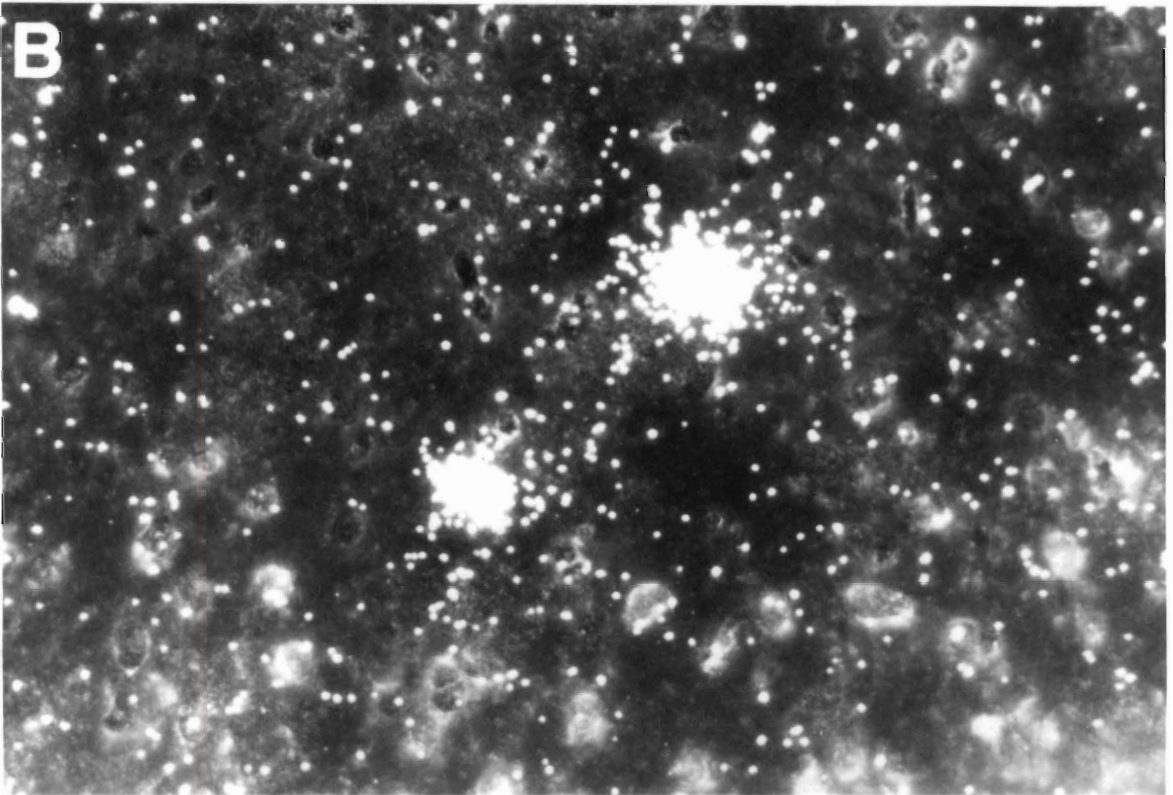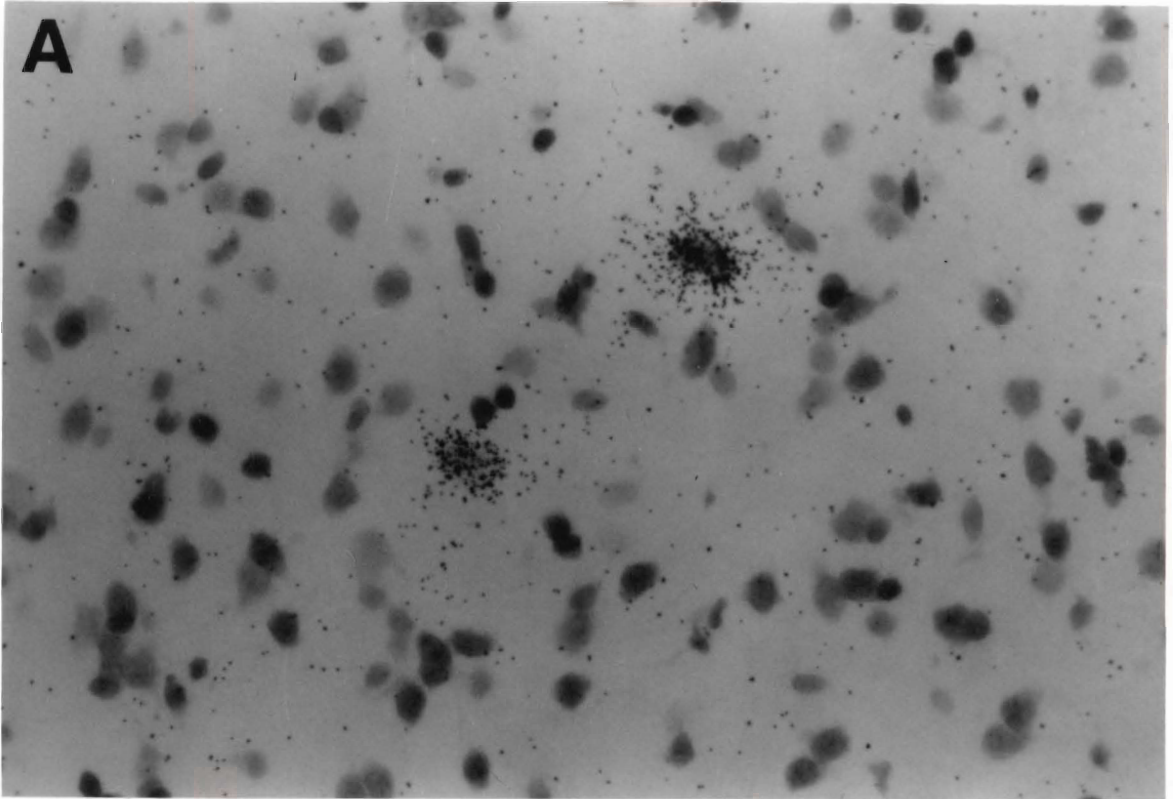
Figure 5.4. Effects of multiple systemic PCP and MK-801 administrations on NPY mRNA in the cortical-callosal border. Animals were treated and tissues processed as described in Material and Methods. Rats were sacrificed 15 h after treatment. These representative sections were taken approximately 0.87μ anterior to bregma. A. control, B. PCP and C. MK-801.
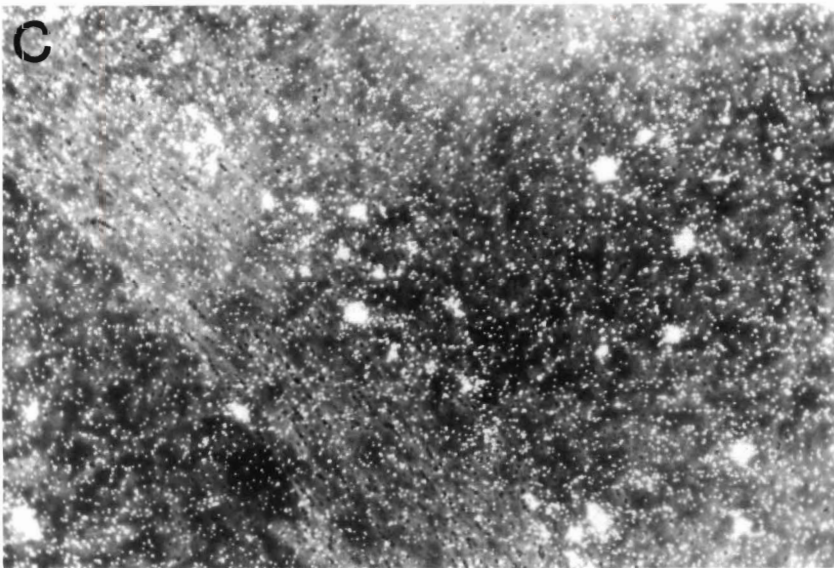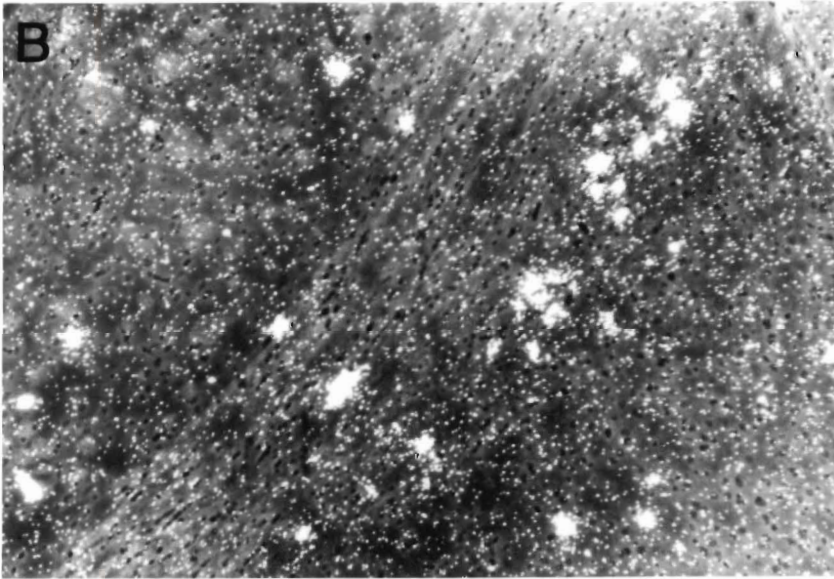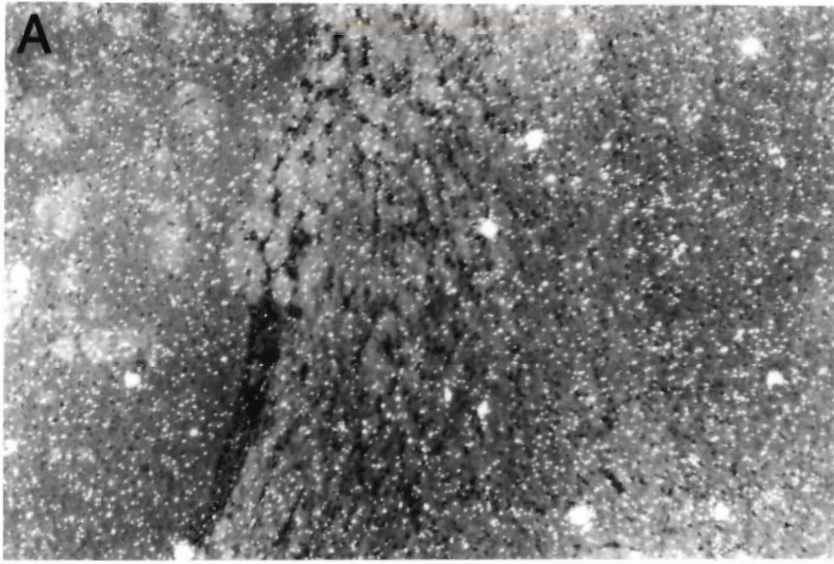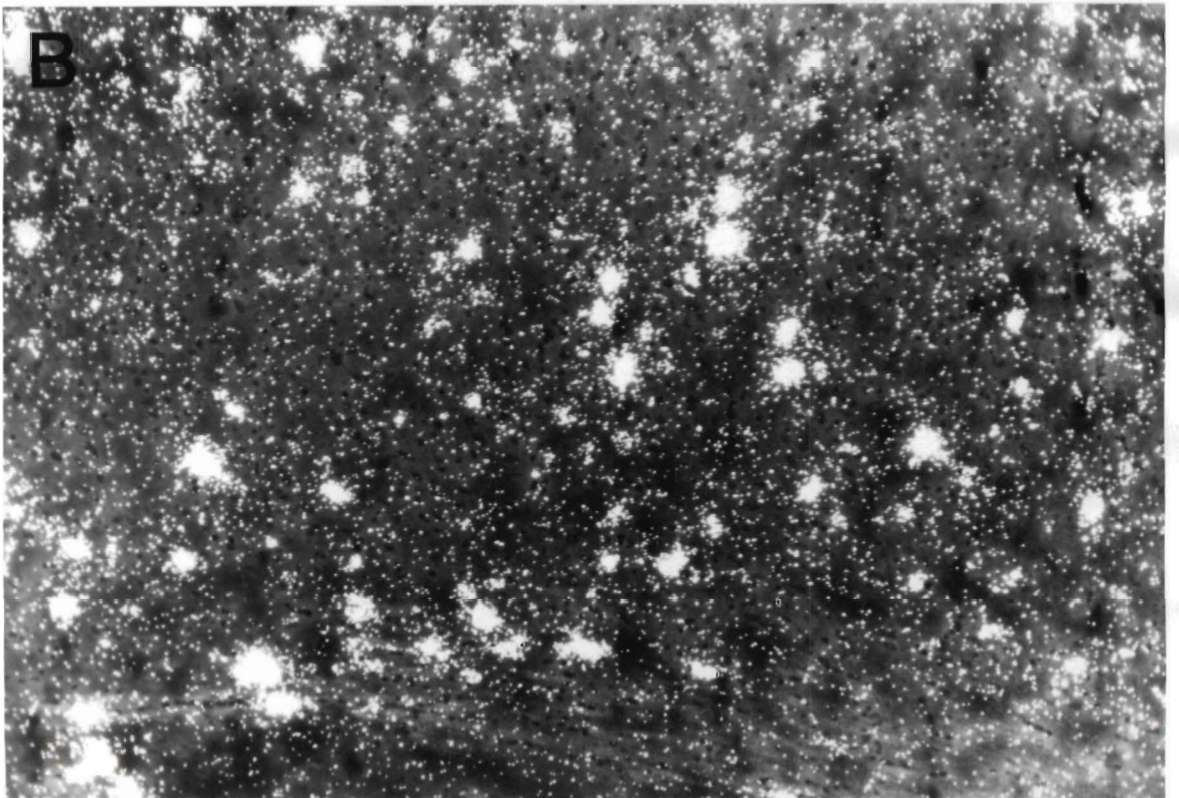
Figure 5.5. Effects of multiple systemic PCP administrations on NPY mRNA in the lateral cortex. Animals were treated and tissues processed as described in Materials and Methods. Rats were sacrificed 15 h after treatment. These representative sections were taken approximately 0.87μ anterior to bregma. A. control, B. PCP.

Discussion

The studies described in previous chapters indicate that central NPY systems are regulated by complex interactions between a number of transmitter systems including dopamine, glutamate and GABA. The studies described in this chapter confirm our previous data and suggest that both peptide synthesis and release may be important components of PCP-induced changes in central NPY systems.

Neuropeptide Y is a relatively large and sticky protein which precludes its measurement by microdialysis with existing technology. The only published report to date of *in vivo* NPY release was by Tatsuoka et al. (1987) who observed decreased striatal NPYLI release in rats after perfusion with amphetamine (10 mM) using push-pull techniques in awake animals. These findings were correlated with increased dopamine release, suggesting that dopamine was inhibitory on striatal NPYLI release. However, the relevance of these findings must be tempered due to the severe tissue damage and stress experienced by conscious animals using this protocol and the extremely high dose of amphetamine employed by these researchers. As described in Figure 5.2, we observed a small but significant increase in striatal NPYLI release after acute systemic PCP administration using push-pull methods in anesthetized animals. Baseline values of released NPYLI in our study correlated well with those reported by Tatsuoka et al. (1987). However, because of the long time periods required to stabilize the baseline, the long half-life of PCP and the high mortality of animals due to the length of the procedure, we were unable to follow the time course for PCP-induced NPYLI release beyond 2-3 h after drug administration. Although we cannot exclude the possibility that

the anesthesia influenced the results obtained with this protocol, based on these and previous data, we suggest that PCP is inducing striatal NPY release, likely through its action as an NMDA antagonist.

Lindefors et al. (1990) found that following dopamine deafferentation, the numerical density of striatal NPY mRNA producing neurons almost doubled (the opposite was true for the fronto-parietal cortex). Data from the mRNA study described in this chapter (Figures 5.3-5.5) suggest that PCP treatment increases NPY synthesis in some brain regions. However, this was a preliminary experiment with only one animal per group and therefore no firm conclusions can be drawn. In addition, the study was performed on animals sacrificed at 15 h after the last treatment to coincide with the radioimmunoassay data. Merchant et al. (1991) have shown that this is not the time of maximal neurotensin mRNA changes after haloperidol treatment; it may also not be the time of maximal NPY changes after treatment with PCP, MK-801, quinpirole or sulpiride treatments. Clearly a time-course study with adequate numbers of animals is the next step in evaluating the effects of these drugs on NPY synthesis. Nevertheless, the NPY mRNA changes observed in the two cortical regions (Figures 5.4 and 5.5) were pronounced enough to suggest that changes in NPY mRNA do occur in response to PCP and MK-801 administration in some structures.

Administration of the selective direct GABA agonists, muscimol and baclofen, had no significant effects by themselves but both drugs completely blocked striatal PCP- and MK-801-induced NPYLI changes. Although these data do not clarify the specific role of GABA-A and -B receptors (indeed they underscore the complexity of the regulation

of NPY systems), they do support our previous hypothesis that GABAergic mechanisms are important in the regulation of striatal NPY systems and suggest that both receptor types contribute to the PCP-induced effects specifically and NMDA effects in general.

Based on the data reported here and by others (Le Moine et al., 1991; Kerkarian-- Le Goff et al., 1991; Kubota et al., 1988; Maura et al., 1988; Midgley et al., 1992; Vuillet et al., 1989) we propose the following possible synaptic arrangement for striatal NPY-containing neurons (Figure 5.6): Neuropeptide Y-containing neurons receive direct synaptic input from GABAergic, dopaminergic and cholinergic neurons and indirect glutamatergic input mediated through GABAergic neurons. Therefore a decrease in glutamatergic activity (as would result from PCP or MK-801 administration in the presense of relatively high basal activity) would indirectly increase NPY release and decrease its tissue content. This action would thus be compensated for by administration of a direct or indirect GABA agonist. Increased NPY synthesis might also be expected to occur in response to a glutamatergic antagonist in order to replenish the NPY lost due to increased neuronal activity and turnover of the peptide. Administration of a dopamine D-2 agonist, according to this scheme, might have an effect similar to a glutamatergic antagonist because of its activity at presynaptic D-2 autoreceptors located on glutamate terminals (Maura et al., 1988). The direct dopaminergic input to striatal NPY systems is likely mediated through the D-1 receptor because no D-2 receptors have been identified on striatal NPY-containing neurons (Kubota et al., 1988; Le Moine et al., 1991). Excitatory cholinergic neurons synapse on NPY-containing neurons but presumably are
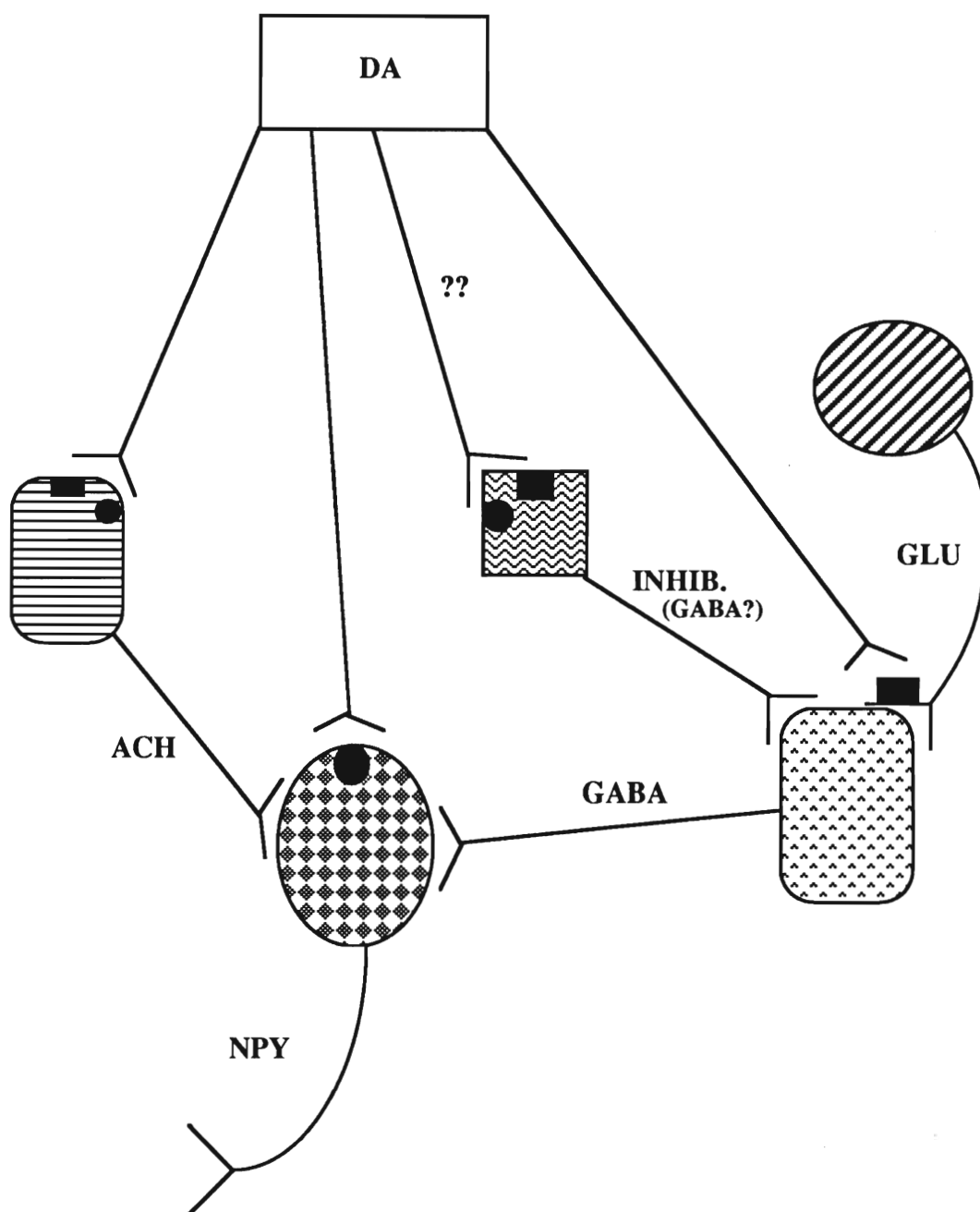
Figure 5.6. Proposed synaptic arrangement of GABAergic, glutamatergic, dopaminergic and cholinergic neurons in relationship to striatal NPY systems. ● D-1 receptors; ■ D-2 receptors.

under the influence of inhibitory dopaminergic receptors (Kerkarian-Le Goff, 1991) and might therefore have low basal activity. Dopaminergic receptors located on striatal cholinergic neurons are principally D-2 receptors, with a small number of D-1 receptors also present (Le Moine et al., 1991). The action of a D-2 antagonist might therefore be to remove the dopaminergic inhibition of cholinergic neurons and increase NPY release (decreasing NPY tissue levels). Conversely, a scheme where dopaminergic neurons synapse on GABAergic interneurons (Le Moine et al., 1991) which then synapse on secondary GABAegic neurons (Aoki and Pickel, 1990) that modulate NPY-containing neurons would be expected to have a similar effect. Synaptic cartoons such as this are speculative and necessarily oversimplified. However, they can be useful tools to assist the observer in understanding results and suggesting future paths for research. It is quite possible, for example, that GABAergic and glutamatergic neurons are involved in parallel processing rather than (or in addition to) in series processing, although this would make the D-2 agonist results more difficult to interpret. In addition, the role of the cholinergic system in the regulation of striatal NPY- containing neurons is presently unclear.

In summary, the results of the studies reported in this dissertation suggest that striatal, accumbens and cortical NPY systems are regulated principally by glutamatergic, dopaminergic and GABAergic activity. They further indicate that glutamatergic regulation of NPY-containing neurons in these structures occurs through the NMDA receptor and is mediated by GABAergic mechanisms. In addition, these results suggest that alterations in both peptide release and synthesis may contribute to the response of NPY systems to NMDA antagonists such as PCP. Clearly, more research is needed to

determine the complex interaction between glutamatergic, dopaminergic, GABAergic and NPY systems, particularly with respect to release and synthesis. One would anticipate that such research will lead to a better understanding of the role of neuropeptide Y in normal CNS functioning and in pathological conditions.

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II.   EDUCATION

      Undergraduate education: Bachelor of History, Cum Laude; 1978
      University of Utah
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      Scanning Electron Microscopy, 1985
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      Colorado State University, Colorado

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      George Washington University, Wash., D.C.

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III.   PROESSIONAL EXPERIENCE

JUL 1988-present. Graduate research under the guidance of Dr. G.R. Hanson, Department of Pharmacology and Toxicology, University of Utah School of Pharmacy, Salt Lake City, Utah.

My research project was a study of the regulation of neuropeptide Y (NPY) systems in the basal ganglia and limbic system of the rat. Techniques employed in the fulfillment of theses goals included radioimmunoassay, *in situ* hybridization, radioenzymatic assay, push-pull and HPLC-ECD. I worked as a teachers assistant (TA) for Common Medicines (Lib. Ed. and Pharmacology, 370) from 1990-1992, and lectured to pharmacy and medical students in 1991 and 1992, respectively. I served as the graduate student representative to the Graduate Training Committee in 1991.

OCT 1984-JUL 1988. Research Microbiologist; U.S. Army Dugway Proving Ground, Dugway, Utah.
The primary technical areas I worked in were image analysis, electron microscopy, fermentation, and downstream product processing. Techniques employed to complete these projects included image analysis, scanning electron microscopy, fermentation and scale up production, downstream product processing, column chromatography, gel electrophoresis, Western blots, ELISA and monoclonal antibody production and characterization. I received an Exceptional performance appraisal 1987.

APR 1979-OCT 1984 Surgical Assistant, Wasatch Springs Animal Hospital, Salt Lake City, Utah.
My responsibilities in this capacity included assisting two veterinarians in surgery, care and treatment of animals, and performing microbiological and biochemical laboratory tests.

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V.   PUBLICATIONS AND PRESENTATIONS

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