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**Attenuation of antagonist-induced impairment of dopamine
receptors by L-prolyl-L-leucyl-glycinamide**

Saleh, Mohamad Iqbal M., Ph.D.

East Tennessee State University, 1988

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ATTENUATION OF ANTAGONIST-INDUCED IMPAIRMENT
OF DOPAMINE RECEPTORS BY L-PROLYL-L-LEUCYL-GLYCINAMIDE

A Dissertation Presented to
the Faculty of the Department of Pharmacology
Quillen-Dishner College of Medicine
East Tennessee State University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

by
Mohamad Iqbal Saleh, M.D.
April, 1988

APPROVAL

This is to certify that the Graduate Committee of
Mohamad Iqbal Saleh

met on the
4th day of April, 1988

The committee read and examined his thesis, supervised his defense of it on an oral examination, and decided to recommend that his study be submitted to the Graduate Council and the Associate Vice-President for Research and Graduate Studies in partial fulfillment of the requirements for the degree Doctor of Philosophy in Biomedical Science.

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ABSTRACT

ATTENUATION OF ANTAGONIST-INDUCED IMPAIRMENT

IN DOPAMINE RECEPTOR ONTOGENY

BY L-PROLYL-L-LEUCYL-GLYCINAMIDE

by

Mohamad Iqbal Saleh, M.D.

It has been shown by others that the prenatal treatment of rats with haloperidol, a dopamine D2 receptor antagonist, leads to a permanent reduction in the number of striatal dopamine D2 receptors in adulthood. Conversely, postnatal treatment of lactating dams with haloperidol from birth for 21 days, leads to an increase in the number of striatal dopamine D2 receptors in the litters, when assessed as adults. The present study was undertaken in order to determine whether chronic, long-term postnatal challenge of rat pups per se, with specific dopamine D1 and D2 receptor antagonists, would modify the ontogeny of the respective receptor types. Since the neuropeptide L-prolyl-L-leucyl-glycinamide (PLG) attenuates the effect of haloperidol on dopamine D2 receptors in adult rats, it was of interest to determine whether PLG would modulate antagonist-induced alterations in the ontogeny of striatal dopamine D1 and D2 receptors. Half of the rats were treated daily for 32 days from birth with SCH-23390 (0.30 mg/kg/d i.p.), a selective dopamine D1 antagonist; or spiroperidol (1.0 mg/kg/d i.p.), a selective dopamine D2 antagonist; or both SCH-23390 and spiroperidol; or saline. The other half of the litters were treated with PLG (1.0 mg/kg/d, i.p.), in combination with the other treatments. Animals were decapitated at 5, 8, and 12 weeks from birth for neurochemical analysis of the striatum. Chronic SCH-23390 treatment produced a 70-80% decrease in the binding of [³H] SCH-23390 (300 pM) to striatal homogenates. The alteration at 5 weeks was associated with a 78% decrease in the B_{max} for [³H] SCH-23390 binding, and no change in the K_D. Similarly, at 5, 8, and 12 weeks, chronic spiroperidol treatment reduced the binding of [³H] spiroperidol (300pM) to striatal homogenates by 70-80%. The alteration at 5 weeks was associated with a 74% decrease in the B_{max} for [³H] spiroperidol binding, and no change in K_D. Furthermore, PLG attenuated these respective changes in dopamine D1 and D2 binding, when assessed at 5 and 8 weeks. These findings demonstrate that the postnatal period is a sensitive and critical time in the development of striatal dopamine D1 and D2 receptors and that PLG is able to attenuate the alterations in ontogeny that are produced by dopamine D1 and D2 receptor antagonists.

DEDICATION

I would like to dedicate this dissertation to my parents.

ACKNOWLEDGEMENTS

I would like to thank Dr. Richard Kostrzewa for his advice, patience and effort. I would also like to thank all the members of my committee: Dr. Donald Hoover, Dr. Michael Miyamoto, Dr. William McCormick and Dr. Ronald Baisden. Finally, I would like to thank Dr. Perter Rice for his help in data analysis, and Dr. Ernest Daingneault, Chairman of the Department of Pharmacology, for his support.

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Chapter I

Introduction

Anatomical Review:

Carlsson et al. (1958) made their important suggestion that dopamine is not only an intermediate precursor of norepinephrine synthesis, but is also a unique monoamine with specific properties and functions in the central nervous system (CNS). Following this suggestion, Ahlström and Fuxe (1964), using a histochemical formaldehyde condensation method, mapped the central monoaminergic systems in great detail. It was found that dopaminergic cell bodies are located mainly in three major areas:

1. Zona compacta of the substantia nigra (A₉)
2. Ventral tegmental area (A₁₀)
3. Arcuate nucleus and the ventral periventricular hypothalamic nucleus (A₁₂)

From the dopamine cells in A₉, the nigrostriatal dopamine system (dorsal component of the mesostriatal dopamine system) arises, and terminates in the caudate nucleus, putamen, globus pallidus and subthalamic nucleus. From the dopamine cells in A₁₀, the mesolimbic dopamine system (ventral component of the mesostriatal dopamine system) arises, and terminates in the nucleus accumbens, olfactory tubercle, nucleus interstitialis stria terminalis, hypothalamus, preoptic area, prefrontal cortex, cingulate

cortex, hippocampus, amygdala, and midbrain; and from the dopamine cells in A₁₂, the tuberoinfundibular and tuberohypophyseal dopamine system arises and terminates in the median eminence, infundibular stalk, and intermediate and neural lobes of the pituitary (Beckstead et al., 1979; and Nauta et al., 1978).

Proposed Function Of Dopamine Systems:

As of now, two major neurological functions can be directly related to dopamine systems:

1. Behavior: Various behaviors can be modulated by dopaminergic transmission. The nigrostriatal pathway is associated with motor behavior, while the mesolimbic, mesocortical pathways are associated with mood behavior, i.e., stereotyped behavior (Creese, 1973), self stimulation behavior (Phillips and Libiger, 1973), conditioned avoidance responding behavior (Ungerstedt, 1971), stimulus control behavior (Ho and Huang, 1975), and feeding and drinking behavior (Ungerstedt, 1971).
2. Neuroendocrine function (tuberohypophyseal dopaminergic system): Neuroendocrine function was recognized from studies on the effect of dopamine on the hypothalamus, gonadal steroids and prolactin secretion (MacLeod 1976).

Dopamine Receptors:

The properties of dopamine receptors were first characterized in 1975 following their identification by

receptor-binding techniques utilizing [³H]-butyrophenones, the potent anti-psychotic drugs used in the treatment of schizophrenia (Snyder et al., 1975; Seeman et al., 1975). These receptors were termed dopamine D₂ receptors, since another type of dopamine receptor had been identified years earlier, even though its functional properties were unknown (Billard et al., 1974).

Later, Cools and Van Rossum (1976) modified the concept of multiple dopamine receptors. Keababian and Calne (1978) followed up on that and classified dopamine receptors into D₁ and D₂ receptors, where dopamine D₁ is the receptor that is linked to dopamine-sensitive adenylate cyclase, and the dopamine D₂ receptor represents the group that is not linked to dopamine sensitive adenylate cyclase. However, this classification is not satisfactory since: 1--Adenylate cyclase does not exist in all dopamine receptor-containing tissue (i.e. pituitary) (Ahn et al., 1979). 2--It is not practically possible to biochemically separate the amount of dopamine receptors linked and nonlinked to adenylate cyclase (Keababian and Calne, 1979). 3--There is no clear relationship between the effect of the dopamine agonists and antagonists on behavior and their effect on adenylate cyclase (McErmed and Miller, 1979).

The gradual discovery of highly specific dopamine receptor agonists and antagonists made it possible to characterize dopamine D₂ receptors (Creese et al., 1977;

Leysen et al., 1978, Laduron and Leysen, 1979) and dopamine D₁ receptors (Iorio et al., 1981; Hyttel, 1983; Iorio et al., 1983). Also, it was then possible to classify dopamine D₁ and D₂ receptors on the basis of specific binding to selective agonists and antagonists.

Selective Dopamine D₂ and D₁ Studies:

Creese et al. (1977) and Laduron and Leysen (1979), using radioligand binding studies with [³H] spiroperidol, a selective dopamine D₂ receptor antagonist, labeled dopamine D₂ receptors in the central nervous system. Klemm et al. (1979) utilized autoradiography with [³H] spiroperidol and localized high densities of dopamine D₂ receptors in the neostriatum (caudate-putamen), nucleus accumbens, olfactory tubercle, and also in the substantia nigra.

The dopamine D₁ receptors have been studied using oxanthenes such as cis-[³H] piflupentixol (Hyttel, 1978) and cis-(2)-[³H] pifluthixol (Hyttel, 1981). However, these radioligands also label dopamine D₂ receptors (Hyttel and Arnt, 1980). SCH-23390[(R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1-H-3-benzazepine] is a new benzazepine which is a selective D₁ antagonist (Iorio et al., 1981; Hyttel, 1983) and shows a potent blockade of the dopamine-stimulated adenylate cyclase (Onali et al, 1984). Radioligand studies have shown that [³H]SCH-23390 labels and binds specifically and with high affinity to dopamine D₁ receptors in the neostriatum (Schulz et al., 1985).

Dopamine Receptor Distribution:

The distribution of dopamine D₁ and D₂ receptors in the brain areas of different species was studied by Hyttel et al. (1978). It was found that there are 2-6 times more dopamine D₁ receptor binding sites than dopamine D₂ receptor binding sites in rat corpus striatum; the same ratio is found in the mouse striatum. In areas of the brain rich in dopamine receptors, the number of dopamine D₁ receptor binding sites is greater than the number of dopamine D₂ receptor binding sites, but in different ratios (Hyttel, 1978; Hyttel and Arnt, 1986).

Today even though there have been intensive studies performed on the function of dopamine D₁ and D₂ receptors, there is still no clear picture of how dopamine D₁ and D₂ receptors and other neuroreceptors interact in the brain to carry out their function.

Dopamine D₁, D₂ Receptor Function And Interaction:

Marked correlation exists between the ability of neuroleptic drugs to displace [³H] spiroperidol from specific binding sites on dopamine D₂ receptors and their anti-psychotic activity (Creese et al., 1979; Seeman, 1980). Therefore, the action of neuroleptics on dopamine D₂ receptor sites can explain some the functions of dopamine receptor activation.

However, it seems that dopamine D₂ receptors do not function independently of dopamine D₁ receptors, and several

studies have provided evidence for dopamine D₁-D₂ receptor interaction. For example, SKF 38393, a dopamine D₁ receptor agonist, stimulates the release of cAMP from superfused striatal tissue slices; this stimulation is antagonized by LY 141865, a selective dopamine D₂ receptor agonist. Furthermore, (-)sulpiride, a dopamine D₂ receptor antagonist, prevents the effect of LY 141865 (Stool and Keabian, 1981). Low doses of spiroperidol can induce abnormal perioral movements in rats; these abnormal movements can be potentiated by SKF 38393, the dopamine D₁ agonist (Rosengarten et al., 1983). It was also found that intravenous administration of SKF 38393 and RU 24926, a dopamine D₂ agonist, produced synergistic effects on the expression of stereotypic behavior and on the firing rate of basal ganglia neurons in rats. Furthermore, simultaneous stimulation of both dopamine D₁ and D₂ receptors produces greater firing rate and behavioral changes similar to those produced by non-selective dopamine receptor agonists (i.e. apomorphine; Walters et al., 1987). In vitro studies have also shown that haloperidol, at concentrations which appear to be selective for dopamine D₂ receptor blockade, increases the release of [³H] acetylcholine (ACh) from superfused striatal tissue slices (Starke et al., 1983); SCH-23390, the dopamine D₁ antagonist, is capable of antagonizing [³H] ACh release. These findings suggest that dopamine D₁ and D₂

receptor interaction may be an important determinant of maintaining balanced behavioral activities.

6-Hydroxydopamine and Dopamine Receptors:

6-Hydroxydopamine, a relatively selective neurotoxin for catecholaminergic neurons, produces destruction of central noradrenergic and dopaminergic fibers after intracerebroventricular administration to rats (Kostrzewa and Jacobowitz, 1974). To diminish noradrenergic fiber destruction, desmethylimpiramine (DMI), an inhibitor of norepinephrine reuptake, may be used as a pretreatment (Breese and Traylor, 1972). Also, by co-administering pargyline, a monoamine oxidase inhibitor, the destructive action of 6-hydroxydopamine on dopaminergic fibers can be potentiated (Breese and Traylor, 1970).

The destruction of dopamine nigrostriatal fibers is associated with specific motor behavioral dysfunctions, i.e., aphagia, adipsia and akinesia (Ungerstedt, 1971; Marshall et al., 1974) and a proliferation of dopamine D₂ receptors (Breese et al., 1984).

These alterations may appear in Parkinson's disease, where the number and function of dopamine cells is reduced in the substantia nigra. Also, after chronic anti-psychotic agents, such as haloperidol, the striatal dopamine receptors are greatly increased in number, and motor behavior dysfunctions are observed (Tarsy and Baldessarini, 1974).

Haloperidol and Dopamine Receptors:

Haloperidol is an anti-psychotic drug that is used chronically in man. Animal studies have shown that chronic injection of haloperidol causes up-regulation (supersensitivity) of striatal dopamine D₂ receptors in adult rats (Muller and Seeman, 1977; Chiu et al., 1981) and in neonatal rats (Rosengarten and Friedhoff, 1979). Furthermore, haloperidol causes a down-regulation (decrease in number) of striatal dopamine D₂ receptors in neonatal rats following prenatal administration (Rosengarten and Friedhoff, 1979). However, haloperidol is a non-selective dopamine D₂ receptor antagonist (70% D₂) (Murrin, 1982); new agents have been found which are more specific antagonists.

Dopamine and Dopamine Receptor Ontogeny:

A--Dopaminergic Input Prior to Birth

Three major dramatic changes in the dopaminergic input to rat striatum occur prior to birth and for the 8 week period after birth:

- 1) Tyrosine hydroxylase activity increases (Coyle et al., 1976).
- 2) There is a gradual increase in dopamine concentration and dopamine uptake (Coyle et al., 1976). The rat striatum contains 12-24% of the adult (i.e., 90 days old) level of dopamine at birth. The dopamine content increases slowly until it reaches adult levels by postnatal day 60, and then remains constant through

adulthood and senescence (up to 20 months of age).

3) There is a rapid increase in the dopamine receptor number up to adult levels by postnatal day 30-35 (Giorgi et al., 1987; Murrin, 1982).

B--Dopamine D₁ Receptor Ontogeny:

Giorgi et al. (1987) found that the density of dopamine D₁ receptors labeled with [³H] SCH-23390 in rat striatum was only 9% of the adult value at birth, and increased rapidly, continuing until day 35, when dopamine D₁ receptor density attained maximum values and then decreased significantly with age; i.e. the B_{max} for dopamine D₁ binding to the striatum at 35 days was 1.75 pmol/mg protein versus 1.2 at 90 days, and 0.89 at 270 days.

C--Dopamine D₂ Receptor Ontogeny:

Murrin (1982) studied the ontogeny of dopamine D₂ receptors using in vivo [³H] spiroperidol binding studies in 5, 15 and 30 day old rat pups. It was found that dopamine D₂ receptors doubled in number between day 5 and day 15, and then gradually increased in number reaching adult levels by day 30. This observation is similar to the in vitro study on dopamine D₂ receptor ontogeny by Pardo and Creese (1977).

Impairment of Dopamine Receptor Ontogeny:

The normal ontogeny of dopamine D₂ receptors can be impaired by prenatal treatment with dopamine receptor antagonists. Rosengarten and Friedhoff (1979) showed that when the neuroleptic haloperidol is administered to pregnant

dams, a persistent decrease in the number of dopamine D₂ receptors occurs in the offspring. Later, it was found that days 15-18 of the gestational period was the critical time for that effect (Rosengarten et al., 1983). This finding was confirmed by Miller and Friedhoff (1986).

It has also been shown that treatment of nursing dams with haloperidol for 21 days after giving birth results in a 40% increase in the number of striatal dopamine D₂ receptors in the littermates two weeks after the last dose of haloperidol has been administered to the dam (Rosengarten and Friedhoff, 1979). Other studies have shown that treatment of neonatal rats with penfluridol, a neuroleptic similar to haloperidol, on alternate days during the first postnatal week does not affect the development of striatal dopamine D₂ receptors, although there is a supersensitive response of these rats to the dopamine receptor agonist, apomorphine, as adults (Coyle et al., 1981).

Until now, there have been no studies to determine the striatal dopamine D₁ and D₂ receptor response to highly selective dopamine D₁ and D₂ antagonists administered either prenatally or postnatally. This type of specific experiment is important to gain more knowledge on the ontogeny of dopamine receptors and their response to chronic blockade by specific antagonists.

Dopamine Receptors And L-Prolyl-L-Leucyl-Glycinamide (PLG):

It is accepted that the hypothalamic tissue of many species, including man, contains a substance that inhibits the release of melanocyte stimulating hormone (MSH) from the pituitary gland (Kastin and Schally, 1966); this neurohormonal substance is called melanocyte stimulating hormone release inhibiting factor (MIF). Nair et al. (1971a) determined the structure of MIF to be L-prolyl-L-leucyl-glycinamide (PLG) and synthesized this substance, which had biological activities similar to those of naturally-occurring bovine MIF. It was also found that PLG has a short biological half-life, approximately 9 minutes (Redding et al., 1973). Several studies have demonstrated that PLG produces a wide variety of behavioral and neurochemical actions in the absence of the pituitary gland. The search for a better and safer treatment for behavior dysfunctions (i.e., psychoses and Parkinson's disease) has brought about the promising role of PLG in specifically modulating dopaminergic processes in the mammalian brain. It was found that PLG potentiates the effects of dihydroxyphenylalanine (L-dopa) on motor performance in both intact and hypophysectomized mice (Plotnikoff, 1971) and in humans (Barbeau, 1975). PLG is also devoid of the adverse neurological side-effects of the L-dopa treatment (Barbeau et al., 1978). Clinical studies indicated that PLG temporarily, but significantly, reduced the intensity of dyskinesic

symptoms associated with chronic anti-psychotic therapy (Ehrensing, 1974; Kastin et al., 1977).

Later, Chiu et al. (1981) showed that PLG was able to attenuate dopamine receptor supersensitivity caused by chronic administration of haloperidol in rats. Bhargava (1981a) also observed that PLG was able to inhibit the development of tolerance to the cataleptic and hypothermic effect of haloperidol in the rat. Chiu et al. (1981) also showed that PLG is able to block the behavioral supersensitivity of dopamine receptors induced by haloperidol.

Other studies have also demonstrated important findings. Bhargava (1981) found that PLG and its cyclic analog, cyclo (Leu-Gly), are able to block the development of analgesic tolerance and dopamine receptor supersensitivity induced by chronic morphine treatment. In addition, Ritzman et al. (1982) found that cyclo (Leu-Gly), when injected into rats prior to chronic exposure to morphine, inhibited morphine-induced increases in behavioral responses to dopamine agonists in addition to the inhibition of some signs of physical dependence.

Further studies were done to determine PLG binding sites in human brain. It was found that [^3H] PLG bound to membrane homogenates with high affinity in a saturable manner. Substantia nigra had the highest level of specific [^3H] PLG binding followed by striatum and hypothalamus. It was

concluded that specific PLG binding sites do exist in the human brain and these binding sites may be strategically localized to be able to modulate the changes in the number of dopamine receptors (Chiu et al., 1980). It has also been found that PLG has distinct binding sites in the rat brain, with the highest density in substantia nigra and striatum. PLG also selectively increases the affinity of the specific binding of the dopamine agonist [³H] apomorphine to dopamine receptors and does not compete for dopamine/neuroleptic receptor binding (Chiu et al., 1981).

It can be concluded that PLG is a neuropeptide that interacts with specific binding sites in the brain and carries out specific functions related to modifying the dopamine system; the mechanism is not yet clear.

Rationale:

This study was undertaken to determine whether postnatal challenge of rat pups with specific dopamine D₁ and D₂ receptor antagonists would modify the ontogeny of the respective receptor populations in the striatum and to determine whether PLG was capable of modulating the changes in the ontogeny of these dopamine receptors.

It would appear that chronic blockade of dopamine receptors may serve as a model by which many neurological and behavioral dysfunctions can be observed. For example, schizophrenia is conventionally treated with dopamine D₂ antagonists. However, protracted treatment with these agents

may lead to tardive dyskinesia in humans and to dopamine D₂ supersensitivity (increase in number) in rats (Chiu, 1981); prolonged treatment of Parkinson's Disease with dopamine agonists leads to dopamine receptor desensitization and worsens the disease manifestations (Rinne et al., 1979). Dopamine desensitization mimics down-regulation of dopamine receptors (decrease in number). In rats, dopamine receptor down-regulation may be caused by prenatal treatment with dopamine D₂ antagonists (Rosengarten and Friedhoff, 1979). Acute and chronic cocaine administration in rats causes dopamine receptor supersensitivity (Taylor et al., 1979), which may be the reason behind severe cravings for cocaine and the resulting abuse of cocaine in humans (Dackis and Marks, 1984). A better understanding of these processes could be of value in promoting the development of a viable treatment for these types of dysfunctions in man. For example, PLG may be of use in antagonizing the dependence and dysfunctions caused by drugs of abuse. PLG may also be of benefit in combination with anti-psychotic drugs, in order to prevent the development of tardive dyskinesia. Similar uses of PLG are suggested for preventing L-dopa-induced side effects in Parkinsonian patients.

In summary, the present study was undertaken to study dopamine receptor ontogeny under chronic blockade with specific antagonists and to test the ability of PLG to attenuate any abnormal changes in that ontogeny.

Chapter II

Material and Method

This project can be divided into two experiments. The first experiment was conducted to determine the effect of chronic blockade with specific antagonists on the ontogeny of dopamine D₁ and D₂ receptors and to test the ability of PLG to attenuate receptor changes produced by the antagonists.

The second experiment can be divided into two studies: The first study was undertaken to test the long-term effect of specific neonatal blockade on dopamine D₁ and D₂ receptors. Rats were treated with specific dopamine D₁ or D₂ antagonists for 32 days, then studied at 12 weeks. The second study was undertaken to determine adult dopamine D₁ and D₂ receptor response to specific blockade; 12 week old rats were treated with specific antagonists for 17 days, then decapitated. The same rats had been treated postnatally for 32 days from the day of birth.

Animals and Treatment:

Timed pregnant Sprague Dawley albino rats were obtained from Charles River Labs (Research Triangle, NC). Animals were housed individually in plastic cages and maintained under standard laboratory conditions (ad libitum access to food and water, temperature of 22[±]1 °C, and 12 hour light-dark cycle, on at 0700 hour). Starting at birth, all neonates were treated once a day for 32 successive days by

intraperitoneal injection (i.p.) with one of the following regimens: (a) saline (0.9%), the SCH-23390.HCl solvent, plus saline containing tartaric acid (0.5%), the spiroperidol solvent, and acetic acid (0.5 mM), the PLG solvent, (b) SCH-23390.HCl (0.30 mg/kg, free base; Research Biochemicals, Inc., Natick, MA) in saline (0.9%) plus saline-tartaric acid/acetic acid, (c) spiroperidol (1.0 mg/kg i.p., Research Biochemicals) in saline-tartaric acid/acetic acid plus saline, (d) SCH-23390 plus spiroperidol, (e) PLG (1.0 mg/kg) in saline-acetic acid, (f) PLG + SCH-23390, (g) PLG + spiroperidol, or (h) PLG + SCH-23390 + spiroperidol. Rats were weaned at 4 weeks and decapitated at 5, 8, or 12 weeks of age. At 12 weeks of age, groups of animals were treated again, once a day for 17 consecutive days. Rats that had been treated neonatally with saline plus saline-tartaric acid/acetic acid or with SCH-23390, or with spiroperidol or with the combination of SCH-23390 plus spiroperidol received the identical treatment at 12 weeks of age. For rats treated as adults, an interval of three days elapsed between the last treatment and the time of decapitation to allow time for elimination of drugs from striatal tissue. After decapitation, brains were immediately removed and the striata were dissected free, frozen on dry ice, and stored at -60° C.

Tissue Preparation:

At the time of assay striata were placed in 30 volumes of 50 mM Tris buffer (pH 7.4) containing 120 mM NaCl, 5 mM

KCl, 1 mM MgCl₂, 10 uM pargyline, 0.1% ascorbic acid and 1 uM ketanserin, the serotonin S₂ receptor antagonist (Janssen Pharmaceutical Co., Beerse, Belgium). After homogenization (setting of 50, 20 s; Tekmar Tissumizer) tissue suspensions were incubated at 37° C for 30 minutes, in order to allow dissociation of any drug residue from the tissue. Samples were centrifuged at 48,000g for 25 min at 10° C in a Beckman L5-75B ultracentrifuge. This step was repeated after resuspending the pellets in 30 volumes of fresh buffer. The final pellets were resuspended in the Tris-salt solution.

D₁ Binding Assay:

To assess total dopamine D₁ receptor binding, the method of Schulz et al. (1985) was employed. Briefly, samples of 0.2 ml of homogenate were added to [³H]SCH-23390 (300 pM, final conc.; Amersham) in Tris-salt solution containing 2 mM CaCl₂. Samples (1 ml incubation mix) were incubated for 15 min at 37° C in a shaking water bath, and then rapidly filtered under partial vacuum on Whatman GF/F glass fiber filters using a Millipore filtration unit. Filters were washed three times with ice-cold Tris-salt solution. After drying, filters were placed in 10 ml of Scintiverse E (Fisher Scientific), and tritium activity was determined in a Beckman LS 9800 liquid scintillation spectrometer. Specific binding of [³H] SCH-23390 was defined as the difference in binding in the presence and absence of SCH-23390 (1 uM).

D₂ Binding Assay:

To assess total dopamine D₂ receptor binding the method of Creese and Snyder (1979) was used. Aliquots of homogenate (0.2 ml) were incubated with [³H] spiroperidol (300 pM, final conc.; Amersham) in Tris-salt solution (1 ml incubation mix) for 15 min at 37° C, and then rapidly filtered on Whatman GF/F glass fiber filters. Specific binding of [³H] spiroperidol represented the difference in binding in the presence and absence of d-butacclamol (1 uM; Research Biochemicals, Inc.).

Kinetics Studies:

In order to determine the B_{max} and K_D values for [³H] SCH-23390 and [³H] spiroperidol receptor binding, Scatchard analysis was used on the binding data from striatal homogenates of 5 week old rats.

For [³H] SCH-23390 kinetic studies in rat striata, which reflect dopamine D₁ binding, rats were treated for 32 days from day of birth with (a) saline, (b) SCH-23390 (0.3 mg/kg i.p), (c) PLG (1.0 mg/kg or (d) SCH-23390 + PLG. The [³H] SCH-23390 concentrations ranged from 50 to 1500 pM (8 concentrations, total). For [³H] spiroperidol kinetic studies in rat striata, which reflect dopamine D₂ binding, rats were treated for 32 days from day of birth with (a) saline, (b) spiroperidol (1.0 mg/kg i.p), (c) PLG (1.0 mg/kg i.p), or (d) spiroperidol + PLG. The [³H] Spiroperidol concentrations ranged from 50 to 1500 pM (8 concentrations, total).

Data Analysis

In the developmental study an analysis of variance (ANOVA) followed by a post-ANOVA Newman-Keuls test was used to analyze the data and to test for significant differences between the treatment groups. To test for the ability of receptor antagonists to produce an upregulation of dopamine D₁ and D₂ receptors an ANOVA was also used. A p value of <0.05 was considered to be the level for statistical significance.

Chapter III

Results

1. Ontogenic Impairment of Striatal D₁ Receptors by Neonatal SCH-23390:

Administration of the dopamine D₁ receptor antagonist, SCH-23390 (0.30 mg/kg, i.p.) to rats, once a day for 32 successive days from birth, resulted in a marked impairment of striatal dopamine D₁ receptors at 5, 8 and 12 weeks (p<0.001) (figs. 1, 2, and 3). In the group of rats treated chronically with SCH-23390, total in vitro binding of [³H]SCH-23390 to striatal homogenates was reduced by about 75% at all time intervals. Groups treated with the combination of SCH-23390 and spiroperidol (1 mg/kg i.p.) exhibited the same degree of impairment of dopamine D₁ receptors binding as those treated with SCH-23390 alone, indicating that dopamine D₂ receptor antagonism in development does not permanently modify the ontogenic impairment of dopamine D₁ receptors by SCH-23390. Also, chronic spiroperidol treatment alone during ontogeny did not alter the development of striatal dopamine D₁ receptors, as assessed at 8 and 12 weeks (figs. 2 and 3). However, spiroperidol altered dopamine D₁ receptor by 20% at 5 weeks (fig. 1).

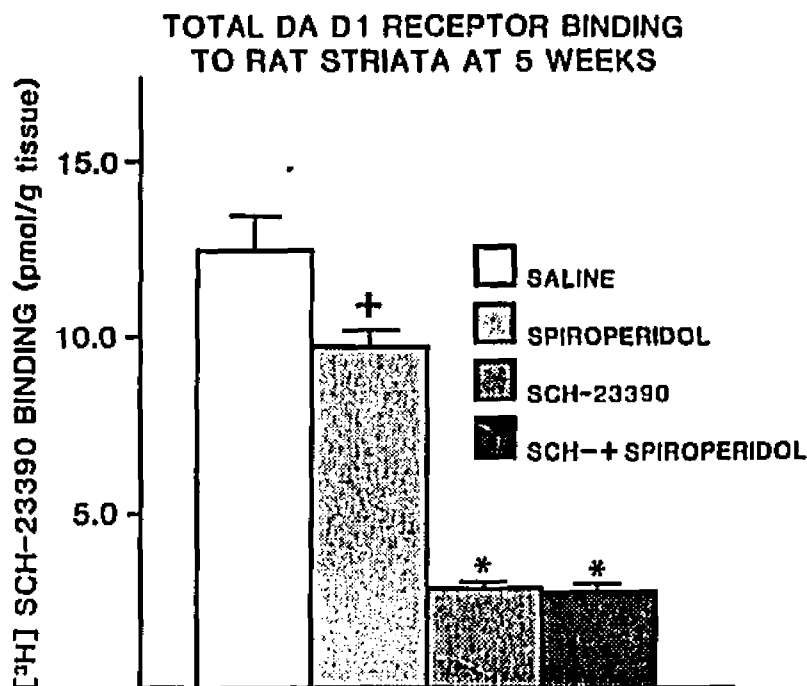


Figure 1. Total [³H]SCH-23390 binding to striatal homogenates of 5 week old rats, chronically treated postnatally with SCH-23390 and/or spiroperidol. Animals were treated i.p. once each day for 32 consecutive days from birth with saline, spiroperidol (1.0 mg/kg), SCH-23390 (0.30 mg/kg), or spiroperidol plus SCH-23390. Each column represents the mean (\pm S.E.M.) specific binding of [³H]SCH-23390 (300 pM; pmol/g tissue). Values represent data from 4 animals per group. +, significantly different from the saline control group, $p < 0.05$; *, $p < 0.001$, compared to the saline control group.

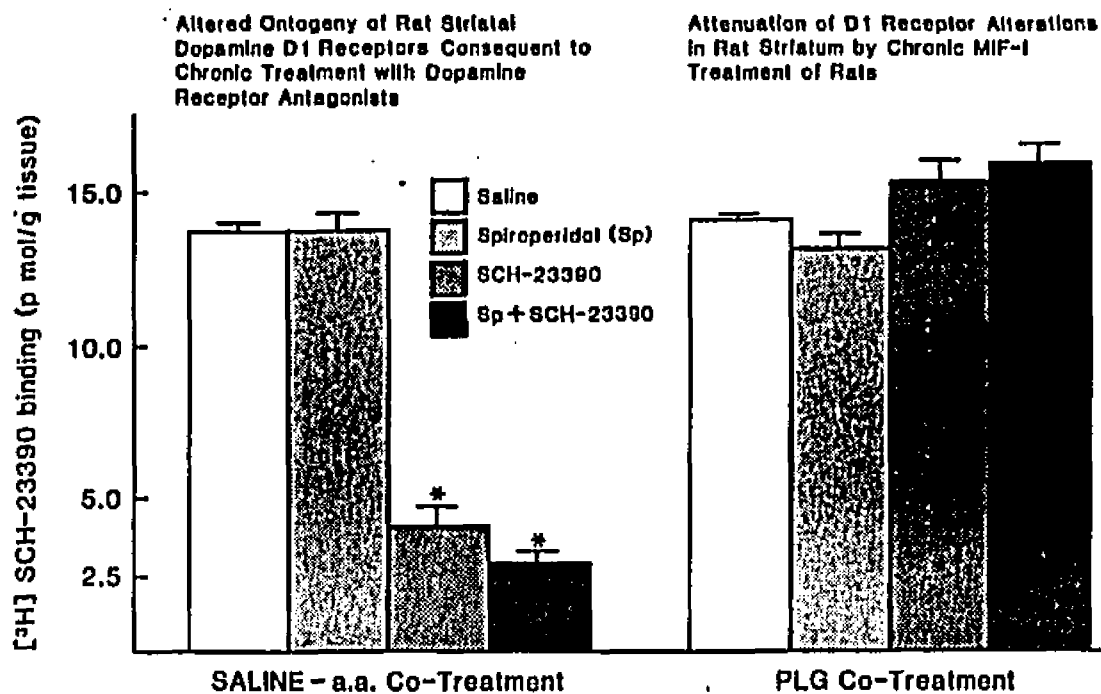


Figure 2. Total [^3H]SCH-23390 binding to striatal homogenates of 8 week old rats, chronically treated postnatally with SCH-23390 and/or spiroperidol. Animals were treated i.p. once each day for 32 consecutive days from birth with saline, spiroperidol (1.0 mg/kg), SCH-23390 (0.30 mg/kg), or spiroperidol plus SCH-23390. Each column represents mean (\pm S.E.M.) specific binding of [^3H]SCH-23390 (300 pM; pmol/g tissue) to striatal homogenates derived from rats at 8 weeks from birth. *, Statistically different from the saline control group ($p < 0.001$).

TOTAL DA D1 RECEPTOR BINDING TO RAT
STRIATA AT 12 WEEKS

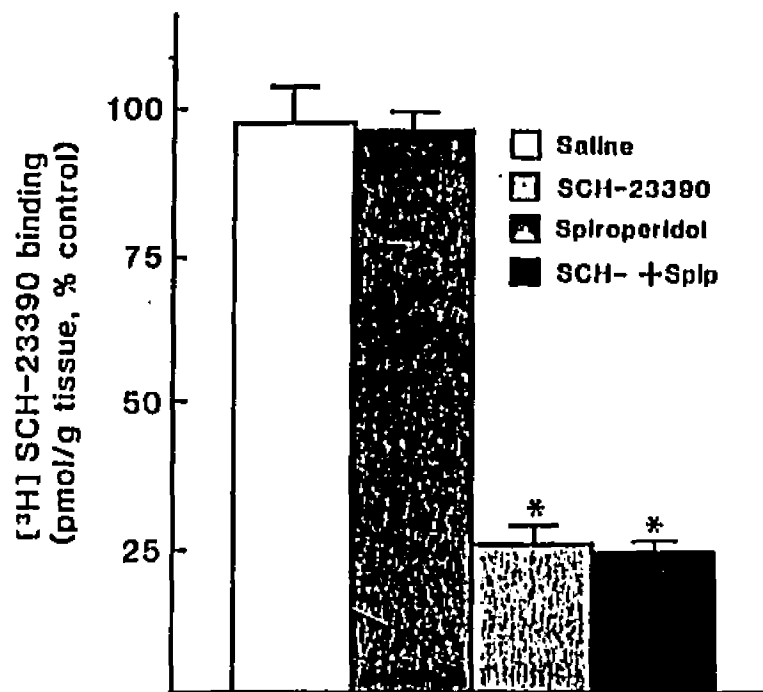


Figure 3. Total [³H]SCH-23390 binding to striatal homogenates of 12 week old rats, chronically treated postnatally with SCH-23390 and/or spiroperidol. Animals were treated i.p. once each day for 32 consecutive days from birth with saline, spiroperidol (1.0 mg/kg), SCH-23390 (0.30 mg/kg), or spiroperidol plus SCH-23390. Each column represents mean (\pm S.E.M.) specific binding of [³H]SCH-23390 (300 pM; pmol/g tissue).

Control group represents [³H] SCH-23390 binding of 12.5 ± 0.8 pmol/g tissue. Values represent data from 4 animals per group.

*, statistically different from the saline control group ($p < 0.001$).

2. Ontogenic Impairment of Striatal Dopamine D₂ Receptors by Neonatal Spiroperidol:

When the dopamine D₂ receptor antagonist, spiroperidol (1.0 mg/kg i.p.), was administered to rats once daily for 32 consecutive days from birth, there was a marked impairment of the development of striatal dopamine D₂ receptors at 5, 8 and 12 weeks ($p < 0.001$) (figs. 4, 5 and 6). Chronic spiroperidol alone reduced total [³H] spiroperidol binding to striatal membranes by 74%, 51% and 70%, at 5, 8, and 12 weeks, respectively. This effect was not modified in the group of rats treated with spiroperidol and SCH-23390, indicating that dopamine D₁ receptor antagonism during development does not modify the ontogenic impairment of dopamine D₂ receptors by spiroperidol. Similarly, chronic SCH-23390 treatment alone during ontogeny did not alter the development of striatal dopamine D₂ receptors at 8 weeks. However, SCH-23390 altered dopamine D₂ receptors by 20% at 5 weeks (fig. 4).

3. Dopamine D₁ Receptor Kinetic Studies:

Dopamine D₁ receptor kinetic studies were performed on striatal tissue from 5 week old rats treated chronically with saline, SCH-23390, PLG, and SCH-23390 + PLG, respectively. It was found that treatments with SCH-23390 and/or PLG did not alter the K_D from the control (K_D of saline group, 348.6 ± 9.1 pM; mean and S.E.M).

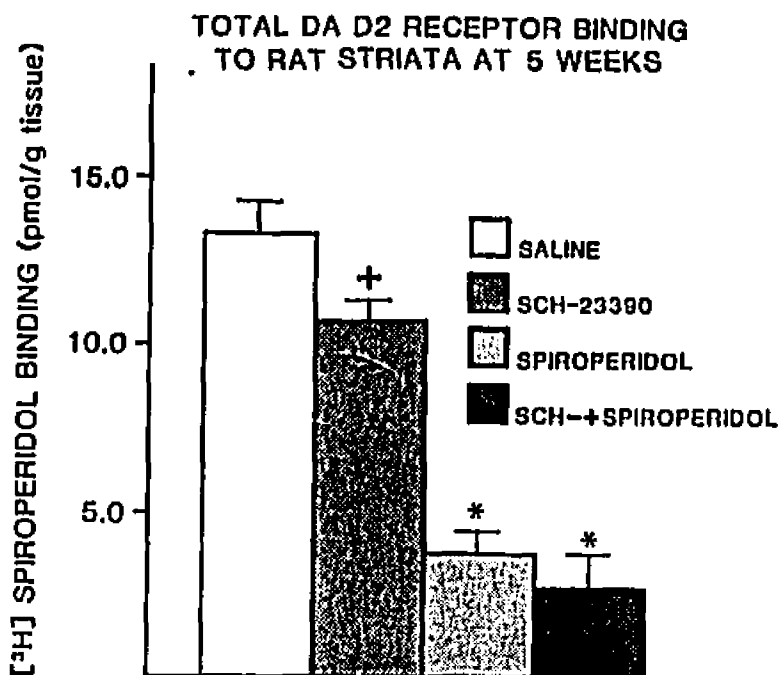


Figure 4. Total [³H]spiroperidol binding to striatal homogenates of 5 week old rats, chronically treated with spiroperidol and/or spiroperidol + SCH-23390. Animals were treated i.p. each day for 32 consecutive days with saline, spiroperidol (1.0 mg/kg), SCH-23390 (0.3 mg/kg), or spiroperidol plus SCH-23390. Each column represents mean (\pm S.E.M.) specific binding of [³H]spiroperidol (300 pM; pmol/g tissue).

Values represent data from 4 animals per group.

+, significantly different from the saline control group, $p < 0.05$;

*, $p < 0.001$, compared to the saline control group.

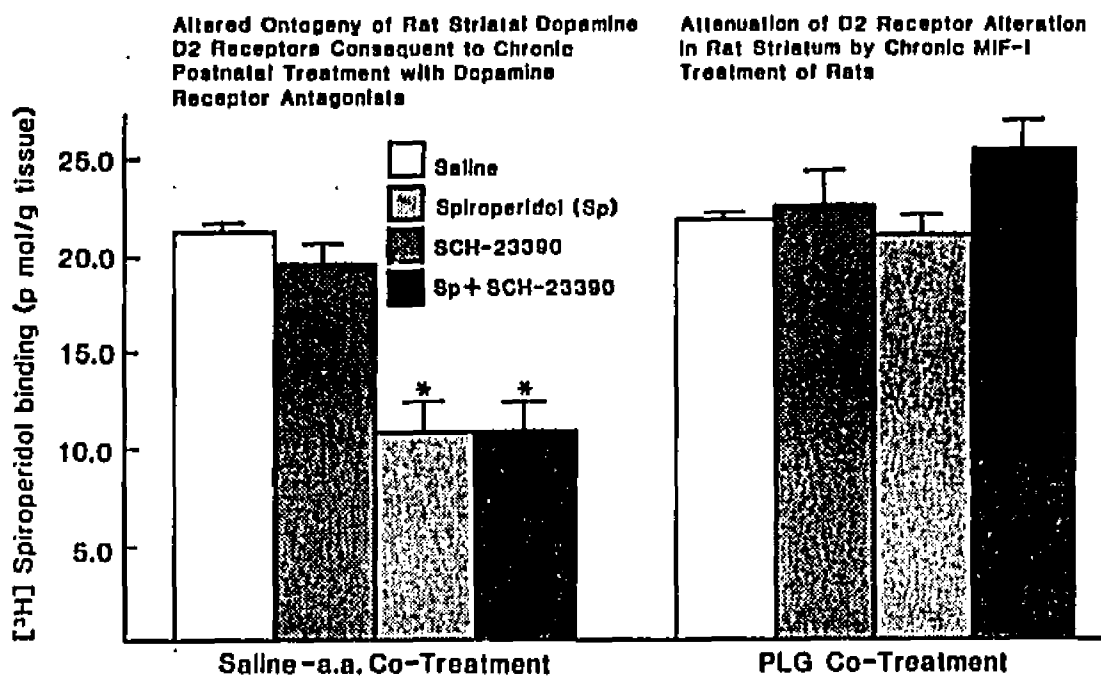


Figure 5. Total [^3H]spiroperidol binding to striatal homogenates of 8 week old rats, chronically treated with spiroperidol and/or spiroperidol + SCH-23390. Animals were treated i.p. each day for 32 consecutive days with saline, spiroperidol (1.0 mg/kg), SCH-23390 (0.3 mg/kg), or spiroperidol plus SCH-23390. Each column represents the mean (\pm S.E.M.) specific binding of [^3H] spiroperidol (300 pM; pmol/g tissue). Values represent data from 4 animals per group.

*, $p < 0.001$, compared to the saline group.

TOTAL DA D2 RECEPTOR BINDING TO RAT
STRIATA AT 12 WEEKS

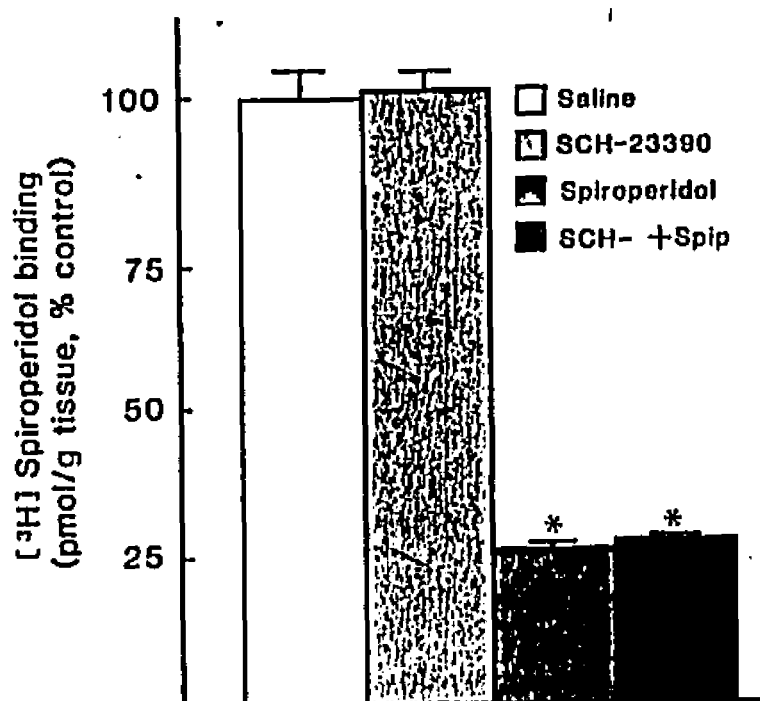


Figure 6. Total [³H]spiroperidol binding to striatal homogenates of 12 week old rats, chronically treated with spiroperidol and/or spiroperidol + SCH-23390. Animals were treated i.p. each day for 32 consecutive days with saline, spiroperidol (1.0 mg/kg), SCH-23390 (0.3 mg/kg), or spiroperidol plus SCH-23390. Each column represents the mean (\pm S.E.M.) specific binding of [³H] spiroperidol (300 pM; pmol/g tissue). Control group represents [³H] spiroperidol binding of 19 ± 0.8 pmol/g tissue. Values represent data from 4 animals per group. *, $p < 0.001$, compared to the saline control group.

However, SCH-23390 treatment effectively reduced the Bmax by 78% from control (Bmax of saline group, 55.0 ± 0.6 fmol/mg tissue) as shown in table 1 and figure 7.

4. Dopamine D₂ Receptor Kinetic Studies:

Dopamine D₂ receptor kinetic studies were performed on 5 week old rats treated chronically with saline, spiroperidol, PLG, and spiroperidol + PLG, respectively. It was found that treatments with spiroperidol and/or PLG did not alter the K_D from the control (K_D of saline group, 168 ± 4.3 pM; mean and S.E.M.). However, spiroperidol treatment effectively reduced the Bmax by 75% from control (Bmax of saline group, 24.6 ± 0.8 fmol/mg tissue) as shown in table 2 and figure 8.

5. PLG Attenuation of Receptor Impairment:

In groups of rats that were co-treated with the neuropeptide PLG (1 mg/kg ip., for 32 days from day of birth), there was complete attenuation of the effects of chronic treatments with the dopamine D₁ and D₂ receptor antagonists.

In rats decapitated at 5 weeks (table 1) and at 8 weeks (fig. 2), the total in vitro binding of dopamine D₁ receptors was similar in the saline control and PLG + SCH-23390-treated groups. These results demonstrate the ability of PLG to prevent the ontogenically impaired development of dopamine D₁ receptors by chronic postnatal SCH-23390 treatment. Also, PLG treatment for 32 days from birth, along with spiroperidol (1 mg/kg i.p.), resulted in normal complement of striatal

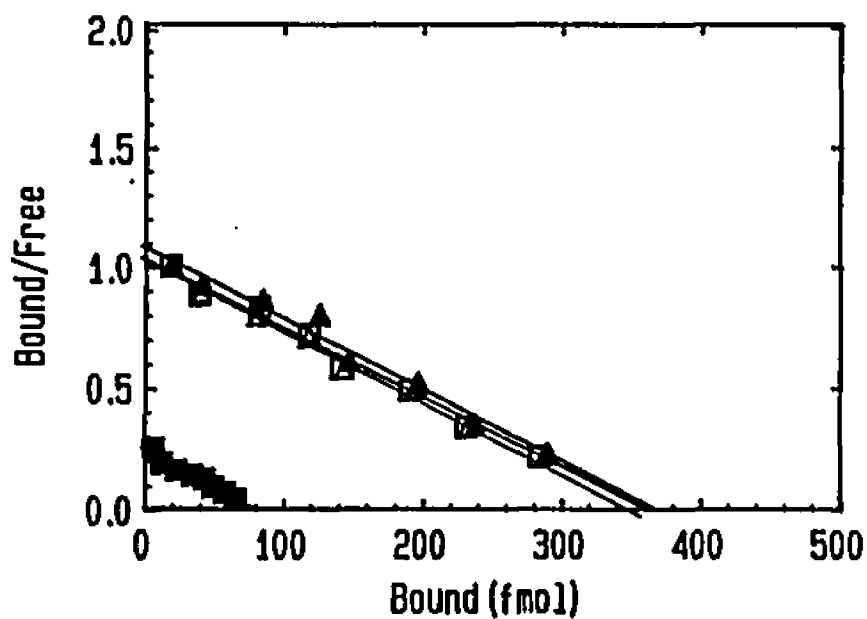


Figure 7. Scatchard analysis derived from [^3H]SCH-23390 binding data of striatum of 5 week old rats. Each treatment group represents the mean binding of 3 animals treated for 32 days postnatally with saline (\triangle), PLG (\blacktriangle , 1 mg/kg), SCH (\blacksquare 0.3 mg/kg), or PLG + SCH-23390 (\square , 1 mg/kg).

Table 1.

Effect of 32 day postnatal treatments on rat striatal dopamine D1 receptors⁺, as determined by Scatchard analysis of [³H] SCH-23390 binding data.

Treatment	Bmax (fmol/mg tissue)	K _D (pM)
Saline	54.9 ± 0.6	348.6 ± 9.1
PLG (1.0 mg/kg/d)	56.6 ± 2.2	334.0 ± 4.8
SCH-23390 (0.30 mg/kg/d)	12.3 ± 0.6*	328.0 ± 5.3
PLG + SCH-23390	54.5 ± 1.8	339.0 ± 3.8

+ Each value is the + S.E.M. of 3 tissues.

* Indicates a significant difference from the saline control group, p < 0.001.

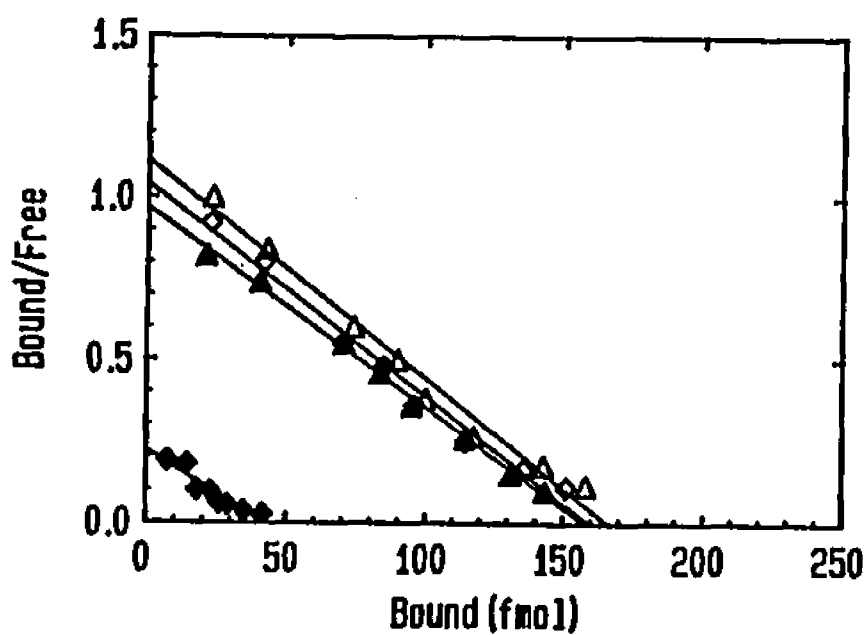


Fig. 8. Scatchard analysis derived from [^3H] spiroperidol binding data of striatum of 5 week old rats. Each treatment groups represent the mean binding of 3 animals treated for 32 days postnatally with saline (Δ), PLG (\blacktriangle , 1 mg/kg), spiroperidol (\blacklozenge , 1 mg/kg), or PLG + spiroperidol (\diamond , 1 mg/kg).

Table 2.

Effect of 32 day postnatal treatments on rat striatal dopamine D2 receptor⁺, as determined by Scatchard analysis of [3H] spiroperidol binding data.

Treatment	Bmax (fmol/mg tissue)	K _D (pM)
Saline	24.6 ± 0.8	168.0 ± 4.3
PLG (1.0 mg/kg/d)	23.9 ± 0.4	159.0 ± 4.9
Spiroperidol (1.0 mg/kg/d)	6.3 ± 0.2*	181.7 ± 3.2
PLG + Spiroperidol	23.6 ± 0.6	162.0 ± 4.5

+ Each value is the mean + S.E.M. of 3 tissues.

* Indicates a significant difference from the saline control group, p <0.001.

dopamine D₂ receptors at 5 weeks (table 2) and at 8 weeks (fig. 8). These results also demonstrate the ability of PLG to prevent the ontogenic impairment (decrease in number) of dopamine D₂ receptors when chronically blocked with a specific antagonist (i.e. spiroperidol).

In conclusion, PLG is able to maintain a normal development of dopamine receptors when these receptors are challenged with chronic specific antagonist treatment.

6. Up-Regulation of Ontogenically Impaired Striatal Dopamine D₁ Receptors After Chronic SCH-23390 Challenge of Adult Rats:

In order to determine whether groups of rats with ontogenically impaired striatal dopamine D₁ receptors could respond to adult challenge with a dopamine D₁ receptor antagonist by up-regulating D₁ receptors, rats at 12 weeks of age were given SCH-23390 (0.30 mg/kg i.p.) once a day for 17 successive days. After a three day drug-free period, rats were decapitated and total in vitro binding of [³H] SCH-23390 to striatal membranes was assessed. It was found that when ontogenically impaired rats were treated for 17 days with SCH-23390, an up-regulation of dopamine D₁ receptors ($p < 0.001$) (fig. 9) resulted. In the group of rats treated during postnatal ontogeny with SCH-23390, total [³H] SCH-23390 in vitro binding to striatal membranes was reduced by 74% at 12 weeks (fig. 3). However, when SCH-23390 was administered for 17 days in these mature rats, the result was a three-fold increase in [³H] SCH-23390 binding, to 75% of

saline control levels. When spiroperidol was administered alone (1.0 mg/kg i.p.), for 17 consecutive days in mature rats, an up-regulation of dopamine D₁ receptors did not result. Furthermore, when spiroperidol was administered in conjunction with SCH-23390 to 12 week old rats for 17 days, the response was the same as after treatment with SCH-23390 alone. Thus, dopamine D₁ receptor antagonism in mature rats results in an up-regulation of striatal dopamine D₁ receptors even when this population of receptors is developmentally reduced in number. Challenge of these dopamine D₁ receptor-impaired adult rats with a dopamine D₂ receptor antagonist did not modify D₁ receptor binding.

7. Lack of Up-Regulation of Ontogenically Impaired Striatal D₂ Receptors After Chronic Spiroperidol Challenge of Adult Rats:

A 17 day treatment with spiroperidol (1.0 mg/kg/d i.p.) in 12 week old rats with ontogenically impaired development of striatal dopamine D₂ receptors failed to change in vitro binding of [³H] spiroperidol to striatal membranes at 12 weeks (fig. 10). Also, when SCH-23390 was administered to mature rats, alone or in combination with spiroperidol, the binding of [³H] spiroderidol was not enhanced.

In groups of rats treated during postnatal ontogeny with spiroperidol, [³H] spiroperidol in vitro binding of dopamine D₂ receptors was reduced by 70% at 12 weeks (fig. 6).

Up-Regulation of Striatal Dopamine D1
Receptors by Chronic SCH-23390
Challenge at 12 Weeks

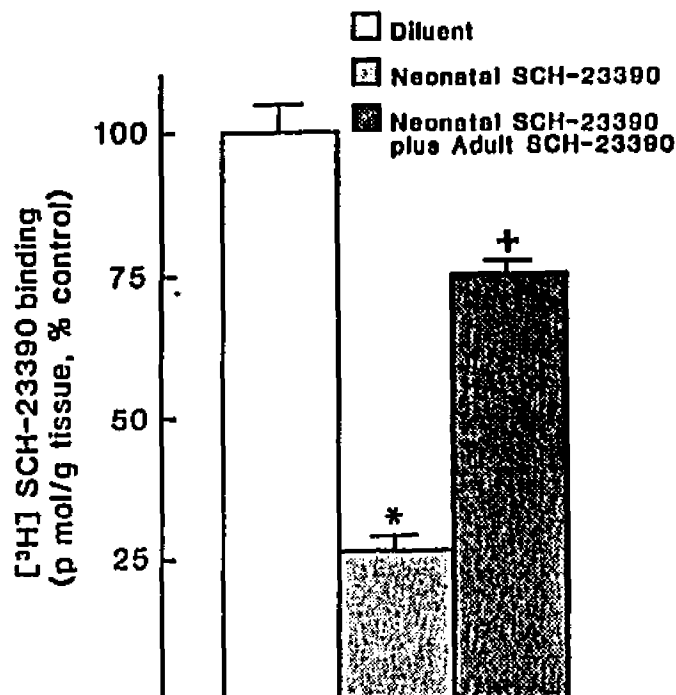


Figure 9. Total [³H]SCH-23390 binding to striatal homogenate of 12 week old rats. Animals were chronically treated with SCH-23390 as neonates, and again as adults. Neonatal rats were treated once each day for 32 consecutive days from birth with diluent or SCH-23390 (0.30 mg/kg i.p.). At 12 weeks after birth the diluent group received an additional 17 daily treatments with diluent, while the SCH-23390 group received an additional 17 daily treatments with SCH-23390. Each column represents the mean (\pm S.E.M.) specific binding of [³H]SCH-23390 (300pM; pmol/g tissue). Control group represents [³H]SCH-23390 binding of 14 ± 0.3 pmol/g tissue.
*, $p < 0.001$, compared to the saline control group.
+, $p < 0.001$, compared to the saline control group and SCH-23390-treated group.

Failure of Striatal Dopamine D2 Receptors
to Up-Regulate in Response to
Chronic Spiroperidol at 12 Weeks

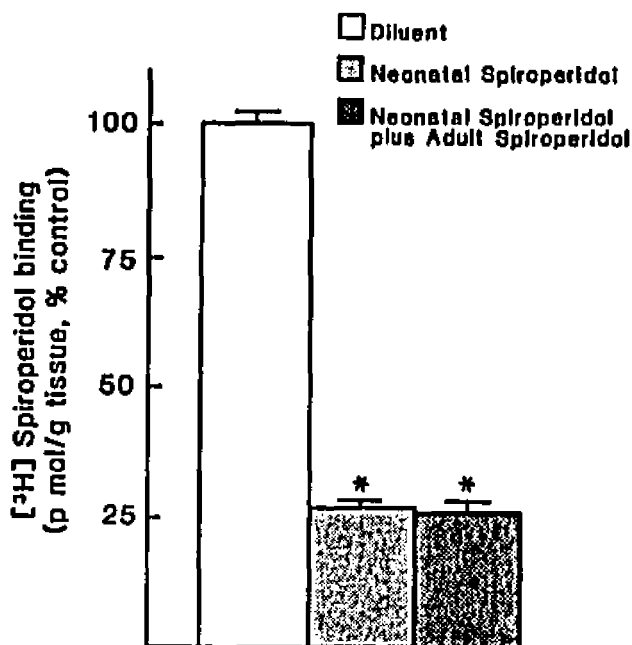


Fig. 10. Total [³H]spiroperidol binding to striatal homogenates of 12 week old rats. Animals were chronically treated with spiroperidol as neonates and again as adults. Neonatal rats were treated once each day for 32 consecutive days from birth with diluent or spiroperidol (1.0 mg/kg i.p.). At 12 weeks after birth, the diluent group received an additional 17 daily treatments with diluent, while the spiroperidol group received an additional 17 daily treatments with spiroperidol (1.0 mg/kg i.p.). Each column represents the mean (+S.E.M.) specific binding of [³H] spiroperidol (300 pM; pmol/g tissue). Control group represents [³H] spiroperidol binding of 13 ± 0.26 pmol/g tissue. *, $p < 0.001$, compared to the saline control group.

Chapter IV

Discussion

1. Ontogenic Impairment of Dopamine D₁ and D₂ Receptors:

The present study has shown that chronic postnatal treatment of rats with highly selective dopamine D₁ or D₂ antagonists results in a persistent impairment of striatal dopamine D₁ and D₂ receptor ontogeny, respectively, as measured at 5, 8, and 12 weeks by total in vitro binding assays. At 5 weeks only, SCH-23390-treatment resulted in a 20% decrease in total [³H] spiroperidol binding to striatum, while spiroperidol treatment resulted in a 20% decrease in total [³H] SCH-23390 binding. This finding demonstrates that the postnatal period is sensitive and critical for normal development of striatal dopamine D₁ and D₂ receptors, and that the chronic blockade of dopamine receptors during this period may lead to permanent impairment in the ontogeny of the receptors.

The impairment in dopamine receptor ontogeny is reflected by the decrease in the total specific binding measured by in vitro assays or, simply a decrease in the number of dopamine receptors. At the time that dopamine D₂ receptors are ontogenically impaired, the dopamine D₁ receptor binding is normal and vice versa. This suggests a functional impairment in the dopamine system, not an anatomical one. In rat striatum there is evidence that

dopaminergic innervation is incomplete at the time of maturation of striatal dopamine D₂ receptors, D₁ receptors, and dopamine-stimulated adenylate cyclase (Giorgi et al., 1987; Pardo et al., 1977). The density of the dopamine D₁ receptors increased rapidly from 9% of the adult level at birth, to a peak value by postnatal day 35 (Giorgi et al., 1987). The dopamine content at birth is 12% of the adult level and increase slowly until reaching adult level by postnatal day 60. The dopamine content is an indication of dopaminergic innervation and presynaptic terminal maturation. Therefore, it can be suggested that the postnatal period is a critical period with regard to dopaminergic development, and antagonistic treatment during this period may alter this development, mainly through postsynaptic receptor sites. The impairment in dopamine receptor ontogeny is apparently due to impairment in the ability of the dopamine receptor cells to express a normal number of dopamine receptors after chronic blockade. Also, specific chronic blockade of dopamine receptors may interfere with signals that are transmitted to the cell nuclei to stimulate greater expression of dopamine receptors (up-regulation). An increase in the number of striatal D₂ receptors was observed when haloperidol was administered to rats for the first three weeks from birth (Rosengarten and Friedhoff, 1979) or when adult rats were chronically treated with haloperidol (Chiu et al., 1981). The same up-regulatory response was observed when dopamine D₁

receptors were chronically blocked by SCH-23390 in adult rats (Creese and Chen, 1985).

In the prenatal study by Rosengarten and Friedhoff (1979) dopamine D₂ ontogeny was permanently impaired following haloperidol treatment during a 3-day critical period (15-18 days of gestation). In their postnatal study dopamine D₂ receptor up-regulation was reported following haloperidol treatment for 3 weeks from birth. There are several differences between the present study and the earlier postnatal study.

In the earlier study, the amount of haloperidol received by each pup in the milk could not be easily quantified, and as the pups approached 21 days of age, any consumption of pelleted food may have reduced milk and drug consumption. Also, haloperidol may have affected the hormonal system in the lactating dams, by blocking D₂ receptors in the pituitary gland, which increases prolactin release and may further dilute the drug in the milk. In the earlier report, serotonin S₂ receptors were not inactivated in the in vitro procedure for [³H] spiroperidol binding, so that serotonin S₂ receptor changes may have artifactually altered results. This does not exclude the possibility of serotonin S₂ sites proliferating as a result of haloperidol treatment, which would make the binding assay results non-specific for dopamine D₂ receptors. In the present study, spiroperidol, a more specific dopamine D₂ receptor antagonist was used, with

the dose kept constant during the treatment period. In addition, ketanserin, a serotonin S₂ receptor antagonist (Leyson et al., 1981) was employed in the in vitro binding assay. Also, in this study neonates were treated daily for 32 days, not only for 21 days; the period between 21 and 32 days could be critical for the development of dopamine receptors. It is suggested that the continuous treatment for 32 days may be needed in order to accumulate enough changes that persistent impairment in dopamine receptor ontogeny can be observed.

Regarding the ability of dopamine D₂ antagonists to alter dopamine D₁ receptor ontogeny by 20% at 5 weeks, it can be suggested that the chronic blockade of dopamine D₂ receptors in the postnatal period is able to impair normal expression of dopamine D₁ receptors. However, this impairment is transient, and dopamine D₁ receptors regain development to normal levels after treatment with dopamine D₂ antagonists is terminated. The same reasoning may be applied to the way dopamine D₁ antagonists affect dopamine D₂ receptors.

In conclusion, the present findings demonstrate that the process of striatal dopamine D₁ and D₂ receptor development can be substantially impaired by chronic antagonist treatment in the postnatal period. It is not known at this time whether there is a specific period during postnatal development when the receptors are particularly susceptible

to antagonists. Additional studies are needed to define the mechanisms that are associated with the altered ontogeny of dopamine receptors.

2. PLG Attenuation of Dopamine Receptor Ontogenic

Impairment:

The ability of PLG to attenuate dopamine D₁ and D₂ ontogenic impairment is a new finding. It has been reported that PLG modulates the neuroleptic-induced up-regulation of dopamine D₂ receptors in adult rats (Chiu et al., 1981; Bhargava, 1984). However, this neuroleptic-induced receptor up-regulation is a relatively short-lived phenomenon, since within 2 weeks after the termination of treatment, dopamine receptor number is at control levels. In the present study, a down-regulation of dopamine D₂ receptors was effected. This change persisted for at least 3 to 4 months, and the reversal or prevention of this persistent change in dopamine D₂ receptor number by PLG is of great importance regarding possible regulatory mechanisms associated with receptor development. It is also of great importance regarding their ontogenic impairment during neurological dysfunction related to the dopamine system.

In addition, the present study demonstrates that PLG is able to attenuate the persistent ontogenic impairment of striatal dopamine D₁ receptors after chronic treatment with SCH-23390. These results highlight the involvement of PLG in

modulating dopamine D₁ and dopamine D₂ receptor development and ontogenic impairment.

It has been reported that, when added in combination with apomorphine, PLG acutely lowers the K_D (an increase in the affinity of the receptor to bind to the agonist) for in vitro binding of [³H] apomorphine to striatal homogenates without affecting [³H] spiroperidol binding (Bhargava, 1983 & 1984; Chiu et al., 1981). It is therefore suggested that PLG may act in vitro to potentiate dopaminergic neurotransmission by interacting with its own binding sites, which are located in an area that allows them to influence dopamine receptor activity. It is also suggested that PLG is able to alter neuronal gene expression and the de novo synthesis of new dopamine receptor protein, since the short half-life of PLG will not allow it to act on or compete for the dopamine receptor sites and to have such a strong ability to modulate dopamine receptor ontogeny. PLG may act directly on the gene that expresses dopamine receptors, in order to modulate any abnormal changes in that expression. It should also be mentioned that dopamine neuronal activities are modulated by the action of neurochemical systems (i.e. GABA, substance P, enkephalin) (Kelley et al., 1982), and a reduction in these neurochemicals may lead to a profound motor behavior dysfunction, i.e. Huntington's Disease (Emson et al., 1980). The possibility needs to be considered that PLG may be indirectly modulating dopamine receptors through an action on

these other neurochemical systems. It may be useful to determine the action of chronic PLG treatment on the above substances, under long-term blockade with dopamine D₁ and D₂ antagonists.

The present results demonstrate that the neuropeptide PLG is able to attenuate the impaired ontogenic development of striatal dopamine D₁ and D₂ receptors that is produced by specific receptor antagonists. Explanation of this phenomenon should lead to a better understanding of dopamine receptor regulation and related neuroreceptor dynamics. These results reinforce the previous suggestion (Chiu et al., 1980, 1981) for the clinical use of PLG to attenuate abnormal dopamine receptor changes i.e. in Parkinson's disease, anti-psychotic therapy, and drug addiction.

3. The Ability of Dopamine D₁ Receptors to Up-Regulate in Adults:

The present study has shown that the postnatal treatment of rats for 32 days from birth with the dopamine D₁ or D₂ receptor antagonists results in persistent ontogenic impairment of both D₁ and D₂ dopamine receptors at 5, 8, and 12 weeks of age. The study has also shown that dopamine D₂ receptors do not up-regulate (increase in number) when rechallenged in adult rats (fig. 8). On the other hand, dopamine D₁ receptors do up-regulate when rechallenged in adult rats (fig. 7).

This up-regulation of ontogenically impaired striatal

dopamine D₁ receptors after chronic treatment of adult rats with SCH-23390 is analogous to the up-regulation of unimpaired striatal dopamine D₁ receptors after SCH-23390 treatment of adult rats (Creese and Chen, 1985). SCH-23390 chronic administration had no effect on striatal dopamine D₂ receptors in either the ontogenically impaired or control rats.

Failure of ontogenically impaired striatal dopamine D₂ receptors to up-regulate in response to challenge of adult rats with spiroperidol contrasts with the up-regulation of these receptors in intact control rats challenged as adults with neuroleptics (Seeman, 1980). It is possible that a higher dose of spiroperidol can stimulate dopamine D₂ up-regulation; however, the challenge doses of receptor antagonists are moderate and identical to the dose of antagonist that was administered during development. Also, in this study, both dopamine D₁ and D₂ receptor binding was ontogenically impaired to the same degree, prior to adult challenge with a receptor antagonist. The differential up-regulation of dopamine D₁ receptors may reflect differences in the ability of the dopamine D₁ and D₂ receptors to respond to receptor antagonism. The adenylate cyclase system that is directly stimulated by dopamine D₁ receptors (Kababian and Calne, 1979) may have the ability to recover some of the normal response of dopamine D₁ receptors (up-regulation) upon challenging treatment. On the other

hand there is either no relationship or a negative relationship between dopamine D₂ receptors and the adenylate cyclase system; dopamine D₂ receptors did not up-regulate. Also there is evidence that dopamine D₂ receptors are modulated by the guanine nucleotide system (Grigoriadis and Seeman, 1985), which may have a different response to challenging treatment from that of the adenylate cyclase system regarding impaired striatal dopamine receptors.

In conclusion, ontogenically impaired dopamine D₁ and D₂ receptors showed different responses to challenging treatment, in which dopamine D₁ receptors up-regulated and dopamine D₂ receptors did not, following treatment with specific antagonists as adults. This may be of importance with regards to the etiology and management of psychiatric disorders related to the dopaminergic system.

Chapter V

Summary

Chronic postnatal treatment of rats with SCH-23390 or spiroperidol, selective dopamine D₁ or D₂ receptor antagonists, causes permanent impairment in the development of the respective striatal receptors. This developmental impairment is reflected by a decrease in the specific binding of [³H] SCH-23390 and [³H] spiroperidol, respectively, to striatal homogenates as assessed at 5, 8 and 12 weeks. As indicated at 5 weeks, the decrease in [³H] SCH-23390 and [³H] spiroperidol binding is associated with a decrease in the B_{max} for the dopamine D₁ and D₂ receptors, respectively. Since the K_D is unchanged, the ontogenic impairment is reflective of a change in number, not the affinity of the dopamine D₁ and D₂ receptor types. These results indicate that the postnatal period is a sensitive and critical one for normal expression of striatal dopamine receptors. The neuropeptide PLG was able to totally attenuate the ontogenic impairment of striatal dopamine D₁ and D₂ receptors that was caused by chronic postnatal treatment with selective dopamine D₁ and D₂ antagonists. These results also indicate that PLG is capable of modulating abnormal developmental changes in striatal dopamine D₁ and D₂ receptors.

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