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UMI

**The Effects of Propofol on
Tyrosine Hydroxylase
in the
Nucleus Accumbens.**

by

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Submitted in partial fulfilment
of the requirements for the degree of
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ABSTRACT

Propofol (2,6 diisopropylphenol) is an intravenous anaesthetic used for the induction and maintenance of general anaesthesia. It also has several non-anesthetic effects. Three days following a six hour infusion of propofol there was a 43% reduction in tyrosine hydroxylase (TH) in the nucleus accumbens (Acb) when compared to the Intralipid® control group. This reduction was not observed at one, five, or seven days post infusion. This reduction was achieved at a dose of 20 mg/kg/hr. A lower dose had no effect while increasing the dosage resulted in no change from controls. The propofol analogue, 4-iodo propofol, has no anesthetic effects but maintains its anxiolytic effects. This compound does not produce a similar drop in TH levels. Reduction of TH in the Acb suggests a decrease in the activation of the dopaminergic system. Overactivity of the dopaminergic neurons in the mesolimbic system is believed to be involved in the manifestation of schizophrenic symptoms. A reduction in TH in the Acb provides evidence that sub-anaesthetic doses of propofol may be an effective anti-psychotic agent.

DEDICATION

I dedicate this thesis to my son, Andrew Gray, who gave the most so I

could accomplish all I could. I love you forever, you will always be my "lovebug".

ACKNOWLEDGMENTS

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LIST OF ABBREVIATIONS

Brain Sites

Acb	Nucleus Accumbens
CP	Caudate Putamen
GPe	External Segment of the Globus Pallidus
GPi	Internal Segment of the Globus Pallidus
IC	Insular Cortex
LFC	Lateral Frontal Cortex
MPF	Medial Prefrontal Cortex
PFC	Prefrontal Cortex
SNc	Substantia Nigra Pars Compacta
SNr	Substantia Nigra Pars Reticulata
STN	Subthalamic Nucleus
VP	Vental Pallidum
VTA	Ventral Tegmental Area

Neurochemicals

GABA	Gamma-aminobutyric acid
TH	Tyrosine Hydroxylase

CHAPTER 1
LITERATURE REVIEW

INTRODUCTION

There continues to be a lot of interest in determining the mechanism of action of

general anesthetics in the central nervous system. It is now widely accepted that the major

hypnotic effect of many anesthetics is achieved through interactions with the gamma-

aminobutyric acid (GABAₐ) receptor. Specifically, anesthetic binding, at clinically relevant

concentrations, to the GABAₐ receptor has been shown to potentiate the GABA mediated

influx of chloride ions into the cell. This modulation of GABAₐ receptor function results

in hyperpolarization of the cell and generalised depression of the CNS. GABAₐ receptor

interaction has been established for a large range of agents such as the benzodiazepines,

barbiturates, inhalation and intravenous anesthetics. Each of which has different binding

sites on the receptor complex (Hill-Venning et al., 1997; Trapani et al., 1998). It has also

been noted that different anesthetic compounds have unique side effects, which outlast

their hypnotic effect (Appadu et al., 1994; Reis et al., 1994). For instance, the general

intravenous anesthetic propofol has several non-anesthetic effects including depression of

the cardiovascular and respiratory centers of the CNS, antiemesis, and behavioral changes,

as well as both anticonvulsant and neuroexcitatory effects (Bryson et al., 1995). This

suggests that various anesthetic agents act at unique sites in the brain, or interact with

different subunit combinations of the GABAₐ receptor. Variations in the subunit make-up

of the GABAₐ receptor have been noted for distinct regions of the brain. For instance, the

most common GABA$_A$ receptor is composed of the subunits, $\alpha1$ $\beta2$ $\gamma2$ and is found

throughout the brain. Other receptor combinations are localised to particular areas as with

the hippocampus, which contains mainly $\alpha2$ $\beta3$ $\gamma2$ subunits and the ventral striatum, which

has high levels of the $\alpha2$ and $\alpha3$ subunits with very few $\alpha1$ (Mohler et al., 1995;

McKernan and Whiting, 1996). These variations in GABA$_A$ receptor subunits may confer

specificity for anesthetic interactions thereby allowing specific agents to have greater

effects on localised sites within the CNS.

One example of this specific effect of an anesthetic agent arose from

immunohistochemical studies previously conducted in our laboratory which indicate that

propofol may have distinct effects in the nucleus accumbens (Acb) of Wistar rats.

Specifically, three days following a six-hour propofol infusion at a subanesthetic dose,

there was a significant reduction in tyrosine hydroxylase (TH) (Kang, 1997), the rate

limiting enzyme in dopamine synthesis (Porter et al., 1989; Tashiro et al., 1990; Unnerstall

and Ladner, 1994). The Acb, a component of the basal ganglia, has been linked to control

of motor behavior (Pennartz et al., 1994). In the rat, the Acb regulates various behavioral

responses associated with exploration such as rearing and locomotion (Arnt, 1985; Kelly

et al., 1988; Churchill et al., 1998). These behaviors are influenced by alterations in

dopamine release from the Acb. Amphetamine, a known dopamine agonist has been

shown to increase the extracellular levels of dopamine and the incidence of exploratory

behaviors (Carr and White, 1987; Antoniou and Kafetzopoulos, 1992; Hurd et al., 1992;

Churchill et al., 1998). Further, behavioral studies conducted in our laboratory indicate a

reduced incidence of these dopamine mediated behaviors directly following injection of

amphetamine in rats treated with propofol three days previously (Kang, 1997). This may

indicate that the reduction in TH enzyme noted in previous studies results in reduced

levels of dopamine and/or dopamine release.

The Acb receives substantial input from both the prefrontal cortex and limbic

system, and is thought to function as a site of integration for motor and limbic

information. It has been suggested that both the prefrontal cortex and limbic system may

be potential sites for neuropathology in schizophrenia (Gray, 1995; Totterdell and

Meredith, 1997). The prefrontal cortex is thought to influence accumbal output indirectly

via glutamatergic efferents to the VTA, which sends major dopaminergic input to the Acb

(Taber et al., 1995). The limbic system on the other hand has direct glutamatergic

projections to the Acb. Disturbance in either of these systems may lead to dysfunction in

dopamine release in the Acb possibly resulting in the manifestation of psychotic symptoms.

Thus, the dopamine input to the Acb is thought to be the final common pathway in

schizophrenia. If propofol administration results in the reduction of dopamine release in

the Acb then it has a potential use as an antipsychotic. The lack of negative side effects

associated with this drug would indicate an ideal treatment for disorders related to

increased mesolimbic dopamine levels, such as schizophrenia.

1.1 PROPOFOL

Propofol (2,6-diisopropylphenol) is an alkyl phenol derivative used for both the

induction and maintenance of general anaesthesia. It is widely used due to the fact that it

induces sedation smoothly and quickly and its actions are short-lived (Barr, 1995; Fulton

and Sorkin, 1995). It is generally well tolerated with few negative side effects.

Subhypnotic doses have been reported to reduce patient anxiety (Pain et al., 1999).

Postoperative benefits include a reduced incidence of nausea and vomiting as well as an

enhanced mood (Borgeat et al., 1994). Propofol is lipid soluble and available as a 1%

solution emulsified in soybean oil, egg phosphatide and glycerol (Intralipid®; Mirenda and

Broyles, 1995).

1.1.1   Pharmacodynamics

In adult humans, sedation with propofol increases in a dose-dependent manner

with a dose of 2 to 2.5 mg/kg necessary to induce anesthesia (Bryson et al., 1995; Fulton

and Sorkin, 1995). Higher doses are required to anesthetize children while adults over the

age of 55 need less. Maintenance of anesthesia can be achieved through continuous

infusion (4 to 12 mg/kg/hr) or with repeated bolus of 25 to 50 mg/kg/hr. Again higher

rates are necessary for children (Bryson et al., 1995). Infusion rates necessary to produce

and maintain sedation and anesthesia in rats are approximately 16 and 25 mg/kg/hr

respectively (Garfield and Bukusoglu, 1996). Prolonged infusion of propofol has not been

shown to lead to the development of drug tolerance, or addiction (Barr, 1995). Propofol

also has several non-anaesthetic effects, which include depression of cardiovascular and

respiratory centers of the CNS, antiemesis, behavioral changes, and anxiolytic properties

as well as both anticonvulsant and neuroexcitatory effects. For reviews see Borgeat et al.,

1994; Barr, 1995; Bryson et al., 1995; and Fulton and Sorkin, 1995. The duration of

some of these effects has been reported to last from days to weeks (Appadu et al., 1994;

Reis et al. 1994; Herrick, 1997).

1.1.2   Pharmacokinetics

Propofol is a weak organic acid which is dissolved in a lipid emulsion (Intralipid®) due

to it's poor solubility in water (Hull, 1991; Doeniicke et al., 1997). In the circulation it is

extensively bound to plasma proteins (96 to 99%). Propofol is rapidly dispersed from the

blood to tissues with distribution first to metabolically active tissues such as the CNS

followed by redistribution to peripheral tissues. Clearance from the brain is also fast

allowing for a shorter mental recovery than with the use of other intravenous anesthetics.

Return of the drug from the peripheral compartment however is slow resulting in a long

terminal elimination half-life (2.4-44.7 hours). Metabolism of the drug to sulphate and

glucuronide conjugates of propofol occurs mainly in the liver. These compounds are

water soluble, and therefore easily secreted in the urine. Total body clearance of propofol

exceeds hepatic blood flow. This suggests that an extrahepatic site for propofol

metabolism must exist although this site has not yet been elucidated (Hull, 1991; Fulton

and Sorkin, 1995; Bryson et al., 1995).

1.1.3   Mechanism of Action

The mechanism of action of most anesthetics is unclear. However, propofol is

believed to exert its anesthetic action through effects on the subcortical GABA system by

binding to a specific site on the GABA$_A$ receptor complex and allosterically modulating

the effects of endogenous GABA. This leads to an increase in chloride ion conductance

resulting in hyperpolarization of the cell and depression of the central nervous system

(Mennini and Gobbi, 1992; Griffiths and Norman, 1993; Tanelian et al., 1993; Orser et al., 1994).

1.1.4   Propofol Analogues

Propofol is associated with a few negative side effects such as pain on injection, apnoea, convulsions and bradycardia. It has been postulated that many of these effects are due to the solubilization of propofol into a fat emulsion (Prankerd and Stella, 1990). Therefore, structural manipulation of the propofol molecule to produce a water soluble compound may eliminate some of these side effects. Trapani et al. (1998) were able to synthesize twenty-seven unique analogues of propofol. Of these, only four (compounds 2-4 and 8) were found that maintained their strong affinity for the $GABA_A$ receptor. Compound number 4, termed 4-iodo propofol, stands out because it is much weaker at directly activating chloride current at the $\alpha 1$ $\beta 2$ $\gamma 2$, the most common receptor subunit in the CNS (Mohler et al, 1995), than the parent compound or 2, 3, and 8. However in the presence of a $\beta 1$ subunit this difference was not observed. In vivo 4-iodo does not produce anesthesia but retains the anxiolytic properties of propofol (Trapani et al, 1998). Receptor specificity along with anxiolytic qualities indicates that 4-iodo propofol may retain the non-anesthetics characteristics of the parent compound.

1.2 GABA and GABA RECEPTORS

GABA is the major inhibitory neurotransmitter in the mammalian central nervous system. It is produced from glutamic acid via a decarboxylation reaction and is found

throughout the brain (Carlson, 1991). Three types of GABA receptors have been

elucidated, GABA$_A$, GABA$_B$, and GABA$_C$. GABA$_A$ and GABA$_C$ receptors form gated

chloride channels, while the GABA$_B$ receptors are coupled to G-proteins and influence

either calcium or potassium channels (Orser et al., 1994; Mohler et al., 1995, Sieghart,

1995). Each receptor is a heteromeric structure consisting of five subunits, which

assemble to form ion channels. To date there are sixteen known receptor subunits (α1-6,

β1-3, γ1-3, δ1, ϵ1, and ρ1,2). Differences in pentamere subunit assembly confer

individual characteristics to receptor subtypes (Jones et al., 1995; Hill-Venning et al.,

1997; Uchidaet al., 1997). The most common GABA receptor in the human CNS consists

of two α1, one β2, and two γ2 subunits. GABA receptor subtypes appear to be specific

for different neuronal circuits. For example, in the forebrain, cholinergic neurons express

the β3 subunit while GABAergic neurons express the β2 subunit. In areas of the brain

which mediate reward, such as the nucleus accumbens, the α2 subunit is highly expressed

(Mohler et al, 1995; McKernan and Whiting, 1996).

1.2.1   GABA$_A$ Receptors

GABA$_A$ receptors, by far the most prominent GABA receptor in the human CNS,

are known to be involved in the regulation of anxiety, memory, muscle tone, and

epileptogenic activity (Mohler et al., 1995). Binding of GABA to the GABA$_A$ receptor

generally results in increased conductance of chloride ions. This produces

hyperpolarization and reduced neuronal excitability (Davies et al., 1997; Sieghart, 1995).

Many important drugs are known to interact with specific sites on the GABA$_A$ receptor

complex. The benzodiazepines and some barbiturates enhance GABA action, while picrotoxin interferes with GABA at this receptor. Most compounds alter GABA action allosterically by causing a conformational change in the receptor thereby altering the properties of other sites (Sieghart, 1995). The GABA$_A$ receptor is known to be important in the action of many general anesthetics such as etomidate, enflurane, isoflurane, propofol and some steroid anesthetics (Tanelian et al., 1993; Trapani et al., 1998).

1.2.2   Propofol and GABA$_A$

Evidence suggests that propofol produces its anesthetic effect by potentiating the GABA response in a dose dependent manner (Sieghart, 1995). It achieves this by increasing GABA binding to its receptor resulting in increased frequency of chloride channel opening. High concentrations of this general anesthetic have also been observed to activate chloride currents in the absence of endogenous GABA (Orser et al., 1994; Jones et al., 1995; Davies et al., 1998). Propofol is thought to act at a site distinct from those of other GABA agonists since binding of other compounds was not affected with concurrent propofol administration. This site has not yet been determined although, it has been suggested that propofol may bind to a site on the channel itself (Concas et al., 1991). Numerous studies have been carried out in order to elucidate subunit dependence for the potentiating effect of propofol on the GABA receptor. The presence of an α and β site appear to be all that is necessary for propofol to enhance GABA activated chloride currents (Sieghart, 1995). Type β subunits have been shown to be necessary for the formation of functional chloride channels and therefore implicated in propofol potentiation

(Uchida et al., 1997; Trapani et al., 1998). Sanna et al. (1995) have shown that the β1 subunit is necessary for direct activation of the chloride current in the absence of endogenous GABA, although GABA enhancement occurs with other β subunits. Presence of an α subunit is also required with the α1 and α2 subunits being the most effective (Jones et al., 1995; Uchida et al., 1997). Propofol potentiation does not appear to be dependent on any other subunit although presence of the γ subunit modulates the strength of the chloride current (Jones et al., 1995). Although no specific binding site for propofol has been found on the GABA_A receptor, studies indicate that the β subunit may mediate the effects of propofol (Trapani et al., 1998).

Propofol is reported to have multiple effects on the GABA_A receptor. Not only can propofol potentiate the GABA current, it causes varying degrees of receptor desensitization (Orser et al., 1994). At low concentrations of propofol (2-100 μM) GABA activated currents are amplified and the probability of channels opening increases. Receptor desensitization at these concentrations is low. Higher concentrations (10-2000 μM) are able to directly activate the receptor in absence of endogenous GABA and desensitization occurs. At extremely high concentrations propofol actually inhibits receptor activation in a noncompetitive manner. This is probably achieved by blocking of the GABA receptor (Orser et al., 1994). Clinically relevant concentrations needed to obtain anesthesia occur in the low range. In cultured cells, propofol potentiation of GABA currents occurs at approximately 20 μM (Sigel and Baur 1988; Frost et al., 1992).

1.2.3   Propofol and Dopamine

It has been observed that propofol increases dopaminergic activities in the cortex in direct proportion to levels of the anesthetic in the blood (Shyr et al., 1997). However, Appadu et al. (1994) have shown that propofol has only a weak affinity for dopamine receptors. This indicates that propofol must exert it's action by some mechanism other than by binding to the dopamine receptor. Shyr et al. (1997) observed that propofol at a concentration of greater than 50 μM actually potentiated the release of dopamine from the striatum. This concentration corresponds with the low concentrations observed which potentiate GABA mediated chloride current. It has also been shown that propofol prevents reuptake of dopamine from the synaptic cleft, possibly by interfering with the dopamine transporter (Keita et al., 1996). This would lead to an increase in dopamine and its metabolites in the synaptic cleft possibly leading to the increased dopaminergic activity noted by Shyr et al., (1997). Build-up of dopamine and dopamine metabolites in the synaptic cleft may result in an overall reduction of dopamine production. A number of possibilities exist for dopaminergic regulation. One potential mechanism is modulation of dopamine via autoreceptors and negative feedback loops. Excess dopamine binding to autoreceptors on the presynaptic terminal may result in a down regulation of catecholaminergic production in the bouton. Likewise autoreceptors located on somatodendrites may modulate dopamine production by affecting the rate of transcription and translation of enzymes essential for the production of dopamine (Palmero-Neto, 1997).

1.3 TYROSINE HYDROXYLASE

Tyrosine hydroxylase (TH) is a homotetramere consisting of subunits which are approximately 60 kilodaltons (kDa) each. It is known to be the rate limiting enzyme in catecholamine production (Markey et al., 1979; D'Andrea et al., 1986; Nagatsu, 1995). In the brain, catecholamines (dopamine, noradrenaline and adrenaline) function as neurotransmitters where they are known to be involved in the pathogenesis of many disorders including Parkinson's disease and schizophrenia (Nagatsu, 1995). TH catalyses the first step in dopamine production by converting tyrosine, which is derived from the diet, into 3,4-dihydroxyphenylalanine (dopa). Decarboxylation of dopa into dopamine is carried out by DOPA decarboxylase (D'Andrea et al., 1986; Carlson, 1991; Kaufman, 1995). It has been found that while most mammals have only one form of the TH enzyme, humans express four isomeres. These extra three forms are the result of alternative splicing of the TH gene. Human TH-1, the most active form, is homologous with the isoform found in other mammals (Lewis et al., 1993; Nagatsu, 1995). The catalytic properties of TH require the presence of tetrahydropteridine and ferrous ions as essential cofactors. The enzyme also requires molecular oxygen as a substrate and is also known as tyrosine 3-monooxygenase (Kaufman, 1995; Nagatsu, 1995; Nagatsu and Stjarne, 1998).

Neuronal TH has been shown to exist in two forms, one being soluble and the other membrane bound. These forms appear to be compartmentalized with the soluble fraction found in the cell bodies and its bound counterpart localized to the axon terminal (Andersson et al., 1992; Kaufman, 1995). Binding of TH to synaptic vesicles in neuronal

terminals is reversible, with the bound component representing the active form of the

enzyme (Nagatsu, 1995). Homogenization procedures used for analysis of TH, has been

shown to solubilize the majority of bound TH (Andersson et al., 1992; Kaufman, 1995).

This supports the idea that TH is synthesized in the soluble form and transported along the

axon bound to a carrier protein . In the rat brain, areas rich in dopamineric terminals such

as the striatum have high TH activity while regions rich in dopaminergic cell bodies have

low TH activity (Kaufman, 1995). This makes sense as dopamine is known to be

synthesized in the nerve terminal.

1.3.1   Regulation of TH

Regulation of TH is achieved by altering the activity of existing TH molecules, in

what is termed short-term regulation, or by changing the amount of TH protein present in

the cell (long-term regulation; Kaufman, 1995; Nagatsu, 1995). Inactivation of the TH

enzyme occurs via end-product inhibition when there is a buildup of free catecholamine in

the terminal. Catecholamines inhibit TH activity by competing with tetrahydrobiopterin

for the same binding site on the TH molecule. Tetrahydrobiopterin is a co-factor

necessary for the correct functioning of the TH enzyme (Haycock, 1989; Nagatsu, 1995;

Lamensdorf and Finberg, 1997). Many effectors can activate TH, including heparin,

phospholipids, high salt concentrations and phosphorylation (Stone, 1980; Richtand et al.,

1985). Phosphorylation, by protein kinases, represents the main pathway for short-term

control of TH activity. There are four different serine residues on the protein which when

phosphorylated induce a conformational change in the inactive protein. This causes the

release of the endogenous inhibitor (excess catecholamine) from the tetrahydrobiopterin site and results in conversion of the molecule from the inactive to active state. Once endogenous catecholamine levels rise, dopamine once again binds to the TH molecule thereby inactivating it (Kaufman, 1995; Nagatsu, 1995). Increased neuronal activity has also been shown to increase TH activity (Nagatsu, 1995). Long-term regulation occurs at the level of transcription and translation of new TH molecules (Nagatsu and Stjarne, 1998). Induction of the TH gene seems to be regulated by three major second messenger signalling pathways involving cyclic adenosine monophosphate (cAMP), diacylglycerol (DAG) and calcium (Kaufman, 1995; Nagatsu, 1995). Osaka and Sabban (1997) have also shown that fibroblast growth factor (FGF-2) causes increased transcription of the TH gene in the presence of a functional cAMP response element (CRE). It appears that the CRE is essential for transcription of the TH gene (Patankar er al., 1997; Tinti et al., 1997).

1.3.2   TH and Disease

Tyrosine hydroxylase is thought to play a significant role in the development of disorders such as depression, schizophrenia, hypertension, hepatic encephalopathy and migraines (D'Andrea et al., 1986). Of particular interest is its possible role in the pathogenesis of neuropsychiatric disease. Nagatsu (1990) found a significant reduction in the activity and levels of TH protein and mRNA in the nigrostriatal dopaminergic neurons of patients with Parkinson's disease. It is possible that this reduction results in manifestation of Parkinsonian symptoms by decreasing endogenous levels of dopamine. No reduction in this enzyme has been observed in the substantia nigra of schizophrenics,

however it is hypothesized that overactivity of TH in the neurons of the VTA is responsible for the increased dopaminergic activity seen in this disorder. Pathology in TH expression/activity has also been suggested in other affective disorders such as manic depression. Other disorders (Segawa's syndrome) which affect the nigrostriatal pathway have been shown to be the result of interference with the synthesis of cofactors necessary for activation of TH (Nagatsu, 1995). The regulation of TH is important in the production of dopamine and therefore any problems encountered in the activation or transcription/translation of this protein could result in disorders attributed to variations in dopamine levels.

1.4 THE BASAL GANGLIA

1.4.1 Neuroanatomy:

The basal ganglia are a collection of subcortical nuclei, which are involved in the regulation of planning and execution of movement (Cote and Crutcher 1991; Middleton and Strick, 1997). Input received from the entire cortical mantle enters the basal ganglia through the striatum which sends output back to the cerebral cortex via the striato-pallido-thalamic and striato-nigro-thalamic pathways (Maurice et al., 1997; Montaron et al., 1996). The components of the basal ganglia can be divided into three groups, input nuclei (striatum), output nuclei, and intrinsic nuclei.

The striatum, consisting of the caudate, putamen and nucleus accumbens (Acb), is a center involved in motor refinement and patterning, as well as a storage of information

regarding movement and other behaviors. These nuclei also facilitate the expression of intentional movements while suppressing those that are unwanted (Lovinger and Tyler, 1996). Distinct areas of the cortex send glutamatergic efferents to separate nuclei in the striatum, conferring a functional organization to the striatum, which is maintained throughout the basal ganglia (Figure 1). In primates, the sensorimotor cortex projects to the putamen forming the sensory motor loop involved in the execution of movement. The association cortex innervates the caudate creating the oculomotor and association loops concerned with the anticipation and planning of movement. The limbic cortex sends axons to the accumbens forming the limbic loop, which primarily deals with the motivational and emotional aspects of motor behavior. In rodents the caudate and putamen form one structure (Parent, 1997).

The majority of the output from the striatal nuclei (striatofugal projections) targets the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr), in what is referred to as the direct pathway. These in turn project to the ventral aspects of the thalamus which relay information to areas of the cortex (Wise et al., 1996). An indirect pathway also exists in which output from the striatum projects to specific intrinsic nuclei; the external segment of the globus pallidus (GPe), the subthalamic nucleus (STN), the substantia nigra pars compacta (SNc), and the ventral tegmental area (VTA). These nuclei are reciprocally linked to the input nuclei allowing for the modulation of transmission of information through the principle loops of the basal ganglia (Parent 1997; Lovinger and Tyler 1996). The ventral pallidum (VP) may also play a role in intrinsic modulation. It receives substantial output from the Acb and in return innervates the STN

Figure 1. Schematic diagram outlining the direct pathway though the basal ganglia based on Martin (1989), Lovinger and Tyler (1996), Montaron et al. (1996), Wise et al. (1996), Maurice et al. (1997), and Parent (1997). Distinct areas of the cortex send glutamatergic efferents to separate nuclei of the striatum, the caudate, putamen, and nucleus accumbens. GABAergic output from these nuclei targets the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). These in turn send GABAergic output to the ventral aspect of the thalamus which relays information to specific areas of the cortex using glutamate as the main neurotransmitter.

CORTEX

Basal Ganglia

Caudate   Putamen   Accumbens

GPi   Snr

THALAMUS

LEGEND

Glutamte →

GABA ·············▶
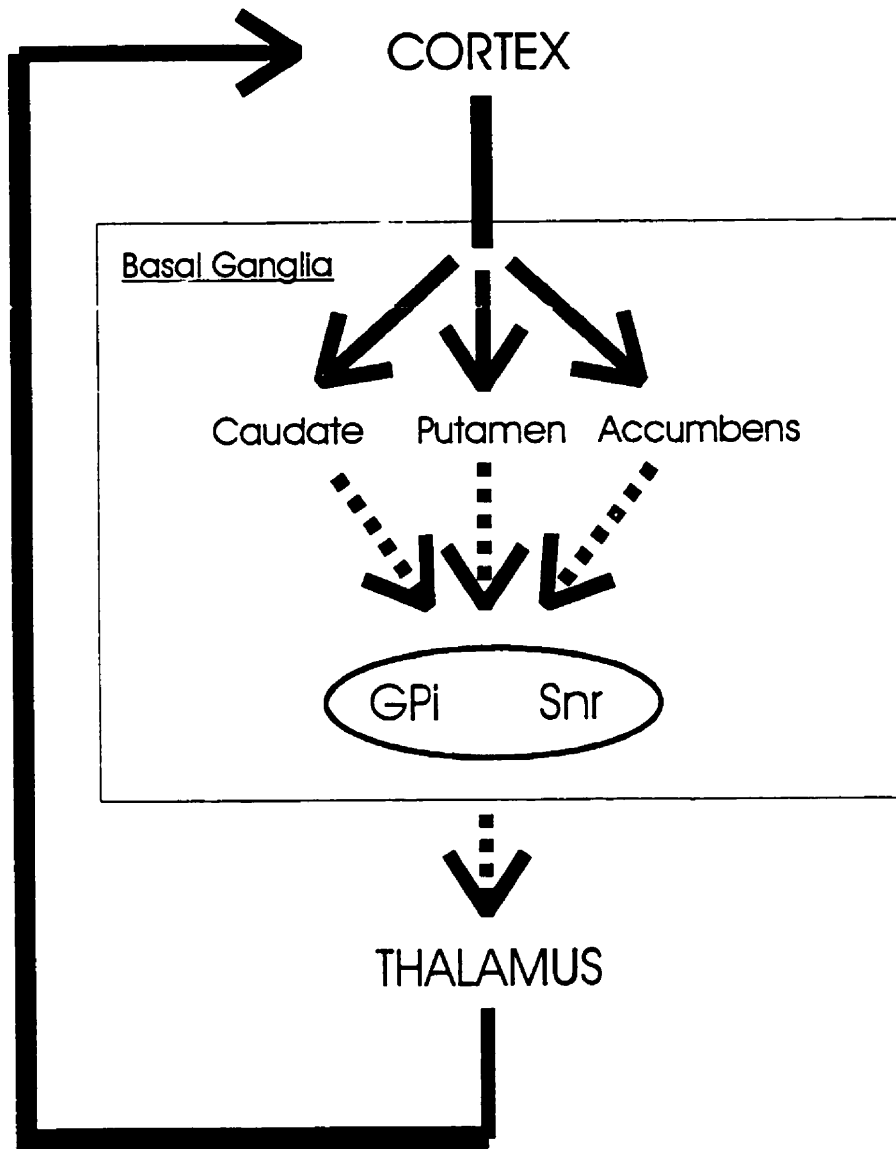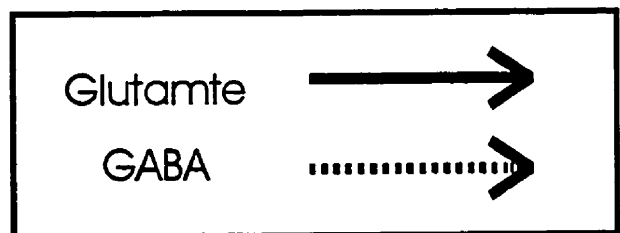
and VTA. The VTA is known to send output back to the nucleus accumbens and VP, forming intrinsic loops which may be involved in the regulation of the limbic striatum (Klitenick et al., 1992; Haber and Fudge, 1997). However, this nucleus (VP) also projects directly to the SNr and thalamus and therefore serves a duel role as an output structure (Maurice et al., 1997).

1.4.2   Chemical Neuroanatomy:

The chemical anatomy of the basal ganglia is very diverse. Neuroactive substances in this region can be divided into two groups, phasic and tonic. The phasic group includes fast acting and short lived small molecule transmitters that exert an all-or-none effect on target neurons. Included are glutamate, acetylcholine (ACh), and gamma-amminobutyric acid (GABA). Tonic substances are neuroactive peptides such as substance P, enkephalin, dynorphin, dopamine, and serotonin. These latter substances exert long-lasting modulatory effects that are mediated by second messenger systems (Mello and Villares, 1997; Parent et al., 1995).

Excitatory glutamatergic projections from the entire cerebral cortex innervate the input nuclei of the basal ganglia. Medium-sized spiny neurons account for 96% of the striatal cell population and can be divided into two subtypes even though almost all use GABA as their main transmitter. Approximately fifty percent of these neurons colocalize enkephalin and project to the GPe corresponding to the indirect pathway. The remaining cells express substance P and dynorphin and send efferents to the GPi and SNr and form the direct pathway (Wise et al., 1996; Parent 1997). Striatal neurons also receive

dopaminergic afferents from cells of the SNc (Lovinger and Tyler, 1996). Four groups of

interneurons exist within the striatum. Large aspiny neurons receive dopaminergic input

from the SNc and form cholinergic synapses with medium spiny neurons. The remaining

interneurons are glutamatergic and can be divided into three groups depending on the

presence or absence of certain proteins and neuropeptides (Parent, 1997; Lovinger and

Tyler, 1996).

The striatum is further broken up into what are called striosomes and matrix

compartments. Striosomes are acetylcholinesterase poor, dopamine rich regions

surrounded by an acetylcholinesterase-rich matrix containing GABAergic projection

neurons . They have been characterised as containing cells that are positive for substance

P, dynorphin, and neurotensin, while the matrix component cell bodies are positive for

enkephalin and somatostatin. Afferents to the striatum innervate compartments based on

function. Sensorimotor areas send axons to matrix, and striosomes receives input from

limbic regions although, these divisions are not strict. Limbic efferents from hippocampal

areas innervate matrix while those from the amygdala project to striosomes (Mello and

Vellares, 1997). Striatal striosomes themselves have been shown to innervate the

dopaminergic cells of the SNc while the matrix component sends axons mainly to the SNr

and GP. The matrix can be further subdivided into the direct and indirect pathways

discussed above (Lovinger and Tyler, 1996; Saint-Cyr et al., 1995; Gorbachecskaya,

1996).

1.4.3 Nucleus Accumbens

The nucleus accumbens is a significant proportion of the ventral striatum involved in processing information from limbic associated cortical areas including the amygdala, insular and entorhinal cortices and the hippocampus (Montaron et al., 1996). In rats it is involved in modulating behaviors which are exploratory in nature such as rearing and locomotion (Churchill et al., 1998). The Acb consists of both a shell and core region, which are thought to subserve both limbic and motor functions respectively (King et al., 1997).

The nucleus accumbens is intricately connected to many regions of the brain as indicated by Figure 2. Glutamatergic afferents from various areas of the PFC directly innervate both core and shell regions of the Acb. The PFC also indirectly influences the Acb via glutamateric input to the VTA which in turn sends dopaminergic efferents to the shell. The pars compacta also sends dopaminergic innervation to the Acb. It is important to note that noradrenergic input into the Acb also arises from the locus coeruleus (Delfs et al., 1998). Noradrenaline also requires TH as the rate limiting enzyme in its synthesis, although noradrenergic fibers account for a very minor input into the Acb (Delfs et al., 1998). The main output of this nucleus is GABAergic projections to the VTA (shell), VP (core), and SNr. (Taber et al., 1995; Gorelova and Yang, 1997; King et al., 1997; Churchill et al., 1998).

Figure 2. Information reaching the input nuclei is modulated by intrinsic nuclei of the basal

ganglia (Taber et al., 1995, Gorelova and Yang, 1997, King et al., 1997). The Acb forms an

intrinsic loop by sending GABAergic efferents to the ventral pallidum (VP) which in turn

innervates the VTA. The VTA then modualtes accumbal output to the substantia nigra (SNr) and

globus pallidus (Gpi)using dopamine as a neurotransmitter. The substantia nigra pars compacta

also sends dopaminergic input to the Acb.

CORTICAL INPUT

22

Acb

SNC   VTA   VP

GPl
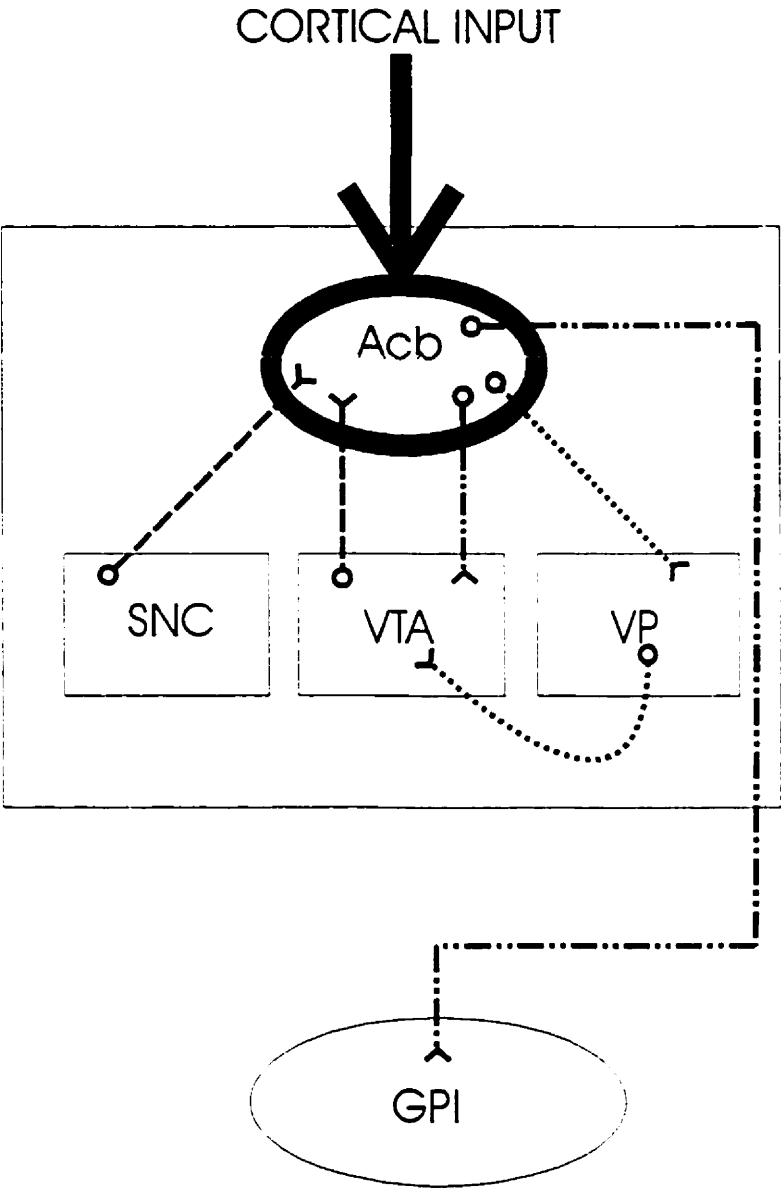
LEGEND

Glutamate	⟶
GABA/ENK	·······⟶
GABA/SP	—·—·⟶
Dopamine	— — ⟶

1.5 SCHIZOPHRENIA

Schizophrenia is a chronic, complex, and disabling neurological disease, characterized by psychotic episodes in which the patient loses his or her hold on reality. It is often associated with hallucinations, delusions, incoherent thinking, disordered memory and confusion. These positive symptoms usually follow negative symptoms, which include social isolation, odd behaviour and ideas, neglect of personal hygiene, and inappropriate expression of emotion. Schizophrenia is known to have an important genetic component and affects approximately 1% of the population worldwide showing no preference between the sexes (Davis, 1993). An additional 2-3% of the population are afflicted with a milder form of the disease known as schizotypical personality disorder (Kirch, 1986). Schizophrenic symptoms have been documented for at least three thousand years, but it was not recognized as a biological disease until the late 19th century. In the early part of the 20th century Eugen Bleuler coined the term "schizophrenic psychoses" to describe the splitting of mental functions seen in this disorder (Tsuang, 1980; Cromwell, 1993). With the inception of psychopharmacology in the 1950's, and the use of what are now referred to as antipsychotics, the investigation into the neurochemistry of schizophrenia was initiated (Oke et al., 1993).

1.5.1 The Dopamine Hypothesis

The dopamine hypothesis of schizophrenia, in its simplest form, states that schizophrenia may be associated with an overactivity of dopamine. Inception of this

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revealed no significant difference in the prolactin levels in schizophrenic patients as compared to healthy controls, indicating that this system is not involved in the symptomology of the disease (Meltazer et al., 1979). The mesocortical dopaminergic system originates in the ventral tegmental area and sends afferents to the cortex, particularly to the prefrontal cortex. The prefrontal cortex is known to be involved in motivation and planning, temporal organization of behaviour, attention, and social behaviour, and therefore may be involved in producing the negative symptoms associated with schizophrenia (Wise et al., 1996). The mesolimbic system has cell bodies located in the ventral tegmental area with projections to the nucleus accumbens and other areas of the limbic system, such as the hippocampus, amygdala, prefrontal, anterior cingulate, and entorhinal cortices. Interest has fallen on the mesolimbic system in the pathology of the positive symptoms of schizophrenia since this system plays a role in emotions and memory. The main site of interest in this system pertaining to schizophrenia is the nucleus accumbens. The accumbens is thought to act as a site of modulation of information coming from the hippocampus and cortex, which are both involved in memory functions (Montaron et al., 1996). Other areas converging on this nucleus may also play a role in the symptomology of this disorder. Both the mesocortical and mesolimbic systems are thought to be involved in cognition (judgment, reasoning, insight, social conscience, and motivation), reward and emotions (Totterdell and Meredith, 1997).

1.5.2   The Neuropathology of Schizophrenia:

Despite intensive study, researchers have been unable to pinpoint the exact

anatomical aberration resulting in the manifestation of schizophrenic symptoms. The most

consistent findings report ventricular enlargement, reduced hippocampal volume and

atrophy of the prefrontal region of the brain (Oke et al., 1993; Stevens, 1997; Buchanan

and Carpenter, 1997). This cortical atrophy is most likely due to a significant lack of

gliosis (Heckers, 1997). The variability of the phenotypic expression of schizophrenia

makes the etiology of this disease difficult to discern. Many varying and unusual

histological findings have been reported in brains of schizophrenic patients, however, none

of these alone is sufficient to diagnose the disease. The manifestation of schizophrenic

symptoms is most likely a response to many different causes (Liddle et al., 1992;

Buchanan and Carpenter, 1997; Goldman-Rakic and Selmon 1997). In order to explain

the basis of this disorder, researchers have attempted to use the symptomology in order to

extrapolate what regions of the brain may be involved. From behavioural observations,

three main areas of the brain, the prefrontal cortex (PFC), the limbic system, and the basal

ganglia have become the main focus for pathology in schizophrenia. However, due to the

intricate connections between these particular areas, the implication of one involves the

others (O'Donnell and Grace, 1998).

1.5.3   The Prefrontal Cortex in Schizophrenia

Numerous studies indicate a prefrontal dysfunction in schizophrenic patients

(Harvey et al., 1993). Positron emission tomography (PET) has shown that the prefrontal

cortex of persons with this disorder have decreased blood flow and thus decreased

metabolism during behavioural tasks (Franzen and Ingvar, 1975). Functional magnetic

resonance imaging (fMRI) also shows impairments in activation of the PFC during verbal

fluency tasks (Yurgelun-Todd et al., 1996). Neurophysiological observations during the

Stroop test, the Continuous Performance Test and the Tower of London task have noted

that patients with frontal lobe damage have impairments similar to schizophrenic patients

(Buchanan et al., 1994; Goldman-Rakic and Selemon, 1997). Each of these tasks require

the proper functioning of the working memory, which is associated with the prefrontal

cortex. Postmortum studies also indicate that there are some cytoarchitectural changes in

the PFC in schizophrenic patients. Kerwin et al. (1990) show a reduction in glutamate

receptor binding and Akil and Lewis (1997) found a reduction in dopaminergic fibers in

the PFC of schizophrenic patients. Clearly, there is some impairment in the ability of the

PFC function in schizophrenia, whether there is an anatomical disturbance or not.

Supporters of the working memory-frontal lobe hypothesis believe this theory may

help in explaining the negative and some of the positive symptoms associated with

schizophrenia. The working memory serves as a computational arena or workspace for

holding items of information. Here, recalled information is manipulated, and associated

with other ideas and incoming stimuli. This enables individuals to hold onto information

which can be used to guide behaviour in various situations. It has been postulated that the

disorganised thought processes observed in schizophrenia may be due to an inability to

hold on to or recall information. This information is necessary to carry out appropriate

situation specific behaviours in the absence of instructive stimuli (Goldman-Rakic and

Selemon, 1997). In the working memory hypothesis it is thought that the dopamine D1

receptor may play an important role in the pathogenesis of schizophrenia. This receptor is

highly expressed in the PFC and has been implicated in the control of working memory.

Using PET, Okubo et al. (1997), examined the distribution of dopamine receptors in

schizophrenic individuals. A marked reduction in radioligand binding to the D1 receptor

in the PFC was found. However, no difference was observed in D1 receptor binding in

the striatum. The authors propose that this reduction in dopamine receptors may

contribute to the negative symptoms and cognitive deficiencies observed in the disease.

1.5.4   The Limbic System in Schizophrenia

The limbic system is a heterogeneous assembly of nuclei, involved in the regulation

of thought, mood, and expression of emotion. It may also play a key role in learning and

memory. Therefore, it is possible that dysfunction in this system may lead to many of the

psychotic symptoms observed in psychiatric disorders. In fact, lesions in this system result

in symptoms more similar to those seen in schizophrenia than lesions of the basal ganglia,

brainstem and parietal and occipital cortices (Bogerts, 1997; Parent et al., 1995). Limbic

structures include regions of the cerebral cortex such as the medial prefrontal, and

temporal lobes, as well as subcortical structures including the hippocampus, amygdala,

Acb and septum (Levitt, 1984; Bogerts, 1993). Cortical areas involved in the limbic

system are known collectively as the limbic association cortex. Lesions here produce

deficits in memory and emotional changes. The limbic association cortex receives input

from the insula and temporal lobe. It then makes connections with the hippocampal

formation and amygdala. Both of these structures make connections with other

subcortical nuclei including the nucleus accumbens, which is a component of the limbic loop of the basal ganglia (Haber and Fudge, 1997). Both the nucleus accumbens and the amygdala receive dopaminergic input from the ventral tegmental area (Reynolds, 1989).

Evidence for the temporolimbic hypothesis of schizophrenia exists in the findings of both anatomical and neurochemical studies. Recently, MRI studies have found a reduction in volume of various limbic structures, including the hippocampal formation, medial temporal lobe and amygdala (Breier et al., 1992; Bogerts et al., 1993; Nasrallah et al., 1994; Rossi et al., 1994; Becker et al., 1996).Using MRI, Woodruff et al. (1993) showed a significant reduction in the mid corpus callosum in schizophrenic patients. The temporal lobes of the brain communicate via the mid corpus callosum. Another study, using PET, correlated increased cerebral blood flow to the parahippocampal region with increasing severity of schizophrenic symptoms (Friston et al., 1992). Dopamine levels in the amygdala have been reported to increase in schizophrenia (Reynolds, 1989), while Kerwin et al. (1998) indicated that there may be dysfunction in the glutamatergic system of the left temporolimbic system. Increased levels of serotonin receptors have been reported in the limbic striatum (nucleus accumbens), limbic cortex, and hippocampus of affected patients (Joyce et al., 1993).

1.5.5   The Basal Ganglia in Schizophrenia

It has been postulated that the basal ganglia may play a major role in schizophrenia (Lauer and Beckmann, 1997; Beckmann and Lauer, 1997). Many schizophrenics exhibit spontaneous involuntary movements, which seem to be compatible with disturbances of

the basal ganglia. Conversely, those with known diseases of the basal ganglia may also display some psychotic symptoms. For instance, Parkinson's patients and schizophrenics with severe negative symptoms both show impairments on frontal lobe tasks similar to those mentioned above. It is also not unusual for Huntington's sufferers to display some positive schizophrenic symptoms (Busatto and Kerwin, 1997). Further evidence for basal ganglia involvement lies in successful treatment with dopamine receptor blockers (Henn, 1982; Weinberger, 1987). Several major dopaminergic pathways transverse the basal ganglia. Therefore because of the high concentration of dopamine receptors it is a prime candidate for the action of neuroleptics. The corticostriatal-thalomocortical loops allow for communication between the basal ganglia and cortex thereby allowing for the modification of cortical information. This places the striatum in a strategic position allowing for the modulation of many types of behaviour thus forming a link between pathology in the basal ganglia and cortical aberrations seen in schizophrenia. These behavioural alterations could contribute to both the positive and negative symptoms seen in this disorder (Busatto and Kerwin, 1997).

Evidence of structural pathology of the basal ganglia in this disorder is limited and conflicting. Reduction in the size of the inner segment of the pallidum has been noted in patients who have not received medication (Bogerts et al., 1985). Medicated patients were found to have bilateral increases in the size of the striatum with increases of the pallidum on the right side only (Brown et al. 1986). There is varying results with respect to the striatum. In two separate studies Bogerts et al. (1985, 1990) found no change in the volume of the striatal components. Heckers et al. (1991) indicate an increased

volume of the caudate and putamen, while an increase in accumbal cell density of schizophrenic patients was observed by Stevens (1982). Due to the limited number of schizophrenic patients, these studies chose different groups of patients to study. For instance, Bogerts et al. (1985) study used never medicated patients while others chose to study those on medication or those that had been off medication for more than six months. The variability of results most likely reflects the differences in the patient groups studied.

Recently, temporal studies using MRI techniques have shown that the volume of the caudate nucleus increased following treatment with typical antipsychotics (Chakos et al., 1998; Busatto and Kerwin, 1997). Typical antipsychotics work by blocking dopamine receptors. This would release the striatum from dopamine's inhibitory influence allowing hypertrophy (Bussatto and Kerwin, 1997). Functional MRI also supports the theory that the basal ganglia are involved in schizophrenic dysfunction. Liddle et al. (1992) positively correlated the occurrence of the positive symptoms of schizophrenia with increases in regional cerebral blood flow (rCBF) to the nucleus accumbens, and temporal regions of the cortex. Changes in rCBF to other regions of the brain include the PFC and caudate, thought to be responsible for the negative symptoms, and the anterior cingulate and insular cortices which may be involved in disorganized thought patterns. These findings support the idea that dysfunction of corticostriato-thalamocorical circuits may be related to the manifestation of schizophrenic symptoms (Busatto and Kerwin, 1997).

1.5.6   The Nucleus Accumbens in Schizophrenia

The Acb, or limbic striatum is a region of the brain thought to be associated with

motivation as well as adaptive learning and behaviours (Meredith, 1996; Gorbachevskaya, 1998). Recently, interest has fallen on this nucleus as playing a central role in the development of schizophrenia as it is the major site of integration between the limbic and motor systems. It has been proposed that pathology in limbic structures can alter dopaminergic input into the accumbens resulting in hyperactivity of the mesolimbic system and manifestation of psychotic symptoms (Gray, 1995; Totterdell and Meredith, 1997). Alternatively, it is possible that abnormalities in the cortical dopaminergic system may result in the disturbance of subcortical dopamine transmission thereby leading to the occurance of schizophrenic behaviours. Control of accumbal dopamine release by the cortex has been shown to occur through both an indirect route, in which the VTA is the primary recipient of cortical efferents, as well as through direct connections from the PFC to various regions of the Acb (Taber et al., 1995; King et al., 1997; Gorelova and Yang, 1997).

One line of convincing evidence for accumbal involvement in schizophrenia involves latent inhibition. Latent inhibition is the difference it takes to exhibit a conditioned response to a stimulus to which one has been pre-exposed compared to a situation in which the conditioned stimulus has not been previously presented. Pre-exposure to a stimulus results in an increase in latent inhibition. In schizophrenia decreased latent inhibition has been shown to be characteristic in patients displaying acute positive symptoms. This process appears to be attenuated due to an inability of schizophrenic patients to ignore irrelevant stimuli which is most likely a result of increased dopaminergic activity (Gray, 1995). The nucleus accumbens has been implicated

in the control of latent inhibition as lesions here have appear to increase this effect and increases in dopamine in this nucleus have been shown to result in the attenuation of latent inhibition (Peters et al., 1993). On the other hand, amphetamine, a known dopamine agonist, injected into the Acb weakens latent inhibition. It is interesting to note that the mesolimbic dopaminergic system has been shown to influence latent inhibition while the nigrostriatal system appears to have no involvement. This is highly consistent with the idea that the basal ganglia, particularly the Acb, is involved in the pathogenesis of schizophrenia (Peters et al., 1993; Gray, 1995, Gray et al., 1995).

1.5.7   Treatment

Over the years, many antipsychotic drugs have been developed to aid in treatment of this disease. These drugs are known to block dopamine receptors. Typical antipsychotics such as chlorpromazine and haloperidol bind to D2 receptors with high affinity. These dopaminergic receptors are found in the midbrain, caudate, and limbic system. Due to the presence of these receptors in the caudate as well as the limbic system these types of antipsychotics also result in extrapyramidal side effects similar to the symptoms seen in patients with Huntington's disease. Atypical antipsychotics, such as clozapine, bind to another group of dopamine receptors, D3 and D4 receptors. These are restricted to the limbic system and cerebral cortex and therefore do not result in as much expression of extrapyramidal side effects (Kandel, 1991). They do however result in agranulocytosis, seizures, low blood pressure, and increase heart rate (Fleischhacker and Hummer, 1997; Malhotra and Pickar, 1999). A major problem encountered with these

treatments is the unwillingness of patients to take the medication due to the side effects experienced. It seems important to continue to seek a treatment which may alleviate some of these side effects to help ensure the long term well being of the patient.

1.6   RATIONALE and OBJECTIVES

Increased dopaminergic output in the nucleus accumbens has been implicated in the pathogenesis of schizophrenia. Current antipsychotics function by nonspecific blockage of dopamine receptors in the CNS. The nonspecific action of these drugs is responsible for the negative side effects seen with antipsychotic administration. Propofol, a general anesthetic has been shown to reduce levels of TH specifically in the nucleus accumbens. Tyrosine hydroxylase is the rate limiting enzyme in dopamine production and therefore it would be expected that reductions in this enzyme may lead to equivalent decreases in dopamine. Behavioral studies indicate a reduction in dopamine mediated behaviors three days following propofol infusion. Propofol has few negative side effects and may therefore represent a superior treatment for the symptoms of schizophrenia.

In order to quantify the latent effects of propofol observed on TH levels in the Acb, the Western blot technique was used. It was hypothesized that three days following propofol infusion, Western blot analysis would indicate a significant reduction in TH specifically in the Acb. A time course study of TH was carried out to determine the duration of this change, while a dose response was done to determine the optimal concentration of propofol necessary to produce this effect. It was found that the desired effect was most pronounced at three days following infusion at a subanesthetic but not

sedative dose. Propofol was also compared to isoflurane under the same conditions to show that the reductions on TH observed with propofol was due to this compound and not a result of general anesthetic effect. The results clearly indicate the reduction is not due to a general anesthetic response.

The propofol analogue, 4-iodo propofol, has been shown to be devoid of anesthetic properties while still maintaining its anxiolytic effect. Therefore, it to may produce a decrease in TH in the Acb and may have a potential use as an antipsychotic. To determine if this analogue acted in similar fashion to the parent compound, 4-iodo was emulsified in the Intralipid® vehicle and infused at a rate of 20 mg/kg/hr for six hours. Although 4-iodo propofol did appear to have anxiolytic properties, there was no reduction in the levels of TH in the Acb following its administration, although there was an increase in TH in the VTA, indicating this compound also has some effect on the mesolimbic dopaminergic system.

CHAPTER 2

MATERIALS AND METHODS

2.1   Animals

A total of 94 Male Wistar rats weighing between 275-325 grams were used for all experiments. Animals were housed two to a cage upon arrival to the university and placed in separate cages following surgical procedures. During recovery from surgery and following experimental conditions, the rats were kept on a 12 hour light-dark cycle and given food and water ad libitum. All experiments and animal care was approved by the Council on Animal Care of the University of Western Ontario.

2.2   Surgical Preparation

Paired male Wistar rats were anaesthetized using sodium pentobarbitol (50 mg/kg, i.p.). A catheter (polyethylene tubing, PE 50, Becton Dickson), containing 30% heparinized saline, was inserted into the right femoral vein and sutured securely into place. An incision was then made at the dorsal aspect of the neck and the cannula guided subcutaneously from the leg to the neck where it was exposed through the incision and sutured securely to the skin surrounding the cut. Incisions were closed using surgical staples and the cannula was plugged. Immediately after the surgery, animals received a prophylactic dose of 0.1 ml Benzathine penicillin G (i.m.; Penlong S; Rogar-STB inc., London, ON, Canada) . The rats were allowed to recover for seven days in a fixed environment with a 12 hour night/day cycle, receiving food and water ad libitum.

2.3 Preparation of 4-iodo Propofol

The propofol analogue, 4-iodo propofol, was generously supplied by Drs. Sanna and Trapani (Dipartimento Farmaco-Chimico, Facolta di Farmacia, Universita degli Studi di Bari, Italy). It arrived as an oil and was stored at 4 °C until needed. Before use the 4-iodo propofol was suspended in the Intralipid® vehicle. Suspension was achieved by adding equal weight of ethanol to the 4-iodo propofol oil and adding a small amount of this mixture to a small amount of Intralipid® in a polystyrene tube (Becton Dickinson, Franklin Lakes, NJ. U.S.A.). This mixture was then vortexed vigorously until suspended. This procedure was repeated until the required amount of the analogue had been suspended in the vehicle. Determination of the exact quantity of 4-iodo propofol to dissolve in each ml of vehicle was determined by adding an amount necessary to achieve the same concentration as the 1% solution. A 1% solution of propofol (MW=178.3) is equivalent to 56 μmol. Thus, 4-iodo propofol (MW=305.2) was also made to 56 μmol. This was equivalent to adding 17.12 mg of 4-iodo propofol to each ml of Intralipid®. To facilitate emulsification in Intralipid® the analogue was first mixed with an equal weight of ethanol and added in small amounts to the Intralipid® mixture. An equal amount of ethanol was added to the Intralipid® control as well to ensure that any changes seen were due to the 4-iodo and not a result of ethanol.

2.3 Infusions

The rats were randomly chosen to undergo a six hour infusion with propofol anaesthetic (1%; Zeneca Pharma, Mississauga, ON, Canada) or the Intralipid® vehicle

alone. Prior to being infused, animals were weighed and the cannulas unplugged and flushed with heparinized saline (30%). Each cannula was then connected, via polyethylene tubing (PE-50), to a 20ml syringe containing propofol or Intralipid®. Syringes were placed into a Harvard pump and the desired rate of administration was set at 15, 20, or 30 mg/kg/hr of propofol or the equivalent volume of Intralipid®. The infusion duration was six hours. Rats were allowed to move freely in their cages and monitored regularly throughout the infusion to ensure patency of the lines. Upon completion of the infusion, the pump w..s turned off, the tubing removed and the cannula replugged. For comparison to the propofol/Intralipid® animals, a second group of male Wistar rats (n=5) were anaesthetized with isofulrane (1.5% in air; Abbott Laboratories Ltd, Montreal, PQ, Canada), or allowed to breath room air only for a six hour period. Initially, the isoflurane was administered in an enclosed chamber. When the rat lost consciousness, the anaesthetic was administered through a nose cone with an attached vacuum line. In both groups, body temperature of the animals receiving anaesthetic was monitored using a rectal thermistor connected to a thermometer (Yellow Springs Instrument Co., Inc., Yellow Springs Ohio, U.S.A.), and maintained at 37.2-37.5 °C using a heating pad (Hamilton Industries, Cincinatti Ohio, U.S.A.).

The infusion of 4-iodo propofol was similar to that of propofol. The infusion rate was set so that each animal received the equivalent molar concentration that a 20 mg/kg/hr infusion of propofol delivered. Since the 4-iodo was emulsified in the Intralipid® at the same concentration as a 1% solution of propofol, the volume of Intralipid® given to these animal was the same as that given to the propofol animals.

2.4   Tissue Preparation

One, three, or seven days following propofol/Intralipid® infusion, and three days

following the isoflurane/air treatment or the 4-iodo propofol infusion ,the animals were

decapitated, the brains quickly removed, frozen on dry ice, and stored at -80°C until

sectioning occurred. Each brain was cut on a freezing microtome into 1 mm sections.

The first section was taken at approximately 3.7-2.7 mm anterior to bregma and bilateral

micropunches of the lateral frontal cortex removed using a 15 gauge needle from which

the tip had been removed and the circumference at the end sharpened. Bilateral samples of

the nucleus accumbens and medial prefrontal cortex were also removed by the micropunch

method at 2.7-1.7 mm anterior to bregma. Samples of the dorsal striatum (caudate

putamen) and insular cortex were removed at 1.7-0.7 mm anterior to bregma. Finally, the

ventral tegmental area (VTA) was micropunched at $^-$4.52-$^-$5.52mm anterior to bregma.

Figure 3 indicates, on schematic drawings of the rat brain, the location of the

micropunches for each area. A stereotaxic atlas of the rat brain was used to ensure that

each region was isolated and punched accurately. During the procedure tissue samples

remained frozen with use of dry ice. Punches were placed in 1.5 ml tubes and stored at -

80 °C until protein levels were determined using Western blot analysis.

2.5   Electrophoresis and Western Blotting

One dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-

PAGE) was performed with the micropunched samples in order to obtain a measure of the

relative amount of TH protein in each sample. Collected tissue samples were removed

from the freezer and 30 µl 1X Laemmli sample buffer was added to each tube. Tissue

samples were boiled for two minutes and homogenized using glass microhomogenizers.

Twenty µl of the homogenate was then loaded into each lane and electrophoresed on a

polyacrylamide gel slab, 1.5 mm in thickness, in the presence of 0.1%

sodiumdodecylsulfate (SDS). A discontinuous buffer system was used with a 10%

separating gel (pH 8.8) and a 4% stacking gel (pH 6.8). Molecular weight standards (5

µl; BioRad, Richmond, CA, U.S.A.) and TH enzyme standards (2-100 ng; Cedarlane,

Hornby, ON, Canada) were also included on each gel slab. The conditions for

electrophoresis were 50 volts (~30 mins) for the stacking gel and 100 volts (~2 hours) for

the separating gel. After electrophoresis on SDS-polyacrylamide gel slabs, the protein on

the slab was transferred to nitrocellulose paper overnight at a constant power of 5 watts

(Bio-Rad Laboratories) using a Trans Blot cell (Bio-Rad Laboratories). The transfer

buffer consisted of 10 volumes of methanol and 90 volumes of an aqueous solution

containing 25 mM Tris base and 192 mM glycine.

2.6   Immunolocalization

        Immunolocalization was carried out on the nitrocellulose paper following the

overnight transfer of protein. The membrane was incubated in a solution containing 5%

nonfat dry milk (Carnation) in phosphate buffered saline (PBS) for one hour at 37°C to

saturate nonspecific binding sites. This was followed by an overnight incubation with a

rabbit anti-rat primary antibody against TH (Eugene Tech International, Inc., Ridgefield

Park, NJ, U.S.A.), also carried out in 5% nonfat dry milk solution. The next morning, the

membrane was washed for 30 minutes (three 10 minute washes) in 1 X PBS and then

incubated for 60 minutes in 10 ml of secondary antibody solution (goat anti-rabbit IgG;

Sigma Chemical Co., St. Louis, MO, U.S.A.) diluted 1:50 000 in 1 X PBS. After a final

wash in PBS (three 15 minute washes), SuperSignal Substrate (5 ml; Pierce, Rockford, IL,

U.S.A.), an enhanced chemiluminescent substrate for the development of immunoblots,

was added. The blots were removed from the substrate after 5 minutes and exposed to x-

ray film at various time intervals. The film was processed with a Kodak M35 X-OMAT

autoprocessor (Eastman Kodak) and the bands representing TH marked.

Immunolocalization of the synaptic vesicle protein, synaptophysin, was also carried out at

the same time using a primary antibody (DAKO Diagnostics, Mississauga, ON, Canada)

which tagged this protein at a dilution of 1:3000. Synaptophysin served as an internal

control.

2.7   Quantification of TH

The developed films were analyzed and TH levels were quantified using Sigma

ScanPro (Jandel Scientific, Chicago, IL. U.S.A.) to scan the image by way of a desktop

scanner (ScanJet 4c/T, Hewlett Packard, SanDiego, CA, U.S.A.). Once scanned the

resolution of the image was increased and it was saved as a bitmap. Densitometry

(Mocha, Jandel Scientific,San Rafael, CA, U.S.A.) was then used to quantify the amount

of TH, or synaptophysin, in each band. This was achieved by outlining each band

separately and determining its area and average intensity. These numbers were then

multiplied together resulting in a representative number with arbitrary units. A correction

was made to adjust for differences in gel loading. The correction was based on the area and intensity of the synaptophysin bands. For each brain sample in a gel the average of the band area multiplied by the intensity was determined for the synaptophysin. A correction factor was determined by calculating the ratio of the synaptophysin band of propofol treated animals to that of control animals. The Intralipid band was then multiplied by the correction factor thereby equalizing the lanes.

The magnitude of the TH standard bands was also determined as the product of area multiplied by intensity. These numbers were then used to plot a standard curve (Figure 4). The corrected TH values were plotted on this standard curve derived from the TH standards of known concentration and the level of TH in each band elucidated.

2.8   Statistical Analysis

Initially, non-parametric Chi-square tests were run to determine statistical significance. One-way ANOVA and the Student Newman-Keuls post-hoc tests were also used to determine statistical significance as all data passed a normality test. A p value of less than 0.05 was considered significant. All values are represented as mean ● standard error of the mean (SEM).

Figure 3. Representative coronal sections illustrating the regions micropunched for Western blot

analysis. Tissue micropunches, 1 mm in thickness, were obtained from the lateral frontal cortex

(LFC), the nucleus accumbens (Acb), the medial prefrontal cortex (MPC), the caudate putamen

(CP), the insular cortex (IC), and the ventral tegmental area (VTA). The figure was adapted from

Paxinos, G. and Watson C., (1982).
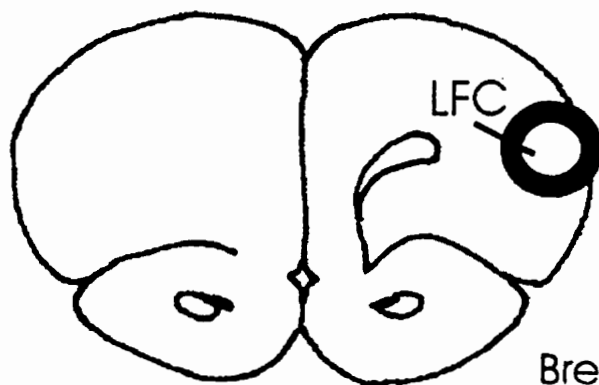
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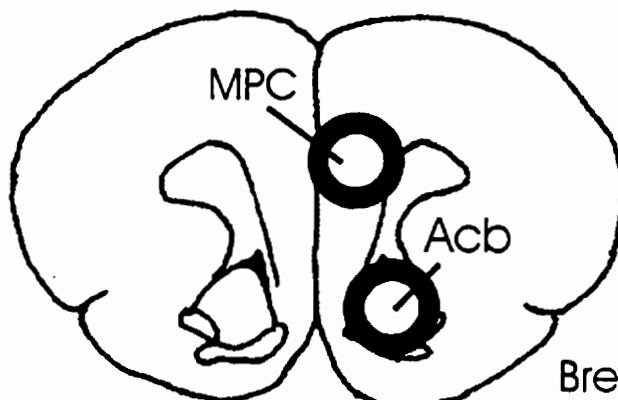
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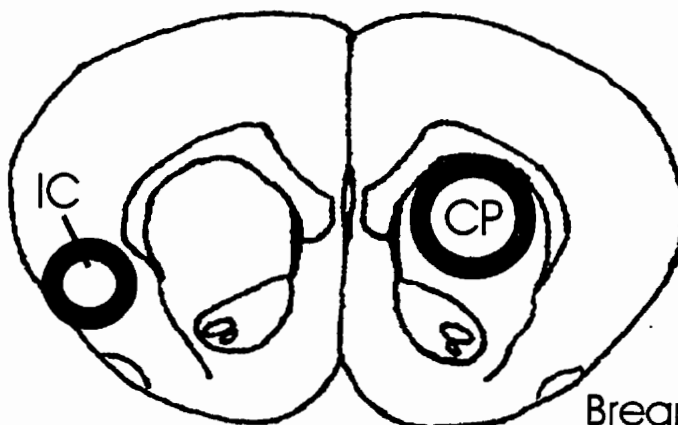
Bregma 1.70
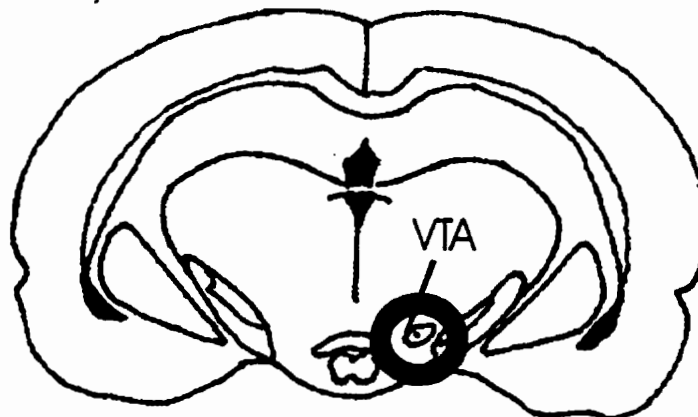
Bregma -4.52

CHAPTER 3

RESULTS

3.1 PROPOFOL

3.1.1 Behavioral Effects of Propofol

To ensure that the rats were maintained at the appropriate level of consciousness

throughout the duration of the infusion, responses of the animals to stimuli were noted.

Following infusion of the sedative dose of 15 mg/kg/hr, all animals were awake and

responded to tapping of the cages. Animals receiving a dose of 20 mg/kg/hr appeared to

be anesthetized but displayed a withdrawal response following pinching of a hind limb

indicating a sub-anesthetic condition. Increasing the administration rate to 30 mg/kg/hr

induced total anesthesia with the animal showing no signs of consciousness following a

pinch test.

3.1.2 TH Immunoblots

A representative immunoblot for propofol and is shown in Figure 4 (pg 47).

Following SDS-Page separation of proteins from the regions previously micropunched,

proteins of interest were localized on nitrocellulose membrane using horseradish

peroxidase linked secondary antibodies. As expected, bands representing TH were

visualized using enhanced chemiluminescesnce at approximately 60 kilodaltons (kDa).

Synaptophysin, a smaller protein was seen at 38 kDa. Synaptophysin, an integral synaptic

vesicle protein present in almost all neurons, was used as an internal control to correct for

Figure 4. Immunoblot for Tyrosine Hydroxylase (TH) after SDS-Page separation of proteins from the nucleus accumbens (Acb) and caudate putamen (CP) of rats three days following infusion with propofol (P) or Intralipid ® (I). Proteins were detected using an affinity purified rabbit anti-TH (1: 2000 dilution), horseradish peroxidase-linked goat anti-rabbit IgG (1: 50 000 dilution), and chemiluminescent detection. Tyrosine hydroxylase is represented by the banding at approximately 60 kDa. The internal control, synaptophysin is visualized at 38 kDa. TH standards are also represented on the blot.

Immunoblot

	Acb		CP		TH Standards (ng)				
	P	I	P	I	5	10	25	50	100

TH 60 kDa

Syn 38 kDa
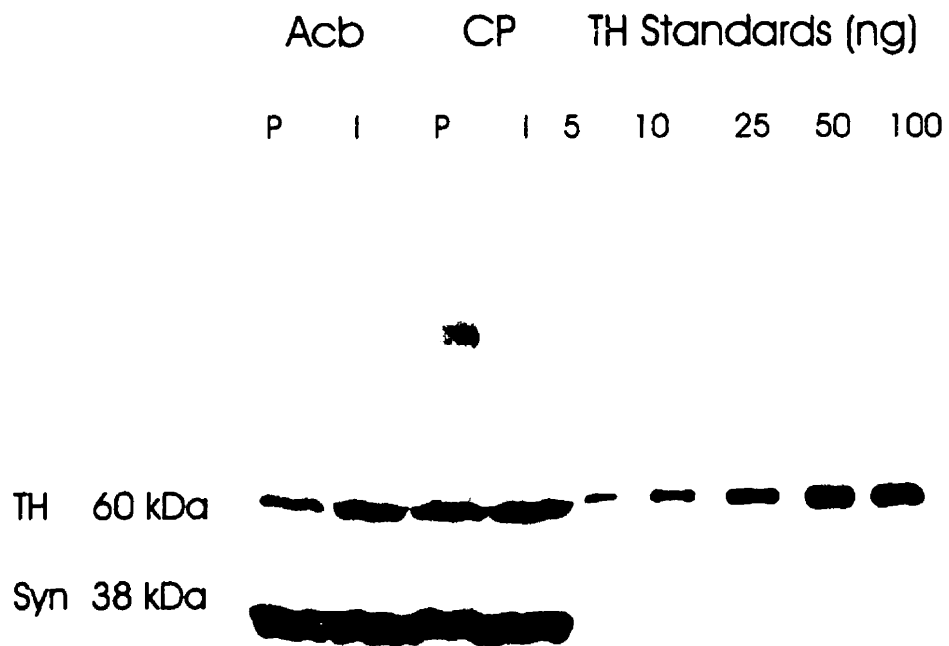
uneven loading of the gel. To ensure synaptophysin levels were not altered by propofol or

Intralipid® a t-test was applied to levels in the Acb of propofol versus Intralipid® animals

three days following propofol infusion. No difference was observed between the two

synaptophysin groups. The immunoblot in Figure 4 indicates reductions in TH levels in

the Acb three days following administration of propofol (P) as compared to the

Intralipid® (I) control. Various concentrations of known TH enzyme were also run on the

gel, and visualized in the same manner as the unknown TH protein. Banding for known

TH concentrations was also visualized at 60 kDa. From these, a standard curve was

generated and used for the determination of TH levels in the samples. A typical standard

curve is shown in Figure 5 (pg 50).

3.1.3   TH in the Nucleus Accumbens

Control levels of TH in the Acb three days following propofol infusion are $22.9 \pm$

4.4 ng (n = 15) with a considerable variation among animals of 10.5 to 51.1 ng. The

variation among animals is depicted in Figure 6 (pg 52). Three days following propofol

infusion, tyrosine hydroxylase levels in the nucleus accumbens decreased significantly

$(13.0 \pm 2.5, p<0.05, n = 15)$ compared to control levels as indicated in Figure 7 (pg 54).

This change is not due to a general anesthetic effect since this decrease was not observed

with the administration of another anesthetic agent, isoflurane. The group receiving

propofol is reduced by 43% when compared to both the Intralipid® and air groups $(22.9 \pm$

4.4 and 34.0 ± 7.5 ng respectively).

The number of animals for measuring TH in the nucleus accumbens is large since

Figure 5. A representative standard curve plotting band density versus protein levels of each TH band with known standards (broken line). A linear regression (solid line) was applied to this plot, allowing for the quantification of TH in bands containing protein samples from micropunched neural regions.

Standard Curve

Tyrosine Hydroxylase (ng)
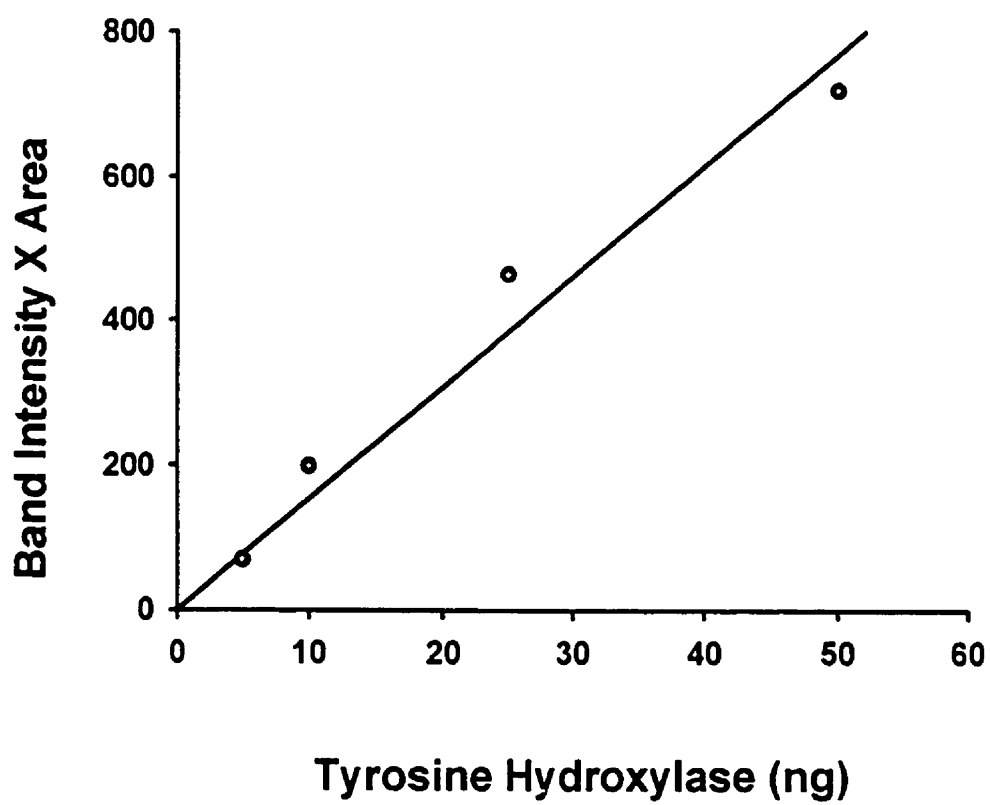
Figure 6. A histogram depicting the variation in TH levels in male Wistar rats. Graph shows the frequency of animals with TH levels in the Acb in 10 ng increments from 0-60. There appears to be a bimodal distribution with peaks between 10-20 ng and 40-50 ng TH. Animals represented are the control group at three days following experimental procedures.

Assay Distribution

Histogram with y-axis labeled "# of Animals" (scale 0 to 10) and x-axis labeled "Tyrosine Hydroxylase (ng)" (scale 10 to 60).
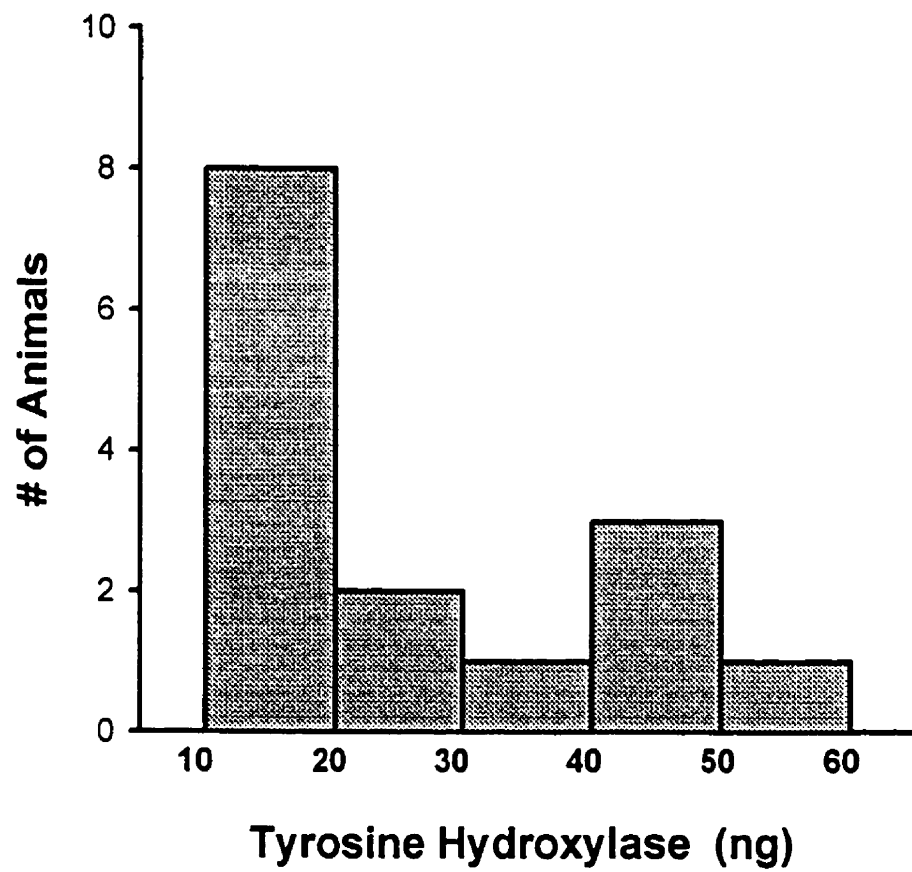
Figure 7. Levels of tyrosine hydroxylase in the nucleus accumbens three days after a six hour exposure to 20 mg/kg/hr propofol (n=15) or 1% isoflurane (n=5) or the combined controls for these anesthetics, (Intralipid®, n=15 and room air, n=5). *TH levels are significantly reduced compared to the controls and isoflurane ($p < 0.05$).

Nucleus Accumbens
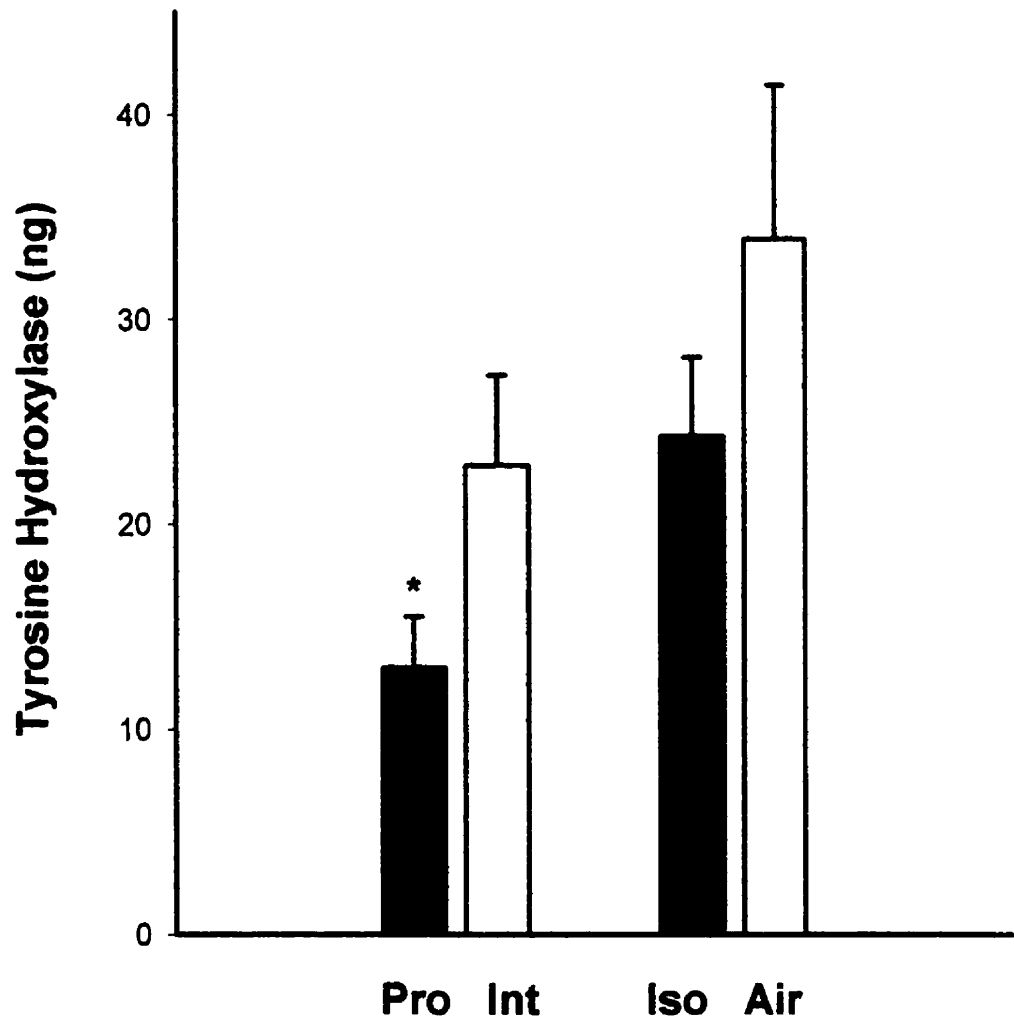
additional groups of animals were done to measure TH in other regions, such as the VTA.

In these groups the TH in the accumbens was also measured. Nevertheless, the first eight

animals measured were enough to show a significant decrease in TH in the Acb of

propofol treated animal in comparison to Intralipid controls with both non-parametric and

parametric tests (15.6 ± 4.4 vs 27.6 ± 5.9).

3.1.4   Time Course: Nucleus Accumbens

To determine the duration of the propofol effect, TH levels were measured in the

nucleus accumbens one, three, five, and seven days following propofol infusion at 20

mg/kg/hr as represented in the time course shown in Figure 8 (pg 57). There is no

significant difference in the levels of TH at day one. However, on the third day post

infusion, levels of TH are significantly reduced (13.0 ± 2.5, $p<0.05$). Five days after

propofol administration, the TH levels appear to be reduced, although there is not a

significant change at this time. By day seven TH has returned to baseline (23.5 ± 5.0 ng).

3.1.5   Dose Response: Nucleus Accumbens

Changing the rate of a six hour infusion from 20 mg/kg/hr to 15 mg/kg/hr

appeared to result in some decrease in TH levels in the Acb, although this reduction is not

significant (Figure 9, pg 59). In addition, the rate of infusion of propofol was increased to

30 mg/kg/hr to determine if a higher dose would result in even further decreases of TH in

the nucleus accumbens or if saturation occurs. The results in Figure 9 indicate that there

is no additional effect of a higher dose of propofol on reductions of TH in the Acb. In

Figure 8. Levels of tyrosine hydroxylase in the nucleus accumbens, one (n=4), three (n=15), five (n=4), and seven (n=6) days following a six hour propofol infusion at 20 mg/kg/hr. * TH levels are significantly reduced (p<0.05) at day three compared to the Intralipid® (Int) controls for all days (n=29).

Time Course:
Nucleus Accumbens
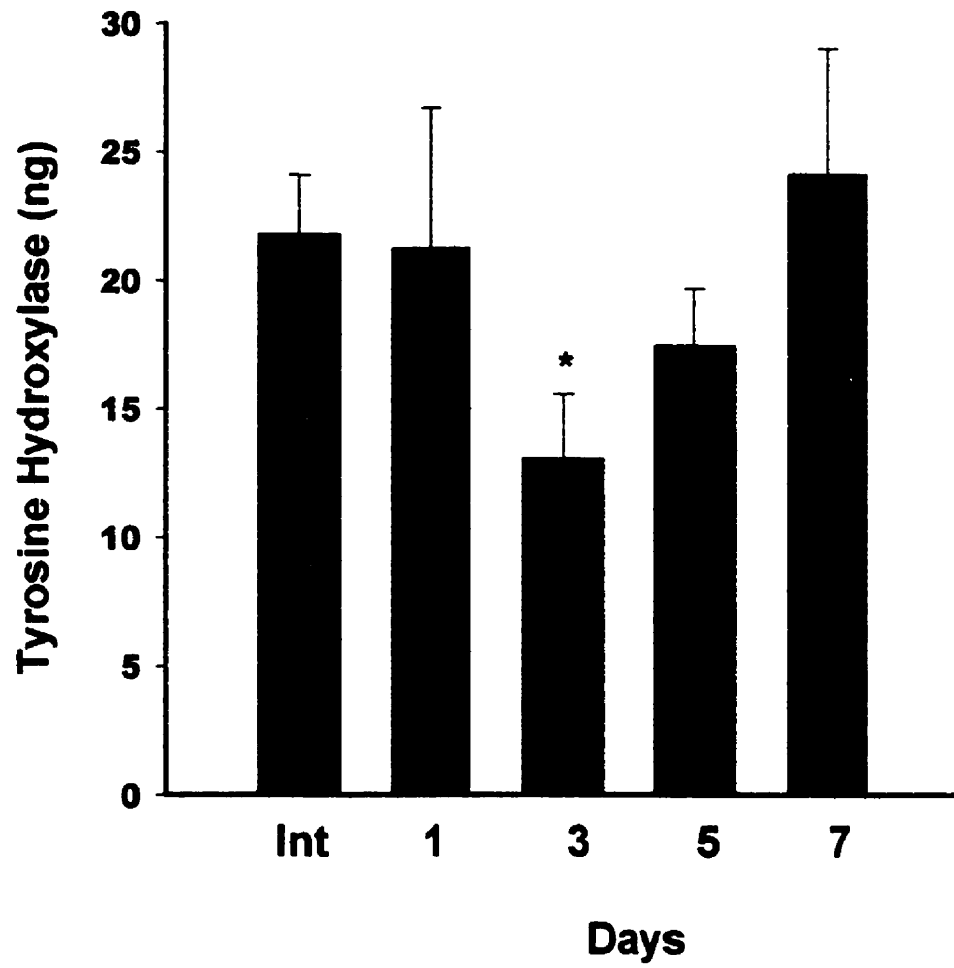
Figure 9. Dose response indicating the levels of tyrosine hydroxylase in the nucleus accumbens three days after various six hour infusions of propofol. A significant reduction (* $p < 0.05$) is seen at 20 mg/kg/hr (n=15). At the lower dose of 15 mg/kg/hr (n=6) no significant decrease in TH is observed. At a higher rate of 30 mg/kg/hr (n=7) the decrease in TH is no longer present.

**Dose Response:
Nucleus Accumbens**
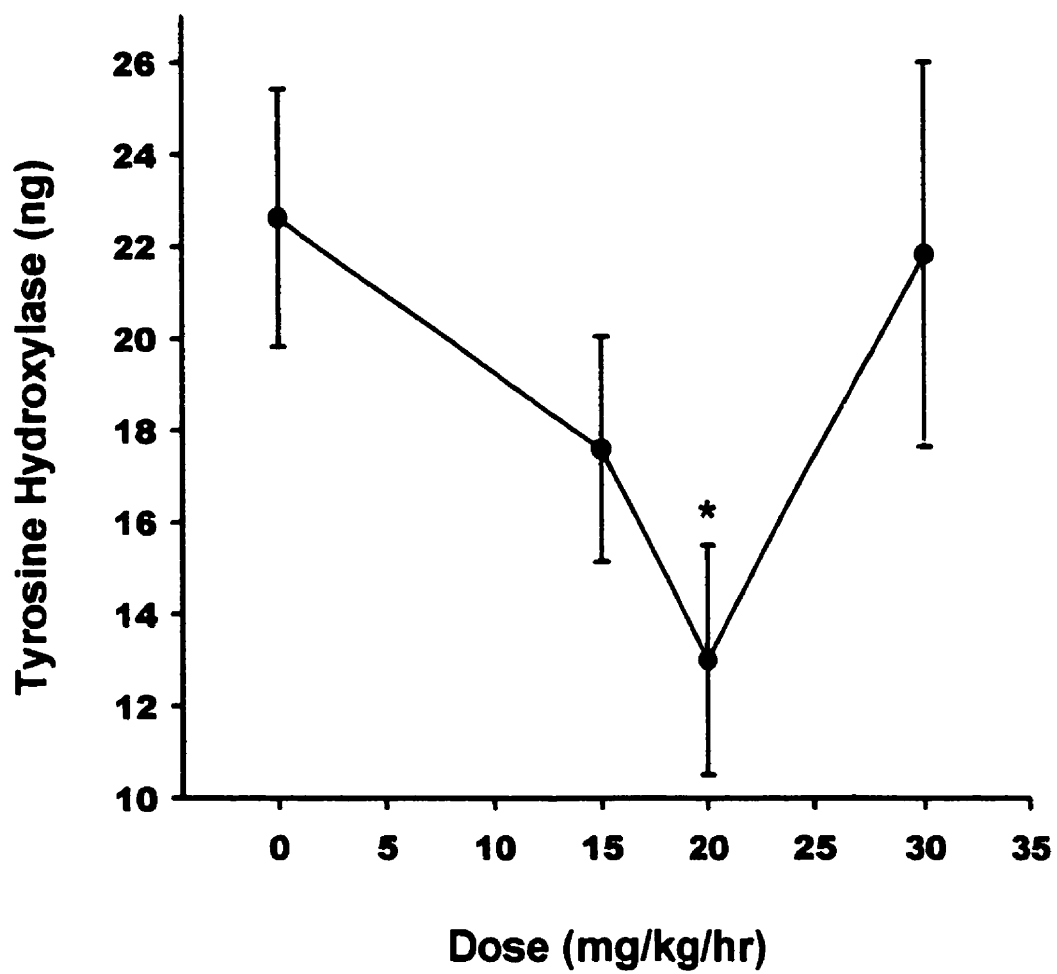
fact, the higher dose gives no significance from controls.

3.1.5   TH in the Caudate-Putamen

Control levels of TH in the CP are 31.3 ± 4.9 ng. These levels are not

significantly different than the control levels in the Acb (22.9 ± 4.4 ng). It is important to

note that tyrosine hydroxylase levels expressed in the caudate-putamen three days after a

six hour propofol infusion show no significant change when compared with isoflurane or

combined control groups (Figure 10, pg 62). This indicates that reduction of TH by

propofol shows specificty for the mesolimbic dopaminergic system As indicated in Figure

11 (pg 64), there does seem to be a trend towards a reduction of TH in the caudate-

putamen one and five days following a six hour administration of propofol anesthetic as

compared to Intralipid® controls (n=4). No change is observed at three (n=15) or seven

(n=6) days following propofol infusion. The trend toward reduction of TH at days one

and five may merely represent the low number of animals in each of these groups.

3.1.7   TH in the Ventral Tegmental Area

Control levels of TH in the VTA are 16.2 ± 3.4 ng. These levels are not

significantly lower than the control group in the Acb (22.9 ± 4.9 ng), but are significantly

lower that the control group for the CP (P<0.05, 31.3 ● 4.9). The VTA is the site of the

dopaminergic cell bodies whose neurons project to the Acb. Therefore, any changes in

TH in the Acb may be a reflexion of alterations in the VTA. The levels of TH in the

ventral tegmental area following a six hour propofol infusion, at 20 mg/kg/hr, were also

Figure 10. Levels of tyrosine hydroxylase in the caudate putamen three days following a six hour infusion of propofol (n=15) or isoflurane (n=5) anesthetics. The control for these anesthetics consists of the combined Intralipid® and room air groups (n=20).

Caudate-Putamen
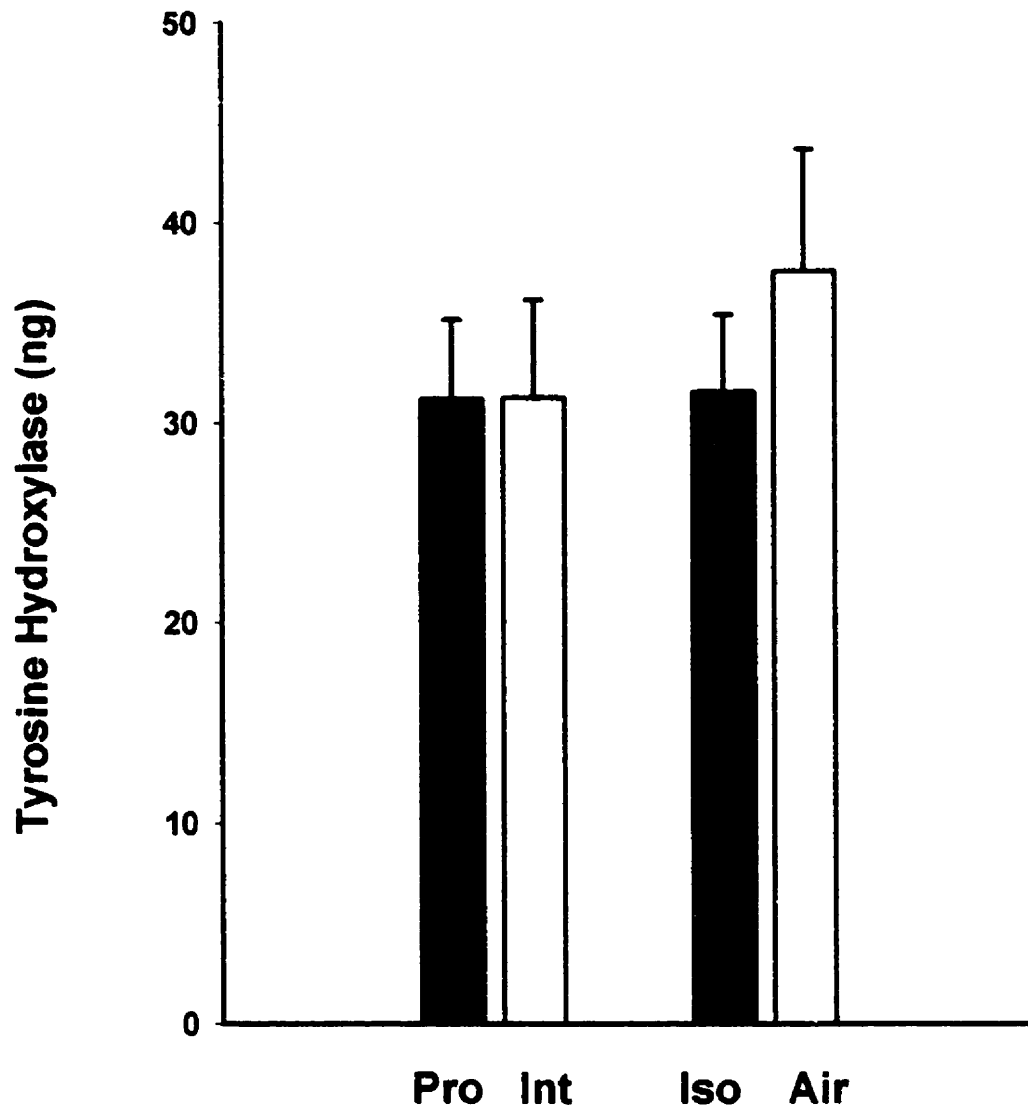
Figure 11. Levels of tyrosine hydroxylase in the caudate putamen one (n=4), three (n=15), five (n=4), and seven (n=6) days following a six hour propofol infusion at 20 mg/kg/hr. TH is not significantly reduced in the dorsal striatum at any time point.

Time Course: Caudate Putamen
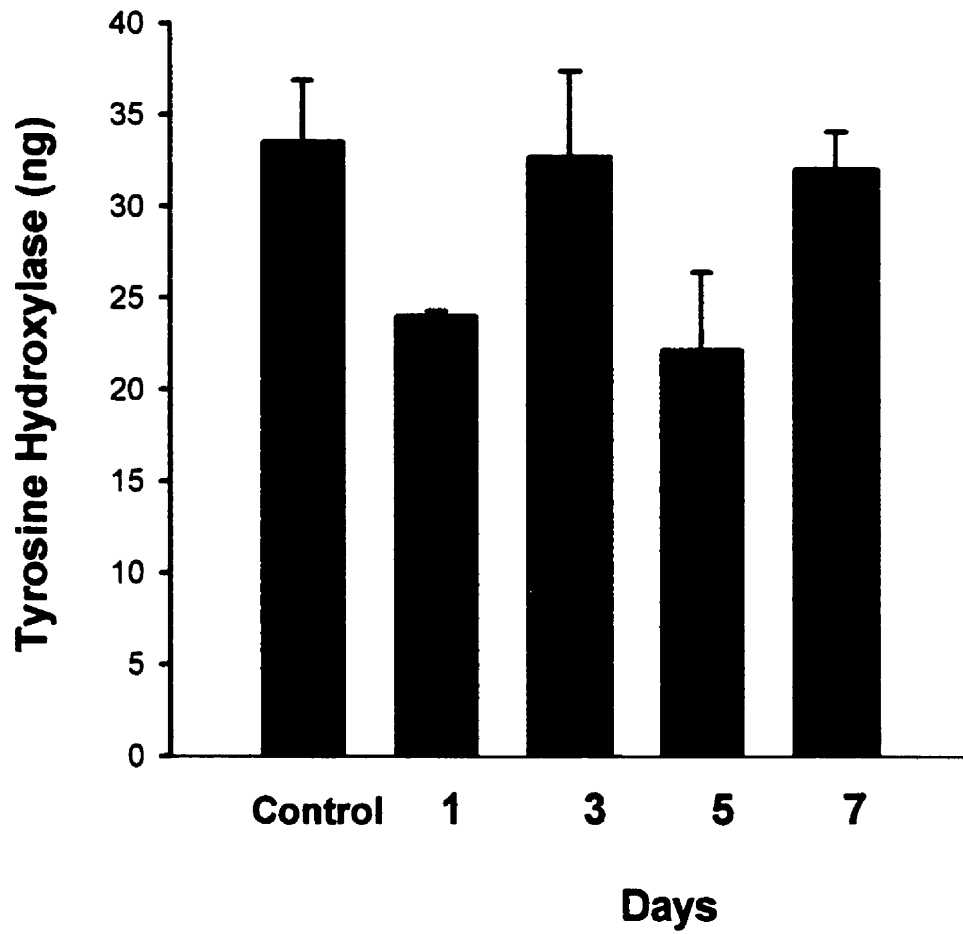
Figure 12. Comparison of tyrosine hydroxylase levels in the nucleus accumbens, caudate

putamen, and ventral tegmental area three days following infusion with propofol, Intralipid®, and

4-iodo propofol. There is a significant reduction in TH in the nucleus accumbens (Acb)

following administration of propofol (n=15) in comparison with both Intralipid® (n=17) and 4-

iodo (n=5) groups (*p<0.05). No change is observed in the caudate putamen (CP). Three days

following infusion of 4-iodo propofol (n=5) there is a significant increase in TH levels in the VTA

(*p<0.05) compared to the Intralipid® (n=7) and propofol (n=4) groups.

Subcortical TH

Legend:
- Intralipid
- propofol
- 4-iodo

Y-axis: Tyrosine Hydroxylase (ng)
X-axis categories: Acb, CP, VTA
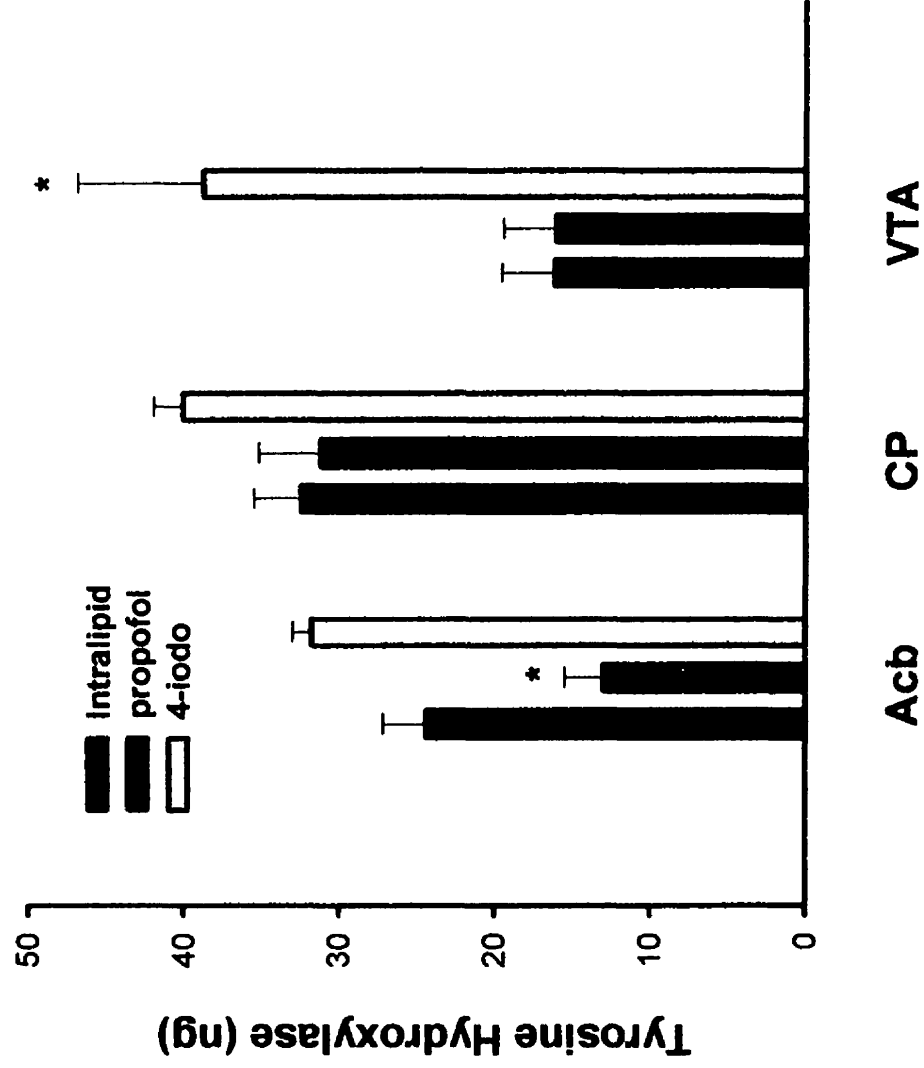
examined (Figure 12, pg 66). With only three animals in each group, there is no

significant change in the levels of TH in the VTA following propofol as compared to

control (16.1 ± 3.4 vs 16.2 ± 3.4 ng).

3.1.8   TH in the Cortex

Cortical punches contained smaller amounts of TH protein than those from

subcortical areas. The Intralipid® controls for the lateral frontal cortex were 13.2 ng and

for the insular cortex 13.1 ng. These levels were significantly different from the Acb, CP,

and VTA (22.9 ± 4.4, 31.3 ± 4.9 and 16.2 ± 3.4 ng). This was expected, as dopaminergic

projections are very diffuse in the cortex and become highly concentrated in the basal

ganglia. Otherwise, there were no significant changes in the levels of TH in either the

lateral frontal cortex or the insular cortex following infusion with propofol when

compared to the Intralipid® control (Figure 13, pg 69).

3.2   4-IODO PROPOFOL

3.2.1   Ethanol in the 4-iodo Preparation

As the 4-iodo propofol required ethanol to be prepared as an emulsion in the

Intralipid®, a calculation of the blood alcohol content was made. Each ml of Intralipid®

contained 17.12 mg ethanol. At an average infusion rate of 0.8 ml/hr each rat recieved

approximately 13 mg of ethanol per hour which should be comparable to 0.005% blood

alcohol content. This calculation does not include any metabolism of alcohol during the

Figure 13. Tyrosine hydroxylase levels in the lateral frontal (LFC) and insular cortices (IC) three days following infusion of propofol (n=7), Intralipid® (n=7), and 4-iodo propofol (n=4). No significant changes in TH levels in the cortical regions are seen.

Cortical TH
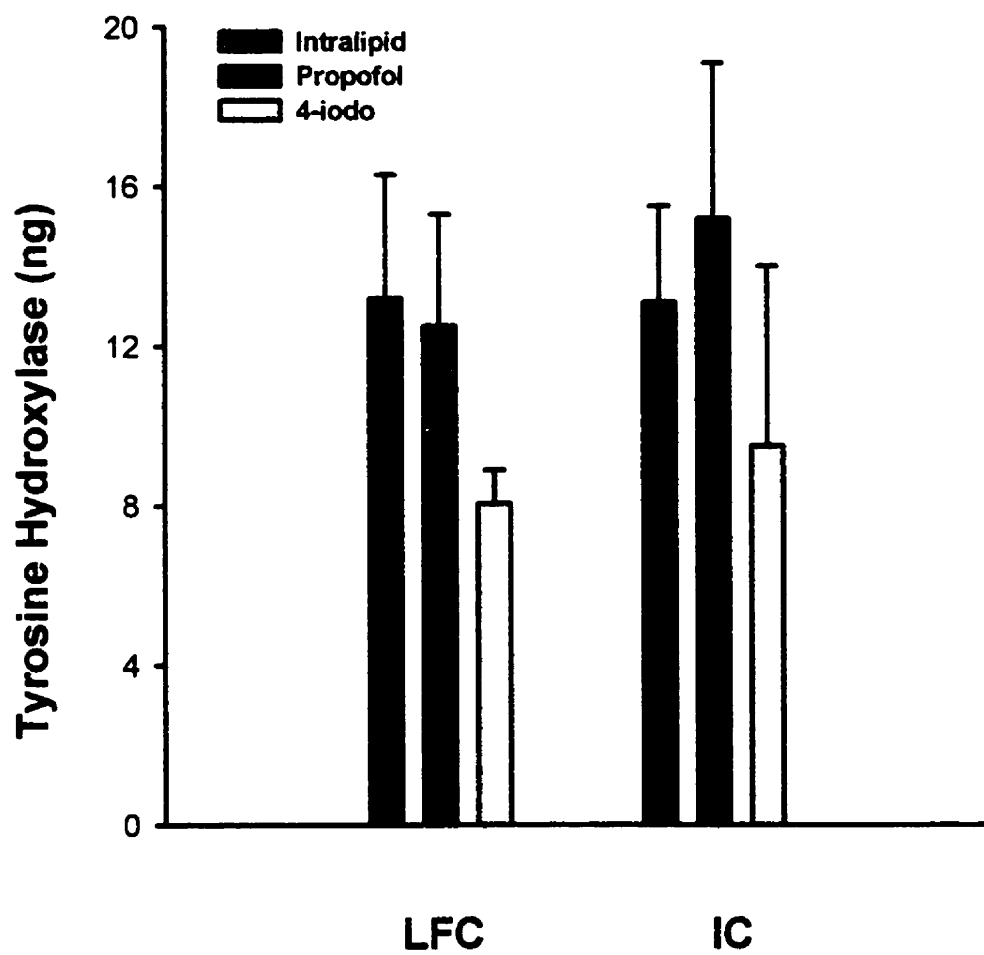
infusion period which would reduce the blood alcohol content even further. Since the TH in the Acb, CP, LFC, and IC for the Intralipid® controls with ethanol were not significantly different from those without, for analysis purpose they were combined.

3.2.2   4-iodo Propofol Effects in the Acb

Unlike infusion with propofol, infusion with the 4-iodo propofol did not result in a reduction of TH in the Acb when compared with the Intralipid® (4-iodo, 31.8 ● 1.2 ng vs Intralipid®, 22.8 ± 4.4 ng ; Figure 12, pg 66). Levels of TH in the Intralipid® group containing ethanol during the 4-iodo series were similar to those found using the original compound and these groups were therefore combined. The significant difference ($p <$ 0.05) in TH observed in the 4-iodo group (31.8 ± 1.2 ng) compared to the propofol (13.0 ± 2.5 ng) was most likely due to differences in the molecular structure of the compounds and not to the inclusion of ethanol in the 4-iodo emulsification.

3.2.3   4-iodo Propofol Effects in the CP

The propofol analogue had no effect on TH levels in the CP when compared to both the Intralipid® and propofol groups (40.1 ± 1.9 vs 31.3 ± 4.9 vs 31.2 ● 4.0 ng respectively). A trend toward increased TH protein in both the Acb and CP is noticeable in Figure 12 (pg 66).

3.2.4   4-iodo Propofol Effects in the VTA

Three days following a six hour infusion of 4-iodo propofol however, there is a

significant increase (2.4 fold) in the levels of TH observed in the VTA when compared to

both the propofol and Intralipid® groups (38.8 ± 8.1, 16.1 ± 3.4, 16.2 ± 3.4, ng

respectively). This increase indicates that the effects of 4-iodo on TH in the striatum differ

from those seen with the parent compound and is likely due to the structual difference

between the two.

3.2.5   4-iodo Propofol Effects in the Cortex

Following a six hour infusion of 4-iodo propofol, there was no significant

differences in TH levels observed in the lateral frontal cortex when compared with either

the Intralipid® control or the propofol groups (8.1 ± 0.9 vs 13.2 ± 3.1 vs 12.5 ± 2.8 ng

respectively; Figure 13 pg 69). Likewise, no significant changes were seen in the insular

cortex (13.1 ± 2.4, 15.2 ● 3.9, 9.5 ± 4.5 ng respectively). The medial prefrontal cortex

was not included in the results as there were no control values available for this region.

CHAPTER 4

DISCUSSION

4.1   Principle Findings

Previous studies carried out in our laboratory using semi-quantitative

immunohistochemistry indicated a selective reduction in TH levels in the nucleus

accumbens of the rat three days following a six hour infusion of propofol anesthetic. The

nucleus accumbens is part of the limbic striatum and thought to be involved in the

integration of information from both the limbic and motor systems. It is thought that

alterations in input to the accumbens, leading to increased dopaminergic output, may

result in the expression of the positive symptoms associated with schizophrenia (Gray,

1995; Totterdell and Meredith, 1997). Tyrosine hydroxylase is the rate limiting enzyme in

dopamine synthesis and therefore, quantification of this enzyme may be a good indicator

of dopamine levels in this region of the brain.

Western blot analysis was used to quantify TH levels. Three days following a six

hour propofol infusion there was a significant reduction (p< 0.05) in TH levels in the Acb.

In comparison, no change in TH was found in the dorsal striatum, VTA, or cortical

regions. The volatile anesthetic isoflurane, also known to produce anesthesia by enhancing

GABA mediated chloride currents, was shown not to significantly alter TH levels. The

optimal dose of propofol to achieve this specific reduction in TH was found to be 20

mg/kg/hr. Lower doses of 15 mg/kg/hr showed no significant reduction in TH while

increasing the infusion rate to 30 mg/kg/hr had no effect.

The propofol analogue, 4-iodo propofol, has been shown to be devoid of anesthetic properties while maintaining its anxiolytic effect (Trapani et al., 1998; Sanna et al., 1999). Therefore it was hypothesized that this compound would also maintain the ability to reduce TH levels specifically in the Acb. A solution of 4-iodo, similar in concentration to that of propofol, was administered to Wistar rats for a six hour time period. Three days following the infusion no alterations in TH levels in the Acb were found. Similarly, no differences were observed in the CP or cortical samples. There was however, a significant increase in TH levels in the VTA of animals infused with 4-iodo propofol when compared with propofol and the Intralipid® control group.

4.2   Detection of Tyrosine Hydroxylase

The immunoblot has become a highly accepted assay for the determination of protein levels in given tissues. Its usefulness lies in the ability of the researcher to isolate a protein of interest using immunochemistry and accurately quantify the amount of protein. Figure 4 represents the outcome of a Western blot assay performed in this study. Following completion of immunochemistry, using a polyclonal primary antibody against rat TH, a horseradish linked bridge, and chemiluminescent detection we observed banding at approximately 60 kDa. Native TH is a large protein consisting of four identical subunits of approximately 60 kDa each (Markey et al., 1979; D'Andrea et al., 1986; Nagatsu, 1995). Homogenization of tissue samples occurs in a high solute concentration, causing protein denaturation. Therefore it was expected that the protein would be visualized at approximately 60 kDa. This result is similar to that reported by others.

Porter et al.(1989) describe a technique used to quantify TH from the median eminence and superior cervical ganglion of the rat. Following tissue isolation, preparation, and Western blot analysis, this group found the molecular weight of denatured TH to be approximately 60 kDa. In order to determine if there were changes in TH activation and phosphorylation in aging rats, Unnerstall and Ladner (1994) isolated TH from the CP and hippocampus. In this study, immunoblots for TH protein were carried out to determine relative protein concentration in the samples compared to known standards. Again, denatured TH protein was visualized at approximately 60 kDa.

Determination of total protein was not carried out on the tissue samples because of the very small amount of tissue obtained from each micropunch. Bilateral punches recovered approximately 2 mg of tissue per animal. To account for possible differences in gel loading, an internal control, synaptophysin, was used. Synaptophysin, an integral membrane protein of synaptic vesicles (Lynch et al., 1994; Galli et al., 1996; Becher et al., 1999), remained consistent in both the experimental and control groups (data not shown). Variations in the levels of synaptophysin between lanes, is an indicator of unequal protein loading. If unequal loading of total protein occurred then the amount of TH protein, calculated without adjustment for loading error, would be inaccurate. Therefore differences in synaptophysin can be used to accurately calculate the amount of TH protein in a given lane by applying a correction to the observed TH value.

4.3   TH Reduction in the Nucleus Accumbens

In all rats, there was a significant reduction ($p < 0.05$) in TH levels in Acb three

days following a six-hour infusion of propofol anesthetic. There was, however, significant variability in TH levels between individual animals. Our data suggests that there are two sub-populations of Wistar rats: a group with low endogenous levels of TH and conversely, one with higher levels. For example, TH levels in the Acb, in animals which were exposed to room air alone appear be clustered around two groups of between 10-20 ng and 40-50 ng. Animals infused with propofol, Intralipid®, or isoflurane showed similar variation in TH levels.

This variation of TH levels in the Acb may be related to the two distinct populations of rats based on previous studies of behavior and mesolimbic dopamine measures (Harris and Nestler, 1995; Desousa et al., 1998). Two distinct populations of rats have been classified according to variations in specific behaviors such as reaction to an acoustic startle, sugar ingestion, and locomotor responses to novel environments. The high responders are classified as animals that consume a lot of sugar, startle easily and actively explore new environments, while low responders appear to stress less easily and show lower interest in consuming sugar over rat chow (Rouge-Pont et al., 1993; Desousa et al., 1998; Sills and Vaccarino, 1998). These differences exist not only within a certain species of rat, but between species as well (Harris and Nestler, 1995). Lucas et al. (1998) further defined these differences neurochemically. High responders have elevated mesolimbic dopaminergic activity while low responders have reduced levels of dopamine. In fact, quantitative microdialysis has shown that basal levels of dopamine are three times higher in the Acb of high reactors than in low reacting rats (Rouge-Pont et al., 1993). Since TH is the rate limiting enzyme in dopamine production, it is possible that the

differences observed in the quantity of this neurotransmitter are a result of varying levels of TH in individual rats.

Propofol binds to the GABAₐ receptor and potentiates the inhibitiory effects of GABA (Orser et al., 1994). This binding to the GABAₐ receptor and transient changes in chloride conductance itself cannot directly account for the reduction seen in TH three days later, as propofol is cleared rapidly from the brain (Fulton and Sorkin, 1995). An indirect mechanism must exist to account for this effect. Figure 14 (pg 77) depicts a circuit diagram of the neurons which may be involved in mediating the decline in TH protein in the Acb. The Acb and VTA are reciprocally linked. The VTA is the major source of dopaminergic innervation to the Acb. The latter nucleus sends GABAergic output back to the VTA. There are two potential mechanisms by which a reduction in TH in the Acb would result. Firstly, binding of propofol, at clinically relevant concentrations, to GABAₐ receptor complexes located in the VTA may result in a substantial inhibition of dopaminergic neural activity leading to a restriction of TH gene transcription. Time is required for TH gene expression and transport from VTA neuron perikarya to the Acb terminals. Thus no reduction in TH enzyme would occur initially and depletion in TH levels would only occur several days later. Alternatively, instead of altering gene transcription and translation propofol may affect enzyme transport to the Acb resulting in diminished TH levels after several days. In either case, a reduction in dopamine output from the Acb would result.

However, the fact that TH in the Acb is reduced but TH levels in the VTA are not, suggests that both TH expression and axonal transport are suppressed. If only TH

Figure 14. Schematic diagram of the neurochemistry of the ventral tegmental area (VTA) accumbal (Acb) pathway, based on Kalivas et al. (1990), Churchill and Kalivas (1994), and Yamada et al. (1996). The Acb receives dopaminergic input from the VTA and in turn sends GABAergic efferents back to the VTA. GABAergic input from the VP also influences the pathway. Propofol may act by enhancing $GABA_A$ receptor function in the VTA resulting in reduced TH levels in the Acb three days following a six hour infusion. Propofol is hypothesised to alter TH protein by repression of TH gene expression and inhibiting TH transport to the terminal.
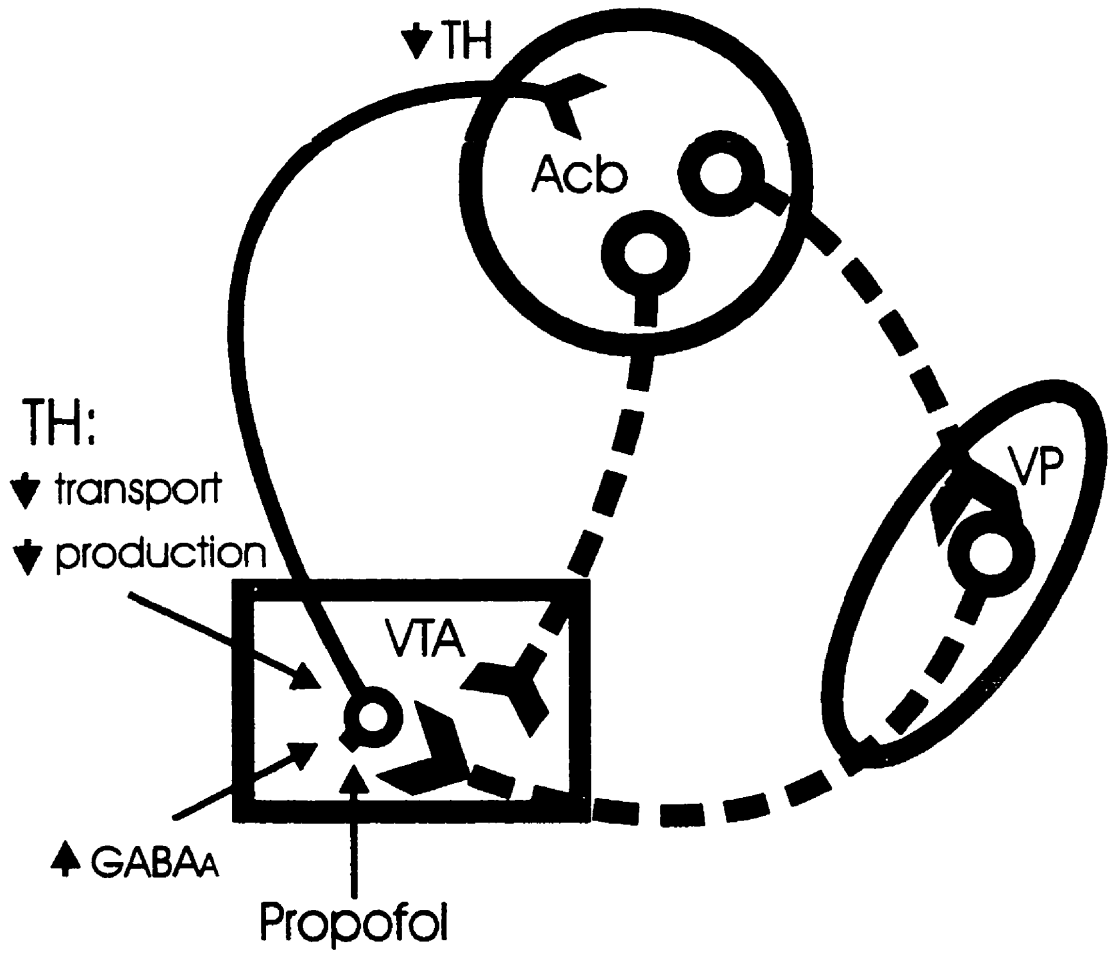
production was supressed, then one would expect a concomitant decrease in TH in the VTA. Conversely, if only TH transport was decreased there should be a back up of the enzyme in the axon raising TH levels in the VTA.

The possibility of propofol altering gene expression or protein transport is supported by several lines of evidence. Interactions of various compounds with the GABA$_A$ receptor produce many acute effects. For example, the neurosteroids allopregnanolone and the progesterone derivative 3 alpha-hydroxy-5 alpha-pregnan-20 one (THDOC) produce anxiolytic, anticonvulsant, and hypnotic/anesthetic effects through amplification of GABA-gated chloride currents (Barbaccia et al, 1998). Alcohol's ability to increase aggressive behavior and disrupt communication between rats is also attributed to GABA$_A$ receptor interaction (Miczek et al., 1997). There are also some long-term neural changes associated with ligand binding to the GABA$_A$ receptor complex. Following pentyleneterazol-induced epileptic seizures Humpel et al. (1993) detected long-term increases in brain-derived neutrotropic factor mRNA and protein. This neuropeptide was markedly increased at one, three and ten days following acute injection of pentylenetetrazol. Alternatively, stimulation of the GABA$_A$ receptor in the hypothalamus resulted in a significant reduction in somatostatin synthesis for up to 24 hours (Llorens-cortes et al., 1992). However, these transient changes in neural activity may result in long term alterations in gene regulation.

Repression and induction of genes and gene products by receptor ligands is well documented. For example, Durham et al. (1997) demonstrate that agonist activation of the serotonin receptor leads to inhibited transcription of the calcitonin gene-related peptide

gene. The bone protein osteocalcin has also been shown to be negatively regulated by

glucocorticoids with increasing levels of glucocorticoids resulting in the development of

osteoporosis (Meyer, 1997; Kearns et al., 1999). Gene repression has also been shown to

exist in the brain (Li et al., 1993; Quinn, 1996; Andrs et al.,1999). In response to trace

amounts of aluminum, Lukiw et al, (1998) demonstrate reduced transcription of RNA

polymerase II and neurofilament light chain, a finding they propose to be linked to the

pathogenesis of Alzheimer's disease. Finally, alterations in the levels of several

neuropeptides in the insular cortex and the amygdala three days following ischemic insult

have been noted by Cheung et al.(1995). The authors indicate that increases in certain

neuropeptides following stroke may afford neuroprotection and aid in the recovery of

injured tissue. Therefore, previous research supports the possibility that the transient

reductions observed in TH in the Acb three days following propofol infusion represents

alterations in TH gene transcription, indirectly resulting from the action of propofol on the

GABA$_A$ receptor complex. Northern blot analysis and in-situ hybridization would enable

quantification and localization of TH mRNA in propofol treated brains to determine the

validity of this proposal.

Another possible mechanism of propofol mediated TH reduction involves its

effects upon presynaptic nerve membranes. Kieta et al. (1996) using in vitro preparations,

in which propofol was applied to striatal synaptosomes indicates that this compound

causes acute dopamine release from the terminal while inhibiting reuptake. Similarly, a

microdialysis study, conducted on rats, showed that intravenous infusion of propofol at

doses producing anesthesia, results in increased dopamine and dopamine metabolites in the

brain (Shyr et al., 1997). These increases were maintained for fourty minutes after the

infusion was stopped. This accumulation of dopamine in the cleft may result in negative

feedback leading to downregulation of TH gene transcription and/or translation.

The effects of propofol on the GABA$_A$ receptor are not consistant with varying

plasma concentrations of the drug. There are three distinct actions of propofol on the

GABA$_A$ receptor. At low clinically relevant concentrations (0.5 - 10 µM), propofol

potentiates the GABA activated currents (Hara et al., 1993; Sanna et al., 1995; Orser et

al., 1994). Direct activation of the chloride channel occurs at approximately 50 µM, while

concentrations outside the clinical range (>100 µM) inhibited channel function by non-

competitive blockage (Orser et al., 1994; Krasowski et al., 1998; Orser et al., 1998). In

this investigation, propofol was administered at the subanesthetic dose of 20 mg/kg/hr for

six hours. This corresponds to, without any metabolism, approximately a 178 µM in the

rat tissues over one hour. In vivo, propofol is extensively bound to plasma proteins (98%)

(Hull, 1991; Fulton and Sorkin, 1995) and therefore a higher concentration in the blood is

required to produce a similar effect. The concentration of 20 mg/kg/hr falls within the

range necessary to produce sub-anesthesia in rats. At this concentration propofol

decreases the levels of tyrosine hydroxylase in the nucleus accumbens. However, 15 or 30

mg/kg/hr had no effect on TH levels. The lower sedative dose may not have been

substantial enough to result in significant potentiation of the chloride current and therefore

reduced TH levels are not seen. Concentrations above the clinical range have been shown

to inhibit chloride conductance. This occurs as a result of receptor desensitization

(Krawoski et al., 1998). Although, a dose of 30 mg/kg/hr, which produces deep

anesthesia, is not above the clinical range there may have been accumulation of propofol in the extracellular fluid which led to receptor desensitization.

In addition to GABA mediated chloride channels, propofol also influences other ion channels including sodium, potassium, calcium, NMDA, and acetylcholine receptor/channels (Orser et al., 1994). Of particular interest is the neuronal nicotinic acetylcholine receptor, which shares extensive homology with GABA$_A$ receptors, as they are members of the same receptor family (Davies et al., 1997; Krasowski et al., 1998). In vivo studies indicate that propofol antagonizes the [alpha]4[beta]2 neuronal nicotinic acetylcholine receptor at clinically relevant concentrations by reducing the open-time of the channel(50 μM) (Wachtel and Wegrzynowicz, 1992; Dilger et al., 1995; Flood et al., 1997; Violet et al., 1997; Nagase et al., 1999). In vitro work has established that these receptors are extremely sensitive to general anesthetics which are thought to exert their effects either by obstructing the pore of the channel or by allosteric modulation (Dilger et al., 1994; Dilger et al., 1995; Violet etal., 1997). Propofol also significantly reduces basal acetylcholine release in the frontal cortex and hippocampus by 70 and 47% respectively, while striatal release is unaffected (Flood et al., 1997; Kikuchi et al., 1998). Acetylcholine is an excitatory neurotransmitter in the CNS and therefore, a reduction in its release along with obstruction of receptors may represent a second pathway by which propofol depresses the CNS (Kikuchi et al., 1998).

Recently, the neuronal nicotinic receptor has been found to participate in regulation of the TH gene. Agonist binding to this receptor induces transcription of the TH gene via a signaling pathway involving cyclic AMP and calcium as second messengers

(Gueorguiev et al., 1998). Therefore, the antagonistic effects of propofol on this receptor could result in lowering expression of the TH gene and consequently TH protein in the cell. It is possible that this effect may not be observable by Western blotting until day three because initial levels of the TH enzyme would dissipate slowly.

In my experiments, there was not a significant reduction in TH observed in isoflurane and Intralipid® treated animals. Nevertheless, a number of recent investigations have demonstrated anesthetic effects on specific neurotransmitter systems. Both propofol and isoflurane are thought to mediate their effects via the GABA$_A$ receptor. However, they each have distinct sites of action on the complex and affect the discharge patterns of neurons in different ways (Krasowski et al., 1998, Antkowiak, 1999). For instance, unlike the GABA-mediated effects of propofol, Ries and Puil (1999) demonstrated an isofluane enhanced potassium leak in thalamocortical neurons which they proposed could account for anesthesia in vivo. However, the lack of a decrease in TH observed in the isoflurane group suggests that propofol acts via a specific pathway.

Isoflurane, a volatile general anesthetic which, like propofol, is known to cause the release of dopamine from nerve terminals in the striatum and prevent reuptake into synaptosomes (Irifune et al., 1997). There have been studies which indicate a possible interaction of isoflurane and other volatile anesthetics with the GABA$_A$ receptor (Sieghart, 1995). Although an exact site of action for these anesthetics has not yet been found, it has been established that isoflurane has a site of action which is distinct from those of intravenous anesthetics (Krasowski et al., 1997). In cultures, isoflurane has been shown to directly open chloride ion channels as well as augment the actions of other

compounds on the GABAₐ receptor. For instance, isoflurane increases the binding affinity of benzodiazepine agonists such as flunitrazepam (Harris et al., 1993; Hall et al., 1994). It has been proposed that general anesthesia caused by the inhalation of isoflurane is the result of increased chloride ion conductance and the resulting hyperpolarization (Sieghart, 1995; Whiting et al., 1995). However, Larsen et al. (1997) as well as Miyazaki et al. (1997) suggest that volatile anesthetics may induce anesthesia via an alternative route. They show that isoflurane administration in vitro results in increased glutamate uptake into astrocytes and that this increased uptake is specific for the excitatory neurotransmitter. The authors propose that a rapid reduction in glutamate, but not GABA, in the synapse may cause a large enough reduction in excitatory potentials to contribute to anesthesia. A third possibility for the mechanism by which isoflurane could produce CNS depression is through interaction with acetylcholine receptors. Isoflurane is known to antagonize nicotinic receptors, which results in reduced excitatory transmission in the CNS possibly contributing to anesthesia (Salord et al., 1997). This may also lead to reductions in TH levels in a mechanism similar to that of propofol anesthetic. Therefore, it is possible that any alteration in TH levels observed following isoflurane inhalation is the result of nicotinic receptor antagonism leading to reduced TH gene expression. Since, both isoflurane and propofol antagonize the nicotinic receptor, but only propofol reduces TH selectively in the Acb, it is likely that nicotinic antagonism is not the mechanism responsible for this effect.

Intralipid® is an intravenous fat emulsion used mainly as a nutrient supply to patients in the intensive care unit who cannot be fed orally (Ulrich, et al., 1996). It is also

used as a vehicle for the delivery of lipid soluble substances such as the general anesthetic propofol. In this study we used Intralipid® as a control to ensure that any reductions in TH seen following propofol infusion were due to the anesthetic and not the vehicle. Figure 7 shows no significant reduction in TH in the Acb three days following infusion with Intralipid®. Little research has been conducted on how this vehicle may affect the CNS, although studies show that it does not interact with the GABA$_A$ receptor complex (Orser et al., 1994). It has been noted, however, that in the gut, Intralipid® increases levels of cholecystokinin (CCK) (Isaacs et al., 1987; Guedon et al., 1988; De Sommer et al., 1990). It is possible that Intralipid® has similar effects on CCK levels in the brain. In the brain, CCK acts as peptide neurotransmitter. It is highly colocalized with dopamine in neurons of the mesolimbic pathway (Vaccarino, 1994; Lanca et al., 1998). CCK, in the mesolimbic system, is a dopamine antagonist and has a bimodal distribution opposite to that of dopamine. Animals with high dopamine concentrations have low CCK levels and vice versa. If Intralipid® acts in the brain, as it does in the gut, to increase CCK levels there may be resultant reduction in dopamine. However Intralipid® did not result in significant lower levels of TH in this study.

Finally, in addition to the Western blot analyses, behavioral studies in our laboratory indicate alterations in dopamine levels may have occurred in the mesolimbic dopaminergic system following propofol infusion (Kang, 1997). Rearing, a known Acb-mediated behavior, was significantly reduced three days following propofol infusion. Additionally, Wahr et al. (1996) found that administration of propofol to patients after surgery resulted in a significantly lower level of extracellular dopamine when compared to

patients given morphine or midazolam. This suggests that, in the long term, propofol leads to reductions in dopamine levels. This possibility could be explored using microdialysis which enables the quantification of neurotransmitter release in conscious, freely moving animals.

4.4   Time Course of TH Levels in the Acb

Prior to day three there are no observed changes in TH levels in the Acb. This is consistent with immunohistochemical results carried out in our laboratory (Kang, 1997). This latency does not necessarily indicate that the TH gene is downregulated three days following propofol infusion, but may be the result of slow turnover of TH present at the time of infusion. Changes in the levels of TH mRNA or TH protein have been shown to occur slowly over 12-24 hours when animals are subject to prolonged stress (2-4 hours, Tank et al., 1998). Stress usually results in an increase in TH via cyclic AMP-and calcium-mediated signaling pathway that activates a cAMP response element protein (CREB) and thereby gene expression (Kaufman, 1995). It is possible that propofol acts either indirectly, via a receptor complex, or directly at any level in this signaling pathway to result in a decrease in TH gene expression. Terminal elimination half life of propofol following long term continuous infusion is between 23.5 to 31.3 hours (Fulton and Sorkin, 1995). The reduction in TH, seen at day three, may be the result of prolonged propofol infusion resulting in a build up of the anaesthetic in the peripheral compartments from which it is slow to return (Bryson et as., 1995). This would allow propofol to have a prolonged effect on the signaling pathway and inhibit induction of the gene for a longer

period. By day seven, the effect of propofol has diminished, which would be expected

since propofol has been cleared from the body.

4.5   TH Levels in the Caudate-Putamen

The caudate-putamen also contains high levels of TH. In response to propofol

infusion however, no significant reduction in TH is observed. Parallel dopaminergic

pathways exist for the caudate and accumbens (Parent, 1995). The accumbens is a

component of the ventral striatal pathway, which is involved in limbic-motor function

while the caudate is part of the dorsal striatum, or striato-nigral tract. Pathologies of these

areas result in different manifestations of disease, for example, damage to the dorsal

striatum is believed to result in Parkinson's disease while increases in dopamine in the Acb

results in schizophrenia (Kandel, 1991; Paxinos, 1995). The failure of propofol to

influence both dopaminergic pathways equally gives further evidence of the specificity of

propofol's actions in the striatum. This specificity may be due, in part, to differences in

receptors in the mesolimbic pathway compared to the nigrostriatal tract. For instance,

subunit conformation of GABAa receptors varies throughout the striatum with those in

the Acb expressing the $\alpha 2$ subunit while the majority of GABAa receptors in the dorsal

striatum contain $\alpha 1$ and $\alpha 3$ subunits (Caruncho et al., 1996; McKernan and Whiting,

1996).

4.6   4-iodo Propofol

Propofol itself has been associated with some adverse effects during administration

including pain on injection, and bradycardia. It has been postulated that the lipid emulsion

may be responsible for these negative effects (Trapani et al., 1998). In an attempt to

reduce these side effects, Trapani et al. (1998) altered the propofol molecule at various

positions hoping to identify a hydrophilic analogue with a better pharmacodynamic profile.

One of these alterations, substitution of a hydrogen ion on the aromatic ring of propofol

with an iodine ion, resulted in a compound which had both increased affinity for the

$GABA_A$ receptor and efficacy at potentiating GABA induced chloride currents. However,

unlike propofol, in the absence of GABA, there was very little ability for 4-iodo propofol

to directly activate chloride currents. This analogue has also been found not to produce

sedation or anesthesia in rats, while still maintaining the anxiolytic properties observed in

the parent compound (Pain et al., 1999; Sanna et al., 1999). Therefore, it was thought

that 4-iodo propofol may have a similar effect on TH levels in the nucleus accumbens, as

the parent compound, without the anesthetic consequences.

The propofol analogue used in this study was received as an oil and therefore it

was necessary to solubilize it into the Intralipid® vehicle. To increase its solubility in the

Intralipid®, 4-iodo was first mixed with an equal weight with ethanol. In humans, the

effects of ethanol become observable at a concentration of 10-300 mM, a level known to

be intoxicating (Tanelian et al., 1993). At a blood alcohol level of 0.005% there were not

any apparent behavioral effects in the Intralipid® group and small animals with rapid

metabolic rates will have much lower blood alcohol content making the effect of ethanol in

this study negligible. There is however, evidence that ethanol's effects are mediated via the

$GABA_A$ receptor complex in much the same way as that of propofol (Ticku, 1990;

Sieghart, 1995;Whitten et al., 1996; Liu and Deitrich, 1998). Although, it is likely that these compounds act at different sites on the complex since, when co-administered, the effect of these drugs are additive (Garfield and Bukusoglu, 1996). In this study however, ethanol seemed to have no effect on TH levels, as this enzyme did not differ between Intralipid® groups of the previous experiment using the parent compound or the present study using the analogue, 4-iodo propofol.

The previous experiments indicate a significant reduction in the levels of TH in the Acb three days following a six-hour propofol infusion. Following infusion with 4-iodo propofol this lessening of TH in the Acb was not observed. There was however, a significant increase in TH levels in the VTA. It was originally thought that since this analogue no longer induced anesthesia while retaining its anxiolytic properties that it may act to reduce TH protein levels in the Acb and potentially be useful as an antipsychotic. Sanna et al. (1999) show that while 4-iodo propofol does directly activate chloride conductance into cells in the absence of GABA, it does so with reduced efficacy at the $\alpha 1$ $\beta 2 \gamma 2$ receptor combination. This combination of subunits is the most common and widespread of the GABA$_A$ receptors in the CNS (Mohler et al., 1995). They also show 4-iodo has subunit specificity, for example, it has higher affinity for the $\beta 2$ subunit than the $\beta 1$. This is suggestive that 4-iodo propofol may have different actions at different regions of the brain depending on what subunit combination is found there. Reduced chloride current may account for the maintenance of the anxiolytic effects while deleting its anesthetic actions. It may be that manipulation of the propofol molecule, while allowing for continued binding to the active site, results in partial blockage of the channel thereby

allowing fewer chloride ions to pass through. A possible binding site for propofol is thought to exist within the channel itself (Tanelian et al., 1993). Therefore, it is plausible that if potentiation of the GABA induced chloride current by propofol is responsible for the earlier reductions in TH seen in the nucleus accumbens then blockage of this current would inhibit this response.

Alternatively, propofol is known to block nicotinic acetylcholine receptors in the CNS (Dilger et al., 1995). It has been suggested that the nicotinic receptor is involved in modulation of the induction of the TH gene. Specifically, receptor agonists induce the gene while antagonists suppress expression. Propofol is known to antagonize this receptor complex (Wachtel and Wegrzynowicz, 1992; Dilger et al., 1995). Manipulation of the parent molecule may have resulted in the inability of 4-iodo propofol to bind to the nicotinic receptor, resulting in continued basal expression of the TH gene.

Tyrosine hydroyxlase levels in the caudate-putamen are not influenced by infusion of 4-iodo propofol. It is interesting to note however, that quantity of TH in the VTA was significantly elevated by 4-iodo propofol but not the parent compound. This again indicates possible differences in the mechanism of action between these two compounds. In contrast to propofol, the analogue increases TH in the VTA while appearing to have no effects in the Acb. Figure 15 illustrates a potential mechanism of action of 4-iodo propofol on ventral striatal circuitry. Again, various possibilities relating to the accumulation of TH in the VTA with no corresponding increase in accumbal TH. The analogue may result in upregulation of the gene with a reduction in transport to the terminals. In a similar fashion, an increase in TH gene products coupled with no change in

transport would also accomplish increased TH in the VTA. Further studies involving the

isolation and quantification of TH mRNA are necessary to elucidate the proper pathway

leading to these changes in TH enzyme.

4.7   Implications for Schizophrenia

Altered regulation of the subcortical dopaminergic systems is thought to be

responsible for the manifestation of psychotic symptoms (O'Donnell and Grace, 1998).

The mesolimbic dopaminergic system and PFC are systems both believed to influence

dopamine release in the basal ganglia via connections with the VTA. The VTA in turn

sends dopaminergic projections to the Acb (Totterdell and Meredith, 1997). Increase

dopaminergic release in the Acb is believed to result in psychotic symptoms (Gray, 1995).

Antipsychotics currently in use target dopaminergic receptors in the brain reducing the

effect of increased dopamine thereby alleviating schizophrenic symptoms. However, this

effect is nonspecific and decreasing dopamine in other systems may be responsible for the

negative side effects associated with antipsychotic use (Fleischhacker and Hummer, 1997).

Propofol is a unique anesthetic agent with few negative side effects which has

been shown to specifically reduce TH levels in the Acb three days following infusion. TH

is the rate limiting enzyme in dopamine production and reductions of this neurotransmitter

following propofol infusion would give this compound a potential use as an antipsychotic.

In fact, behavioral studies support the concept of a concomitant decrease in dopamine

from the Acb following propofol infusion (Kang, 1997). Unfortunately, the reduction in

TH occurs only with administration of a sub-anesthetic dose not a sedative dose. The

anesthetic properties of this drug are not compatible with antipsychotic treatment.

Therefore, elucidation of a propofol analogue with no anesthetic properties which

maintains the ability to reduce TH, or a determination that longer term application of

lower doses of propofol is effective is necessary to develop this further.

Figure 15. The proposed effect of 4-iodo propofol on the neurochemistry of the ventral tegmental area (VTA), nucleus accumbens (Acb) circuit. The circuit diagram is based on Kalivas et al. (1990), Churchill and Kalivas (1994), and Yamada et al. (1996). The Acb receives dopaminergic input from the VTA and in turn send GABAergic efferents back to the VTA. It is suggested that 4-iodo propofol acts on the $GABA_A$ receptor complex. 4-iodo causes increases in TH protein in the VTA but not the Acb. This may be accomplished by increasing TH gene expression while reducing transport to the terminals. Alternatively, there may be no changes in mRNA levels with blockage of protein transport.
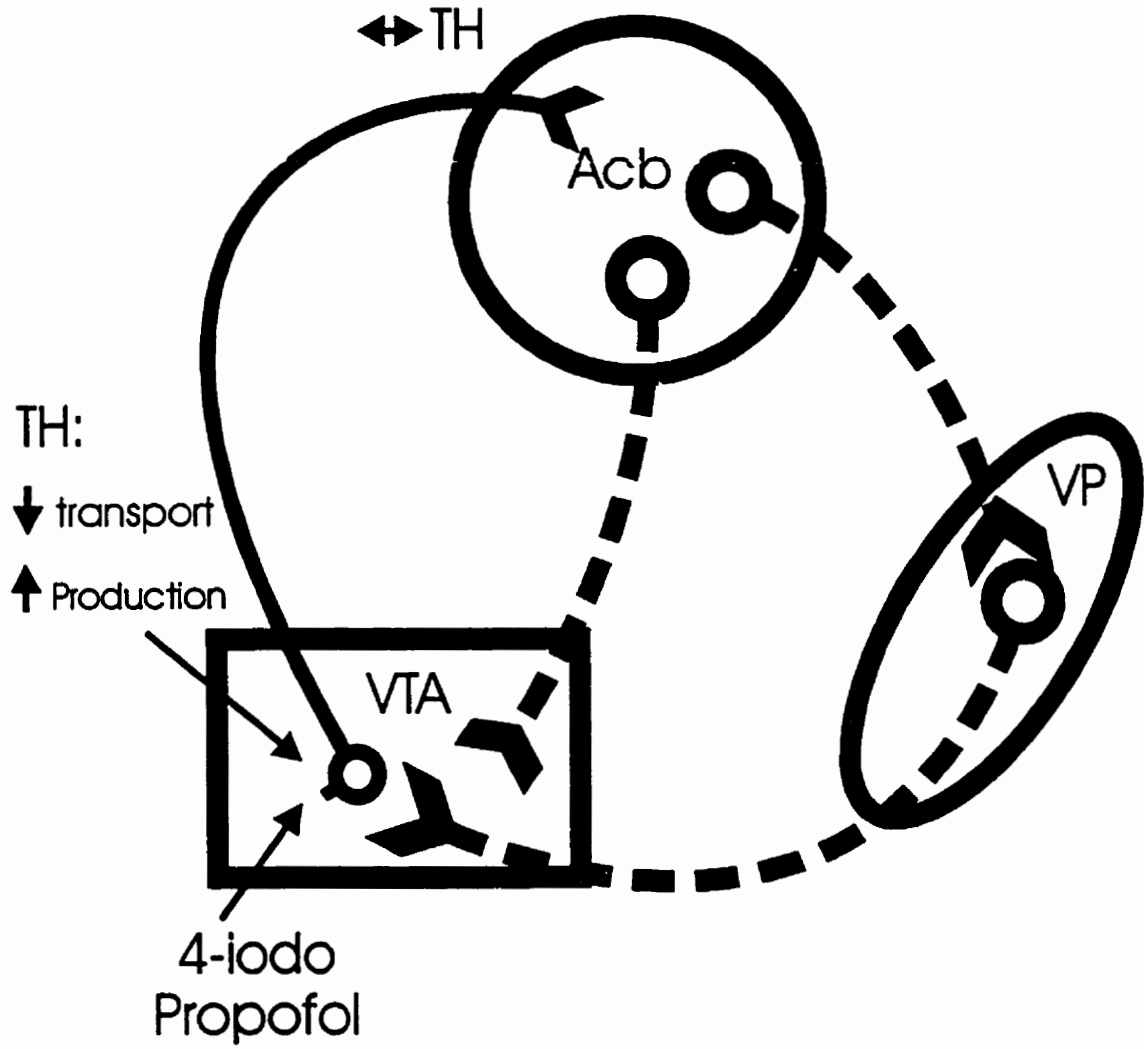
SUMMARY AND CONCLUSIONS

The general anesthetic, propofol, has been demonstrated to produce its anesthetic action by binding to the GABAₐ receptor complex and potentiating the GABA mediated chloride current (Hill-Venning et al., 1997). Semi-quantitative immunohistochemical studies carried out in our laboratory indicated a reduction in TH three days following a six hour propofol infusion. Western blot analysis was utilized to quantify these changes.

A significant reduction (43%) in TH values was found in the ventral, but not dorsal striatum, indicating a specificity of this compound for GABAₐ receptor complexes located in this neuronal region. In addition, a time course study was carried out and TH levels in the Acb were quantified at one, three, five, and seven days following propofol administration. Reductions in TH were observed at three days only indicating an indirect mechanism of action. It is possible that propofol indirectly represses gene expression via an unknown mechanism. Another possibility is that administration of propofol interferes with transport of the TH protein from the cell bodies in the VTA to the terminals located in the Acb. In order to elucidate propofol's exact mechanism of action assays such as Northern blot analysis and in situ hybridization can be used. Northern blots would allow for the quantification of TH mRNA levels in specific regions of the brain while in situ hybridization aids in the localization of potential changes in mRNA within nuclei.

The reduction in TH protein within the Acb was observed to be dependent on the specific dose of 20 mg/kg/hr. Infusion at rates of 15 mg/kg/hr did not affect TH levels significantly while higher doses at a rate of 30 mg/kg/hr resulted in a reversal of the effect.

Experiments using a lower dose of propofol infused for shorter time periods over many days may overcome this and enable treatment of psychotic symptoms without anesthesia.

Unlike its parent compound, 4-iodo propofol does not reduce the levels of tyrosine hydroxylase in the Acb. This implies that conformational changes in the molecule may have altered its mechanism of action, and that the ability of propofol to reduce TH in this nucleus may somehow be attached to its anesthetic properties. The analogue, 4-iodo propofol, appeared to act opposite to that of propofol by increasing TH in the VTA but not the Acb. It is possible that 4-iodo may act specifically at a different combination of GABAₐ receptors than does propofol. The results obtained from this study indicate that the actions of 4-iodo propofol do not render it a candidate for use as an antipsychotic. However, other propofol analogues may conserve the ability to reduce TH in the Acb while maintaining little or none of its anesthetic actions.

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