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Regional Differences in Central Adrenergic Neuronal Activity During Anesthesia

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REGIONAL DIFFERENCES IN CENTRAL ADRENERGIC NEURONAL ACTIVITY DURING ANESTHESIA

by

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VITA

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A B B R E V I A T I O N S

α	alpha
AMPT	alpha-methyl-para-tyrosine
^{14}C	carbon 14 label isotope
C	centigrade
cm	centimeter
CO_2	carbon dioxide
CPM	counts per minute
DA	dopamine
DPM	disintegrations per minute
DPS	dorsal periventricular system
DTB	dorsal tegmental bundle
EDTA	ethylenedinitrilo-tetraacetic acid
EEG	electroencephalography
g	gravitational force
G	grams
GABA	gamma-amino butyric acid
GHB	gamma-hydroxybutyrate
^{3}H	tritium
HCl	hydrochloric acid
5-HT	serotonin
HVA	homovanilic acid
kg	kilogram
M	molar
MAC	minimun alveolar concentration

ABBREVIATIONS (cont.)

MFB	medial forebrain bundle
mg	milligram
min	minute
ml	milliliter
mm	millimeter
N	normal
NE	norepinephrine
ng	nanogram
nM	nanometer
N_2O	nitrous oxide
O_2	oxygen
p	probability
PCPA	parachlorophenylalanine
SE	standard error of the mean
torr	millimeter mercury
uMHg	micrometer mercury
VPS	ventral periventricular system

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Biophysical Anesthetic Mechanism

The study of the mechanism of anesthetic action has been approached by a surprisingly diverse group of disciplines. These studies have arisen from almost every type of laboratory in the biological and chemical sciences. The resulting milieu is clouded and filled with many diverse theories. With this in mind, I have taken the liberty to group these theories into two subgroups, the protein theories and the lipid theories, in an attempt to gain semblance. First, a few basic concepts should be considered to help gain insight into these theories which suggest a unitary molecular site of anesthetic action.

General anesthesia is produced by a wide variety of compounds with no common chemical structure or chemical activity. Some examples of the diverse chemical natures of agents that produce general anesthesia would include aliphatic, aromatic, halogenated hydrocarbons, alcohols, aldehydes, ketones, esters, and ethers (Kaufman, 1977). Even the nitrogen in the air we breathe has anesthetic

properties (Paton, 1967; Winter, et al., 1975). Importantly, it has been suggested that a specific receptor does not play a significant role in anesthetic mechanisms. This has been proposed largely for two reasons. First, the molecular size differential between a small molecule (e.g., N_2O) and a large molecule (e.g., a steroidal anesthetic) makes the feasibility of such a relationship unlikely. Second, stereoisomerism is an important criterion for receptor delineation: stereoisomers of most anesthetics are equipotent (Kendig, et al., 1973).

For many years it has been known that hydrostatic pressure can modify an organism's response to drugs (Cattel, 1936). In fact, anesthesia itself is generally reversed with increased atmospheric pressure. Johnson and Flagler (1950) first made the observation that tadpoles narcotized with a 2 - 5% ethanol would resume swimming in an apparently normal manner following application of pressure. Similar hyperbaric reversal of anesthesia has been demonstrated for ether, halothane, barbiturates, and nitrous oxide. Even though much work remains to be done in this field, pressure reversibility of anesthesia is an important aspect in the consideration of anesthetic biophysical models.

Lipid Theory

The first and certainly the most successful biophysical theory of anesthetic mechanisms arose from the observations of Meyer (1899) and Overton (1901). This theory, the lipid solubility theory of anesthetics, was best stated in Meyer's 1937 paper: "Narcosis commences when any chemically indifferent substance has attained a certain molar concentration in the lipids of the cell. This concentration depends on the nature of the animal or cell, but is independent of the narcotic."

In 1954, Mullins reformulated the lipid theory of anesthesia resulting in the critical volume theory which, even today, acts as a basis for lipid theories of anesthesia. The critical volume hypothesis is based on a simple model of solvents. It accounts accurately for both the relative potencies of gaseous anesthetics and for pressure reversal of anesthesia. Simply stated the theory proposes that anesthesia commences when a certain critical volume fraction of an inert substance is attained in membranes. The critical volume of an anesthetic entering the membrane can be thought of as that volume which fills up the free volume of the membrane. The resulting compression caused by a volume increase in the membrane lipid bilayer influences the membrane proteins essential to

neural function. Mullins believed the resultant protein modification would effect sodium or potassium channels, neurotransmitter receptor conformation, or enzyme systems that affect neurotransmitters. Highly specific supportive evidence to affirm this theory comes from the theory's capability to predict both the excitation of the nervous system at high pressure (high pressure neurological syndrome; Miller, 1974) and the pressure reversibility of anesthesia. Three subsequent interpretations of the critical volume theory have recently surfaced: (a) the fluidized lipid hypothesis, (b) the phase transition hypothesis and (c) the lateral phase separation hypothesis.

(a) Fluidized Lipid Hypothesis:

The original critical volume hypothesis proposed by Mullins suggested that the anesthetic filled up the "free volume" in the membrane in such a way as not to allow for protein accommodation necessary for nerve function. Work by Hubbell et al. (1970), and Seeman (1975) has not supported this "free volume" concept. Instead, the critical volume of anesthetic entering the membrane was thought to expand the membrane. The resulting disorder caused by the volume increase in the membrane lipid bilayer would result

in decreased membrane viscosity. Although this theory modification does not address itself directly to the problem of how a fluidized membrane might interfere with nerve performance, and therefore anesthesia, it does correct the critical volume theory in the light of the new data. Gage and Hamill (1976) attempted to link the fluidized membrane with its physiological performance. He suggested a decrease in membrane viscosity as affecting the function of the membrane ionophores and thus membrane polarization.

(b) Phase Transition Hypothesis:

The phase transition hypothesis of Lee (1976) has an advantage over the fluidized lipid hypothesis in that it provides a direct mechanism by which the anesthetic agents effect nerve function. He postulates an annulus lipid structure in the gel state gating the sodium channel. The excitable protein is "controlled" by this lipid annulus and thus this lipid hypothesis has a direct link between lipid and protein mechanisms. Specifically, this theory states that "an anesthetic agent 'melts' (phase transition) the lipid annulus stabilizing the channel in its closed state."

(c) Lateral Phase Separation:

The last of the three modifications dealing with

the critical volume theory was originally proposed by Shimshick and McConnel (1973), however Trudell (1977) later incorporated Shimshick's theory into a unitary theory of anesthesia based on lateral phase separation. As defined by Shimshick, "lateral phase separation occurs in phospholipid bilayer membranes when highly ordered gel phase phospholipids and disordered fluid-phase phospholipids coexist." The borders between solid and fluid-phase in a phospholipid bilayer are called lateral phase separations. Accordingly, the phospholipid bilayer is able to accommodate volume change by transforming high volume disordered chains to lower-volume ordered chains (Shimshick and McConnell, 1973). This is termed lateral compressibility. As stated previously, proteins have been shown to change conformation during normal function (Low and Somero, 1975) and during anesthesia (Seeman, 1972). This evidence suggests that lateral compressibility is necessary for some cellular processes. Steer and Levitzki (1973) and Bretscher and Raff (1975) have in fact described phase transition in lipid surrounding enzyme systems and membrane proteins in mammals. The lateral phase theory goes on to suggest that application of an anesthetic agent to the membrane bilayer results in a disordered configuration of fatty-acid chains near the protein with no lateral

compressibility. Once disordered by the anesthetic agent the protein would require a large amount of energy in order to expand or change configuration. In effect, the lipid is not able to assume its lower volume solid phase necessary for protein rearrangement. When a sufficient number of nerve membrane protein molecules are functionally blocked the cell or organism is then anesthetized.

Various models dealing with protein conformation changes necessary for nerve function are discussed in the section on protein theories of anesthesia. It is important to note at this time that each of the protein theories to be discussed in that section could be adequately explained in terms of lateral compressibility. One theory unrelated to a protein mechanism was proposed by Taupin and McConnel (1975) and later by Van der Bosch and McConnel (1975) suggesting that lateral phase separation blocks exocitosis without protein involvement.

Protein Related Biophysical Anesthetic Mechanism Theories

The rationale of an anesthetic-protein interaction in the past has been questioned. This was due to the well known fact that anesthetics are highly lipid soluble (hydrophilic) while protein molecules are generally polar (hydrophobic) making protein an unlikely locus for anesthetic action. With current knowledge of protein structure many amino acid residues have been considered to be hydrophobic when in peptide side chains. These amino acids are glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, and methionine. If anesthetic agents do work directly upon protein it would be these lipid soluble amino acids of the side chains that interact with the anesthetic agents.

Further support for a direct anesthetic-protein interaction came from Balasubramanian and Wetlaufer (1966). They confirmed that gaseous anesthetics interact with protein and changed the conformation of the protein side chains without affecting the basic α-helical configuration of the peptide chains. Schoenborn (1968) and Schoenborn et al. (1965) have further delineated these sites. For historical continuity, each theory will be discussed.

a) Surface Tension Theory:

The first of the protein theories began possibly

with Traube (1904) and Lillie (1909). They observed that anesthetic potency could be related to the ability to lower surface tension. To elucidate the mechanism Clements and Wilson (1962) reformulated earlier theories of Lillie (1909) and Warburg (1921) describing the interaction of general anesthetics with surface films of protein. An alteration of the surface protein was proposed to cause a change in neural transmission and thus cause anesthesia. The surface tension theory is, historically, the origin of the current day protein conformational change theory to be discussed later.

b) Colloid Theories:

A second protein theory had its origin in the late eighteenth century. Proposed by Claude Bernard in 1875, and formalized by Bancroft and Richter in 1931, the colloid theory suggested reversible aggregation of all colloids causing anesthesia. Reversible cessation of protoplasmic streaming has been demonstrated in slime mold following the addition of chloroform, cyclopropane and ethylchloride (Seifritz, 1950). Heilbrunn (1952) observed that viscosity of the cytoplasm of slime molds and algae was decreased by low anesthetic concentrations and increased by high concentrations. Bruce and Christiansen

(1966) showed that diethyl ether and halothane inhibited protoplasmic streaming in the amoeba.

The colloid theory culminated in the current day microtubule theory. Proposed in 1968 by Allison and Nunn, microtubule inhibition was suggested as the underlying mechanism by which the anesthetic agent produced viscosity changes and anesthesia. Subsequently, Allison et al. (1970) demonstrated that five inhalation anesthetics produce reversible dispersion of microtubules in heliozoa at concentrations of 2 to 4 MAC (minimum alveolar concentration) and nitrous oxide at concentrations below 1 MAC.

The relevance of these findings to the understanding of anesthesia is questionable. Other investigators have failed to show an effect of pentobarbital and halothane on microtubules in mouse optic nerve (Sauberman and Gallagher, 1973). It was also demonstrated that squid axons continued to conduct action potentials following removal of 95% of the axoplasm which suggests that microtubules are not necessary for axonal conduction. Colchicine, podophyllotoxin, vinblastine, and griseofulvin each have the ability to disrupt the microtubule system. Katz (1972) found that colchicine and podophyllotoxin decrease transmitter release from frog neuromuscular junction, however, as these drugs were shown to act on nerve terminal membranes, he concluded that the effects on

transmitter release were independent of microtubular effects. Further, colchicine and vinblastine inhibited acetycholine-induced catecholamine release from the perfused bovine adrenal gland and blocked transmission through the superior cervical ganglia of the cat (Trifaro et al., 1972), actions which were attributed to the anti-cholinergic effects of these drugs and not to microtubular actions. Further problems shrouding this theory include its inability to account for pressure reversibility of anesthesia (Salmon, 1975) and the ability of anesthetics to release calcium with only incidental effects on microtubules. Supporters will, however, cite variability of microtubules within individual cells (Behnke and Forer, 1967) and between cell types and species as the cause of the discrepancies (Olmsted et al., 1971). As a result of the conflicting data and a general lack of investigative support, the microtubule theory of anesthetic action has not been well accepted.

c) Protein Conformational Change Theory:

The most favorable protein related anesthetic mechanism theory revolves around protein conformational changes as a result of anesthesia. This theory stems from both the surface tension theory (previously discussed) and from a bacterial enzyme model.

Photobacterium phosphoreum possess a light producing system which is sensitive to anesthesia. This light production results from the interaction of the enzyme luciferase with reduced flavin mononucleotide and oxygen. The light emission is proportional to the reaction velocity (Johnson and Eyring, 1948). It has been shown that a wide variety of anesthetic agents, including halothane, chloroform, cyclopropane, and ether at equipotent clinical anesthetic doses produces an inhibition of luminescence of this type which is reversible by application of hyperbaric pressure (Johnson et al., 1942; White and Dundas, 1970; Halsey and Smith, 1970). The anesthetic produced inhibition of luminescence is believed to be due to a conformational change of the luciferase enzyme.

Seeman (1972) and Low and Somero (1975), who have demonstrated membrane expansion associated with anesthesia, found that the increase in membrane volume was about 10 times the occupying volume of the anesthetic in the membrane protein suggesting conformational changes in the membrane protein. Eyring (1973) suggested this conformational change resulted from membrane unfolding such that the membrane occupied a larger volume. In this connection, Eyring (1973) has described the effect of anesthetics on protein conformational change in terms of a thermodynamic change. Hill (1974) proposed a thermodynamic description

of general anesthesia in terms of the "Gibbs" free energy of the anesthetic site. The advantage of Hill's theory is that the outcome of the interaction can be predicted without knowledge of the mechanistic details of that process.

Thermodynamic analysis of firefly luminescence has been applied by Ueda and Kamaya (1973) to the anesthetic action of methoxyflurane, chloroform, halothane, enflurane and fluroxene suggesting their site of action at a hydrophobic site on luciferase inducing a structural membrane "unfolding."

There are many ways by which protein conformational change may affect neural function. Protein conformational change or "unfolding" may (a) prevent the association of protein into ion channels, (b) depress the transmitter release by preventing vesicular and presynaptic membrane fusion, (c) inhibit rate limiting enzymes, or (d) affect the postsynaptic membrane receptor. Any or all of these effects may be involved.

Although not as well supported as the lipoid anesthetic model, the protein-based anesthetic interaction model is certainly viable. It is also clear that no single theory will explain the effects of all anesthetic agents. A hydrophobic protein site of action can explain many of the physiochemical relationships cited.

An important common denominator between all theories developed that must be stressed is that, at best, all of these theories are descriptive in nature. They describe the effect of anesthetics rather than a mechanism by which these effects are produced.

Other Proposed Biophysical Mechanisms of Anesthetic Action

Two seemingly important models for anesthetic action were proposed independently by Miller (1961) and Pauling (1961) and were popularized in the past decade. These theories fit neither the protein nor the lipid theories of anesthesia. Pauling and Miller each suggested that the site of action of anesthetics lies in the aqueous water phase of the nerve cell. Both investigators established a relationship between anesthetic potency and highly ordered water molecules orientated around aqueous solutions of gases (i.e., hydrated crystals). Pauling (1961) suggested the formation of mixed hydrates of solutes in brain fluid and charged side-chains of protein which are capable of forming stable hydrate substructures. These hydrated microcrystals, or "clathrates," once formed, were to result in increased neural impedance, ionophore blockage, or altered enzymatic activity. Both theories have died from lack of evidence that clathrates form at normal body temperatures.

Macromolecular Site of Anesthetic Action

The theories of anesthesia developed thus far have proposed or alluded to a specific biomolecular locus of anesthetic action. However, it becomes apparent from a review of the literature that sufficient evidence exists to imply that anesthetics have their primary neuronal action in the synaptic region at the macromolecular level (Alper and Flacke, 1969; Brooks and Eccles, 1947; de Jong et al., 1968a; de Jong and Nace, 1967; De Robertis, 1971; Halsey and Smith, 1970; Ho and Keats, 1969; Karis et al., 1966, 1967; Larrabee and Posternak, 1952; Paton and Speden, 1965; Richards, 1972, 1973). Investigations into anesthetic effects on synaptic elements have raised several important questions: Is the anesthetic action on the synapse due to a direct lipid and/or protein interaction as suggested in the previously discussed theories? Do anesthetics affect pre- or postsynaptic elements or both?

It is widely believed that for a general anesthetic to have effect on a synapse it must directly or indirectly cause the depression of nerve impulse conduction, depress presynaptic transmitter release, result in postsynaptic membrane conductance changes, or induce a combination of these effects ultimately leading to a depression of certain synapses in the central nervous

system. Balasubramanian and Wetlaufer (1966), Ho and Keats (1969), Musick and Hubbard (1972) and others consider these basic nerve functions to be performed by membrane lipoproteins. When considering the argument between lipid or protein anesthetic interaction, Woodbury et al. (1976) wittily suggested "the parallelism between the effects of anesthetics on lipids and proteins suggest that research in these two fields are feeling different parts of the same elephant." As to which mechanism is actually responsible for the anesthetic induced reduction in synaptic transmission, it is too early to postulate.

There are many reputed central neurotransmitter systems (i.e., cholinergic, adrenergic, gabaminergic, and serotonergic). Whether anesthetic agents affect all transmitter system synapses uniformly or only select transmitter systems in a discrete fashion has not been determined.

This dissertation examines the effects of certain anesthetic agents on the central adrenergic nervous system. A review of the known central adrenergic pathways follows.

Adrenergic Neuropathways

Dopamine (DA) and norepinephrine (NE), each a catecholamine, are considered to be putative neurotransmitters in the mammalian central nervous system. Norepinephrine was first detected in the mammalian brain by von Euler (1946). Vogt (1954) later found that the concentration of NE in brain was not evenly distributed and did not parallel brain vasculature. Von Euler further demonstrated a close correlation between the content of NE present and the proportion of nonmyelinated to myelinated nerve fibers. Further, hypothalamic NE concentration was found to be unaltered by cervical sympathectomy. These early findings led investigators to propose a role for NE as a central neurotransmitter.

Dopamine, initially believed to be present in mammalian brain solely as a precursor of NE (Carlsson, 1966), was found to be present in whole brain in concentrations higher than NE (Montagu, 1957; Weil-Malherbe and Bone, 1957). Later DA studies demonstrated a distribution markedly different from that of NE (Cooper et al., 1974).

Many studies have been subsequently performed to establish the role of both NE and DA as central adrenergic neurotransmitters. Specific areas of catecholamine research establishing their putative roles in the central

nervous system have been extensively reviewed in the literature. (For reviews of specific areas cf.: Biochemistry, Molinoff and Axelrod (1971); Function, Hornykiewicz (1966), and Marley and Stephenson (1972); Metabolism, Axelrod (1966 and 1971), Glowinski and Baldessarini (1966), and Kopin (1972); Pharmacology, Salmoiraghi et al. (1965), and Sulser and Sanders-Bush (1971); Receptors, Curtis and Crawford (1969), and Triggle (1972); Release, Glowinski (1970), and Smith et al. (1977); Synthesis, Kopin (1968), Axelrod (1971), and Costa and Meed (1974); Transport and Storage, Glowinski (1970), and Shore (1972); Uptake, Iversen (1970 and 1971)).

Instrumental in outlining the central anatomical pathways of the catecholaminergic neurons have been fluorescence methodologies. First developed by a Swedish research group headed by Eranko (1955) and later improved by Falck and Hillarp (Falck et al., 1962; Falck, 1962) catecholamine neurons were shown to fluoresce when exposed to vaporized formalin. Lindvall and Bjorklund (1974) and Lindvall et al. (1974) have recently introduced the use of glyoxylic acid instead of formaldehyde to produce monamine fluorescence of greater intensity. Since the amount of fluorescence produced is proportional to the amount of catecholamine present, several techniques are usually

employed to alter endogenous catecholamine levels and therefore change fluorescence visibility. This includes drugs such as reserpine and monoamine oxidase inhibitors, lesioning to increase proximal axonal amine content (Anden et al., 1974; Ungerstedt, 1971), and cytotoxic compounds for system delineation (Snyder et al., 1970a; Ungerstedt, 1968; Baumgarten et al., 1971).

Recent developments have led to the introduction of a highly sensitive and selective immunocytochemical peroxidase-antiperoxidase system to identify and localize enzymes necessary for catecholamine production. A comparison of data from existing techniques leads to the delineation of distinct dopaminergic and noradrenergic pathways (cf. below).

Dopaminergic Pathways:

The majority of dopaminergic cell bodies lie in mesencephalon and give rise to an estimated 500,000 terminals (Cooper et al., 1974). Three primary dopaminergic pathways have been proposed. Each of the three pathways has been established with a large variety of techniques, including fluorescence histochemistry, biochemistry, stains of terminal degeneration, and electron microscopy (for a more extensive review of the literature on techniques specific for DA cf. Moore and Dominic,

1971; Carpenter and Peter, 1972).

a) The Nigrostriatal System:

The first dopaminergic pathway to be discussed is the nigrostriatal system (i.e., cell groups A8 and A9, of Dahlstrom and Fuxe, 1964). The substantia nigra is the largest nuclear mass in the mesencephalon (Carpenter and Peter, 1972). The cell bodies lie primarily in the zona compacta of the substantia nigra and project through the medial forebrain bundle to the striate and amygdaloid nuclei. Specific fibers in the caudal two-thirds of the nigra project to portions of the putamen, while rostral parts of the nigra project to the caudate nucleus.

It has been suggested that this nigro-neostriatal dopaminergic system is related to the extrapyramidal motor system. As to whether this is its sole action is yet to be determined. Neurophysiologic data in support of this pathway has shown that iontophoretically applied DA to neurons of the cat caudate nucleus causes depression of spontaneous neuronal firing (Connor, 1972). An identical response was elicited by electrical stimulation of the substantia nigra. Both nigral stimulation and iontophoretic DA application were shown to be blocked by α-methyldopamine. It has been suggested that DA has an inhibitory action at the caudate nucleus when released from cells originating in the substantia nigra. In contrast, York

found iontophoretically applied DA caused a facilitation of neuronal firing in the cat putamen. Electrical stimulation of the substantia nigra elicited a similar facilitation thus both facilitatory and inhibitory actions of DA have been demonstrated (York, 1970).

b) The Mesolimbic System:

The second of the three DA pathways to be discussed is the mesolimbic dopaminergic pathway. The cell bodies for these nerves arise in the medial mesencephalon just dorsal to the interpeduncular nucleus (cell group A10, Dahlstrom and Fuxe, 1964); these fibers ascend medially to the crus cerebri into the medial forebrain bundle innervating the tuberculum offactorium and the nucleus accumbens. These nerve bundles run lateral to noradrenergic nerve tracts innervating the neostriatum. The function of the mesolimbic dopamine system is at present unknown.

c) The Tuberoinfundibular System:

The cell bodies of the third known dopaminergic system lie primarily in the arcuate nucleus of the hypothalamus (Dahlstrom and Fuxe, 1964, cell groups #A11-12). They innervate the external layer of the medial eminence. The dopaminergic neurons in this last system behave differently from those found in the nigrostriatal

or mesolimbic system. The tuberoinfundibular system is insensitive to the normal destructive action of 6-hydroxydopamine and when activated for any length of time becomes markedly depleted of transmitter stores. It has been suggested that the neurons in the system behave in a manner similar to that of neurosecretory neurons.

Noradrenergic Pathways:

Noradrenergic cells give rise to large collateral networks of fibers innervating many brain areas. As an example of this system's diffuse nature it has been shown that in the case of the locus coeruleus one noradrenergic cell body sends tracts to both the cerebral cortex and the cerebellum. The central noradrenergic system has both ascending and descending tracts, most of which originate in the pons and medulla oblongata. Noradrenergic fibers have been shown to innervate the thalamus, the preoptic area, the amygdaloid nucleus, the hippocampus, cingulate gyrus, the pyriform cortex, the striatal terminalis, the septal areas, the neocortex, and several hypothalamic nuclei. The ascending noradrenergic system has its cells of origin in the medulla and pons, Dahlstrom and Fuxe (1964), areas A-1, A-2, A-5, A-6, and A7.

Due to the diffuse projections of the noradrenergic systems it is easier to construct a general picture of this

system by considering the four major noradrenergic conduction pathways: (a) the central tegmental tract, (b) dorsal tegmental bundle, (c) the periventricular fiber system, and (d) the medial forebrain bundle. A brief description will follow for each. A more complete description is included in the works of Dahlstrom and Fuxe (1964), Ungerstedt (1971), and Lindvall and Bjorklund (1974).

(a) The central tegmental tract (CTT)

The central tegmental tract is usually considered to be a highly heterogeneous system of longitudinally running fibers in the reticular formation. The ascending system is comprised of cells originating in three locations: (1) spinal cord, (2) the medullary reticular formation (groups A1 and A2), and (3) the pons (from cell group A5 situated lateral to the superior olivary nucleus, A7, the subcoeruleus, and A6, the locus coeruleus). Lindvall and Bjorklund (1974) also reported vertical T-shaped noradrenergic fibers in this pathway interconnecting the CTT with the periventricular grey. Chu and Bloom (1974) and Lindvall and Bjorklund (1974) have suggested that these vertically arranged fiber systems represent collateral branches from the CTT to give rise to an extensive terminal system distributed in the locus coeruleus, dorsal raphe nucleus, and the periventricular grey system.

(b) Dorsal tegmental bundle (DTB)

The dorsal tegmental bundle is an ascending homogeneous group of noradrenergic fibers arising from the locus coeruleus (A6). Soon after the DTB emerges from the locus coeruleus, the crossing fibers deviate medially from the DTB. Along its course the tract ascends lateral to the medial longitudinal fasciculus. While still in the mesencephalon, the DTB gives off fibers just rostral to the decussation of the superior cerebellar peduncles (i.e., at the level of the inferior colliculus) to the tegmental radiation. At the meso-diencephalon junction the DTB runs ventrolateral to the periventricular grey of the third ventricle. Dopaminergic A11 cells of the caudal thalamus have been observed to project axons into the DTB. The DTB then courses through the hypothalamus giving rise to several branches that innervate the thalamus (Lindvall et al., 1974). Past the hypothalamus the DTB merges with the medial forebrain bundle. Shortly before the DTB joins the medial forebrain bundle, it gives rise to fibers that run with the nigrostriatal DA system.

(c) The periventricular system:

The periventricular system is composed of two subsystems, the dorsal periventricular system (DPS) and

the ventral periventricular system (VPS). The DPS extends from the periventricular and periaqueductal grey of the medulla oblongata through the pons and mesencephalon to the rostral portions of the diencephalon and septum. Some of the cells of origin are located in the locus coeruleus and are quite different from other catecholamine systems. Many of the cell bodies are distributed diffusely from the pons through the mesencephalon to the posterior thalamus. In addition, fibers from the cell groups A2, A4, and area postrema may be present (Dahlstrom and Fuxe, 1964). This system has been shown to project to the medial thalamic, epithalamic, pretectal, and hypothalamic regions. Projections to the tegmental radiations, medial forebrain bundle, and dorsal raphe nucleus have also been demonstrated. Lindvall and Bjorklund (1974) have suggested that this system is composed of relatively short fiber systems that originate at different levels of the DPS and run for some distance along the bundle to terminate either locally in the central grey or at higher levels. This system, they suggest, can be regarded as an adrenergic component of the dorsal longitudinal fasciculus. (The dorsal longitudinal fasciculus carries fibers from the dorsal tegmental nucleus. Their function seems to be a rhinencephalic relay to brain stem nuclei.)

The second periventricular system, the VPS, is

less well defined. It is formed at the meso-diencephalic junction from converging tegmental radiation fibers as is the medial forebrain bundle. It is believed to run from the interpeduncular nucleus rostrally to the mamillothalamic tract. At this location, fibers from the median forebrain bundle join the VPS. The VPS then moves dorsal-rostrally through the posterior hypothalamic area where fiber from the DPS join to form a broad ascending hypothalamic catecholamine system which seems to serve as a connection between the dorsomedial and paraventricular hypothalamic nuclei.

(d) The medial forebrain bundle (MFB)

The MFB is a heterogeneous system containing both NE and DA pathways. Caudally, the MFB is formed at the meso-diencephalic junction in the area medial to the substantia nigra from fibers in the tegmental radiations (as previously stated these include fibers from the DTB, CTT, and the DPS). The converging fibers give rise to the ascending MFB system. Fibers from A10, the mesolimbic DA system, and the A8 and A9 cell groups, and substantia nigral system, are also present. Lindvall et al. (1974) suggested that the substantia nigra DA projection through the MFB courses to the limbic cortex. At the rostral level of the hypothalamus the DTB joins the MFB. Reflecting their diverse origins the NE fibers of the MFB have

widely varying terminations to most brain regions. Those fiber tracts are discrete when entering the MFB, remain together in this fiber bundle. For additional information on the numerous MFB projections see Lindvall and Bjorklund (1974).

Anesthetic Agents:

Ketamine

Ketamine, a phencyclidine derivative, is a short-acting intravenous or intramuscular anesthetic. Ketamine is unusual in the type of anesthesia elicited, and unique in that it is the only clinically available drug in its class. This agent is considered to be a "disassociative anesthetic" and is termed such because it produces a state of central detachment from the environment.

Ketamine is generally a rapid acting anesthetic producing a state characterized by profound analgesia, catatonia, normal pharyngeal-laryngeal reflexes, normal or slightly enhanced skeletal muscle tone, cardio-vascular and respiratory stimulation and occasionally a transient, minimal, respiratory depression. Autonomic and reflex functions are largely unaffected. Airway preservation, by maintenance of normal respiratory reflexes, and respiratory stimulation permits short procedures to be done with relative ease without the need for mechanical ventilation (Doubrava and Larson, 1971).

The action of ketamine on the cardiovascular system is mediated by three proposed mechanisms; first, there is a general stimulation of the central vasomotor center; second, a peripheral release of norepinephrine, and third, there is evidence that baroreceptor activity

is decreased, resulting in the increased blood pressure and heart rate.

The dose of ketamine needed to produce surgical anesthesia is related to the developmental stage of the central nervous system. This is not only true phylogenetically, but is also true in man where a larger dose is needed in young children than in adults (Doubrava and Larson, 1971). In view of the disassociative state produced by this anesthetic and its phylogenetic dose relationship, it has been presumed that the sensory isolation must occur in the association areas of the brain (Doubrava and Larson, 1971).

Toxicity of ketamine develops in rats at a dose 100 X the anesthetic dose, and in humans at approximately 20 X the anesthetic dose. Toxic doses of ketamine are capable of uncoupling the excitation contraction process of the cardiac muscle and triggering CNS convulsions which can result in death.

Ketamine is rapidly absorbed and distributed into most body tissues with relatively higher concentrations in body fat, liver, lung and brain. It is known not to be metabolized in the brain (Cohen and Trevor, 1974) and believed mainly to be N-demethylated and hydroxylated in the liver to form water soluble products. Ninety-one percent of an intravenous ketamine

dose can be recovered in the urine in the form of metabolites. These metabolites are less than 1/6th as potent as ketamine itself and are believed not to contribute to the anesthetic effects (Doubrava and Larson, 1971). Repetitive ketamine injections do not produce visible tachyphylaxis. A 5-fold increase in ketamine metabolism caused by enzyme induction with phenobarbital and a 35% prolongation of ketamine's half life caused by microsomal enzyme inhibition with SKF-525-A have been shown not to affect the duration of anesthesia (Cohen and Trevor, 1974). Termination of anesthetic action is considered to be due to redistribution, as is the case for the short-acting thiobarbiturates.

The use of ketamine for anesthesia is accompanied by a risk of CNS excitation in the post anesthetic period. This CNS excitation is termed "emergence excitatory phenomena" and includes restlessness, agitation, increased motor activity, crying, screaming, and visual hallucinations (Domino et al., 1965; Becsey et al., 1972). These side effects have added great impetus to study the effects of ketamine on central neurotransmitter systems. Specifically the catalepsy and hallucinogenic effects of this drug have stirred a fortuitous interest in these central neuronal systems, i.e., the cholinergic, adrenergic, and serotonergic. Literature on catalepsies

and movement disorders continually suggests that these defects may be related to a cholinergic-dopaminergic imbalance (Arvidsson et al., 1966; Munkvad et al., 1968; Corrodi et al., 1972). Serotonin and tryptamine have also been considered to play a role in catalepsy as demonstrated by intracerebral injections (Michaux, 1962; Ernst, 1969). Hallucinations and central excitation alone and associated with psychotropic drugs have been tentatively linked with the noradrenergic and serotonergic systems (Sulser and Sanders-Bush, 1971; Weiss and Laties, 1969; Schildkraut and Kety, 1967; Snyder et al., 1970b; Sung et al., 1973). With regard to the adrenergic system, several ketamine effects have tentatively been identified.

It has been generally accepted that the adrenergic neuronal reuptake system, a high affinity neuronal transport system for NE, functions as the primary means of terminating the biologically active neurogenically released transmitter at peripheral and central synapses (see Iversen, 1971). This type of high affinity system is not unique to NE containing neurons. Similar systems have been described in brain for neurons containing DA (Snyder and Coyle, 1969), gamma-aminobutyric acid (GABA) (Iversen and Neal, 1968), and serotonin (5-HT) (Shaskam and Snyder, 1970). Several studies have assessed ketamine's ability to block these specific reuptake

systems and therefore potentiate transmitter action. Smith et al. (1975) demonstrated *in vitro* in rat cerebral cortex tissue that ketamine competitively inhibited the high affinity transport system for the uptake of NE into adrenergic nerve terminals. Ketamine, *in vitro*, also was found to cause release of stored NE. However, ketamine's potency in this respect was estimated at 1/15 the potency of desmethylimipramine, suggesting minor physiologic significance to this action. This same group more recently assessed ketamine's *in vitro* ability to block uptake of DA, GABA and 5-HT and compared ketamine's relative potency on each system. Smith et al. found ketamine to be most effective in blocking 5-HT reuptake and 1/4 as effective in blocking NE and DA reuptake. GABA was least affected (Azzaro and Smith, 1976).

Phencyclidine, the parent drug of ketamine, failed to gain clinical usage due to a very high incidence of the post anesthetic emergence phenomena. It is of interest to note that ketamine, which is considerably less potent than phencyclidine in its behavioral effects, is also about 100 times less potent than phencyclidine in inhibiting synaptosomal NE uptake (Smith et al., 1975). It has now also been demonstrated that phencyclidine inhibits DA and 5-HT synaptosomal uptake in approximately

the same proportion (Smith et al., 1975). Even though ketamine has been demonstrated to competitively block reuptake of these amines, ketamine is not actively accumulated in rat brain slices (Cohen and Trevor, 1974).

Ketamine's effect on central DA metabolism was first examined on whole brain samples. These early studies report no change in endogenous DA levels following ketamine. More recent studies, realizing that endogenous levels may be maintained with marked changes in amine metabolism (turnover), have also measured DA's major metabolite, homovanillic acid (HVA). A rise in this metabolite is believed to indicate an increase in central DA activity; a fall in this metabolite is taken to mean a decrease in central DA activity (Cooper et al., 1974). Unfortunately most of the data was derived from whole brain studies which limits their value. Biggio et al. (1974) found ketamine does not influence whole brain DA levels, however, he reported finding a significant increase in HVA at 60 minutes post ketamine administration and suggested the possibility of an increase in DA turnover. Sung et al. (1973), using enzyme inhibition to estimate DA turnover, found that 40mg/kg of ketamine resulted in a decreased rate of turnover at two hour post ketamine administration followed by increased turnover four and six hours post administration. The turnover rate

at six hours post ketamine administration was elevated 200%. Although the duration of anesthesia was not reported, it might be assumed from the given dose and animal species that this large increase in turnover occurred approximately five hours post anesthesia. The relevance of this increase in turnover to anesthesia should be questioned. In 1976, Glisson et al. reported endogenous NE and DA levels in rat whole brain and select brain regions of rabbit. Again, it was demonstrated that no effect of ketamine could be seen in whole brain on endogenous amine levels; however, in select areas ketamine caused a significant rise in DA levels at 10 and 30 minutes post ketamine administration. Ylitalo et al. (1976) measured both DA and HVA in rats post ketamine and reported significant increases in striatal HVA 15 minutes and 1 hour after ketamine administration. These values returned to normal 3 hours post drug administration.

The effects of ketamine on norepinephrine levels are less consistent than for dopamine. Norepinephrine whole brain levels have been reported to increase (Ylitalo et al., 1976), decrease (Sung et al., 1973), and remain unchanged (Biggio et al., 1974) during ketamine anesthesia. All experiments were carried out on rats; Ylitalo and Biggio used a 100mg/kg dose while Sung used a 40mg/kg dose; in all of these experiments amine levels were examined at

approximately the same time periods.

Investigators have used drugs known to have effects on specific central transmitter systems in an attempt to delineate relationships between these transmitter systems and ketamine. Conflicting results have been found with cholinergic and anticholinergic agents. Authier et al. (1972) used the anticholinergic agent procyclidine in rabbits and found enhanced ketamine-induced catalepsy. Hatch (1974) found that atropine, another anticholinergic agent, prevented ketamine catalepsy in dogs, however, mecamylamine, a ganglionic blocker, was shown to enhance ketamine catalepsy. In the case of the serotonergic system, Monachon et al. (1972) found in cats that methiothepin, an antiserotonergic compound, prevented ketamine catalepsy and prolonged ketamine anesthesia. With respect to the adrenergic system, pimozide, an antidopaminergic compound, was shown by Hatch and Ruch (1974) to enhance ketamine catalepsy, and more importantly, antagonize ketamine anesthesia.

Other investigations have attempted to localize ketamine's effect on brain by characterizing and localizing gross changes in electroencephalographic (EEG) activity. Ketamine, unlike many other anesthetics, is epileptogenic in nature. The increase in EEG activity resulting from ketamine's epileptic nature make this form

of study more logical than for EEG depressing anesthetics. Corssen et al. (1968) first suggested ketamine to be a dissociative anesthetic based on his findings that ketamine electrographically dissociated the neocortex from the limbic system, and the thalamocortical system from the midbrain reticular system. Also of importance is that this group suggested that ketamine affected two areas of the brain differentially, the cerebral cortex and the limbic system.

Of the EEG effects induced by ketamine, theta activity has been characterized by both Domino et al. (1965) and Massopust et al. (1972). The synchronous theta activity seen in both the thalamus and the neocortex has led Domino et al. and others to speculate that ketamine might act on the diffuse thalamic projection system at either the thalamic or cortical level. Massopust, on the other hand, has suggested a temporal relationship between theta activity and analgesia from data he obtained in rhesus monkey. This group further suggests that the beginning of theta activity and analgesia correlates well with anesthetics other than ketamine which also are potent analgesics (i.e., nitrous oxide and cyclopropane).

Halothane

The introduction of halothane into clinical practice has met with unprecedented success. In an attempt to find a non-inflammable volatile anesthetic agent Suckling (1957) examined a number of highly stable fluorinated hydrocarbons which led to the introduction of halothane.

Halothane is a heavy colorless liquid at room temperature. It has a gram molecular weight of 197.39 and a boiling point of 50.2^{o}C (at one atmospheric pressure). With a vapor pressure of 241 mmHg at 20^{o}C, halothane is extremely suitable for vaporization.

Halothane is a potent nonirritating anesthetic characterized by a low post anesthetic incidence of complications. Halothane blood solubility coefficient is 2.3 (blood:gas, partition ratio) resulting in rapid induction of anesthesia, as compared to ether. Halothane does have a moderate affinity for fat, however, considering the relatively low blood flow through adipose tissue, this affinity becomes important only after prolonged periods of anesthesia.

Halothane has been shown to have profound effects on circulation which can lead to moderate or marked arterial hypotension. Price (1960) observed decreased contractile force, stroke volume and cardiac output with

halothane-oxygen anesthesia. This myocardial depression is directly related to the dose of halothane (Mahaffey et al., 1961; Wenthe et al., 1962). Depression of the S-A node has been demonstrated by Flacke and Alper (1962). Like chloroform, halothane sensitizes the ventricular conducting tissues to the actions of catecholamines increasing the arrhythmic potential. Vascular smooth muscle is also directly affected by halothane. Direct vasodilatation has been reported on vascular smooth muscle in skeletal muscle and skin (Black and McArdle, 1962), as well as splanchnic, hepatic and renal vasculature (Epstein et al., 1962). This vasodilatation is accompanied by a diminished blood flow as a result of lower systemic pressure.

It has been suggested by Black and McArdle (1962) that halothane blocks the effects of noradrenaline at its effector site. In this way halothane interferes with the ability of the efferent sympathetic nervous system to antagonize a fall in systemic blood pressure.

Halothane has minimal neuromuscular blocking action and therefore at surgical levels must be supplemented by specific neuromuscular blocking agents when good muscle relaxation is needed. Slight potentiation of the non-depolarizing agents and slight antagonism of depolarizing agents has been reported (Katz and Gissen, 1967).

Shivering is common during post anesthesia recovery and is attributed to heat loss presumably from cutaneous vasodilation.

One obvious disadvantage of halothane's clinical use for surgery is that it is a relatively poor analgesic agent. For this reason, halothane is usually supplemented with nitrous oxide.

To a large extent halothane is eliminated unchanged through the lungs. It has been estimated that a minimum of 60% of administered halothane is eliminated unchanged within 30 hours. Up to 20% is metabolized by the liver microsomal enzyme system and excreted in the urine (Van Dyke and Chenoweth, 1965).

The toxicology of halothane stems from halothane's limited metabolism. Hepatic damage as a result of halothane anesthesia is believed to result from an accumulation of metabolites in the liver. As of yet, the toxic metabolite or metabolites have not been identified. Halothane has been shown to undergo both oxidation and dehalogenation to form trifluoroacetic acid and bromide and chloride radicals. Stenger and Johnson (1972) have pointed out that animals pretreated with phenobarbital, which caused induction of

microsomal enzymes and therefore increased the fraction of halothane metabolized, increased hepatic damage as well. More recently, it has been suggested by Van Dyke (1978) that in the presence of low oxygen tension the pathway of metabolism of halothane is altered resulting in the formation of toxic intermediates. This toxicity, however, is relevant to chronic rather than acute exposure.

The relationship of central catecholamine function to halothane anesthesia has been studied primarily from two directions: (a) halothane's effect on catecholamine metabolism; and (b) the effect of pharmacologically altered amine metabolism on halothane anesthesia.

(a) Halothane's effect on central amine metabolism:

Nikki and co-workers (1971) found halothane to increase rat whole brain DA levels and decrease 5-HT levels without altering brain NE levels after 30 minutes of anesthesia. Anden et al. (1974) found no effect of halothane or pentobarbitol anesthesia on rat whole brain DA metabolism or turnover. This group also investigated anesthetic effects on haloperidol-induced increase in DA turnover. In rat whole brain, these authors demonstrated that pentobarbital but not halothane resulted in a decrease in whole brain DA turnover. Ngai et al. (1969a) reported that halothane had no effect on NE or DA turnover.

However, he did report a decrease in the NE rate constant with a concomitant increase in endogenous content suggesting altered NE metabolism. Consistent with this finding, Li et al. (1964) showed an increase in NE content in the heart with halothane anesthesia. Further support comes from Persson and Waldeck (1971) who reported a decrease in whole brain NE turnover with halothane and Lidbrink et al. (1972) who demonstrated a similar effect in the neocortex.

Ngai et al. (1969b) also investigated halothane's effect on neuronal uptake; unlike ketamine, halothane did not significantly alter this process.

(b) Altered amine metabolism and MAC:

The minimum alveolar concentration of an anesthetic (MAC) necessary to prevent movement in response to a painful stimulus in 50% of the subjects tested, has proven to be the most readily measurable and accurate index of anesthetic potency for inhalation anesthetics (Markel and Eger, 1963; Eger et al., 1963). Alterations in potency of an inhalation anesthetics are readily measured in terms of altered MAC.

Sympathominergic drugs have been studied in relation to anesthesia for a multiplicity of reasons. Of interest to many has been the altered states of central nervous system excitation produced by amphetamines,

catecholamines, depleters (reserpine), and precursor therapy (L-DOPA) in relation to anesthesia.

The effect of both acute and chronic amphetamine administration has been evaluated on halothane MAC. Acute administration of amphetamine at doses of 0.1 to 1.0 mg/kg were shown to increase halothane MAC in dogs from 20% to 100% above controls in a dose related manner. In contrast, chronic doses of 5 mg/kg over a seven day period produced a 21% decrease in halothane MAC over control (Johnston et al., 1972). Johnston and colleagues (1974), in a further attempt to relate these changes to catecholamine function, used alpha-methyl-p-tyrosine (AMPT), reserpine, and parachlorophenylalanine (PCPA) in conjunction with amphetamine and determined halothane MAC. They found that pretreatment of dogs with either reserpine or AMPT greatly reduced the amphetamine precipitated increase in halothane MAC, while PCPA, a drug affecting serotonin, had no effect, suggesting that catecholamines and not serotonin were responsible for the amphetamine-induced increase in halothane MAC. Reserpine and AMPT, catecholamine depleters, were shown to decrease halothane MAC when used alone.

L-Dopa, a precursor for both central adrenergic transmitters, DA and NE, when administered results predominantly in an increase in DA rather than NE (Everett and

Brocherding, 1970; Liu et al., 1971) Johnston et al. (1975) found that both acute and chronic L-dopa in doses of 5 to 25 mg/kg reduced halothane MAC in a dose dependent fashion. In view of these results Battista et al. (1973) and Johnston (1975) suggested the decrease in halothane MAC to be a result of increased central inhibition as a consequence of increased DA central content. This group further suggests that NE is excitatory in nature centrally and therefore an increase in NE centrally results in an increased halothane MAC.

Nitrous Oxide

Nitrous oxide (N_2O), one of the first known anesthetic agents, was first prepared by Priestly in 1776. Not until 1868 did its use become fashionable in clinical practice when Andrews combined oxygen with nitrous oxide to lay the foundation for its present day use.

Commercially, nitrous oxide is produced by heating ammonium nitrate to a temperature above 245°C, which results in two products, N_2O and H_2O. Many of the early toxic effects attributed to N_2O were shown to be a result of ammonia and nitric acid impurities. As a result, commercially obtained N_2O is carefully processed to a high degree of purity and marketed in steel cylinders under pressure in a liquid state.

At room temperature and one atmospheric pressure, N_2O is a colorless, sweet smelling and nonirritating gas. Nitrous oxide is neither inflammable nor explosive but will support combustion of other agents, even in the absence of oxygen, due to its decomposition at temperatures over 450°C to oxygen and nitrogen.

The blood/gas solubility coefficient of nitrous oxide is 0.47. It is rapidly absorbed from the alveoli and does not combine chemically within the body and is therefore eliminated as rapidly as it is absorbed (Dripps et al., 1972).

Today more general anesthesia techniques are based

on the use of nitrous oxide than on that of any other single agent (Dripps et al., 1972). Although N_2O is a potent analgesic agent, it lacks potency as an anesthetic agent. Smith (1971) has estimated that a 101% concentration would be needed for 1 MAC (as compared to 0.74 for halothane), however, 20 volumes % is reported to be equivalent to 15 mg of morphine sulphate in analgesic effectiveness (Goodman and Gilman, 1975).

Nitrous oxide has gained wide clinical acceptance for current day anesthesia. This can be attributed to nitrous oxide's analgesic ability and more importantly its capacity to significantly reduce the amount of volatile inhalation anesthetic needed to achieve adequate levels of anesthesia. Munson et al. (1965) reported that a 72 percent alveolar concentration of the N_2O reduced the MAC of fluroxene anesthetic over 75% (i.e., from 3.4 to 0.8%). Saidman and Eger (1964), using a 65% concentration of nitrous oxide, found a 60% reduction in halothane MAC. This increases the clinical margin of safety and effectively decreases the toxic side effects of these potent volatile anesthetic agents.

As mentioned previously, toxic effects attributed to N_2O in the early literature have been found to have resulted from impurities. Current day manufacturing standards have resulted in nitrous oxide gaining the

reputation of the least toxic gas anesthetic available (Wytie and Churchill-Davidson, 1972). Nitrous oxide is believed not to be metabolized *in vivo*. It is eliminated through the lung (90%) as well as through the skin (10%). Various forms of anoxia have been reported in conjunction with nitrous oxide use. Two causes have been cited. First, when nitrous oxide is used alone in concentrations above 80%, required oxygen content of 20% cannot be provided. Second, diffusion hypoxia occurs when concentrations above 75% of nitrous oxide are used over long periods of time (over 90 minutes). During anesthesia recovery, rapid elimination of nitrous oxide through the lungs can result in decreased oxygen availability if inspired oxygen concentration is not kept above 25% (Fink, 1955; Frumin and Edelist, 1969).

The amount of research dedicated toward exploring the central mechanisms of nitrous oxide has been surprisingly small. This fact may be attributed to nitrous oxide's lack of potency.

Investigation into nitrous oxide's effect on monosynaptic and polysynaptic spinal reflex actively has shown each to be depressed approximately to the same degree (de Jong et al., 1967; de Jong et al., 1968b). Although research initially suggested the spinal cord as a possible site of analgesic action by nitrous oxide,

later studies by the same investigators in decerebrate cats with intact spinal cords and without the use of an anesthetic agent such as halothane, as was used in initial experimentation has shown only slight effects on spinal cord synaptic transmission (de Jong et al., 1970).

The cardiovascular system has been shown to be relatively unaffected by nitrous oxide. At high levels of nitrous oxide a decrease in ventricular contractile force in a sympathectomized preparation was demonstrated by Craythorne and Darby (1965). In the nonsympathectomized animal a reflex increase in sympathetic tone results with nitrous oxide; however, no effect was seen on blood pressure, cardiac output, or stroke work. Additional work by Lundborg et al. (1966) demonstrated an increase in peripheral vascular resistance with nitrous oxide suggesting that nitrous oxide exerts only reflex stimulation of the sympathetic nervous system. Recent publications indicate that nitrous oxide, in the presence of halothane, results in increased sympathetic activity mediated from suprapontine origins. In fact, when cats were sectioned midcollicularly, nitrous oxide had a depressant effect on peripheral sympathetic activity (Fukunaga and Epstein, 1973). It is of interest to note that this effect was not found when enflurane, another volatile halogenated anesthetic, was used (Smith et al., 1978).

The relationship of limbic neuronal firing to nitrous oxide-oxygen anesthesia has been examined by Babb et al. (1975), in seven patients implanted with fine wire microelectrodes. Unlike ketamine which has been shown to increase limbic neuronal activity, or sodium thiopental which has been shown to decrease limbic neuronal activity, nitrous oxide was shown to have little or no effect on limbic neuronal activity as shown by electroencephalographic activity. Importantly, this suggests that different anesthetics may have several different mechanisms of action on synaptic transmission in the central nervous system (Babb et al., 1975; Galindo, 1975). Babb further suggests that thiopental enhances the activity of central inhibitory interneurons.

Nitrous oxide analgesia has been compared experimentally to analgesia produced by aspirin, alcohol and narcotics in an attempt to characterize its nature. Unlike aspirin and alcohol, nitrous oxide resembles pharmacologically the opiates in inhibition of pain. Nitrous oxide analgesia is reversible in a dose related fashion (5-30 mg/kg) by naloxone; its euphoric action can also mimic narcotic euphoric effects (Berkowitz et al., 1977). Berkowitz and co-workers have suggested three important areas of research needed to help clarify

mechanisms of analgesia: (1) Alterations in metabolism of NE, DA or 5-HT; (2) Alterations of receptors or post synaptic events in relation to these or other transmitter systems; (3) Release mechanisms for the endogenous opiate like substances, endorphine and enkephalin, need to be established in relation to the stated transmitter systems.

CHAPTER II

METHODS

Animals

Specific animal needs of the research involved in this dissertation required: (1) that the animals be easy to handle, (2) that they have large lateral ventricles adequate in size to facilitate intraventricular administration of ^{14}C-tyrosine necessary for isotopic determination of catecholamine turnover, (3) that the brain size be sufficiently large so that the catecholamines could be assayed on brain regions of a single animal in order to avoid the increased error encountered when brain parts are pooled, (4) and lastly, for administration of the volatile inhalation anesthetics, animals large enough for tracheal intubation were needed. To meet these specific requirements, adult male albino New Zealand rabbits were chosen. Male animals were used exclusively to prevent possible estrus effects on the experimental results. Animals were obtained from a single breeding source, as it has been shown that turnover levels can be significantly affected when different sources are used (Costa, E., 1971). All animals were housed in individual rabbit cages located

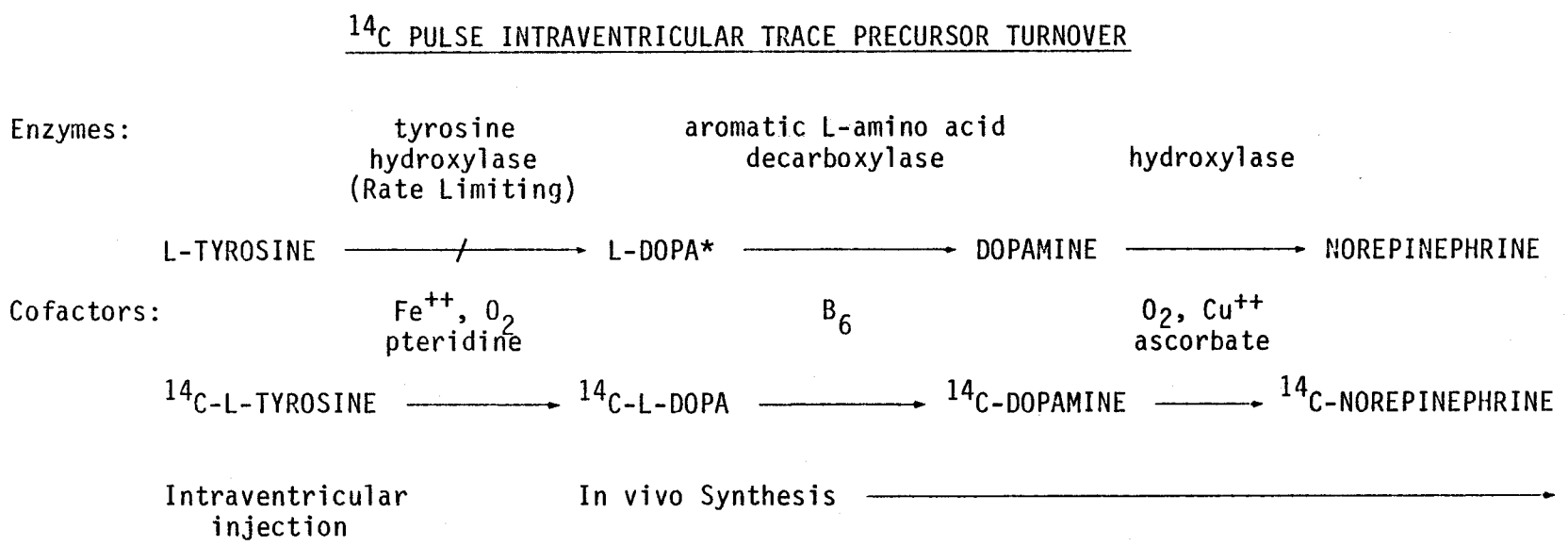
within the Animal Research Facility. These facilities are maintained at 22 degrees Centigrade with a 12 hour alternating light-dark cycle. Food and water was provided to the animals ad lib. All animals were housed in these conditions for 2 weeks prior to use. The brain areas used in this study were the mesencephalon, thalamus, hypothalamus, and cortex. These areas were chosen because: (1) they have sufficient content of the amines under study to prevent the necessity of brain part pooling, (2) they are major anatomical subsections of the brain, and (3) for literature comparison, these areas are frequently studied.

Basic Turnover Techniques

In establishing the turnover techniques to be used in this dissertation, many turnover methods were considered (e.g., Isotopic: Labeling of stores with either labeled precursor or labeled transmitter; Nonisotopic: Inhibition of biosynthesis or measuring end product formation (Costa and Neff, 1970)). After an in-depth evaluation of all methods available, the isotopic trace precursor method was found to be the most reliable.

Basic to this measure of turnover is the metabolic pathway of endogenous catecholamine synthesis (cf. Figure #1). L-tyrosine, the precursor to both DA and NE, enters the central nervous system (CNS) by transversing the blood-brain barrier. Once in the nerve cell, L-tyrosine is converted to L-3,4-dihydroxyphenylalanine (L-dopa) by the enzyme tyrosine hydroxylase. This conversion of tyrosine to L-dopa is considered to be the rate limiting process which controls the amount of transmitter synthesized by the nerve cell, L-dopa is then converted to dopamine and/or norepinephrine depending on whether the cell is dopaminergic or noradrenergic in nature. During "steady state" neuronal activity, the amount of NE or DA synthesized is considered to be equal to that degraded (Costa, 1971), thus a constant endogenous level of neurotransmitter is maintained. It is important to

Figure 1



*L-DOPA = L-3,4 dihydroxyphenylalanine

Figure #1 Diagram of the biosynthesis of dopamine and norepinephrine in the central nervous system. Tyrosine, which is permeable to the blood brain barrier, is supplied to the brain from the blood. Radioactively labeled tyrosine (^{14}C-L-Tyrosine) is introduced intraventricularly and is subsequently metabolized *in vivo* to labeled dopamine and norepinephrine and to their respective metabolites parallel to the non-labeled endogenous amines.

understand that basic to the concept of turnover is the primary assumption that the amount of transmitter released (neuronal activity) is proportional to the amount degraded. Since the amount of transmitter formed is equal to that degraded, it has been ascertained that both synthesis and degradation are proportional to transmitter release. These premises are the foundation upon which the concept of turnover has been established (Costa, 1971; Costa and Neff, 1970; Cooper et al., 1974). As described, turnover is an estimate of neuronal activity, is based on steady state kinetics, and is calculated from the rate of synthesis or degradation of the transmitter under study.

Preliminary Studies:

Initially several preliminary studies were undertaken to establish dopamine and norepinephrine turnover methodologies which would be of sufficient sensitivity so that individual brain parts could be used without brain pooling and to be able to determine dopamine and norepinephrine turnover in four brain regions simultaneously. After careful screening, an isotopic precursor method was chosen using labeled L-tyrosine for the precursor (used because it precedes the rate limiting enzymatic step that controls the synthesis rate).

Two commonly used isotopically labeled tyrosine forms are available, ^{3}H and ^{14}C. ^{3}H-tyrosine has a higher

specific activity than ^{14}C-tyrosine, however, the ^{3}H atoms easily exchange with H^{+} atoms particularly at a low pH. This makes the ^{3}H labeled form unreliable for use in these experiments which required acid extraction. The ^{14}C-L-tyrosine form was found to be stable at low pH and used throughout these experiments (483 mCi/mmol, Amersham-Searle Co.).

The intraventricular injection technique used was previously described by Glisson (1971). Dye and isotopic distribution using the intraventricular injection route showed an acceptable distribution to all brain areas under study and was identical to that reported by Glisson (1971).

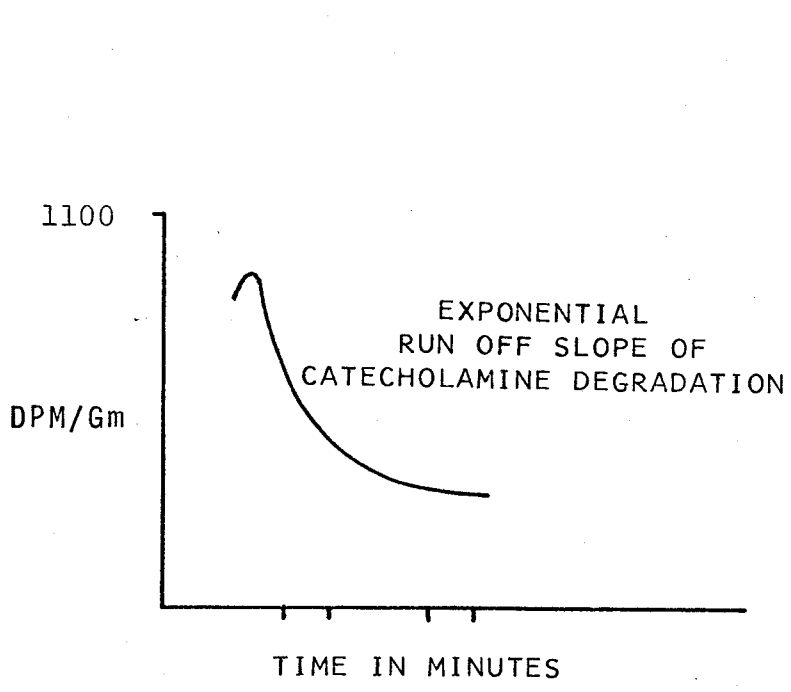
It should be noted that initially the caudate nucleus was to be included in this study, however, it was found that this brain area, which is in juxtaposition to the isotopic injection site, had an unacceptable difference in isotope concentration between the left and right sides. The magnitude of such unequal isotopic distribution for this particular area would result in an erroneous determination.

Based upon results of preliminary trials the following procedure for isotopic intraventricular injection was adopted: Four hours before the turnover experiment, each animal was gently restrained and the hair

clipped from the top of the head. Lidocaine (1%) was injected intradermally into the scalp (0.25cc). The analgesic field was tested by a pin prick; in the absence of analgesia the animals blink. After the loss of pain sensation midline longitudinal incision was made through the scalp exposing the skull. Bone wax was applied to the skull to stop any bleeding from the bone. The coronal and sagital sutures were visualized. Using dividers a point 4.0 mm caudal to the coronal suture and 4.5 mm lateral to the sagital suture was identified and marked. A small hole was then made using a blunted 18 gauge needle by gentle hand rotation. Special care was taken never to penetrate the dura mater. Bone wax was then applied to seal the hole until time of the intraventricular injection. The surgical procedure itself produced no signs of stress during or after establishing the intraventricular injection site. The animals were then left undisturbed until the start of the experiment. Intraventricular injections were made through the injection site using a 250 μl syringe fitted with a blunt 27 gauge stainless steel needle 6 mm in length. Using gentle pressure, two and one half μCi of ^{14}C-L-tyrosine was delivered slowly in a 50 μl volume. Once the isotope was delivered the hole was immediately resealed with bone wax. All injections were visually confirmed for

intraventricular placement during dissection of the brain. This type of injection is referred to as delivering a "pulse dose." The amount of L-tyrosine injected into each rabbit was 883 ng. The quantity of isotopic tyrosine injected in proportion to the whole brain content of tyrosine must be minute so that the endogenous tyrosine pool is not significantly altered. This small amount is frequently referred to as a "trace dose." Generally the above described technique is referred to as an isotopic pulse intraventricular trace precursor technique (Costa, 1971).

^{14}C-L-tyrosine enters the metabolic scheme as is shown in figure #1. The rate of synthesis of ^{14}C-L-DOPA from ^{14}C-L-tyrosine parallels that of endogenous L-DOPA from endogenous L-tyrosine. The labeled transmitter synthesized is stored and released from the granular pool as is the endogenous transmitter (Costa, 1971; Costa and Neff, 1970). The rate of synthesis, or degradation, can be determined by measuring the content of labeled transmitter in whole or brain parts at different time periods (Brodie et al., 1966; and figure #2). For these determinations, specific portions of the synthesis or degradation curves are used. The degradation slope (run off slope) is best suited for turnover determination due to its long stable exponential decay.



Diagramatic Representation of a Typical Exponential Isotopic Catecholamine Run Off Slope

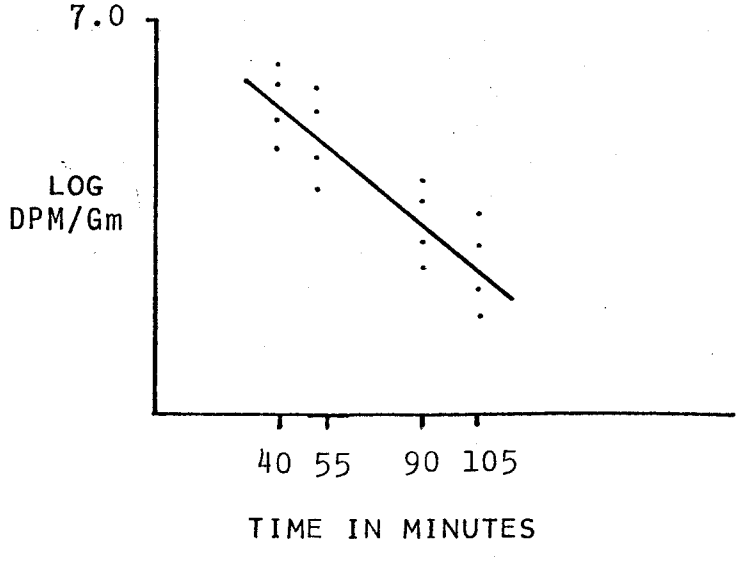


FIGURE #2

Figure #2 Diagramatic representation of a typical exponential isotopic catecholamine run off slope as determined by sampling the isotopically labeled dopamine or norepinephrine in brain regions at the four designated time coordinates. When disintegrations per minute / gram (DPM/Gm) are plotted against time an exponential function is found (above). When the DPM/Gm values are converted to natural logarithm and plotted, a straight line function is derived (below).

Preliminary studies demonstrated that run off slopes varied in time with different brain regions. Based upon the results, four time periods were chosen which allowed for the simultaneous turnover measurement of dopamine and norepinephrine in the four brain areas used. These time coordinates were 40, 55, 90, 105 minutes.

Turnover Calculations:

Calculation of turnover is based on two factors: (1) the "rate constant" as determined by the run off slope and (2) the level of endogenous transmitter. The rate constant is defined as the fraction of the neurotransmitter pool that is lost per unit time and is frequently referred to as the "fractional rate constant" (for additional information see Brodie et al., 1966; Costa, 1971). Once the rate constant and endogenous amine level have been determined, turnover is then calculated as the product of the rate constant times the endogenous level.

The limiting problems concerning measurement of catecholamine turnover <u>in vivo</u> involve: (1) establishment of steady state conditions, (2) the number of time points that can be measured in a single animal, (3) unknown cytological factors, (4) enzyme and substrate controlling mechanisms are not fully understood, to name a few. A comprehensive discussion of these and other limitations

can be found in articles by Costa (1971) and Costa and Neff (1970).

Specific Methodologies

The specific methodologies used in this dissertation to measure dopamine and norepinephrine turnover in the various brain areas are as follows. All experiments were started at approximately 9:30 a.m. to minimize chronobiological variation. A total of 52 animals was used to establish the awake control data. Four animals were used for each run, where one animal was used for each time period. Awake control subgroups were staggered between each anesthetic series to help control for seasonal variation. In each anesthetic group sixteen animals were used, four at each time period.

Tissue Samples:

Animals were sacrificed by decapitation. The brains were removed rapidly, rinsed well with saline to remove excess isotope and placed on an ice cold petri dish for dissection. Surface blood vessels and connective tissue were removed. The left and right cerebral hemispheres were separated. A cut was made through the left and right cerebral peduncles, freeing the left and right hemispheres. With careful teasing, the hippocampus was removed exposing the caudate nucleus from head to tail. Using a pair of toothed iris forceps, the caudate nucleus was teased away from the ventricular wall. A cut just

lateral to the internal capsule was made to remove the lenticular nuclei. The remaining tissue was considered as cortex. Next, the midbrain-diencephalon region was obtained by transecting the lower brain stem on a line from the caudal end of the inferior colliculus to the rostral end of the pons, thereby isolating the midbrain-diencephalon from the rest of the brain stem. The midbrain was separated from the diencephalon by a transverse cut. The diencephalon portion was cut transversely to facilitate dissection of the thalamus and hypothalamus. A transverse section was made separating the dorsal thalamus from the more ventral hypothalamus. These dissections were easily made and identical areas were always removed as demonstrated by the consistent brain part weights (See Appendix A).

Catecholamine extraction:

Immediately following removal, each tissue was weighed and placed in a 15 ml ground glass homogenizing tube containing 6 ml ice cold 0.4 N perchloric acid, 0.02% ascorbic acid and 0.2% EDTA. The tissue was thoroughly homogenized and poured into a 15 ml thick walled conical centrifuge tube stored on ice. An additional 2 ml of 0.4 N perchloric acid were added to the homogenizing vessel as a wash and then transferred to the centrifuge tube. The tubes were placed in a refrigerated centrifuge

held at 0°C and centrifuged for 20 minutes at 3200 x g. Following centrifugation, the supernatant was filtered using Whatman #1 filter paper. The pellet was resuspended in 2 ml of ice cold 0.4 N perchloric acid and centrifuged for reextraction. The supernatant was filtered and combined with the original supernatant. The tubes were sealed and stored frozen (-25°C) until catecholamine separation by column chromatography (Glisson, 1971).

Column preparation:

The column assemblies were adapted from 1.0 ml disposable syringes. It was necessary that the internal diameter of the syringe barrel be 4.2 - 4.5 mm. The upper lip of the syringe was cut off and a 2 cm piece of tygon tubing affixed to the top of the syringe barrel in such a way that one centimeter of tubing extended above the top of the barrel (Glisson, 1971). This "column assembly" was then submerged in a beaker containing distilled water. It was necessary that the actual packing of the column with resin be done under airtight conditions, as air bubbles will disrupt the uniform packing of the resin. Air bubbles were removed from the empty column assembly by inserting a stirring rod inside the column and working it up and down a few times. With the column completely filled with water, a small ball of Pyrex glass wool was inserted into the column and forced to the tip with a

stirring rod. This piece of glass wool serves to trap the resin within the column. A G 50w X 4 200-400 mesh resin cleaned and cycled to the H^+ form was suspended in 0.01 M phosphate buffer and 0.1% EDTA and placed in a 2ml pipet. The tip of the filled pipet was inserted under water into the tygon tubing attached to the top of the column. The resin was then allowed to flow into the column. After the resin bed had settled to a height of 50mm, another ball of Pyrex glass wool was inserted into the top of the column. The freshly packed columns were then mounted onto the column board.

The column board consisted of 20ml graduated glass syringes mounted through a horizontal board attached to ring stands. A plastic 3 way stopcock was attached to the tip of this syringe. To the side of this syringe, a 20ml syringe barrel was mounted in a similar fashion. This barrel served as a reservoir through which the tissue extract and other reagents could be added to the column. The reservoir and syringe were connected through a series of plastic stopcocks. On the open end of the stopcock attached to the glass syringe, a plastic fitting was placed. The fitting allowed for an air-tight seal to be made between the tygon tubing on the resin column and the stopcock on the glass syringe. The fitting was made by removing the metal insert of a disposable needle and using

the upper plastic portion as the fitting. Prior to the attachment of the freshly packed resin column, distilled water was added to the reservoir and drawn through the stopcock assembly into the syringe to remove any air from the system. With the distilled water slowly dripping through the stopcock, the resin column was seated tightly on to the stopcock fitting. The column was mounted in such a way as to allow for liquids to be drawn into the glass syringe from the reservoir and then to flow through the resin column. With the resin column mounted on the column assembly, the resin was then cycled from the H^+ form to the Na^+ form by passing 20 ml 0.1M sodium phosphate buffer containing 0.1% EDTA at pH 6.5 through the resin column at a flow rate of one drop every 10 seconds (.3mls/min)(Glisson, 1971). After the buffer had passed through the column the pH of the effluent was checked. A pH of 6.5 indicated that the column was cycled and ready to receive the tissue extract.

Catecholamine Ion Exchange Column Chromatography:

Before adding the tissue extract to the column, the pH was adjusted to 6.5 using 10 N potassium carbonate. A digital pH meter, magnetic stirrer and an automatic buret were used. At pH 6.5, potassium perchlorate precipitates out of the neutralized extract. Placing the neutralized samples into a refrigerator for 10 minutes

increases the precipitation rate. Following cooling, the extract was centrifuged at 1500 g for 10 minutes to pack the precipitant. The supernatant was poured into the reservoir of a column assembly. The extract was drawn into the glass syringe and allowed to flow through the resin at a rate of one drop every 10 seconds. After the extract passed through the column, the resin was washed with 40 mls of distilled water. The flow rate for the water wash was twice as fast as that of the tissue extract. Care must be taken in handling the water wash as well as all column effluents, as the water will contain considerable amounts of ^{14}C products washed from the resin. Following the wash, 1 N hydrochloric acid was added to the reservoir. During the elution and fractionation of the norepinephrine and dopamine (isotopic and endogenous), the flow rate for the hydrochloric acid (HCl) was again controlled at 1 drop every 10 seconds. The first 3.5 ml HCl passed through the column was collected and discarded by appropriate isotopic waste procedure. The next 6 mls of acid passed through the column containing the norepinephrine were collected in small shell vials that had been washed and rinsed in distilled water. An additional 13 ml HCl fraction was collected and contained the dopamine. All vials were sealed and frozen at -25^{o} C until samples were thawed for further processing.

Catecholamine quantification:

Before either norepinephrine or dopamine can be quantitatively measured, they must be converted to their respective fluorophores. As fluorophores dopamine and norepinephrine can be measured using a spectrophotofluorometer. Since norepinephrine and dopamine are converted at different optimum pH's to two different fluorophores, the method is highly specific for each compound (Carlsson and Lindqvist, 1962; Carlson and Waldeck, 1958).

The conversion scheme for norepinephrine as modified from Glisson (1971) is shown in table #1. Upon completion of the chemical procedure shown in table #1, the samples were poured into reading tubes and read in an AMINCO spectrophotofluorometer equipped with an ellipsoidal mirror condensing system at 400 nM excitation and 505 nM emission wavelengths. The fluorophore was found to be stable for one half hour. The samples were read within this time period. Spectral curves were plotted to insure sample purity. Maximum sensitivity of these methods was found to be 500 picogram (NE). Calculation of endogenous norepinephrine was done using the mathematical equation as shown in table #2.

For dopamine, it was necessary to develop new assay techniques to be able to measure the concentrations in a single brain region. This was accomplished in two

TABLE #1

Conversion of Norepinephrine to Norepinephrinolutine
(Fluorometric Conversion)

Reagent	Standards 1X	Standards 2X	Faded Blank	Sample	Internal Standard
H_2O	3.8	3.7	1.4	1.4	1.3
PO_4 Buffer 0.1 M 6.5 pH	0.5	0.5	0.5	0.5	0.5
NE Std. 1 ug/ml	0.1	0.2	...	...	0.1
Neutral Sample Eluate	...	...	2.5	2.5	2.5
0.5 % Zinc Sulphate	.05	.05	.05	.05	.05
0.25 % $K_3Fe(CN)_6$	.05	.05	.05	.05	.05
Wait Five Minutes					
5N NaOH + 2% Ascorbic Acid	.5	0.5	...	0.5	0.5
5N NaOH	...	...	.45	...	...
Wait Ten Minutes					
2% Ascorbic Acid	...	...	.05	...	...

Table #1 (continued)

Read samples within 30 minutes in a spectrophotofluorometer at 400/505 nM.

All units are ml.

Table #1 The chemical scheme for the conversion of norepinephrine to norepinephrinochrome and then to norepinephrinolutine. This conversion is specific for norepinephrine (cf. text) and results in a fluorescent molecule that can be assayed quantitatively in a spectrofluorometer.

TABLE #2

Calculation of Endogenous NE and DA

Tissue Catecholamine Concentration (ug/g) =

$$\frac{(\text{Samp.FR.} - \text{T.Blk.FR.})}{(\text{Std.FR.} - \text{RB.FR.})} \times \text{Std.Conc.} \times \frac{\text{Vol.Samp.}}{\text{Fct.Samp.}} \times \text{RF}^{-1} \times \text{BPW}^{-1}$$

Where:

Std.FR. = Standard fluorometric reading

RB.FR. = Reagent blank fluorometric reading

Samp.FR. = Sample fluorometric reading

T.Blk.FR. = Tissue blank fluorometric reading

Std.Conc. = Standard concentration in ug.

Vol.Samp. = The total volume of sample. For the NE sample this is the 6 mls. column eluate plus the K_2CO_3 volume used to neutralize it to 6.5 pH. For DA this would be the 1 ml. volume used to dissolve the freeze dried column extract.

Fct.Samp. = The fractional amount (in mls.) of the Vol.Samp. used in the determination.

RF = The recovery fraction as determined by the "recovery standards" for that particular run (ug recovered/concentration added).

BPW = The brain part weight in grams wet weight.

steps. First, (a) the 13 ml 1N HCl column elution volume containing the dopamine was reduced in volume to concentrate the eluate. The second procedure (b) used to improve dopamine sensitivity was to develop a micro scheme for fluorophore conversion. Each will be discussed separately.

(a) A freeze drying technique was employed to reduce the 13 ml dopamine samples to dryness. Dopamine was found to be chemically stable throughout this process. The freeze dryer was obtained from the Virtis Company Model #10-010 and was equipped with a 150 liter/min vacuum pump and a McLeod gauge, so that an accurate measure of internal vacuum could be determined.

Freeze drying was accomplished by the following procedure. The instrument was started and allowed to equilibrate to a temperature of -65^{o} centigrade and an internal vacuum of 0.05 uM Hg. An acetone-dry ice bath was prepared. Each dopamine sample eluate was placed in a 100ml round bottom freeze-drying flask that had been previously cleaned with concentrated potassium hydroxide solution, and finally repeatedly rinsed with distilled water. Each sample was shell-frozen by gentle rotation in the bath until solidly frozen. Samples were placed on the freeze dryer and the vacuum allowed to reequilibrate

to 0.05 uMHg. between sample placement. This procedure was repeated until all samples were frozen and placed on the freeze dryer. A maximum of 12 DA samples could be run at any one time.

An additional freeze dryer recovery control was added at this point of processing. Since dopamine underwent both exchange column chromatography separation and freeze drying, a known standard was introduced prior to freeze drying so that recovery could be monitored for both operations. To this end two standards were prepared for each run (i.e., 2 standards with 10 samples) by adding a known concentration of dopamine to 13 mls of 1 N HCl. Freeze dried dopamine recovery was demonstrated both fluorometrically and isotopically to be 90% $\pm$ 5 down to 10ng. It took 12 hours to reduce all samples to dryness. The samples were removed from the freeze dryer and 1 ml of 0.01 N HCl was added to each flask to redissolve the crystalline dopamine. One third of the HCl was placed in a scintillation vial with 10 ml of Handiflour^{R}. The vial was placed in a Packard Tri-Carb scintillation counter and the radioactivity measured. The remaining 0.66ml was used for the DA fluorophore conversion.

(b) The dopamine fluorophore quantification method was adapted from the trihydroxy indole method of Carlsson

and Waldeck (1958). The method was modified to reduce the total reaction volume so that the concentration of the dopamine fluorophore would be greater per unit volume.

During preliminary studies, it was determined that the total reaction volume could be successfully reduced from 6mls to 1.22 mls resulting in a 4.9 fold increase in fluorophore concentration per unit volume. The chemical conversion scheme is shown in table #3.

After cooling the samples for one hour they were poured into reading tubes and read in a spectrophotofluorometer at 330 nM excitation and 380 nM emission, wavelengths uncorrected. Spectral scans were plotted to confirm the existence of uncontaminated dopamine in the sample. Calculation of endogenous dopamine was done using the mathematical equation as shown in table #2.

In effect, freeze drying and the micro-volume fluorophore conversion increased the method's sensitivity by 65 X. It should be noted that accompanying this increase in sensitivity was an accompanying increase in tissue blank fluorescence. To this end extreme care must be taken in cleaning and rinsing all glassware used and resin should be carefully cleaned by cycling to minimize blank fluorescence. Maximum final sensitivity of the dopamine methods was determined to be 1ng.

TABLE #3

Conversion of Dopamine to Dopaminolutine
(Fluorometric Conversion)

Reagent	Standards 1X	Standards 2X	Standards 4X	Reagent Blank	Sample	Blank
H_2O	0.45	0.2	0.6	0.7	0.4	0.4
Citrate Phosphate Buffer	0.1	0.1	0.1	0.1	0.1	0.1
DA Std. 100 ng/ml	0.25	0.5	...	...	...	...
DA Std. 1 ug/ml	...	...	0.1	...	...	...
Freeze Dried Sample	...	...	...	...	0.3	0.3
Na_2SO_3 + NaOH	...	...	...	...	...	0.1
Boil Blank ONLY for Twenty Minutes						
Iodided Solution	0.02	0.02	0.02	0.02	0.02	0.02
Wait Five Minutes						
Na_2SO_3 + NaOH	0.1	0.1	0.1	0.1	0.1	...
Wait Five Minutes						
5N Acetic Acid	0.3	0.3	0.3	0.3	0.3	0.3

Table #3 (continued)

Boil for Thirty Minutes Cool for Thirty Minutes (Read 330/380nM)

All units are ml.

Table #3 The chemical scheme to convert dopamine to dopaminochrome and on to dopaminolutine. Dopaminolutine is fluorescent and can be quantified with a spectro-fluorometer. This procedure is specific for dopamine in that any possible norepinephrine contamination will not affect the amount of fluorescence produced (cf. text).

Calculation of the Isotopic Catecholamine Run Off Slope:

1) Determination of NE and DA disintegration per minute (DPM) per gram of tissue (i.e., DPM/G): The amount of ^{14}C NE and DA produced in vivo was determined by measuring individually the NE and DA counts (CPM) per sample as formulated in table #4.

2) Plotting and regression analysis of the isotopic data: Eight turnover plots can then be generated of 16 points each from the 128 (8 X 16) separate DPM/G determinations. Each plot is constructed separately. First, the log DPM/G were determined (either natural or common log may be used; however, if common log is used a correction factor is necessary), and the corresponding time coordinates converted to fractions of hours. With the abscissa as time in hours and the ordinate as log DPM/G, each point can then be appropriately plotted. A sample plot is shown in figure #2. Regression analysis was performed on the isotopic data to determine the slope (i.e., the run off slope or rate constant) and its respective correlation coefficient (r). The correlation coefficient indicates the probability of fit of the data points. Only regression lines that demonstrated a significant fit of $p < .05$ were used. The regression lines were then plotted and the slope determined. The calculated slope or run off slope is the rate at which the ^{14}C trans-

TABLE #4

Calculation of the Isotopic Catecholamine Run Off Slope

$$DPM/G = \frac{(CPM-BG) \times Vol.\ Samp.}{BPM \times CE \times Fct.\ Samp. \times Col.\ R.}$$

Where:

CPM = Counts per minute for each sample as counted in a refrigerated Tri-Carb liquid scintillation spectometer.

BG = Background counts.

Vol. Samp. = The total volume of sample. For the NE sample this is the 6ml column eluate before neutralization. For DA this would be the 1ml volume used to dissolve the freeze dried column extract.

BPW = Brain part weight in grams (wet weight).

CE = Counting efficiency as determined by a sealed ^{14}C DA and ^{14}C NE standard which was counted simultaneously with each set of samples.

Fct. Samp. = The fractional amount (in mls) of the Vol. Samp. used for scintillation counting. For DA Fct. Samp. = 0.3mls, for NE Fct. Samp. = 1.0ml.

Col. R. = Column recovery

mitter is leaving the nerve terminal under study. Once both the endogenous level and the run off slope have been determined, the turnover rate of NE and DA can be calculated as their product (Brodie et al., 1966).

Computer Calculation of Data:

Data handling initially presented itself as a problem. The large amount of data, for example for control sets there were 320 samples and each sample had 16 data bits (i.e., volume, Fluorometer reading, etc.) totalling over 5,000 numbers, had to be recorded and entered into the appropriate phase of calculation. Data recording and repetitive calculations, however, lends itself readily to computer adaptation. To facilitate data handling a computer based storage, calculation, and statistical analysis system was developed. The system was created to be interactive (i.e., run from a computer based time sharing terminal). The computer language used was Pl-1. All programs were designed to both store and output all data entered and values calculated so that errors due to manual handling of the data were eliminated. All programs were system tested for accuracy by at least two different people on separate occasions.

All data was stored on a disk file system with a back-up tape system to prevent loss of data in the event

of a computer breakdown. Statistical programs used in this dissertation were adapted from existing documented computer statistical systems. Using the adapted programs, determination of mean, standard deviation, standard error, variance, range, maximum, minimum, t-test comparison, plotting, regression analysis, and slope comparison by covariance analysis could be carried out (Snedecor and Cochran, 1967, pp. 421-425).

Monitoring Techniques: (cf appendix B for values)

It was necessary to insure "normal physiological function" (cf. below) in all experimental animals. "Normal physiological function" in these experiments is taken to mean stable blood chemistries, core temperature, and blood pressure throughout the experimental procedures. All experimental animals were equipped with an indwelling arterial and venous femoral catheter. To avoid pain to the animals, these catheters were positioned through a small incision, one half inch in length, following local infiltration of 1% lidocaine (0.25 ml).

One ml blood samples were drawn at regular intervals for blood chemical analysis. A model 165 Corning Blood gas analyzer was used to determine blood pH, PaO_2, $PaCO_2$, HCO_3, and bases of excess by standardized procedures. An average of six arterial blood samples were drawn per animal 20 minutes apart or as needed. In the

halothane experiments a 100 ul capillary tube was filled with arterial blood and sealed immediately upon acquisition of the 1ml sample for subsequent determination of blood halothane (cf. Lowe, 1964).

Blood pressure was monitored via the femoral arterial cannula connected to a Hewlett Packard pressure transducer and displayed on a recording polygraph. Blood pressure and heart rate were recorded just prior to each blood sample. Both systolic and diastolic pressures were obtained. Where necessary mean blood pressure is considered to be 1/3 X pulse pressure plus diastolic pressure where pulse pressure is equal to systolic pressure minus diastolic pressure.

Core body temperature was approximated by monitoring rectal temperature continuously with the aid of Yellow Springs Telethermometer. It was found that normal body temperature ($39^{o}C$) could be maintained easily in anesthetized animals by maintaining a $27^{o}C$ room temperature. An auxiliary heating pad was seldom used.

Volatile and Gaseous Anesthetic Delivery System for Halothane and Nitrous Oxide:

To deliver halothane, a volatile anesthetic, and nitrous oxide (N_2O), a gaseous anesthetic, to the rabbits under study, it was necessary to create an anesthetic

delivery system to maintain consistent blood anesthetic levels in animals from experiment to experiment. Spontaneous breathing anesthesia delivery systems were disregarded. A delivery system where ventilation and delivered anesthetic concentration could be controlled minute to minute was decided upon.

For this purpose, a semi-closed pediatric anesthetic circuit was chosen and modified to allow for ventilation of two rabbits simultaneously. There are two distinct advantages of this system: (1) continuous control of physiologic blood gases and anesthetic concentration, (2) with a semi-closed system and a proper evacuation system, there is no escape of the anesthetic gases into the room to present a health hazard to laboratory personnel (OSHA Standards).

The delivery system was comprised of five major on line components (figure #3). (a) An Ohio Medical Metamatic veterinary ventilator was used to circulate oxygen and anesthetic through the system and ventilate the animals. This ventilator is designed for anesthetic gas delivery and allows for a wide variety of ventilation patterns either volume or pressure limited. (b) A series of flow gauges and valves were used to regulate gas flow and mixture. A Foregger system was used. (c) A halothane vaporizer (Foregger Fluomatatic) which compensates for gas

ANESTHESIA CIRCUIT

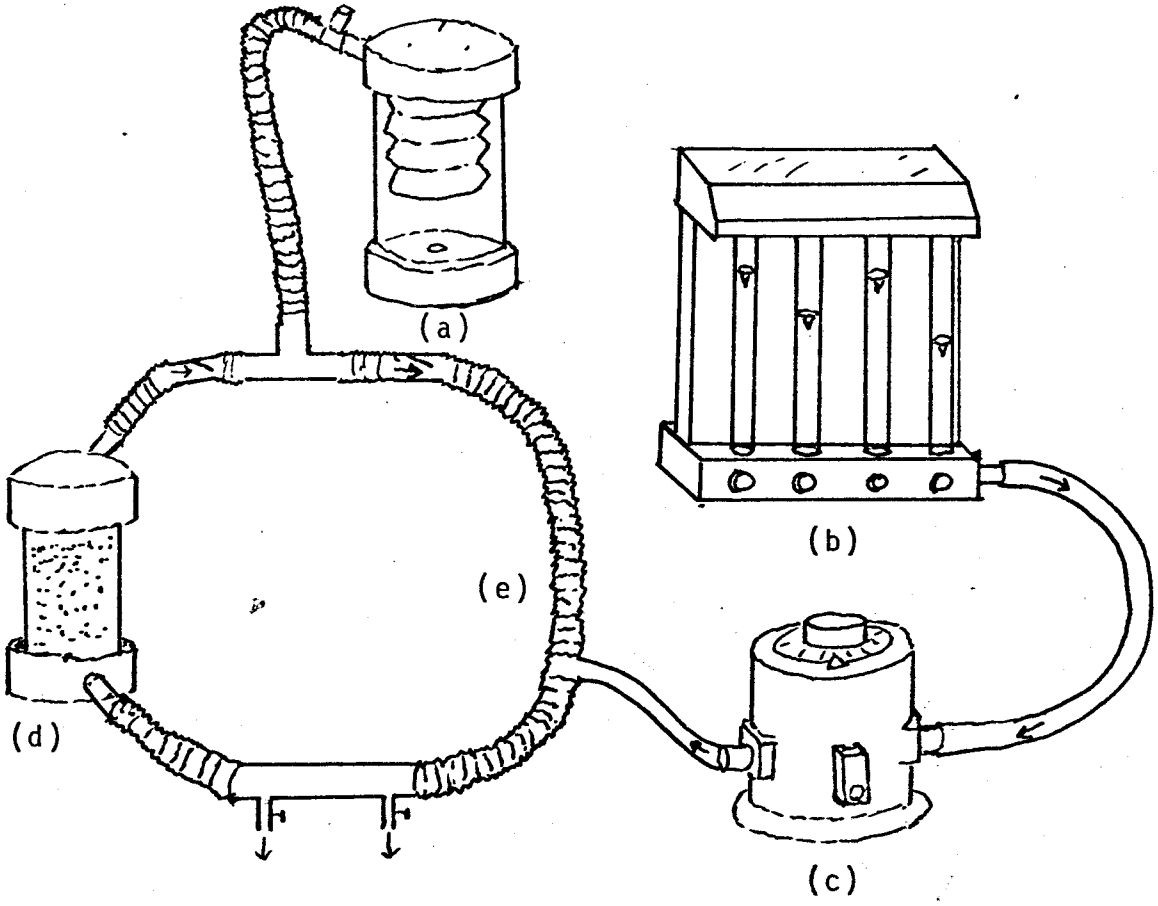


FIGURE # 3

Figure #3 Diagram of the anesthesia circuit used in conjunction with the nitrous oxide, halothane, and halothane - nitrous oxide combination studies. Shown: (a) an Ohio Medical Metamatic Ventilator, (b) a series of flow gauges, (c) a halothane vaporizer, (d) a carbon dioxide absorber and (e) corrugated anesthetic tubing equiped with two "one way" valves.

flow, ambient temperature, and barometric pressure to deliver the precise concentration desired was used to deliver halothane. (d) A carbon dioxide (CO_2) soda lime absorber was used to remove expired CO_2. Finally, (e) disposable corrugated anesthetic tubing and two one way valves were used to complete the circuit and maintain a "one way" flow and screw clamps were interposed between the animals and the circuit to adjust for difference in animal compliance.

Tracheal Intubation:

Tracheal intubation in the rabbit is difficult but can be accomplished consistently and nontraumatically once the skill has been refined. First the animals were pre-oxygenated for 5 minutes with 100% O_2 via an animal mask. The ventilatory system was checked for leaks and primed with 100% O_2 during this period. A cuffed endotracheal tube of appropriate size was chosen. It was found in this study that for every 1 kg of body weight, 1 mm in tube size was needed. The endotracheal tube was placed on an introducer so that the introducer protruded 1/2 inch past the end of the endotracheal tube. The tube was lubricated with lidocaine 2% jelly. To aid endotracheal intubation, pancuronium bromide, 0.126 ug/kg was administered by lateral ear vein injection. When the

animal was sufficiently paralyzed, laryngoscopy was performed and the vocal cords visualized. The introducer with the endotracheal tube in place was then inserted so that the introducer could be seen to pass the vocal cords. The introducer was then held in place and the endotracheal tube slowly inserted to a predetermined length. The endotracheal tube was then connected to the anesthetic circuit and the cuff filled until no backflow around the tube could be heard. While the animal equilibriated (approximately 10 minutes), a femoral cut down was performed as previously described. Blood gases, blood pressure, heart rate, and body temperature were monitored and recorded. Ventilator rate and/or tidal volume was adjusted if necessary to maintain PCO_2 at 35-40 torr. When all recorded paramters were determined to be normal the experimental procedure was begun. If for any reason "normal physiological function" was not established, the animal was eliminated and a repeat experiment performed.

Drug Scheduling

Three anesthetic agents were studied: ketamine, halothane and nitrous oxide. These three agents have different clinical uses within the field of anesthesia. Each anesthetic was chosen for its dissimilarities from the other two so that, hopefully, different actions on central adrenergic neuronal transmitters could be demonstrated.

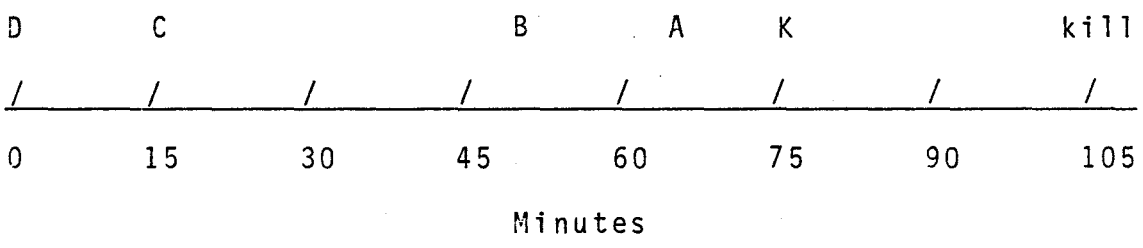
Ketamine:

Ketamine, a short acting "dissociative" anesthetic, is characterized by both analgesia and narcosis. It was used at a dose of 40mg/kg which corresponds well with the reported ketamine doses for rabbits in the literature (Sung et al., 1973; Biggio et al., 1974). The ketamine dose used in humans is 2.0 - 8.0 mg/kg. Interestingly, a scan of the literature indicates that the lower an animal is on the evolutionary scale, the larger the ketamine dose needed for anesthesia. It appears that the dose needed for ketamine anesthesia is inversely related to phylogeny (Doubrava and Larson, 1971).

In this study, ketamine was given to 20 rabbits all weighing between 2.5 and 3.5 kg by lateral ear injection. Preliminary studies showed that cardiovascular depression occurred if ketamine was injected at a concen-

tration of 100 mg/ml. The injection concentration decided upon for experimentation was 50 mg/ml. The ketamine infusion rate was held constant at 1.0 ml/min. At this concentration and infusion rate, no respiratory or cardiovascular depression was noted. A control subgroup was administered saline in place of ketamine.

All ketamine experiments were begun between 9:00 and 10:00 a.m. Four animals, one for each of the four time points (40, 55, 90, and 105 min.) were handled simultaneously. Each animal was prepared with an intraventricular injection site as previously described, allowed 1.5 hours recovery, then injected with the ^{14}C-L-tyrosine using the schedule shown below.



Where:

A is the ^{14}C-tyrosine injection time point for 40 min.
B is the ^{14}C-tyrosine injection time point for 55 min.
C is the ^{14}C-tyrosine injection time point for 90 min.
D is the ^{14}C-tyrosine injection time point for 105 min.
K is the ketamine injection time point.

The thirty minute time period was used for ketamine because this is the normal anesthetic time expected

for this agent at this dose. Each animal was monitored for body temperature and heart rate. Blood gases were drawn as previously described (cf. appendix A for monitoring values). Animals were sacrificed by decapitation. Tissue dissection, extraction, fluorometric and isotopic procedures were carried out as discussed in the previous sections. Catecholamine data obtained from the sham injected control animals was not found to differ from the awake control group.

Ketamine Turnover:

In this study ketamine anesthesia was produced using the single intravenous injection technique. To measure ketamine turnover using this technique, it is necessary to correct the isotopic data such that it reflects ketamine's action over the entire turnover time course, 0 - 105 minutes, rather than the actual 30 minute ketamine anesthesia time. To accomplish this correction the formula shown below was applied to each of the ketamine data points. The correction was achieved by applying the formula: $\frac{\text{(control DPM - ketamine DPM)}}{\text{ketamine fractional time}}$ where the ketamine fractional time for the 40 minute point equals 0.75; for the 55 minute point, 0.545; for the 90 minute point, 0.33; and for the 105 minute point, 0.285. The fractional times were derived by dividing the

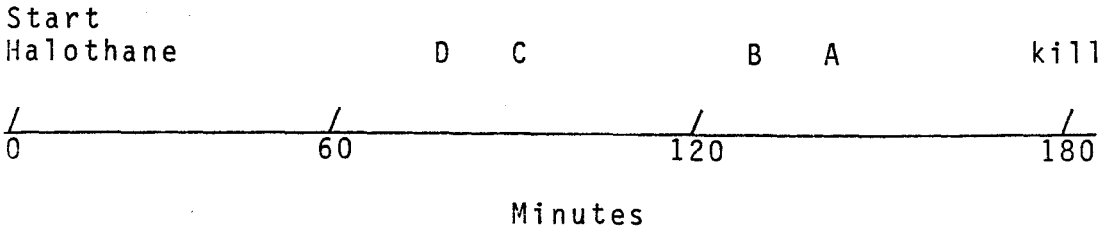
30 minute ketamine anesthesia time by the total time for each period, e.g., $\frac{30 \text{ min.}}{40 \text{ min.}} = 0.75$ for the 40 minute time period. Using this corrected isotopic data, ketamine turnover was calculated according to the formula shown in the section Turnover Calculations.

Halothane:

Halothane is one of the most widely used inhalation anesthetics today. It is a halogenated, volatile liquid at room temperature and requires a vaporizer for controlled use. Halothane produces profound narcosis but unlike ketamine results in little, if any, analgesia at clinical concentrations. Some degree of muscle relaxation is common.

All animals were prepared as previously described. Each was intubated and ventilated. Two animals were handled simultaneously. Halothane was added into the anesthetic circuit using a Foregger Fluomatic vaporizer. Two percent halothane was used continuously. When the depth of anesthesia was established, femoral, arterial and venous catheters were implanted. The animals were allowed to stabilize. Blood gases were determined and modifications of the respiratory rate or tidal volume were made if needed according to PCO_2 values. Each animal was maintained on 2% halothane for a 3 hour period. The

isotopic injection was adjusted so that each isotope time period ended simultaneously with the anesthesia period as shown below.



Where:

A is the ^{14}C-tyrosine injection time point for 40 min.

B is the ^{14}C-tyrosine injection time point for 55 min.

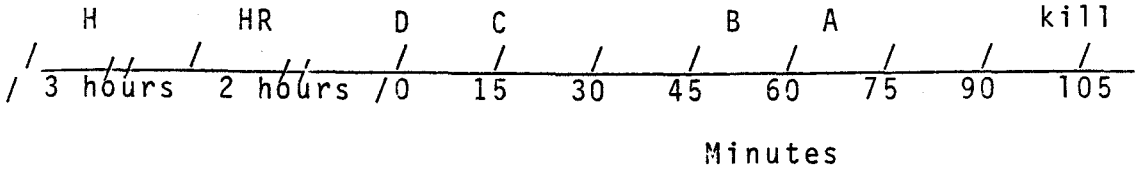
C is the ^{14}C-tyrosine injection time point for 90 min.

D is the ^{14}C-tyrosine injection time point for 105 min.

Blood halothane levels were determined to insure a consistent blood halothane on each animal and between animals. This level was 12.3 mg% ± 0.85. It should be noted that the halothane vaporizer used was rated for a minimum flow of 4 liters/minute. This flow rate is geared to human use. The considerable excess of gas necessarily input into the system was vented through an exhausted pressure sensitive pop-off valve.

A halothane recovery study was also performed. Sixteen animals were used. Each group was treated with 2% halothane in precisely the same manner as described before except these animals were allowed to recover for a

two hour period (cf. below).



Where:

A is the ^{14}C-tyrosine injection time point for 40 min.

B is the ^{14}C-tyrosine injection time point for 55 min.

C is the ^{14}C-tyrosine injection time point for 90 min.

D is the ^{14}C-tyrosine injection time point for 105 min.

H is the 3 hour halothane period.

HR is the 2 hour halothane recovery period.

During the two hour period, the animals were maintained in a 100% oxygen environment. Although continuous blood pressure monitoring was impossible due to animal movement, blood pressure measurements and blood gas determinations were intermittently made throughout the recovery period to insure "normal physiologic parameters." Blood samples were taken prior to sacrifice to determine the blood halothane level two hours post anesthesia. Turnover calculation for both the halothane and the halothane recovery data was done as shown in the Turnover Calculation section.

Nitrous Oxide Studies:

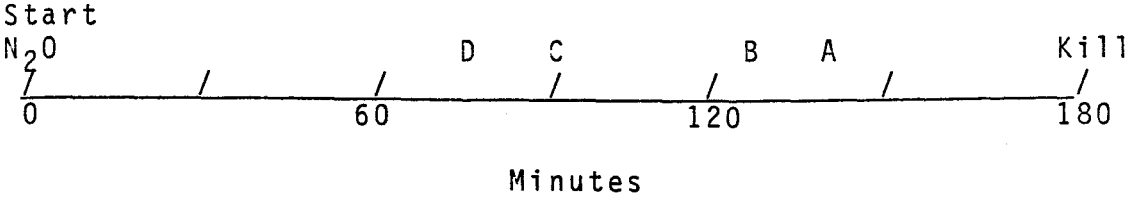
Nitrous oxide (N_2O), a gas at standard temperature and pressure, is supplied in a compressed gas cylinder and was connected to the anesthesia flow valve assembly by appropriate plumbing. This gas is clinically used to produce profound analgesia. In addition, its use in balanced anesthesia decreases the concentration of halogenated anesthetic needed. Deep narcosis or muscle relaxation is not seen at concentrations below 80%.

For these studies, a 2 liter flow of nitrous oxide mixed with a 3 liter oxygen flow was used. In this way, a 40% nitrous oxide concentration was delivered into the anesthesia circuit.

Animals were prepared as previously described. With the aid of pancuronium bromide, intubation was performed and the animals equilibrated on the 40% nitrous oxide and O_2 gas mixture. The femoral cut down was performed and venous and arterial lines implanted. Gas samples were drawn after a 10 minute equilibration period and ventilatory parameters were adjusted if needed. Any animals which did not demonstrate "normal physiological function" were eliminated and repeat experiments performed.

The intraventricular injection site was prepared as described earlier. Physiologic parameters were monitored at the predetermined intervals. ^{14}C-tyrosine

injections were made at 40, 55, 90 and 105 minutes prior to the 3 hours nitrous oxide termination point. The time sequence is shown below.



Where:

A is the ^{14}C-tyrosine injection time point for 40 minutes.

B is the ^{14}C-tyrosine injection time point for 55 minutes.

C is the ^{14}C-tyrosine injection time point for 90 minutes.

D is the ^{14}C-tyrosine injection time point for 105 minutes.

Two animals were ventilated simultaneously on the anesthesia circuit. It was found necessary to run two animals each day on two consecutive days. The remaining processing was handled simultaneously. All phases of the nitrous oxide experimentation were identical to that described for halothane except for the drug delivered. Once sacrificed, all animal tissues and data calculations were treated as previously described.

Halothane and Nitrous Oxide:

In this study halothane and nitrous oxide were used in combination. This halothane-nitrous oxide combination is widely used as a basal anesthesia resulting in

excellent narcosis and muscle relaxation and profound analgesia.

Animals were handled as before. Induction and intubation were facilitated with the use of pancuronium bromide. Animals were ventilated on halothane (2%) and nitrous oxide (40%) in combination. Femoral arterial and venous lines were implanted and the animals allowed to equilibrate. Blood gases were monitored and ventilatory parameters adjusted if necessary. The three hour drug and turnover schedule used was a previously described in both halothane and nitrous oxide sections.

CHAPTER III

RESULTS

Introduction:

This study investigated the effects of ketamine, halothane, nitrous oxide and halothane-nitrous oxide in combination on dopamine and norepinephrine turnover and their endogenous levels to determine if these anesthetic agents have common and/or unique effects specific to any or all of the brain regions studied. The four brain areas considered were the mesencephalon, thalamus, hypothalamus and cortex.

Dopamine and norepinephrine turnover and related values are shown in tabular form for the awake (unanesthetized) control and experimental groups in tables 5, 6, 8, 9, 11, 12, 14 and 15. These values include: (a) the endogenous content in ug/g, (b) the rate constant, which is the rate of isotopic amine disappearance as described by the first derivative of the natural log function, (c) the turnover time, which is the mean length of time necessary for one molecule to turnover and is calculated as the reciprocal of the rate constant, and (d) the turnover value itself, which

expresses the amount of amine in nanograms which is degraded per hour. Awake (unanesthetized) control endogenous and turnover levels for norepinephrine and dopamine determined in this study are consistent with published endogenous and turnover values (Glisson et al., 1972; Brodie et al., 1966; Costa and Neff, 1970).

Two comparisons are important in this study. First, values for each anesthetic agent have been compared to values obtained from awake unanesthetized animals to determine alterations from normal amine metabolism as a result of the anesthetic agent. Second, the effects of each anesthetic agent were compared with these and the other anesthetic agents to determine likeness and dissimilarities between them.

Mesencephalon

Turnover and Endogenous Level as Compared to Awake Control:

The mean endogenous dopamine level in the mesencephalon (table #5) for the awake control was found to be 0.333 ug/gram of tissue. Ketamine and nitrous oxide administration each resulted in a drop in dopamine from control levels. With ketamine a 55% decrease ($p<0.01$) in endogenous dopamine occurred, while nitrous oxide had only a slight reducing effect (2%) (cf. table #5 and figure #4). Halothane and halothane in combination with nitrous oxide resulted in a significant increase in endogenous dopamine compared to awake control. The halothane group induced a 51% increase ($p<0.05$) in DA levels and halothane plus nitrous oxide in combination resulted in a 203% increase in endogenous dopamine ($p<0.01$) (figure #4).

The endogenous norepinephrine awake control level in mesencephalon was found to be 0.323 ug/gram (table #6). As in the case of dopamine, a decrease in endogenous norepinephrine content occurred with ketamine and nitrous oxide. Ketamine resulted in a 56% decrease ($p<.01$) and nitrous oxide caused an 11% decrease in norepinephrine (figure #4). Halothane alone and in

DOPAMINE

MESENCEPHALON

	ENDOGENOUS ug/gram	RATE CONSTANT	TURNOVER TIME	TURNOVER ng/g/hr
AWAKE CONTROL	0.333±.15	1.190	0.840	396
KETAMINE ANESTHESIA	0.151±.05	0.747	1.339	248
HALOTHANE ANESTHESIA	0.503±.08	1.520	0.657	506
NITROUS OXIDE ANESTHESIA	0.326±.04	1.410	0.709	470
HALOTHANE PLUS NITROUS OXIDE ANESTHESIA	1.010±.13	1.440	0.627	1450

TABLE #5

Table #5 Effects of ketamine (40 mg/kg) halothane (2%), nitrous oxide (40% in oxygen), and the combination of halothane (2%) plus nitrous oxide (40%) on mesencephalic dopamine. Endogenous content is expressed as the mean ± the standard deviation, the rate constant is the rate of disappearance of isotopic dopamine over time (also called the run off slope), turnover time is shown in fractions of hours, and turnover rate is expressed in nanograms per gram of brain (wet weight) per hour. The number of animals in the control group was 52; in each experimental group 16 animals were used.

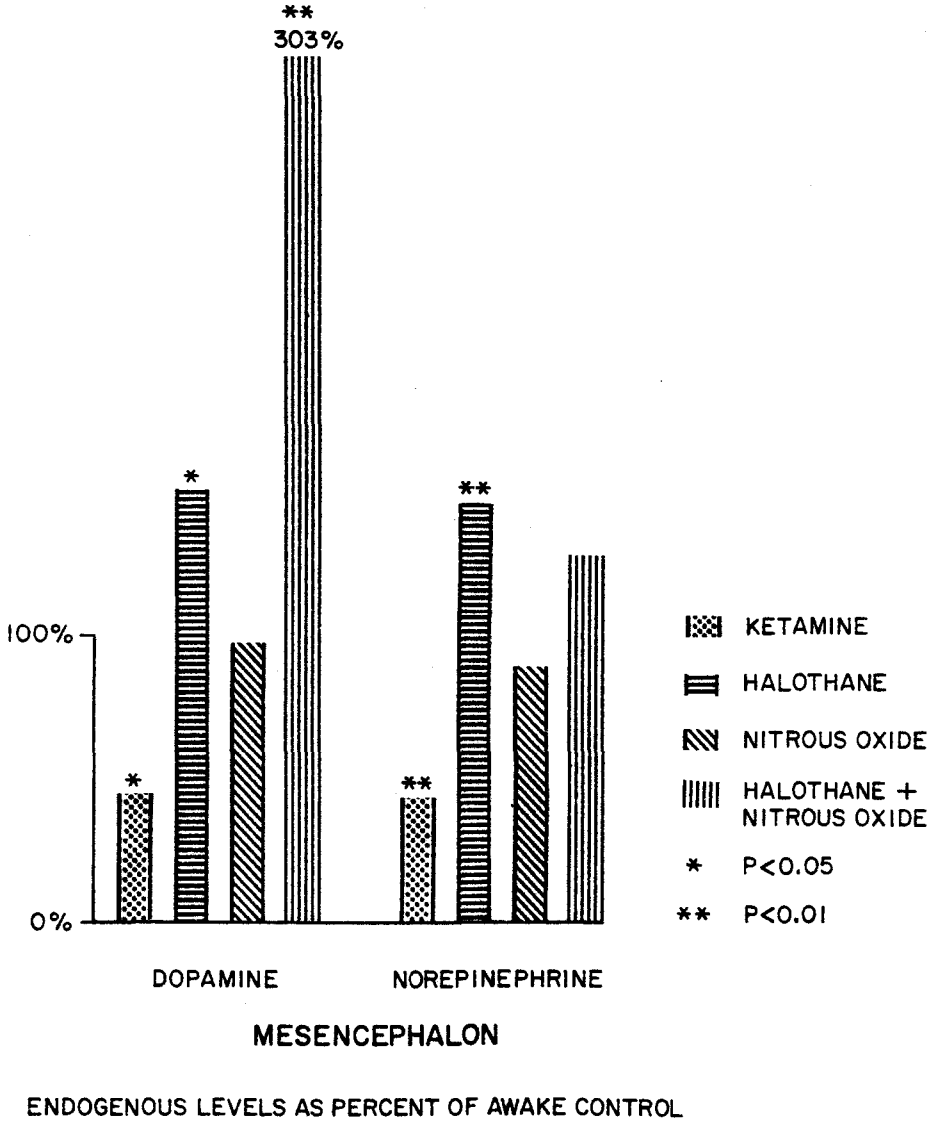

**
303%
*
**
100%
*
**
0%
KETAMINE
HALOTHANE
NITROUS OXIDE
HALOTHANE +
NITROUS OXIDE
* P<0.05
** P<0.01
DOPAMINE
NOREPINEPHRINE
MESENCEPHALON
ENDOGENOUS LEVELS AS PERCENT OF AWAKE CONTROL

Figure #4

Figure #4 Endogenous dopamine and norepinephrine effects in the mesencephalon graphed as a percent of awake control. Plots are shown for ketamine (40mg/kg), halothane (2%), nitrous oxide (40% in oxygen), and the combination of halothane (2%) plus nitrous oxide (40%). Statistical testing was accomplished by Student's T test and significance indicated in terms of probability (p).

NOREPINEPHRINE

MESENCEPHALON

	ENDOGENOUS ug/gram	RATE CONSTANT	TURNOVER TIME	TURNOVER ng/g/hr
AWAKE CONTROL	0.323±.08	0.929	1.076	300
KETAMINE ANESTHESIA	0.143±.06	0.625	1.625	202
HALOTHANE ANESTHESIA	0.467±.04	1.074	0.931	347
NITROUS OXIDE ANESTHESIA	0.289±.04	0.730	1.370	236
HALOTHANE PLUS NITROUS OXIDE ANESTHESIA	0.370±.06	0.806	1.240	260

TABLE # 6

Table #6 Norepinephrine effects by ketamine (40 mg/kg), halothane (2%), nitrous oxide (40% in oxygen), and the combination of halothane (2%) plus nitrous oxide (40%) in the mesencephalon. Endogenous content is expressed as the mean ± the standard deviation, the rate constant is the rate of disappearance of isotopic norepinephrine over time (ie. run off slope), turnover time is shown in fractions of hours, while turnover rate is expressed in nanograms per gram of brain (wet weight) per hour. The number of animals in the control group was 52; in each experimental group 16 animals were used.

combination with nitrous oxide resulted in a 45% ($p<0.01$) and 28% increase, respectively, in endogenous norepinephrine levels over control (figure #4).

Control dopamine turnover in the mesencephalon (table #5) was found to be 396 ng/g/hr. Ketamine reduced dopamine turnover significantly 37% ($p<0.01$). Halothane, nitrous oxide, and halothane-nitrous oxide in combination increased dopamine turnover. For either agent alone the effects were not as dramatic as when combined. Halothane alone resulted in a 28% increase and nitrous oxide alone resulted in a 19% increase in turnover. When used in combination the resulting increase was a significant ($p<0.01$) 266% increase (cf. table #5 and figure #5).

Norepinephrine turnover in the mesencephalon differed in pattern from that of dopamine. Halothane was the only agent that caused an increase in norepinephrine turnover (15%). Ketamine, nitrous oxide, halothane-nitrous oxide in combination all resulted in a decrease in norepinephrine turnover. Nitrous oxide resulted in a 21% decrease, halothane in combination with nitrous oxide resulted in a 13% decrease in turnover and ketamine caused a 33% decrease which was significant, $p<0.01$ (cf. figure #5).

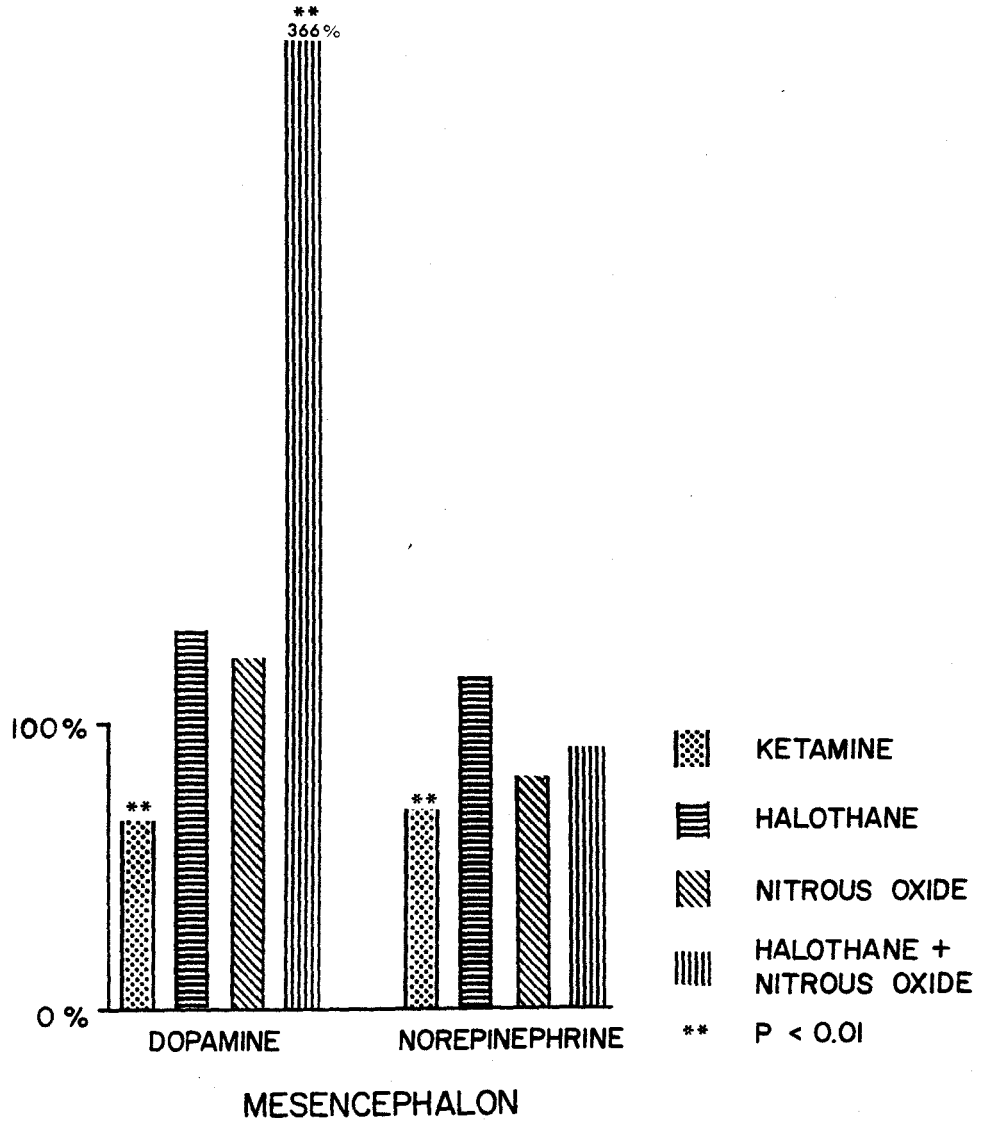

**
366%
100%
0 %
**
**
DOPAMINE
NOREPINEPHRINE
KETAMINE
HALOTHANE
NITROUS OXIDE
HALOTHANE +
NITROUS OXIDE
** P < 0.01
MESENCEPHALON
TURNOVER AS PERCENT OF AWAKE CONTROL

Figure #5

Figure #5 Anesthetic effects on mesencephalic dopamine and norepinephrine graphed as a percent of awake control. Data is shown for ketamine (40 mg/kg), halothane (2%), nitrous oxide (40% in oxygen) and the combination of halothane (2%) plus nitrous oxide (40%). Statistical comparisons to awake control were made by covariance analysis and are reported in terms of probability (p).

Summary of mesencephalic data:

1) Ketamine caused significant decreases in endogenous dopamine and norepinephrine content and turnover.
2) Halothane produced in a significant increase in endogenous dopamine and norepinephrine levels.
3) The combination of halothane plus nitrous oxide resulted in a significant increase in endogenous and turnover levels.

Comparison of Turnover and Endogenous Levels Between Anesthetics:

The second important goal encompassed by this dissertation is to compare the NE and DA data of each anesthetic agent to the data of all other anesthetic agents. This comparison will indicate the similarities and dissimilarities in change of amine metabolism between individual anesthetics.

When ketamine, halothane, nitrous oxide, and halothane plus nitrous oxide groups were compared to one another, all norepinephrine endogenous and turnover level comparisons were significantly different with one exception. Halothane vs. halothane plus nitrous oxide endogenous levels when tested were not significantly

different (table #7). In the case of dopamine for these same group comparisons, all endogenous levels were significantly different, however, only ketamine vs. halothane, nitrous oxide, and halothane plus nitrous oxide in combination resulted in significant differences for the dopamine turnover data (table #7).

MESENCEPHALON

ANESTHETIC GROUP	DOPAMINE Endogenous	DOPAMINE Turnover	NOREPINEPHRINE Endogenous	NOREPINEPHRINE Turnover
KET vs HAL	P<0.01	P<0.01	P<0.01	P<0.01
KET vs N_2O	P<0.01	P<0.01	P<0.01	P<0.01
KET vs HAL + N_2O	P<0.01	P<0.01	P<0.01	P<0.01
HAL vs N_2O	P<0.01	NS	P<0.01	P<0.01
HAL vs HAL + N_2O	P<0.01	NS	NS	P<0.01
N_2O vs HAL + N_2O	P<0.01	NS	P<0.01	P<0.01

KET = Ketamine HAL = Halothane N_2O = Nitrous Oxide

TABLE #7

Table #7 Statistical cross comparisons of endogenous levels (Student's T test used) and turnover rates (covariance analysis was used) for dopamine and norepinephrine in the mesencephalon. Significance is indicated in terms of probability (p); insignificant cross comparisons are indicated by NS. Cross comparisons for the anesthetic groups ketamine (40mg/kg), halothane (2%), nitrous oxide (40%), and the halothane-nitrous oxide combination are shown.

Thalamus

Turnover and Endogenous Levels as Compared to Awake Control:

Control dopamine turnover in the thalamus was found to be 422 ng/g/hr (table #8). Ketamine and nitrous oxide cause dopamine turnover to slow to 361 ng/g/hr and 388 ng/g/hr, respectively. This 14% and 8% drop in dopamine turnover was not significant (figure #6). Halothane alone resulted in an increase in dopamine turnover to 517 ng/g/hr (23%; $p<0.01$). When halothane-nitrous oxide combination was used, no significant effects were found, seemingly a cancellation of halothane's increase with nitrous oxide's decrease.

Noradrenergic turnover was affected to a greater extent than was the dopamine turnover. Control norepinephrine turnover was found to be 146 ng/g/hr (table #9). Ketamine had the least effect on thalamic turnover. Turnover accelerated from 146 ng/g/hr to 156 ng/g/hr or 7% (figure #6). Both halothane and nitrous oxide caused large increases in norepinephrine turnover in this brain area. Nitrous oxide resulted in a 28% increase, while halothane caused a 154% rise in turnover (i.e., 146 ng/g/hr to 371 ng/g/hr; table #9). When these agents were used in combination, proportionately

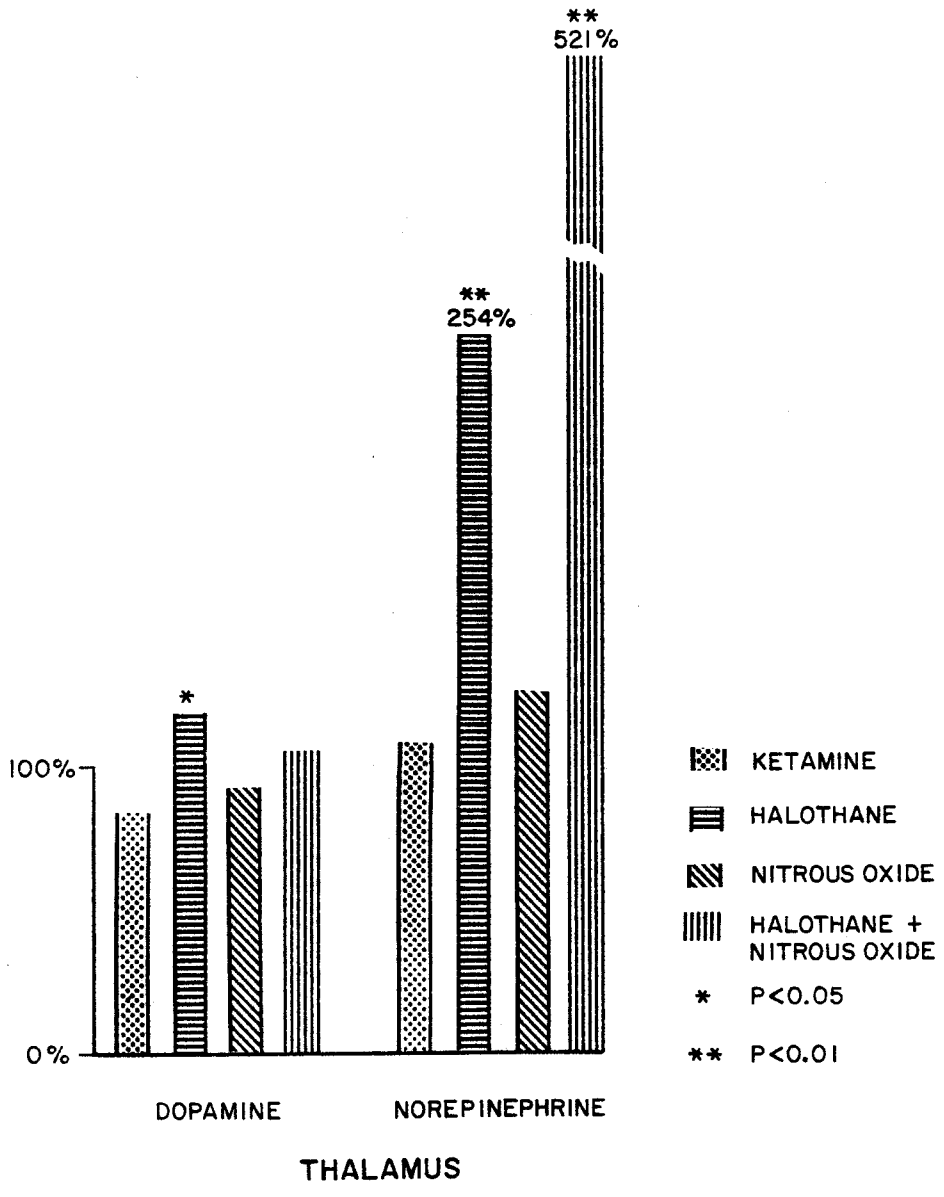
DOPAMINE

THALAMUS

	ENDOGENOUS ug/gram	RATE CONSTANT	TURNOVER TIME	TURNOVER ng/g/hr
AWAKE CONTROL	0.380±.16	1.110	0.900	422
KETAMINE ANESTHESIA	0.238±.04	0.951	1.051	361
HALOTHANE ANESTHESIA	0.364±.06	1.360	0.735	517
NITROUS OXIDE ANESTHESIA	0.286±.04	1.020	0.980	388
HALOTHANE PLUS NITROUS OXIDE ANESTHESIA	1.092±.13	1.180	0.847	448

TABLE # 8

Table #8 Effects of ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%) and the combination of halothane-nitrous oxide on thalamic dopamine. Endogenous content is expressed as the mean $\pm$ the standard deviation, the rate constant is the rate of disappearance of ^{14}C-dopamine over time (i.e., run off slope), turnover time is shown in fractions of hours and turnover rate is expressed in nanograms per gram of brain (wet weight) per hour. The number of animals in the control group was 52; in each experimental group 16 animals were used.



THALAMUS

TURNOVER AS PERCENT OF AWAKE CONTROL

Figure #6

Figure #6 Anesthetic effects on thalamic dopamine and norepinephrine turnover graphed as a percent of awake control. Data is shown for ketamine (40 mg/kg), halothane (2%), nitrous oxide (40% in oxygen), and the combination of halothane (2%) plus nitrous oxide (40%). Statistical comparisons to awake control were made by covariance analysis and are indicated in terms of probability (p).

NOREPINEPHRINE
THALAMUS

	ENDOGENOUS ug/gram	RATE CONSTANT	TURNOVER TIME	TURNOVER ng/g/hr
AWAKE CONTROL	0.377±.14	0.386	2.591	146
KETAMINE ANESTHESIA	0.185±.05	0.413	2.421	156
HALOTHANE ANESTHESIA	0.463±.04	0.983	1.017	371
NITROUS OXIDE ANESTHESIA	0.308±.04	0.497	2.012	187
HALOTHANE PLUS NITROUS OXIDE ANESTHESIA	0.370±.07	2.020	0.495	761

TABLE # 9

Table #9 Norepinephrine effects by ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%) and the combination of halothane - nitrous oxide in the thalamus. Endogenous content is expressed as the mean ± the standard deviation, the rate constant is the rate of disappearance of ^{14}C-norepinephrine over time (ie. run off slope), turnover time is shown in fractions of hours, while turnover rate is expressed in nanograms per gram of brain (wet weight) per hour. The number of animals in the control group was 52; in each experimental group 16 animals were used.

a larger than additive increase in norepinephrine turnover resulted. The resultant increase was to 761 ng/g/hr or a highly significant 421% increase over control (figure #6).

For the awake control, thalamic endogenous dopamine was found to be 0.380 ug/gram of tissue (table #8). Administration of ketamine caused endogenous dopamine levels to decrease from 0.380 to 0.238 ug/gram or 37% (table #8 and figure #7). This did not prove to be significant. Halothane and nitrous oxide separately also had no significant effect on dopamine endogenous levels. However, when in combination, halothane and nitrous oxide resulted in a dramatic rise in endogenous dopamine. The level rose from 0.380 ug/g control to 1.092 ug/g, a 187% increase (cf. table #8 and figure #7). This finding is strikingly similar to that found in the mesencephalon.

Endogenous norepinephrine levels in the thalamus were found to be 0.377 ug/g. Halothane anesthesia resulted in a 22% rise in norepinephrine (table #9, figure #7). Nitrous oxide, on the other hand, caused an 18% fall in the amine content. These two anesthetic agents, when combined, seemed to be additive resulting in a net cancellation of the individual effects. The endogenous norepinephrine level with the halothane and

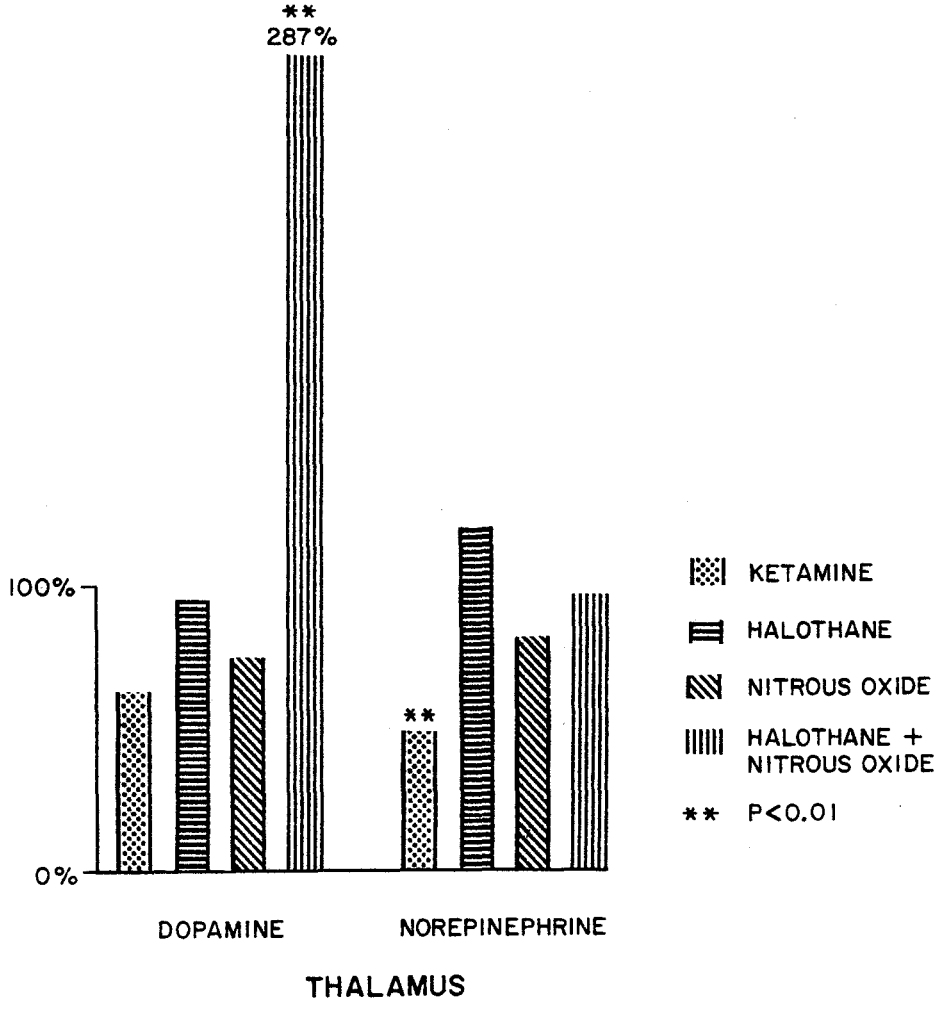

**
287%
100%
0%
**
KETAMINE
HALOTHANE
NITROUS OXIDE
HALOTHANE +
NITROUS OXIDE
** P<0.01
DOPAMINE
NOREPINEPHRINE
THALAMUS
ENDOGENOUS LEVELS AS PERCENT OF AWAKE CONTROL

Figure #7

Figure #7 Effects on endogenous dopamine and norepinephrine in the thalamus graphed as a percent of awake control. Plots are shown for ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%) and the combination of halothane (2%) plus nitrous oxide (40%). Statistical testing was accomplished by Student's T test and significance indicated in terms of probability (p).

nitrous oxide combination was 0.370 ug/g which was a 2% change from control (cf. table #9 and figure #7). In the case of ketamine, a 51% ($p<0.01$) decrease in norepinephrine from control occurred.

Summary of thalamic data:

1) Halothane increased norepinephrine and dopamine turnover.
2) Halothane plus nitrous oxide increased norepinephrine turnover and increased dopamine endogenous levels.
3) Ketamine decreased endogenous norepinrphrine levels.

Comparison of Turnover and Endogenous Levels Between Anesthetics:

When the groups ketamine, halothane, nitrous oxide, and halothane-nitrous oxide were compared to one another to examine their likeness and dissimilarities it was found that all comparisons were significantly different in the case of both dopamine and norepinephrine endogenous data. Only two group comparisons, ketamine vs. halothane and halothane vs. nitrous oxide, were found to be significantly different for the dopamine turnover. For norepinephrine turnover, all groups were again significant except for ketamine vs. nitrous oxide

(table #10).

THALAMUS

ANESTHETIC GROUP	DOPAMINE Endogenous	DOPAMINE Turnover	NOREPINEPHRINE Endogenous	NOREPINEPHRINE Turnover
KET vs HAL	P<0.01	P<0.01	P<0.01	P<0.01
KET vs N_2O	P<0.05	NS	P<0.01	NS
KET vs HAL + N_2O	P<0.01	NS	P<0.01	P<0.01
HAL vs N_2O	P<0.05	P<0.05	P<0.01	P<0.01
HAL vs HAL + N_2O	P<0.01	NS	P<0.01	P<0.01
N_2O vs HAL + N_2O	P<0.01	NS	P<0.05	P<0.01

KET = Ketamine HAL = Halothane N_2O = Nitrous Oxide

TABLE # 10

Table #10 Statistical cross comparisons of endogenous levels (Student's T test was used) and turnover rates (covariance analysis was used) for dopamine and norepinephrine in the thalamus. Significance is indicated in terms of probability (p); insignificant cross comparisons are indicated by NS. Cross comparisons for the anesthetic groups ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%) and halothane-nitrous oxide are shown.

Hypothalamus

Turnover and Endogenous Levels as Compared to Awake Control:

In the hypothalamus the majority of the changes seen in catecholamine metabolism as a result of the anesthetic agents tested were found to be dopaminergic. Control dopamine turnover in the hypothalamus, shown in table #11, was 535 ng/g/hr. Ketamine, halothane, nitrous oxide, and halothane-nitrous oxide in combination resulted in a significant increase over the control level. Ketamine and nitrous oxide raised hypothalamic dopamine turnover 39% and 32% ($p<0.01$) respectively (cf. figure #8). Halothane and halothane-nitrous oxide resulted in larger increases in dopamine turnover, 89% ($p<0.01$) for halothane alone and 162% ($p<0.01$) for halothane in combination with nitrous oxide (cf. figure #8).

Relative to dopamine, norepinephrine turnover (shown in table #12) was essentially unaffected in the hypothalamus by each of the anesthetic agents. Awake control norepinephrine turnover was found to be 889 ng/g/hr. Ketamine anesthesia resulted in a 30% drop in norepinephrine turnover while halothane and nitrous oxide caused a 32% and 31% decrease respectively when

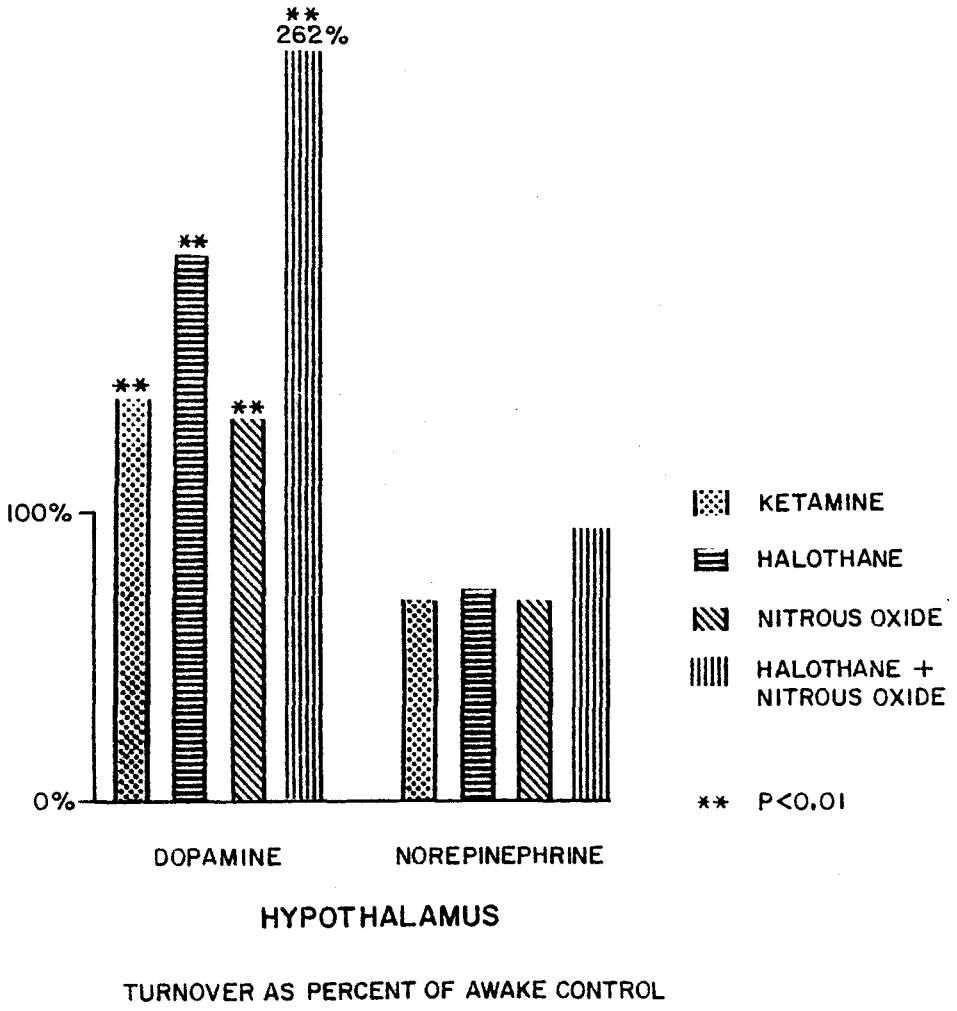
DOPAMINE

HYPOTHALAMUS

	ENDOGENOUS ug/gram	RATE CONSTANT	TURNOVER TIME	TURNOVER ng/g/hr
AWAKE CONTROL	0.601±.19	0.890	1.124	535
KETAMINE ANESTHESIA	0.309±.06	1.236	0.809	743
HALOTHANE ANESTHESIA	1.154±.19	1.648	0.607	1012
NITROUS OXIDE ANESTHESIA	0.828±.13	1.175	0.851	706
HALOTHANE PLUS NITROUS OXIDE ANESTHESIA	2.070±.22	2.330	0.429	1400

TABLE # 11

Table #11 Effects of ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%) and the combination of halothane-nitrous oxide on hypothalamic dopamine. Endogenous content is expressed as the mean ± the standard deviation, the rate constant is the rate of disappearance of ^{14}C-dopamine over time (i.e., run off slope), turnover rate is expressed in nanograms per gram of brain (wet weight) per hour. The number of animals in the control group was 52; in each experimental group 16 animals were used.



HYPOTHALAMUS

TURNOVER AS PERCENT OF AWAKE CONTROL

Figure #8

Figure #8 Hypothalamic effects on dopamine and norepinephrine turnover expressed as a percent of awake control. Data is shown for ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%) and the combination of halothane (2%) plus nitrous oxide (40%). Statistical comparisons to awake control were made by covariance analysis and are indicated in terms of probability (p).

NOREPINEPHRINE
HYPOTHALAMUS

	ENDOGENOUS ug/gram	RATE CONSTANT	TURNOVER TIME	TURNOVER ng/g/hr
AWAKE CONTROL	0.823±.20	1.080	0.926	889
KETAMINE ANESTHESIA	0.293±.02	0.751	1.332	618
HALOTHANE ANESTHESIA	0.910±.08	0.735	1.361	605
NITROUS OXIDE ANESTHESIA	0.661±.06	0.745	1.342	613
HALOTHANE PLUS NITROUS OXIDE ANESTHESIA	0.742±.10	1.030	0.971	848

TABLE # 12

Table #12 Effects on norepinephrine by ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%) and the combination of halothane (2%) plus nitrous oxide (40%) in the hypothalamus. Endogenous content is expressed as the mean ± the standard deviation, the rate constant is the rate of disappearance of the ^{14}C-norepinephrine over time (ie. the run off slope), turnover time is shown in fractions of hours, and turnover rate is expressed in nanograms per gram of brain (wet weight) per hour. The number of animals in the control group was 52; in each experimental group 16 animals were used.

compared to awake control. Halothane when used in conjunction with nitrous oxide had little effect. A 5% drop in NE turnover was seen (cf. figure #8).

The mean control endogenous dopamine level in the hypothalamus was found to be 0.601 ug/g of tissue (table #11). Ketamine, the only treatment group to lower endogenous dopamine levels, resulted in a mean value of 0.309 ug/g or a 49% decrease (figure #9). Halothane and nitrous oxide alone and in combination resulted in dramatic increases in endogenous dopamine levels. A 92% ($p<0.05$) increase (0.601 ug/g to 1.154 ug/g; table #11) occurred with halothane anesthesia (figure #9). Nitrous oxide increased dopamine levels 38% over control. This effect is seen in other brain areas. Nitrous oxide in combination with halothane significantly increased endogenous dopamine levels to 2.07 ug/g (table #11) or a 245% increase over control (cf. figure #9).

Norepinephrine endogenous levels in the hypothalamus were generally unaffected by the anesthetic agents tested. Ketamine, the only anesthetic to have significant effect upon endogenous norepinephrine levels resulted in a 64% drop from control (0.823 ug/g to 0.293 ug/g; cf. table #12 and figure #9). In the case of halothane a nonsignificant 11% increase in endogenous

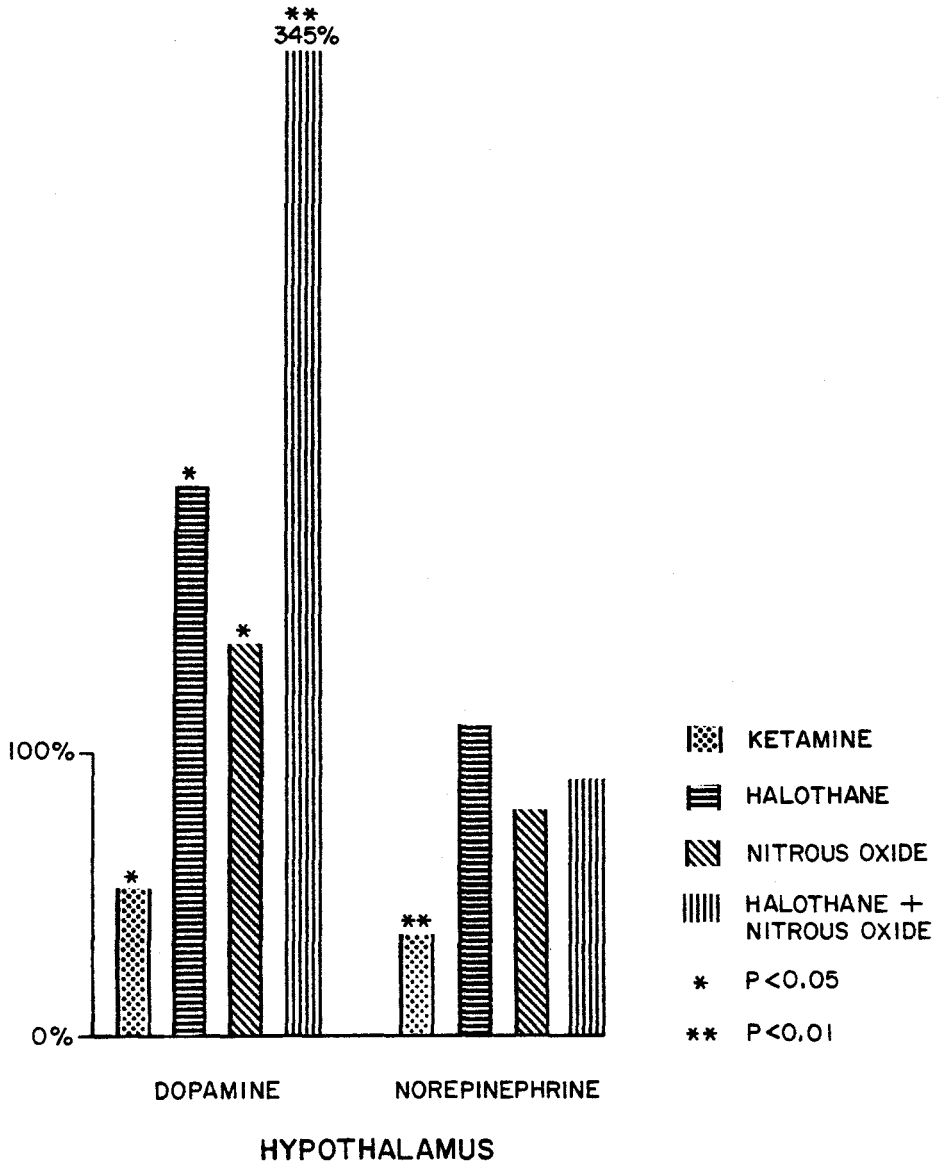

**
345%
*
*
*
100%
0%
**
KETAMINE
HALOTHANE
NITROUS OXIDE
HALOTHANE + NITROUS OXIDE
* P<0.05
** P<0.01
DOPAMINE
NOREPINEPHRINE
HYPOTHALAMUS
ENDOGENOUS LEVELS AS PERCENT OF AWAKE CONTROL

Figure #9

Figure #9 Effects on endogenous dopamine and norepinephrine in the hypothalamus graphed as a percent of awake control. Plots are shown for ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%) and the combination halothane - nitrous oxide. Statistical testing was accomplished by Student's T test and significance indicated in terms of probability (p).

norepinephrine levels resulted (see table #12 and figure #9) while with nitrous oxide a 20% decrease was found. When the two agents were used in combination the effect resultant was a nonsignificant 10% drop in endogenous norepinephrine levels.

Summary of hypothalamic data:

1) Ketamine resulted in a significant decrease in both dopamine and norepinephrine endogenouse levels. Dopamine turnover was significantly increased.
2) Halothane significantly increased both turnover and endogenous dopamine levels.
3) Nitrous oxide increased dopamine turnover and endogenous levels.
4) The combination of halothane-nitrous oxide resulted in increased turnover and endogenous dopamine levels.

Comparison of Turnover and Endogenous Levels Between Anesthetics:

When the groups ketamine, halothane, nitrous oxide, and halothane-nitrous oxide were compared to one another to examine their likenesses and dissimilarities, it was found that for both dopamine and norepinephrine all endogenous level comparisons were found to be

significantly different from one another except for nitrous oxide vs. halothane-nitrous oxide, norepinephrine only. For dopamine turnover all comparisons were significant except for ketamine vs. nitrous oxide. For norepinephrine turnover distinct alterations of metabolism appeared only for the halothane-nitrous oxide group when compared to either halothane or nitrous oxide (cf. table #13).

HYPOTHALAMUS

ANESTHETIC GROUP	DOPAMINE Endogenous	DOPAMINE Turnover	NOREPINEPHRINE Endogenous	NOREPINEPHRINE Turnover
KET vs HAL	P<0.01	P<0.01	P<0.01	NS
KET vs N_2O	P<0.01	NS	P<0.01	NS
KET vs HAL + N_2O	P<0.01	P<0.01	P<0.01	NS
HAL vs N_2O	P<0.01	P<0.01	P<0.01	NS
HAL vs HAL + N_2O	P<0.01	P<0.01	P<0.01	P<0.05
N_2O vs HAL + N_2O	P<0.01	P<0.01	NS	P<0.05

KET = Ketamine HAL = Halothane N_2O = Nitrous Oxide

TABLE # 13

Table #13 Statistical cross comparison of endogenous levels (Student's T test was used) and turnover rates (covariance analysis was used) for dopamine and norepinephrine in the hypothalamus. Significance is indicated in terms of probability (p); insigifnicant cross comparisons are indicated by NS. Cross comparisons for the anesthetic groups ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%) and the combination of halothane (2%) plus nitrous oxide (40%) in combination are shown.

Cortex

Turnover and Endogenous Levels as Compared to Awake Control:

Control dopaminergic turnover in the cortex was found to be 112 ng/g/hr (table #14). Ketamine, which caused a decrease in dopamine turnover in the mesencephalon and thalamus, also caused a decrease in the cortex. Dopamine turnover in the ketamine group was found to be 72 ng/g/hr, which is a significant 36% decrease (cf. table #14 and Figure #10). Halothane also decreased dopamine turnover. A dramatic drop from 112 ng/g/hr was seen. This was a significant 76% drop as is shown in figure #10. Nitrous oxide had little effect on dopamine turnover, however, in combination with halothane, dopamine turnover increased to 258 ng/g/hr, a marked 111% increase over control (cf. figure #10 and table #14).

Dramatic changes were seen in cortical norepinephrine turnover by all anesthetics. Awake control norepinephrine turnover shown in table #13 was 80 ng/g/hr. Ketamine, halothane, nitrous oxide, and halothane-nitrous oxide (seen in figure #10) resulted in increased turnover levels from awake control. Ketamine caused a 120% increase to 175 ng/g/hr. Halothane and nitrous

DOPAMINE

CORTEX

	ENDOGENOUS ug/gram	RATE CONSTANT	TURNOVER TIME	TURNOVER ng/g/hr
AWAKE CONTROL	0.105±.10	1.070	0.935	112
KETAMINE ANESTHESIA	0.079±.04	0.689	1.451	72
HALOTHANE ANESTHESIA	0.184±.03	0.259	3.860	27
NITROUS OXIDE ANESTHESIA	0.133±.02	1.030	0.971	108
HALOTHANE PLUS NITROUS OXIDE ANESTHESIA	0.362±.04	0.712	1.404	258

TABLE # 14

Table #14 Effect of ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%), and the combination of halothane-nitrous oxide on cortical dopamine. Endogenous content is expressed as the mean ± the standard deviation, the rate constant is the rate of disappearance of ^{14}C-dopamine over time (i.e., the run off slope), turnover rate is expressed in nanograms per gram of brain (wet weight) per hour. The number of animals in the control group was 52; in each experimental group 16 animals were used.

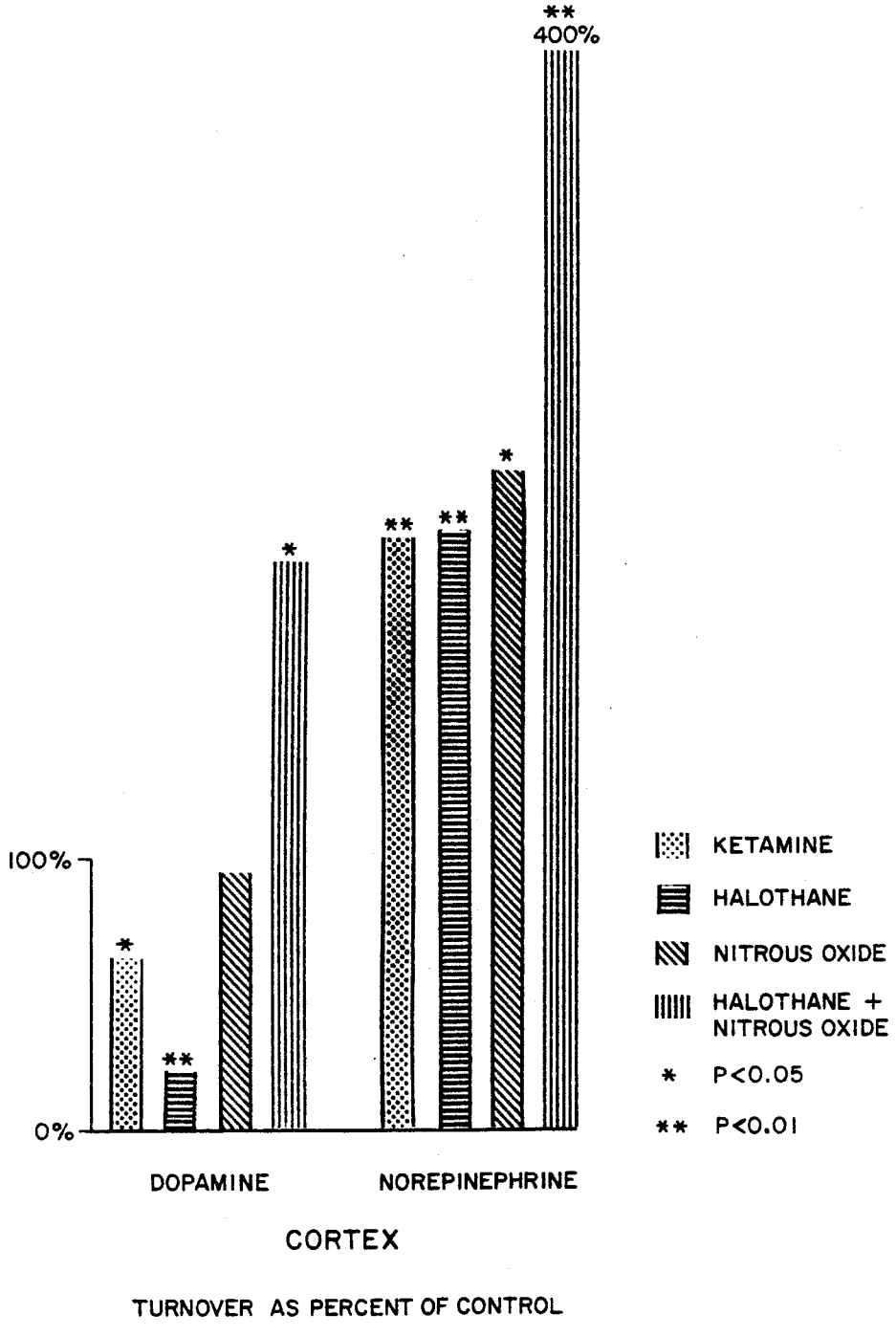

**
400%
100%
0%
*
**
DOPAMINE
NOREPINEPHRINE
CORTEX
TURNOVER AS PERCENT OF CONTROL
KETAMINE
HALOTHANE
NITROUS OXIDE
HALOTHANE + NITROUS OXIDE
* P<0.05
** P<0.01

Figure #10

Figure #10 Cortical effects on dopamine and norepinephrine turnover expressed as a percent of awake control. Bar graphs are shown for ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%) and the combination halothane - nitrous oxide. Statistical comparisons to awake control were made by covariance analysis and are indicated in terms of probability (p).

oxide increased norepinephrine turnover 122% and a 145%, respectively (cf. table #15 and figure #10). When nitrous oxide and halothane were used in combination, turnover increased 300% ($p<0.01$, figure #10).

For the awake control, cortical endogenous dopamine was found to be 0.105 ug/gram of tissue (table #14). Ketamine was the only anesthetic to lower dopamine endogenous levels in the cortex, and this was a nonsignificant 25% decrease (cf. table #14 and figure #11). On the other hand, halothane, nitrous oxide and the two agents in combination increased cortical dopamine endogenous levels, halothane alone increased dopamine from 0.105 ug/g control to 0.184 ug/g, a 75% increase ($p<0.01$). Nitrous oxide alone effected a 22% increase in dopamine levels. Most significant was the finding that nitrous oxide in combination with halothane produced a dramatic 245% increase in dopamine level (figure #11).

Awake control endogenous norepinephrine levels in the cortex were found to be 0.160 ug/gram of tissue. As was demonstrated in the other three brain areas, ketamine caused a significant decrease in endogenous norepinephrine level. As seen in table #15 ketamine anesthesia resulted in a significant fall to 0.075 ug/gram of tissue, a 54% drop (figure #11). Halothane

NOREPINEPHRINE

CORTEX

	ENDOGENOUS ug/gram	RATE CONSTANT	TURNOVER TIME	TURNOVER ng/g/hr
AWAKE CONTROL	0.160±.07	0.498	2.001	80
KETAMINE ANESTHESIA	0.075±.01	1.093	0.915	175
HALOTHANE ANESTHESIA	0.255±.02	1.109	0.902	177
NITROUS OXIDE ANESTHESIA	0.186±.03	1.210	0.826	194
HALOTHANE PLUS NITROUS OXIDE ANESTHESIA	0.196±	2.002	0.500	320

TABLE # 15

Table #15 Effect of ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%), and the combination of halothane-nitrous oxide on cortical dopamine. Endogenous content is expressed as the mean ± the standard deviation, the rate constant is the rate of disappearance of ^{14}C-dopamine over time (i.e., the run off slope), turnover rate is expressed in nanograms per gram of brain (wet weight) per hour. The number of animals in the control group was 52; in each experimental group 16 animals were used.

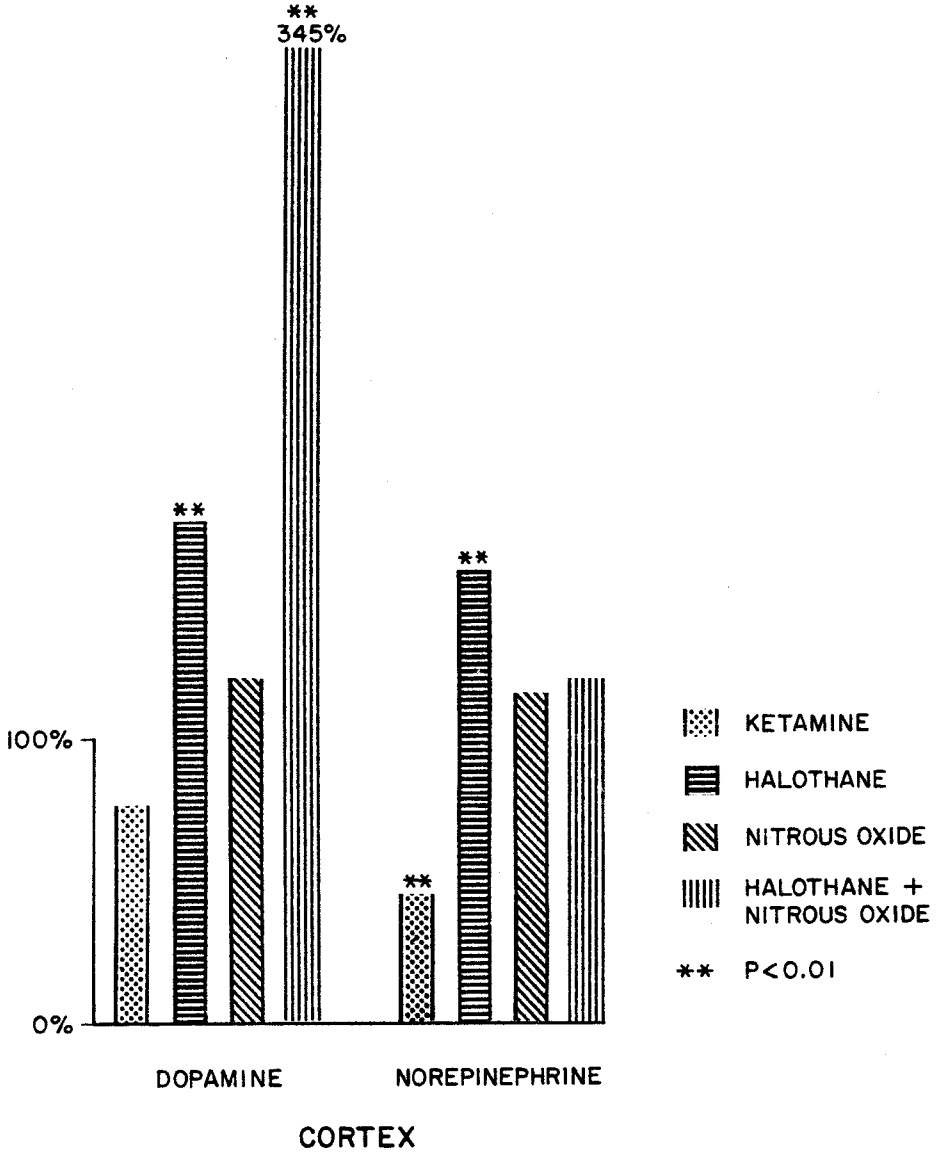

**
345%
**
**
**
100%
0%
KETAMINE
HALOTHANE
NITROUS OXIDE
HALOTHANE +
NITROUS OXIDE
** P<0.01
DOPAMINE
NOREPINEPHRINE
CORTEX
ENDOGENOUS AS PERCENT OF CONTROL

Figure #11

Figure #11 Effects on endogenous dopamine and norepinephrine in the cortex graphed as a percent of awake control. Plots are shown for ketamine (40 mg/kg), halothane (2%), nitrous oxide (40%), and the combination halothane - nitrous oxide. Statistical testing was accomplished by Student's T test and significance indicated in terms of probability (p).

alone increased endogenous norepinephrine levels a significant 59%. Nitrous oxide alone did not significantly change endogenous cortical norepinephrine levels. The use of nitrous oxide in combination with halothane increased the endogenous norepinephrine levels from 0.160 control, to 0.196 ug/g, a 23% increase.

Summary of cortical data:

1) Ketamine decreased dopamine turnover and increased norepinephrine turnover. Norepinephrine endogenous levels were increased.
2) Halothane increased norepinephrine turnover and endogenous levels and dopamine endogenous levels. Dopamine turnover was decreased.
3) Nitrous oxide increased only norepinephrine endogenous levels.
4) The combination of halothane-nitrous oxide increased dopamine and norepinephrine turnover. Dopamine endogenous levels were also significantly increased.

Comparison of Turnover and Endogenous Levels Between Anesthetics:

When ketamine, halothane, nitrous oxide, and halothane-nitrous oxide groups were compared to one another, all dopamine endogenous and turnover level

comparisons were significantly different with the exception of ketamine vs. halothane-nitrous oxide, turnover only (table #16). In the case of dopamine for these same group comparison, all endogenous data was significantly different except for nitrous oxide vs. halothane-nitrous oxide. With respect to the norepinephrine comparisons, only ketamine, halothane, and nitrous oxide vs. halothane-nitrous oxide were found significant.

Halothane Recovery:

When turnover and endogenous levels were assayed, in animals allowed to recover for two hours post halothane anesthesia, it was found that dopamine turnover in the mesencephalon, thalamus, hypothalamus, and cortex returned to levels 16%, 17%, 6%, 2% different from awake control, respectively. None of these values demonstrated a significant difference from control (cf. figure #12).

In the case of norepinephrine, mesencephalic turnover returned to a level 9% below control, while hypothalamic levels returned to a level 5% above control. A 45% decrease in turnover was demonstrated for norepinephrine turnover in the thalamus, however, this decrease was not significant when compared to awake control. The cortex showed a 101% increase in norepinephrine turnover ($p<0.01$) post anesthesia. This finding is similar to that effect seen for all anesthetics studied.

CORTEX

ANESTHETIC GROUP	DOPAMINE Endogenous	DOPAMINE Turnover	NOREPINEPHRINE Endogenous	NOREPINEPHRINE Turnover
KET vs HAL	P<0.01	P<0.01	P<0.01	NS
KET vs N_2O	P<0.01	P<0.01	P<0.01	NS
KET vs HAL + N_2O	P<0.01	NS	P<0.01	P<0.01
HAL vs N_2O	P<0.01	P<0.01	P<0.01	NS
HAL vs HAL + N_2O	P<0.01	P<0.01	P<0.01	P<0.01
N_2O vs HAL + N_2O	P<0.01	P<0.01	NS	P<0.01

KET = Ketamine HAL = Halothane N_2O = Nitrous Oxide

TABLE # 16

Table #16 Statistical cross comparisons of endogenous levels (Student's T test was used) and turnover rates (covariance analysis was used) for dopamine and norepinephrine in the cortex. Significance is indicated in terms of probability (p); insignificant cross comparisons are indicated by NS. Cross comparisons for the anesthetic groups ketamine (40mg/kg), halothane (2%), nitrous oxide (40%), and the combination halothane (2%) plus nitrous oxide (40%) are shown.

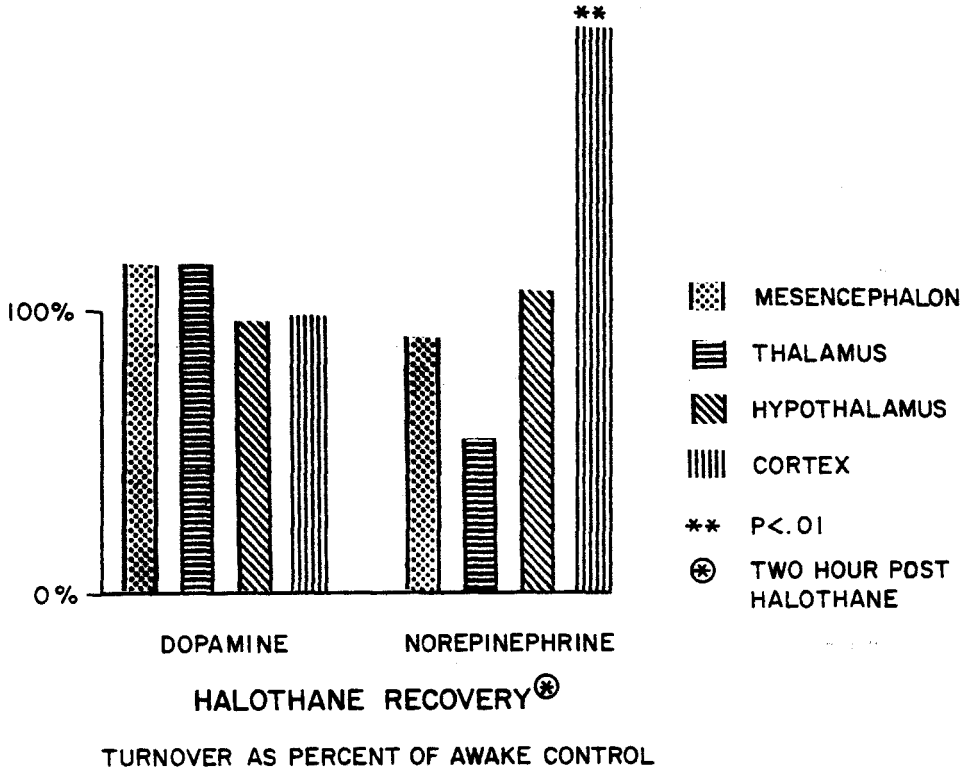

**
100%
0%
DOPAMINE
NOREPINEPHRINE
MESENCEPHALON
THALAMUS
HYPOTHALAMUS
CORTEX
** P<.01
⊛ TWO HOUR POST HALOTHANE
HALOTHANE RECOVERY⊛
TURNOVER AS PERCENT OF AWAKE CONTROL

Figure #12

Figure #12 Effects on dopamine and norepinephrine turnover levels in the mesencephalon, thalamus,hypothalamus, and cortex two hours post halothane anesthesia graphed as a percent of awake control. Statistical comparisons to awake control were made by covariance analysis and reported in terms of probability (p). Sixteen animals were used for the halothane recovery group.

With regard to endogenous levels, only the mesencephalon and cortex showed significant changes in dopamine when compared to control levels. The mesencephalon showed a 51% increase ($p<0.05$) while the cortex showed a 93% increase ($p<0.01$). All brain areas studied were found to have significant decreases in norepinephrine levels post anesthesia. These decreases ranged from 63% to 73% of control (cf. figure #13).

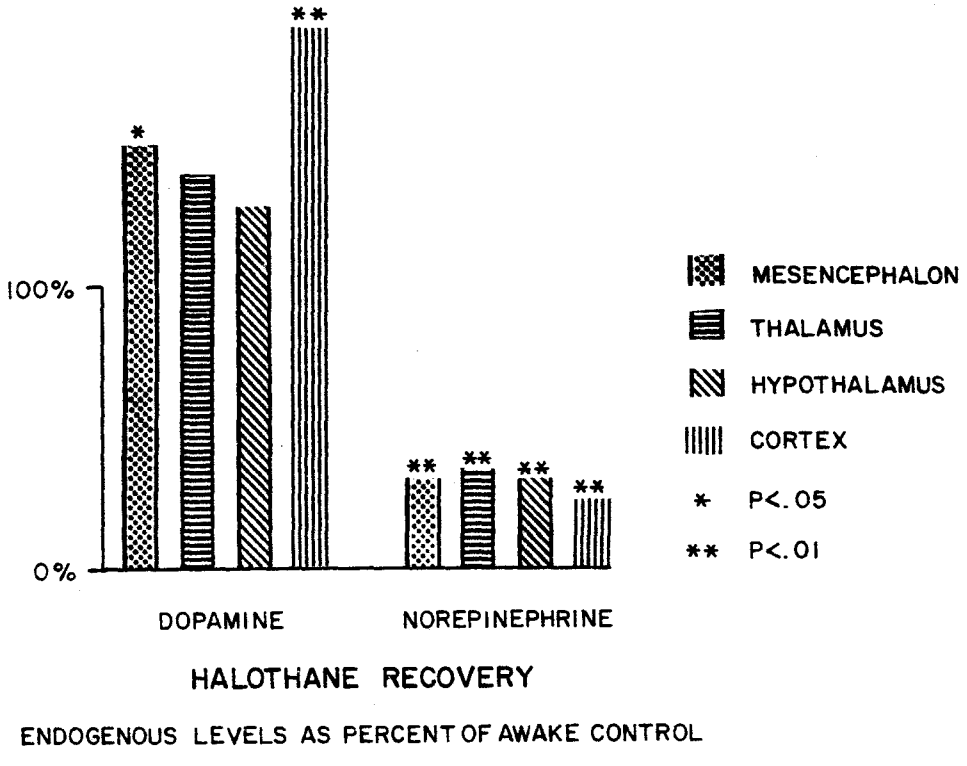

100%
0%
*
**
**
**
**
**
DOPAMINE
NOREPINEPHRINE
MESENCEPHALON
THALAMUS
HYPOTHALAMUS
CORTEX
* P<.05
** P<.01
HALOTHANE RECOVERY
ENDOGENOUS LEVELS AS PERCENT OF AWAKE CONTROL

Figure #13

Figure #13 Effects on dopamine and norepinephrine endogenous levels in the mesencephalon, thalamus, hypothalamus, and cortex two hours post halothane anesthesia graphed as a percent of awake control. Statistical comparisons were made by Student's T test and reported in terms of probability (p). Sixteen animals were used for the halothane recovery group.

CHAPTER IV

DISCUSSION

The mechanism by which anesthetic agents produce central nervous system anesthesia has been formally queried since the late eighteen hundreds when Meyer (1899) and Overton (1901) first proposed the lipid theory of anesthesia. Adding great impetus to this search over the years has been the belief that knowing the central mechanism of anesthesia would allow the tailoring of a more perfect anesthetic agent. Unfortunately, a careful examination of the research findings has left more questions unanswered than answered.

Two basic approaches to the research of anesthesia have evolved. The first, micromolecular, is limited in scope to the molecular interaction of anesthetics within the cell. Theories arising from these works have generally tied the primary anesthetic effect to a direct action on nerve cell components. These theories have been discussed at length in the Introduction of this dissertation under the Biophysical Anesthetic Mechanism.

The second approach, macromolecular, considers

that anesthetics affect the central synapses. Of importance in this concept is how anesthetics affect the central transmitter systems. Since comprehensive investigation into the effect of anesthetic agents on central dopaminergic and noradrenergic nerve activity had not been carried out, the aim of this study was to investigate anesthetic action in the central adrenergic nervous system with particular attention to the following questions:

1) Is the central adrenergic nervous system affected by anesthetic agents as determined by adrenergic amine turnover?
2) If affected, are neurons of the same type (i.e., dopamine or norepinephrine) affected in an identical manner in all brain regions?
3) Do clinically different anesthetics have different effects centrally on adrenergic neurotransmitters?

To answer these questions three clinically different anesthetics, ketamine, halothane, and nitrous oxide as well as the combination of halothane-nitrous oxide were studied to determine their effect on dopamine and norepinephrine turnover in four brain areas. The turnover data was used as an index of neuronal activity for these two adrenergic transmitter systems

(Costa and Neff, 1970).

With respect to the above three questions the results of this study provide the following answers and allow several conclusions to be drawn.

Is the central adrenergic nervous system affected by anesthetic agents as determined by adrenergic turnover?

Simply summarized, the turnover results from this study show that ketamine anesthesia is associated with significant dopamine decreases in the mesencephalon and cortex with an increase in the hypothalamus; halothane caused similar dopamine changes in the hypothalamus and cortex but differed in that a significant increase in thalamic dopamine turnover was found with no change seen in the mesencephalon. Nitrous oxide results were markedly different than those produced by ketamine and halothane in that only one area was affected (i.e., an increase in the hypothalamus). Combination halothane plus nitrous oxide resulted in significant increases in dopamine turnover in the mesencephalon, hypothalamus, and cortex or three out of four areas studied.

For norepinephrine, ketamine was associated with an increased turnover in the cortex and a decreased turnover in the mesencephalon. Halothane influenced norepinephrine turnover similarly in the cortex, did

not produce any changes in the mesencephalon, as did ketamine, and increased norepinephrine turnover in the thalamus. Nitrous oxide affected norepinephrine only in the cortex (an increase). The combination of halothane-nitrous oxide resulted in significant increases in the thalamus and cortex.

These data indicate that anesthesia by ketamine, halothane, nitrous oxide and the combination of halothane and nitrous oxide is associated with altered dopamine and norepinephrine turnover when compared to awake control.

Importantly, these results suggest why conflicting findings on whole brain were reported. With ketamine anesthesia, Biggio et al. (1974) suggested an increased dopamine turnover while Sung et al. (1973) reported a decrease. Both research groups used whole brain samples. Our ketamine data clearly shows both an increase (hypothalamus) and decrease (mesencephalon and cortex) in dopamine turnover. With respect to halothane, our data generally support findings of Nikki et al. (1971) and refute that of Anden et al. (1974) and Ngai et al. (1969a), which reported halothane to have no effect on dopamine and norepinephrine turnover in whole brain. Our findings further suggest that individual dopamine and norepinephrine transmitter systems operate independently of one another as both systems demonstrated distinctly different turnover changes

in conjunction with the same anesthetic agent. This is demonstrated by the finding that both ketamine and halothane resulted in decreased cortical dopamine turnover, while both increased norepinephrine turnover. Similar results were obtained by Biggio et al. (1974) and Nikki et al. (1971).

Endogenous amine results obtained in this study also support involvement of the adrenergic system with anesthesia (significant change was observed for all anesthetic groups except for nitrous oxide), however, such data cannot reflect neuronal activity. It is this lack of correlation between endogenous levels and neuronal activity that has led researchers to determine turnover rather than just the endogenous content of a transmitter to predict central neuronal activity (Costa, 1971).

<u>If affected, are neurons of the same type (i.e., dopamine or norepinephrine) affected the same in all brain regions</u>?

Early attempts to ascertain the involvement of the central adrenergic system with anesthesia were carried out on whole brain samples with little success. Both Anden et al. (1974) and Ngai et al. (1969a) found no effect by halothane anesthesia on norepinephrine

and dopamine. Present results offer a reason for the early failures. With all anesthetics studied: ketamine, halothane, nitrous oxide, and halothane-nitrous oxide, the change in NE and DA turnover in individual brain regions was non-uniform. Increased turnover in one brain area with decreased turnover in another area, approximately in proportion, as was the case with halothane dopamine turnover, would mask by cancellation anesthetic effects on turnover when whole brain samples are used as in the earlier studies.

Importantly, this study has provided evidence of non-uniform regional changes in dopamine and norepinephrine neuronal activity associated with anesthesia. The concept that anesthetics have regionally different central actions was previously proposed by Roizen (1976) on the basis of endogenous catecholamine data and local changes in glucose metabolism (Shapiro et al., 1978). Likewise, Ngai et al. (1978) proposed regional effects for halothane and cyclopropane with respect to acetylcholine turnover. Clearly, the evidence supports regional effects of anesthetics on neural activity, however the effect varies from region to region. The meaning of these regional differences relative to anesthesia remains at present elusive.

To postulate that the demonstrated regional

turnover changes for dopamine and norepinephrine are related, at least temporally to anesthesia, one should expect these turnover changes to revert to awake control levels post anesthesia. A test of this hypothesis demonstrated two hours post halothane anesthesia that turnover changes seen during anesthesia do in fact revert to values not significantly different from the awake control. Only cortical norepinephrine turnover did not return to the pre-anesthetic level. Interestingly, the only effect common to all agents during anesthesia was this increase in cortical norepinephrine turnover. The relationship of this effect to the increased norepinephrine turnover post anesthesia is unknown.

Do clinically different anesthetics have different effects centrally on adrenergic neurotransmitters?

The anesthetics used were chosen for their differences in clinical effect. There are now many classes of anesthetic agents (e.g., dissociative, inhalation, narcotic, barbiturate) each with its own clinically unique type of anesthesia. Some agents have good hypnotic action, while having little to no analgesic activity (e.g., barbiturates), while others produce good analgesia at clinical doses (e.g., N_2O). It is unknown whether or not all anesthetics have common effects centrally, although common sense might dictate that

clinically different anesthetics should result in different central effects as well. To test this hypothesis, individual ketamine, halothane, nitrous oxide, and halothane-nitrous oxide turnover and endogenous amine data from the same brain region were cross-correlated. By design, a significant difference in turnover activity between the two anesthetic agents being compared indicated a different effect of each agent on that amine system. And conversely, a lack of significance between the effects of different anesthetics was interpreted to indicate a similarity in effect on that brain region. This type of a comparison is problematic and generally limited in scope for the following reasons. It is difficult to provide evidence of equal anesthetic depth between intravenous and inhalation anesthesia. Nitrous oxide at the concentration used is only analgesic, not anesthetic. It is also well documented that the addition of nitrous oxide to halothane increases halothane's potency. Therefore, in this study where the same concentration of halothane is used with and without nitrous oxide the levels of anesthesia of these two groups must be different.

Even though there are differences in anesthetic level these comparisons allow one to determine qualitative differences between these clinically different

anesthetics. This is an important step in determining similarities of effect on central neurotransmitter systems by anesthetic agents.

Ketamine vs. Halothane:

Ketamine anesthesia has been termed "dissociative" and is characterized by possible increases in EEG activity, profound analgesia, normal muscle tone, and catatonia. Halothane is a potent anesthetic marked by poor analgesia, depressed EEG activity, minimal depression of neuromuscular junctions, and causes profound narcosis. These two agents present themselves as clinically different anesthetics.

When ketamine turnover was compared with that of halothane, dopamine turnover levels were significantly different in all four brain regions. Norepinephrine turnover comparisons were significantly different in two areas, the mesencephalon and thalamus, and not different (or similar) in the other two areas, hypothalamus and cortex. The conclusion derived was that ketamine and halothane have dissimilar patterns of effect on regional amine metabolism. This conclusion is supported by multi unit electrophysiological findings which also show regional differences for these two anesthetics (Domino, 1968; Massopust et al., 1972).

Ketamine vs. Nitrous Oxide; Ketamine vs. Nitrous Oxide-Halothane:

Nitrous oxide, when used in clinical concentrations, is a potent analgesic agent with few other clinically significant effects. Nitrous oxide is therefore similar to ketamine as an analgesic.

Comparison of dopamine and norepinephrine turnover data for ketamine and nitrous oxide demonstrated some similarities for these two agents. Thalamic and hypothalamic dopamine changes were similar, while norepinephrine turnover was significantly different in three out of the four areas studied, the thalamus, hypothalamus, and cortex. Further, when ketamine data is compared to the halothane-nitrous oxide combination there were fewer areas of significant difference compared to halothane alone and more areas of difference when compared to nitrous oxide alone, which is qualitatively what one might expect. It should be noted that this comparison is based on the overall number of significant findings.

Halothane vs. Nitrous Oxide:

When halothane and nitrous oxide were compared one might anticipate different effects in central adrenergic activity since these are two quite different clinical agents. When turnover data was compared some

areas showed similarity of effect and some statistically different effects. The thalamus, hypothalamus, and cortex showed significantly different dopamine changes, while in the case of norepinephrine, turnover comparisons showed different effects in the mesencephalon and thalamus. It can be concluded that halothane and nitrous oxide each alter regional adrenergic neuronal activity in a different fashion.

Halothane vs. Halothane-Nitrous Oxide; Nitrous Oxide vs. Halothane-Nitrous Oxide:

Comparison of the halothane-nitrous oxide combination to halothane alone and nitrous oxide alone provided an interesting finding. The overall effect on turnover due to halothane-nitrous oxide combination was greater than either agent alone or the sum of the individual turnover effects. In the mesencephalon and thalamus, the magnitude of change indicated a supra-additive effect, while in cortex a complete reversal in the direction of change occurred. These findings are novel insofar as central adrenergic neural activity is involved, although in the periphery several authors have suggested that the combination of halothane plus nitrous oxide results in measured effects attributable to neither agent alone. Hornbein et al. (1969) and Bahlman et al. (1971) measured cardiovascular and

respiratory parameters, while Smith et al. (1970) recorded sympathetic out flow. Both groups found a disproportionate increase in response when nitrous oxide was added to halothane anesthesia. Fukunaga and Epstein (1973) found a large increase in sympathetic out flow when nitrous oxide was added to halothane, however, if the same animal had a mid-collicular lesion, addition of nitrous oxide was then found to cause a decrease in sympathetic activity. These authors postulated a supra-collicular site as responsible for the combined effects.

Although evidence of central and peripheral supraadditive anesthetic actions has been demonstrated, the mechanism and/or significance of these actions remain unknown.

It can be concluded from this study that the clinically different anesthetics ketamine, halothane, nitrous oxide, and the combination halothane-nitrous oxide do produce individual (supraadditive) effects on regional central catecholamine turnover.

Anesthesia in Relation to Transmitter Systems - a Perspective:

When one considers change in adrenergic turnover relative to anesthesia, it is important to consider the

direction of the observed change. Conceptually, it would seem easier to relate a decrease rather than increase in neuronal activity to a state of anesthesia. This stems from the long standing, and generally unproven, hypothesis that the CNS is deactivated during anesthesia. This concept began to change when Winters et al. (1967) reclassified anesthetic agents with respect to EEG activity during surgical anesthesia. He found that not all agents acted in the classical sense to cause EEG depression. Ether, barbiturate, and halothane did act in this fashion, while such agents as nitrous oxide, gama hydroxybutyrate, phencyclidine, ketamine and enflurane all could increase brain EEG activity at clinical doses.

It has been considerably more difficult to obtain a good neurochemical correlate similar to that of EEG activity to relate to anesthesia. Recent evidence has related cholinergic neuronal activity to both EEG activity and anesthesia (Ngai et al., 1978). Anesthetics shown to decrease EEG activity have also been shown to decrease acetylcholine turnover, while other anesthetics known to increase EEG activity have been shown in some cases to increase acetylcholine turnover (Ngai et al., 1978). Moreover, clinical and experimental evidence utilizing various cholinergic agonists and

antagonists have demonstrated that cholinergic manipulation will affect the anesthetic process (Winter, 1976; Horrigan, 1978). Importantly, physostigmine has been shown in humans to reduce the recovery time post anesthesia (Smith et al., 1976).

The central cholinergic system probably does not act as an independent neurochemical system (Sethy et al., 1976). The complex neuronal circuitry containing known and unknown transmitters that appear to work in concert have yet to be sufficiently unraveled. Results from this study suggest the premise that reported increases and decreases in cholinergic activity with anesthesia might be the result of induced changes from stimulatory or inhibitory neurotransmitter systems.

Serotonin turnover has been generally shown to decrease with anesthesia (Sung et al., 1973; Bourgoin et al., 1975) and gamma-amino butyric acid (GABA), a known central inhibitory transmitter, was proposed by Cheng and Brunner (1975) to cause post synaptic inhibition and anesthesia through increased GABA concentration in the cortex. Other work related to GABA has been carried out using gamma-butyrolactone, a precursor to GABA, and gamma-hydroxybutyrate (GHB), a metabolite of GABA and an anesthetic agent itself. Gessa et al. (1966) demonstrated that GHB-induced anesthesia was

associated with significant increases in brain dopamine. A concomitant decrease in acetylcholine turnover with GHB anesthesia was suggested by the data of Sethy et al. (1976). Further gamma-butyrolactone was demonstrated to increase central dopamine content presumably by increasing tyrosine hydroxylase activity (Murrin and Roth, 1976).

Johnston et al. (1975) and Batista et al. (1973) have postulated dopamine to be an inhibitory transmitter in relation to anesthesia. In general, results from this study show that anesthetics outlined by Winters et al. (1967) to decrease EEG activity (halothane in this study) caused increased dopamine turnover in almost all brain areas studied. Conversely a decrease in dopamine turnover was seen with anesthetics which caused increased EEG activity (ketamine and nitrous oxide in this study). This inverse relationship between EEG activity and acetylcholine turnover (Ngai et al., 1978) and dopamine turnover (this study) could relate to an inhibitory effect of dopamine on the central cholinergic neurotransmitter system with resultant anesthesia. Such an acetylcholine inhibition by dopamine is known to exist in the nigrostriatal system (Cooper et al., 1974). In the same context, nitrous oxide when added to halothane anesthesia is known to increase halothane's

potency (as previously discussed). This dopamine based theory of anesthesia would predict a potentiation of the halothane induced increase in dopamine turnover when nitrous oxide is added to halothane. This was demonstrated in this study.

Further support of dopamine involvement during anesthesia comes from studies by Fyrö et al. (1975), who have shown in humans that halothane-nitrous oxide anesthesia increases homovanillic acid (HVA) output into the cerebral spinal fluid strongly suggesting an increase in dopamine turnover and therefore dopaminergic nerve activity.

Additional supportive evidence comes from studies using anti-dopaminergic compounds such as pimozide, which was shown to antagonize certain types of anesthesia (Hatch, 1974) while drugs known to elevate dopamine concentration such as L-dopa have been shown to increase anesthetic potency (Johnston et al., 1975).

Thus accumulating evidence from both animal and human studies support the concept of increased neuronal activity in a proposed central dopamine inhibitory system during anesthesia. The validity of this hypothesis must await future experimental results.

SUMMARY

To summarize the findings of this study, it was demonstrated that the anesthetic agents ketamine, halothane, nitrous oxide and the combination of halothane - nitrous oxide significantly alters dopamine and norepinephrine turnover in select brain regions. In brief, ketamine results in a significant decrease in both mesencephalic and cortical turnover and an increase in hypothalamic dopamine turnover. Ketamine decreased cortical norepinephrine turnover. Halothane results were different from those found with ketamine. Increased thalamic and hypothalamic dopamine was found with a concomitant decrease in cortical dopamine turnover. Halothane increased both thalamic and cortical norepinephrine turnover. Two hours post halothane anesthesia, turnover rates in all brain areas affected were found to have returned to control except for cortical norepinephrine. Nitrous oxide had only two effects on catecholamine turnover. An increase in hypothalamic dopamine and an increase in cortical norepinephrine were found. Of interest were the large changes in turnover found when halothane - nitrous oxide combination was used. This combination resulted in a dramatic increase in dopamine turnover in the mesencephalon, hypothalmus and cortex. Large increases in thalamic and cortical norepinephrine were also found.

Several conclusions were drawn from this data (1) It was determined that adrenergic turnover is effected by the anesthetics ketamine, halothane, and nitrous oxide at clinical doses. This suggests a role for these neurotransmitter systems in anesthesia. (2) Dopamine and norepinephrine metabolism were found to be independently altered by these anesthetic agents, indicating a possible distinct role for each neurotransmitter. (3) Effects of these anesthetics were found not to be uniform throughout the brain. This could account for many of the discrepancies in previous turnover studies using whole brain. (4) The reversal of the halothane effects two hours post anesthesia denotes a strong temporal relationship between the state of anesthesia and the alteration of adrenergic turnover. (5) Anesthetic agents which are clinically different were shown to also differ in their effect on central adrenergic turnover. (6) The data from this study coupled to evidence obtained from animal and human studies supports the concept of <u>increased</u> neuronal activity during anesthesia in proposed inhibitory transmitter systems.

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APPENDIX A

B R A I N W E I G H T S
(in grams)

	MES.	THAL.	HYPO.	CORT.
Awake Control	0.75±.02*	0.55±.02	0.35±.01	1.98±.05
Ketamine	0.74±.03	0.56±.02	0.33±.02	1.99±.03
Halothane	0.72±.02	0.58±.02	0.34±.01	1.95±.06
Nitrous Oxide	0.75±.02	0.58±.02	0.36±.02	2.08±.05
Halothane + Nitrous Oxide	0.70±.03	0.54±.04	0.32±.03	2.05±.04
Halothane Recovery	0.71±.04	0.60±.03	0.33±.01	2.14±.06

* Mean ± SE
MES. = Mesencephalon
THAL.= Thalamus
HYPO.= Hypothalamus
CORT.= Cortex

APPENDIX B

M O N I T O R I N G V A L U E S

Parameter	Range of Values
1) PaO_2	350 - 450 torr
2) $PaCO_2$	34 - 39 torr
3) pH	7.34 - 7.40
4) Temperature	39 - 39.5°C
5) $\bar{X}$ Arterial Blood Pressure	92 - 110 torr
6) Halothane Blood Levels	12.3 ± 0.8 mg%

Note: All values shown are within normal limits for rabbit. The PaO_2 range for ketamine was 86 - 100 torr due to room air spontaneous ventilation. Halothane blood levels are consistent with published values.

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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