

**AN IN VITRO AUTORADIOGRAPHIC INVESTIGATION
OF DOPAMINE RECEPTORS IN THE BRAIN
OF THE SPONTANEOUSLY HYPERTENSIVE RAT**

BY

GILBERT J. KIROUAC

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

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Department of Anatomy

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Margrit for her continual support

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ABSTRACT

Recent evidence points to a brain dopamine insufficiency in both human essential hypertension and in the spontaneously hypertensive rat (SHR). To localize this dopamine insufficiency, the density of D1 and D2 dopamine receptors was assessed in the brain of the SHR. *In vitro* receptor autoradiography of D1 and D2 receptors was carried out on adjacent sections of selected brain regions (anteromedial prefrontal cortex, cingulate cortex, lateral septal nucleus, nucleus accumbens, caudate-putamen, globus pallidus, amygdaloid complex). Brain sections from 5 and 15-week-old SHRs and their controls the Wistar-Kyoto rat (WKY) were incubated with 1 nM [³H]SCH 23390 (D1 receptor antagonist) or 15 nM [³H]sulpiride (D2 receptor antagonist), and exposed along with radioactive standards ([³H]Microscales, Amersham) to ³H-Hyperfilm. The optical density of the autoradiograms was quantified using computer-assisted densitometry. These experiments showed a significant increase in [³H]SCH 23390 binding in the nucleus accumbens and caudate-putamen of 5 and 15-week-old SHRs compared to WKYs. [³H]SCH 23390 binding was also increased in the lateral septal nucleus of 5-week-old SHRs and in the globus pallidus of 15 week-old-SHRs. Binding of [³H]sulpiride was increased in the nucleus accumbens of 5-week-old rats. The results indicate that an increase in dopamine receptor density may be associated with genetic hypertension. Up-regulation of both D1 and D2 dopamine receptors in prehypertensive SHRs suggests that dopamine may be responsible for the pathogenesis of the hypertension in SHRs and that it is not secondary to the rising blood pressure.

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LIST OF ABBREVIATIONS

BP	blood pressure
CNS	central nervous system
SHR	spontaneously hypertensive rat
WKY	Wistar-Kyoto rat
cyclic AMP	cyclic adenosine monophosphate

1. INTRODUCTION

Cardiovascular diseases such as coronary heart disease and stroke are the most common cause of death in affluent societies. A major risk factor in the development of these diseases is hypertension (McNeil, 1989). Since the recognition of hypertension as an important disease entity, an enormous effort has been made at understanding its etiology and pathophysiology with the hope of providing more effective antihypertensive agents (MacMahon, 1989).

Human essential hypertension is a condition in which a chronic elevation of blood pressure (BP) occurs without any definable cause. It accounts for the majority of hypertensive individuals seen in clinics. Epidemiological studies have shown that genetic and environmental factors play an important role in the development of essential hypertension (Folkow, 1982). In an attempt to produce an appropriate animal model to study human hypertension, Okamoto and Aoki (1963) inbred Wistar rats with higher than normal BPs. Post-weanling offsprings of these rats spontaneously developed high BP and, therefore, were referred to as the spontaneously hypertensive rat (SHR). The SHR is generally considered to be a suitable experimental model for the study of essential hypertension because SHRs and hypertensive humans share the same circulatory hemodynamics (Trippoda & Frohlich, 1981).

The hemodynamic pattern of the developing hypertension in humans and SHRs is characterized by an initial increase in cardiac output (Lundin & Hallback-Nordlander, 1980). The enhanced cardiac output, which precedes the establishment of the hypertension, is believed to cause structural and functional changes in the

heart and blood vessels. These changes result in a shift in the hemodynamics from a high output to high resistance pattern (Folkow, 1982, 1987). The resulting elevated total peripheral resistance maintains the BP at a new and hypertensive level.

The early rise in cardiac output in hypertension-prone individuals may be due to hyperreactivity of the cardiovascular system to environmental stimuli (Folkow, 1982). The hyperreactivity may in fact represent one of the inherited predisposing factors in genetic hypertension (Pickering & Gerrin, 1990). The anatomical substrates responsible for the integration of cardiovascular reactivity are found in forebrain limbic structures (Smith & DeVito, 1984). A neurotransmitter system of great importance for the normal function of the forebrain is the dopaminergic system. Mogenson (1987) has proposed that dopamine in the forebrain plays a neuromodulatory role in which dopamine influences the output of limbic and striatal structures. Consequently, changes in dopaminergic activity could lead to a dysfunction of the neural mechanisms controlling cardiovascular reactivity. Indeed, evidence points to a dopamine insufficiency in both human and rat models of genetic hypertension. This is provided by studies in which dopamine and its agonists have antihypertensive effects in both human hypertension and SHRs (Stumpe et al., 1977; Kolloch et al., 1981; Nagahama et al., 1984). However, the type and location of brain dopamine receptors mediating dopamine's hypotensive effect remains to be determined. This thesis examined these questions using *in vitro* receptor autoradiography of D1 and D2 dopaminergic receptors in the brain of prehypertensive and hypertensive SHRs and their normotensive controls the Wistar-Kyoto rats (WKY).

2. REVIEW OF THE LITERATURE

2.1 Hyperkinetic Circulation in Hypertension

The SHR is considered the best model for studying clinical hypertension because of the similarities between the two in their hemodynamics and the progression of the hypertension (Trippoda & Frohlich, 1981). A most important observation for understanding the pathogenesis of hypertension is that human patients with borderline or labile hypertension and young prehypertensive SHRs have a hyperkinetic circulation (Conway, 1984; Folkow, 1982; Lundin & Hallback-Nordlander, 1980). The early hemodynamic pattern characterizing this hyperkinetic circulation involves an enhanced cardiac output and a normal peripheral vascular resistance. With progression of the hypertension, the cardiac output returns to normal levels while the peripheral resistance gradually rises to a new elevated level. Vascular hypertrophy and an increased in arteriolar vasoconstriction combined with a decrease in cardiac responsiveness, causes the hemodynamics to shift from a high output to a high resistance pattern (Folkow, 1982). As suggested by Folkow (1982), the hypertension becomes self-sustained through a process of structural reinforcement of precapillary arterioles.

Of particular importance in understanding the role of the hyperkinetic state in hypertension is that the cardiovascular system of humans with hypertension and SHRs is hyperreactive to a variety of environmental stimuli (Hallback & Folkow, 1974; McMurtry & Wexler, 1981; Pickering & Gerrin, 1990). For example, young prehypertensive SHRs exposed to a stressful environment respond with a greater increase in cardiac output, heart rate, and mean arterial BP than do their

normotensive controls. This cardiovascular hyperreactivity precedes the onset of hypertension and may represent one of the inherited predisposing factors in genetic hypertension. More direct evidence supporting this hypothesis comes from studies in which social and sensory deprivation of young SHRs attenuated the development of hypertension (Hallback, 1975) while stimulation aggravated it (Okamoto, 1969). A clinically interesting report in which adolescents from hypertensive families respond to forced arithmetic with an accentuated heart rate and BP further supports the hyperreactivity hypothesis (Falkner et al., 1979).

The hyperkinetic circulation observed in early hypertension is to a great extent mediated by the autonomic nervous system (Julius & Esler, 1975; Lundin & Hallback-Nordlander, 1980). Cardiovascular hyperreactivity to environmental stimuli must originate from higher levels of the brain where sensory, motor, and autonomic responses are integrated and initiated. Regions of the forebrain, which include the limbic system and hypothalamus, are particularly important in integrating and coordinating behavioral and neurohumoral responses to emotional arousal (Smith & DeVito, 1984; Mogenson, 1987). From an anatomical viewpoint, limbic structures are situated between the sensory cortices and the hypothalamus which monitors and regulates internal body functions. Clearly, if we are to link hypertension to dysfunctions of the central mechanisms controlling an organism's responsiveness to the environment and its emotional arousal, we must examine the brain centers and the neural mechanisms that integrate both emotions and autonomic responses.

2.2 Emotional Arousal and Blood Pressure

An organism's BP fluctuates with different levels of arousal and intensity of behavioral actions. These fluctuations represent the summation of changes in the resistance of various vascular beds and cardiac output that occur to meet the specific metabolic needs imposed by the performance of a behavior (Cohen & Obrist, 1975). For example, BP is more elevated in rats during eating and drinking than during grooming (LeDoux et al., 1982). A link between emotions and changes in autonomic function has been debated for nearly a century (Eckman et al., 1983). One indisputable fact is that emotional arousal, especially of an aversive nature, is accompanied by dramatic adjustments in the autonomic nervous system. Such emotional arousal, often referred as a defense reaction, can be elicited in humans and animals by natural stimuli (Folkow, 1983, 1987) or by electrical brain stimulation in experimental animals (Smith & DeVito, 1984). The defense reaction induced by electrical brain stimulation produces escape or fight behavior and a variety of autonomic responses that include profound cardiovascular and respiratory adjustments. The cardiovascular response accompanying a defense reaction consists of vasodilation in skeletal muscles and in the myocardium with concomitant vasoconstriction in the viscera. These circulatory adjustments result in an increase in cardiac output, heart rate, and arterial BP, which together serve to increase blood flow to the skeletal muscles and the heart (Yardley & Hilton, 1986; Smith et al., 1990). This type of cardiovascular reaction can be elicited by the electrical stimulation of specific brain regions including the lateral hypothalamic area, mesencephalic central gray matter, and the amygdaloid complex (Yardley & Hilton,

1986; Maskati & Zbrozyna, 1989; Smith et al., 1990). From these electrical stimulation studies, we can conclude that these brain centers are involved in the integration of autonomic responses that accompany emotional arousal. To exert their influence on the autonomic nervous system, these same brain centers must also have access to the effector organs, namely the heart and blood vessels.

2.3 Brain Regions Innervating the Autonomic Nervous System

The motor-neurons involved in cardiovascular regulation are the sympathetic preganglionic neurons located in the intermediolateral cell column of the spinal cord (Strack et al., 1988) and the vagal parasympathetic preganglionic neurons located in the nucleus ambiguus of the medulla (Plecha et al., 1988). The neural outflow from these preganglionic cells is regulated by a variety of cell groups located throughout the brain. Axoplasmic transport studies have provided a tentative picture of possible brain centers regulating the autonomic nervous system. However, these studies are limited by their inability to provide any information on the functional nature of the connections. For example, if a brain region is shown to project to the upper thoracic intermediolateral cell column, it is not possible to determine whether this pathway regulates sympathetic preganglionic neurons involved in cardiovascular, pupillomotor, or pilomotor functions.

To circumvent this problem, the transneuronal viral cell labelling technique was developed (Martin & Dolivo, 1983). Pseudorabies virus injected into specific ganglia known to innervate the heart or specific vascular beds, is retrogradely transported in the axoplasm of sympathetic preganglionic neurons and transferred

transneuronally to second order neurons that innervate that same preganglionic neuron. The intraneuronal accumulation of the virus is then demonstrated using immunohistochemistry. As a result of this technical improvement in neuronal tract-tracing, a more accurate picture of the brain centers innervating the sympathetic preganglionic neurons has emerged (Strack et al., 1989). The paraventricular hypothalamic nucleus, A5 noradrenergic cell group, caudal raphe nuclei, and the rostral ventromedial and ventrolateral medulla were found to innervate all spinal cord levels. More important for this discussion, the lateral hypothalamic area, mesencephalic central gray matter, zona incerta, and posterior periventricular hypothalamic area in addition to the five areas listed above were found to innervate the stellate ganglion, the major source of sympathetic fibers innervating the heart. Therefore, these brainstem and forebrain areas have direct access to sympathetic preganglionic neurons innervating the heart and should have the largest influence on the cardiovascular system.

The inputs to the vagal preganglionic nucleus, the nucleus ambiguus, have been studied using the retrograde transport of the lectin horseradish peroxidase and by the anterograde transport of ^3H -leucine (ter Horst et al., 1984; Holstege, 1991). These studies showed that the nucleus ambiguus receives projections from the paraventricular hypothalamic nucleus, dorsomedial hypothalamic nucleus, lateral hypothalamic area, central nucleus of the amygdala, zona incerta, and bed nucleus of the stria terminalis. Therefore, many of the same regions of the brain that innervate the sympathetic preganglionic neurons also innervate the parasympathetic preganglionic neurons. These anatomical studies suggest which forebrain centers

have the necessary connections to influence the cardiovascular system. In an attempt to determine whether brain centers have a role in BP regulation or hypertension, neuroscientists have stimulated or lesioned these centers and observed the resulting cardiovascular effects.

2.4 Role of Forebrain Structures in Cardiovascular Reactivity

Work in the past two decades has identified a wide variety of brain regions and pathways involved in cardiovascular regulation. The circuitry responsible for mediating tonic and reflex changes in BP levels appear to be located in the brainstem (Ciriello et al., 1986; Reis et al., 1988; Chalmers & Pilowsky, 1991) while the systems responsible for the integration of behaviorally coupled changes in BP involves mostly the forebrain (Follow, 1987; Loewy, 1991). These forebrain regions include nuclei in the hypothalamus, septal area, prefrontal cortex, and amygdala. A picture has emerged in which a set of interconnected forebrain nuclei exert their effects on BP via connections with cardiovascular centers in the brainstem and spinal cord. Therefore, these forebrain structures can be viewed as the command centers for the neural pathways controlling the circulation.

2.4.1 Paraventricular Nucleus of the Hypothalamus

Several hypothalamic nuclei are connected with cardiovascular centers in the brainstem and spinal cord. One of these, the paraventricular nucleus of the hypothalamus, is involved in a variety of autonomic and endocrine functions. The magnocellular portion of the paraventricular nucleus consists of vasopressin and

oxytocin containing neurons that send their axons to the posterior pituitary gland (Cunningham & Sawchenko, 1991). The parvocellular component of the paraventricular nucleus projects directly to the ventrolateral medulla, the anatomical site for the brainstem vasomotor center, and to the intermediolateral cell column of the spinal cord, site of preganglionic neurons that innervate the heart and arterioles (Luitien et al, 1985; Strack et al. 1990a, 1990b). Depending on whether the paraventricular nucleus is electrically or chemically stimulated, increases or decreases in BP have been reported (Ciriello & Calaresu, 1980; Porter & Brody, 1986; Yamashita, 1987). Moreover, bilateral electrolytic lesions of the paraventricular nucleus in young SHR's attenuated the development of hypertension (Ciriello et al., 1984).

2.42 Cerebral Cortex

Recently, a role in the control of the autonomic nervous system for the prefrontal cortex and insular cortex, which form part of the primitive association cortex or limbic cortex, has received some attention. The prefrontal cortex projects to the nucleus of the solitary tract (Van der Kooy et al., 1984; Neafsey et al., 1986) and the insular cortex projects heavily to both the lateral hypothalamic area and the amygdala (Yasui et al., 1991). Stimulation of the medial prefrontal cortex and insular cortex produced both pressor and depressor responses (Terreberry & Neafsey, 1984; Burns & Wyss, 1985; Powell et al., 1985; Ruggiero et al., 1987; Hardy & Holmes, 1988; Yasui et al., 1991). In addition, cooling of prefrontal cortical areas reversed the hypertension produced by mineralocorticoid-salt treatment or aortic

constriction (Szilagyi et al., 1987). Whether these limbic cortical areas are involved in the hypertension of SHRs remains to be determined.

2.43 Lateral Hypothalamic Area

Anatomical studies have established that the lateral hypothalamic area projects extensively to forebrain, brainstem, and spinal cord areas implicated in autonomic function (Hosaya & Matsushita, 1981; Berk & Finkelstein, 1982; Holstege, 1987). The paraventricular nucleus of the hypothalamus, bed nucleus of the stria terminalis, nucleus of the solitary tract, mesencephalic central gray matter, parabrachial nucleus, ventrolateral medulla and the intermediolateral cell column of the spinal cord all receive significant fiber projections from the lateral hypothalamic area. The lateral hypothalamic area and its interconnections with other brain nuclei form part of a central autonomic network (Loewy, 1991). Electrical stimulation of the lateral hypothalamic area produced a defense reaction with its distinctive pattern of cardiovascular changes, which include vasodilation in skeletal muscle and myocardium; vasoconstriction in the splanchnic region, kidney, and skin; and increases in cardiac output, heart rate, and arterial BP (Haifa et al., 1989; Yardley & Hilton, 1986). The hemodynamic pattern induced by stimulation of the lateral hypothalamic area is similar to the cardiovascular hyperreactivity seen in SHRs which suggests that they are both mediated by the same neural substrates.

2.44 Amygdaloid Complex

Neuroanatomical and physiological studies have shown that the basolateral nucleus of the amygdala receives sensory information from the visual and auditory association cortices and from the hippocampus (Herzog & Van Hoesen, 1976; Rosene & Van Hoesen, 1977; Turner et al., 1980). The basolateral nucleus projects to the central nucleus of the amygdala, septal area, and nucleus accumbens (Krettek & Price, 1978). The central nucleus in turn gives rise to extensive and widespread projections to cardiovascular sites in the hypothalamus and brainstem (Hopkins & Holstege, 1978; Krettek & Price, 1978; Schwaber et al., 1980). This anatomical network suggests that the amygdala may be an important link between the sensory cortex and centers known to have a role in cardiovascular regulation. Considering that the SHR exhibits an exaggerated cardiovascular reactivity to environmental stimuli and that the amygdala is in a central anatomical position between the sensory cortex and cardiovascular centers, suggests a role for the amygdala in the pathophysiology of hypertension. Support for this notion is provided by studies in which electrical stimulation of the amygdala's basolateral nucleus and central nucleus in awake animals produced a defense reaction (Iwata et al., 1987; Haifi et al., 1989). Indeed, lesions of the same regions of the amygdala attenuated the development of hypertension in SHRs (Galeno et al., 1982). The defense reaction elicited by stimulation of the amygdala is similar to that elicited from the lateral hypothalamic area with some differences (Hilton & Zbrozyna, 1963). Stimulation of the amygdala produced a less intense, later onset, and more gradual cardiovascular response (Hopkins & Holstege, 1978; Schwaber et al., 1980; Price & Amaral, 1981). This

suggests that the effects are mediated indirectly by neural connections to the hypothalamus or brainstem. In addition, there are numerous projections from the basolateral nucleus of the amygdala to the nucleus accumbens where locomotion and other complex behaviors can be elicited using electrical and chemical stimulation (Mogenson, 1987). As a consequence of its strategic connections, the amygdala may be viewed as an important link between the limbic system and the hypothalamic and brainstem centers involved in cardiovascular function.

2.5 Forebrain Dopaminergic Systems

Using the classical formaldehyde method, Dahlstrom and Fuxe (1964, 1965) were the first neuroscientists to describe the details of the mesotelencephalic dopaminergic system. Since their pioneering work, a complete description of the brain dopaminergic system has been made possible by the introduction of the new and more sensitive fluorescence and immunohistochemical techniques.

The ascending mesotelencephalic dopaminergic system is subclassified into the mesostriatal and mesolimbocortical systems (Bjorklund & Lindvall, 1984). The mesostriatal system is further subdivided into a dorsal and ventral component (Bjorklund & Lindvall, 1984). The dorsal component of the mesostriatal dopaminergic system originates from the A9 dopamine cell group located primarily in the pars compacta of the substantia nigra and partially from the A8 dopamine cell group in the retrorubral field (Fuxe, 1965; Beckstead et al., 1979). The dopamine containing axons of the nigral dopaminergic cells travel in the medial forebrain bundle to innervate the caudate-putamen. The ventral component of the

mesostriatal dopaminergic pathway originates from the A10 dopamine cell group located in the ventral tegmental area and from the medial part of the substantia nigra (Fallon & Moore, 1978; Lindvall & Stenevi, 1978; Beckstead et al., 1979). The ventral component innervates, also via the medial forebrain bundle, the ventral striatum including the nucleus accumbens, olfactory tubercle, and the bed nucleus of the stria terminalis. A large proportion of dopamine containing neurons in the ventral tegmental area also contain the neuropeptides neurotensin and cholecystokinin (Seroogy et al., 1987, 1988). The functional significance of this co-localization remains to be determined. However, these neuropeptides are co-released with dopamine and appear to modulate the release of dopamine (Hole et al., 1986; Ruggeri et al., 1987).

The mesolimbocortical dopaminergic system originates mostly from the A10 cell group and partially from the A9 cell group (Lindvall, 1975; Lindvall & Stenevi, 1978; Fallon & Moore, 1978; Fallon et al., 1978; Swanson, 1982). The fibers of the mesolimbocortical system ascend in the medial forebrain bundle and innervate the lateral septal nucleus; amygdaloid complex; hippocampus; anterior olfactory nuclei; olfactory bulb; and the supragenual prefrontal, pregenual anteromedial prefrontal, cingulate, periform and entorhinal cortices. It is evident from the list of cortical areas innervated by dopamine that dopaminergic cells innervate almost exclusively areas of the limbic cortex, a phylogenetically older cortical area involved in the integration of behavioral and neuroendocrine responses (Mogensen, 1987; Loewy, 1991).

Dopaminergic neurons are also found throughout the hypothalamus. Periventricular cell bodies belonging to the A12 and A14 dopamine cell groups are located along the entire dorsoventral border of the periventricular grey in the anterior hypothalamic and preoptic areas (Hokfelt et al., 1976; Lindvall et al., 1984). The periventricular system gives rise to fibers that remain close to their cell of origin. The incertohypothalamic dopaminergic system originates from the A13 dopamine cell group in the medial zona incerta and also gives rise to short local fibers (Bjorklund et al., 1975; Hokfelt et al., 1976). The tuberoinfundibular and tuberohypophyseal dopaminergic systems originate in the A12 dopamine cell group located in the arcuate nucleus and the ventral periventricular hypothalamic nucleus (Smith & Fink, 1975; Lofstrom et al., 1976). The tuberoinfundibular system innervates the median eminence and has an inhibitory effect on the secretion of hypothalamic hormones from the median eminence, including prolactin, luteinizing hormone-releasing hormone, and thyrotropin-releasing hormone (Fuxe et al., 1985). The tuberohypophyseal system innervates the entire pars intermedia and the posterior lobe of the pituitary gland and may modulate the secretion of vasopressin from the pituitary gland (Fuxe et al., 1985). Finally, a diencephalospinal dopaminergic system originates from the the A11 dopamine cell group located in the dorsal hypothalamic area and projects to the dorsal horn and intermediolateral cell column of the spinal cord (Swanson et al., 1981; Blessing & Chalmers, 1979).

2.6 Function of the Mesotelencephalic Dopaminergic System

The projections of the mesotelencephalic system contain both dopaminergic and non-dopaminergic neurons (Swanson, 1982; Loughlin & Fallon, 1983). However, a major portion of neurons in the mesostriatal system are dopaminergic and it has been estimated that less than 5% of A9 or A10 neurons projecting to the dorsal striatum are non-dopaminergic (Van der Kooy et al., 1981). Similarly, the projection to the nucleus accumbens has been estimated to comprise only 10-15% non-dopaminergic neurons (Swanson, 1982).

The mesencephalic dopaminergic system provides a striking example of how a small number of a single source of divergent neurons can exert global control of brain function. In the rat, bilateral lesions of this system produces a severe and generalized state of behavioral unresponsiveness which include such diverse symptoms as akinesia, catalepsy, sensory inattention, aphagia, and adipsia. To explain this behavioral unresponsiveness, Mogenson (1987) has provided strong evidence that activation of mesotelencephalic dopaminergic neurons has a modulating or "gating" influence on the relay of information in the nucleus accumbens from the limbic system and neocortex to extrapyramidal motor areas. Evidence for this comes from studies in which activation of nucleus accumbens neurons by stimulation of the amygdala or hippocampus is reduced by the stimulation of dopaminergic cells in the ventral tegmental area (Yim & Mogenson, 1982; Yang & Mogenson, 1984). A similar attenuating effect of excitatory inputs to the nucleus accumbens neurons is produced by the exogenous application of dopamine to nucleus accumbens neurons (Yim & Mogenson, 1982; Yang &

Mogenson, 1984). Moreover, nigrostriatal dopaminergic neurons have similar attenuating effects on the excitatory responses of caudate-putamen neurons to stimulation of the neocortex (Hirata et al., 1984; Vives & Mogenson, 1984; Abercombie & Jacobs, 1985; Toan & Schultz, 1985). Indeed, the constellation of motor symptoms characterizing Parkinson's disease which include akinesia, rigidity, and tremor, have been attributed to a dysfunction of a dopamine-dependent gating mechanism. In this case, dopamine insufficiency produces an abnormal flow of information from the cerebral cortex via the striatum to motor systems.

Mogenson (1987) suggests that electrophysiological responses of nucleus accumbens neurons to dopamine or ventral tegmental area stimulation reflect important limbic-motor integrative activity that contribute to behavioral response initiation (Mogenson & Yim, 1981; Yim & Mogenson, 1983; Mogenson, 1984). He goes on to propose that a nucleus accumbens dopamine-dependent gating mechanism may play a role in translating the intent of a behavioral response into such important actions as feeding, drinking, and the defense reaction. In his theoretical framework, the mesotelencephalic dopaminergic system is viewed as a level-setting system which determines the threshold for an organisms behavioral responding. Forebrain limbic structures, especially the amygdala, have prominent connections with the cardiovascular centers in the hypothalamus and brainstem. Reduction in dopaminergic activity could result in a disinhibition of forebrain activity and consequently cause a reduction in the threshold for behavioral responding. This could make an organism hyperreactive to the environment. The emerging role for dopamine in the modulation of output from limbic structures and the striatum, in

addition to the role of forebrain structures in cardiovascular regulation, suggests that the mesotelencephalic dopaminergic systems may play an important role in BP regulation and possibly hypertension.

2.7 Brain Dopamine Receptors

In the early 1970's, biochemical studies indicated that only one dopamine receptor type existed in the CNS. At that time, the ability of dopamine to stimulate adenylate cyclase activity had been demonstrated by several laboratories (Brown & Makman, 1972; Kebabian & Calne, 1979). The classical dopamine receptor agonists and antagonists displayed appropriate effects in the adenylate cyclase model (Iverson, 1975). However, several dopaminomimetic ergots (e.g., bromocriptine) exhibited inappropriate activity in this biochemical assay system. For example, ergots were found to block the ability of dopamine to increase adenylate cyclase activity in the anterior pituitary gland (Kebabian et al., 1977). To explain these unexpected findings, it was postulated that the dopamine receptor capable of stimulating adenylate cyclase, the D1 receptor, was distinct from the dopamine receptor in the anterior pituitary gland, the D2 receptor. Furthermore, it was proposed that the physiological effects of dopamine on D1 receptors were due to an increase in cyclic AMP production while the physiological effects of dopamine on D2 receptors were the consequence of an inhibition of cyclic AMP (Stoof & Kebabian, 1981; Cote et al., 1982; Swennen & Denef, 1982).

The localization of dopamine receptor subtypes in the brain can provide some clues about their physiological functions. Dopamine receptors are located

throughout the CNS where dopamine-containing fiber terminal or cell bodies are found. Using the most selective D1 and D2 antagonists available, Wamsley and colleagues (Gehlert & Wamsley, 1985; Dawson et al., 1986) used *in vitro* autoradiography to localize and quantify dopamine receptor density in the rat brain. The highest concentration of D1 and D2 receptors was found in the caudate-putamen, nucleus accumbens, olfactory tubercle, and island of Calleja. In these areas, D1 receptor binding was about two-fold that of D2 receptor binding. Moderately high D1 receptor binding was found in most nuclei of the amygdala while no D2 binding was demonstrated in the amygdala. Very low to moderate levels of both D1 and D2 receptor binding was demonstrated in the remainder of brain areas innervated by dopaminergic fibers or containing dopaminergic cell bodies. One notable exception is the absence of any significant binding in the hypothalamus despite its relatively high concentrations of dopamine-containing fibers and somata.

The mechanisms by which dopamine exerts its effects on neuronal activity remains unclear. Histochemical and biochemical evidence has shown that dopamine receptors are located on both postsynaptic and presynaptic structures (Kebabian & Calne, 1979). Most reports support a neuromodulatory role for dopamine because when dopamine is iontophoretically applied to postsynaptic neurons, it produced slow membrane depolarizing or hyperpolarization accompanied by a decrease in postsynaptic neuronal activity (Kitai et al., 1976; Bernardi et al., 1978; Herrling & Hull, 1980; Mercuri et al., 1985; Yim & Mogenson, 1988). Others have reported membrane depolarization with an increase in action potential discharge, suggesting

that dopamine can also function as an excitatory neurotransmitter (Kitai et al., 1976). Both D1 and D2 receptors have been demonstrated on presynaptic nerve terminals in the striatum (Carlsson, 1977; Schwarcz et al., 1978; White & Wang, 1986; Goldstein et al., 1990). They are called presynaptic receptors when found on non-dopaminergic terminals and autoreceptors when located on dopaminergic terminals. Activation of dopamine autoreceptors has been shown in biochemical studies to reduce the release and synthesis of dopamine (Starke et al., 1978; Westfall et al., 1976) and to decrease terminal excitability of dopaminergic neurons (Tepper et al., 1986).

A neuromodulatory role for dopamine has emerged in which dopamine, by acting on presynaptic nerve terminals of non-dopaminergic fibers, affects the release and turnover of other neurotransmitters in the striatum. For example, the dopamine agonist apomorphine, acting on D2 presynaptic receptors, inhibited the *in vivo* release of acetylcholine from the striatum (Bartholini et al., 1974). In support of dopamine's inhibitory effect on acetylcholine release, selective D2 receptor blockade increased acetylcholine content in the striatum (Wong et al., 1983; Eurard et al., 1980). Similarly, dopamine acting on D2 receptors inhibited the release and caused an accumulation of beta-endorphin in the mediobasal hypothalamus (Vernes et al., 1985; Nohtomi et al., 1984). Dopamine has also been implicated in the release and turnover of other neurotransmitters including gamma-aminobutyric acid, glutamate, noradrenaline, serotonin, enkephalin, somatostatin, substance P, and cholecystokinin (Sonsalla et al., 1984; Hong et al., 1985; Hutchinson et al., 1986; Thal et al., 1986; Tossman & Ungerstedt, 1986). The receptor subtype mediating these effects remains

to be determined. Clearly, the accumulated evidence points to a dual role for dopamine. First, dopamine produces membrane depolarization of postsynaptic neurons by a direct action on neuronal cell bodies and dendrites. Secondly, dopamine attenuates the postsynaptic effects of other neurotransmitters by acting on presynaptic terminals of afferent inputs causing an inhibition of neurotransmitter release. The functional implications of dopamine's neuromodulatory role are being actively investigated by several laboratories around the world.

2.8 Dopamine and Hypertension

Recent evidence suggests that central dopaminergic systems play an important role in cardiovascular functioning and hypertension. Initially, a role for brain dopamine in BP regulation was suggested by the observation that intracerebroventricular administrations of dopamine in conscious rats produced a decrease in BP and heart rate (Kondo et al., 1981a, 1981b). Apart from a direct action on dopamine receptors, dopamine could stimulate adrenoceptors directly or could be converted to norepinephrine and exert its effects indirectly (Goldberg, 1979). However, a direct effect of dopamine in the CNS is suggested by the observation that inhibition of dopamine- β -hydroxylase, the enzyme that converts dopamine to norepinephrine, did not affect the hypotensive effect of centrally applied dopamine (Zanberg et al., 1979). Furthermore, in anesthetized and unanesthetized cats, dogs, and rats, a variety of dopamine antagonists but not adrenoceptor antagonists blocked dopamine's centrally mediated cardiovascular effects (Heise, 1976; Day & Roach, 1976; Lang & Woodman, 1979; Kondo et al.,

1981a, 1981b). These results suggest that dopamine receptors rather than adrenoceptors mediate dopamine's centrally mediated cardiovascular actions.

The anatomical pathways and neural mechanisms mediating dopamine's hypotensive effect remain unknown. As discussed earlier, dopaminergic cell bodies and fibers are located in a wide variety of brain regions. Moreover, the physiology of dopamine receptors is extremely complex and poorly understood. However, the recognition that dopamine plays an important role in modulating neural mechanisms in the forebrain and the discovery that many of these forebrain structures are involved in cardiovascular regulation, points to the possibility that dopamine modulates the output of forebrain structures to the heart and blood vessels.

A consistent side-effect of dopaminergic drugs is the reduction of BP. Clinical trials using the oral administration of the dopamine agonist bromocriptine to patients with essential hypertension were successful at returning BPs to normotensive levels (Stumpe et al., 1977; Kolloch et al., 1981). Similarly, intravenous administrations of bromocriptine in SHRs decreased their BPs to levels comparable to their normotensive controls the WKYs (McMurtry et al., 1979; Sowers, 1981; Nagahama et al., 1984). Bromocriptine was acting centrally because metoclopramide, a dopamine antagonist that crosses the blood brain barrier, blocked bromocriptine's hypotensive effect while domperidone, a dopamine antagonist that does not cross the blood brain barrier, had no effect (Nagahama et al., 1984). An interesting and well documented observation is that bromocriptine given to humans with essential hypertension attenuated the cardiovascular hyperreactivity to physical or mental stress (Kolloch et al., 1980, 1981; Sowers et al., 1982a, 1982b). Consistent

with these clinical observations, bromocriptine given intravenously to SHR_s suppressed BP responses to immobilization stress in SHR_s (Sowers, 1981). As discussed earlier, both humans with essential hypertension and SHR_s have an increase cardiovascular reactivity to environmental stimuli. This reactivity may play a key role in the pathogenesis of hypertension (Folkow, 1987). These results not only link dopamine to the maintenance of hypertension but also link dopamine to the enhanced cardiovascular reactivity characterizing both human essential hypertension and spontaneous hypertension in rats. Whether dopamine, cardiovascular reactivity, and hypertension are causally related remains to be determined: clearly the evidence supports such a relationship.

The antihypertensive effects observed following administration of dopamine agonists led researchers to propose that hypertensive humans and SHR_s suffered from a central dopaminergic insufficiency. Two lines of evidence support this hypothesis. First, hormonal factors under inhibitory dopaminergic control are elevated in individuals with hypertension. Plasma prolactin concentration is elevated in both humans with essential hypertension and SHR_s (Stumpe et al., 1977; Sowers et al., 1979). It also has been reported that plasma levels of vasopressin and thyroid-stimulating hormone are increased in SHR_s (Kojima et al., 1975; Crofton et al., 1978). These peptide abnormalities are consistent with a reduced dopaminergic activity of the tuberoinfundibular system and has been suggested to represent a widespread central dopaminergic insufficiency (Fuxe et al., 1985). A second line of evidence supporting the dopamine insufficiency hypothesis is provided by studies in which injections of dopamine into the brain of SHR_s produced a greater hypotensive

effect than in normotensive controls (Kawebe et al., 1983; Hutchinson & Mok, 1984; Mok & Sim, 1987; Mok et al., 1990). Pretreatment with metoclopramide, a relatively selective D2 dopamine receptor antagonist, attenuated the hypotensive effect while blockade of adrenoreceptors had no effect (Kawebe et al., 1983; Mok et al., 1990). From these microinjection studies, it is possible to conclude that the exogenous application of dopamine replenished the depleted endogenous stores of dopamine, allowing BP to return to a normotensive level.

Attempts at identifying the dopamine insufficiency led researchers to examine dopamine receptor density and dopamine content in the brain of the SHR. While some laboratories found no difference in striatal and cortical dopamine levels (Versteeg et al., 1976; Le Fur et al., 1981; Fuller et al., 1983; Howes et al., 1984; Yu et al., 1990) others reported increased levels in the striatum, frontal cortex, and hypothalamus (Versteeg et al., 1976; Fuxe et al., 1979a, 1979b; Van den Buuse et al., 1984; Henley & Bellush, 1989). Attempts at demonstrating differences in dopamine receptor density between SHRs and WKYs produced similar discrepancies. Several reports supported an increase in maximum binding capacity (B_{max}) for both D1 and D2 dopamine receptors in the striatum (Le Fur et al., 1981; Chiu et al., 1982; Bhargava, 1984; Lim et al., 1989, 1990). However, a recent report by Watanabe et al. (1989) using the more specific D1 and D2 receptor antagonists SCH 23390 and sulpiride, respectively, demonstrated no difference in dopamine receptor density in the striatum of SHRs and WKYs. There are also scattered reports of an increase in dopamine receptors in the nucleus accumbens, frontal cortex, and hypothalamus (Le Fur et al., 1981; Bhargava, 1984; Lim et al., 1989).

The studies examining dopamine content and receptor density in the brain of SHR_s are largely inconsistent and inconclusive for a variety of reasons. First, most of the studies involving dopamine receptor binding assays were done with non-selective antagonists. For example, spiperone, the most commonly used D₂ antagonist in these studies, binds to serotonergic and adrenergic receptors (Seeman et al., 1984). Secondly, these studies were conducted on SHR_s at ages that ranged from prehypertension to full-blown hypertension. Age-related changes could make comparisons between studies involving animals of different ages impossible. Thirdly, despite the many studies assessing dopamine receptor density and dopamine content in the striatum, different studies have examined different brain regions. Comparisons between different reports studying different regions of the brain provide misleading conclusions. These methodological shortcomings have made the interpretation of these studies practically impossible. However, a preponderance of investigations support an increase in both D₁ and D₂ receptor density in the caudate-putamen of SHR_s. This conclusion lends support to the notion that SHR_s suffer from an attenuated activity of central dopaminergic neurons which results or leads to an up-regulation of dopamine receptors (Creese & Snyder, 1979; Snyder, 1979). Since dopamine has a hypotensive effect, up-regulation of dopamine receptors in SHR_s could be responsible for SHR_s supersensitivity to exogenous administrations of dopamine.

3. AIMS AND OBJECTIVES

The well documented antihypertensive effects of dopamine and its agonists has prompted researchers to search for dopamine's site of action. Several investigators have examined dopamine content and dopamine receptor density in the brains of SHRs. Unfortunately, the use of non-selective D1 and D2 dopamine receptor antagonists used in receptor binding assays and the variety of ages and brain regions examined, have provided conflicting results. However, a preponderance of studies involving receptor binding assays of membrane homogenates point to an up-regulation of both D1 and D2 dopamine receptors in the dorsal striatum. Up-regulation of dopamine receptors in the brain of the SHR would explain their supersensitivity to dopamine administrations into the brain and the antihypertensive effects of dopamine agonists.

Several questions concerning the involvement of dopamine receptors in genetic hypertension remain to be answered:

- (1) is the density of dopamine receptors altered in the brain of SHRs compared to their controls the WKYs?
- (2) which receptor type is altered?
- (3) in what brain region are dopamine receptors altered?
- (4) do changes in dopamine receptor density precede the onset of hypertension or are the changes secondary to the rise in BP?

To answer these questions, an assessment of dopamine receptor density was conducted using the sensitive *in vitro* receptor autoradiography method. Because ligand binding is done *in situ*, which results in a higher anatomical resolution than

achieved with dissected brain tissue, *in vitro* receptor autoradiography is several-fold more sensitive than receptor binding assays of membrane homogenates (Kuhar, 1985). D1 and D2 dopamine receptor density in selected regions of the brain was compared in SHRs and WKYs at prehypertensive (5-week-old) and hypertensive (15-week-old) stages. Adjacent brain sections were studied to compare D1 and D2 receptor binding in the same anatomical region.

4. METHODS

4.1 Animals

Studies were performed on 5 and 15-week-old male SHR and WKY rats (Charles River, New York). The animals were housed in a temperature controlled environment (22 ± 1 C°) with free access to food and water and on a 12 hours/12 hours dark-light cycle.

4.2 Blood Pressure Assessment

To confirm whether SHRs or WKYs were hypertensive or normotensive, systolic BP was measured using the tail-cuff method. Rats were placed in a small animal restrainer and warmed with a lamp. An inflatable occlusion cuff containing a photoelectric sensor was placed on the animal's tail. The cuff was inflated and the pressure slowly released so that the pulse signal could be detected and amplified with a IITC Model 59 pulse amplifier (Woodland Hills, California). The mean of five readings was recorded as the rat's systolic BP.

4.3 Dopamine Receptor Autoradiography

Rats were deeply anesthetized using 5 mg/100 g b.wt. sodium pentobarbital (Abbott Laboratories, Toronto) and perfused transcardially with 0.9% ice-cold saline. The rat's brain was quickly dissected out, blocked using a stereotaxic frame with the incisor bar placed 5 mm above the interaural line, and frozen on a cryostat chuck using crushed dry-ice. Four adjacent 20 μ m sections from selected stereotaxic levels were cut using a Leitz cryostat at -15 C°. Sections were thaw mounted on chrome

alum/gelatin-coated slides to produce four series of identical slides. Slides were stored overnight at $-20\text{ }^{\circ}\text{C}$ in a slide box containing desiccant and subsequently kept at $-70\text{ }^{\circ}\text{C}$ until all brains had been cut and dried adequately. To assess D1 and D2 specific binding, the slides were thawed and incubated at room temperature for 30 min in a media containing 50 mM Tris buffer (pH 7.7) and either 1 nM [^3H]SCH 23390 (85.6 Ci/mmol, Dupont-NEN, Boston) or 15 nM [^3H]($-$)sulpiride (71.4 Ci/mmol, Dupont-NEN, Boston), respectively. To assess non-specific binding, a second series of sections was incubated with the same radioligands as for specific binding but with the addition of the D1 and D2 receptor agonists 10 μM fluphenazine (Sigma, St Louis) or 1 μM haloperidol (Sigma, St Louis), respectively. The sections were then rinsed in ice-cold buffer (2 x 5 min for SCH 23390, 4 x 1 min for sulpiride). The sections were dried with a cold stream of air. Autoradiograms were produced by placing the labelled tissue along with [^3H]Microscales (Amersham, Oakville) against sheets of Hyperfilm- H^3 (Amersham, Oakville) in tightly sealed cassettes and stored at $+4\text{ }^{\circ}\text{C}$. The X-ray film was exposed to SCH 23390 labelled tissue for 3 weeks and to sulpiride labelled tissue for 6 weeks. The films were developed by dipping them at room temperature in the following solutions: 5 min in D-19 developer (Kodak, Rochester), 30 sec in distilled water, 5 min in fixer (Kodak, Rochester), and 30 sec in distilled water. For anatomical localization of labelled receptor areas, one series of slides of exposed tissue was fixed in paraformaldehyde and stained for Nissl bodies using thionin. Autoradiograms were cut out of the autoradiographic sheets and placed between glass slides for protection. The autoradiograms were coded to keep the experimenter blind during assessment.

4.4 Image Analysis System

For densitometric quantitation, the optical density of tritium-sensitive film is scanned electronically. The optical density of a film area is measured by the proportion of a constant light source that passes through the tissue image. The image is transmitted from a video camera to a video monitor and digitized by a computer. The computer creates a gray scale for each pixel from which total binding readings can be taken from anatomical areas to give radioactivity per mg of tissue as calculated from standards.

The instrumentation for computer-assisted microdensitometry consisted of a light-box (Logan Electric, Chicago), a COHU 4810 series CCD video camera (San Diego), an electrohome ECM 1312 U high resolution color monitor (Kitchener, Ont) and a Mind microcomputer (Winnipeg). The Targa-M8 frame grabber software (Truevision, Indianapolis) stores a single image from the video camera and converts this image to a set of digital values. Interaction with the image displayed on the video monitor was accomplished using a Numonics Model 2210 digitizing tablet (Montgomeryville). Optical density measurements were taken using the JAVA video analysis software (Jandel Scientific, Corte Madera). This system can detect 256 levels of gray.

4.5 Computer-Assisted Densitometry

For each autoradiogram, the respective scales of standards were first analyzed to measure the mean optical density for each standard spot minus background values of the surrounding film. These values were then plotted against the corresponding

amounts of standard radioactivity (nCi/mg) provided by the manufacturer (Amersham). The best statistical fit for the relationship was calculated. Subsequent analysis of tissue autoradiogram was restricted to values belonging to the pseudolinear portion of the calibration curve. With the computerized system, an autoradiographic image of a selected brain region was captured on video and digitized. Using a cursor, four background values of the film were taken and the mean of these values was used to estimate the level of nonspecific radioactivity. Several optical density measurements were then taken from selected brain areas (anteromedial prefrontal cortex, cingulate cortex, nucleus accumbens, caudate-putamen, lateral septal nucleus, amygdaloid complex) to calculate the tissue mean optical density. Total specific binding was estimated by subtracting the mean background optical density value from the mean tissue optical density value. This product, the final tissue optical density, was then converted into radioactivity per mg tissue by extrapolating values from the respective standard calibration curve. For calibration of the radioactive standards, the correspondence in nCi/mg protein of gray matter given by the manufacturer was used. Accordingly, the specific radioactive activity was further converted into fmol/mg protein.

4.6 Statistical Analyses

Data are presented as mean \pm standard error. Spontaneously hypertensive rats were compared to WKYs with Student's t-test (two-tailed). The correlation coefficient and best statistical fit were calculated for each [^3H]Microscale standards.

5. RESULTS

The age-related changes in body weight, kidney to body weight ratio and heart weight to body weight ratio, and systolic BP are shown in Table I. Five-week-old SHR and WKYs had similar BPs while body weight was significantly elevated in WKYs. Heart weight to body weight ratio was significantly elevated in the 5-week-old SHR. Kidney weight to body weight ratio was not significantly different between 5-week-old SHR and WKYs. At 15 weeks the BP, kidney and heart weight to body weight ratios were significantly elevated in SHR. However, body weight was not different in 15-week-old rats.

Figures I and II are examples of autoradiograms showing specific and non-specific binding of [³H]SCH 23390 and [³H]sulpiride. The presence of the D1 and D2 agonists fluphenazine and haloperidol completely blocked the binding of the radioactive ligands. Correlation coefficients for optical density of each autoradiographic image of the standard and the standard radioactivity ranged from $r = +0.94$ to $+0.97$. Clearly, the optical density of the film increased as the radioactivity of the standard increased. The best statistical fit for this relationship was calculated to produce a standard calibration curve. Accordingly, correspondence between the optical density values measured on film for the images of brain tissue and the respective amount of radioactivity could be deduced from this calibration curve. The amount of tissue radioactivity was converted to fmol of ligand bound to mg tissue according to the batch analysis data provided by the manufacturer (Amersham, Oakville).

Table I. General characteristics of experimental animals.

	5-week-old		15-week-old	
	WKY	SHR	WKY	SHR
Systolic Blood Pressure (mmHg)	82 ± 1	79 ± 1	119 ± 1	168 ± 2*
Body Weight (gm)	99 ± 2	82 ± 2*	311 ± 3	287 ± 7
Kidney Weight/Body Weight (mg/100gm)	947 ± 20	998 ± 24	694 ± 20	776 ± 11*
Heart Weight/Body Weight (mg/100gm)	413 ± 7	467 ± 9*	329 ± 3	395 ± 11*

Values are means ± SE (n = 6).

Significantly different (p < 0.005) from WKY.

Figure I. Autoradiogram of [^3H]SCH 23390 binding in the forebrain.

A. Autoradiogram of [^3H]Microscale standard strip. Note that the grain density increases with the amount of radioactivity in the standard. B. Photomicrograph showing non-specific binding of [^3H]SCH 23390. C. This photomicrograph shows the grain distribution representing total specific binding of [^3H]SCH 23390 in the caudate-putamen (CP) and nucleus accumbens (NA) of the striatum.

nCi/mg

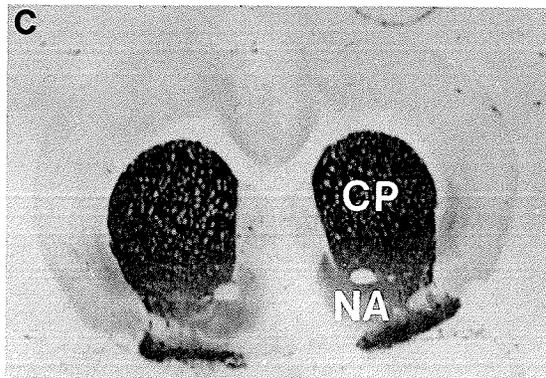
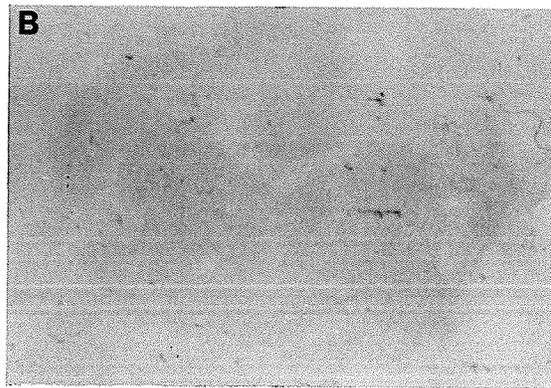
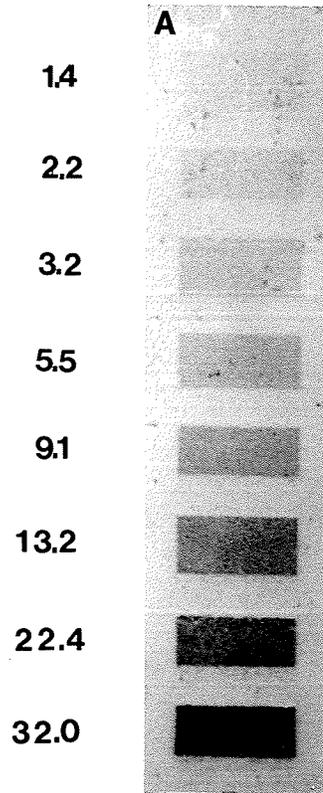


Figure II. Autoradiogram of [^3H]sulpiride binding in the forebrain.

A. Autoradiogram of [^3H]Microscale standard strip. Note that the grain density increases with the amount of radioactivity in the standard. B. Photomicrograph showing non-specific binding of [^3H]sulpiride. C. This photomicrograph shows the grain distribution representing total specific binding of [^3H]sulpiride in the caudate-putamen (CP) and nucleus accumbens (NA) of the striatum.

nCi/mg

1.4

2.2

3.2

5.5

9.1

13.2

22.4

32.0

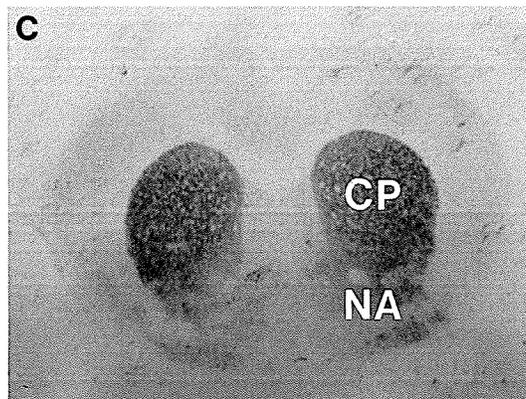
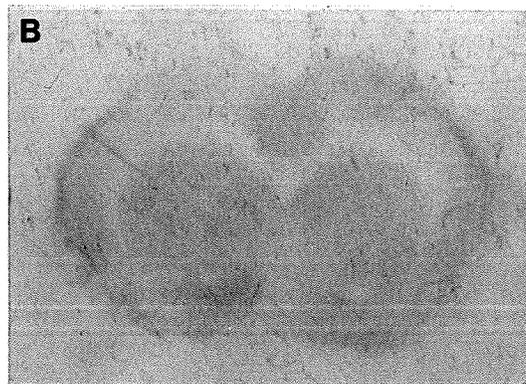
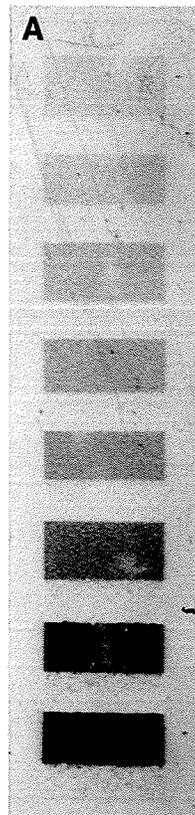


Table II shows the binding of [^3H]SCH 23390 and [^3H]sulpiride in the brain of 5-week-old SHR and WKY. Binding density of [^3H]SCH 23390 was significantly greater in the caudate-putamen, nucleus accumbens, and lateral septal nucleus of SHR. However, the binding density of [^3H]sulpiride was significantly increased only in the nucleus accumbens of SHR. The data for D2 receptor binding in the anteromedial prefrontal cortex, cingulate cortex, and amygdaloid complex was not computed because [^3H]sulpiride binding was at same level as non-specific D2 receptor binding.

Table III shows the binding of [^3H]SCH 23390 and [^3H]sulpiride in the brain of 15-week-old SHR and WKY. Similar to the binding in 5-week-old rats, [^3H]SCH 23390 was significantly higher in the caudate-putamen and nucleus accumbens of SHR. However, at the age of 15 weeks, the globus pallidus but not the lateral septal nucleus showed significantly greater [^3H]SCH 23390 binding in SHR. Binding of [^3H]sulpiride was not different between SHR and WKY at the age of 15 weeks.

Figures III and IV summarize the binding data in the caudate-putamen and the nucleus accumbens, respectively. For the caudate-putamen, [^3H]SCH 23390 binding was increased by 30% in 5-week-old SHR and 40% in 15-week-old SHR (Figure III). In the nucleus accumbens, [^3H]SCH 23390 binding was greater in SHR by 70% at the age of 5 weeks and 60% at the age of 15 weeks (Figure IV). Binding of [^3H]sulpiride was greater by 70% in 5-week-old SHR but was not significantly different at 15 weeks of age (Figure IV).

Table II. [³H]SCH 23390 (D1 antagonist) and [³H]sulpiride (D2 antagonist) binding density in the brain of 5-week-old rats.

	D1		D2	
	WKY	SHR	WKY	SHR
Caudate-putamen	247 ± 18	319 ± 9**	107 ± 5	99 ± 4
Nucleus Accumbens	133 ± 14	225 ± 21***	25 ± 2	43 ± 4**
Lateral Septal Nucleus	12 ± 4	27 ± 1*	12 ± 2	12 ± 2
Globus Pallidus	46 ± 9	51 ± 4	8 ± 2	7 ± 2
Anteromedial Prefrontal Cortex	52 ± 9	54 ± 4	NA	NA
Cingulate Cortex	34 ± 9	39 ± 5	NA	NA
Amygdaloid Complex	29 ± 4	36 ± 4	NA	NA

Values expressed in fmol/mg tissue are means ± SE (n = 6); NA, data not available.

* (p < 0.05), ** (p < 0.005), *** (p < 0.0001) significantly different from WKY.

Table III. [³H]SCH 23390 (D1 antagonist) and [³H]sulpiride (D2 antagonist) binding density in the brain of 15-week-old rats.

	D1		D2	
	WKY	SHR	WKY	SHR
Caudate-putamen	195 ± 8	268 ± 15**	106 ± 9	108 ± 8
Nucleus Accumbens	91 ± 10	143 ± 16*	27 ± 3	36 ± 4
Lateral Septal Nucleus	8 ± 2	17 ± 6	12 ± 3	19 ± 5
Globus Pallidus	14 ± 3	27 ± 4*	16 ± 4	14 ± 2
Anteromedial Prefrontal Cortex	27 ± 5	40 ± 4	NA	NA
Cingulate Cortex	18 ± 6	29 ± 3	NA	NA
Amygdaloid Complex	12 ± 4	21 ± 4	NA	NA

Values expressed in fmol/mg tissue are means ± SE (n = 6); NA, data not available

* (p < 0.05), ** (p < 0.005) significantly different from WKY.

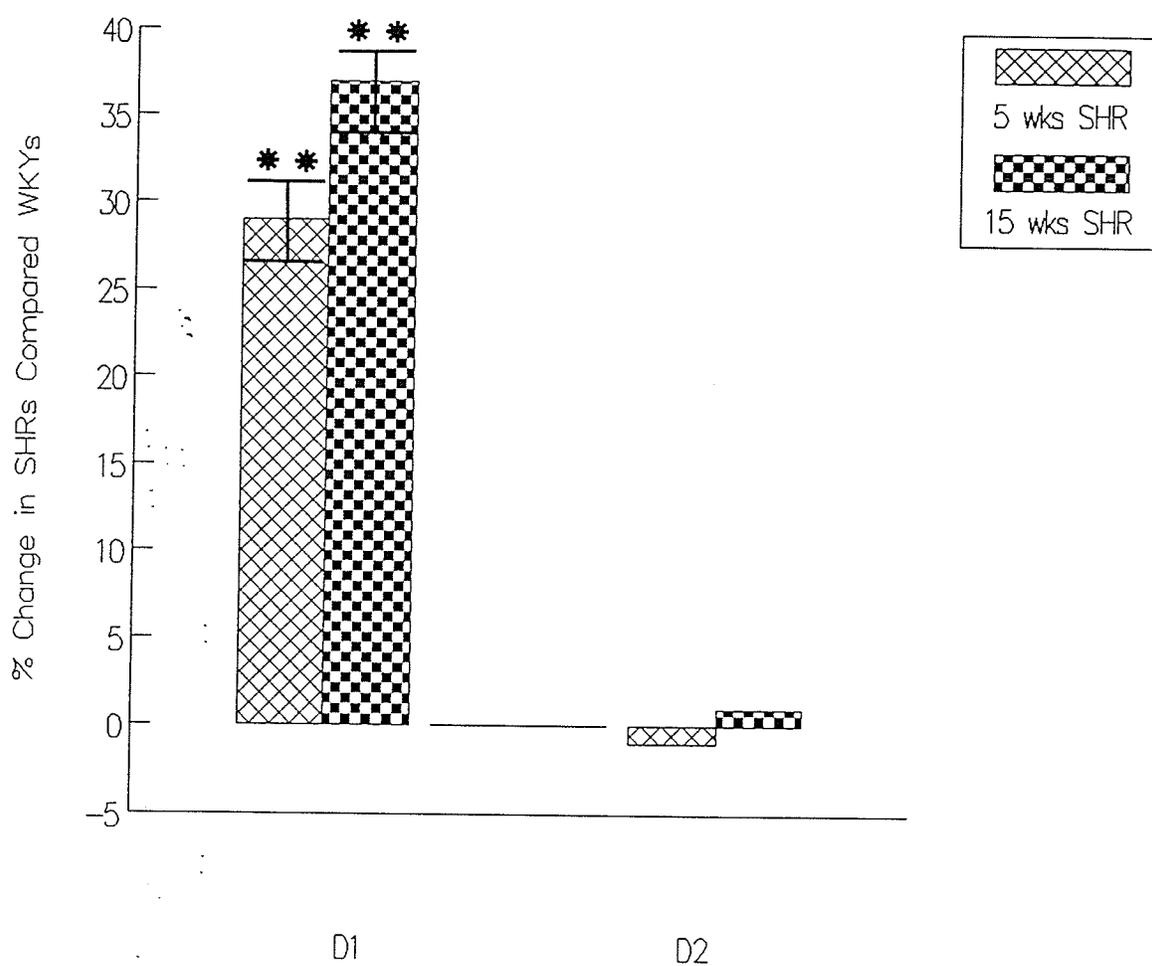


Figure III. Percent change in D1 and D2 receptor density in the caudate-putamen of SHRs compared to WKYs. ** significantly different from WKYs ($p < 0.005$).

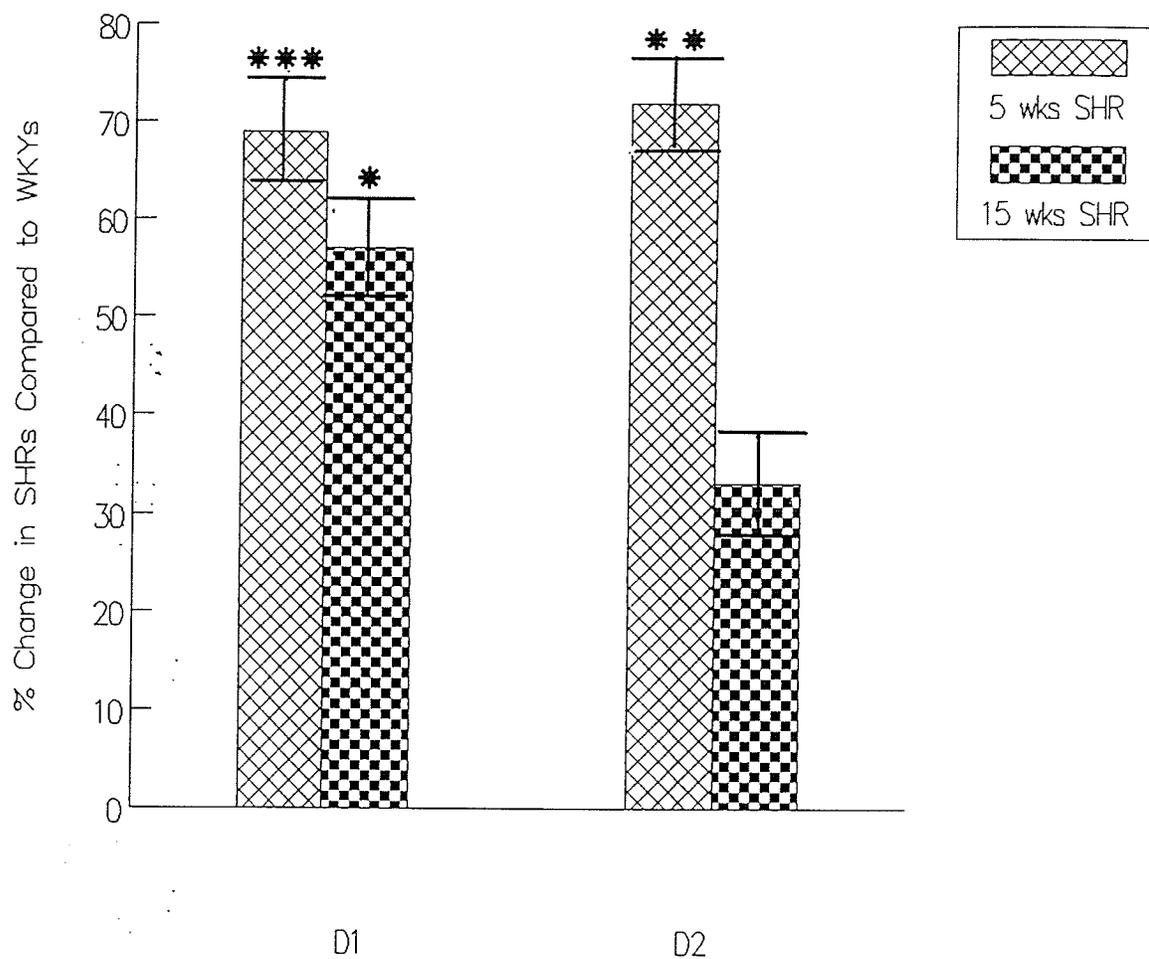


Figure IV. Percent change in D1 and D2 receptor density in the nucleus accumbens of SHRs compared to WKYs. Significantly different from WKY * ($p < 0.05$), ** ($p < 0.005$), *** ($p < 0.0001$).

6. DISCUSSION

It is clear from the data that at the age of 5 weeks, SHR_s were prehypertensive and that at the age of 15 weeks, SHR_s were hypertensive. This is in accordance with the well documented observation that a gradual rise in BP occurs in post-weanling SHR_s (Trippoda & Frohlich, 1981; Folkow, 1982). Blood pressure increases with age in both SHR_s and WKY_s but it does so at a faster rate and achieves a higher level in SHR_s. In the WKY, mean BPs reach a maximum level of about 120 mmHg at the age of 6-10 weeks, whereas in the SHR, mean BP increases until the age of 20-28 weeks and reaches levels as high as 200 mmHg (Trippoda & Frohlich, 1981). Therefore, the 15-week-old rats in the present investigation were in the developmental phase of hypertension and would have reached higher levels if allowed to age. The data in this paper is also in agreement with previously published results showing that WKY_s are slightly heavier than the same age SHR_s (Trippoda & Frohlich, 1981). However, BP in SHR_s has been shown to be independent of body weight (Folkow, 1982). An increase in kidney and heart weight in the hypertensive rats indicates that secondary effects, such as organ hypertrophy, has occurred.

A now well established effect of dopaminergic drugs are their hypotensive actions. The success of bromocriptine in lowering BP in both humans with essential hypertension and SHR_s suggests that hypertensive humans and rats suffer from a dopaminergic insufficiency that can be corrected by exogenous administrations of a dopamine agonist (Stumpe et al., 1977; McMurtry et al., 1979; Kolloch et al., 1981; Sowers, 1981; Nagahama et al., 1984). The central dopamine insufficiency

hypothesis is supported by the demonstration that SHR's exhibit a greater hypotensive effect to microinjections of dopamine into the brain (Kawebe et al., 1983; Hutchinson & Mok, 1984; Mok & Sim, 1987; Mok et al., 1990). The most sensible approach to explain the SHR's supersensitivity to exogenous dopamine is that dopamine receptors in the brain of SHR's are up-regulated. The increase number of dopamine receptors would result in more neurotransmitter molecules binding to the neural substrates controlling the cardiovascular system and therefore exerting a greater hypotensive effect. Despite conflicting reports, several investigators found support for up-regulation of both D1 and D2 dopamine receptors in the striatum of SHR's (Le Fur et al., 1981; Chiu et al., 1982; Bhargava, 1984; Lim et al., 1989, 1990).

The experiments in this thesis investigated dopamine receptor density in a variety of brain centers. Strong support for up-regulation of D1 dopamine receptors, as demonstrated by the increased binding of the D1 antagonist [^3H]SCH 23390, was found in the caudate-putamen and nucleus accumbens of both prehypertensive and hypertensive SHR's. Increased binding of the D1 antagonist was also demonstrated in the lateral septal nucleus of prehypertensive SHR's and in the globus pallidus of hypertensive SHR's. Other areas, for example the amygdaloid complex, showed similar increases in the binding of the D1 antagonist. Because the data showed a high amount of variability, no significance difference was found. Increased D2 receptor binding density, as demonstrated by binding of [^3H]sulpiride, was found only in the nucleus accumbens of prehypertensive SHR's.

Folkow (1982) has integrated an enormous amount of literature on hypertension and has proposed a teleological hypothesis for hypertension. He provides strong evidence that SHR and humans with essential hypertension inherit a predisposing hyperreactivity to the environment. During a prehypertensive stage, susceptible individuals have exaggerated cardiovascular responses to demanding or stressful situations. This cardiovascular hyperreactivity includes an enhanced cardiac output, heart rate, and arterial BP. Over time, this hyperkinetic circulation will lead to structural and functional alterations in the myocardium and blood vessels and these alterations will serve to maintain BP at an ever increasing level. Many of these structural and functional changes are being continually added to an ever increasing list of "causes of hypertension". However, most of these changes are secondary to increases in BP and are probably not important in the pathogenesis of the disease.

The functional implications for hypertension of up-regulation of dopamine receptors in the striatum of prehypertensive SHR is speculative. However, the striatum forms an interface between higher levels of the brain, namely the cerebral cortex and limbic structures, and the motor systems involved in the production of behavioral and autonomic responses (Mogenson, 1987). The striatum's nodal position suggests that it has a major role in the limbic-motor integration that underlies complex behavioral responses. Of particular interest for a discussion on hypertension is the nucleus accumbens. Its anatomical connections place it strategically between the amygdala and the lateral hypothalamic area (Krettek & Price, 1978; Nauta et al., 1978; Groenewegen & Russchen, 1984). Electrical

stimulation of the basolateral nucleus of the amygdala and the lateral hypothalamic area produce a defense reaction with a similar cardiovascular response as observed in SHRs exposed to alerting and noxious stimuli (Iwata et al., 1987; Haifi et al., 1989). Moreover, the defense reaction elicited from the amygdala is believed to be mediated by unknown relays to the lateral hypothalamic area and the brainstem (Hopkins & Holstege, 1978; Schwaber et al., 1980; Price & Amaral, 1981). The nucleus accumbens may serve as this critical relay because it receives a large number of projections from the basolateral nucleus of the amygdala and the nucleus accumbens in turn projects to the lateral hypothalamic area (Krettek & Price, 1978; Nauta et al., 1978; Groenewegen & Russchen, 1984). Consequently, dopamine by altering the relay of information in the nucleus accumbens from the amygdala to the lateral hypothalamic area, may modulate the cardiovascular and behavioral responses of the defense reaction and the cardiovascular hyperreactivity characterizing SHRs. Indeed electrical stimulation of the nucleus accumbens inhibited the cardiovascular components of the defense reaction elicited by stimulation of the amygdala and hypothalamus (Haifa et al., 1989).

Mogenson (1987) has provided strong evidence for a neuromodulatory role for dopamine in the nucleus accumbens. First, electrical stimulation of the ventral tegmental area, the major source of dopaminergic inputs to the nucleus accumbens, produced both excitatory and inhibitory responses on neurons in the nucleus accumbens (Mogenson & Yim, 1981). The postsynaptic responses in the nucleus accumbens were not blocked by dopamine antagonists (Mogenson & Yim, 1981). Because of the inability of dopamine antagonists to block the postsynaptic effects

produced by stimulation of the ventral tegmental area, dopamine must have a neuromodulatory and not a direct neuromediating role in the nucleus accumbens. Secondly, stimulation of the ventral tegmental area or iontophoretic application of dopamine in the nucleus accumbens, attenuated the excitatory responses of nucleus accumbens neurons produced by electrical stimulation of the hippocampus and the basolateral nucleus of the amygdala (Yim & Mogenson, 1982; Yang & Mogenson, 1984; Yim & Mogenson, 1988). The attenuating effects on nucleus accumbens neurons by the electrical stimulation of the ventral tegmental area was blocked by the D2 receptor antagonists sulpiride and haloperidol but not D1 receptor antagonists. This suggests that dopamine released in the nucleus accumbens has a modulating effect on afferent inputs to the nucleus accumbens. Thirdly, the excitability of axonal terminals in the nucleus accumbens was enhanced by stimulation of the ventral tegmental area (Yang & Mogenson, 1986). This excitability was blocked by the D2 antagonist sulpiride but was unaffected by the D1 receptor antagonist SCH 23390. Since neurotransmitter release is dependent on the amplitude of the action potential arriving at a fiber terminal, then moderated depolarization of axon terminals will increase the resting membrane potential and reduce the quantal release of the transmitter substance (Takeuchi & Takeuchi, 1962; Kusano et al., 1967). From Mogenson's experiments, one can conclude that dopamine released in the nucleus accumbens acts on presynaptic terminals of non-dopaminergic neurons to modulate the release of neurotransmitters. Indeed, dopamine by acting on D2 presynaptic receptors, has been shown to reduce the release of the excitatory amino acid glutamate in the striatum (Mitchell & Doggett,

1980; Rowland & Roberts, 1980; Godukhin et al., 1984). Therefore, it is probable that dopamine has little if any direct postsynaptic effect on nucleus accumbens neurons but has a neuromodulatory effect on afferent inputs to the nucleus accumbens.

In this autoradiographic receptor binding study, I found an up-regulation of D1 receptor in the caudate-putamen and nucleus accumbens of both prehypertensive and hypertensive SHR. In addition, I demonstrated an up-regulation of D2 receptors in the nucleus accumbens of prehypertensive SHR. This is in agreement with previous reports demonstrating an up-regulation of D1 and D2 receptors in the striatum of hypertensive SHR (Le Fur et al., 1981; Chiu et al., 1982; Bhargava, 1984; Lim et al., 1989, 1990). More importantly, this is the first report showing an up-regulation of D1 and D2 receptor in the striatum of prehypertensive SHR. Up-regulation of dopamine receptors in prehypertensive SHR provides evidence that these changes are not secondary to an increase in BP and points to an altered dopamine function as an etiological factor in genetic hypertension.

7. CONCLUSIONS

As described earlier, a great deal of evidence has accumulated implicating hyperreactivity of the cardiovascular system to environmental situations as an initiating cause for genetic hypertension (Folkow, 1982). This hyperactivity appears to be mediated by projections from the amygdala to the hypothalamus and the brainstem. The nucleus accumbens forms an important anatomical and functional interface between the amygdala and the hypothalamus (see Figure 5). Dopamine released in the nucleus accumbens is believed to modulate the flow of information from limbic structures (Mogenson, 1987). More specifically, dopamine acts on presynaptic terminals in the nucleus accumbens to attenuate the release of neurotransmitters and thereby reducing the neurotransmitter's postsynaptic actions on neurons in the nucleus accumbens. Removal of this presynaptic inhibition leads to an increase in neuronal activity and output of the nucleus accumbens. Because of the plasticity of dopamine receptors, up-regulation of dopamine receptors reflects a decrease in the release of the dopamine in the striatum (Goldberg, 1979). An attenuated release of dopamine would result in the removal of some of dopamine's inhibitory influence on inputs arriving at the nucleus accumbens. The resulting increase in activity of the amygdala-accumbens-hypothalamus circuitry could be responsible for the cardiovascular hyperreactivity believed to be an etiological factor in genetic hypertension.

Experiments should be designed to answer several questions concerning dopamine's role in hypertension. First, is the release of dopamine in the striatum of SHRs different from WKYs and are these changes present in prehypertensive

animals? Secondly, is the function of neurotensin and cholecystokinin, which are neuropeptides co-localized and co-released with dopamine, also altered in SHRs? Finally, because dopamine presynaptically inhibits the release of neurotransmitters in the nucleus accumbens and because SHRs suffer from a dopamine insufficiency, is the release of neurotransmitters in the nucleus accumbens altered in SHRs?

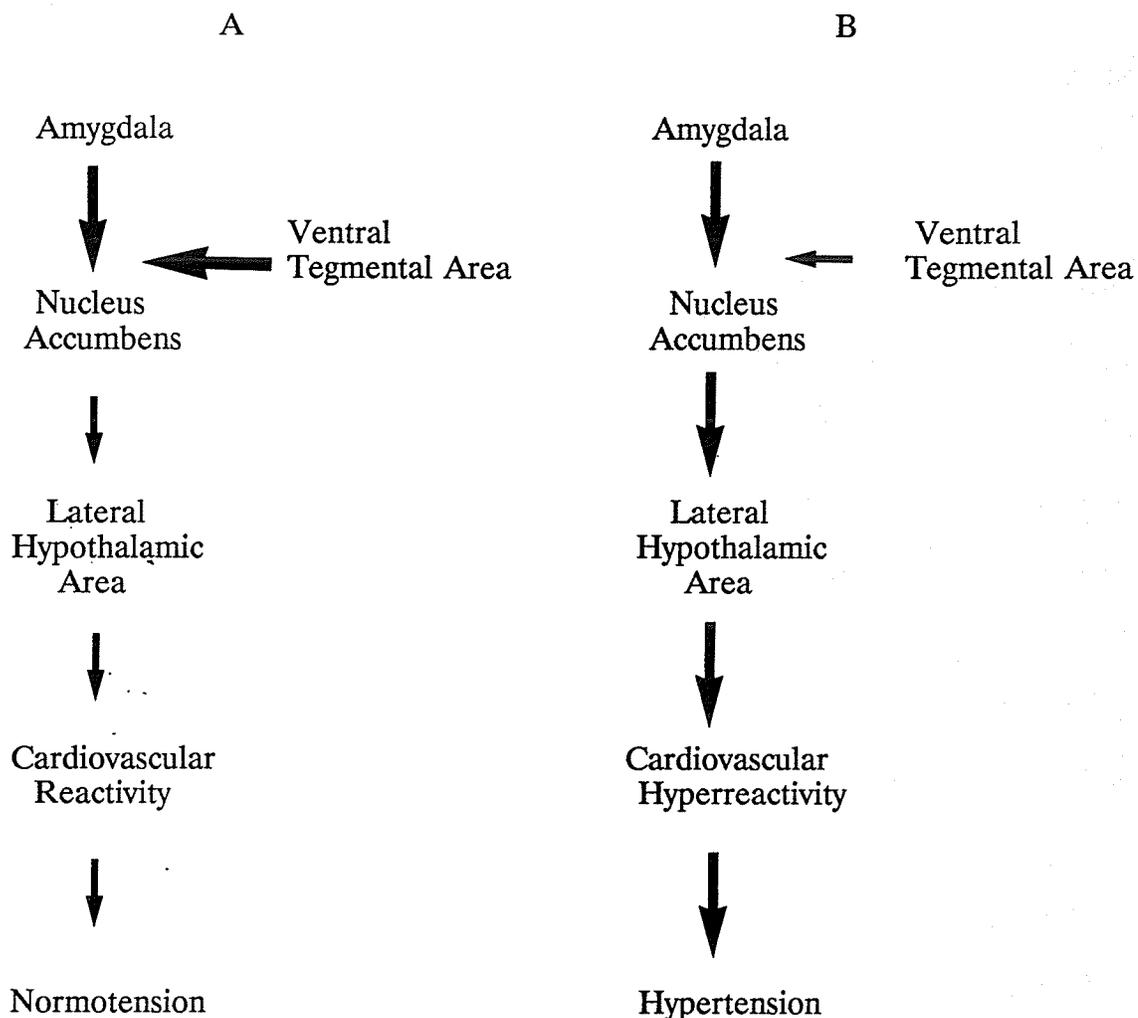


Figure V. Diagrammatic representation of dopamine's involvement in hypertension.

A. Dopaminergic projections to the nucleus accumbens attenuate the relay of information from the amygdala to the lateral hypothalamic area allowing normal reactivity and normotension. B. Dopamine insufficiency leads to an over-activity of the amygdala-accumbens-hypothalamus circuitry responsible for the cardiovascular responses accompanying emotional arousal leading to hyperreactivity and hypertension.

8. REFERENCES

- Beckstead, R.M., Domesick, V.B., & Nauta, W.J.H. (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res.*, 175, 191-217.
- Bernardi, G., Marciani, M.G., Morocutti, C., Pavone, F., & Stanzione, P. (1978) The action of dopamine on rat caudate neurones intracellularly recorded. *Neurosci. Lett.*, 8, 235-240.
- Bertholini, G., Lloyd, K.G., & Stadler, H. (1974) Dopaminergic regulation of cholinergic neurons in the striatum: Relation to parkinsonism. *Adv. Neurol.*, 5, 11-17.
- Bhargava, H.N. (1984) Effect of cyclo(leucyl-glycine) on [³H]spiroperidol binding in the corpus striatum and hypothalamus of spontaneously hypertensive rats. *Eur. J. Pharmacol.*, 100, 109-112.
- Bjorklund, A., & Lindvall, O. (1984) Dopamine-containing systems in the CNS. In A. Bjorklund & T. Hokfelt (Eds.) *Handbook of chemical neuroanatomy. Vol 2. Classical neurotransmitters in the CNS* (pp 55-122). Elsevier, Amsterdam.
- Bjorklund, A., Lindvall, O., & Nobin, A. (1975) Evidence of an incertohypothalamic dopamine neurone system in the rat. *Brain Res.*, 89, 29-42.
- Blessing, W.W., & Chalmers, J.P. (1979) Direct projection of catecholamine (presumably dopamine) -containing neurons from hypothalamus to spinal cord. *Neurosci. Lett.*, 11, 35-40.

- Brown, J.H., & Makman, M.H. (1972) Stimulation by dopamine of adenylate cyclase in retinal homogenates and of adenosine 3',5'-cyclic monophosphate formation in intact retina. *Proc. Natl. Acad. Sci. USA*, 69, 539-543.
- Burns, S.M., & Wyss, J.M. (1985) The involvement of the anterior cingulate cortex in blood pressure control. *Brain Res.*, 340, 71-77.
- Carlson, A. (1977) Dopaminergic autoreceptors: Background and implications. *Adv. Biochem. Psychopharmac.*, 16, 439-441.
- Chalmers, J. & Pilowsky, P. (1991) Brainstem and bulbospinal neurotransmitter systems in the control of blood pressure. *J. Hypertens.*, 9, 675-694.
- Ciriello, J., & Calaresu, F.R. (1980) Role of paraventricular and supraoptic nuclei in central cardiovascular regulation in the cat. *Am. J. of Physiol.*, 239, R137-R142.
- Ciriello, J., Caverson, M.M., & Polosa, C. (1986) Function of the ventrolateral medulla in the control of the circulation. *Brain Res. Rev.*, 11, 359-391.
- Chiu, P., Rajakumar, G., Chiu, S., Kwan, C-Y., & Mishra, R.K. (1982) Enhanced [³H]spiroperidol binding in striatum of spontaneously hypertensive rat (SHR). *Eur. J. Pharmacol.*, 82, 243-244.
- Cohen, D.M., & Obrist, P.A. (1975) Interactions between behavior and the cardiovascular system. *Cir. Res.*, 37, 363-369.
- Conway, J. (1984) Hemodynamic aspects of essential hypertension in humans. *Physiol. Rev.*, 64, 617-660.

- Cote, T.E., Eskay, R.L., Frey, E.A., Grewe, C.W., Munemura, M., Stoof, J.C., Tsuruta, K., & Keibian, J.W. (1982) Biochemical and physiological studies of the beta-receptor and the D-2 dopamine receptor in the intermediate lobe of the rat pituitary gland: A review. *Neuroendocrinology*, 35, 217-224.
- Creese, I., & Snyder, S.H. (1979) Nigrostriatal lesions enhance striatal ^3H -apomorphine and ^3H -spiroperidol binding. *Eur. J. Pharmacol.*, 56, 277-281.
- Crofton, J.T., Share, L., Shade, R.E., Allen, C., Tarnowski, D. (1978) Vasopressin in the rat with spontaneous hypertension. *Am. J. Physiol.*, 235, H361-H366.
- Cunningham, E.T. Jr., & Sawchenko, P.E. (1991) Reflex control of magnocellular vasopressin and oxytocin secretion. *Trends Neurosci.*, 14, 406-411.
- Dahlstrom, A., & Fuxe, K. (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand.*, 232, 1-55.
- Dawson, T.M., Gehlert, D.R., McCabe, R.T., Barnett, A., Wamsley, J.K. (1986) D-1 dopamine receptors in the rat brain: A quantitative autoradiographic analysis. *J. Neurosci.*, 6, 2352-2365.
- Day, M.D., & Roach, A.G. (1976) Cardiovascular effects of dopamine after central administration into conscious cats. *Brit. J. Pharmacol.*, 58, 505-515.
- Eckman, P., Levinson, R.W., & Friesen, W.V. (1983) Autonomic nervous system activity distinguishes among emotions. *Science*, 221, 1208-1210.

- Euvar, C., Ferland, L., DiPaolo, T., Beaulieu, M., Labrie, F., Oberlander, C., Raynaud, J.P., & Boissier, J.R. (1980) Activity of two new potent dopaminergic agonists at the striatal and anterior pituitary levels. *Neuropharmacology*, 19, 379-386.
- Falkner, B., Onesti, G., Angelakos, E.T., Fernandes, M., & Langman, C. (1979) Cardiovascular response to mental stress in normal adolescents with hypertensive parents. *Hypertension*, 1, 23-30.
- Folkow, B. (1982) Physiological aspects of primary hypertension. *Physiol. Rev.*, 62, 347-504.
- Folkow, B. (1987) Psychosocial and central nervous influences in primary hypertension. *Circulation*, 76 (suppl. I), I10-I19.
- Fallon, J.H., Koziell, D.A., & Moore, R.Y. (1978) Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal cortex and entorhinal cortex. *J. Comp. Neurol.*, 180, 509-532.
- Fallon, J.H., & Moore, R.Y. (1978) Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. *J. Comp. Neurol.*, 180, 545-580.
- Fuller, R.W., Hemrick-Luecke, S.K., Wong, D.T., Pearson, D., Threlkeld, P.G., & Hynes, M.D. (1983) Altered behavioral response to a D2 agonist, LY141865, in spontaneously hypertensive rats exhibiting biochemical and endocrine responses similar to those in normotensive rats. *J. Pharmacol. Exper. Ther.*, 227, 354-359.

- Fuxe, K. (1965) Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. *Acta Physiol. Scand.*, suppl. 247, 34-85.
- Fuxe, K., Agnati, L.F., Kalia, M., Goldstein, M., Andersson, K., & Harfstrand, A. (1985) Dopaminergic systems in the brain and pituitary. In E. Flückiger, E.E. Müller, & M.O. Thörner (Eds.) *Basic and clinical aspects of neuroscience: The dopaminergic system* (pp 11-25), Springer, New York.
- Fuxe, K., Ganten, D., Jonsson, G., Agnati, L.F., Andersson, K., Hokfelt, T., Bolme, P., Goldstein, M., Hallman, H., Unger, T., & Rascher, W. (1979) Catecholamine turnover changes in hypothalamus and dorsal midline area of the caudal medulla oblongata of spontaneously hypertensive rats. *Neurosci. Lett.*, 15, 283-288.
- Fuxe, K., Ganten, D., Jonsson, G., Bolme, P., Agnati, L.F., Andersson, K., Goldstein, M., & Hokfelt, T. (1979) Evidence for a selective reduction of adrenaline turnover in the dorsal midline area of the caudal medulla oblongata of young spontaneous hypertensive rats. *Acta Physiol. Scand.*, 107, 397-399.
- Galeno, T.M., Van Hoesen, G.W., Maixner, W., Johnson, A.K., Brody, M.J. (1982) Contribution of the amygdala to the development of spontaneous hypertension. *Brain Res.*, 246, 1-6.
- Gehlert, D.R., & Wamsley, J.K. (1985) Dopamine receptors in the rat brain: Quantitative autoradiographic localization using [³H]sulpiride. *Neurochem. Int.*, 7, 717-723.

- Godukhin, O.V., Zharikova, A.D., & Budantsev, A.Y. (1984) Role of presynaptic dopamine receptors in regulation of the glutamatergic neurotransmission in rat neostriatum. *Neuroscience*, 12, 377-383.
- Goldberg, L.H. (1979) The dopamine vascular receptor. In A.S. Horn, J. Korf, & B.H.C. Westerink (Eds.) *The Neurobiology of dopamine* (pp 541-551). Academic Press, London.
- Goldstein, M., Harada, K., Meller, E., Schalling, M., & Hokfelt, T. (1990) Dopamine autoreceptors: Biochemical, pharmacological and morphological studies. *Ann. N.Y. Acad. Sci.*, 604, 169-175.
- Groenewegen, H.J., & Russchen, F.T. (1984) Organization of the efferent projections of the nucleus accumbens to pallidal, hypothalamic, and mesencephalic structures: A tracing and immunohistochemical study in the cat. *J. Comp. Neurol.*, 223, 347-367.
- Haifa, A., Maskati, A., & Zbrozyna, A.W. (1989) Stimulation in prefrontal cortex area inhibits cardiovascular and motor components of the defense reaction in rats. *J. Auton. Nerv. Sys.*, 28, 117-126.
- Hallback, M. (1975) Consequence of social isolation on blood pressure, cardiovascular reactivity and design in spontaneously hypertensive rats. *Acta Physiol. Scand.*, 93, 455-465.
- Hallback, M., & Folkow, B. (1974) Cardiovascular responses to acute mental "stress" in spontaneously hypertensive rats. *Acta Physiol. Scand.*, 40, 684-698.
- Hardy, S.G.P., & Holmes, D.E. (1988) Prefrontal stimulus-produced hypotension in rat. *Exp. Brain Res.*, 73, 249-255.

- Heise, A. (1976) Hypotensive action by central α -adrenergic and dopaminergic receptor stimulation. In A. Scriabine, & C.S. Sweet (Eds.) *New antihypertensive drugs* (pp 135-145). Spectrum Publications, New York.
- Henley, W.N., & Bellush, L.L. (1989) Central catecholaminergic responses in hypoxic moderation of spontaneous hypertension. *Brain Res. Bull.*, 22, 963-968.
- Herzog, A.G., & Van Hoesen, G.W. (1976) Temporal neocortical afferent connections to the amygdala in the rhesus monkey. *Brain Res.*, 115, 57-69.
- Herrling, P.L., & Hull, C.D. (1980) Iontophoretically applied dopamine depolarizes and hyperpolarizes the membrane of cat caudate neurons. *Brain Res.*, 192, 441-462.
- Hokfelt, T., Johansson, O., Fuxe, K., Goldstein, M., & Park, D. (1976) Immunohistochemical studies on the localization and distribution of monoamine neuron systems in the rat brain. I. Tyrosine hydroxylase in the mes- and dicephalon. *Med. Biol.*, 54, 427-453.
- Hole, K., Russo, P.V., & Mandell, A.J. (1986) The influence of neuropeptides on amine synthesis: Cholecystokinin-8 and neurotensin reduce striatal tyrosine hydroxylase activity. *Neurochem. Int.*, 9, 493-498.
- Holstege, G. (1991) Descending motor pathways and the spinal motor system: Limbic and non-limbic components. *Prog. Brain Res.*, 87, 307-421.
- Hong, J.S., Yoshikawa, K., Kanamatsu, T., & Sabol, S.L. (1985) Modulation of striatal enkephalinergic neurons by antipsychotic drugs. *Fed. Proc.*, 44, 2535-2539.

- Hopkins, D.A., & Holstege, G. (1978) Amygdaloid projections to the mesencephalic, pons and medulla oblongata in the cat. *Exp. Brain Res.*, 32, 529-547.
- Howes, L.G., Rowe, P.R., Summers, R.J., & Louis, W.J. (1984) Age related changes of catecholamines and their metabolites in central nervous system regions of spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats. *Clin. Exp. Hypertens.*, A6, 2263-2277.
- Hutchinson, J.S., & Mok, J.S.L. (1984) Neuropeptide abnormalities suggest a dopaminergic basis for high blood pressure in the spontaneously hypertensive rat. *Clin. Exp. Hypertens.*, A6, 2055-2058.
- Hutchison, J.B., Strupish, J., & Nahorski, S.R. (1986) Release of endogenous dopamine and cholecystokinin from rat striatal slices: Effects of amphetamine and dopamine antagonists. *Brain Res.*, 370, 310-314.
- Iverson, L.L. (1975) Dopamine receptors in the brain. *Science*, 188, 1084-1089.
- Iwata, J., Chida, K., & LeDoux, J.E. (1987) Cardiovascular responses elicited by stimulation of neurons in the central nucleus of amygdala in awake but not anesthetized rats resemble conditioned emotional responses. *Brain Res.*, 418, 183-188.
- Julius, S. & Esler, M. (1975) Autonomic nervous cardiovascular regulation in borderline hypertension. *Am. J. Cardiol.*, 36, 685-696.
- Kababian, J.W., & Calne, D.B. (1979) Multiple receptors for dopamine. *Nature*, 277, 93-96.

- Kebabian, J.W., Calne, D.B., & Kebabian, P.R. (1977) Lergortile mesylate: An in vivo dopamine agonist which blocks dopamine receptors in vitro. *Commun. Psychopharmacol.*, 1, 311-318.
- Kawabe, H., Kondo, K., & Saruta, T. (1983) Effect of the intracerebroventricular injection of dopamine on blood pressure in the spontaneously hypertensive rat. *Clin. Exp. Hypertens.*, A5, 1703-1716.
- Kitai, S.T., Sugimori, M., & Kocsis, J.D. (1976) Excitatory nature of dopamine in the nigro-caudate pathway. *Experimental Brain Res.*, 24, 351-363.
- Kojima, A., Takahashi, Y., Ohno, S-I., Sato, A., Yamada, T., Kubota, T., Yamori, Y., & Okamoto, K. (1975) An elevation of plasma TSH concentration in spontaneously hypertensive rats (SHR). *Proc. Soc. Exp. Biol. Med.*, 149, 661-663.
- Kolloch, R., Kobayashi, K., & DeQuattro, V. (1980) Dopaminergic control of sympathetic tone and blood pressure: Evidence in primary hypertension. *Hypertension*, 2, 390-394.
- Kolloch, R., Stumpe, K.O., Ismer, U., Kletzky, O., & DeQuattro, V. (1981) Central dopaminergic mechanisms in young patients with essential hypertension. *Clin. Sci.*, 61, 231s-234s.
- Kondo, K., Ebihara, A., Suzuki, H., & Saruta, T. (1981a) Role of dopamine in the regulation of blood pressure and the renin-angiotensin-aldosterone system in conscious rats. *Clin. Sci.*, 61, 235s-237s.

- Kondo, K., Suzuki, H., Handa, M., Nagahama, S., & Saruta, T. (1981) Effects of intracerebroventricular administration of dopamine and metoclopramide on blood pressure in rats. *Arch. Int. Pharmacodyn.*, 250, 273-278.
- Krettek, J.E., & Price, J.L. (1978) Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. *J. Comp. Neurol.*, 178, 225-254.
- Krettek, J.E., & Price, J.L. (1978) A description of the amygdaloid complex in the rat and cat with observations on intra-amygdaloid axonal connections. *J. Comp. Neurol.*, 178, 225-280.
- Kuhar, M.J. (1985) Receptor localization with the microscope. In H.I. Yamamura, S.J. Enna, & M.J. Kuhar (Eds.) *Neurotransmitter receptor binding* (pp 153-177). Raven, New York.
- Kusano, K., Livenwood, D.R., Werman, R. (1967) Correlation of transmitter release with membrane properties of the presynaptic fibre of the squid giant synapse. *J. Gen. Physiol.*, 50, 2579-2601.
- Lang, W.J., & Woodman, O.L. (1979) Cardiovascular responses produced by the injection of dopamine into the cerebral ventricles of the unanaesthetized dog. *Brit. J. Pharmacol.*, 66, 235-140.
- LeDoux, J.E., Del Bo, A., Tucker, L.W., Harshfield, G., Talman, W.T., & Reis, D.J. (1982) Hierarchic organization of blood pressure responses during expression of natural behaviors in rat: Mediation by sympathetic nerves. *Exp. Neurol.*, 78, 121-133.

- Le Fur, G., Guilloux, F., Kabouche, M., Mitrani, N., Ferris, O., & Uzan, A. (1981) Central dopaminergic neurons during development of genetic and DOCA-salt hypertension in the rat. *Dev. Brain Res.*, 1, 153-163.
- Lim, D.K., Ito, Y., Hoskins, B., Rockhold, R.W., Ho, I.K. (1989) Comparative studies of muscarinic and dopamine receptors in three strains of rat. *Eur. J. Pharmacol.*, 165, 279-287.
- Lim, D.K., Yu, Z.J., Hoskins, B., Rockhold, R.W., & Ho, I.K. (1990) Effects of acute and subacute cocaine administration on the CNS dopaminergic system in Wistar-Kyoto and spontaneously hypertensive rats: Dopamine receptors. *Neurochem. Res.*, 15, 621-627.
- Lindvall, O. (1975) Mesencephalic dopaminergic afferents to the lateral septal nucleus of the rat. *Brain Res.*, 87, 89-95.
- Lindvall, O., Bjorklund, A., & Skagerberg, G. (1984) Selective histochemical demonstration of dopamine terminal systems in rat di- and telencephalon: New evidence for a dopaminergic innervation of hypothalamic neurosecretory nuclei. *Brain Res.*, 306, 19-30.
- Lindvall, O., & Stenevi, U. (1978) Dopamine and noradrenaline neurons projecting to the septal area in the rat. *Cell Tissue Res.*, 190, 383-407.
- Loewy, A.D. (1991) Forebrain nuclei involved in autonomic control. *Prog. Brain Res.*, 87, 253-268.

- Lofstrom, A., Jonsson, G., & Fuxe, K. (1976) Microfluorometric quantitation of catecholamine fluorescence in rat median eminence. I. Aspects on the distribution of dopamine and noradrenaline nerve terminals. *J. Histochem. Cytochem.*, 24, 415-429.
- Loughlin, S.E., & Fallon, J.H. (1983) Dopaminergic and non-dopaminergic projections to amygdala from substantia nigra and ventral tegmental area. *Brain Res.*, 262, 334-338.
- Lundin, S. & Hallback-Nordlander, M. (1980) Background of hyperkinetic circulatory state in young spontaneously hypertensive rats. *Cardiovas. Res.*, 14, 561-567.
- MacMahon, S. (1989) The effects of antihypertensive drug treatment on the incidence of stroke and of coronary heart disease. *Clin. Exp. Hypertens.*, A11, 807-823.
- Martin, X., & Dolivo, M. (1983) Neuronal and transneuronal tracing in the trigeminal system of the rat using the herpes suis. *Brain Res.*, 273, 253-276.
- Maskati, H.A.A., & Zbrozyna, A.W. (1989) Cardiovascular and motor components of the defense reaction elicited in rats by electrical and chemical stimulation in amygdala. *J. Auton. Nerv. Sys.*, 28, 127-132.
- McGeer, P.L., McGeer, E.G., & Suzuki, J.S. (1977) Aging and extrapyramidal function. *Arch. Neurol.*, 34, 33-35.
- McMurtry, J.P., Kazama, N., & Wexler, B.C. (1979) Effects of bromocriptine on hormone and blood pressure levels in the spontaneously hypertensive rat. *Proc. Soc. Exp. Biol. Med.*, 161, 186-188.

- McMurtry, J.P., & Wexler, B.C. (1981) Hypersensitivity of spontaneously hypertensive rats (SHR) to heat, ether, and immobilization. *Endocrinology*, 108, 1730-1736.
- McNeil, J.J. (1989) Analysis of antihypertensive treatment outcome and stroke. *Clin. Exp. Hypertens.*, A11, 761-777.
- Mercuri, N., Bernadi, G., Calabresi, P., Cotugno, A., Levi, G., & Stanzione, P. (1985) Dopamine decreases cell excitability in rat striatal neurons by pre- and postsynaptic mechanisms. *Brain Res.*, 358, 110-121.
- Mitchell, P.R., & Doggett, N.S. (1980) Modulation of striatal [³H]glutamic acid release by dopaminergic drugs. *Life Sci.*, 26, 2073-2081.
- Mogenson, G.J. (1987) Limbic-motor integration. *Prog. Psychobiol. Physiol. Psychol.*, 12, 117-169.
- Mogenson, G.J., Jones, D.L., & Yim, C.Y. (1980) From motivation to action: Functional interface between the limbic system and the motor system. *Prog. Neurobiol.*, 14, 69-97.
- Mogenson, G.J., & Yim, C.Y. (1981) Electrophysiology and neuropharmacological-behavioral studies of the nucleus accumbens: Implications for its role as a limbic motor interface. In R.B. Chronister & J.F. DeFrance (Eds.) *The neurobiology of the nucleus accumbens* (pp 210-229). Haer Institute for Electrophysiological Research, Maine.

- Mok, J.S.L., Hutchinson, J.S., & Sim, M.K. (1990) Effects of dopamine on blood pressure and heart rate after microinjection into the hypothalamus of spontaneously hypertensive and normotensive Wistar-Kyoto rats. *Clin. Exp. Hypertens.*, A12, 15-36.
- Mok, J.S.L., & Sim, M.K. (1987) The actions of dopamine on the blood pressure and heart rate of conscious hypertensive rats: Evidence for reduced dopaminergic activity in rats of the Japanese strains. *Clin. Exp. Hypertens.*, A9, 1615-1635.
- Nagahama, S., Chen, Y-F., & Oparil, S. (1984) Mechanism of the depressor effect of bromocriptine in the spontaneously hypertensive rat. *J. Pharmacol. Exp. Ther.*, 228,370-375.
- Nauta, W.J.H., Smith, G.P., Faull, R.L.M., & Domesick, V.B. (1978) Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. *Neuroscience*, 3, 385-401.
- Neafsey, E.J., Hurley-Gius, K.M., & Arvanitis, D. (1986) The topographical organization of neurons in the rat medial frontal, insular, and olfactory cortex projecting to the solitary nucleus, olfactory bulb, periaqueductal gray and superior colliculus. *Brain Res.*, 377, 261-270.
- Okamoto, K. (1969) Spontaneous hypertension in rats. *Intern. Rev. Exptl. Pathol.*, 7, 227-270.
- Okamoto, K. & Aoki, K. (1963) Development of a strain of spontaneously hypertensive rats. *Japan. Circulation J.*, 27, 282-293.
- Pickering, G.W. (1968) High blood pressure. Churchill Livingstone, London.

- Pickering, T.G., & Gerrin, W. (1990) Cardiovascular reactivity and the role of behavioral factors in hypertension: A critical review. *Ann. Beh. Med.*, 12, 3-16.
- Plecha, D.M., Randall, W.C., Reis, G.S., & Wurtser, R.D. (1988) Localization of vagal preganglionic somata controlling sinoatrial and atrioventricular nodes. *Am. J. Physiol.*, 255, R703-R708.
- Porter, J.P., & Brody, M.J. (1986) A comparison of the hemodynamic effects produced by electrical stimulation of subnuclei of the paraventricular nucleus. *Brain Res.*, 375, 20-29.
- Powell, D.A., Buchanan, S., & Hernandez, L. (1985) Electrical stimulation of insular cortex elicits cardiac inhibition but insular lesions do not abolish conditioned bradycardia in rabbits. *Behavioral Brain Res.*, 17, 125-144.
- Price, J.L., & Amaral, D.G. (1981) An autoradiographic study of the projections of the central nucleus of the monkey amygdala. *J. Neurosci.*, 1, 1242-1259.
- Reis, D.J. & Ledoux, J.E. (1987) Some central neural mechanisms governing resting and behaviorally coupled control of blood pressure. *Circulation*, 76, (suppl I), I2-I9.
- Reis, D.J., Morrison, S., & Ruggiero, D.A. (1988) The C1 area of the brainstem in tonic and reflex control of blood pressure. *Hypertension*, 11, (suppl I), I8-I13.
- Robbins, T.W., & Everett, B.J. (1982) Functional studies of the central catecholamines. *Int. Rev. Neurobiol.*, 25, 303-366.

- Rowland, G.J., & Roberts, P.J. (1980) Activation of dopamine receptors inhibits calcium dependent glutamate release from cortico-striatal terminals *in vitro*. Eur. J. Pharmacol., 62, 241-242.
- Rosene, D., & Van Hoesen, G.W. (1977) Hippocampal efferents reach widespread areas of cerebral cortex and amygdala in the rhesus monkey. Science, 198, 315-317.
- Ruggeri, M., Ungerstedt, U., Agnati, L.F., Mutt, V., Harfstrand, A., & Fuxe, K. (1987) Effects of cholecystokinin peptides and neurotensin on dopamine release and metabolism in the rostral and caudal part of the nucleus accumbens using intracerebral dialysis in the anesthetized rat. Neurochem. Int., 10, 509-520.
- Ruggiero, D.A., Mraovitch, S., Granata, A.R., Anwar, M., & Reis, D.J. (1987) Role of insular cortex in cardiovascular function. J. Comp. Neurol., 257, 189-207.
- Schwaber, J.S., Kapp, B.S., and Higgins, G. (1980) The origin and extent of direct amygdala projections to the region of the dorsal motor nucleus of the vagus and the nucleus of the solitary tract. Neurosci. Lett., 20, 15-20.
- Schwarcz, R.I., Creese, I., Coyle, J.T., & Snyder, S.H. (1978) Dopamine receptors localized on cerebral cortical afferents to rat corpus striatum. Nature, 271, 766-768.
- Seeman, P., Ulpian, C., Wreggett, K.A., & Wells, J.W. (1984) Dopamine receptor parameters detected by [³H]spiperone depend on tissue concentration: Analysis and examples. J. Neurochem., 43, 221-235.

- Seroogy, K., Ceccatelli, S., Schalling, M., Hokfelt, T., Frey, P., Walsh, J., Dockray, G., Brown, J., Buchan, A., & Goldstein, M. (1988) A subpopulation of dopaminergic neurons in rat ventral mesencephalon contains both neurotensin and cholecystokinin. *Brain Res.*, 455, 88-98.
- Seroogy, K.B., Mehta, A., & Fallon, J.H. (1987) Neurotensin and cholecystokinin coexistence within neurons of the ventral mesencephalon: Projections to forebrain. *Exp. Brain Res.*, 68, 277-289.
- Smith, O.A., & DeVito, J.L. (1984) Central neural intergration for the control of autonomic responses associated with emotion. *Ann. Rev. Neurosci.*, 7, 43-65.
- Smith, O.A., DeVito, J.L., & Astley, C.A. (1990) Neurons controlling cardiovascular responses to emotion are located in lateral hypothalamus-perifornical region. *Am. J. Physiol.*, 259, R943-R954.
- Smith, G.C., & Fink, G. (1975) Experimental studies on the origin of monoamine-containing fibers in the hypophyseal complex of the rat. *Brain Res.*, 43, 37-51.
- Snyder, S.H. (1979) Receptors, neurotransmitters, and drug responses. *N. Engl. J. Med.*, 300, 465-472.
- Sonsalla, P.K., Gibb, J.W., & Hanson, G.R. (1984) Opposite responses in the striato-nigral substance P system to D-1 and D-2 receptor activation. *Eur. J. Pharmacol.*, 105, 185-187.
- Sowers, J.R. (1981) Effects of bromocriptine on responses to stress in spontaneously hypertensive rats. *Hypertension*, 3, 544-550.

- Sowers, J.R., Resch, G., Tempel, G., Herzog, J., & Colantino, M. (1979) Hyperprolactinaemia in the spontaneously hypertensive rat. *Acta Endocrinol.*, 90, 1-70.
- Sowers, J.R., Golub, S., Berger, M.E., & Whitfield, L.A. (1982a) Dopaminergic modulation of pressor and hormonal responses in essential hypertension. *Hypertension*, 4, 424-430.
- Sowers, J.R., Nyby, M., & Jasberg, K. (1982b) Dopaminergic control of prolactin and blood pressure: Altered control in essential hypertension. *Hypertension*, 4, 431-438.
- Starke, K., Reimann, N., Zumstein, A., & Hertting, G. (1978) Effect of dopamine receptor agonists and antagonists on release of dopamine in the rat caudate nucleus *in vitro*. *Naunyn-Schmeideberg's Arch. Pharmac.*, 305, 27-36.
- Stoof, J.C., & Kababian, J.W. (1981) Opposing roles for the D-1 and D-2 dopamine receptors in efflux of cAMP from rat neostriatum. *Nature*, 294, 366-368.
- Strack, A.M., Sawyer, W.B., Marubio, L.M., & Loewy, A.D. (1988) Spinal origin of sympathetic preganglionic neurons in the rat. *Brain Res.*, 455, 187-191.
- Strack, A.M., Sawyer, W.B., Hughes, J.H., Platt, K.B., & Loewy, A.D. (1989) A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. *Brain Res.*, 491, 156-162.
- Stumpe, K.O., Kolloch, R., Higuchi, M., Kruck, F., & Vetter, H. (1977) Hyperprolactinaemia and antihypertensive effect of bromocriptine in essential hypertension. *Lancet*, 2, 211-214.

- Swanson, L.W. (1982) The projections of the ventral tegmental area and adjacent regions: A combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res. Bull.*, 9, 321-353.
- Swanson, L.W., Sawchenko, P.E., Berod, A., Hartman, B.K., Helle, K.B., & Vanorden, D.E. (1981) An immunohistochemical study of the organization of catecholaminergic cells and terminal fields in the paraventricular and supraoptic nuclei of the hypothalamus. *J. Comp. Neurol.*, 196, 271-285.
- Swennen, L., & Denef, C. (1982) Physiological concentrations of dopamine decrease cAMP levels in cultured rat anterior pituitary cells and enriched populations of lactotrophs: Evidence for a causal relationship to inhibition of prolactin release. *Endocrinology*, 111, 398-405.
- Szilagy, J.E., Taylor, A.A., & Skinner, J.E. (1987) Cryoblockade of the ventromedial frontal cortex reverses hypertension in the rat. *Hypertension*, 9, 576-581.
- Takeuchi, A., & Takeuchi, N. (1962) Electrical changes in pre- and postsynaptic axons of giant synapse of *Loligo*. *J. Gen. Physiol.*, 45, 1181-1193.
- Tepper, J.M., Sawyer, S.F., Young, S.J., & Groves, P.M. (1986) Autoreceptor-mediated changes in dopaminergic terminal excitability: Effects of potassium channel blockers. *Brain Res.*, 367, 230-237.
- ter Horst, G.J., Luiten, P.G.M., & Kuypers, F. (1984) Descending pathways from hypothalamus to dorsal motor vagus or ambiguus nuclei in the rat. *J. Auton. Nerv. Sys.*, 11, 59-75.

- Terreberry, R.R., & Neafsey, E.J. (1984) The effects of medial prefrontal cortex stimulation on heart rate in the awake rat. *Soc. Neurosci. Abstr.*, 19, 614.
- Thal, L.J., Laing, K., Horowitz, S.G., Makman, M.H. (1986) Dopamine stimulates rat cortical somatostatin release. *Brain Res.*, 372, 205-209.
- Tossman, U., & Ungerstedt, U. (1986) The effect of apomorphine and pergolide on the potassium evoked overflow of GABA in rat striatum studied by microdialysis. *Eur. J. Pharmacol.*, 123, 713-722.
- Trippodo, N.C., & Frohlich, E.D. (1981) Similarities of genetic (spontaneous) hypertension: Man and rat. *Circ. Res.*, 48, 309-319.
- Turner, B.H., Mishkin, M., & Knapp, M. (1980) Organization of the amygdalopetal projections from modality-specific cortical association areas in the monkey. *J. Comp. Neurol.*, 191, 515-543.
- Van den Buuse, M., Versteeg, D.H.G., & De Jong, W. (1984) Role of dopamine in the development of spontaneous hypertension. *Hypertension*, 6, 899-905.
- Van der Kooy, D., Coscina, D.V., & Hattori, T. (1981) Is there a non-dopaminergic nigrostriatal pathway? *Neuroscience*, 6, 345-357.
- Van der Kooy, D., Koda, L.Y., McGinty, J.F., Gerfen, C.R., & Bloom, F.E. (1984) The organization of projections from the cortex, amygdala, and hypothalamus to the nucleus of the solitary tract in the rat. *J. Comp. Neurol.*, 224, 1-24.
- Vermes, I., Tilders, F.J.H., & Stoof, J.C. (1985) Dopamine inhibits the release of immunoreactive beta-endorphin from rat hypothalamus in vitro. *Brain Res.*, 326, 41-46.

- Versteeg, D.H., Palkovits, M., Van der Gugten, J., Wijnen, H.L.J.M., Smeets, G.W.M., & De Jong, W. (1976) Catecholamine content of individual brain regions of spontaneously hypertensive rats (SH-rats). *Brain Res.*, 112, 429-434.
- Watanabe, M., Tsuruta, S., Inoue, Y., Kinuya, M., Ogawa, K., Maniya, G., & Tatsunuma, T. (1989) Dopamine D1 and D2 receptors in spontaneously hypertensive rat brain striatum. *Can. J. Physiol. Pharmacol.*, 67, 1596-1597.
- Westfall, T.C., Besson, M.J., Gioreguieff, M.F., & Gloninski, J. (1976) The role of presynaptic receptors in the release and synthesis of ^3H -dopamine by slices of rat striatum. *Naunyn-Schmeideger's Arch. Pharmacol.*, 305, 27-36.
- White, F.J., & Wang, R.Y. (1986) Electrophysiological evidence for the existence of both D1 and D2 dopamine receptors in the rat nucleus accumbens. *J. Neurosci.*, 6, 274-280.
- Wong, D.T., Bymaster, F.P., Reid, L.R., Fuller, R.W., Perry, K.W., & Kornfeld, E.C. (1983) Effect of a stereospecific D-2 dopamine agonist on acetylcholine concentration in corpus striatum in rat brain. *J. Neural Trans.*, 58, 55-67.
- Yang, C.R., & Mogenson, G.J. (1984) Electrophysiological responses of neurons in the nucleus accumbens to hippocampal stimulation and the attenuation of the excitatory responses by the mesolimbic dopaminergic system. *Brain Res.*, 324, 69-84.
- Yardley, C.P., & Hilton, S.M. (1986) The hypothalamic and brainstem areas from which the cardiovascular and behavioral components of the defence reaction are elicited in the rat. *J. Auton. Nerv. Sys.*, 15, 227-244.

- Yasui, Y., Breder, C.D., Saper, C.B., & Cechetto, D.F. (1991) Autonomic responses and efferent pathways from the insular cortex in the rat. *J. Comp. Neurol.*, 303, 355-374.
- Yim, C.Y., & Mogenson, G.J. (1982) Responses of nucleus accumbens neurons to amygdala stimulation and its modification by dopamine. *Brain Res.*, 239, 401-405.
- Yim, C.Y., & Mogenson, G.J. (1988) Neuromodulatory action of dopamine in the nucleus accumbens: An *in vivo* intracellular study. *Neuroscience*, 26, 403-415.
- Yu, Z.J., Lim, D.K., Hoskins, B., Rockhold, R.W., & Ho, I.K. (1990) Effects of acute and subacute cocaine administration on the CNS dopaminergic system in Wistar-Kyoto and spontaneously hypertensive rats: Levels of dopamine and metabolites. *Neurochem. Res.*, 15, 613-619.
- Zandberg, P., DeJong, W., DeWied, D. (1979) Effect of catecholamine-receptor stimulating agents on blood pressure after local application in the nucleus tractus solitarii of the medulla oblongata. *Eur. J. Pharmacol.*, 55, 43-56.