ABSTRACT

D. Paul Shackelford Jr. CENTRAL ALPHA ADRENERGIC INVOLVEMENT IN THE HEMODYNAMIC EFFECTS OF ESTROGEN. (Under the direction of S. Gregory Iams, Ph.D). Department of Biology, July 1987.

Estrogen treatment in individuals genetically predisposed to hypertension is known to cause a reduction in systolic blood pressure, wnile estrogen treatment in normotensive individual will cause a mild elevation or no change depending, on the type of estrogen used. The effects of the estrogens on the cardiovascular system are thought to be mediated in part by estrogen adrenergic interaction in regions of the brain that regulate cardiovascular function. The purpose of this study was to compare the effects of mestranol and estradiol on blood pressure in normotensive as well as hypertensive rats and to correlate observed changes in blood pressure with changes in central alpha adrenoceptors. Female spontaneously hypertensive rats (SHR) and normotensive Sprague Dawley (SD) rats (30 days of age) were were injected S.C. biweekly for 12 weeks with sesame oil vehicle, estradiol or mestranol [50ug/100g body weight]. Animals were sacraficed by decapitation brains were removed and quick frozen. Alpha-1 and alpha-2 adrenoceptors in the frontal cortex (FC), rostral hypothalamus (RH), caudal hypothalamus (CH), the region of the locus coeruleus (LC) and the region of the nucleus tractus solitarius (NTS) were assayed using [3H]-prazosin

and [3H]-clonidine. Mestranol and estradiol treatment attenuated the development of hypertension in the SHR by 15% and 30% relative to sesame oil control (170+2mmHg). Sprague Dawley blood pressure (132+2mm Hg) were not influenced by either estrogen treatment. Estradiol treatment in the SHR had no effect on alpha receptor sub population in any of the brain region examined. Alpha-1 adrenoceptors from the FC of mestranol treated SHR were significantly ($p<0.05$) reduced. No other brain regions were effected. Estradiol treatment in the SD resulted in elevated alpha-1 adrenoceptors in the NTS. Alpha-2 adrenoceptors were unaffected by estradiol treatment. Mestranol caused a significant reduction in alpha-1 adrenoceptors in the LC, and a significant reduction in alpha-2 adrenoceptors in the FC, LC and NTS of the SD. Estrogen treatment caused shifts in alpha receptors in several brain regions of the SD with out effecting blood pressure. Conversly estrogen treatment attenuated the development of blood pressure in the SHR while having no effect on alpha receptors in blood pressure regulatiory regions. This lack of correlation between estrogen induced changes in blood pressure and alterations in alpha adrenoceptors in blood pressure regulatory sites of the brain suggest that the hemodynamic effects of the estrogens are not mediated by estrogen induced changes in central alpha adrenoceptors.

CENTRAL ALPHA ADRENERGIC INVOLVEMENT IN THE HEMODYNAMIC EFFECTS OF ESTROGENS

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By

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ABBREVIATIONS

AII	Angiotension II
ADH	Antidiuretic Hormone
BP	Blood Pressure
BW	Body Weight
CH	Caudal Hypothalamus
CNS	Central Nervous System
DMV	Dorsal motor nucleus of the vagus
E2/4H	Estrogen 2/4 hydroxylase
ERT	Estrogen Replacement Therapy
FC	Frontal Cortex
IC50	Average Inhibitory Concentration
icv	intracerebroventricular
Kd	Dissociation Constant
LC	Region of the Locus Coeruleus
mmHg	millimeters of mercury
NE	Norepinephrine
NTS	Region of the Nucleus Tractus Solitarius
OC	Oral Contraceptives
PB	Parabracila Nucleus
PRA	Plasma Renin Activity
PRS	Plasma Renin Substrate
RH	Rostral Hypothalamus
SD	Sprague Dawley
SHR	Spontaneously Hypertensive Rat
WKY	Wistar Kyoto rats

INTRODUCTION

Hypertension is the chronic elevation of arterial pressure caused by dysfunction of central nervous system (CNS) and/or peripheral blood pressure regulatory mechanisms. The mechanisms responsible for the maintenance of arterial pressure in a normotensive range are complex and integrated. Many of the peripheral mechanisms function autonomously in that they respond directly to pressure changes without the involvement of the CNS. Examples of such mechanisms include metabolic regulation of local blood flow, and the renal servo-controller mechanism that regulates pressure about a set point by altering blood and extracellular fluid volumes. The CNS is involved in pressure regulation in part through its involvement in reflex control. The baroreceptors, chemoreceptors and the cardiac receptors send information to the brain. This information is then processed and effector response stimuli is fed out through sympathetic and vagal efferents to regulate vascular tone, heart rate and contractility. The CNS also influences blood pressure through its ability to regulate various hormones which alter the set point at which the renal servo-controller functions. Alteration or dysfunction of any of these mechanisms that control blood pressure may result in pathologic hypertension.

Hypertension and other forms of cardiovascular disease are more prevalent in males than in females. However this difference begins to disappear at the onset of menopause (34,78). One of the major physiologic differences observed in pre and post

menopausal females is the reduction in circulating levels of estradiol and progesterone. These data suggest that the female sex steroids in some way protect against cardiovascular diseases, specifically hypertension. In the early 1960's the use of oral contraceptives composed of synthetic estrogens and progesterones became common practice. Soon thereafter, laboratory researchers reported that oral contraceptives caused an elevation of arterial pressure in experimental animal models (10). Clinical investigators found similar results in humans, including development of overt hypertension in some individuals (51,88,92,98). When epidemiologic data became available it also confirmed the findings of laboratory and clinical researchers demonstrating a positive correlation between oral contraceptive use and cardiovascular disease (77,84). By the late 1960's it became very clear that female sex steroids had some influence on blood pressure and general cardiovascular function. However, the exact role that estrogens and progestins play in cardiovascular function was unclear, even more confusing was how these sex steroids influence blood pressure.

Involvement of Estrogens in Blood Pressure Regulation

Since Brownrigg's (10) first report in 1962 that oral contraceptives (OC) may induce hypertension, attention has been focused on estrogen and progesterone's role in blood pressure regulation. It is now a well established fact that the use of OC

will cause a slight elevation of blood pressure in most individuals. In general, the use of OC causes a 5 mmHg rise in systolic pressure and a 1 to 2 mmHg rise in diastolic pressure (77), and is associated with a three to sixfold increase in the risk of overt hypertension (77,84).

Both the estrogen and progesterone components of OC have been implicated as the hypertensive factor. Analysis of data collected between 1970 and 1980 shows that the estrogen (Ethinyl Estradiol) content of OC dropped from 100ug to 30ug. This was associated with an 80% decrease in the cardiovascular risk factors associated with OC use (84). However, some of the decrease may also be associated with changes in the progesterone content. The risk factors for stroke and myocardial infarction are two times greater in women using an OC with 3 to 4 mg norethindrone acetate than women using an OC with 1 to 2 mg of norethindrone acetate, with similar dose responses observed with other synthetic progesterones (84).

Data obtained from noncontraceptive estrogen replacement therapy (ERT) is generally contradictory to the OC data. Epidemiologic data have failed to establish any type of relationship between ERT and hypertension. (61). This discrepancy may be related to the age difference in the two populations. In two well designed clinical trials, ERT was reported to cause a slight but significant drop in blood pressure in treated subjects (55,58). In a study conducted by Lind (55), 49 previously untreated post menopausal women were divided into a

placebo group or one of six hormone treated groups given either, 1.25 mg conjugated equine estrogen, 1.5 mg estrone sulfate, 2 mg estradiol or each of these three estrogens with 5 mg norethindrone acetate for six months. During the treatment period systolic blood pressure declined in 75% of the individuals on hormone replacement therapy, but returned back to control levels three months after treatment had stopped. It is interesting to point out that the addition of the progesterone, norethindrone acetate, had no apparent blood pressure effect in this experiment, suggesting that estrogens are the hemodynamic regulatory factor. This idea is further supported by investigations into how blood pressure is affected during the menstrual cycle. Eiff et. al. (22) reported a decreased blood pressure response to emotional stimuli in women that were in the first phase of the menstrual cycle when estradiol levels are high. Moreover Greenburg et. al. (36) were unable to demonstrate a clear cut rise in blood pressure during the leuteal phase when progesterone levels are highest.

The clinically available estrogen preparations used for ERT can be divided into three groups in order of clinical preference; native human estrogens, conjugated equine estrogens such as those used in the Lind study, and synthetic estrogens such as those used in oral contraceptives. Since these estrogens differ in their potency and ability to inhibit follicle stimulating hormone and stimulate hepatic protein synthesis (60), it is possible that they would have different cardiovascular effects. It is clear

that the synthetic estrogens are potentially harmful to the cardiovascular system, while it would appear that the natural estrogens are in some way mildly protective.

PROPOSED MECHANISMS

The involvement of estrogens in the long-term regulation of blood pressure has been established, however the mechanisms responsible for this influence are not clear. Initially The effects of estrogens on blood pressure was thought to be caused by steroidial influence on volume regulating hormones such as Angiotension II and ADH, however recent evidence suggest that this is not the case (6,29,48,82,83). Several other possibilities have been suggested which involve direct alterations of the vasculature, as well as alterations in sympathetic nervous system regulation of the cardiovascular system.

Direct Influence on the Cardiovascular System

A direct influence of the estrogens on the cardiovascular system has been proposed. Estrogens have been demonstrated to affect vascular protein synthesis (26,67), alter endothelium dependent relaxation (63,97) and vascular adrenergic receptors (19,49). Reduced vascular collagen deposition, relative to elastin (26) and decreased synthesis of vascular contractile proteins (67) have been reported in estradiol treated rats. These alterations in

vascular proteins may change vascular compliance. Recently Williams et. al. (97) and Miller et al. (63) demonstrated that chronic estrogen treatment augments the endothelial dependent relaxation to acetylcholine in SHR (Fig.1) thoracic aortic and rabbit ovarian arterial rings. This augmented endothelium response may reflect an alteration in vascular reactivity which is complimented by the effects on vascular proteins. Altered vascular catecholamine responsiveness has also been reported in response to acute estrogen treatment. Kondo et. al.(49), demonstrated that acute i.v. infusion of high doses of estradiol hemisuccinate attenuated the pressure response to norepinephrine (NE), while Colucci et. al. (19), reported an increased vascular catecholamine sensitivity after estrogen treatment. These contradictory results may be due to the difference in estrogen preparations used as well as differing doses. It is doubtful that these responses are physiologically relevant, given the low levels of the estrogens found in the blood, even the high serum titers of estrogens created by oral contraceptive therapy do not reach the levels used by Kondo (49). Preliminary studies by Iams and McConnaughey (unpublished data) indicate that vascular adrenergic receptors are influenced by testosterone but the influence of estrogens is uncertain. At this time, it is doubtful that the blood pressure effects of estrogen are attributable to altered vascular catecholamine responsiveness, however the hemodynamic effects may be related to the effects of estrogens on vascular reactivity and compliance.

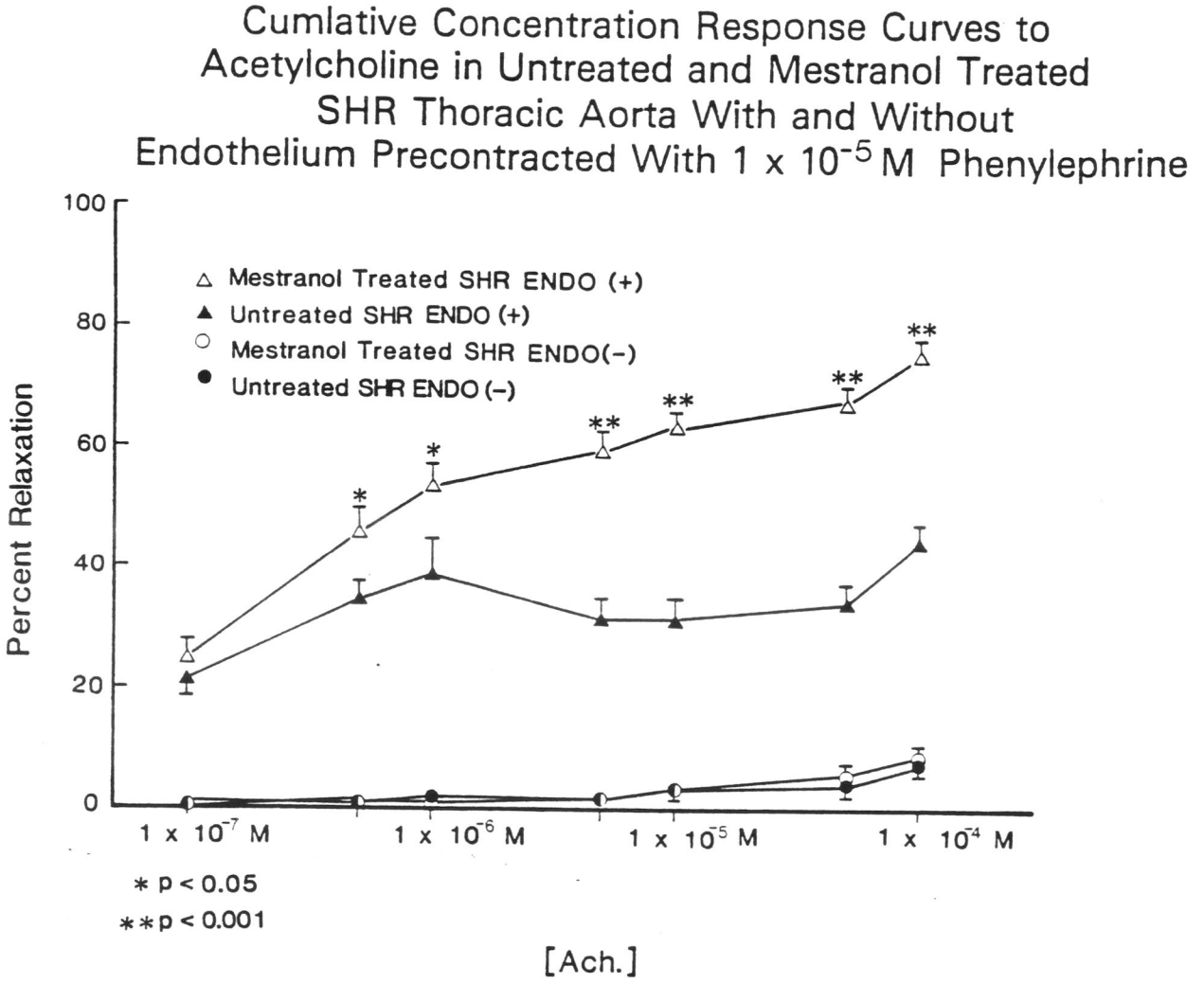


Figure 1. Thoracic aorta relaxation responses to acetylcholine in phenylephrine precontracted vessels from control and mestranol treated animals (98).

Involvement of the Sympathetic Nervous System

In 1980 Bunag (11) suggested that the hemodynamic effects of the oral contraceptive Enovid, may be due to sympathetic hyperactivity. In this experiment rats with hypertension induced by one kidney one clip surgery developed higher blood pressure if they were treated with Enovid. This difference was abolished by ganglionic blockade indicating that an increased sympathetic drive is necessary for the maintenance of the elevated blood pressure in the Enovid treated animals. The idea that female sex steroids, specifically estrogens, may alter sympathetic drive is supported by several studies involving catecholamine metabolism (1,20,24,95) as well as changes in adrenoceptor densities (14,44,89,90,91,95) and responsiveness (14,31,32,87).

Cardiovascular Regulatory Brain Regions That Contain Estrogen Binding Sites

Estrogens are thought to play a role in modulating several physiologic systems through the CNS, least of which is the cardiovascular system. Steroid hormones are lipophilic and therefore not restricted by the blood brain barrier. Estrogen binding sites have been demonstrated in all regions of the brain including the preoptic area, the median basal area and the paraventricular nucleus of the hypothalamus as well as the locus coeruleus, the parabracial nucleus, the dorsal motor nucleus of the vagus, and the nucleus tractus solitarius of the medulla.

Most of these estrogen binding areas are known to influence the cardiovascular system (81).

The nucleus tractus solitarius (NTS) is located about the caudal portion of the fourth ventricle just below the obex, about the area postrema. This medullary nucleus is the primary site of termination for the carotid sinus nerve, the aortic depressor nerve and nerves arising from cardiac receptors (46). These nerves exclusively convey cardiovascular information. Destruction of the NTS or sectioning of the nerves leading into the NTS will cause hypertension and tachycardia (57) while microinjection of NE. or electrical stimulation will cause hypotension and bradycardia (45).

The dorsal motor nucleus of the vagus (DMV) is located lateral to the NTS, about the caudal portion of the fourth ventricle just below the obex. This nucleus is the site of origin for the vagus. An increase in efferent vagal tone will cause a decrease in heart rate while vagal transection or destruction of the DMV is known to cause an elevation in heart rate (17). As with the NTS, the significance of estrogen binding sites in this area is uncertain.

The region of the locus coeruleus (LC), centered around the anterior portion of the fourth ventricle, is the largest NE producing nucleus of the brain. This nucleus has been implicated in regulation of the cardiovascular and endocrine systems as well as behavior (72,27). Estrogens are thought to modify the firing rate to this nucleus by altering the NE concentration. The LC is

known to respond to visual, tactile, and somatosensory stimuli (2) as well as alterations in blood pressure (23). Electrical stimulation of this brain region has been reported to cause an elevation in arterial pressure and trigger the release of ADH (37,86). However, lesioning of the LC with 6-hydroxydopamine also caused an increase in blood pressure (71). These conflicting studies suggest a complicated role for the LC in the regulation of blood pressure, moreover the exact role played by this nucleus has yet to be discovered.

The parabracial nucleus (PB) located just lateral to the locus coeruleus, also contains estrogen binding sites. Electrical stimulation of this region has been demonstrated to increase blood pressure and cause the release of ADH (86). Lesioning of the PB has been demonstrated to alter cardiovascular responses elicited by stimulation of other brain regions (92). Estrogens may function in this region to alter ADH release although the specific significance of estrogen receptors in this region is uncertain.

The paraventricular nucleus of the hypothalamus (PVH) located just lateral to the anterior portion of the third ventricle. This nucleus is classically known for its synthesis of oxytocin and ADH. Not surprisingly, this nucleus contains estrogen binding sites. This is possibly related to estrogen regulation of oxytocin synthesis. However, this nucleus has also been implicated in the regulation of the cardiovascular system. Electrical stimulation of this nucleus has been shown to cause an

elevation of blood pressure and an increase in heart rate (53). Electrolytic lesion and chemical destruction of this nucleus will prevent and/or reverse the hypertension caused by lesioning of the NTS (99) or baroreceptor denervation (100), as well as alter the development of hypertension in the spontaneously hypertensive rat (3,18).

All of the afore mentioned brain nuclei that contain estrogen binding sites are reported to have direct reciprocal connections (Fig. 2) with each other, as demonstrated by electrophysiological and horseradish peroxidase studies (13). Alterations in catecholamine metabolism and/or adrenergic receptors in one or all of these nuclei by estrogens could directly affect tonic and reflex control of the cardiovascular system.

Altered Catecholamine Metabolism; Catechol Estrogens

Estrogen induced alterations in catecholamine metabolism have been reported by several investigators (1,20,24). Crowley et. al. (20) found that estradiol differentially influenced both NE and dopamine turnover and metabolism in several discrete nuclei in the rat brain, including the PVH, supraoptic (SON) and anterior hypothalamic nuclei. Estrogens are metabolized by various routes however one of these leads to the formation of a catechol group on the "A" ring of the steroid causing the formation of the catechol estrogens. Initially the formation of 2 or 4 (Fig.3) hydroxyestrogens occurs via the enzyme estrogen

NEURAL PATHWAYS THAT CONTROL CIRCULATION

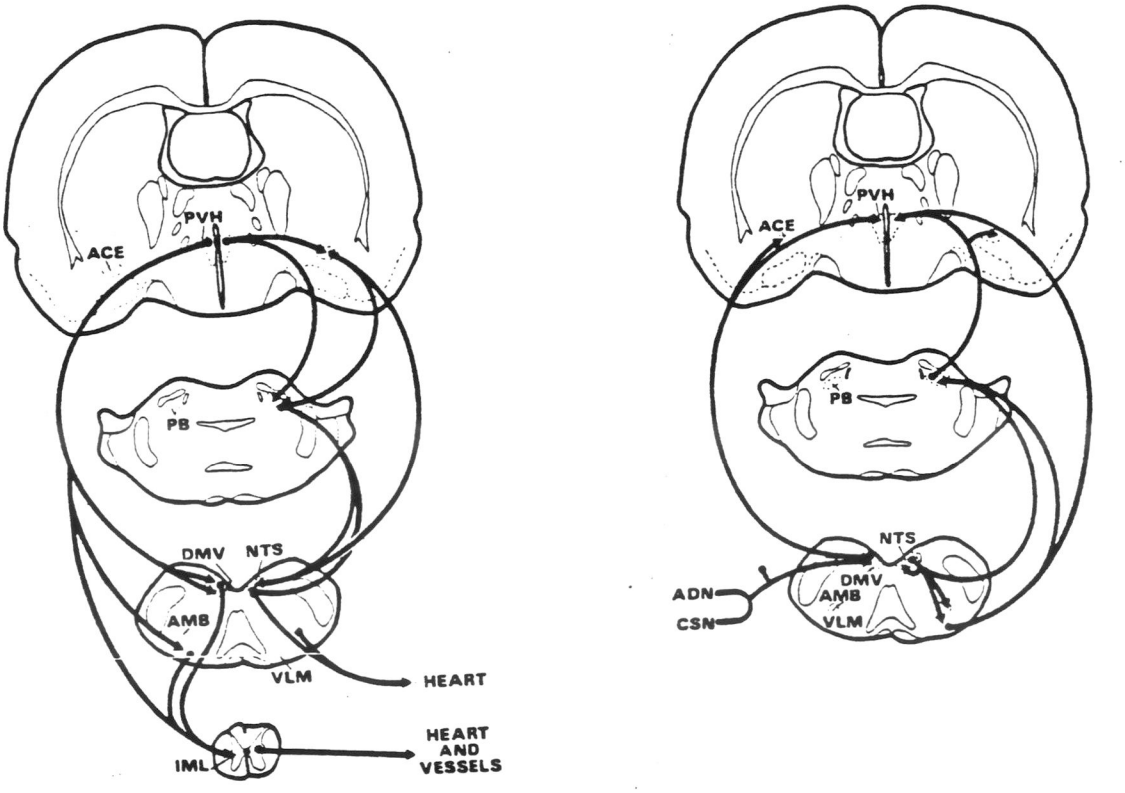
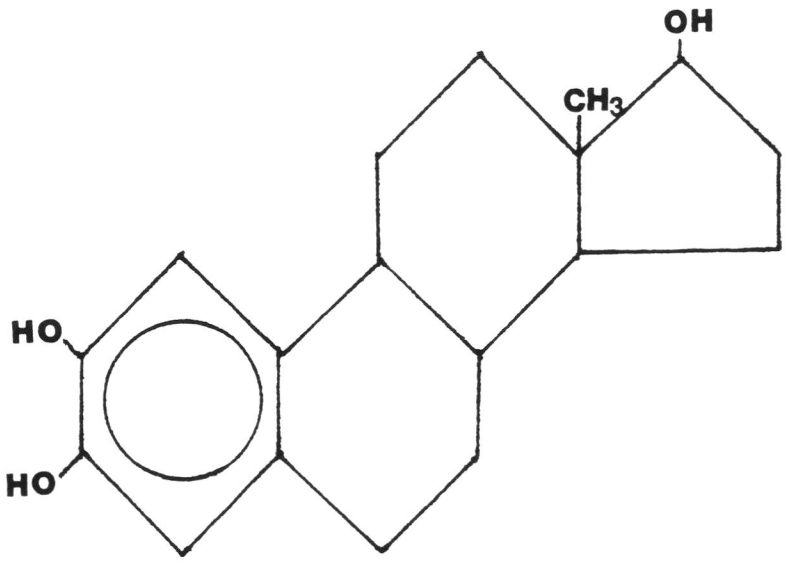
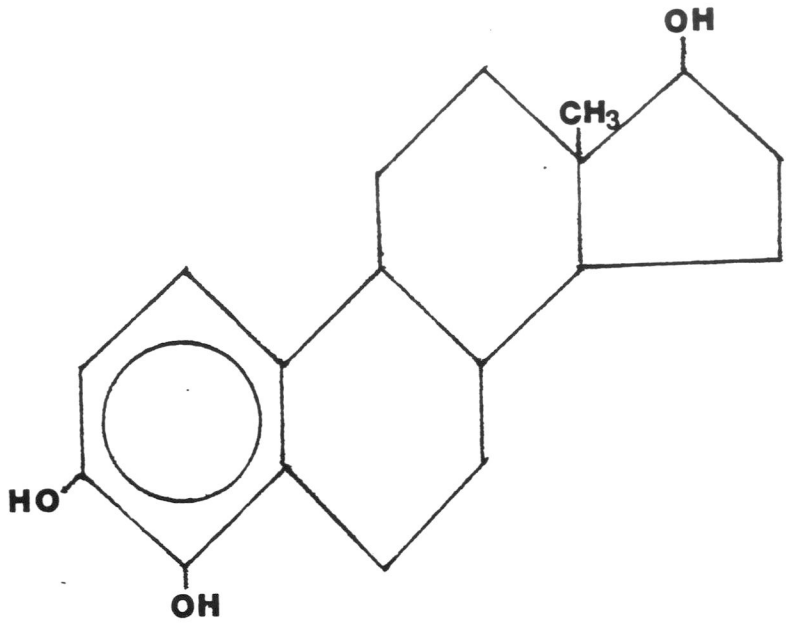


Figure 2. Wiring diagram of major ascending and descending cardiovascular pathways. Abbreviations: central nucleus of the amygdala (ACE), aortic depressor nerve (ADN), nucleus ambiguus (ABM), carotid sinus nerve (CSN), dorsal mortor nucleus of the vagus (DMV), intermediolateral nucleus (IML), nucleus of the solitary tract (NTS), parabracial nucleus (PB), paraventricular nucleus of the hypothalamus (PVH), ventrolateral medulla (VLM).

Figure 3 Catechol Estrogens



2-Hydroxyestradiol



4-Hydroxyestradiol

Estrogen metabolites that inhibit tyrosine hydroxylase and catechol-o-methyl transferse.

2/4 hydroxylase (E2/4H). These catechol estrogen metabolites are unique in their ability to still bind estrogen receptors and act as a catecholamine. These compounds are capable of altering catecholamine synthesis by competitive inhibition of tyrosine hydroxylase (28), or inhibit degradation by competing with catechol-O-methyl transferase (5). This proposes a new dimension to the theory of estrogen action. Not only will estrogens selectively influence protein synthesis in the classical manner, now they are thought to directly and immediately alter catecholamine levels in tissues with high levels of E2/4H. Weisz and Crowley (95) showed that E2/4H was regionally distributed in the rat brain and not a general constitute enzyme. They found elevated levels of this enzyme in the acurate nucleus of the median eminence, a brain region implicated in prolactin release. Elevated levels of E2/4H were also found in the SON and PVH, correlating with the altered NE levels found by Crowley et al. (20). However, i.c.v. infusion of both the 2 and 4 hydroxyestorgen metabolite failed to produce the significant increase in NE content in the hypothalamus and cerebral cortex seen with 17-beta estradiol infusion (73). This lack of activity observed may be due to the rate at which the catechol estrogens are metabolized.

Central Adrenoceptors

Estrogens are known to directly influence the adrenergic system, not only by altering catecholamine metabolism but by a

direct effect on adrenergic receptors. Much of the earlier work has concentrated on the beta adrenoceptor. Fregly et. al., Carlberg et. al. and Thrasher et. al. (14,31,32,87) reported that rats chronically treated with ethinyl estradiol (5 to 6 weeks) showed a decreased beta adrenoceptor responsiveness to isoproterenol infusion. Altered beta adrenoceptor responsiveness was determined by changes in heart rate, water intake, and tail temperature. Wagner et. al. (90) found reduced beta adrenoceptor responsiveness to isoproterenol in the cerebral cortex of rats chronically (14days) treated with ethinyl estradiol. In this study isoproterenol stimulated cAMP accumulation was significantly lower in brain slices from estrogen treated animals. Wagner et. al.(90) also demonstrated reduced beta receptor density as a result of ethinyl estradiol treatment in the cerebral cortex, corpus striatum, olfactory bulb, and the hypothalamus. However, these results may be questioned. Their animals were ovarectomized with one group receiving estrogen replacement therapy. The results therefore may be attributed to the decreased estrogen titers in the ovarectomized rats rather than from the increased estrogen titers in the experimental group. Wagner et. al.(91) later reported that female rats had reduced isoproterenol stimulated adenylate cyclase and beta receptor density in the cerebral cortex, when compared to males and that these differences could be abolished by ovarectomy. These results matched those in an earlier study in which he found that the animal with higher estrogen titers had lower beta

receptor responsiveness and lower beta receptor density. Both Carlberg et. al. and Wagner et. al. (14,91) suggest that their results may reflect down regulation of the beta receptor due to increased NE levels in estrogen treated animals as a result of the known decrease in NE turnover (1,20,24).

Studies involving the alpha adrenoceptor have not been quite so extensive. To date, only acute estrogen treatment has been directly evaluated. Two studies that either utilized (72 Hr) estradiol treatment or hormonal variations during the short rat estrus cycle failed to establish clear cut consistent alterations in alpha or beta receptor populations in the hypothalamus. Wilkerson et. al.(95) and Vascus et. al. (89), using normotensive animals, reported that hypothalamic beta receptor densities were elevated in response to acute estradiol treatment while alpha receptors were unaffected. Wilkerson (95) also demonstrated that neither receptor population was significantly altered throughout the estrus cycle, with the exception of lowered alpha receptor affinity on the day of proestrus, when estradiol levels are elevated. However, this observation was not repeated with estrogen treatment of ovarectomized animals.

Recently, attempts to determine the influence of estrogens on central alpha-2 receptor density and responsiveness have given us greater insight as to the effect of estrogens on a sub population of alpha adrenoceptors. Chalmers et. al. (15) demonstrated that contraceptive steroid treatment enhanced the sedative response to the centrally acting alpha 2-adrenoceptor

agonist clonidine. This altered sedative response was associated with a dose related decrease in diastolic blood pressure. Chalmers went on to suggest that this altered response may result from changes in alpha-2 adrenoceptor densities. This was based on estrogens ability to alter adrenoceptor densities in platelets (79) and urethra (52), as well as estrogens ability to alter catecholamine metabolism. In 1985 Johnson et. al. (44) published an autoradiographic study that showed an increased [3H]-para amino clonidine receptor density in response to 48 hr. estradiol benzoate treatment, in several estrogen binding hypothalamic nuclei of the guinea pig brain. This study correlates with Chalmers suggestion that there is an increased alpha 2-adrenoceptor density based on the observed increase in clonidine responsiveness. The study by Johnson is important in that it is the first attempt to demonstrate the effects on estrogen treatment on a subpopulation of alpha receptors and show where in the brain the effect occurs. However such autoradiographic studies lack the sensivity to accurately quantify receptor numbers and are unable to account for differing affinity states.

HYPOTHESIS

It is clear that estrogens influence the central adrenergic system through a variety of mechanisms. Changes in various parameters of the sympathetic nervous system may account for part of the hemodynamic effects of the estrogens. Alterations in the firing rate of sympathetic nerves may feed out to increase or decrease vascular tone, or cause shifts in cardiovascular reflex responses. These estrogen induced alterations most likely originate in regions of the brain that contain estrogen binding sites. A vast number of brain regions known to be involved in cardiovascular regulation have been shown to contain estrogen binding sites. Therefore, it seems plausible that the hemodynamic effects of the estrogens are mediated in part by estrogen induced alteration in adrenergic parameters in one or all of these brain regions. This study was undertaken to explore the effects of mestranol and estradiol, on blood pressure in normotensive and hypertensive rats as well as alpha adrenoceptors subpopulations in hypothalamic, and medullary brain regions known to bind estrogens and regulate blood pressure.

MATERIALS AND METHODS

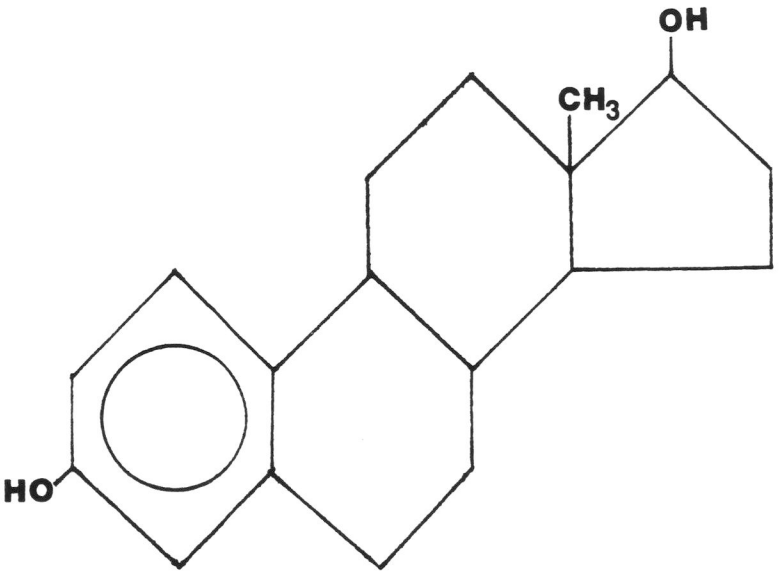
Treatment of animals

Female Sprague Dawley (SD) and spontaneously hypertensive rats (SHR) 30 days of age were obtained from the Animal Resource Center of East Carolina University School of Medicine. The animals were maintained at 24 C+1 C on a 12:12 light cycle with standard lab chow and tap water ad libitum. Both SD and SHR were divided into 3 groups (16 animals/group) according to body weight and blood pressure such that no significant differences in blood pressure or body weight existed between groups. Animals received subcutaneous injections of 50ug estradiol/100g body weight (Bw), 50ug mestranol/100g Bw, (Fig. 4) or sesame oil vehicle twice a week for 12 weeks. Systolic blood pressures were measured with tail cuff plethysmography (12) under mild restraint in unanesthetized animal prewarmed (38±5 C for 5 minutes) animals at 3 to 4 week intervals.

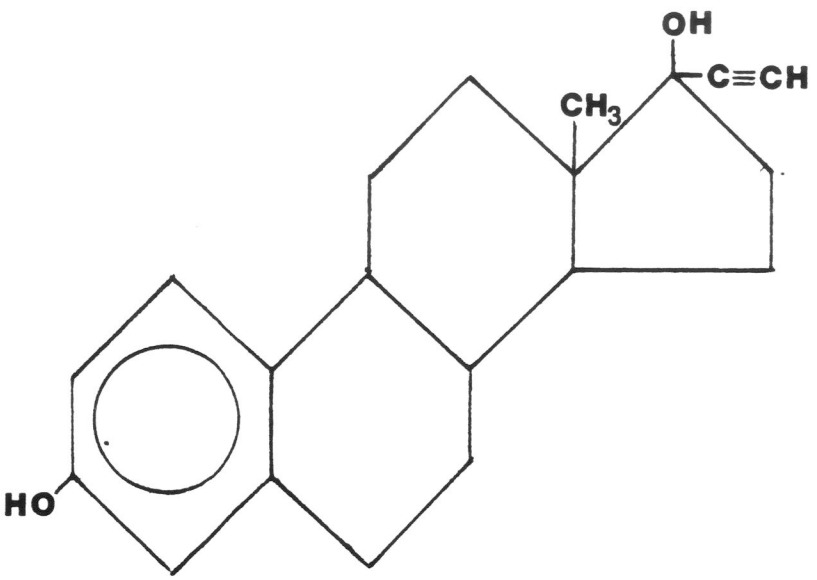
Dissection

At the end of 12 weeks of treatment animals were sacrificed by decapitation between 0800 and 1100 hours. Brains were immediately removed and quick frozen on a bed of dry ice and then stored at -70 C. Brain regions were dissected over a dry ice cold plate as described by Pullen et. al. (75) as represented in Figure 5. Briefly; The area of the frontal cortex (FC) known for its higher brain functions, consisted of tissue from both poles

Figure 4 Estrogen Steroids

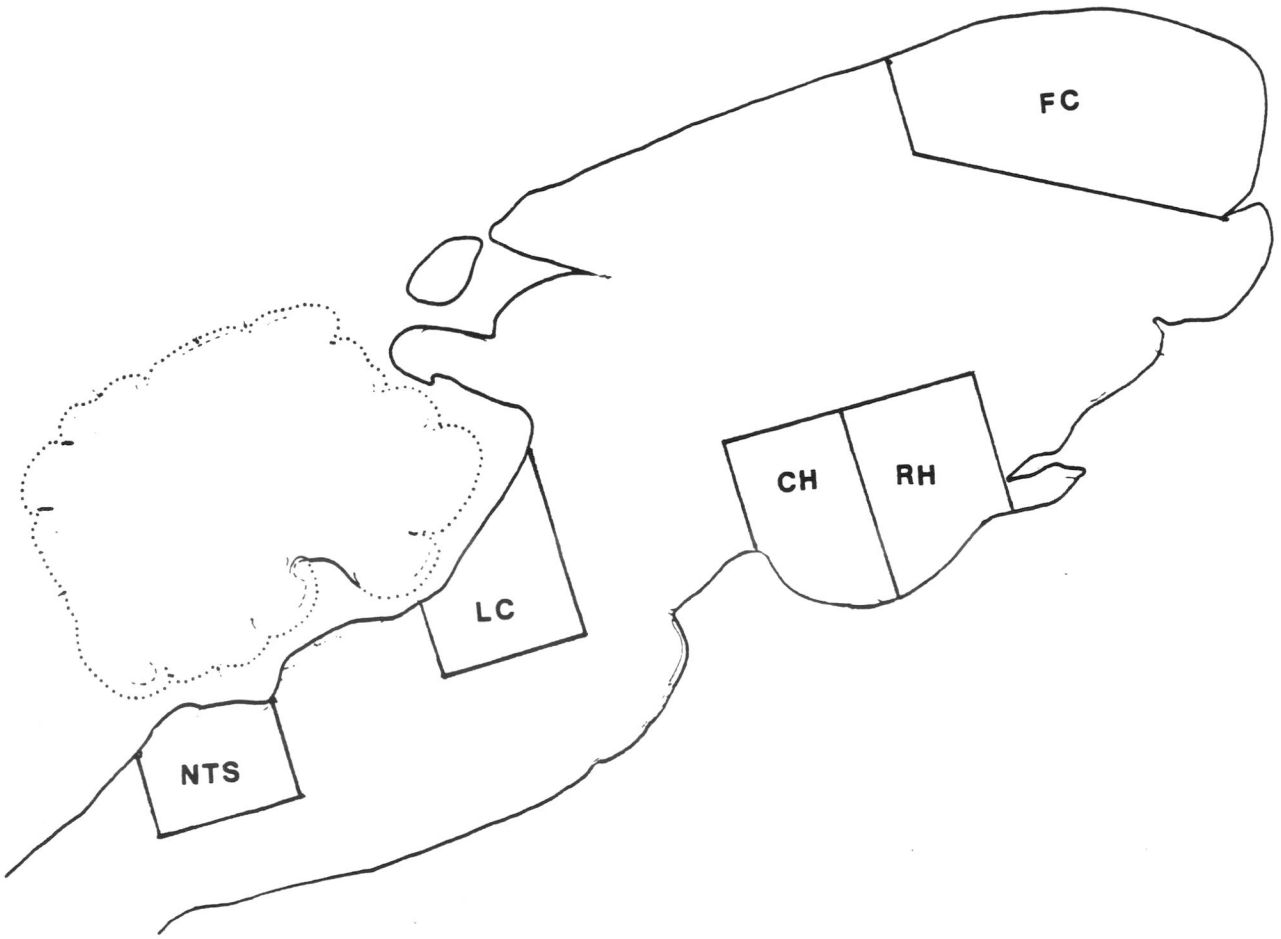


Estradiol



Mestranol

Figure 5 Brain Regions



Approximate location of dissected brain regions. Frontal cortex (FC), Rostral hypothalamus (RH), Caudal Hypothalamus (CH), region of the Locus coeruleus (LC), and the region of the nucleus tractus solitarious (NTS).

of the telencephalon dorsal to the corpus callosum. The initial hypothalamic section consisted of tissue from the preoptic area to the mammillary area in the rostral caudal direction, and extended 2mm laterally on either side of the midline and 2 mm deep. The hypothalamic section was then divided into rostral (RH) and caudal (CH) sections by a coronal cut through the median eminence. The RH contains the PVH, the supraoptic nuclei, the acurate nuclei, a portion of the preoptic area and a portion of the ventral medial nucleus. The CH contains the mammillary nuclei, median fore brain bundle and the posterior nucleus of the hypothalamus. The area of the locus coeruleus (LC) consisted of a 3x2x2 mm block of tissue dissected from the midline at the rostral border of the 4th ventricle. This section contains the nucleus locus coeruleus, parabracial nucleus and the dorsal ralphe. The area of the nucleus tractus solitarius also consisted of a 3x2x2 mm section of tissue obtained from the midline of the caudal portion of the 4th ventricle, just below the obex. This section contains the nucleus tractus solitarius, the solitary tract and the dorsal motor nucleus of the vagus. Consistency of dissection was determined by tissue weights.

Tissue preparation

Tissues from two rats were pooled and homogenized on ice in 10 volumes of ice cold homogenization buffer (50mM Tris/300mM sucrose pH 7.4 at 25 C) using the Brinkmann Polytron PT-10 on

setting 7 for two 10 sec. pulses. Homogenates were then separated into aliquots for alpha-1 adrenoceptor and alpha-2 adrenoceptor assays and stored at -25 C until time of the assay. Proteins were determined by the method of Lowry et. al.(56) prior to receptor assays to insure that the optimal amount of protein was used in the receptor assay and prevent waste of the tissue homogenate. Lowry protein determination was used because of its high sensitivity and the small amount of tissue homogenate required for consistent and accurate protein determination.

Alpha Adrenoceptor Characterization

Alpha-1 adrenoceptor binding was measured using the antagonist [3H]-prazosin, while alpha-2 adrenoceptors were measured using the agonist [3H]-clonidine. Alpha adrenergic receptors were characterized utilizing saturation analysis, displacement characterization and linearity with protein. Receptor saturation analysis was carried out using the method of Greenburg et. al. (35) as described by McConnaughey et. al. (62). Briefly; 500ul of ice cold receptor buffer (50mM Tris HCl, 10mM $MgCl_2$ pH 7.4 at 25 C), approximately 300ug of protein and varying concentrations (0.5-10nM) of the [3H]-ligand made up fresh in distilled deionized water, were added to 13x100mm borosilicate culture tubes on ice. The binding reaction was initiated by placing the tubes in 30 C shaking water bath for 10 minutes. The reaction was terminated by placing the tubes on ice. Nonspecific binding was determined by duplicate incubations

in the presence of 10^{-4}M phentolamine for alpha-1 or clonidine for alpha-2 respectively. Membrane bound ligand was separated from unbound radioligand by rapid filtration through Whatman GF/C glass filters, utilizing a millipore filter manifold. Each filter was rinsed with 15 ml of ice cold receptor buffer. The filters containing the membrane bound radioligand were then placed in 7 ml plastic counting viles and allowed to air dry overnight. Liquiscint scintillation fluid (5 ml) was then added to each vile. The contents of the viles were then thoroughly mixed for 10 to 15 seconds using a vortex mixer. Radioactivity was then measured by liquid scintillation spectrophotometry utilizing a Packard beta counter.

Displacement characterization was carried out by competitive binding between the [3H]-ligand and an unlabeled competitive binding drug as described by Greenburg (35). Briefly; to 500ul of receptor buffer were added approximately 300ug of protein, a saturable concentration of the [3H]-ligand (5nM clonidine and 5nM prazosin) and varying concentrations (10^{-3} to 10^{-10}) of the unlabeled competitive binding drug. The binding reaction was initiated by placing the culture tubes in 30 C shaking water bath for 10 minutes. The reaction was terminated by placing the tubes on ice. Membrane bound radioligand was separated from unbound radioligand as described above. Radioactivity was then determined by liquid scintillation spectrophotometry.

Linearity with protein was determined by successively increasing the concentration of protein while maintaining a constant saturable concentration of the labeled ligand. Briefly; 500 ul of ice cold receptor buffer, 50ug to 300ug of protein and a saturable concentration of radiolabeled drug were added to 13x100 mm borosilicate culture tubes on ice. The binding reaction was initiated by placing the tubes in a 30 C shaking water bath for 10 minutes, and terminated by placing the tubes on ice. Nonspecific binding was determined by duplicate incubations in the presence of 10^{-4}M phentolamine or clonidine respectively. Membrane bound radioligand was separated from unbound ligand as described above. Radioactivity was then determined by liquid scintillation spectrophotometry.

Alpha Adrenoceptor Binding

Total apparent adrenoceptor numbers were determined directly by the method of Williams and Lefkowitz (96) as described by Blumenthal et.al. (8). Briefly; 500 ul of ice cold receptor buffer, approximately 300ug of protein and a saturable concentration [3H]-prazosin or [3H]-clonidine were added to 13x100 mm borosilicate culture tubes on ice. The binding reaction was initiated by placing the tubes in a 30 C shaking water bath for 10 minutes, and terminated by placing the tubes on ice. Nonspecific binding was determined by duplicate incubations in the presence of 10^{-4}M phentolamine or clonidine respectively.

Membrane bound radioligand was separated from unbound radioligand as described above. Radioactivity was then determined by liquid scintillation spectrophotometry previously described. Receptor affinities (Kd) were calculated from the average inhibitory concentration (IC50) by the method of Cheng and Pursoff 1973 (16). IC50 were determined by competitive displacement binding between the [3H]-ligand and an unlabeled competitive binding drug as previously described.

Statistics and Calculations

Mean blood pressures, body weights and total apparent receptor numbers were calculated and significance determined using one way analysis of variance. Significance was determined as $p<0.05$. Significance within strains and between groups was determined using Dunkans multiple range test. All described tests were performed using the SPSS pc+ statistical package.

Total apparent adrenergic receptor numbers were then calculated using the following formulas;

Specific Activity of the [3H]-Ligand = Ci/mmole

[Ci/mmole] x [2.22×10^{12} DPM/Ci] = DPM/mmole

Assuming a 60% counting effency, [DPM/mmole] x .6 = CPM/mmole

Total CPM applied/ [CPM/mmole] = Total mmoles applied

mmole applied x 1000 = pmoles appled

Total CPM - Non Specific binding CPM = delta CPM

$$\frac{(\text{pmoles 3[H]-Ligand}) \times [\text{detla CPM}]}{[\text{Total CPM applied}] \times \text{mg Protein}} \times 1000 = \text{fmol/mg Protein}$$

Receptor affinities were determined by displacement analysis. Kd was calculated by the following formula;

Drug concentration to give 50% inhibition of receptor binding = IC50

[3H]-ligand concentration = [s]

half maximal velocity = Km

dissociation constant = Kd

$$\frac{IC50}{1+[s]/Km} = Kd$$

RESULTS

Receptor Characterization

Radiolabeled prazosin and clonidine demonstrated characteristics of selective alpha-1 and alpha-2 adrenoceptor binding respectively in all five brain regions. Radiolabeled prazosin binding of the alpha-1 adrenoceptor was linear between 200 mg and 600 mg of protein (Fig. 6). Specific [3H]-prazosin binding was found to be saturable (Fig. 7) with increasing concentrations of [3H]-prazosin (1-10nM) and demonstrating a Kd of 2.8+0.09 nM. Alpha-1 adrenoceptor binding with [3H]-prazosin was also displaceable with the alpha antagonist phentolamine in the concentration range of 10^{-9} to 10^{-4} M (Fig. 8). Radiolabeled clonidine binding of the alpha-2 adrenoceptor was linear from 200 mg protein to 600 mg protein (Fig. 6). Binding of [3H]-clonidine was found to be saturable (Fig. 7) with increasing concentration of [3H]-clonidine (0.5-5nM) demonstrating a Kd of 3.5+0.1 nM. Alpha-2 adrenoceptor binding with [3H]-clonidine was found to be displaceable with clonidine in the range of 10^{-8} to 10^{-4} M (Fig. 8)

Body weights.

Body weights are presented in Table 1, and growth curves presented in Figures 9 and 10. Mestranol caused an immediate reduction body weight in both strains of animals while estradiol caused no significant effect on body weight of the SHR until week

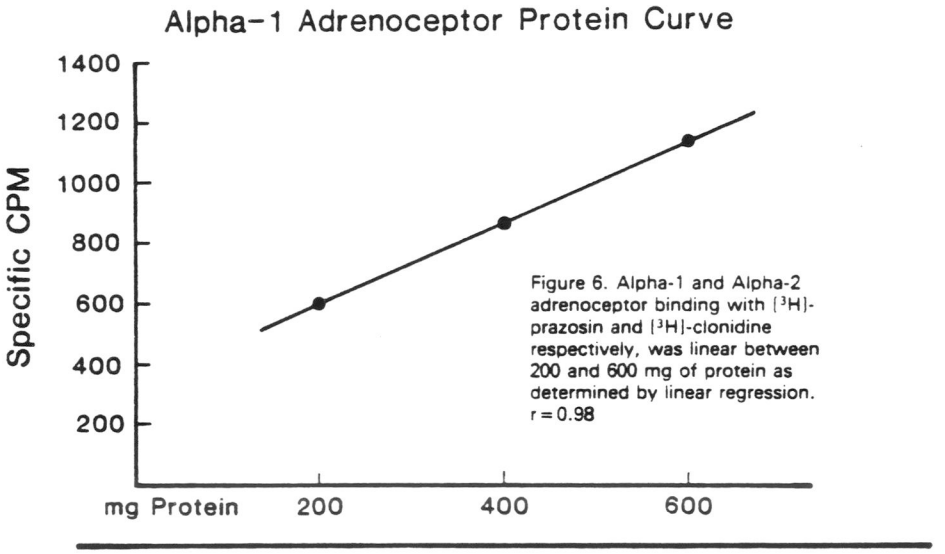
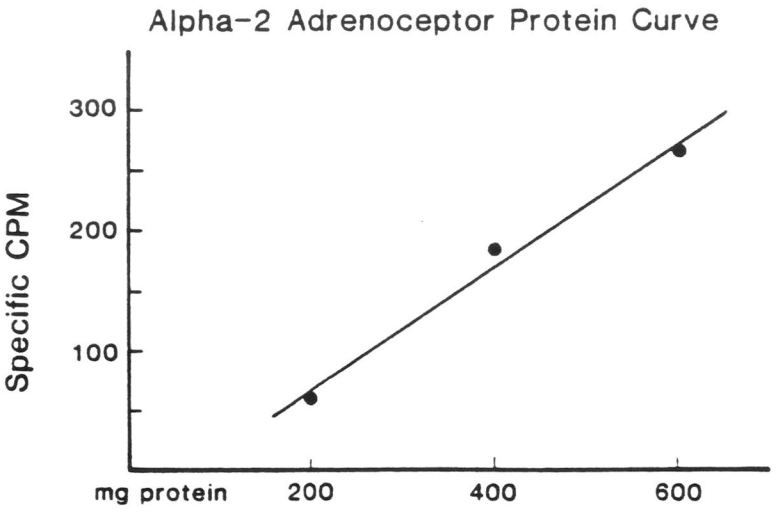


Figure 6. Alpha-1 and Alpha-2 adrenoceptor binding with [^{3}H]-prazosin and [^{3}H]-clonidine respectively, was linear between 200 and 600 mg of protein as determined by linear regression. $r = 0.98$



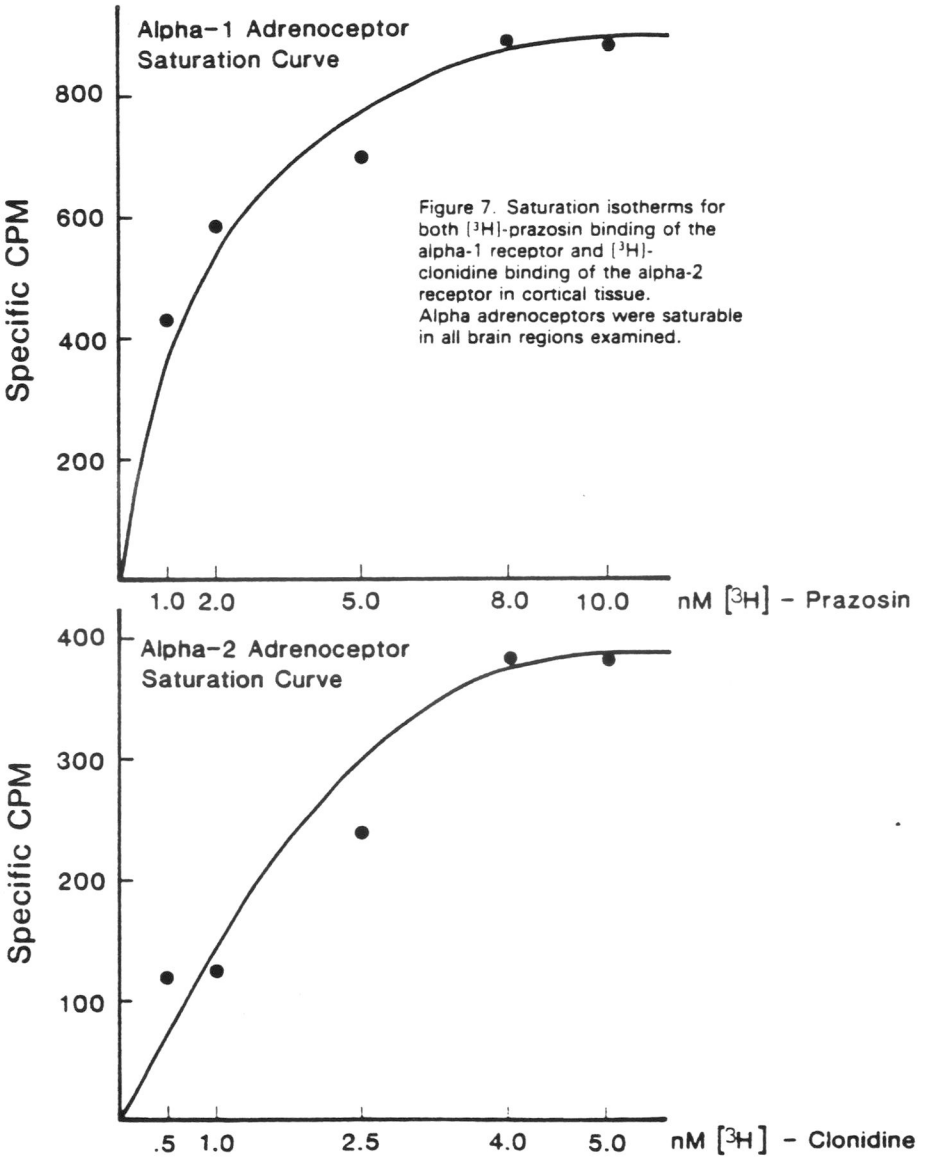
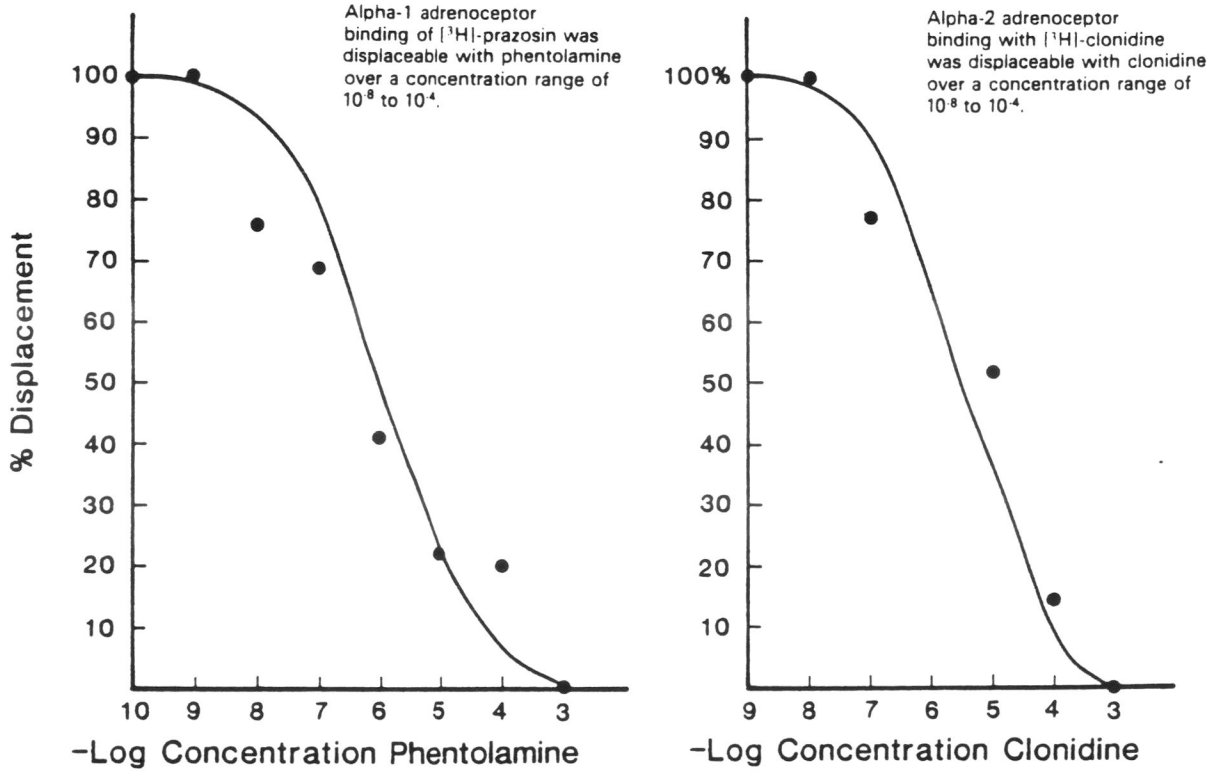


Figure 7. Saturation isotherms for both [^{3}H]-prazosin binding of the alpha-1 receptor and [^{3}H]-clonidine binding of the alpha-2 receptor in cortical tissue. Alpha adrenoceptors were saturable in all brain regions examined.

Figure 8. Displacement Curves



eight and on SD until week ten. In general SHR body weights are 35 to 40% lower than SD body weights. This interstrain variation is maintained with drug treatments indication that the animals body weights were effected equally by the drug treatments, i.e. mestranol caused the same percent reduction in body weight in both strains. Estradiol and mestranol caused a significant ($p<0.05$) depression in body weight relative to intrastrain controls in both SD and SHR after 12 weeks of treatment, however the body weights of the mestranol treated animals were also significantly lower than the estradiol treated animals.

Blood Pressure

Sprague Dawley control animals maintained normotensive systolic pressure levels of (131+2 mm Hg). Both estradiol and mestranol failed to cause any significant effect on resting blood pressure in the SD rats when compared to controls (Fig. 11). Spontaneously hypertensive rat control animals developed hypertension as expected (170+2 mm Hg systolic) which was significantly higher than the blood pressure of normotensive SD controls by a factor of 30%. Both estradiol and mestranol treatment significantly lowered blood pressure in the SHR (Fig. 12), however depression in blood pressure caused by mestranol was significantly greater than that of estradiol (136+2 mm Hg vs 153+2 mm Hg). Blood pressures of the estradiol treated SHR were in the borderline hypertensive range (153+3 mm Hg) while the systolic blood pressures of the mestranol treated animals were in

Table 1.
SPRAGUE-DAWLEY BLOOD PRESSURES AND BODY WEIGHTS
WEEK 12

	Control (n=16)	Estradiol (n=16)	Mestranol (n=16)
Blood pressure (mmHg)			
SD	131+2	134+1	132+1
SHR	170+2	153+3*	136+2*
Body Weight (grams)			
SD	278+7	256+7*	179+4*
SHR	177+3	148+2*	114+1*

Blood pressures and body weights taken prior to sacrifice. * Indicates significance from control at $P<0.05$.

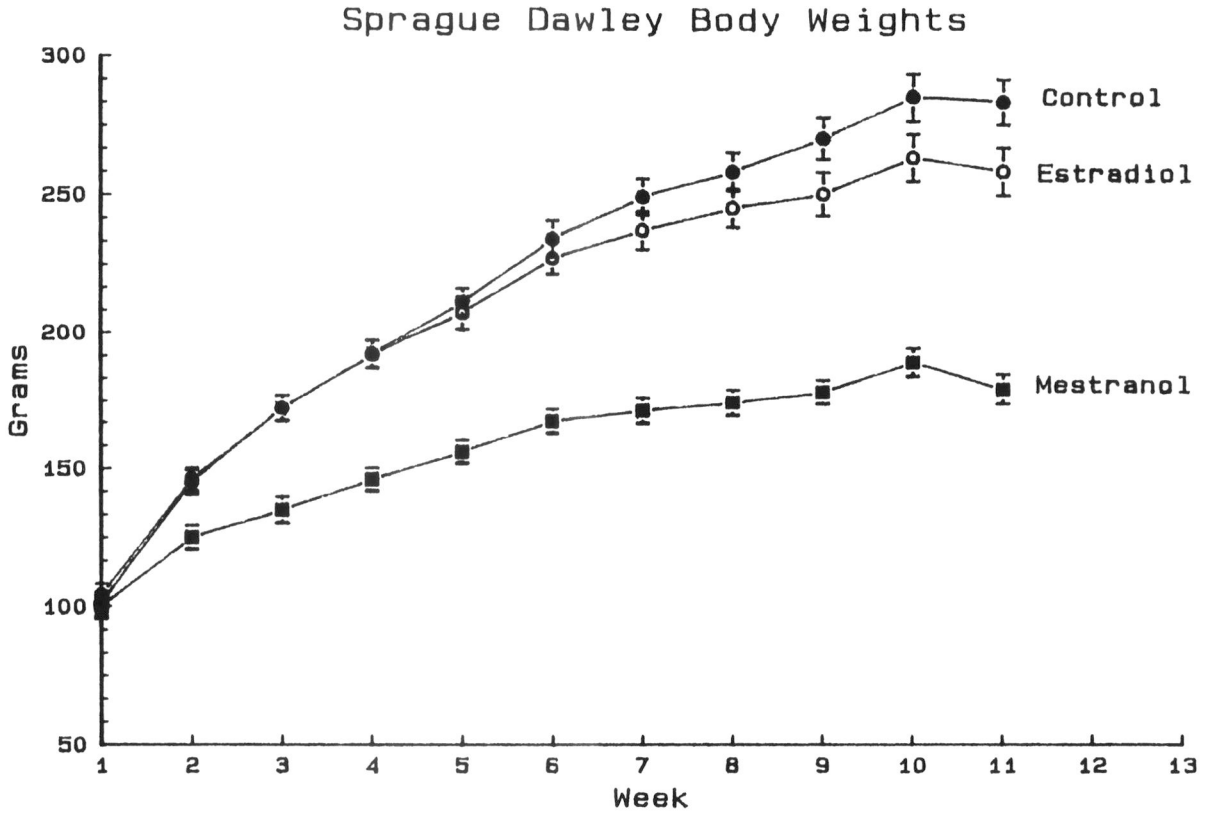


Figure 9. Weekly body weights of the normotensive Sprague Dawley rats treated with estradiol, mestranol or sesame oil control.

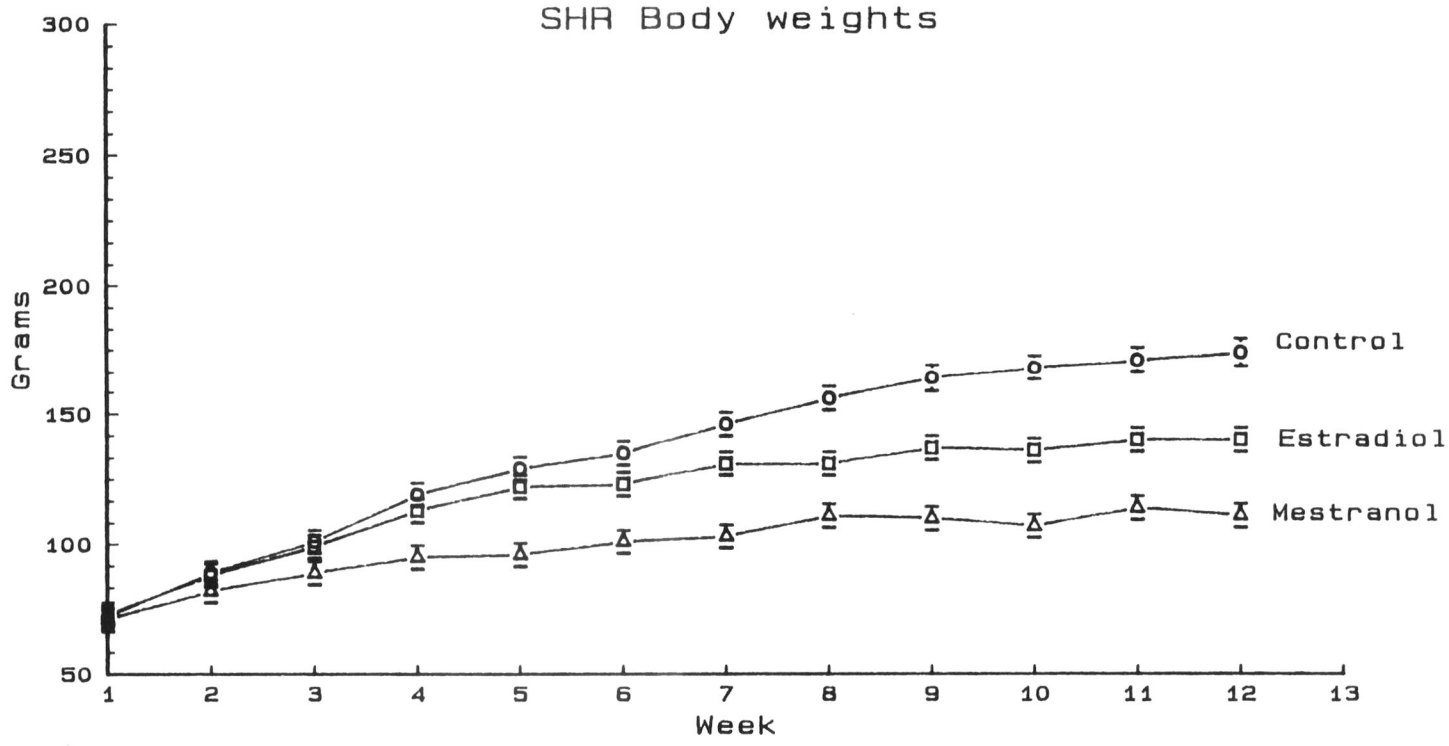


Figure 10. Weekly body weights of the spontaneously hypertensive rats treated with estradiol, mestranol or sesame oil control.

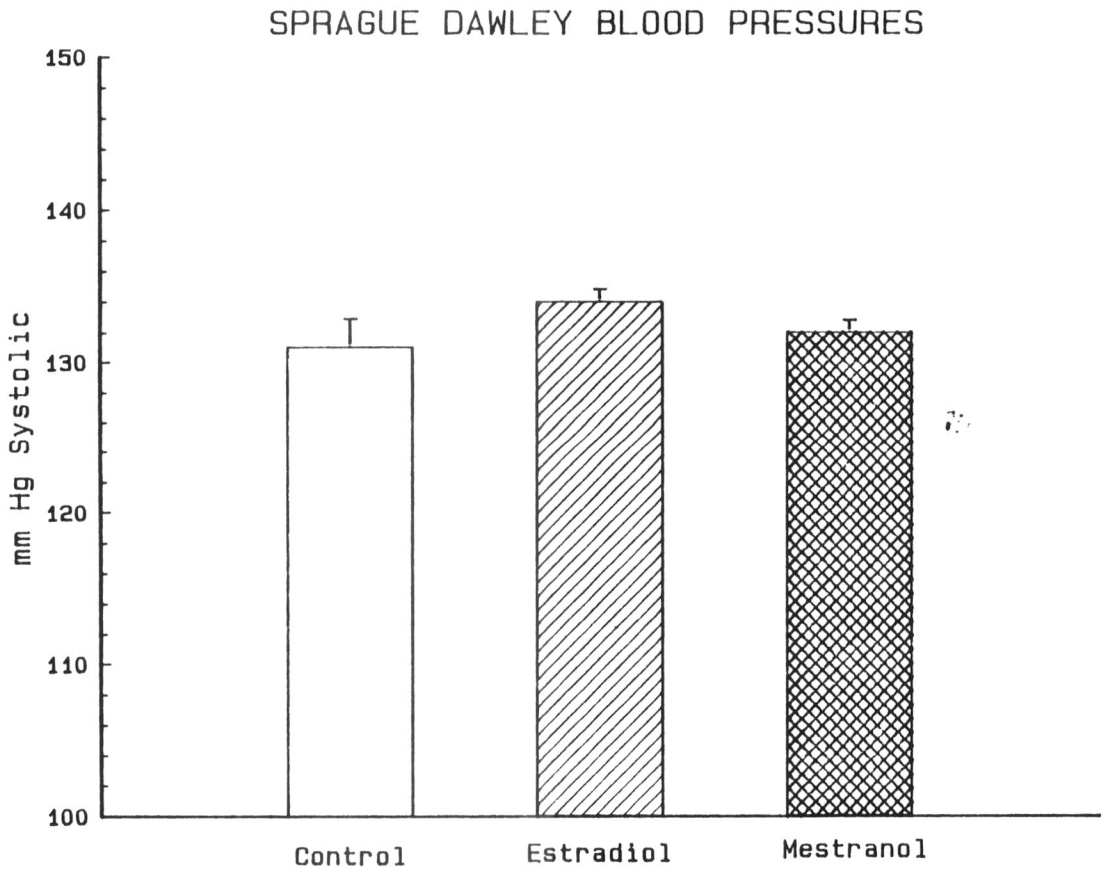


Figure 11. Systolic blood pressures of the normotensive Sprague Dawley rats after 12 weeks of treatment with estradiol (single hash bars), mestranol (cross hash bars), or sesame oil control (open bars). Systolic blood pressure was determined by the tail cuff method. Asterisk (*) indicates significance from control $p<0.05$.

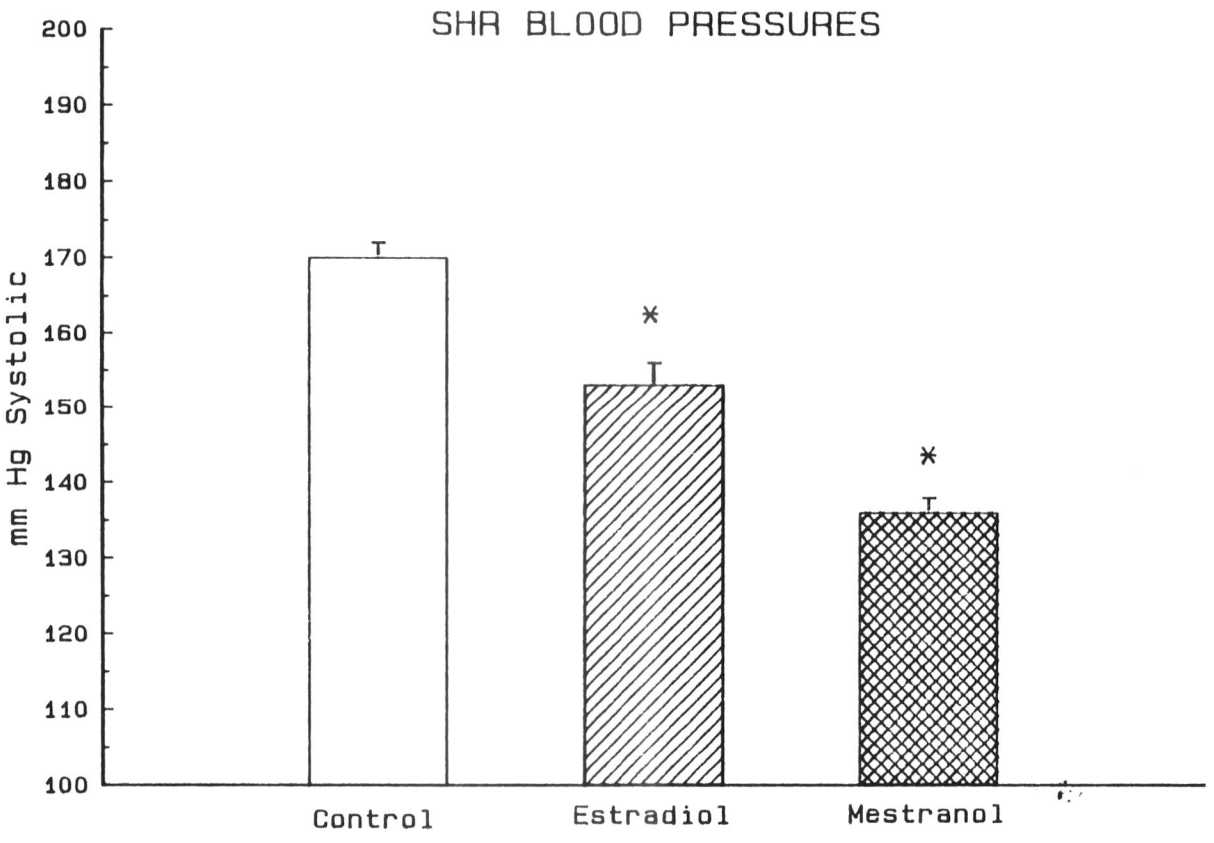


Figure 12. Systolic blood pressures of the spontaneously hypertensive rats after 12 weeks of treatment with estradiol (single hash bars), mestranol (cross hash bars), or sesame oil control (open bars). Systolic blood pressure was determined by the tail cuff method. Asterisk (*) indicates significance from control $p<0.05$.

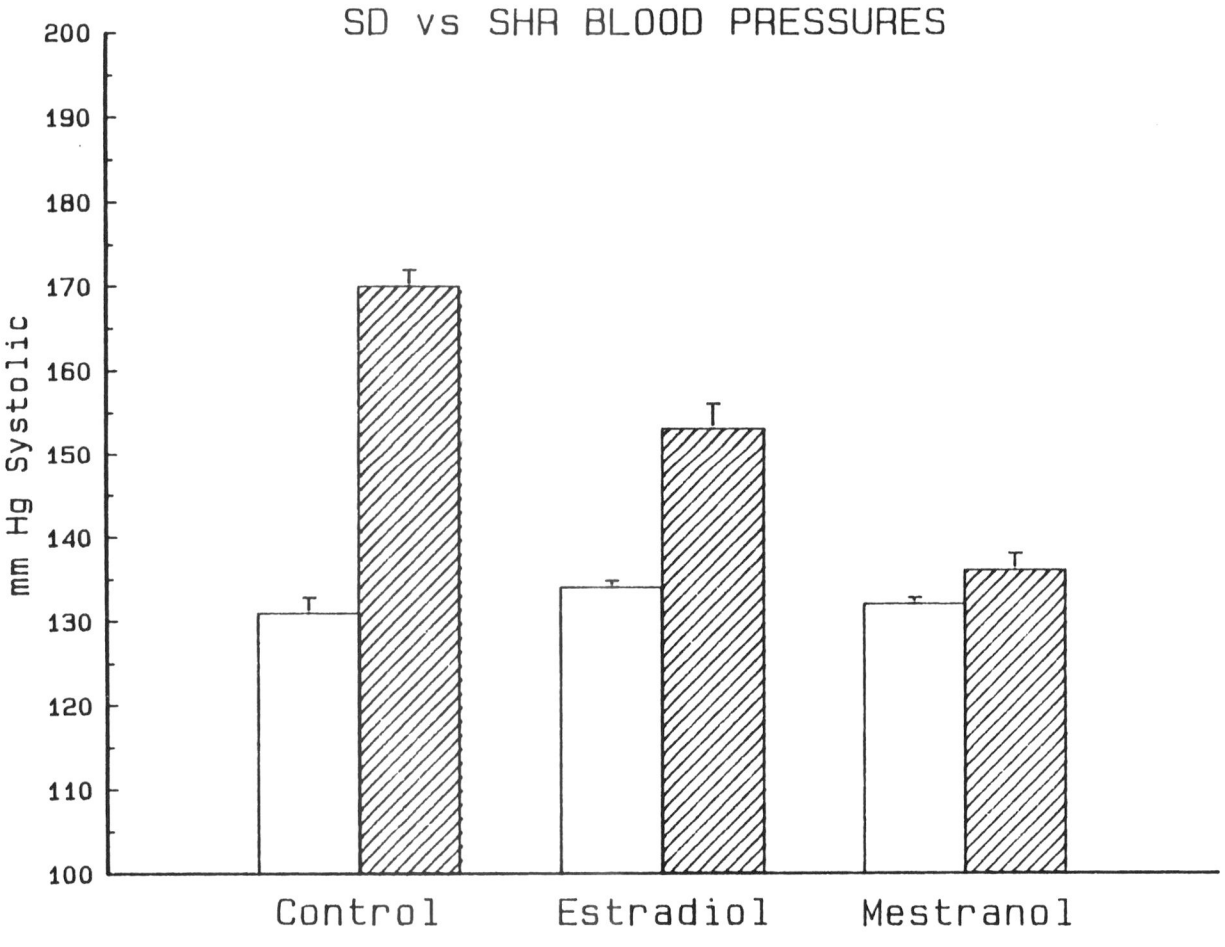


Figure 13. Systolic blood pressures of normotensive Sprague Dawley rats (open bar) compared to spontaneously hypertensive rats (single hash bar). Both strains of animals treated with estradiol, mestranol or sesame oil control as determined by the tail cuff method.

the normotensive range comparable to SD control and treated animals (Fig. 13). However, while the mestranol induced depression in blood pressure in the SHR was significantly lower than control SHR it was still significantly higher than normotensive control SD animals.

Alpha Adrenoceptor Binding

In the Sprague Dawley rats alpha-1 and alpha-2 adrenoceptor affinities were assessed in all brain regions by displacement analysis and found to be unaltered by either estradiol or mestranol treatment (Table 2). A typical displacement curve is presented in Figure 14. Spontaneously hypertensive rats alpha adrenoceptor affinities were not assessed due to a lack of tissue. However, there is little evidence to suggest that there might be any affinity alterations caused by the estrogens.

Interstrain comparison of apparent alpha-1 adrenoceptors from control animals indicates that there was no significant difference between strains in the five brain regions examined (Fig 15). Apparent alpha-2 adrenoceptors of the RH of the SHR control animals were significantly higher (27.1 ± 3.1 vs 15.8 ± 2.1) than those in the RH of the SD control animals, while alpha-2 adrenoceptors in the other brain regions of the SHR were not significantly different from SD control animals (Fig. 16).

Sprague Dawley total alpha-1 adrenoceptor values are presented in Table 3 and Figure 17. Mestranol treatment caused a significant ($p<0.05$) reduction in SD alpha-1 adrenoceptor

Table 2.
SPRAGUE DAWLEY ALPHA-1 ADRENOCEPTOR AFFINITIES [Kd]

	Control	Estradiol	Mestranol
Frontal Cortex	2.6	2.8	2.5
Rostral Hypothalamus	3.6	3.0	3.8
Caudal Hypothalamus	2.7	2.8	-
Locus Coeruleus	2.6	2.7	2.6
Nucleus tractus Solitarius	-	2.6	2.9

* Indicates significance from control at $P<0.05$.

SPRAGUE-DAWLEY ALPHA-2 ADRENOCEPTOR AFFINITIES [Kd]

	Control	Estradiol	Mestranol
Frontal Cortex	2.7	2.5	3.2
Rostral Hypothalamus	3.7	3.2	3.5
Caudal Hypothalamus	3.9	3.7	3.7
Locus Coeruleus	3.7	4.0	3.7
Nucleus tractus Solitarius	3.0	3.6	3.5

* Indicates significance from control at $P<0.05$.

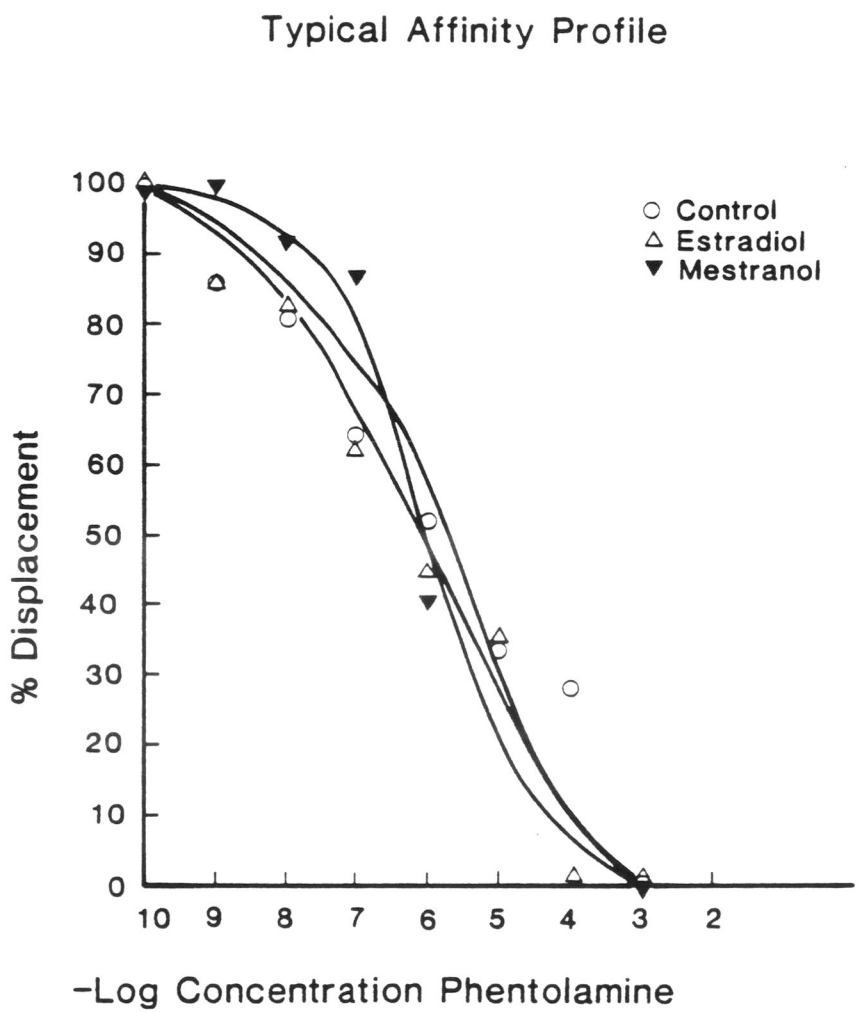


Figure 14.
Dispalcement analysis of alpha-1 adrenoceptors of the locus coeruleus to determine shifts in affinity. This is a representative graph of displacement analysis for both alpha-1 and alpha-2 adrenoceptors in the five brain regions examined.

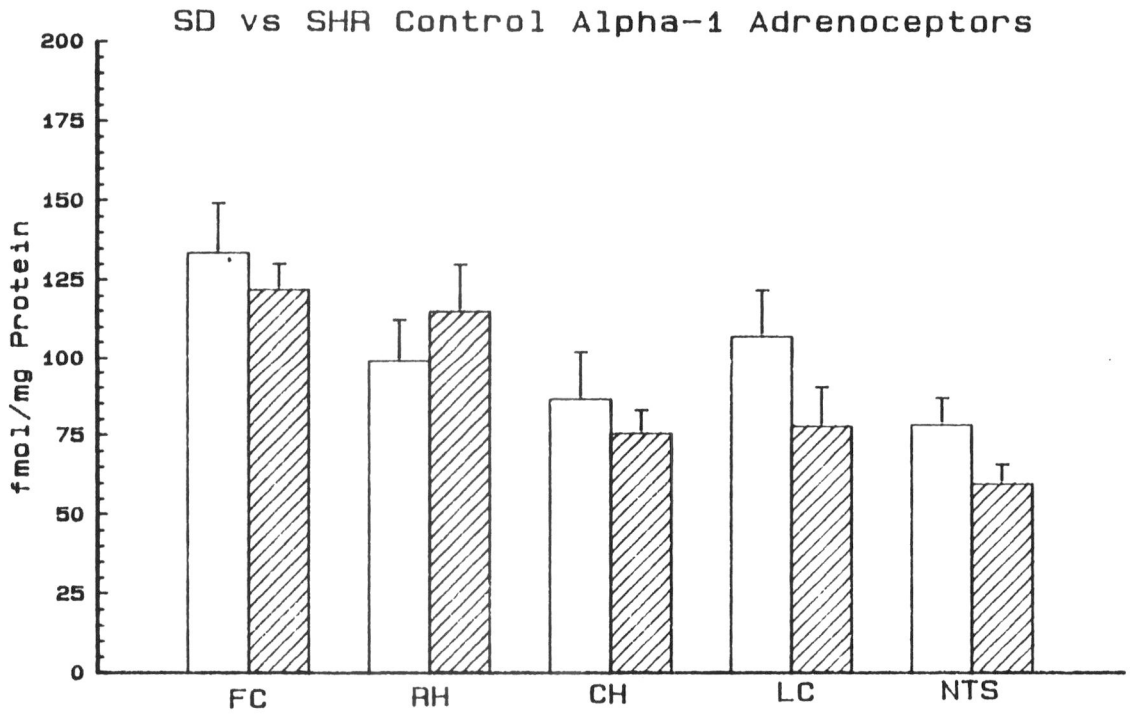


Figure 15. Sprague Dawley (open bar) and spontaneously hypertensive rat (single hash bar) control alpha-1 adrenoceptors after 12 weeks. Abbreviations: Frontal cortex (FC) rostral hypothalamus (RH), caudal hypothalamus (CH), region of the locus coeruleus (LC) and region of the nucleus tractus solitarius (NTS). Asterisk (*) indicates significance from control $p<0.05$

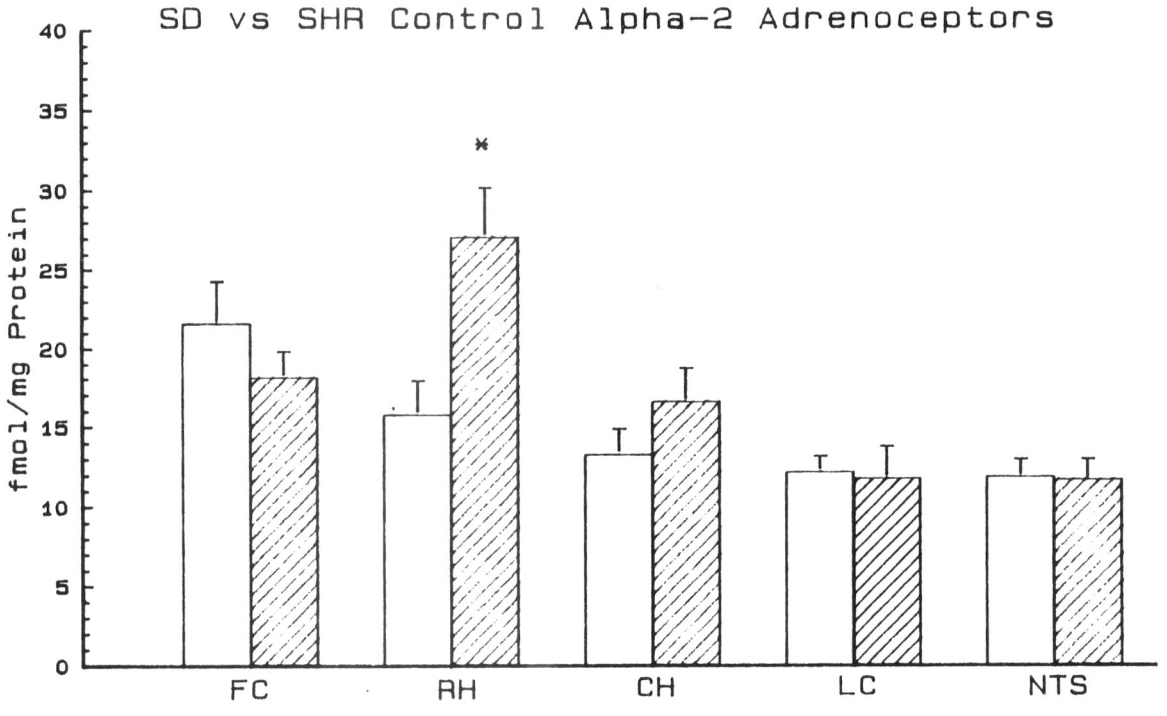


Figure 16. Sprague Dawley (open bar) and spontaneously hypertensive rat (single hash bar) control alpha-2 adrenoceptors after 12 weeks. Abbreviations: Frontal cortex (FC) rostral hypothalamus (RH), caudal hypothalamus (CH), region of the locus coeruleus (LC) and region of the nucleus tractus solitarius (NTS). Asterisk (*) indicates significance from control $p<0.05$

density in the LC relative to control and in the CH, and LC relative to estradiol treated animals. Estradiol treatment caused a significant elevation of SD alpha-1 adrenoceptor density in the NTS (113.1±12.3 vs 78.5±8.6). Total apparent alpha-2 adrenoceptor values are presented in Table 4 and Figure 18. Mestranol treatment also caused a significant reduction in alpha-2 adrenoceptor binding in the FC, LC and NTS as compared to control. The mestranol induced depression in the NTS was found significantly different from estradiol treated animals. Estradiol had no significant effect on alpha-2 adrenoceptors in any of the five brain regions examined.

Total apparent alpha-1 adrenoceptor values from the SHR are presented in Table 5 and Figure 19. Spontaneously hypertensive rat alpha-1 adrenoceptors were not significantly affected in the five brain regions examined by either mestranol or estradiol after 12 weeks of drug treatment. Total apparent alpha-2 adrenoceptor values are presented in Table 6 and Figure 20. Mestranol caused a significant depression in alpha-2 adrenoceptor binding only in the FC (10.9±1.2 vs 18.1±1.8). The binding was unaffected in the other brain regions. Estradiol treatment had no significant effect on alpha-2 adrenoceptors in any of the five brain regions examined.

Table 3.
SPRAGUE-DAWLEY ALPHA-1 ADRENOCEPTORS
[fmol/Mg Protein]

	Control (n=8)	Estradiol (n=8)	Mestranol (n=8)
Frontal Cortex	133.6+15.5	142.5+15.5	98.7+10.7
Rostral Hypothalamus	99.1+13.2	121.6+12.7	81.7+9.0
Caudal Hypothalamus	86.5+15.7	118.4+16.4	64.7+13.8
Locus Coeruleus	107.0+14.7	101.4+8.3	42.8+3.2*
Nucleus Tractus Solitarius	78.5+8.6	113.1+12.3	85.4+9.9

* Indicates significance from control at P<0.05.

Table 4.
SPRAGUE DAWLEY ALPHA-2 ADRENOCEPTORS
[fmol/mg Protein]

	Control (n=8)	Estradiol (n=8)	Mestranol (n=8)
Frontal Cortex	21.6+2.7	16.9+1.1	15.1+1.9
Rostral Hypothalamus	15.8+2.1	19.1+2.4	18.7+2.4
Caudal Hypothalamus	13.3+1.6	14.1+0.5	12.7+1.0
Locus Coeruleus	12.3+1.0	10.2+0.9	8.3+0.7*
Nucleus tractus Solitarius	11.9+1.1	13.4+1.0	7.6+0.8*

*Indicates significance from control at P<0.05.

Table 5.
SHR ALPHA-1 ADRENOCEPTORS
[fmol/mg Protein]

	Control (n=8)	Estradiol (n=7)	Mestranol (n=8)
Frontal Cortex	121.8+8.2	115.2+10	102.5+7.2
Rostral Hypothalamus	115+15	114+17	114+19
Caudal Hypothalamus	75.8+7.5	97.6+9.4	97.6+7.7
Locus Coeruleus	78.4+12.4	97.6+10.2	64.4+9.9
Nucleus tractus Solitarius	61.0+6.1	69.6+2.3	57.9+5.2

*Indicates significance from control at P<0.05.

Table 6.
SHR ALPHA-2 ADRENOCEPTORS
[fmol/mg Protein]

	Control (n=8)	Estradiol (n=7)	Mestranol (n=8)
Frontal Cortex	18.1+1.8	14.6+0.9	10.9+1.2*
Rostral Hypothalamus	27.1+3.1	29.3+3.4	22.3+3.3
Caudal Hypothalamus	16.6+2.1	15.3+1.9	14.4+1.0
Locus Coeruleus	11.8+1.9	13.0+1.4	10.3+1.2
Nucleus tractus Solitarius	11.7+1.3	14.4+0.9	12.4+0.7

*Indicates significance from control at P<0.05.

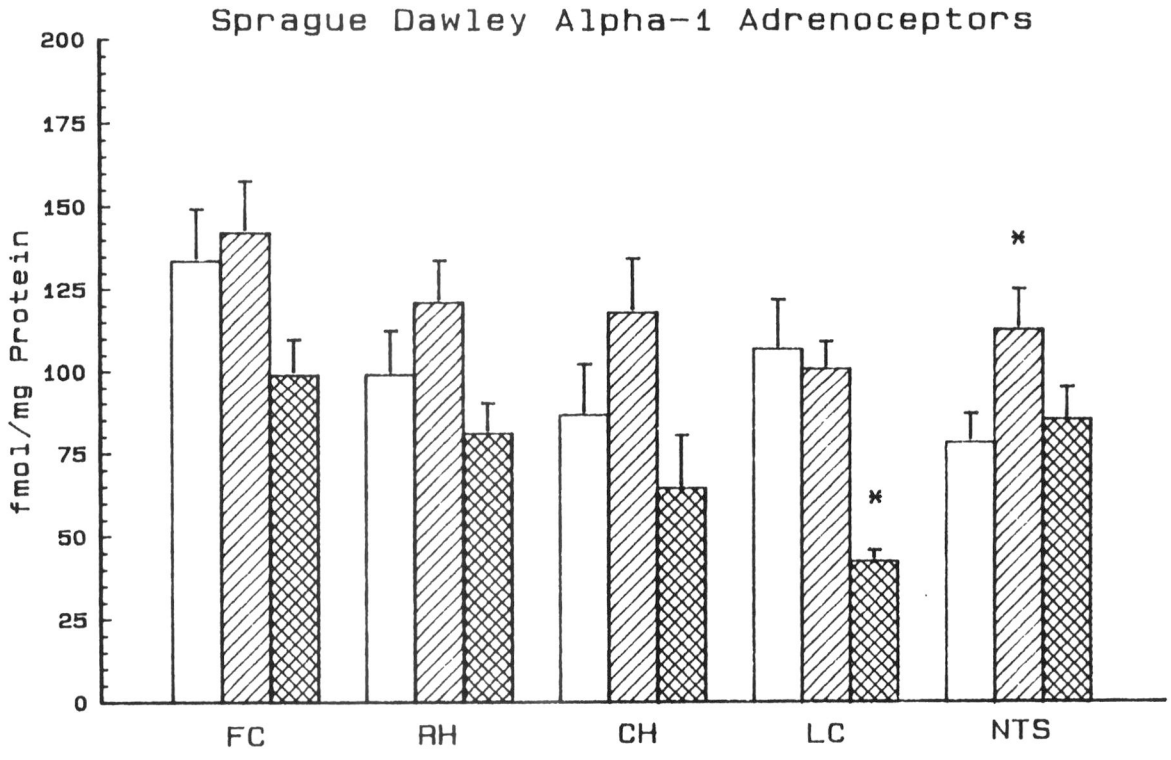


Figure 17. Sprague Dawley alpha-1 adrenoceptors after 12 weeks of treatment with either sesame oil vehicle (open bar), estradiol (single hash bar) or mestranol (cross hash bar). Abbreviations: Frontal cortex (FC) rostral hypothalamus (RH), caudal hypothalamus (CH), region of the locus coeruleus (LC) and region of the nucleus tractus solitarius (NTS). Asterisk (*) indicates significance from control $p<0.05$

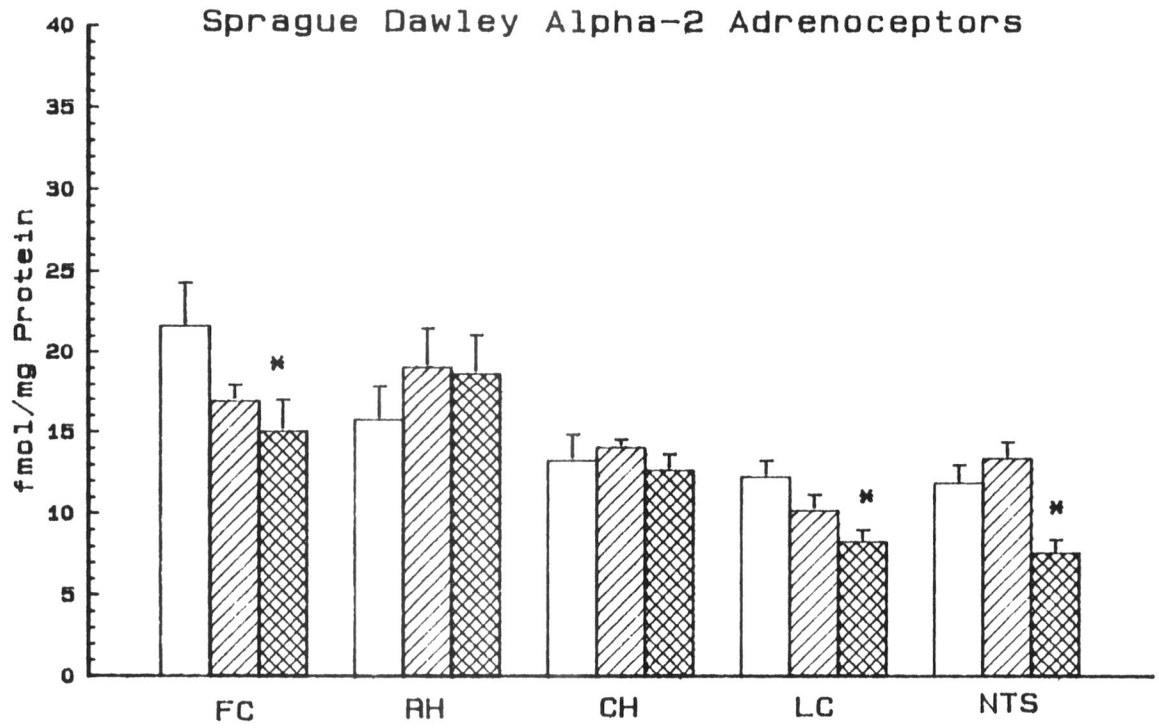


Figure 18. Sprague Dawley alpha-2 adrenoceptors after 12 weeks of treatment with either sesame oil vehicle open bar), estradiol (single hash bar) or mestranol (cross hash bar). Abbreviations: Frontal cortex (FC) rostral hypothalamus (RH), caudal hypothalamus (CH), region of the locus coeruleus (LC) and region of the nucleus tractus solitarius (NTS). Asterisk (*) indicates significance from control $p<0.05$

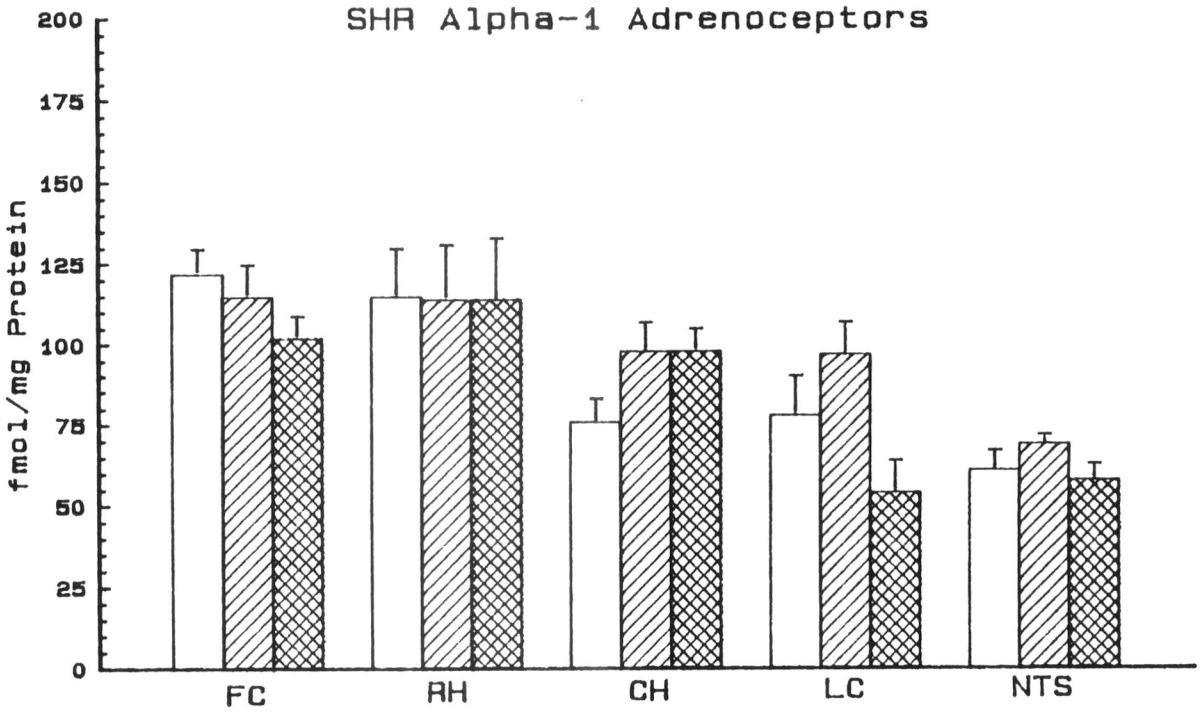


Figure 19. Spontaneously nypertensive rat alpha-1 adrenoceptors after 12 weeks of treatment with either sesame oil vehicle open bar), estradiol (single hash bar) or mestranol (cross hash bar). Abbreviations: Frontal cortex (FC) rostral hypothalamus (RH), caudal hypothalamus (CH), region of the locus coeruleus (LC) and region of the nucleus tractus solitarius (NTS). Asterisk (*) indicates significance from control $p<0.05$

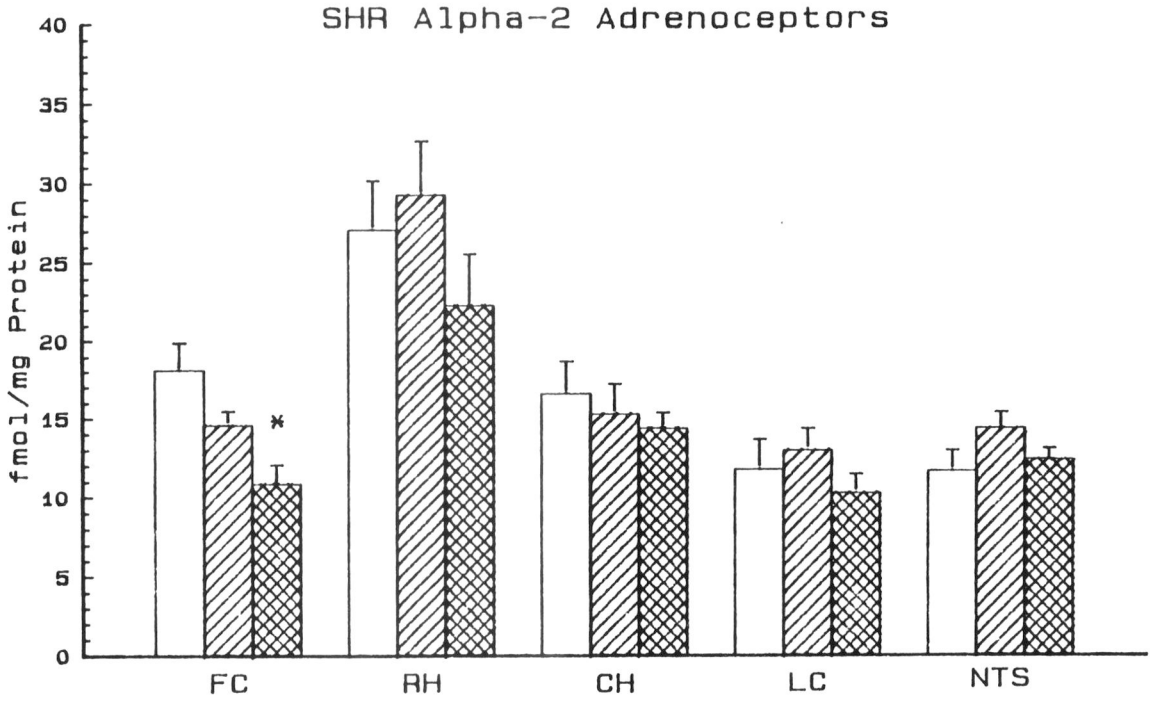


Figure 20. Spontaneously hypertensive rat alpha-2 adrenoceptors after 12 weeks of treatment with either sesame oil vehicle open bar), estradiol (single hash bar) or mestranol (cross hash bar). Abbreviations: Frontal cortex (FC) rostral hypothalamus (RH), caudal hypothalamus (CH), region of the locus coeruleus (LC) and region of the nucleus tractus solitarius (NTS). Asterisk (*) indicates significance from control $p<0.05$

DISCUSSION

In this study we found that estrogens, while having no influence on blood pressure in normotensive Sprague Dawley rats were effective in preventing the development of hypertension in SHR. Further more, natural and synthetic estrogens have differing effects on central alpha adrenoceptors sub populations. However the observed hemodynamic effects attributed to the estrogens do not correlate with estrogen induced changes in alpha adrenoceptors in brain regions that regulate blood pressure and contain estrogen binding sites.

The specific physiologic role that estrogens play in influencing the cardiovascular system is uncertain. Females have a lower incidence of hypertension and general cardiovascular disease prior to menopause, than males indicating that female sex steroids are in some way biologically protective during the reproductive years. Postmenopausal females have an increasing incidence of cardiovascular disease and hypertension relative to age matched males. The major difference pre and post menopausally is the ovarian steroid titers. However pharmacolocgic doses of ovarian steroids, in the form of oral contraceptives, are known to increase the risk of cardiovascular disease and overt hypertension (84). Several investigators have suggested that the estrogen component of oral contraceptives is the hemodynamic agent (22,55,84). Yet postmenopausal estrogen replacement therapy (ERT) with native human estrogens or conjugated equine estrogens, given to prevent osteopetrosis and

as treatment for hot flushes is associated with a protective influence on the cardiovascular system (47).

To explore the hemodynamic effects of estrogens two experimental models were chosen. Spontaneously hypertensive rats were used as the hypertensive model while Sprague Dawley rats were used as the normotensive model. The traditional normotensive control animals are the Wistar Kyoto (WKY), the rat from which the SHR was developed (42). We chose to use the SD over the WKY for our normotensive controls because of this familial relationship. Estrogen treatment in the WKY has been shown to cause a reduction in systolic pressure just as in the SHR, although the reduction was not as great (39). This is similar to the effects of estrogens on borderline hypertensive individuals (74). Estrogen treatment in the SD animals for 12 weeks is known to have no significant effect on systolic pressures (30) which is similar to normotensive humans on estrogen treatment (Fig. 13). Therefore in estrogen related experiments the WKY appears be a poor choice for control based on blood pressure responses. Many studies have been conducted that suggest the difference in pressure between the SHR and the WKY may be related to differences in central alpha adrenoceptors. These studies demonstrate elevated alpha-1 adrenoceptors in the cortex, hypothalamus and medulla of the SHR relative to the WKY animals (33,66,75). When receptors values in SD and SHR control animals were compared in this we found that study demonstrate alpha-2 adrenoceptors from the SHR controls were significantly

higher than SD controls only in the RH (Fig. 16). Alpha-1 adrenoceptor numbers were not significantly different in the five brain regions examined (Fig. 15). Therefore, we conclude that the SD is a better control animal for our study. Not only do the animals respond to estrogen treatment in a similar fashion to humans but there appears to be fewer differences between the animals in terms of central alpha adrenoceptors.

The blood pressure results of this study are comparable to those observed clinically in human females using estrogens. Individuals genetically predisposed to hypertension experience a 4 to 5 mm Hg reduction in systolic blood pressure in response to estrogen treatment in addition to the reduction in pressure attributable to the antihypertensive therapy that they were already receiving (74). Spontaneously hypertensive rats treated with estradiol experienced a 10% reduction in pressure relative to control SHR while mestranol treatment caused a 20% reduction in systolic pressure relative to control. These results are similar to those of Hoeg et. al. (39) and Iams et al. (42) who also demonstrated a reduction in SHR systolic pressure in response to estrogen treatment.

Normotensive animals respond differently to estrogen treatment. Sprague Dawley animals treated with relatively low doses of mestranol in their diet for 12 weeks fail to develop hypertension yet continued treatment up to six months leads to the development of a borderline hypertension (26). Similarly we were unable to demonstrate any significant elevation in resting

blood pressure in SD rats after 12 weeks of estrogen treatment. Our experimental results with the normotensive SD are also similar to normotensive humans on estrogen replacement therapy (ERT). Pfeffer et. al. (74) showed that normotensive females on ERT experienced a slight but nonsignificant elevation in resting systolic blood pressure. The results of our study and those of Pfeffer indicate that naturally occurring estrogens are not detrimental to the cardiovascular system of normotensive subjects however they do not indicate if estrogens have a protective role.

The attenuation of blood pressure in the SHR was independent of body weight as demonstrated here and by Hoeg et.al. (39). Estradiol and mestranol treatment caused a significant reduction in body weight in both SD and SHR while only attenuating pressure development in the SHR (Table 1.). The effects of estrogen on body weight are well documented, however the mechanisms involved are uncertain (39,42,101).

The central alpha adrenergic system is involved in the tonic and reflex control of the cardiovascular system. Estrogens have been found to effect the central adrenergic system, and are thought to influence the sympathetic function. Therefore, it seems possible that the hemodynamic effects of the estrogens, maybe mediated in part by alterations in sympathetic control of the cardiovascular system. This effect may originate from estrogen induced changes in central alpha adrenergic receptors in regions of the brain known to regulate blood pressure and to contain estrogen binding sites.

Alpha adrenoceptors have been divided in to two sub classes designated as alpha-1 and alpha-1 adrenoceptors. Alpha-1 adrenoceptors have been generally considered stimulatory, while alpha-2 adrenoceptors are generally considered inhibitory by modulating a feedback response. Originally the receptors were thought to be anatomically separated into postsynaptic alpha-1 and presynaptic alpha-2 feedback receptors (50). Later it was suggested that the receptors be classed according to physiologic function and ligand specificity (7). Alpha-1 stimulation has been shown to cause cell depolarization and therefore increase cell firing rates (68,79), while alpha-2 stimulation has been shown to cause cell hyperpolarization and therefore reduce the overall firing rate (9,65). Alpha-1 and alpha-2 adrenoceptors are also thought to have opposing effects on the cellular protein phosphorylation system (25). Therefore in any study of the alpha adrenergic phenomenon particularly in the CNS both receptors should be examined. Changes in one or both of the receptor populations numbers may reflect variations in sympathetic firing rate.

Elevated noradrenergic stimulation is reported to cause a down regulation of both alpha-1 and alpha-2 adrenoceptors (38,64). Yet evidence from this study and by others (43,54) indicates that alpha adrenoceptors may be differentially regulated in response to various stimuli. In our study mestranol caused a significant reduction in both subpopulations alpha adrenoceptors in the LC and a selective reduction in alpha-2

adrenoceptors in the FC and NTS. Estradiol caused a significant elevation in alpha-1 adrenoceptors of the NTS. Uniyal (43) demonstrated that alpha-2 adrenoceptors from the PVH could be selectively up or down regulated by food deprivation, while alpha-1 adrenoceptors were uninfluenced by food deprivation. The specific effect on alpha-2 adrenoceptors was dependent on the time in the light cycle and length of food deprivation. Maggi et. al. (59) reported that alpha-2 adrenoceptors could be selectively up regulated by beta adrenoceptor stimulation with isoproterenol. Long-term activation of the beta adrenergic system, while down regulating beta receptors, caused a reversible increase in the number of alpha-2 adrenoceptors in rat cerebral cortex.

The general down regulation of both alpha-1 and alpha-2 adrenoceptors in the LC of the mestranol treated SD rats may reflect an increase in noradrenergic stimulation in this region due to changes in catecholamine metabolism, or to specific mestranol induced effects elsewhere in the brain. Estradiol 2/4 hydroxylase, the enzyme responsible for conversion of estradiol to catechol estrogens, has been localized in the LC (95). The catechol estrogens are known to inhibit both catechol-o-methyl transferase (5), and tyrosine hydroxylase (28), enzymes involved in catecholamine metabolism. Regional alterations in these enzymes are thought to cause altered catecholamine levels in discrete regions of the brain (1,20,24). General down regulation

of alpha adrenoceptors in the LC may reflect high catecholamine levels in this area of the brain as a result of mestranol induced changes in catecholamine metabolism. The LC is reported to have direct reciprocal connections to the FC and NTS (13), brain regions where alpha-2 adrenoceptors were selectively down regulated by mestranol treatment. The specific reduction in alpha-2 adrenoceptor binding in these brain regions may significantly alter noradrenergic traffic to the LC from these brain regions thus cause the general down regulation of alpha adrenoceptors observed. However a direct effect of mestranol on the LC can not be ruled out. The mestranol induced down regulation of alpha-2 adrenoceptors in the FC of both strains and the NTS of the SD may reflect selective receptor regulation by mestranol. General alpha adrenoceptor regulation as a result of altered catecholamine levels or changes in nerve traffic would effect both alpha adrenoceptors (38,64). The specific down regulation of alpha-2 adrenoceptors in these brain regions suggest a direct effect of mestranol on the alpha-2 adrenoceptor, possibly through receptor synthesis, and independent of noradrenergic metabolism and/or noradrenergic nerve traffic.

Alpha adrenoceptors are reported to be involved in the regulation of steroid dependent processes thought to be centered in the hypothalamus. Alpha-2 stimulation with clonidine is associated with a prolongation of the lordosis response in estrogen primed guinea pigs (21), while alpha blockade reduces the lordosis response (70). The interaction between estrogens

and neurotransmission is not unidirectional. As mentioned previously, estrogens interfere with catecholamine metabolism (4,5,28,73,95) and are also known to effect norepinephrine uptake (69). However in this study alpha adrenoceptor binding in the RH and CH of both SD and SHR was unaffected by long-term chronic estrogen treatment. This finding was somewhat surprising because Johnson (44) using autoradiographic techniques found a significant increase in alpha-2 adrenoceptor density in several preoptic nuclei of the rostral hypothalamus in response to acute estrogen treatment. However Wilkerson (96) and Vascus(89) demonstrated that alpha adrenoceptors in the cortex and hypothalamus were unaffected by estrogen treatment. They used the general alpha adrenoceptor ligand [3H]-dihydroergocryptine which does not distinguish between alpha-1 and alpha-2 adrenoceptors and therefore might not have been able to pick up subtle changes that could occur in alpha receptors sub populations. Our inability to find any changes in alpha adrenoceptors in the RH and CH may be due to the large amounts of tissue used which could mask discrete changes in alpha receptors. It may be necessary to measure binding in isolated nuclei in order to truly determine estrogen effect on alpha adrenoceptors.

The physiologic significance of the selective alpha-2 receptor alterations in the FC and NTS and the general alpha adrenoceptor alteration in the LC of the SD remains to be explained since no changes in resting blood pressure were observed. The LC and NTS have been implicated in the regulation

of cardiovascular function and have been demonstrated to contain estrogen binding sites (81). Electrical stimulation of the LC is known to cause the release of ADH and elevate resting blood pressure (86), however the specific role of the LC in cardiovascular regulation remains uncertain. The region of the NTS taken also contains the DMV. This region is the site of termination of many visceral afferents arising from cardiac receptors, chemoreceptors and baroreceptors, as well as the origin of the vagus. Electrical stimulation of the NTS or its afferent neurons is known to cause a depressor response while destruction of this region will cause hypertension (57). The alpha-2 down regulation by mestranol and the alpha-1 upregulation by estradiol in the NTS of the SD may play a role in the reported effects of both estradiol an mestranol on blood pressure development and reflex control of the cardiovascular system (30,39,42,54). Central alpha-2 stimulation with the antihypertensive clonidine is thought to reduce sympathetic tone (41) and cause vagal activation (40). However, in normotensive subjects clonidine has little effect on resting blood pressure levels (80). Huchet reported that central alpha-2 blockade with the alpha-2 antagonist yohembine failed to cause any significant effect on resting blood pressure levels in dogs. However yohembine treatment significantly inhibited the hypotensive effect of carotid sinus nerve stimulation (41). On the other hand, central alpha-1 adrenoceptor stimulation, by injection of phenylephrine into the cisterna magna, will cause a significant

elevation in resting blood pressure and a reduction in the hypotensive response of carotid sinus nerve stimulation. These data indicate that alpha-2 adrenoceptors play a role in the reflex control of blood pressure but have minimal influence over resting blood pressure. Therefore the reduction in alpha-2 adrenoceptors in the LC and NTS of the mestranol treated SD may not affect resting blood pressure. Alpha-1 adrenoceptors, however, are reported to play a role in the maintenance of resting blood pressure and in reflex control (40,41). We did find an elevation in alpha-1 adrenoceptors of the NTS in estradiol treated SD but no pressure changes. Thus increases in alpha-1 receptors in other regions of the brain involved in blood pressure control may be required before changes are seen in blood pressure levels.

Our SD blood pressure data is similar to that of Fowler, showing no significant difference in blood pressure after 12 weeks of mestranol treatment (30). However, he did demonstrate that continued treatment with mestranol will result in elevated blood pressure. The reduction in alpha-2 adrenoceptors in the LC and NTS of the SD after 12 weeks of treatment found in our study, occur prior to the elevation in pressure observed by Fowler (30), and may be required for the development of the elevated pressure levels. Pullen et. al. (75) found elevated alpha-1 adrenoceptors in the RH, CH, LC and NTS of four week old SHR prior to the development of hypertension, relative to age matched WKY controls. At 12 weeks of age SHR blood pressures were

hypertensive yet alpha-1 adrenoceptors of the LC and NTS were not significantly different from age matched WKY controls while RH and CH alpha-1 adrenoceptors remained significantly elevated. These results suggest that the receptor differences in the LC and NTS may be involved in development of the hypertension but not in the maintenance. However it should be noted that we observed no difference in adrenergic receptor levels between control SD and SHR, except for alpha-2 adrenoceptors of the RH (Fig. 16).

The fact that we observed no significant changes in pressure in the SD rats in response to estrogen treatment while we observed estrogen induced shifts in central adrenoceptors in several brain regions suggest that no direct correlation exist between central alpha adrenoceptors and resting blood pressure. However the receptor differences observed may alter cardiovascular reflexes or be involved in the development of elevated pressure levels in SD animals treated with mestranol for six months (30). A lack of correlation is farther suggested by the data from the SHRs. While estrogen treatment caused a significant reduction in resting blood pressure levels, adrenergic receptors were unaffected, suggesting that alpha adrenoceptors are not involved in attenuation of resting blood pressure in treated SHR. These data indicate that the protective effects of the estrogens on the cardiovascular system may not be directly related to changes in central alpha adrenoceptors as originally proposed.

Estrogens may not affect alpha adrenoceptors but may alter receptor transducer mechanisms. Recently Studer et. al. (85) suggested that the alpha adrenergic differences observed in male and female rat hepatocytes was a result of differences in the calcium linked transducer mechanism and not due to alterations in the receptor itself. More work needs to be completed before a definitive answer can be reached. Therefore, the effects on estrogens on the central alpha adrenergic system of the SHR may be mediated by changes in the transducer mechanism coupled to the receptor while both mechanisms may be acting in the SD.

SUMMARY

While it is clear that estradiol and mestranol share several estrogenic properties it should be noted that they influence central alpha adrenoceptors differently, and therefore should not be used as interchangeable estrogens. Both estradiol and mestranol attenuate hypertension in the SHR while having in influence on normotensive SD after 12 weeks of treatment. Estrogen induced changes in central alpha adrenoceptors on normotensive SD rats and the lack of change observed in central alpha adrenoceptors of the SHR indicate that shifts in central alpha-adrenoceptors are minimally involved in the estrogens effects on resting blood pressure.

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