erodimeric receptors for laminin and type IV collagen. J. Cell Biol. *107* (1988) 1241–1252.
144 Townes, P., and Holtfreter, J., Directed movements and selective adhesion of embryonic amphibian cells. J. exp. Zool. *128* (1955) 53–120.
145 Troccoli, N. M., and Hausman, R. E., Retina cognin does not bind to itself during membrane interaction in vitro. Cell Differ. *22* (1988) 225–232.
146 Tuszynski, G. P., Rothman, V., Murphy, A., Siegler, K., Smith, L., Smith, S., Karczewski, J., and Knudsen, K. A., Thrombospondin promotes cell-substratum adhesion. Science *236* (1987) 1570–1573.
147 Vaughan, L., Huber, S., Chiquet, M., and Winterhalter, K., A major six-armed glycoprotein from embryonic cartilage. EMBO J. *6* (1987) 349–353.
148 Volk, T., and Geiger, B., A-CAM: a 135 kd receptor of intercellular adherens junctions. I. Immunoelectron microscopic localization and biochemical studies. J. Cell Biol. *103* (1986) 1441–1450.
149 Wewer, U. M., Liotta, L. A., Jaye, M., Ricca, G. A., Drohan, W. N., Claysmith, A. P., Rao, C. N., Wirth, P., Coligan, J. E., Albrechtsen, R., Mudry, M., and Sobel, M. E., Altered levels of laminin receptor mRNA in various human carcinoma cells have different abilities to bind laminin. Proc. natl Acad. Sci. USA *83* (1986) 7137–7141.
150 Williams, A. F., and Barclay, A. N., The immunoglobulin superfamily – domains for cell surface recognition. A. Rev. Immun. *6* (1988) 381–405
151 Yamada, K. M., Fibronectin domains and receptors, in: Fibronectin, pp. 47–121. Ed. D. F. Mosher. Academic Press, New York 1989.
152 Zinn, K., McAllister, L., and Goodman, C. S., Sequence analysis and neuronal expression of Fasciclin I in grasshopper and Drosophila. Cell *53* (1988) 577–587.
153 Bieber, A. J., Snow, P. M., Hortsch, M., Patel, N. H., Jacobs, J. R., Traquina, Z. R., Schilling, J., and Goodman, C. S., Drosophila neuroglian: a member of the immunoglobulin superfamily with extensive homology to the vertebrate neural adhesion molecule L1. Cell (1989) in press.

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Steroid hormones and the cardiovascular system: Direct actions of estradiol, progesterone, testosterone, gluco- and mineralcorticoids, and soltriol [vitamin D] on central nervous regulatory and peripheral tissues

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Summary. Knowledge of steroid hormone sites of action and related effects in cardiovascular and neural regulatory tissues is reviewed. Evidence for nuclear receptor sites is derived mainly from autoradiographic studies with relatively intact tissues and some biochemical studies with tissue homogenates.
In the heart and in the walls of blood vessels, estradiol, dihydrotestosterone, corticosterone, aldosterone, dexamethasone, and soltriol (vitamin D) show nuclear binding. In the brain and spinal cord, neuronal regions associated with cardiovascular regulation contain nuclear receptors in specific patterns for each steroid hormones, including progesterone and soltriol. These data indicate that all steroid hormones exert direct actions on the cardiovascular system at its different levels of organization, thus enabling adjustment to the changing demands during reproduction (gonadal steroids), stress (adrenal steroids), and solar seasons (vitamin D-soltriol).
Key words. Estradiol; progesterone; dihydrotestosterone; adrenal steroids; soltriol; vitamin D; cardiovascular system; brain; spinal cord.

Introduction

Blood flow varies with the needs of the organism and its individual tissues. Changes in blood flow can be accomplished by adjusting the functions of cardiovascular tissues through peripheral messengers and neural factors, 'regulated according to certain pre-programmed priorities'[16] for different physiological conditions of procreation and survival. Such vital conditions include procurement of food, competition for partner, nest building and territorial defense, estrus and mating, pregnancy, lactation, and maternal care. Induction and control of these conditions require actions of sex steroid hormones, adrenal steroid hormones, and the seasonal steroid hormone soltriol[97].
Steroid hormone regulation of cardiovascular functions is exerted at different levels of organization. Receptors for steroid hormones can be demonstrated in neural regions of the brainstem and spinal cord, and in the heart and in walls of blood vessels including capillaries. In addition to direct effects, indirect effects on cardiovascular functions may be exerted through steroid hormone actions on endocrine and metabolism controlling organs, such as pituitary, adrenal, liver and kidney. Effects of steroid hormones on the cardiovascular system, thus, appear to be extensive and complex, and to affect all phases of life. Such conclusions can be supported through existing information, albeit incomplete, on steroid hormone receptor distribution, steroid hormone effects on glucose, protein, and lipid metabolism, on monoamine and peptide messenger related receptor production, on liver, kidney and pituitary-endocrine func-

tions, and on age- and sex-related cardiovascular 'diseases'.

Evidence will be reviewed from autoradiographic, immunohistochemical, and biochemical studies concerning information about cardiovascular sites of receptors for sex and adrenal steroid hormones, and the steroid hormone of sunlight, soltriol (1,25-dihydroxycholecalciferol, vitamin D). Observations will be included from related clinical, physiological, pathological, and epidemiological studies.

Estradiol (fig. 1)

Clinical and experimental reports suggest the presence of sex differences and indicate a role for sex steroid hormones in the etiology of cardiovascular diseases. The published results are, however, contradictory, and it is difficult to establish causative relationships with respect to the etiology of pathologic conditions. This was the conclusion drawn by McGill and Stern in an extensive review of the literature on sex and arteriosclerosis[66].

Estrogens, when given in high doses, lower LDL and raise HDL in both men and women. When estrogens are given in physiological doses, effects on serum lipid concentrations and on atherosclerotic lesions are absent or negligible. The risk of myocardial infarction seems to be increased among oral contraceptive users, but postmenopausal estrogen replacement therapy has neither an adverse nor protective effect on the risk of myocardial infarction. Similarly, there is no evidence that estrogen therapy improves the prognosis in men who have experienced myocardial or cerebral infarction, although high doses increase the incidences of cardiovascular complications[66]. No clear pattern of association is apparent between free or total estrogen blood levels and ischemic heart disease[19]. But there are other clinical and experimental reports that attribute to estrogens and androgens a protective or deleterious role in cardiovascular physiology and pathology[66].

Several reports testify to effects of estrogen on tissues of the arterial wall. For instance, after treatment with the antiestrogen tamoxifen, incubation of rabbit aorta with ^{14}C proline shows a reduced presence of hydroxyproline in collagen and elastin, but an increase after testosterone and progesterone treatment. Receptors are found to be present in the cytosol and to 'translocate' to the nucleus after administration of the steroid, suggesting direct effects of the hormones on arterial wall to alter collagen and elastin synthesis[32]. In another study by Fischer and Swain[33], ovariectomy in rabbits fed an atherosclerotic diet resulted in a significantly greater degree of atherosclerosis and collagen synthesis in the aortic arch, when compared to intact or ovariectomized animals that had received estradiol. Tissue deposition of cholesterol paralleled the degree of atherosclerosis.

It is well established that vascular responses to oxytocin, vasopressin, norepinephrine and epinephrine vary with the blood concentrations of steroid hormones[2, 60, 61, 84, 89]. These variations involve both release of messenger and receptor-mediated action. The

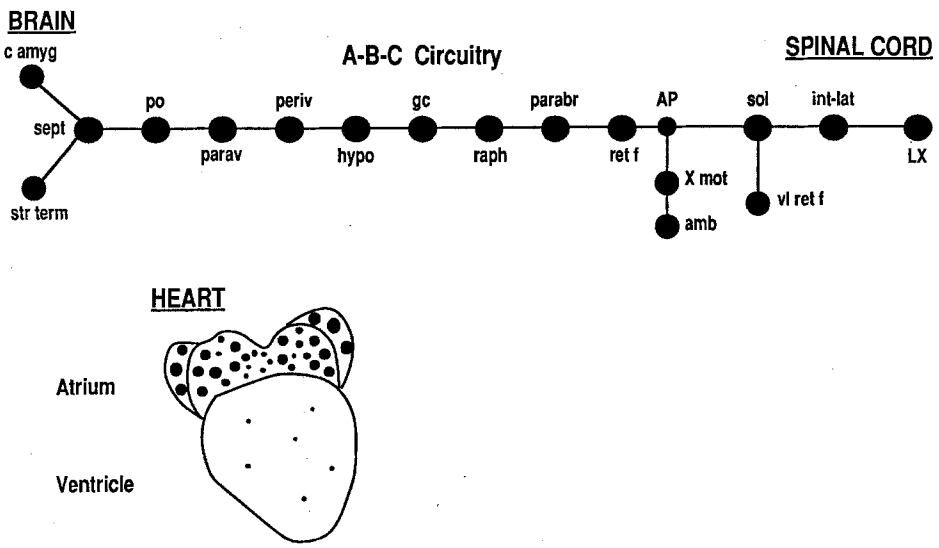

Figure 1. Estradiol sites of action in the heart and central neuronal regions involved in the control of cardiovascular functions. In brain and spinal cord, sites implicated in cardiovascular central regulation correspond to those that had been identified earlier as the estradiol controlled A-B-C (Allocortex-Brainstem-Core) circuitry of endocrine-autonomic integration and regulation[101]. Estradiol nuclear binding is also observed in atrial cardiomyocytes[108], in muscle and connective tissue cells in walls of large, intermediate and small arteries, and in capillary pericytes[105]. Dots indicate sites with nuclear binding of ^3H estradiol.

Not indicated are sensory labeled neurons in spinal ganglia and substantia gelatinosa in spinal cord and medulla oblongata, which may be components of sympathetic reflex arcs. Other effects of estradiol on cardiovascular function, not depicted, include modulation of angiotensin II receptor densities in pituitary and adrenal[17], production of HDL and LDL by the liver, and probably electrolyte balance by kidney.

Abbreviations: c amyg = nucleus (n.) centralis amygdalae; str term = bednucleus of the stria terminalis; sept = septum; po = preoptic area; parav = n. paraventricularis; peri = n. periventricularis; hypo = hypothalamus; cg = central gray; parabr = n. parabrachialis; ret f = reticular formation; sol = n. tractus solitarii; Xmot = dorsal motor nucleus of vagus nerve; ambig = n. ambiguus; int-lat = n. intermediolateralis; LX = lamina X of spinal cord.

sex-related difference in vasopressin release in rats is abolished by gonadectomy and restored by treatment of females with ovarian hormones and of males with testosterone[84]. A sexual dimorphism is also recognizable in deoxycorticosterone (DOC)-salt hypertension, a form of hypertension that requires the presence of vasopressin[22]. While there is no elevation of plasma vasopressin levels, pressure responsiveness to vasopressin is greater in male rats than in female rats that are in diestrous, proestrous, or metestrous phase of the estrous cycle. The pressure responsiveness of female rats in estrus, however, is identical to that in males[84]. The changes in vascular response can be monitored through microscopic observations of mesenteric arterioles, where pretreatment with estrogen results in an enhancement of the constrictor actions of catecholamines and neurohypophyseal hormones, probably by changing the reactivity of arteriolar smooth muscle[2,89].

It remains to be explained why pressure responsiveness to vasopressin is greater in female rats in estrus, compared to the other stages of the estrous cycle, while DOC-salt hypertension is exacerbated in ovariectomized females but prevented by treatment with estradiol. Similarly unexplained is the situation in the non-castrated male rat, in which the pressure responsiveness to vasopressin is high, while after castration the DOC-salt hypertension is attenuated[84].

When ^{3}H estradiol is injected into animals, nuclear accumulation and retention of radioactivity can be observed in reproductive organs, but also in many other tissues not traditionally categorized as 'reproductive'[90,105]. Many of the components of the cardiovascular system have been identified as target tissues for estradiol, showing nuclear accumulation and retention of the hormone in the fashion characteristic of 'reproductive organs' such as the uterus, oviduct, vagina, ovarian granulosa cells, Leydig cells in the testis[92], and epithelial cells in the epididymis[82], which defies a distinction between 'reproductive' and 'non-reproductive' organs.

Heart muscle has been used by biochemists as a control non-target tissue for estradiol. When studied by autoradiography, heart muscle shows no or little nuclear binding in the ventricle, but nuclear labeling is conspicuous in auricles and other parts of the atrium, as well as in the wall of large blood vessels[108]. Evidence for nuclear receptors is also seen in muscle cells in the tunica media of intermediate and small arteries, and in capillary pericytes. Under the same experimental conditions no nuclear concentration of radiolabeled ligand could be detected in endothelial cells. The presence of receptors for estradiol in atrial myocytes was then interpreted to indicate a stimulatory action of estradiol on the production of 'atrial granules' and the secretion of a postulated heart hormone[108], identified as atrial nutriuretic factor[23].

In the brain and spinal cord, sites of nuclear labeling with ^{3}H estradiol could be found in anatomically defined regions soon after the introduction of the dry-mount and thaw-mount autoradiographic techniques[52,90,93]. The development of these techniques has been prerequisite for successful steroid hormone autoradiography[103]. A central nervous system pattern of neuronal estradiol targets has been established in rodents[52,90,93,109] and primates[51] that has challenged existing concepts of a 'sex center' and of a 'hypophyseotrophic area', and suggested the presence of 'estrogen-neuron-systems or circuits'[90,93,94] throughout the brain stem, with involvement of the amygdala, limbic cortex, and spinal cord.

Many of the regions that contain estradiol target neurons have been implicated in the regulation of cardiovascular functions[3,7,16,18,36,62,64,72,88]. In the spinal cord, estrogen target neurons are found in the nucleus intermediolateralis and in lamina X. In the brain stem from caudal to rostral, estrogen target neurons are numerous in the nucleus of the solitary tract, dorsal motor nucleus of the vagus, nucleus ambiguus, nucleus reticularis parvocellularis and ventrolateral reticular formation, more rostral in the parabrachial nuclei with the Kölliker-Fuse nucleus, raphe nuclei, central gray, nuclear groups in the basal hypothalamus, paraventricular and periventricular nuclei in the anterior hypothalamus, preoptic region, lateral septum, bednucleus of the stria terminalis and nuclei in the amygdala[93,109].

Such widespread distribution of specific neurons seems to defy the concept of a medullary cardiovascular center, proposed by Dittmar in 1870[24], which, like other 'center' concepts in neuroendocrinology[93,94] has largely been abandoned. But, important neuronal groups reside in the caudal medullary region. Since this region does not act independently and is an important component of a more comprehensive neuronal system, the idea of circuits rather than center(s) is more appropriate, even though nodal points within this regulatory circuitry can be identified. It must also be recognized that the system is open, both to the outside and to the inside, via neural and humoral sensory afferents.

The thoraco-lumbar intermediolateral nucleus is the origin of preganglionic fibers that contribute to the sympathetic innervation of the heart. This nucleus is sex dimorphic, at least in the cat, where the number of neurons has been reported to be higher in males than in females[44]. Sites of central cardiovascular regulation and input to the intermediolateral nucleus have been identified through the use of electrophysiological, autoradiographic and other histochemical tracing techniques[16,18,36,62]. These regulatory structures include serotoninergic fibers from the midline raphe nuclei, catecholamine fibers from the subceruleal region, the nucleus of the solitary tract and the lateral reticular nucleus. In addition, projections to the intermediolateral nucleus are noted from the parabrachial nuclei with the Kölliker-Fuse nucleus, as well as the hypothalamic paraventricular nucleus, which is closely linked to the bednucleus of the stria terminalis and the central nucleus of the amygdala. Close connections exist between the nucleus of the

solitary tract and the nucleus ambiguus, the dorsal motor nucleus of the vagus, the nucleus parvocellularis, the n. reticularis gigantocellularis, the central gray, components of the hypothalamic paraventricular nucleus[1,46], and many others, which can not be covered here in more detail. In all of these regions, nuclear binding of estradiol has been reported in rats and mice[52,93,104,109], indicating involvement of this hormone in central cardiovascular regulation. The estradiol target neurons in spinal ganglia, substantia gelatinosa, lamina X, and nucleus intermediolateralis are likely to contribute to a suggested polysynaptic pathway for spinal sympathetic reflexes[70]. Included in the neuronal groups of the brain stem are catecholamine neurons. Co-localization of radiolabeled estradiol by autoradiography and catecholamine fluorescence[39,45] or antibodies to dopamine beta hydroxylase[78] have established a direct cellular relationship between these messengers. This provides an anatomical basis for the well-known effects of estradiol on catecholamine blood and tissue levels during the estrous and menstrual cycle, pregnancy, and menopause and the influence of sex steroid on events outside of reproduction that are recognized to be under the control of catecholamines, including feeding and drinking, memory, aggression, depression and other mood changes, vomiting, thermoregulation, blood pressure regulation[2,60,61,69,84], and others.

Between 50 and 80% of estrogen target neurons contain catecholamine fluorescence in the regions of the nucleus (n.) reticularis lateralis (group A1), n. tractus solitarii (group A2), in the pons adjacent to the n. olivarius superior (group A5), in the locus ceruleus (group A6), and in the vicinity of the lemniscus lateralis (group A7). In addition, a small number of dopamine neurons in the hypothalamic arcuate and periventricular nucleus (group A12) are labeled with estradiol[45]. These data indicate that the many autonomic-endocrine effects of catecholamines can be modulated or controlled by estradiol. Correspondence of the anatomical distribution of estrogen target neurons, similar to those studied in detail for catecholamines, is apparent for serotonin in various raphe nuclei, as well as for peptide hormones, such as neurotensin in the n. tractus solitarii, n. parabrachialis and other nuclei[48], oxytocin in the anterior hypothalamus[49], endorphin and enkephalin[49,79] in the arcuate nucleus and somatostatin, GABA[79], and atrial natriuretic peptide[8] in the periventricular hypothalamus. This list is incomplete. Anatomical correspondence exists between estradiol targets and sites of production of several other brain messengers associated with central regulation of circulation[35,73]. Therefore, cellular coexistence with estradiol nuclear receptors can be expected in the future to be demonstrable for many of the production sites of brain messengers as well as for peptides in the peripheral cardiovascular system[115]. While colocalization alone does not prove effects of the steroid on manufacture and secretion of the related messengers, such effects are strongly suggested based on demonstrations of estrogen effects on the secretion of oxytocin, vasopressin, dopamine, serotonin, norepinephrine, GABA, enkephalin, and others.

Relationships in the hypothalamus between ANF neurons and estradiol target neurons are of particular interest, since in the heart co-localization between ANF and estradiol has been demonstrated in the atrium[6]. In the brain, a region in the anterior hypothalamus has received special attention. Injection of ANF to the 'anteroventral third ventricle region', which includes the vascular organ of the lamina terminalis and the pars suprachiasmatica of the preoptic nucleus[12,15], produces an increase in blood pressure and heart rate[85]. This area is thought to be involved in the regulation of blood pressure and fluid and electrolyte balance[14,85]. Estradiol target neurons exist in the suprachiasmatic portion of the preoptic nucleus and in and around the vascular organ of the lamina terminalis[90,93]. Caudal to this region, bordering the optic recess of the third ventricle, ^{3}H estradiol and antibodies to ANF have been co-localized in identical neurons in the ventral periventricular nucleus of the preoptic and anterior hypothalamus[8].

It is noteworthy that the anatomical distribution of cardiovascular regulatory neuronal groups throughout the brain stem corresponds to the A-B-C (Allocortex-Brainstem-Core) circuitry of autonomic-endocrine integration and regulation as conceptualized by Stumpf and Jennes in 1984[101]. All of these brain stem-limbic cortex regions are characterized by a conspicuous presence of perikarya and projections of multiple kinds of peptidergic and aminergic neurons, many of which concentrate radiolabeled estradiol in their nuclei. This suggests that these neurons can be acted upon genomically according to changing blood levels of estradiol, again indicating a direct involvement of estradiol in autonomic and cardiovascular regulations.

Estradiol may affect cardiovascular regulation indirectly through actions at the pituitary level, where all endocrine cell types display nuclear binding[91], and at the level of the adrenal cortex, which contains receptors for estradiol[105]. Chronic estrogen treatment has been shown to decrease angiotensin II receptor density in the adrenal cortex[17], suggesting that estradiol modulates target cell responsiveness to angiotensin II through differential down-regulation of receptors.

Nuclear receptors for estradiol have also been identified in liver and kidney. Estradiol actions on the liver modulate the manufacture of proteins for transport of hormones and metabolites to and from target tissues[11]. Low and high density lipoproteins provide an example of estrogen-mediated effects that involve liver function. Oral administration of estradiol in postmenopausal women increases plasma concentrations of high-density lipoprotein cholesterol and decreases those of low-density lipoprotein cholesterol, in an apparent dose-dependent fashion[31]. An elevation of plasma high-density lipo-

protein cholesterol upon estrogen therapy has been observed also in males with prostatic carcinoma[68]. There are many reports describing similar observations.

In the kidney, nuclear binding of estradiol has been observed in proximal tubules, intertubular connective tissue, and in ladder cells in the medulla[105]. Estrogen effects on proximal tubule cells involves regulation of 1a-hydroxylation of 25-hydroxycholecalciferol. Secondarily, 1,25-dihydroxycholecalciferol may then affect functions of the heart, blood vessels, and regulatory neurons (see below). In addition, estrogen actions on the kidney are likely to influence electrolyte balance.

Together, the available data indicate that estradiol effects components of the cardiovascular system at all levels of organization, including the heart and walls of blood vessels, the spinal cord, medulla and rostral brain stem, and organs that influence water balance and transport of messengers. These effects probably include modulation of electrolyte secretion in the kidney, indicated by nuclear labeling of cells in the proximal tubules, and effects on secretion of vasopressin through nuclear receptors in the paraventricular and supraoptic nuclei, and in pituicytes in the posterior pituitary. Co-localization of estradiol and ANF in the atrium[6] suggests effects on secretory responses of atrial cardiomyocytes. Co-localization of ANF and estradiol in identical neurons[8] within the hypothalamus further suggests a regulatory action of estradiol on central ANF secretion, the function of which is still to be clarified. Changes in the amount of monoamine and peptide hormone receptors through estradiol may influence tissue responses, for instance, to oxytoxin, vasopressin, norepinephrine, and other messengers.

Progesterone (fig. 2)

Compared to estradiol, little is known about progesterone. The role of progesterone and its metabolites in reproduction is still poorly understood. It is recognized that progestagens are important modulators of estrogen effects, either enhancers or inhibitors, depending on dose ratios between the two steroid hormones and duration of treatment. Until the late seventies, several investigators questioned the presence of progesterone receptors in the brain even though progestin nuclear binding had been demonstrated by autoradiography in castrated, untreated and estrogen-primed rats and guinea pigs[76].

Progestin nuclear receptor sites in the forebrain that may be related to cardiovascular regulation, include the medial nucleus of the amygdala, the preoptic suprachiasmatic nucleus, the periventricular hypothalamic nucleus, and the hypothalamic infundibular region with the arcuate and the ventromedial nuclei. In the hindbrain, recent studies with ^{125}I progestin revealed nuclear receptors in the nucleus of the solitary tract, the dorsal motor nucleus of the nervus vagus, the ventrolateral and ventral reticular formation in the medulla (involving the nucleus reticularis gigantocellularis, the magnocellular and parvocellular lateral reticular nucleus), the nucleus ambiguus, scattered neurons in the nucleus reticularis parvocellularis, and in the substantia gelatinosa of the trigeminus (unpublished). These data suggest that progesterone as well is involved in the central nervous regulation of cardiovascular functions.

Nuclear concentration of radiolabeled progestin is absent in the heart of mice, under conditions in which strong nuclear labeling is recognizable in uterine muscle cells. In the wall of blood vessels and in endothelial cell, no nuclear accumulation of radiolabeled hormone could be detected in the limited samples studied. Further information is needed.

Testosterone (fig. 3)

While there is little doubt that testosterone affects the cardiovascular system, it is not clear to what degree

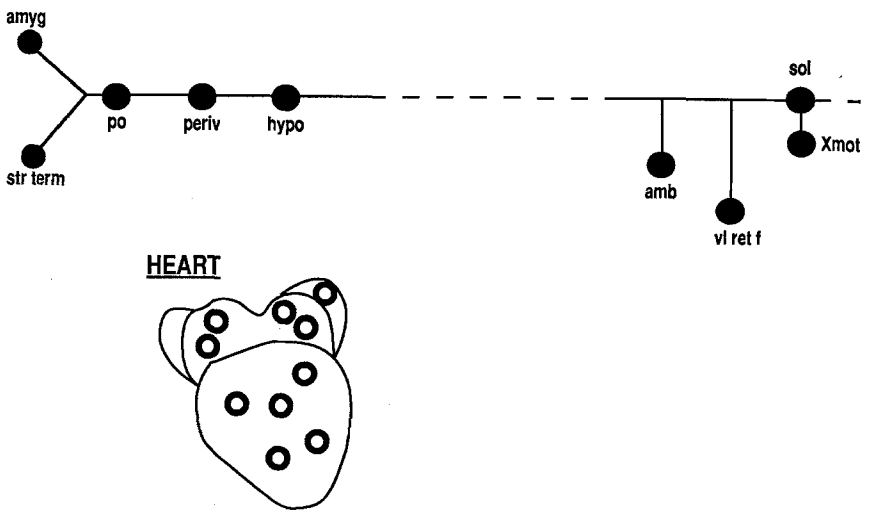

Figure 2. Progestin sites of action in heart and regulatory neurons are incompletely studied. Results from autoradiographic experiments indicate presence of nuclear receptors in the medulla oblongata and in periventricular regions of the forebrain (abbreviations see fig. 1). In cardiomyocytes a conspicuous cytoplasmic accumulation of hormone or metabolite has been observed under conditions in which strong nuclear labeling of myometrial cells exists (unpublished).

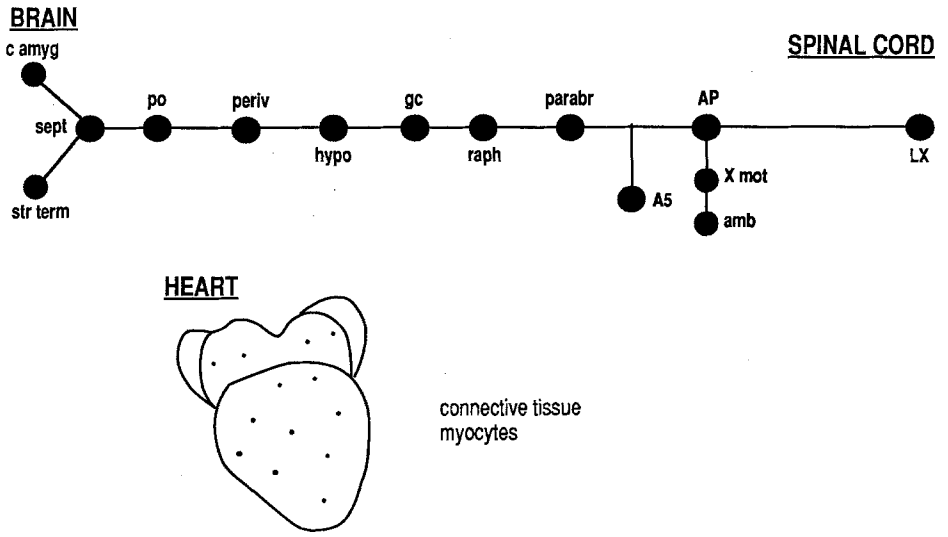

Figure 3. Dihydrotestosterone sites of action in heart and central neuronal regions include target neurons in brain and spinal cord (abbreviations see fig. 1), myocytes and connective tissue in heart atrium and ventricle, and the wall of large arteries[65]. Information on arterioles and capillaries is lacking.

testosterone itself or its metabolites, dihydrotestosterone (DHT) or estradiol, contribute to the effects, or toward which tissues the effects are directed.

In studies published in the literature, low serum testosterone levels have been repeatedly associated with low serum levels of high density lipoprotein cholesterol (HDL-C)[42]. The administration of androgen and some progestins decreases serum LDL levels[38,41]. In men with severe ischemic heart disease, total and free testosterone levels are significantly lower, compared to controls[19]. Measurements of endogenous testosterone taken from a cohort of 1132 men between 30 and 79 years of age resulted in an inverse relationship between testosterone and blood pressure[53].

Results obtained in clinical studies have been supported by animal research. In one study, left ventricular filling and function were impaired in isolated working heart preparation taken from gonadectomized, sex hormone deficient animals, when coronary flow, myocardial oxygen consumption and pump and muscle function were measured. These alterations in function were associated with depressed cardiac myosine ATPase activity[81]. In studies with spontaneously hypertensive stroke-prone rats, blood pressure decreased after gonadectomy and increased significantly after hormone replacement with dihydrotestosterone[59]. In the same study, changes in heart and body weight and in ventricular myosine isoenzyme pattern were noted. In another study, 'a vigorous response to testosterone administration' was observed in the ventricular myocardium of male mice, with accretion of RNA and protein, and increased activities of mitochondrial cytochrome C oxidase and several lysosomal hydrolases[55]. The authors of this study concluded that androgens may regulate proteins associated with the inner mitochondrial membrane in a manner similar to thyroid hormones, which would serve to augment the respiratory capacity of heart mitochondria. In addition, it was proposed that 'testosterone enhances autophagy, and possibly pinocytosis to some extent', in ventricular myocytes[55].

Autoradiography and biochemistry have provided information concerning specific sites of testosterone action. The presence of 'specific androgen receptor in rat heart muscle' has been reported in homogenates[58], and nuclear binding of ^{3}H dihydrotestosterone has been demonstrated in autoradiograms of baboon heart myocytes in autoradiograms and in baboon heart homogenates[65,67]. According to Krieg et al.[58], the heart contains 'an androgen receptor and enzyme pattern which favors the accumulation of testosterone instead of 5α-dihydrotestosterone'. Evidence from autoradiographic studies with ^{3}H dihydrotestosterone is summarized in figure 3. Nuclear uptake and retention has been demonstrated in atrial and ventricular cardiomyocytes and in the wall of blood vessels in the baboon[65,67]. Neuronal nuclear labeling is present within areas of the central nervous system concerned with the regulation of cardiovascular function such as in the rat spinal cord in lamina X, and in the lower brain stem in the area postrema, dorsal motor nucleus of the vagus, nucleus ambiguus, and more rostrally, in the region of the catecholamine group A5, raphe nuclei, in the central gray of the midbrain, basal hypothalamus, periventricular nucleus, preoptic region, bed nucleus of the stria terminalis, dorsolateral septum, and amygdala[77,95].

^{3}H dihydrotestosterone nuclear binding has been co-localized with monoamine and peptide messengers and has been found to be different from that of estradiol. Dihydrotestosterone and catecholamine co-localization has been reported to exist in 50–80% of catecholamine neurons in the pons at the dorsolateral corner of the fourth ventricle (group A4), adjacent to the n. olivaris

superior (group A5), in the locus ceruleus (group A6), in the region of the lemniscus lateralis (group A7), and in the n. arcuatus and n. periventricularis hypothalami[45]. The distribution of DHT target neurons strongly suggests effects of this androgenic metabolite of testosterone on central regulatory neurons. Since aromatization of testosterone and related nuclear binding of testosterone-derived estradiol exist in various forebrain regions[82], estradiol-mediated testosterone effects must also be considered. Aromatization-related effects of testosterone in cardiovascular tissues, especially in aged males, remain a possibility, until conclusive data to exclude such effects become available. To date, no indications can be found in the literature for aromatization of testosterone in heart tissues. In the rat heart, no evidence for aromatization of testosterone has been obtained under conditions when regions in the brain showed clear nuclear labeling with testosterone-derived estrogen[82].

Adrenal steroids (fig. 4)

While the existence of different types and distribution of receptors for gluco- and mineralcorticosteroids and for corticosterone is still debated in the literature, there appears to be abundant evidence that adrenal steroids affect the cardiovascular system probably involving blood electrolytes and the renin-angiotensin system.

Autoradiographic studies with corticosterone, aldosterone, and dexamethasone[100, 105, 106] show a wide distribution of cells with nuclear binding of hormone. Nuclear binding of all three hormones has been observed in heart muscle[98] and exists to varying degrees in all cell types of the cardiovascular system. In the heart, after injection of ^{3}H dexamethasone, in addition to nuclear concentration and retention, elevated levels of extranuclear radioactivity have been noted in both atrium and ventricle, with higher levels in the atrium than in the ventricle[100]. This observation suggests the presence of cytoplasmic receptors and related nongenomic effects in addition to existence of nuclear receptors.

In surgically obtained human arteries, the presence of receptors for gluco- and mineralcorticosteroid has been demonstrated by sucrose density gradient analysis of homogenates[80].

Aldosterone nuclear binding exists in most or perhaps all tissues that contain glucocorticoid nuclear receptors. It has been found by autoradiography in heart muscle cells, walls of blood vessels, and in many regions of the brain[95]. Intracerebroventricular infusion of aldosterone selectively attenuates the pressor response produced by the injection into the lateral ventricle of arginine vasopressin, but not of angiotensin II, suggesting a central inhibitory effect of aldosterone[47].

There is a wide representation of target cells for adrenal steroids in the spinal cord and brain stem that differs in localization from those for sex steroids. The distribution of target neurons has been reported for corticosterone and aldosterone in brain and spinal cord[9, 27, 28, 30, 95]. Dexamethasone target sites correspond to some degree, but largely differ, from those for corticosterone[100]. Unlike corticosterone, dexamethasone enters the brain slowly via the ventricular system and dexamethasone target neurons are found in select regions of the hypothalamus. Identification of target sites for dexamethasone and cortisol is still under investigation.

In atrial myocytes, ^{3}H dexamethasone has been co-localized with antibodies to ANF[98], suggesting a direct effect on functions of these myoendocrine cells. The effect how-

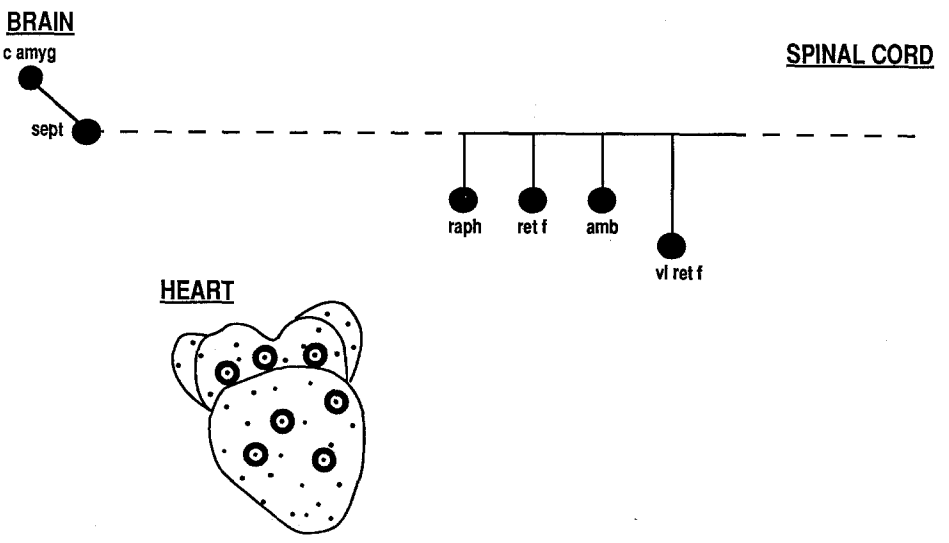

Figure 4. Corticosteroid sites of action in heart and central neuronal regions include certain groups of target neurons in the hindbrain and forebrain (abbreviations as in fig. 1), myocytes and fibroblasts in the atrium and ventricle of the heart and all cell types in large arteries, arterioles and capillaries. These sites include both gluco- and mineralcorticosteroids, which bind to similar tissues with varying intensities. The available information is incomplete.

ever appears to be less selective and more extensive than the effect of estradiol, since dexamethasone also shows distinct nuclear binding in ventricular myocytes.
Other experimental evidence is in agreement with autoradiographic findings indicating that gluco- and mineralcorticoids regulate the synthesis and release of atrial natriuretic factor in vivo. Adrenalectomy abolishes the acute natriuretic effect of ANF which was restored 50% by combined therapy with gluco- and mineralcorticoids[34]. These authors concluded that the presence of steroids is necessary for the NaCl-stimulated ANF release. It is of interest that certain nucleotide sequences in the ANF gene bind a glucocorticoid receptor complex[40].

Soltriol (vitamin D, 1,25-dihydroxycholecalciferol) (figs 5 and 6)

Through evidence provided by autoradiographic studies, new and unexpected targets for vitamin D, designated as the steroid hormone soltriol, have been discovered[96]. These targets include receptors in cardiomyocytes and in endothelial cells of certain blood vessels. Our demonstration of specific nuclear binding sites in heart muscle in 1981 have been followed in subsequent biochemical receptor assays with tissue homogenates[86,112,113]. In autoradiographic in vivo studies with ^{3}H soltriol in mice, a strong nuclear labeling was observed in atrial myoendocrine cells (fig. 6), with only weak or absent nuclear labeling in ventricular cardiomyocytes in the same animal. This pattern indicates a preferential distribution of soltriol receptors in ANF-producing cells, similar to that found with estradiol, and suggests involvement of soltriol, together with estradiol and adrenal steroids, in the regulation of ANF secretion.
Neurons in the central nervous system that contain nuclear receptors for soltriol are found in regions of the spinal cord and brain stem that are associated with cardiovascular regulation. These areas include in the spinal cord the intermediolateral nucleus and lamina X[99], in the lower brain stem the nucleus ambiguus and the ventrolateral reticular formation (area of group A1), in the pons the parabrachial nuclei and dorsal raphe nucleus, in the hypothalamus the infundibular nucleus, ventromedial nucleus, periventricular nucleus, parvocellular paraventricular nucleus, as well as the bed nucleus of the stria terminalis and the central nucleus of the amygdala[102].
Nuclear labeling with soltriol has also been observed in the posterior pituitary in pituicytes, thought to be involved in the regulation of vasopressin release. In addition, conspicuous nuclear labeling has been reported in kidney podocytes, and macula densa cells. Other portions of the nephron are labeled as well to varying degrees. Involvement of soltriol in the regulation of electrolyte homeostasis appears likely based on these demonstrations[110].
Nuclear receptors for soltriol have also been found in epinephrine and norepinephrine cells in the adrenal medulla[20], indicating soltriol effects in the manufacture and activity of enzymes that are involved in the production of norepinephrine and epinephrine, and perhaps other messengers produced in the adrenal medulla. Soltriol-related activation of the adrenal medulla is likely to be important for the seasonal adaptation and regulation of endocrine functions.
The autoradiographic data and associated new concepts about vitamin D action are supported by experimental and clinical reports in the literature. In rats maintained on a vitamin D-deficient diet for 9 weeks, increases in systolic blood pressure and serum creatine phosphokinase are observed, even when serum calcium is maintained at 9.0 mg/dl[114]. Effects of sunlight on the cardiovascular system, probably related to vitamin D actions,

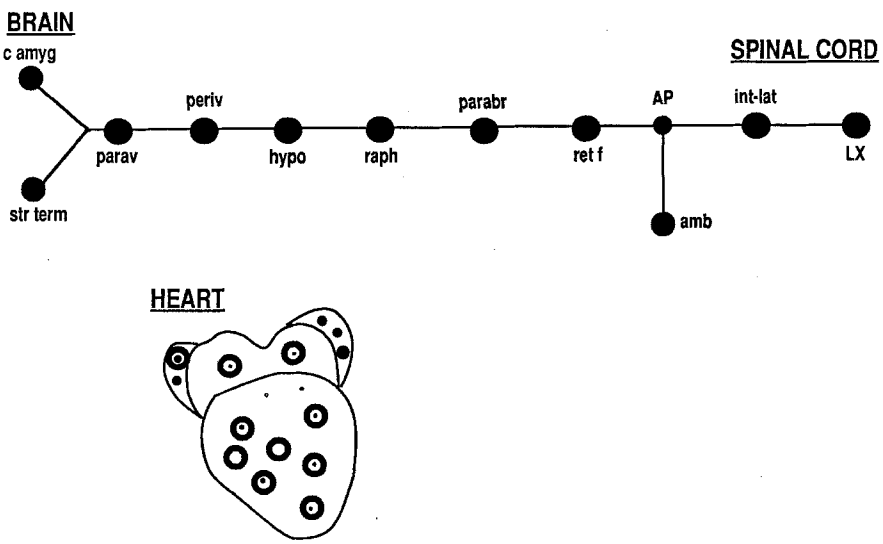

Figure 5. Soltriol (1,25 dihydroxycholecalciferol) sites of action in heart and central neuronal regions include target neurons in the spinal cord, hindbrain, and forebrain, in cardiomyocytes, and in endothelial cells of blood vessels. The available information is incomplete.

Figure 6. Autoradiogram of mouse atrium (A) with nuclear concentration of ^{3}H soltriol in myoendocrine cells, but weak or inconspicuous nuclear labeling in adjacent ventricle (V) myocytes. 4 µm, × 560, exposure time 180 days. (Stumpf, W. E., Bidmon, H. J., and Schleicher, G., unpublished).

have been seen in a number of clinical observations. In a study with 24 young, healthy males, after a series of irradiations with UV light, a beneficial influence on cardiovascular regulations was noted[13]. In the UVB (280–315 nm wavelength) treated males, bradycardia both at rest and during physical stress were observed. The respiratory minute volume decreased at the same time. These effects could be achieved only if the radiation spectrum had a sufficient amount of UVB. Similar effects of UV radiation have been noted earlier in children, resulting in improved cardiovascular response to physical challenge with increased vagal tone, resembling those after physical training[54]. Ultraviolet radiation led to a reduction of the systolic and diastolic blood pressure gradually developing after 24 hours and persisting for several days[75]. A similar lowering of blood pressure has been noted after carbon arc radiation, lasting 1–2 days. This response was accompanied by an increase in cardiac output averaging 21%[50].

Direct effects of soltriol on cardiomyocytes, perhaps in association with effects of sex and adrenal steroids, may underlie the observed seasonal changes of the endocrine heart in *Bufo arenarum*[5]. During hibernation, the number of cells engaged in ANF production diminishes significantly, and ultrastructurally, the number of atrial granules is significantly higher during summer compared to winter.

Large doses of vitamin D lead to the development of medial sclerosis in arteries. This effect was reported as early as 1928 by Kraitmair and Moll[57]. During intoxication, arterial blood pressure increases while extensive medial sclerosis with calcification does not occur until after withdrawal of the agent, when serum calcium levels are normal[4,37]. In addition to a medial calcific mass, histological changes have been reported in elastic fibers and in smooth muscle cells in the intima[29,43].

Overdosis of vitamin D given during early childhood may be a cause for a specific syndrome characterized by supravalvular aortic stenosis, mental retardation, and characteristic facies[83,111]. While the pathologic changes have been ascribed to sequelae of abnormal calcium levels[10], direct effects of 1,25 dihydroxycholecalciferol are likely to play a primary role and must be considered in the etiology of the described vascular changes. A direct effect has been proposed in the studies of essential hypertension, in which soltriol changes the blood pressure in a manner opposite to that seen in calcium loading, so that the effect of calcium might depend on suppression of endogenous 1,25 dihydroxycholecalciferol[74].

Vitamin D is generally believed to exert its main effects through the regulation of systemic and cellular calcium levels. This concept has been contested[96,97]. Vitamin D* has been recognized as having extensive actions as a somatotrophic activator and regulator for the seasonal adjustment of vital functions for the development, maintenance and procreation of life. Information from our studies has pointed to a direct and primary interaction of soltriol with many central and peripheral components of the cardiovascular system. These receptor-mediated actions appear not to be linked to primary changes in vitamin D-dependent calcium binding protein or other vitamin D-dependent calcium related mechanisms. The new observations and the development of new concepts thus open up new directions for cardiovascular research related to effects of soltriol and sunlight.

Conclusions

A review of nuclear binding of steroid hormones in cardiovascular and related neural tissues indicates an extensive presence of receptors for all of the steroid hormones. The evidence suggests regulatory genomic steroid effects at central and peripheral organizational levels that occur *simultaneously and differentially* at any given time. The differential intensity of nuclear steroid binding in the various target cell populations probably parallels grada-

* The traditional designation vitamin D for 1,25 dihydroxycholecalciferol is incorrect. $1,25(OH)_2$ Vitamin D_3 is not a vitamin, but a steroid hormone. Therefore, the term soltriol[96] is used in analogy to estradiol and cortisol and in consideration of being a hormonal messenger of sunlight.

tions of transcriptional responses. Such differential multiple activation of heterogeneous systems (MAHS) has been proposed to occur in the various estrogen target cell populations in the brain and elsewhere [107]. Differential target cell actions of a steroid hormone may be assessed by quantification of nuclear steroid molecules, in conjunction with measurements of the activity of related enzymes, the amount of related product, and the number of related messenger RNA molecules. Such comparative studies with individual cells or defined cell populations in vivo remain a technical challenge.

Within the heart, direct genomic effects appear to exist for estradiol, dihydrotestosterone (perhaps also for testosterone), gluco- and mineralcorticoids, and soltriol. Experiments with radiolabeled progestagen show a distinct cytoplasmic but no nuclear accumulation of the radiolabeled hormone or metabolite in cardiomyocytes, while myometrial cells in the same animal display a strong nuclear concentration. This result argues for a predominant nongenomic progestin action in the heart. However, nuclear progestagen receptors may be expressed under certain conditions and their presence in the heart cannot be excluded. It is noteworthy that radiolabeled digitonin, a steroid with estrogenic properties, concentrates in the cytoplasm of cardiomyocytes, similar to progestin, in our autoradiographic studies with rats [98]. Cytoplasmic presence of radiolabeled hormone or metabolite has been noted, in addition to nuclear accumulation, in experiments with adrenal steroids, dihydrotestosterone, and soltriol. These demonstrations suggest for these steroid hormones varying degrees of nongenomic actions, in addition to the well-known nuclear effects.

In the wall of large arteries, nuclear labeling has been reported for estradiol, dihydrotestosterone, and corticosteroids. Soltriol may also have direct or indirect effects, since sclerotic changes in the media have been observed after intoxication with vitamin D. Capillary pericytes are targets for estradiol and corticosteroids. Information for other steroids is missing. Endothelial cells show distinct nuclear labeling in certain capillary beds in autoradiograms with tritiated soltriol and corticosteroids, but not with estradiol for which nuclear receptor levels may be low or absent under the conditions of the experiments.

In the nervous system, many sites are recognized as being involved in cardiovascular regulation. Among the most frequently and intensely steroid hormone-labeled regulatory structures are in the medulla the nucleus ambiguus, the nucleus of the solitary tract, the motor nucleus of the vagus, the ventrolateral reticular formation, the parabrachial nuclei, and at the most rostral end of the brain stem the region of the preoptic recess organ. Both, the area postrema and the region of the optic recess have received special attention in recent years as regulatory sites for blood pressure and heart rate [14, 25, 63, 87]. In certain neurons of these regions, estrogen, progestagen, dexamethasone, soltriol, and androgen binding sites have been noted in our autoradiographic studies. Therefore, production of peptides with cardiovascular effects in the anterior hypothalamus and in the caudal medulla may be directly affected by these steroid hormones.

In the concept of the hierarchical nature of cardiovascular control [71], individual levels of regulation are recognized, such as, the sympathetic final common pathway from the intermediolateral column in the spinal cord, the parasympathetic final common pathway from the nucleus ambiguus and the dorsal motor nucleus of the vagus in the caudal medulla oblongata, the more extensive vasomotor area in the medulla oblongata, the pontine-mesencephalic level, the central hypothalamic level, the anterior hypothalamic-septal region of the optic recess of the third ventricle with the organum vasculosum of the lamina terminalis, the limbic cortex, and the neocortex. It is of interest that the sites identified as being involved in central cardiovascular regulation are all components of the brain stem A-B-C (allocortex-brainstem-core) circuitry of autonomic-endocrine regulation and integration [101] which contains peptidergic and aminergic neurons acted upon by estradiol and known to be involved as well in the control of respiration, gastro-intestinal function, temperature, and secretion by endocrine glands. The regulation of cardiovascular function is linked to all of these autonomic-endocrine nervous components that integrate diverse information, including neural input from sensory perception. The latter involves most likely steroid target cells in the substantia gelatinosa of the spinal cord and trigeminus, and other dorsal regions of the brain stem.

In the anesthesized dog, unilateral stimulation of vagal afferents produces an increase in metabolic activity in the nucleus of the solitary tract, area postrema, parabrachial nuclei, inferior olivary nuclei, external cuneate nuclei, and other areas [56]. High resolution studies with ^{14}C 2-deoxyglucose, using the dry-mount autoradiographic technique, reveal a population of high-activity neurons in the medulla of resting unanesthesized rats, which probably represents cardiovascular pacemaker neurons [26].

Although information on steroid hormone sites of action in the cardiovascular system is incomplete, it is apparent from the reviewed data that all steroid hormones exert direct effects through nuclear receptors on multiple specific components of the cardiovascular system in a pattern characteristic for each hormone. A number of target regions stand out that appear to be acted upon simultaneously by several steroids. These areas include in the brain the nucleus ambiguus, the area postrema, the parabrachial nuclei, the ventrolateral reticular formation, and the periventricular hypothalamic nucleus. Muscular and connective tissue components of the heart and the wall of blood vessels contain nuclear receptors for estradiol, dihydrotestosterone, and adrenal steroids, as well as for soltriol.

The observations and related concepts presented here have consequences for our understanding of physiology

and pathology. Steroid hormones appear to play a dominant role in cardiovascular functions by genomically inducing changes in all structural components, allowing the organism to cope with specific conditions of reproduction (gonadal steroids) and stress (adrenal corticosteroids), both of which are modulated by seasonal changes (soltriol). Thus, by the steroidal endocrine status, cardiovascular functions can be regulated to optimize performance. The steroidal endocrine status determines the level and capacity of situational responses by modifying the secretion of peptide-monoamine messengers and altering the readiness (tonus) and sensitivity of effector tissues through changes in steroid hormone regulated trophic states and receptor levels. All of the steroid hormones appear to act simultaneously but in different and varying capacities with resultant cooperative, alternative, or antagonistic action. The understanding of this interaction remains a challenge.

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1 Akmayev, I. G., Vikhreva, O. V., and Konovalova, L. K., The origin of the hypothalamic-vagal descending pathway: An experimental ultrastructural study. Brain Res. *230* (1981) 342–345.
2 Altura, B. M., Sex and estrogens and responsiveness of terminal arterioles to neurohypophyseal hormones and catecholamines. J. Pharm. exp. Ther. *193* (1975) 403–412.
3 Amendt, K., Czachurski, J., Dembowsky, K., and Seller, H., Bulbospinal projections to the intermediolateral cell column; a neuroanatomical study. J. aut. nerv. Syst. *1* (1979) 103–117.
4 Angell-James, J. E., Pathophysiology of aortic baroreceptors in rabbits with vitamin D sclerosis and hypertension. Circ. Res. *34* (1974) 327–338.
5 Aoki, A., Maldonado, C. A., and Forssmann, W. G., Seasonal changes of the endocrine heart, in: Functional Morphology of the Endocrine Heart, pp. 61–68. Eds W. G. Forssmann, D. W. Scheuermann and J. Alt. Steinkopf Verlag Darmstadt/Springer Verlag, Berlin 1989.
6 Back, H., Forssmann, W. G., and Stumpf, W. E., Atrial myoendocrine cells (cardiodilatin/atrial natriuretic polypeptide-containing myocardiocytes) are target cells for estradiol. Cell Tiss. Res. *255* (1989) 673–674.
7 Bard, P., Anatomical organization of the central nervous system in relation to control of the heart and blood vessels. Phys. Rev., Suppl. *4* (1960) 3–26.
8 Bidmon, H. J., Sar, M., Gutkowska, J., and Stumpf, W. E.,Co-localization of ^{3}H estradiol and atrial natriuretic peptide in rat hypothalamus by combined autoradiography immunohistochemistry. Submitted (1989).
9 Birmingham, M. K., Sar, M., and Stumpf, W. E., Localization of aldosterone and corticosterone in the central nervous system assessed by quantative autoradiography. Neurochem. Res. *9* (1984) 333–335.
10 Bonucci, E., and Sadun, R., Experimental calcification of the myocardium. Am. J. Path. *71* (1973) 167–184.
11 Boullion, R., Van Baelen, H., and De Moor, P., Physiology and pathophysiology of vitamin D-binding protein, in: Binding Proteins of Steroid Hormones. Colloque INSERM, John Libbey Eurotext Ltd, vol. 149 (1986) 333–356.
12 Brody, M. J., Fink, G. D., Buggy, J., Haywood, J. R., Gordon, F. J., and Johnson, A. K., The role of the anteroventral third ventricle (AV3V) region in experimental hypertension. Circ. Res. *43*, Suppl. 1 (1984) I2–I13.
13 Bühring, M., Rödiger, E., Reuter, H., and Bechhofer, G., Cardiovascular advantages of an insolation with UVB? in: Human Exposure to Ultraviolet Radiation: Risks and Regulations, pp. 113–116. Eds W. F. Passchier and B. F. M. Bosnajakovic. Elsevier Science Publishers B.V., 1984.
14 Buggy, J., and Fisher, A. E., Anteroventral third ventricle site of action for angiotensin induced thirst. Pharmac. biochem. Behav. *4* (1976) 651–660.
15 Buggy, J., Huot, S., Pamnani, M., and Haddy, F., Periventricular forebrain mechanisms for blood pressure regulation. Fedn Proc. *43* (1984) 25–31.
16 Calaresu, F. R., Faiers, A. A., and Mogenson, G. J., Central neural regulation of heart and blood vessels in mammals. Progr. Neurobiol. *5* (1975) 1–35.
17 Carrière, P. D., De Léan, A., Gutkowska, J., Genest, J., and Cantin, M., Chronic estradiol treatment decreases angiotensin II receptor density in the anterior pituitary gland and adrenal cortex but not in the mesenteric artery. Neuroendocrinology *43* (1986) 49–56.
18 Cechetto, D. F., Central representation of visceral function. Fedn Proc. *46* (1987) 17–23.
19 Chute, C. G., Baron, J. A., Plymate, S. R., Kiel, D. P., Pavia, A. T., Lozner, E. C., O'Keefe, T., and MacDonald, G. J., Sex hormones and coronary artery disease. Am. J. Med. *83* (1987) 853–859.
20 Clark, S. A., Stumpf, W. E., Bishop, C. W., DeLuca, H. F., Park, D. H., and Joh, T. H., The adrenal: a new target organ of the calciotropic hormone 1,25 dihydroxyvitamin D_3. Cell Tiss. Res. *243* (1986) 299–302.
21 Crofton, J. T., Baer, P. G., Share, L., and Brooks, D. P., Vasopressin release in male and female rats: effects of gonadectomy and treatment with gonadal steroid hormones. Endocrinology *117* (1985) 1195–1200.
22 Crofton, J. T., Share, L., Shade, R. E., Lee-Kwon, W. J., Manning, M., and Sawyer, W. H., The importance of vasopressin in the development and maintenance of DOC-salt hypertension in the rat. Hypertension *1* (1979) 31–38.
23 De Bold, A. J., Borenstein, H. B., Veress, A. T., and Sonnenberg, H., A rapid and potent natriuretic response to intravenous injection of atrial myocardial extracts in rats. Life Sci. *28* (1981) 89–94.
24 Dittmar, C., Ein neuer Beweis für die Reizbarkeit der centripetalen Fasern des Rückenmarks. Ber. Verh. Sächs. Ges. Wiss. Leipzig, math.-phys. Kl. 22 (1870) 18–48.
25 Diz, D. I., and Jacobowitz, D. M., Cardiovascular actions of four neuropeptides in the rat hypothalamus. Clin. Exper. Theory Practice *A6* (10&11) (1984) 2085–2090.
26 Duncan, G. E., Kaldas, R., Mitra, K. E., Breese, G., and Stumpf, W. E., High-activity neurons in the reticular formation of the medulla oblongata: a high-resolution 2-deoxyglucose study. Neuroscience (1990) in press.
27 Duncan, G. E., and Stumpf, W. E., Target neurons for [^{3}H]-corticosterone in the rat spinal cord. Brain Res. *307* (1984) 321–326.
28 Duncan, G. E., and Stumpf, W. E., A combined autoradiographic and immunocytochemical study of ^{3}H corticosterone target neurons and catecholamine neurons in rat and mouse brain stem. Neuroendocrinology *40* (1985) 262–271.
29 Eisenstein, R., and Zeruolis, L., Vitamin D-induced aortic calcification. Archs Path. *77* (1964) 27–35.
30 Ermisch, A., and Rühle, H. J., Autoradiographic demonstration of aldosterone concentrating neuron populations in rat brain. Brain Res. *147* (1978) 154–158.
31 Fahraeus, L., The effects of estradiol on blood lipids and lipoproteins in postmenopausal women. Obstet. Gynec. *72* Suppl. 5 (1988) 185–225.
32 Fischer, G. M., Bashey, R. I., Rosenbaum, H., and Lyttle, C. R., A possible mechanism in arterial wall for mediation of sex difference in atherosclerosis. Exp. molec. Path. *43* (1985) 288–296.
33 Fischer, G. M., and Swain, M. L., Effects of estradiol and progesterone on the increased synthesis of collagen in atherosclerotic rabbit aortas. Atherosclerosis *54* (1985) 177–185.
34 Garcia, R., Debinski, W., Gutkowska, J., Kuchel, O., Thibault, G., Genest, J., and Cantin, M., Gluco- and mineralcorticoids may regulate the natriuretic effect and the synthesis and release of atrial natriuretic factor by the rat atria in vivo. Biochem. biophys. Res. Commun. *131* (1985) 806–814.
35 Gardiner, S. M., and Bennett, T., Brain neuropeptides: actions on central cardiovascular control mechanisms. Brain Res. Rev. *14* (1989) 79–116.
36 Gebber, G. L., Brainstem systems involved in cardiovascular regulation, in: Nervous Control of Cardiovascular Function, pp. 346–368. Ed. W. C. Randall. Oxford University Press, New York 1984.

37 Gillman, T., Grant, R. A., and Hathorn, M., Histochemical and chemical studies of calciferol-induced vascular injuries. Br. J. exp. Path. *41* (1960) 11–18.

38 Goldberg, R. B., Rabin, D., Alexander, A. N., Doelle, G. C., and Gertz, G. S., Suppression of plasma testosterone leads to an increase in serum total and high density lipoprotein cholesterol and apoproteins A-I and B. J. clin. Endocr. Metab. *60* (1985) 203–211.

39 Grant, L. D., and Stumpf, W. E., Hormone uptake sites in relation to CNS biogenic amine sites, in: Anatomical Neuroendocrinology, pp. 445–464. Eds W. E. Stumpf and L. D. Grant. Karger, Basel 1975.

40 Greenberg, B. D., Bencen, G. H., Seilhamer, J. J., Lewicki, J. A., and Fiddes, J. C., Nucleotide sequence of the gene encoding human atrial natriuretic factor precursor. Nature *312* (1984) 656–658.

41 Hämäläinen, E., Adlercreutz, H., Ehnholm, C., and Puska, P., Relationship of lipoproteins and apolipoproteins to sex hormones and binding capacity of sex hormone binding globulin (SHBG) in healthy Finnish men. Metabolism *36* (1986) 335–346.

42 Hämäläinen, E., Tikkanen, H., Härkönen, M., Näveri, H., and Adlercreutz, H., Serum lipoproteins, sex hormones and sex hormone binding globulin in middle-aged men of different physical fitness and risk of coronary heart disease. Atherosclerosis *67* (1987) 155–162.

43 Hass, G. M., Trueheart, R. E., Taylor, C. B., and Stumpe, M., An experimental histologic study of hypervitaminosis D. Am. J. Path. *34* (1958) 395–431.

44 Henry, J. L., and Calaresu, F. R., Topography and numerical distribution of neurons of the thoraco-lumbar intermediolateral nucleus in the cat. J. comp. Neurol. *144* (1972) 205–213.

45 Heritage, A. S., Stumpf, W. E., Sar, M., and Grant, L. D., Brainstem catecholamine neurons are target sites for sex steroid hormones. Science *207* (1980) 1377–1379.

46 Hornby, P. J., and Piekut, D. T., Anatomical evidence for interaction of $ACTH^{1-39}$ immunostained fibers and hypothalamic paraventricular neurons that project to the dorsal vagal complex. Histochemistry *90* (1988) 201–206.

47 Janiak, P., and Brody, M. J., Central interactions between aldosterone and vasopressin on cardiovascular system. Am. J. Phys. *255* (1988) (1Pt 2):R166–173.

48 Jennes, L., Stumpf, W. E., and Kalivas, P. W., Neurotensin: topographical distribution in rat brain by immunohistochemistry. J. comp. Neurol. *210* (1982) 211–224.

49 Jirikowski, G. F., Stumpf, W. E., and Pilgrim, C., Hypothalamic estradiol target neurons immunoreactive for neuropeptides. Verh. anat. Gesellsch. *35* (1987) 87–92.

50 Johnson, J. R., Pollock, B. E., Mayerson, H. S., and Laurens, H., The effect of carbon arc radiation on blood pressure and cardiac output. Am. J. Physiol. *114* (1987) 594–602.

51 Keefer, D. A., and Stumpf, W. E., Estrogen localization in primate brain, in: Anatomical Neuroendocrinology, pp. 153–165. Eds W. E. Stumpf and L. D. Grant. Karger, Basel 1975.

52 Keefer, D. A., Stumpf, W. E., and Sar, M., Topographical localization of estrogen-concentrating cells in the rat spinal cord following ^{3}H-estradiol administration. Proc. Soc. exp. Biol. Med. *143* (1973) 414–417.

53 Khaw, K.-T., and Barrett-Connor, E., Blood pressure and endogenous testosterone in men: an inverse relationship. J. Hypertension *6* (1988) 329–332.

54 Klein, E., and Weis, V., Statistische Untersuchungen über die Steigerung der Leistungsfähigkeit durch Ultravioletbestrahlung. Arbeitsphysiologie *15* (1953) 85–92.

55 Koenig, H., Goldstone, A., and Lu, C. Y., Testosterone-mediated sexual dimorphism of the rodent heart. Circ. Res. *50* (1982) 782–787.

56 Kostreva, D. R., Functional brain mapping of cardiovascular reflexes using [^{14}C] deoxyglucose in the dog, in: Cerebral Blood Flow: Effects of Nerves and Neurotransmitters, pp. 75–86. Eds D. D. Heistad and M. L. Marcus. Elsevier North Holland, Amsterdam 1982.

57 Kreitmair, H., and Moll, T. H., Hypervitaminose durch grosse Dosen Vitamin D. Münch. med. Wschr. *75* (1928) 637–639.

58 Krieg, M., Smith, K., and Bartsch, W., Demonstration of a specific androgen receptor in rat heart muscle: relationship between binding, metabolism, and tissue levels of androgens. Endocrinology *103* (1978) 1686–1694.

59 Lengsfeld, M., Morano, I., Ganten, U., Ganten, D., and Rüegg, C., Gonadectomy and hormonal replacement changes systolic blood pressure and ventricular myosin isoenzyme pattern of spontaneously hypertensive rats. Circ. Res. *63* (1988) 1090–1094.

60 Lloyd, S., The vascular responses of the rat during the reproductive cycle. J. Physiol. *148* (1959) 625–632.

61 Lloyd, S., Changes in the vascular responses of the rat during pregnancy. J. Physiol. *149* (1959) 586–592.

62 Loewy, A. D., and McKellar, S., The neuroanatomical basis of central cardiovascular control. Fedn Proc. *39* (1980) 2495–2503.

63 Mangiapane, M. L., Skoog, K. M., Rittenhouse, P., Blair, M. L., and Sladek, C. D., Lesion of the area postrema region attenuates hypertension in spontaneously hypertensive rats. Circ. Res. *64* (1989) 129–135.

64 Manning, J. W., Intracranial representation of cardiac innervation, in: Nervous Control of the Heart, pp. 16–33. Ed. W. C. Randall. Williams and Wilkins Co., Baltimore 1965.

65 McGill, H. C. Jr, and Sheridan, P. J., Nuclear uptake of sex steroid hormones in the cardiovascular system of the baboon. Circ. Res. *48* (1981) 238–244.

66 McGill, H. C. Jr, and Stern, M. P., Sex and atherosclerosis, in: Atherosclerosis Reviews, vol. 4, pp. 157–235. Eds R. Paoletti and A. M. Gotta, Jr. Raven Press, New York 1979.

67 McGill, H. C. Jr, Anselmo, V. C., Buchanan, J. M., and Sheridan, P. J., The heart is a target organ for androgen. Science *207* (1980) 775–777.

68 Moorjani, S., Dupont, A., Labrie, F., Lupien, P. J., Gagne, C., Brun, D., Giguere, M., Belanger, A., and Cusan, L., Changes in plasma lipoproteins during various androgen suppression therapies in men with prostatic carcinoma: effects of orchiectomy, estrogen, and combination treatment with luteinizing hormone-releasing hormone agonist and flutamide. J. clin. Endocr. Metab. *66* (1988) 314–322.

69 Nakayama, M., Inao, H., Yagi, K., and Maeyama, M., The effects of sex steroids on blood pressure in SHR and normotensive Wistar rats. Jap. Heart J. *19* (1978) 563–564.

70 Petras, J. M., and Cummings, J. F., Autonomic neurons in the spinal cord of the Rhesus monkey; a correlation of the findings of cytoarchitectonics and sympathectomy with fiber degeneration following dorsal rhizotomy. J. comp. Neurol. *146* (1972) 189–218.

71 Preiss, C. N., Concepts of cardiovascular regulation: past, present and future, in: Nervous Control of the Heart, pp. 154–197. Ed. W. C. Randall. Williams and Wilkins Co., Baltimore 1965.

72 Randall, W. C., Nervous Control of Cardiovascular Function. Oxford Univ. Press, New York 1984.

73 Reid, J. L., and Rubin, P. C., Peptides and central neural regulation of the circulation. Physiol. Rev. *67* (1987) 725–749.

74 Resnick, L. M., Nicholson, J. P., and Laragh, J. H., 1,25 Dihydroxyvitamin D in essential hypertension, in: Vitamin D. A Chemical, Biochemical and Clinical Update, pp. 1115–1116. Walter de Gruyter & Co., Berlin 1985.

75 Rothman, S., Physiology and Biochemistry of Skin. University of Chicago Press, Chicago 1954.

76 Sar, M., and Stumpf, W. E., Neurons in the hypothalamus concentrate ^{3}H-progesterone or metabolites of it. Science *182* (1973) 1266–1268.

77 Sar, M., and Stumpf, W. E., Distribution of androgen-concentrating neurons in rat brain, in: Anatomical Neuroendocrinology, pp. 120–133. Eds W. E. Stumpf and L. D. Grant. Karger, Basel 1975.

78 Sar, M., and Stumpf, W. E., Estradiol concentration in dopamin-ß-hydroxylase containing neurons in lower brain stem demonstrated by combined autoradiography and immunohistochemistry. Exp. Brain Res., Suppl. 3 (1981) 18–28.

79 Sar, M., and Stumpf, W. E., Simultaneous localization of steroid hormones and neuropeptides in the brain by combined autoradiography and immunohistochemistry. Meth. Enzymol. *103* (part H) (1983) 631–638.

80 Sasano, H., Watanabe, N., and Sasano, N., Corticosteroid binding in human artery. Tohoku J. exp. Med. *150* (1986) 117–125.

81 Schaible, T. F., Malhotra, A., Ciambrone, G., and Scheuer, J., The effects of gonadectomy on left ventricular function and cardiac contractile proteins in male and female rats. Circ. Res. *54* (1984) 38–49.

82 Schleicher, G., Stumpf, W. E., Gurley, J. M., and Drews, U., Differential nuclear binding of ^{3}H testosterone and its metabolites to androgen and estrogen receptors in brain, pituitary, heart, kidney and accessory sex glands of the mouse. J. Steroid Biochem. *33* (1989) 581–587.

83 Seelig, M. S., Vitamin D and cardiovascular, renal, and brain damage in infancy and childhood. Ann. N. Y. Acad. Sci. *147* (1969) 539–582.

84 Share, L., Crofton, J. T., and Ouchi, Y., Vasopressin: Sexual dimorphism in secretion, cardiovascular actions and hypertension. Am. J. med. Sci. *31* (1988) 314–319.

85 Sills, M. A., Nguyen, K. Q., and Jacobowitz, D. M., Increases in heart rate and blood pressure produced by microinjections of atrial natriuretic factor into the AV3V region of rat brain. Peptides *6* (1985) 1037–1042.
86 Simpson, R. U., Evidence for a specific 1,25-dihydroxyvitamin D_3 receptor in rat heart. Circulation *68* (1983) 239.
87 Skoog, K. M., and Mangiapane, M. L., Area postrema and cardiovascular regulation in rats. Am. J. Physiol. *254* (1988) H963–H969.
88 Smith, O. A., Anatomy of central neural pathways mediating cardiovascular functions, in: Nervous Control of the Heart, pp. 34–53. Ed. W. C. Randall. Williams and Wilkins Co., Baltimore 1965.
89 St-Louis, J., Parent, A., Lariviére, R., and Schiffrin, E. L., Vasopressin responses and receptors in the mesenteric vasculature of estrogen-treated rats. Am. J. Physiol. *251* (1986) H885–H889.
90 Stumpf, W. E., Estradiol concentrating neurons: Topography in the hypothalamus by dry-mount autoradiography. Science *162* (1968) 1001–1003.
91 Stumpf, W. E., Cellular and subcellular ^{3}H-estradiol localization in the pituitary by autoradiography. Zeitschr. Zellforsch. *92* (1968) 23–33.
92 Stumpf, W. E., Nuclear concentration of ^{3}H-estradiol in target tissues. Dry-mount autoradiography of vagina, oviduct, ovary, testis, mammary tumor, liver and adrenal. Endocrinology *85* (1969) 31–37.
93 Stumpf, W. E., Estrogen-neurons and estrogen-neuron systems in the periventricular brain. Am. J. Anat. *129* (1970) 207–218.
94 Stumpf, W. E., The brain: An endocrine gland and hormone target, an introduction, in: Anatomical Neuroendocrinology, pp. 2–8. Eds W. E. Stumpf and L. D. Grant. Karger, Basel 1975.
95 Stumpf, W. E., Binding of estrogens, progestagens, androgens and corticosteroids in the central nervous system, in: Interaction Between the Nervous and the Endocrine Systems, pp. 103–134. Eds A. A. J. Verhofstad and J. A. M. van Kemenade. Univ. Nijmegen, Nijmegen, Holland, 1979.
96 Stumpf, W. E., Vitamin D-soltriol, The heliogenic steroid hormone: Somatotrophic activator and modulator. Discoveries from histochemical studies lead to new concepts. Histochemistry *89* (1988) 209–219.
97 Stumpf, W. E., The endocrinology of sunlight and darkness: complementary roles of vitamin D and pineal hormones. Naturwissenschaften *75* (1988) 247–251.
98 Stumpf, W. E., Back, H., and Forssmann, W. G., Steroid hormones and the endocrine heart, in: Functional Morphology of the Endocrine Heart, pp. 95–102. Eds W. G. Forssmann, D. W. Scheuermann and J. Alt. Steinkopff Verlag, Darmstadt, and Springer Verlag, New York 1988.
99 Stumpf, W. E., Clark, S. A., O'Brien, L. P., and Reid, F. A., $1,25(OH)_2$ Vitamin D_3 sites of action in spinal cord and sensory ganglion. Anat. Embryol. *177* (1988) 307–310.
100 Stumpf, W. E., Heiss, C., Sar, M., Duncan, G. E., and Craver, C., Dexamethasone and corticosterone receptor sites: Differential topographic distribution in rat hippocampus revealed by high resolution autoradiography. Histochemistry *92* (1989) 201–210.
101 Stumpf, W. E., and Jennes, L., The A-B-C (Allocortex-Brainstem-Core) circuitry of endocrine-autonomic integration and regulation. Peptides *5*, Suppl. 1 (1984) 221–226.
102 Stumpf, W. E., and O'Brien, L. P., $1,25(OH)_2$ vitamin D_3 sites of action in the brain: an autoradiographic study. Histochemistry *87* (1987) 393–406.
103 Stumpf, W. E., and Roth, L. J., High resolution autoradiography with dry-mounted, freeze-dried, frozen sections: Comparative study of six methods using two diffusible compounds, ^{3}H-estradiol and ^{3}H-mesobilirubinogen. J. Histochem. Cytochem. *14* (1965) 274–287.
104 Stumpf, W. E., and Sar, M., Hormone architecture of the mouse brain with ^{3}H estradiol, in: Anatomical Neuroendocrinology, pp. 254–261. Eds W. E. Stumpf and L. D. Grant. Karger, Basel 1975.
105 Stumpf, W. E., and Sar, M., Autoradiographic localization of estrogen, androgen, progestin and glucocorticosteroid in "target tissues" and "non-target tissues", in: Receptors and Mechanism of Action of Steroid Hormones, pp. 41–84. Ed. J. Pasqualini. Marcel Dekker, New York 1976.
106 Stumpf, W. E., and Sar, M., Glucocorticosteroid and mineralcorticosteroid hormone target sites in the brain: Autoradiographic studies with corticosterone, aldosterone and dexamethasone, in: Interactions within the Brain-Pituitary Adrenocortical System, pp. 137–147. Eds M. T. Jones, M. F. Dallman, B. Gillham and S. Chattopadhyay. Academic Press, New York 1979.
107 Stumpf, W. E., and Sar, M., Anatomical relationships between estrogen target sites and peptidergic-aminergic neurons: Multiple activation of heterogeneous systems (MAHS). Exp. Brain Res., Suppl. 3 (1981) 18–28.
108 Stumpf, W. E., Sar, M., and Aumüller, G., The heart: A target organ for estradiol. Science *196* (1977) 319–321.
109 Stumpf, W. E., Sar, M., and Keefer, D. A., Atlas of estrogen target cells in rat brain, in: Anatomical Neuroendocrinology, pp. 104–119. Eds W. E. Stumpf and L. D. Grant. Karger, Basel 1975.
110 Stumpf, W. E., Sar, M., Narbaitz, R., Reid, F. A., DeLuca, H. F., and Tanaka, Y., Cellular and subcellular localization of $1,25(OH)_2$ vitamin D_3 in rat kidney: comparison with localization of parathyroid hormone and estradiol. Proc. natl. Acad. Sci. USA *77* (1980) 1149–1153.
111 Taussig, H. B., Possible injury to the cardiovascular system from vitamin D. Ann. int. Med. *65* (1966) 1195–1200.
112 Walters, M. R., Cuneo, D. L., and Jamison, A. P., Possible significance of new target tissues for 1,25-dihydroxyvitamin D_3. J. Steroid Biochem. *19* (1983) 913–920.
113 Walters, M. R., Wicker, D. C., and Riggle, P. C., 1,25-Dihydroxyvitamin D_3 receptors identified in the rat heart. J. molec. cell. Cardiol. *18* (1983) 67–72.
114 Weishaar, R. E., and Simpson, R. U., Vitamin D_3 and cardiovascular function in rats. J. clin. Invest. *79* (1987) 1706–1712.
115 Wharton, J., and Gulbenkian, S., Peptides in the mammalian cardiovascular system. Experientia *43* (1987) 821–832.

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