is that sphingomyelinase synthesis is impaired in animals receiving AY-9944. An experimental animal analog of Niemann-Pick disease produced in this fashion has distinct advantages over attempts to mimic lipid storage diseases by the use of compounds that inhibit the activity of sphingolipid hydrolases (*9*).

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The Heart: A Target Organ for Estradiol

Abstract. *Autoradiographic studies of rat heart reveal that tritiated estradiol concentrates in cell nuclei of the myocardium of the atria and auricles, similar to the myometrium of the uterus. This suggests that estrogen has a direct effect on atrial myocytes through which its "protective" action may be mediated. Cardiac glycosides that are known to exert estrogen-like effects on classical estrogen target tissues, such as uterine muscle, endometrium, vagina, and mammary gland, probably act on atrial muscle through a genomic, steroid hormone-like mechanism of action.*

Cardiovascular diseases are more frequent in men than in women. This has been ascribed to stress in males as well as protective effects of sex hormones in females. Clinical and experimental evidence suggest that estrogen mitigates or delays the occurrence of hypertension, coronary artery disease, paroxymal tachycardia, myocardial ischemia, and certain pathologic changes in the electrocardiogram (*1*). Whether in some of these conditions estrogen plays a role by a primary—that is, direct—action on heart muscle is not known. The sites of action of estrogen on the cardiovascular system have not been clearly identified, and the mechanisms of action are little understood. Autoradiographic studies with [^{3}H]estradiol, by means of special techniques developed in our laboratory (*2*), demonstrated target sites for estrogen not only in such target tissues as uterine muscle but also in a number of presumed nontarget tissues, as well as in the walls of blood vessels and in heart muscle (*3*). In the past, biochemists have used "heart muscle" as a control nontarget tissue in their investigations of estradiol receptors (*4*). In our study, serial section autoradiograms of rat heart were prepared 1 hour after the intravenous injection of [^{3}H]estradiol in order to identify the types of target cells for the hormone and to determine their topographic distribution.

Two 26-day-old intact and six 26-day-old female Holtzman rats, ovariectomized for 9 days, were each injected intravenously with 0.5 μg per 100 g of body weight of 17β-[2,4,6,7-^{3}H]estradiol (specific activity 91 c/mmole), dissolved in 10 percent ethanol in isotonic saline. The specificity of estradiol localization was tested by injecting 50 μg of unlabeled 17β-estradiol into two immature ovariectomized rats 5 minutes prior to the injection of 17β-[^{3}H]estradiol. One additional male rat was injected with [^{3}H]estradiol in order to ascertain whether estradiol target cells exist in the male as well as in the female. One hour after injection of the 17β-[^{3}H]estradiol the rats were killed. The heart was removed, mounted on tissue holder, and frozen in −180°C liquefied propane. Serial frozen sections (4 μm) were cut (wide range cryostat, Harris Manufacturing Co., Inc., North Billerica, Mass.) and thaw-mounted or dry-mounted on photographic emulsion (Kodak NTB-3) coated slides. After autoradiographic exposure for several months, the slides were photographically processed and stained with methyl green pyronin as described (*2*).

After the injection of [^{3}H]estradiol, muscle cells of the left and right auricles and atria (Fig. 1, a and b) in both the female and male rat show concentration and retention of radioactivity in nuclei (Fig. 2 a, b, and d). The muscle cells of the ventricles (Fig. 2c) do not show such nuclear concentration, even after autoradiographic exposure times of more than 1 year. The concentration of radioactivity in nuclei of atrial myocytes can be prevented if unlabeled 17β-estradiol is injected prior to 17β-[^{3}H]estradiol (Fig. 2e). Muscle cells of the auricles concentrate more radioactivity than those of the atria. In the myoctes of the atria, including the auricles, nuclear labeling is not uniform. Variations exist not only among cells, but also within the same cell: that is, in some myocytes, labeled and unlabeled nuclei are seen side by side.

In our preparations, the sino-atrial node and the atrial conducting system could not be identified. Since, throughout the atrium, labeled muscle cells are found, it can be assumed that the cells of the sino-atrial node and the atrial conducting system do not behave differently regarding [^{3}H]estradiol uptake, compared to the other cells. In the ventricles, the easily recognizable Hiss bundle and Purkinje fibers, similar to other ventricular cells, do not concentrate radioactivity. Cells in the tunica media of the arteria pulmonalis and the aorta show weak nuclear labeling as do some of the connective tissue cells at the base of the tricuspid and mitral valves. Radioactively labeled fibroblasts are also found in the extramyocardial area at the base of the heart in the vicinity of the unlabeled autonomic ganglion cells. The separation between labeled atrial and unlabeled ventricular myocytes is abrupt in the area of the auricles, but somewhat transitional in the centrally located atrioventricular contact zone.

The autoradiographic studies reveal that the myocardium cranial to the atrioventricular skeleton contains target cells for estradiol. This atrio-auricular part of the heart is derived embryologically from the primitive paired atrium and sinus venosus, which are located extrapericardially at the eight-somite stage

(5). Only later in development do the primitive atria and sinus venosi fuse and assume an intrapericardial position. These special extrapericardial embryological conditions may provide clues for an explanation of why the atria and not the ventricles have developed an apparent selective sensitivity to estrogen—and, perhaps other steroid hormones. The presence of estrogen uptake and concentration suggests that estrogen has a specific effect on atrial muscle. Differences between atrial and ventricular muscle cells are apparent physiologically and morphologically. In the atrium, the speed of conduction and rhythmicity are faster than in the ventricle. Compared to ventricular myocytes, atrial myocytes are smaller, transverse tubules are less well developed or absent, the Golgi complex is larger, and dense core atrial specific granules are found. Up to now, the function of these granules has remained obscure (6). They resemble secretory granules in certain endocrine cells, and it has been suggested that the specific granules are related to a secretory function in myocardium (7). It is quite possible that some of the specific properties of the atrial myocardium, such as the manufacture of "atrial granules," may be dependent on or influenced by a direct genomic action of sex steroids. Our observations and postulated effects of estrogen on atrial muscle cells demand further elucidation, not only because of the known sex differences in cardiovascular physiology and pathology but also because of the widespread use of estrogens, for example, in contraceptive pills during the reproductive period and for hormonal substitution during the postreproductive period in female life, as well as in the treatment of prostatic tumors in men.

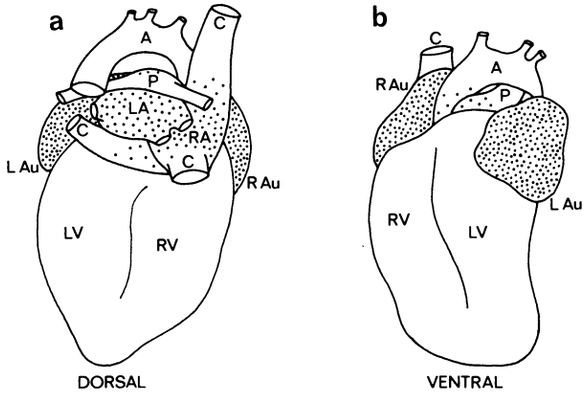

Fig. 1. In the auricles and atria of the female and male rat heart, muscle cell nuclei concentrate estradiol, similar to the muscle cells of the uterus. In (a) and (b) the intensity of the stippling indicates the regional distribution of estrophilic myocytes.

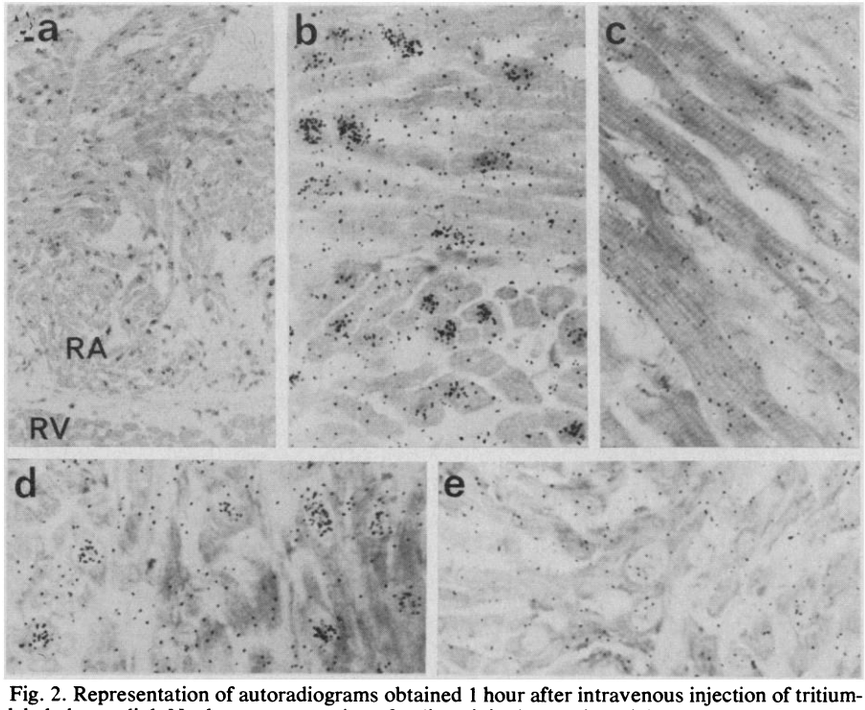

Fig. 2. Representation of autoradiograms obtained 1 hour after intravenous injection of tritium-labeled estradiol. Nuclear concentration of radioactivity is seen in atrial myocytes (a, b, and d), while the ventricular myocytes (c) do not show concentration. Competition by prior treatment with unlabeled estradiol inhibits the nuclear uptake of radioactivity in atrial myocytes in contrast to the control without such treatment. Sections, 4 μm; (a) magnification $\times$160; (b–e) $\times$520; exposure times 290 days (a–c), 160 days (d and e). Abbreviations: *A*, aorta; *C*, vena cava; *LA*, left atrium; *LAu*, left auricle; *LV*, left ventricle; *P*, arteria pulmonalis; *RA*, right atrium; *RAu*, right auricle; and *RV*, right ventricle.

In addition to the heart, other portions of the cardiovascular system must be considered as sites where estradiol may exert its effects, including the tunica media of arteries, the pericytes of capillaries, the proximal tubules and intertubular connective tissue in the kidney, and the adrenal cortex. Furthermore, estrogen may influence cardiac function through its action on neurons in brain regions that have been associated with its regulation. These include the motor nucleus of the vagus nerve, the nucleus of the solitary tract, as well as various nuclei in the hypothalamus and the amygdala. In all these structures, estrogen has been found to be taken up and retained by target cell nuclei, in a fashion characteristic for the uterus (3, 8).

Digitalis and allied cardiac glycosides have been in therapeutic use for several centuries. The mechanism of action of these steroid derivatives is still not clear, and many hypotheses have been advanced. In the light of the present observation of [^{3}H]estradiol localization and the known estrogenic effect of cardiac glycosides it is probable that digitoxin and its congeners act preferentially at atrial muscle cells through genomic stimulation. Accordingly, we are proposing the concept of genomic cardiac glycoside action for further testing.

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Hypothalamic Stimulation Facilitates Contralateral Visual Control of a Learned Response

Abstract. *Rats that ate during hypothalamic stimulation were trained to press a lever for food only while receiving light signals from head-mounted lights. During stimulation, they pressed if the signal was visible to the eye contralateral to the electrode, but ignored the signal if it was visible only to the ipsilateral eye.*

Behavioral studies using both electrical stimulation and lesions have provided evidence suggesting that the lateral hypothalamus influences behavior, in part, by controlling the utilization of sensory information by systems that release and guide responses. One example is Bandler and Flynn's (*1*) demonstration, in cats, that unilateral hypothalamic stimulation facilities lunging toward a mouse seen by the contralateral eye. Another is Marshall and Teitelbaum's (*2*) report that unilateral hypothalamic lesions produce a contralateral neglect of olfactory, tactile, and visual stimuli.

In most of these studies, a sensory stimulus is presented to one side of the animal, evoking a reflex response directed toward the same side. Thus, it is difficult to determine whether it is really the sensory input or only the motor system that has been affected by the hypothalamic manipulation. By using an arbitrary, learned, stimulus-response connection, we have been able to show that lateral hypothalamic stimulation in rats produces a contralateral visual facilitation that cannot be attributed to motor potentiation. Rats were trained to use a visual discrimination to control a lever press response. During brain stimulation, the animal's responding was controlled solely by information received through the eye contralateral to the electrode.

We used four male albino rats that were induced to eat when stimulated through electrodes implanted in the lateral hypothalamus. Two of the rats had two electrodes each, bilaterally placed. The others had one electrode each. The stereotaxic coordinates for the six electrode sites, verified by subsequent histology as lateral hypothalamus, were 1.5 to 2.8 mm posterior to bregma, 1.5 mm lateral to the midline, and 8.5 mm below the level skull surface. Stimulation consisted of monophasic negative pulses, 0.2 msec in duration, delivered through monopolar stainless steel electrodes at a frequency of 100 pulses per second (*3*).

While deprived of food, the animals were trained to press a lever for 45-mg food pellets on a fixed ratio of three presses for each pellet. Then, while being stimulated, they were taught to press only while signal lights were on. Two tiny light bulbs (*4*) on stiff wire stalks were attached to the top of the rat's head. They were mounted on the miniature electrical connector between the electrodes and wires from the stimulator. For each animal, the stalks were bent to position the bulbs approximately 8 mm lateral to each eye. The bulbs were painted opaque black except for a 1.5-mm spot aimed at a point on the rat's head 5 mm below and 5 mm behind each eye, to ensure that each eye could receive light only from its own bulb (*5*). The visual discrimination was established by making food available only when both lights were on. Training continued until the rats pressed eight times as often when the lights were on as they did when the lights were off.

After this training, each animal was tested with only one light at a time. The hypothalamus was stimulated for 30-second periods separated by 60-second intervals. Left and right lights were presented in random order, switched while stimulation was off. Each electrode was tested in one continuous session of 20 stimulation periods, 10 with each light.

The results were the same for all six of the electrodes. During stimulation, the rats pressed the lever only when the contralateral light was on. When the stimulation was off, they made few or no responses. Lever press rates for the unilateral light test were compared with rates for the last session of the two-light discrimination training (Fig. 1). The animals performed the discrimination during stimulation as though they were using only the eye contralateral to the electrode. If the contralateral light was on, they pressed. If the contralateral light was off, they did not press, regardless of whether the ipsilateral light was on or off.

This finding is not a result of one light's being more visible than the other, because the animals with two bilateral electrodes (5L,R; 17L,R) used the left eye during stimulation through the right electrode, and the right eye during stimulation through the left electrode. In addi-

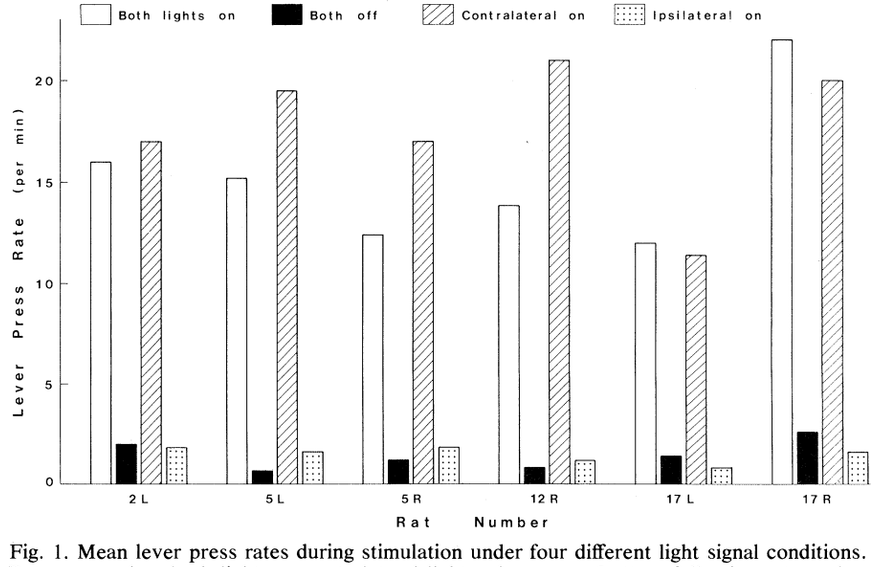

Fig. 1. Mean lever press rates during stimulation under four different light signal conditions. Rats press when both lights or contralateral light only are on. Letters following rat number indicate whether the electrode was on the left or right side of the brain. Each bar represents either 5 or 6 minutes of responding.