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Serum Prolactin Levels in Rats Following Isoproterenol-Induced Myocardial Infarction (39112)

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We have been using the potent beta-adrenergic stimulating agent, isoproterenol, to induce acute myocardial infarction in nonarteriosclerotic and arteriosclerotic male and female Sprague–Dawley rats (1–4). Male rats die in much greater numbers than females, following isoproterenol-induced myocardial infarction. Males also have a different pathophysiologic response pattern, for example, myocardial repair, ECG, serum enzyme, glucose and lipid changes, during the immediate post-infarction phase. In man, there is also a sex-related difference in the mortality rate from acute myocardial infarction. Because women are less prone to myocardial infarction during their reproductive years and become more prone after the menopause, it has been proposed that estrogen treatment might reduce the risk of a second myocardial infarct in males. However, clinical tests of this proposal had to be halted because estrogen treatment of the male was fraught with complications (5, 6). Because of the analogous situation in our rats with drug-induced myocardial infarction, that is, superior survival and myocardial repair in females vs males, we have elected to alter the hormonal milieu of these animals prior to an isoproterenol-induced myocardial infarct. Further, the suggestion has been made that high prolactin levels during the stress of a myocardial infarction might contribute to cardiac arrhythmias (7). Prolactin has been implicated in promoting salt retention in rats (8, 9), sheep (10), and man (11, 12), and high doses of prolactin lead to increased blood volume and alterations in blood pressure in rats (9). It has also been demonstrated that there is a dichotomous response between males and females in their secretion of prolactin. In the present investigation we monitored the prolactin levels in nonarteriosclerotic virgin, arteriosclerotic breeder (13), and neonatally sterilized male and female rats during the acute necrosis and repair phases of isoproterenol-induced myocardial infarction to determine whether (a) there are dynamic changes in prolactin production during myocardial infarction induced in rats, (b) whether this response is different in male vs female rats, (c) whether this response may be altered by manipulation of sex hormones, and (d) whether the presence of preexisting arterial disease affects any of these conditions.

Materials and methods. Six groups of Sprague–Dawley rats were used in this experiment: (A) nonarterioscleroitc male and female virgin rats; (B) arteriosclerotic male and female breeder rats; and (C) neonatal sterilized male and female virgin rats. (Repeatedly bred male and female rats will develop a Cushingoid spectrum of degenerative changes, for example, hyperglycemia, hyperlipidemia, hypercalcemia, hypertension, arteriosclerosis, etc., spontaneously, without surgical or dietary manipulation. The severity of these degenerative changes parallels the frequency and number of breedings and is best correlated with changes in adrenocortical steroid production. Virgin rats do not develop any of these untoward changes (13)). All of the animals were maintained on a 14-hr light, 10-hr dark schedule, fed commercial rat chow (Teklad) and water *ad libitum*. The experimental animals received two injections of isoproterenol subcutaneously, 24 hr apart and administered at 6 AM colony time. The nonarteriosclerotic (virgin) animals received 50 mg per 100 g body weight, and the arteriosclerotic (breeder) animals received 25 mg per 100 g body weight. This regimen has been demonstrated to produce an equivalent degree of

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TABLE I. SURVIVAL RATES (DAY 12) FOR MALE AND FEMALE RATS SUBJECTED TO ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN RELATION TO EARLY (DAY 1) CIRCULATING LEVELS OF PROLACTIN

	Virgin (no arteriosclerosis)		Breeder (arteriosclerosis)		Neonatal sterile (no arteriosclerosis)	
	Prolactin (ng RP-1/ml) (average mean ± SE)	Survival (%) (average)	Prolactin (ng RP-1/ml) (average mean ± SE)	Survival (%) (average)	Prolactin (ng RP-1/ml) (average mean ± SE)	Survival (%) (average)
Males	60 ± 4	42[b]	44 ± 10	51[b]	100 ± 9	79[b, d]
	(25)[a]	[210][c]	(20)	[170]	(15)	[86]
Females	109 ± 14	90	115 ± 12	97	265 ± 50	91
	(20)	[90]	(25)	[87]	(20)	[89]

[a] ( ) = number of serum samples of prolactin analyzed during the first 24 hr of acute myocardial ischemia.

[b] $P < 0.01$ compared with females.

[c] [ ] = number of animals treated with isoproterenol (Day 0).

[d] $P < 0.01$ (chi-square test) compared with other males.

myocardial necrosis in the nonarteriosclerotic (virgin) and arteriosclerotic (breeder) animals, despite the disparity in dose per 100 g body wt (1–4). The total dose per animal is approximately equivalent, since breeder rats weigh considerably more than their virgin counterparts. Animals were sacrificed by decapitation at 2, 4, 8, 12, and 24 hr following each injection of isoproterenol on Days 1 and 2 (acute necrosis phase). On Days 4 through 12 (repair phase), the animals were sacrificed between 8 and 10 AM to preclude the effects of diurnal variation.

Neonatal sterilization of the animals in Group C was accomplished by the subcutaneous injection of either 1.25 mg testosterone proprionate (TP, females) or 0.12 mg estradiol benzoate (EB, males) when the animals were 5 days old (1, 14). (A single injection of the opposite sex steroid in 5-day-old male and female rats will lead to gonadal atrophy and permanent sterilization (14, 15)). Litter mate controls received injections of the sesame oil vehicle. Any animals which did not respond to the neonatal sterilization were excluded from the experimental results.

Serum was collected at autopsy, stored at −20°C, and subsequently assayed for prolactin by a double antibody radioimmunoassay using materials and methods provided by the NIAMD Hormone Distribution Program, N.I.H., U.S. Public Health Service. All results are reported in terms of the NIAMD Rat Prolactin RP-1 standard. The presence or absence of arterial disease for each animal was confirmed at autopsy or by subsequent histopathologic examination.

Statistical analysis of results was performed using a one-way analysis of variance, chi-square test, or Student's t test, as appropriate (16).

Results. Mortality from isoproterenol-induced myocardial infarction is invariably higher among males than females (1–4). In this experiment, there was also a significant difference ($P < 0.01$), calculated from a chi-square test, between the sexes in the numbers surviving the drug-induced infarct (Table I). There was no significant difference in mortality between the various groups of females or between the nonarteriosclerotic (virgin) and arteriosclerotic (breeder) male rats. However, the sterilized EB males had a significantly improved ($P < 0.01$), chi-square test) survival rate when compared either to the arteriosclerotic (breeder) males (79% vs 51%) or to the nonarteriosclerotic (virgin) males (79% vs 42%).

Serum prolactin levels became acutely elevated in both the sterilized EB male controls (Fig. 1) and the sterilized TP female controls (Fig. 2) as compared with the nonarteriosclerotic (virgin) and arteriosclerotic (breeder) control groups. The serum prolactin levels attained after the first and second injection of isoproterenol were considerably higher in females, compared to males.

Following the first injection of isopro-

terenol, prolactin levels in the sterilized EB males and the nonarteriosclerotic (virgin) males became elevated threefold within the first 4 hr (Fig. 1). However, the arteriosclerotic (breeder) males did not show any real change in prolactin levels until 24 hr after the first injection, at which time the nonarteriosclerotic (virgin) males also had elevated prolactin levels, and the sterilized EB males manifested prolactin values that were in the high normal range. There was an initial elevation in serum prolactin in all three male groups following the second injection of isoproterenol. This was rapidly followed by a drop to subnormal levels in the nonarteriosclerotic (virgin) and sterilized EB males while the prolactin levels in the arteriosclerotic (breeder) males returned to normal. Prolactin levels were relatively normal in the nonarteriosclerotic (virgin) and arteriosclerotic (breeder) males from Days 4 to 12, they were significantly depressed in the sterilized EB males on Days 4 to 6.

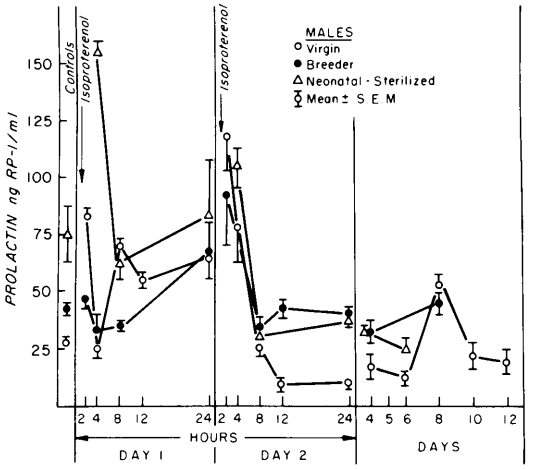

FIG. 1. Changes in serum prolactin levels (mean ± SE) of nonarteriosclerotic (virgin), arteriosclerotic (breeder), and nonarteriosclerotic, neonatal sterilized male rats. Each point is the mean value of five animals except for controls, which are the mean for a total of 15 animals sacrificed in groups of five on three different days during the time course of the experiment. The experimental animals were sacrificed at intervals following one (Day 1) or after a second injection (Day 2) of isoproterenol.

Prolactin levels were initially depressed in the arteriosclerotic (breeder) females after the first injection of isoproterenol (Fig. 2) but did become elevated 24 hr later. By direct contrast, the prolactin levels in the nonarteriosclerotic (virgin) and sterilized TP females, although initially unchanged, did become elevated at 12 hr, returning toward normal levels by 24 hr. Following the second injection of isoproterenol, all three groups of females displayed markedly disparate re-

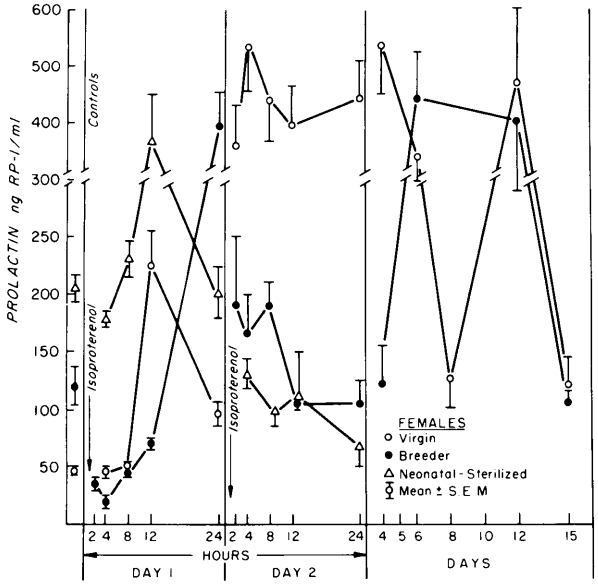

FIG. 2. Changes in serum prolactin levels (mean ± SE) of nonarteriosclerotic (virgin), arteriosclerotic (breeder), and nonarteriosclerotic, neonatal sterilized female rats.

sponses. In the arteriosclerotic, female breeders, the prolactin levels were elevated at 24 hr on Day 1, but quickly returned to normal on Day 2, remained normal through Day 4, and then became highly elevated from Days 6 to 12. The prolactin levels in the sterilized TP females were depressed for the 24 hr following the second injection, while the prolactin values measured in the nonarteriosclerotic (virgin) females were highly elevated until Day 8. The nonarteriosclerotic (virgin) females also had a transient rise in serum prolactin on Day 12, returning to high normal levels on Day 15.

Discussion. The superior survival of the neonatal sterilized rats is particularly intriguing. We have also obtained improved survival when surgically castrated male rats were subjected to isoproterenol-induced myocardial infarction (17). Since we found no change in the survival rate of the hormonally sterilized TP females, as well as in ovariectomized females in ancillary experiments (18), we suggest that it is unlikely that the female hormones, estrogen and progesterone, are responsible for the female rat's superior ability for cardiac repair and survival after myocardial infarction. The lack of protection and perhaps even deleterious effects of estrogens and progestogens in females taking contraceptive pills, and in males treated with estrogen as prophylactic treatment against a possible second myocardial infarct, is disconcerting. Since surgically castrated or hormonally sterilized rats show improved survival, it may be the comparatively high levels of circulating androgens which are responsible for the poor myocardial repair and survival of male rats (1–4). Unfortunately, no information is available as yet as to the actual circulating levels of steroids and gonadotropins in the estrogen-sterilized male rat. However, the organ weights in such animals reported by Kincl *et al.* (15) indicate that gonadotropin and androgen activity is lower in EB-sterilized males than in intact males. If this is the case, then it would follow that our EB-sterilized males did indeed have reduced circulating androgen levels, and this would argue in favor of our concept that it may be androgenicity that is deleterious to male victims of myocardial infarction.

Horrobin (19) and others (20, 21) have found that prolactin is greatly increased in the circulation of patients being admitted into a coronary care unit during exercise, stress, surgery, and after myocardial infarction. Because prolactin can affect electrolyte metabolism, Horrobin has suggested that the increased production of prolactin during myocardial infarction may contribute to cardiac dysrhythmia through its effects on potassium metabolism (19). It has been shown that patients receiving prolactin-releasing drugs such as methyldopa, phenothiazines, reserpine, and tricyclic depressants have suffered fatal dysrhythmias (22), and that some of the cardiac problems connected with digitalis therapy are due to its ability to stimulate estrogen production (23), which, in turn, causes increased prolactin secretion. Pertinent to our experimental findings, high doses of prolactin will cause bradycardia (24). Our data is in accord with the clinical and experimental findings of Horrobin and others (19–21), that is, our finding of a dynamic increase in prolactin production, especially in female rats, during an acute isoproterenol-induced myocardial infarction. (Horrobin *et al.* (7) found the highest prolactin levels in a woman immediately after myocardial infarction.) The superior survival of female rats, both nonarteriosclerotic (virgin) and arteriosclerotic (breeder), may be a result of their significantly higher prolactin levels (Table I), which, at these high levels, would induce a sinus bradycardia, and thus dampen the tachycardia-inducing effects of isoproterenol. It is generally accepted, both in animals and in man, that males show decreased prolactin release in response to stimuli, for example, stress (25). Neill (25) has suggested that there is a prolactin "surge center" located in the hypothalamus, which controls Prolactin Inhibiting Factor (PIF), and that the function of this "surge center" is stimulated by estrogens and suppressed by androgens. Further, male rats are unable to respond with a prolactin surge even when treated with estrogens, probably due to the long-term sexual differentiation of hypothalamic centers. Androgenicity per se, in this frame of reference, may interfere with the survival from cardiac ischemia due to androgen suppression of prolactin release.

In connection with the above, it is in-

triguing that the male and female rats that were hormonally sterilized when they were but 5 days old manifested the most prompt and greatest increase in prolactin in association with their superior survival. The delayed prolactin response in the arteriosclerotic male and female breeders may be due to some effect of their arterial disease on the hypothalamus and its releasing centers. We have found definitely aberrant prolactin levels in these arteriosclerotic rats. If elevated prolactin levels have an ameliorative effect on the pathogenesis of myocardial infarction, they do so during the early stages of myocardial ischemia, that is, Day 1. We have found a clear division of animals in their response to the initial challenge of isoproterenol. Those which cannot withstand the intense stimulation of increased cardiac work, induced by isoproterenol, succumb; those able to compensate survive with little or no evidence of cardiac damage. (If the surviving animals are given a second dose of isoproterenol (Day 2), they almost universally proceed to inexorable infarction.) Therefore, the prompt increase in prolactin on Day 1 in the hormonally sterilized animals, despite progressively dwindling levels and responsiveness on Days 2 through 5, indicate that the salutary effects of prolactin, if any, were operative during the earliest stages of myocardial infarction.

Summary and conclusions. Circulating prolactin levels were monitored in nonarteriosclerotic, arteriosclerotic, and hormonally sterilized male and female Sprague–Dawley rats during the acute necrosis and repair phases of myocardial infarction induced by isoproterenol. Male rats are particularly prone to succumb to acute myocardial ischemia but reduction of androgen levels by neonatal sterilization improved survival considerably. Circulating prolactin levels are greatly increased, particularly in females, during acute myocardial ischemia. Since androgens suppress the hypothalamic center for prolactin release, prolactin levels were delayed and transitory in males. It is suggested that the superior survival of female rats may be related to their greater production of prolactin during acute stages of myocardial ischemia, which would dampen the tachycardia-inducing effects of the potent beta-adrenergic stimulating agent, isoproterenol.

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