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The Effects of Ovariectomy and Progesterone on Peripheral Aromatization in the Female Rhesus Monkey

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We investigated the acute effects of surgery, i.e. ovariectomy, the long-term effects of ovariectomy, and the effects of progesterone on the peripheral aromatization of androstenedione in rhesus monkeys (*Macaca mulatta*). For the acute effects of surgery, 7 rhesus monkeys were given a pulse of [^{3}H]androstenedione/[^{14}C]estrone 2 weeks before and immedately after ovariectomy. In each case all urine was collected for 4 days and analyzed for radioactivity as estrone glucuronide and the peripheral aromatization calculated from the isotope ratios. Similarly, 5 monkeys were studied before and 18 months after ovariectomy. The acute effects of surgery resulted in a significant decrease in the peripheral aromatization of androstenedione to estrone from a mean ± SE of 0.94 ± 0.26 to 0.61 ± 0.19%, $P = 0.0452$. Conversely, the long-term effects of ovariectomy resulted in a significant increase in peripheral aromatization from 0.38 ± 0.06 to 0.67 ± 0.12%, $P = 0.0207$. In 7 monkeys the peripheral aromatization was measured before and 10 days after the administration of progesterone, 100 mg in oil. There was no difference in peripheral aromatization before, 0.62 ± 0.04% and after progesterone, 0.58 ± 0.05%, $P = 0.10$. We conclude that the acute stress of ovariectomy, or possibly the loss of ovarian aromatizing tissue, results in a decline in peripheral aromatization, but ovariectomy will have the long-term effect of an increase in aromatization, and that the presence or absence of progesterone does not play a role.

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INTRODUCTION

Since peripheral aromatization is such an important source of estrogens in men and post-menopausal women, changes in peripheral aromatization can have important effects on the hormonal milieu.

In women peripheral aromatization increases with age, but does not appear to be related to the menopause, *per se* [1]. Stress from acute illness results in an increase in circulating estrogens in men and women [2, 3] although the source of the increase in estrogens is uncertain. The increase may be secondary to the reported increase in the peripheral aromatization following major surgery, hysterectomy, in women [4] but an increase in the circulating levels of the precursor androstenedione cannot be excluded [2]. In addition, it has been reported that progestins inhibit aromatase activity *in vitro* [5, 6]. Whether circulatory progesterone plays a role in the increase in aromatization seen with aging or stress is uncertain.

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The rhesus monkey has been shown to be a good model for studies of reproductive hormones in the human [7]. We have shown that peripheral aromatization in the rhesus monkey is at the same level as in men and women [8]. We have already used the rhesus monkey to study the changes in peripheral aromatization under a number of conditions as a model for the human [8, 9]. Therefore, we chose this primate model to study the acute and chronic effects of ovariectomy equating acute ovariectomy with acute stress. In addition, we studied the effects of progesterone on peripheral aromatization. We report here our studies of the effects of acute and chronic ovariectomy and progesterone administration on peripheral aromatization in the rhesus monkey.

MATERIALS AND METHODS

All experiments were performed in adult female rhesus (*Macaca mulatta*) monkeys which were housed in individual cages and fed monkey chow (Purina Co., St Louis, MO) *ad lib*. The protocol was approved by the Institutional Animal Care and Use Committee.

Reagents were prepared as described previously [10–12]. [7-^{3}H]testosterone ([^{3}H]T) (sp. act. 25 mCi/mmol) and [4-^{14}C]estrone (sp. act. 54 mCi/mmol) were obtained from New England Nuclear Corp. (Boston, MA). [7-^{3}H]androstenedione ([7-^{3}H]A) was formed from [7-^{3}H]T by Jones' oxidation [13]. The [7-^{3}H]A so formed was purified by thin layer chromatography and was >98% pure by reverse isotope dilution and crystallization. All labeled androgens and estrogens were purified, and the purity was checked before use [14]. Nonradioactive estrone (E_1) and estradiol (E_2) were obtained from Steraloids Co. (Wilton, NH) and crystallized from methanol before use. Silica gel HF254 was obtained from Brinkmann (Westbury, NY).

To measure peripheral aromatization each monkey was given a pulse injection i.v. of [^{3}H]A and [^{14}C]E_1, and then all urine was collected for 96 h and stored frozen at −15°C until analyzed.

Monkeys were tranquilized with ketamine, 5 mg/kg, and maintained on halothane/nitrous oxide anesthesia during ovariectomies, but for pulse injections the monkeys were tranquilized with ketamine only.

In 7 monkeys, peripheral aromatization was measured initially following the administration of [^{3}H]A/[^{14}C]E_1 i.v. and the collection of all urine for 96 h. Then 7–14 days later the animal had both ovaries removed and while the incision was being sutured, the monkey received [^{3}H]A/[^{14}C]E_1 i.v. and all urine was again collected.

In 5 monkeys control studies were done when the ovaries were present and then 12–18 months after ovariectomy studies were repeated.

Peripheral aromatization was measured in 7 monkeys before and again 10 days after receiving 100 mg progesterone-in-oil i.m. To ensure adequate progesterone receptor levels the monkeys were implanted with a single silastic capsule containing estradiol [15].

The urine samples were analyzed as described previously [16, 17]. Unconjugated steroids were extracted using cyclohexane–ethylacetate (1:2, v/v) and the urine was incubated with β-glucuronidase. The hydrolyzed estrone was extracted with cyclohexane–ethylacetate (1:2, v/v) and purified by alkaline partition and multiple chromatographic and derivatization steps.

The E_1 radioactivity was measured using a liquid scintillation spectrometer. In these samples, ^{3}H ranged from 150 to 500 dpm, and ^{14}C ranged from 3000 to 8000 dpm. Each sample was counted for 50 min, and the counting errror was <2%. $[\rho]^{A,E_1}_{BM}$ (the percentage of A injected converted to E_1) was calculated as $(100*[^3H]E_1/[^{14}C]E_1)_{urine}/([^3H]A/[^{14}C]E_1)_{infused}]$ [16].

Paired *t*-tests were used to compare the peripheral aromatizations before and after ovariectomy and progesterone administration.

RESULTS

As noted in Table 1, 5 of the 7 monkeys showed a decrease in $[\rho]^{A,E_1}_{BM}$, and the overall decrease was significant, $P = 0.0452$, during and immediately after the surgical stress of ovariectomy compared to the control values.

Table 1. Peripheral aromatization, $[\rho]^{A,E_1}_{BM}$, in 7 monkeys measured before and immediately after ovariectomy

Monkey No.	$[\rho]^{A,E_1}_{BM}$ Before ovariectomy (%)	After ovariectomy (%)
38	0.72	0.70
55	0.84	0.84
42	1.30	0.68
44	0.73	0.75
65	1.26	0.55
66	1.01	0.30
67	0.69	0.44
Mean	0.94	0.61
SE	0.10	0.19

All 5 of the monkeys studied showed an increase in $[\rho]^{A,E_1}_{BM}$ 12–18 months after ovariectomy compared to the individual $[\rho]^{A,E_1}_{BM}$ value measured before ovariectomy (Table 2). This increase was significant at $P = 0.0207$.

The administration of progesterone increased the aromatization in 2 monkeys, decreased it in 4, and caused no change in 1 (Table 3). These changes were not significant, $P = 0.643$.

DISCUSSION

Although a previous study reported an increase in peripheral aromatization as a result of surgical stress in some women [4], our results showed a decrease in aromatization in the rhesus monkey following surgery. In our study, the surgery was ovariectomy, and it is possible that the acute loss of the ovaries was responsible for the decline in peripheral aromatization. However, there are a number of factors that suggest this is not the major cause for the decline; male monkeys have slightly greater peripheral aromatization than female [8], but if the ovary were a major contributor to peripheral aromatization, then one would expect the reverse; ovarian blood flow is only a small percent of cardiac output, and thus only a small fraction of circulating androstenedione would be available for aromatization; and we had shown that most of the peripheral aromatization in female rhesus monkeys

Table 2. Peripheral aromatization, $[\rho]^{A,E_1}_{BM}$, in 5 monkeys before and >12 months after ovariectomy

Monkey No.	$[\rho]^{A,E_1}_{BM}$ Control (%)	After progesterone (%)
17	0.33	0.75
33	0.54	1.09
37	0.18	0.38
30	0.46	0.58
35	0.37	0.57
Mean	0.38	0.67
SE	0.06	0.12

Table 3. Peripheral aromatization, $[\rho]^{A,E_1}_{BM}$, in 7 monkeys before and 10 days after injection of progesterone-in-oil 100 mgs i.m.

Monkey No.	$[\rho]^{A,E_1}_{BM}$ Before ovariectomy (%)	After ovariectomy (%)
2	0.78	0.39
4	0.51	0.51
5	0.60	0.51
6	0.68	0.53
10	0.65	0.60
25	0.62	0.70
45	0.48	0.80
Mean	0.62	0.58
SE	0.04	0.05

occurred in tissues other than the ovary [18]. Nevertheless, without actual measurements across the rhesus ovary, we cannot exclude the possibility that ovarian removal is responsible, in part, for the decline in peripheral aromatization. Since both the pre- and post-surgery studies were done under the same type of anesthesia, it is unlikely that the decrease was secondary to anesthesia. That in itself is a stress, but would have been similar in each case. It is possible that surgery results in a shift in blood flow from tissues with high levels of aromatase activity to tissues with low levels of aromatase activity.

Although glucocorticoids and norepinephrine have been shown to result in marked increases in aromatase activity *in vitro* [19–22], glucocorticoids have not been shown to have an effect, *in vivo* when administered in high doses [23, 24]. We did find a decrease in aromatization in cynomologous monkeys after dexamethasone administration [25] so that our results after surgery may be a result of the increase in glucocorticoids.

We were unable to show that menopausal state had an effect on peripheral aromatization [1], and previously Siiteri could find no effect of ovariectomy on peripheral aromatization in women [26]. Nevertheless, peripheral aromatization did increase in the monkeys when measured at least 12 months after ovariectomy. It is unlikely that age alone was responsible for the increase in aromatization since the time interval was too short. It is unlikely that a change in estrogen level was responsible for the increase since estrogen levels were not high and the animals were not cycling before the ovariectomy. In our colony the monkeys rarely cycle normally for some months after arrival and these were ovariectomized during that time. We thought it possible, despite the lack of ovulation, that progesterone might play a role in inhibiting aromatase activity as has been reported [5, 6], but raising progesterone levels did not result in a change in aromatization. This is further evidence that progesterone does not effect peripheral aromatization although it has been reported that progestins inhibit aromatization in the ovary [5, 6], but not in adipose stromal cell cultures [27].

It should be noted that the mean value for peripheral aromatization was similar in the monkeys following acute or chronic ovariectomy, but the control values were different in each set. The same technicians were involved in the processing of the samples, and the techniques did not change, so that does not explain the difference. Also the ovariectomy studies were done at the same time that the progestrone studies were done but the latter showed no change in aromatization. In addition peripheral aromatization was measured on 2 separate occasions in 5 monkeys previously ovariectomized and the percent aromatizations were similar with a mean difference of 0.06%, $P = 0.46$, for the studies. Thus, it is unlikely that laboratory variation was responsible for the differences.

It would appear, therefore, that acute ovariectomy, either through operative stress and/or removal of aromatizing tissue results in a decrease in aromatization which increases thereafter with time. The exact mechanism(s) for these changes remains uncertain, but does not involve changes in progesterone levels.

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