Progesterone receptors and ventilatory stimulation by progestin

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BRODEUR, PENELOPE, MARY MOCKUS, ROSANN MCCULLOUGH, AND LORNA GRINDLAY MOORE. *Progesterone receptors and ventilatory stimulation by progestin.* J. Appl. Physiol. 60(2): 590–595, 1986.—Progestin is thought to be a ventilatory stimulant but its effectiveness in raising ventilation is variable in humans and other species. We hypothesized that the level of progesterone receptors was an important determinant of the ventilatory response to progestin. Since estradiol induces progesterone receptor formation, we compared the ventilatory effect of the synthetic progestin medroxyprogesterone acetate (MPA) given in combination with estradiol with the effects of estradiol alone, MPA alone, or vehicle (saline) in ovariectomized rats. Animals receiving MPA alone had low numbers of progesterone receptors (2.43 pmol/g uterine wt) and had no change in ventilation, arterial P_{CO_2}, or P_{O_2}. MPA administration raised ventilation 23 ± 5%, lowered arterial P_{CO_2} 3.2 ± 0.9 Torr (both $P < 0.01$) and tended to raise arterial P_{O_2} when given in combination with estradiol to animals with increased numbers of progesterone receptors (4.85 pmol/g uterine wt). Estradiol alone produced the highest number of progesterone receptors (12.3 pmol/g uterine wt) but had no effect on ventilation or arterial P_{CO_2} and decreased arterial P_{O_2}. Combined estradiol plus MPA treatment produced a greater fall in arterial P_{CO_2} than did treatment with MPA alone, estradiol, or saline (all $P < 0.05$). These results suggest that both an elevation in progestin levels and progesterone receptor numbers are required to stimulate ventilation.

hormones; gender; ventilatory control; estrogen

PROGESTIN HAS BEEN IMPLICATED in the increased ventilation observed during pregnancy, in the luteal phase of the menstrual cycle, and in men after the administration of progesterone or the synthetic progestin, medroxyprogesterone acetate (MPA) (7, 16, 28). However progestins do not increase ventilation in several experimental animal species. Only sheep given very high doses and guinea pigs have a ventilatory response to progestin, and it has no apparent effect in the rat, cow, goat, and pony (2, 10, 13, 27, 29).

Steroid actions in target tissues are mediated by specific receptors. We hypothesized that the level of progesterone receptors was a determinant of the ventilatory response to progestin. Progesterone receptors are normally present in target tissues at only very low levels unless induced by estradiol acting through estrogen receptors (4, 5, 14, 26). We therefore examined the effect of MPA on ventilation when given in combination with estradiol to induce the formation of progesterone receptors. These animals were compared with animals that received either MPA alone, estradiol alone, or vehicle (saline) to determine whether elevations in progesterone receptor numbers influenced the ventilatory response to MPA. We chose the rat as an experimental animal because it was among the species previously reported not to respond to progestin administration. We reasoned that a ventilatory response to progestin might be elicited in the presence of elevated progesterone receptor numbers.

METHODS

Hormone treatment. Forty-two 250-g female Sprague-Dawley rats were ovariectomized to remove the primary source of endogenous steroids and allowed to recover for 2 wk. Females were chosen so that the uterus could be used as a marker tissue for subsequent assay of progesterone receptor numbers. Treatment consisted of daily intramuscular 0.1-ml injections of either saline, 1 μg estradiol alone (Sigma, 0.01 mg/ml, 1% ethanol in saline), 2 mg MPA alone (UpJohn, medroxyprogesterone acetate, 20 mg/ml in saline) for 4 days, or 1 μg estradiol for 4 days and then 1 μg estradiol plus 2 mg MPA for an additional 4 days. The progestin MPA was chosen because it has strong binding affinity for progesterone receptors (20) and is the progestin most frequently used in humans. Nine animals were treated with hormones for subsequent assay of progesterone receptor numbers; three received estradiol alone, three received MPA alone, and three received estradiol plus MPA. Previous studies have shown that saline treatment alone is ineffective in inducing progesterone receptors (11). Ventilation and blood gas studies were carried out in 33 animals; eight received saline, nine received estradiol alone, eight received MPA alone, and eight received estradiol plus MPA. Saline treatment was used for 4 days to control for possible effects of repeated handling during the period of ventilation measurements. Subsets of three animals received estradiol alone and three animals received saline for 8 days to control for the longer course of treatment in the estradiol plus MPA group. These hormone doses were approximately physiological and were in accord

with other studies (10, 33). Body weights did not change with treatment nor differ among the groups (Table 1).

Progesterone receptor measurements. Animals were killed with an overdose of pentobarbital sodium. The uterus was removed within 3 min and immediately placed on ice, weighed, dropped into liquid N_2, and kept frozen at −70°C until ready for assay. Uteri were pooled from the animals in each treatment group to increase the amount of tissue available for assay. We justify our single measurement in each treatment group insofar as our purpose was to confirm the large, already well-established effects of estradiol and MPA on progesterone receptor numbers under our experimental conditions (4, 11, 14, 26, 33).

Frozen uteri were homogenized and suspended in phosphate buffer (5 mM sodium phosphate, 10 mM thioglycerol, 10% glycerol, pH 7.4 at 4°C) until the cells were greater than 90%-disrupted, as seen by phase microscopy and dye exclusion. The homogenate was centrifuged and the supernatant cytosol was used immediately in sucrose density gradients for assay of progesterone receptors. The average protein content of cytosols was 2 mg/ml. We used a standard technique for measuring progesterone receptors (26, 33) which has been validated previously as being highly specific for progesterone receptors (18). Briefly, to determine specific binding, a radiolabeled synthetic progestin, R5020 ([6,7-^{3}H] 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione, 87 Ci/mmol; New England Nuclear, Boston, MA), was added to the undiluted cytosol and incubated at 40°C for 3 h. This synthetic progestin has a high affinity for progesterone receptors and does not bind to progesterone-binding serum proteins (22). Nonspecific binding was determined by treating a parallel set of samples with a 100-fold excess of the unlabeled synthetic progestin. Excess unbound steroid was removed after incubation by treatment with dextran-coated charcoal. After centrifugation, the supernatant was sedimented through a continuous 5–20% sucrose/phosphate buffer gradient. ^{14}C-labeled bovine serum albumin (New England Nuclear, Boston, MA) was added as an internal sedimentation marker. Fractions (0.2 ml) were collected and the radioactivity in each fraction was measured. When the fraction number was plotted against the counts per fraction, one curve was generated for the cytosols incubated only in the synthetic progestin and one curve was generated for the cytosols incubated with radiolabeled synthetic progestin plus a 100-fold excess of the unlabeled synthetic progestin (Fig. 1). The area between the two curves represents the specific binding to receptors. The number of specific receptors was calculated using the specific activity of the radiolabel and the efficiency for tritium (46%) and carbon-14 (89%) and expressed in picomoles per gram uterine tissue.

Ventilation and blood gas measurements. Catheterization of one femoral artery was carried out in each animal prior to initiating treatment. Under pentobarbital anesthesia using sterile technique, a polyvinyl catheter (TW, Norton, ID 0.023 in., OD 0.038 in.) was inserted in the femoral artery and the tip advanced into the abdominal aorta. Catheter placement was confirmed at autopsy. The catheter was secured and tunneled subcutaneously to a point between the scapulae where the free end was stored in a cap sutured to the skin. Skin incisions were closed using 3-0 prolene and sprayed with adhesive bandage. Surgery took ~1 h. Prophylactic penicillin (Tonecil, 0.1 mg/100 g body wt im) was given postoperatively. The animals were allowed to recover for 1 wk. Full mobility was regained and animals appeared to adjust well to the presence of the interscapular cap. Catheter patency was maintained by flushing every 2–3 days with a heparin-saline solution.

A 3-liter whole-body plethysmograph similar to that described by Bartlett and Tenney (1) was used to measure ventilation. We modified their design by having only a single animal chamber to which a differential pressure transducer (Statham PM5 ± 1D-350, Gould Medical Products, Oxnard, CA) was attached. The plethysmograph contained inlet and outlet ports and a rubber stopper through which was inserted a mercury thermometer, a glass calibration syringe (1 ml), and a pliable plastic sleeve. The exposed portion of the indwelling arterial catheter was passed through the plastic sleeve to permit the rat unrestrained movement and to allow withdrawal of arterial samples.

The catheterized unrestrained rat was placed in the chamber ventilated with 100% humidified room air. Once the animal was in a quiet, awake condition, the chamber's inlet and outlet ports were occluded and the pressure changes within the chamber due to the warming and wetting of air at inspiration and the cooling and drying of air at expiration were recorded for 30–40 s. During each measurement, a known volume of air was withdrawn from the chamber and injected rapidly at the end of an inspiration for calibration purposes. At the same time, air temperature within the chamber was read to the nearest 0.1°C. This procedure was repeated until three tracings of good quality were obtained. Tidal volume was calculated using the equation of Drorbaugh and Fenn (3)

TABLE 1. *Group characteristics before and after 2–4 days of saline or hormone treatment*

	Saline (n = 8)		Estradiol (n = 9)		MPA (n = 8)		Estradiol + MPA (n = 8)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Tidal volume, ml BTPS	2.86±0.15	2.94±0.11	2.97±0.15	3.02±0.15	2.74±0.17	2.99±0.18*	2.54±0.08	2.88±0.11*
Frequency, breaths/min	87±7	93±6	80±7	81±7	91±5	91±5	86±5	93±6*
pH_a, units	7.48±0.01	7.48±0.01	7.49±0.01	7.49±0.01	7.48±0.02	7.47±0.01	7.47±0.01	7.47±0.01
Hct, %	34±1	36±2	34±2	34±1	36±1	34±2	37±2	34±1
Body wt, g	324±17	305±19	297±12	295±11	305±13	301±14	293±10	296±8

Values are means ± SE. MPA, medroxyprogesterone acetate; pH_a, arterial pH; Hct, hematocrit. *Paired t test, $P < 0.05$.

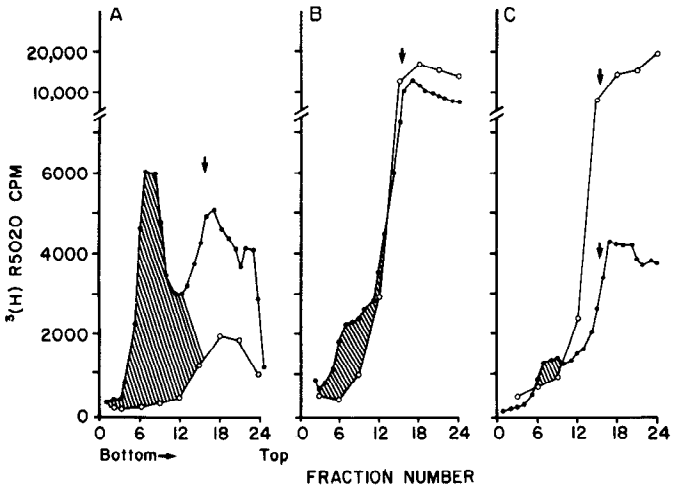

FIG. 1. Progesterone receptors in uterine cytosol from animals treated with estradiol alone (*A*), estradiol plus medroxyprogesterone acetate (MPA) (*B*), or MPA alone (*C*). Cytosols were incubated with radiolabeled synthetic progestin (*closed circles*) alone or together with unlabeled synthetic progestin in a 100-fold excess (*open circles*) and then sedimented through sucrose density gradients, fractionated, and counted. Counts per minute for each fraction are shown starting at bottom of gradient. *Shaded area* between curves in each panel encompasses high affinity sites or those not displaced in presence of a 100-fold excess of synthetic progestin. Peak specific binding in each treatment group occurs in 7–8S region identified by reference to sedimentation of known molecular weight standard, [^{14}C]bovine serum albumin, at 4.6S as shown by *arrows*.

and respiratory frequency was determined from the pressure trace. Results were averaged from the three tracings at a given measurement time. Arterial blood (0.5 ml) was withdrawn immediately after the final ventilatory trace to measure arterial P_{O_2} and P_{CO_2}, and pH using Corning (Medfield, MA) electrodes. The total volume of blood withdrawn (2 ml over ~1 wk) did not change hematocrit in any group (Table 1).

Measurements of ventilation and blood gases were conducted 4 days and, again, 1 day before initiating treatment. The values varied among individual rats but were the same on the two pretreatment days (mean ± SD of differences between the 2 days for $\dot{V}E = -3 \pm 24$ ml·min^{-1}·100 g body wt^{-1}, for arterial CO_2 tensions (Pa_{CO_2}) = 0.4 ± 3.0 Torr, for arterial O_2 tension (Pa_{O_2}) = −1.3 ± 5.1; all P = NS) and therefore the values were averaged for each animal. The pretreatment values were the same among the four treatment groups (Fig. 2, Table 1) and were within the range of normal values for unanesthetized rats in Denver (30). Ventilation and blood gas measurements were then made on the 2nd and 4th days of treatment. In most animals (76%) complete determinations were obtained on both days. The $\dot{V}E$, Pa_{O_2}, and Pa_{CO_2} tensions varied among individual animals but did not differ between the 2nd and 4th days of treatment (mean ± SD of differences between the 2 days for $\dot{V}E = 1 \pm 18$ ml·min^{-1}·100 g body wt^{-1}, for Pa_{CO_2} = 0.5 ± 3.6 Torr, for $Pa_{O_2} = -1.3 \pm 4.0$ Torr; all P = NS) or within any treatment group (data not shown). Therefore, the values were averaged from the 2nd and 4th days of treatment for each animal. In animals with missing data, values were taken from the single day on which measurements were obtained. Values from the 6th and 8th days of saline or estradiol treatment were also similar and hence were averaged for each animal.

Statistics. $\dot{V}E$, Pa_{O_2}, and Pa_{CO_2} tensions agreed well within animals as shown by the small mean differences between the two pretreatment and the two treatment days. To minimize the influence of interindividual variation on the experimental effect, we assessed the effect of treatment by comparing the change with treatment rather than absolute values. Pretreatment values were compared with treatment values within each group using paired *t* tests. Comparisons of the change with treatment among groups were performed using one-way analysis of variance with Student-Newman-Keuls or Scheffe multiple comparisons. Values are reported as means ± SE. Comparisons were considered significant when the two-tailed $P < 0.05$.

RESULTS

The numbers of specific progesterone receptors present with hormone treatment were calculated as 12.3 pmol/g uterine wt in estradiol-treated animals (*A*), 4.85 pmol/g uterine wt in estradiol plus MPA-treated animals (*B*), and 2.43 pmol/g uterine wt in MPA-treated animals (*C*) (Fig. 1). The high number of progesterone receptors in estradiol compared with MPA-treated animals (Fig. 1, *A* and *C*) was consistent with the induction of progesterone receptors by estradiol as studied in several animal species and cell culture models (4, 5, 14, 26). The intermediate progesterone receptor numbers in the estradiol plus MPA treatment group cytosols probably represented receptor turnover caused by MPA treatment (33).

Saline or estradiol treatment did not change ventilation (Fig. 2), tidal volume or respiratory frequency (Table 1). Arterial CO_2 and O_2 tensions and pH were also unchanged after saline administration. Pa_{O_2} fell but Pa_{CO_2} and pH remained the same with estradiol treatment (Fig. 2, Table 1). Extended (6–8 days) treatment with saline or estradiol also had no effect on ventilation, arterial blood gas tensions, or pH (Table 2).

Animals receiving MPA alone had an increase in tidal volume but $\dot{V}E$, respiratory frequency, Pa_{CO_2} and Pa_{O_2} tensions, and pH did not change (Table 1, Fig. 2). In contrast, animals that were treated with estradiol plus MPA increased their minute ventilation 23 ± 5% above pretreatment values (Fig. 2) due to increases in both tidal volume and respiratory frequency (Table 1). Pa_{CO_2} fell and Pa_{O_2} tended to increase ($P = 0.08$) but pH did not change after combined estradiol plus MPA treatment (Table 1, Fig. 2).

$\dot{V}E$, Pa_{O_2}, Pa_{CO_2}, and pH values after treatment did not differ among the groups. Estradiol plus MPA treatment prompted a greater rise in Pa_{O_2} and a greater fall in Pa_{CO_2} than did saline or estradiol treatment alone (both $P < 0.05$). Combined estradiol plus MPA treatment produced a greater fall in Pa_{CO_2} than did MPA treatment alone ($P < 0.05$). The changes in ventilation with treatment did not differ ($P = 0.18$) among the groups.

DISCUSSION

This study showed that the administration of the synthetic progestin, MPA, failed to increase ventilation

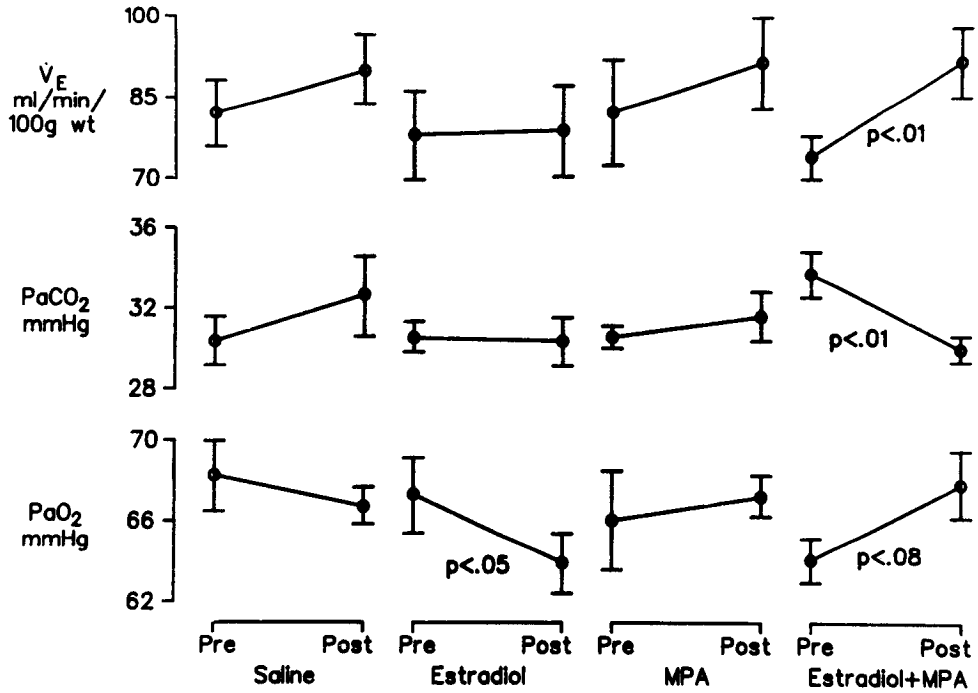

FIG. 2. Comparing values before pretreatment (pre) and during treatment (post), only 8 animals that were pretreated with estradiol and then with estradiol plus medroxyprogesterone acetate (MPA) increased their minute ventilation ($\dot{V}E$), decreased their arterial P_{CO_2} (Pa_{CO_2}) and tended to increase their arterial P_{O_2} (Pa_{O_2}) tensions. Treatment with MPA alone ($n = 8$) had no effect on $\dot{V}E$, Pa_{CO_2} or Pa_{O_2}. Treatment with saline or estradiol alone ($n = 8$ and $n = 9$, respectively) had no effect on $\dot{V}E$ or Pa_{CO_2}. Saline treatment also had no effect on Pa_{O_2} and estradiol treatment decreased Pa_{O_2}. Changes in ventilation did not differ among groups ($P = 0.18$). Estradiol plus MPA treatment produced a greater fall in Pa_{CO_2} than did treatment with MPA alone, estradiol or saline (all $P < 0.05$) and a greater Pa_{O_2} rise than observed with estradiol or saline treatment (both $P < 0.05$).

TABLE 2. *Ventilation and arterial gas tensions before and after 6–8 days saline or estradiol treatment*

	Saline ($n = 3$)		Estradiol ($n = 3$)	
	Pre	Post	Pre	Post
$\dot{V}E$, ml BTPS·min^{-1}· 100 g body wt^{-1}	78±8	81±4	57±4	61±4
Pa_{CO_2}, Torr	33±2	32±2	33±1	36±3
Pa_{O_2}, Torr	67±1	67±2	64±1	66±3
pH_a, units	7.48±0.01	7.49±0.01	7.48±0.01	7.49±0.03

Values are means ± SE. $\dot{V}E$, minute ventilation; Pa_{CO_2}, arterial CO_2 tension; Pa_{O_2}, arterial O_2 tension; pH_a, arterial pH.

or alter blood gas tensions when given alone to animals whose progesterone receptor numbers were low. However, when given to animals with elevated progesterone receptor numbers, MPA administration did stimulate ventilation and lower Pa_{CO_2}. Estradiol treatment alone increased progesterone receptor numbers but had no effect on $\dot{V}E$ or Pa_{CO_2} and actually decreased Pa_{O_2}. The explanation for the lack of pH change with estradiol plus MPA treatment is not clear unless the ability of the rat to rapidly decrease its bicarbonate levels (21) resulted in pH compensation by the time our measurements were made. Comparison among groups did not reveal any differences in the values of $\dot{V}E$, Pa_{O_2}, or Pa_{CO_2} after treatment. However, interindividual variation in absolute values could have obscured differences in group response to treatment. We therefore elected to compare the change with treatment among groups. Only the combination of estradiol plus MPA treatment produced a pattern of changes in ventilation and arterial gas tensions consistent with ventilatory stimulation. The group comparisons also revealed that MPA combined with estradiol produced a greater fall in Pa_{CO_2} than when MPA was given alone and a greater fall in Pa_{CO_2} and rise in Pa_{O_2} than in the estradiol or saline control groups. One problem in our measurements was that, even though pretreatment values were not different among the four groups, there was a trend toward lower $\dot{V}E$ and Pa_{O_2} values and higher Pa_{CO_2} levels in the estradiol plus MPA group. To test if initial values influenced the ventilatory response to treatment, we examined animals in the other groups whose ventilatory values fell within the range of the estradiol plus MPA animals and the relationship between pretreatment values and ventilatory response to treatment among the saline controls. Two-thirds of the animals in the other treatment groups had pretreatment values in the range of the estradiol plus MPA group but did not show a pattern of ventilation and blood gas changes consistent with ventilatory stimulation. Among the saline controls, the initial Pa_{O_2} but not the $\dot{V}E$ or Pa_{CO_2} correlated with the change with treatment. Thus the evidence available suggests that pretreatment values were not the major determinant of ventilatory response to treatment. We therefore concluded that ventilatory stimulation occurred with combined estradiol plus MPA treatment but not with either hormone alone, suggesting that an elevation both in MPA levels and in progesterone receptor numbers is required for progestin to stimulate ventilation.

It is generally appreciated that steroid receptors are required to elicit a response to a circulating hormone. Progestin receptors require induction by increased estradiol levels acting through estrogen receptors (4, 5, 14, 26). Subsequent administration of progestin causes translocation of the hormone-receptor complex to the cell nucleus where increased specific protein synthesis leads to the physiological and metabolic changes characterized as a response to progestin. The apparent decrease in progesterone receptors after estradiol plus MPA treatment, relative to the numbers present with estradiol

treatment alone, was most likely due to the activation of the hormone-receptor complexes in the nucleus, and thus does not necessarily represent a true reduction in the total numbers of receptors present. Although progesterone receptors exist in a range of target tissues including the uterus, breast, hypothalamus, pituitary, and aorta (4, 5, 8, 9, 12, 14), it is not known whether they are present in tissues involved in ventilatory control. Since the locus of the effects of progestin on ventilation is also unknown and because limitations of assay sensitivity require tissues with high receptor numbers, we chose the uterus as a convenient target tissue to serve as a marker for possible changes in receptor numbers elsewhere.

It is possible that some of the variability in ventilatory response to progestin observed in previous studies is attributable to differences in progesterone receptor numbers. After MPA treatment, men with obesity-hypoventilation syndrome experience a greater fall in Pa_{CO_2} than normal males (28, 31, 32, 35). While the magnitude of their P_{CO_2} fall may be a result of their initial hypercapnia, the greater amount of adipose tissue present in the obese state may increase the aromatization of endogenous steroids to estradiol, raise estradiol levels (23), and possibly progesterone receptor numbers as well. The increased ventilation in the luteal phase of the menstrual cycle and during pregnancy occurs after, or in association with, a continued rise in estradiol levels. However, normal males consistently respond to progestin in the absence of elevated estrogen levels and presumably progesterone receptor numbers (28, 35). In contrast, in most experimental animal studies, progestin has been administered to males without supplemental estrogen and no ventilatory stimulation has been observed. In the guinea pig, where a ventilatory response to progestin has been observed, females were studied and an increase in ventilation was observed more consistently, and correlated with serum progesterone levels only when estradiol was combined with progesterone treatment (10).

The mechanism by which progestin influences ventilation is unknown. An increase in ventilation appears to be a primary effect of MPA, with no evidence for blood or cerebrospinal fluid acidification or alterations in metabolic rate and body temperature acting as the stimulus to ventilation (28, 35). It is unknown whether the locus of progestin's effects on ventilation are central or peripheral. Central mechanisms are supported by cross-circulation studies in dogs in which the presence of peripheral chemoreceptors was not required for the acute hyperventilation induced by progestin (17). However, the ventilatory response to hypoxia increases with progestin administration to men, and during the luteal phase of the menstrual cycle and during pregnancy in women (15, 19, 24, 25, 28, 34, 35). Thus, while inferences about the site of progestin's actions or ventilation from whole-body stimulus response tests are limited, the available evidence is not sufficient to exclude a peripheral site of action.

Most nonrespiratory effects of progestin have been shown to be hormone-receptor mediated. Our results are consistent with the idea that increased ventilation in response to progestin is a direct, hormone receptor-mediated phenomenon. Further studies are required to determine whether larger doses and/or longer treatment would produce greater ventilatory stimulation and significant differences in absolute values among groups. The sites at which progestin and progesterone receptors influence ventilation also require localization. These studies may be important for improving the therapeutic efficacy of progestin as a treatment for diseases characterized by marked hypoventilation and for understanding the mechanisms responsible for gender differences and hormonal influences on ventilatory control.

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