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Progesterone stimulates respiration through a central nervous system steroid receptor-mediated mechanism in cat

(respiratory control/phrenic nerve activity/blood pressure/RU 486)

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ABSTRACT **We have examined the effect on respiration of the steroid hormone progesterone, administered either intravenously or directly into the medulla oblongata in anesthetized and paralyzed male and female cats. The carotid sinus and vagus nerves were cut, and end-tidal P_{CO_2} and temperature were kept constant with servo-controllers. Phrenic nerve activity was used to quantitate central respiratory activity. Repeated doses of progesterone (from 0.1 to 2.0 μg/kg, cumulative) caused a sustained (>45 min) facilitation of phrenic nerve activity in female and male cats; however, the response was much more variable in females. Progesterone injected into the region of nucleus tractus solitarii, a respiratory-related area in the medulla oblongata, also caused a prolonged stimulation of respiration. Progesterone administration at high concentration by both routes also caused a substantial hypotension. Identical i.v. doses of other classes of steroid hormones (17β-estradiol, testosterone, and cortisol) did not elicit the same respiratory effect. Pretreatment with RU 486, a progesterone-receptor antagonist, blocked the facilitatory effect of progesterone. We conclude that progesterone acts centrally through a steroid receptor-mediated mechanism to facilitate respiration.**

It has been known since the turn of the century that women hyperventilate during pregnancy (1, 2) and during the luteal phase of the menstrual cycle (3). Because the level of circulating progesterone correlates positively with increased ventilation, progesterone has been proposed as the ventilatory stimulant in these conditions (4)—a contention further supported by studies in which exogenously administered progesterone caused hyperventilation in normal male subjects (5, 6) and in patients with breathing disorders (7–12).

Even though many animal species hyperventilate during pregnancy when endogenous progesterone levels are elevated (13–15), most fail to respond in a like manner to exogenously administered progesterone under experimental conditions (5, 15–17). Such data has been interpreted to mean that progesterone is not the respiratory stimulant during pregnancy and the luteal phase of the menstrual cycle. However, a reasonable explanation exists for this apparent discrepancy. Estrogen levels increase during pregnancy, and estrogen causes induction, or upregulation, of progesterone receptors within certain regions of the brain (18, 19). Thus, progesterone released endogenously during pregnancy would be expected to elicit a greater response than exogenously administered progesterone to animals not pretreated with estrogen. Furthermore, a mild stimulation of respiration induced by exogenous progesterone could possibly have been obscured by concomitant hypocapnia and negative respiratory feedback.

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Therefore, to properly characterize the effect of exogenously administered progesterone on respiration the studies must be done in animals where respiratory feedback mechanisms have been either eliminated or controlled. Under such conditions we show progesterone to be a powerful respiratory stimulant. That a specific steroid receptor mechanism mediates the facilitatory effect of progesterone on respiration is also supported by our data.

MATERIALS AND METHODS

Studies were done on 18 female and 12 male adult cats. All were anesthetized i.v. with chloralose (40 mg/kg) and urethane (240 mg/kg); because this combination provides a long-lasting anesthesia (20), no further anesthetic was administered during the study. Femoral arterial pressure was measured with a strain gauge. Temperature was monitored with a rectal thermistor and maintained at 37°C by an electronic servo-control circuit and dc heating pad. The trachea was cannulated, and airway P_{CO_2} was measured with an infrared CO_2 analyzer.

Respiratory feedback from the arterial chemoreceptors and the pulmonary mechanoreceptors was blocked by cutting the carotid sinus and vagus nerves. A dorsal laminectomy was performed in one cat, and the spinal cord was transected at C_7/T_1 to eliminate input from visceral or somatic receptors. One phrenic nerve root (C_5) was exposed, cut distally, desheathed, and placed in a bipolar platinum recording electrode that was built into a small acrylic platform placed in a tissue well adjacent to the nerve. Because the electrode had no fixed external attachments (the electrical wires were flexible and moved freely), it was possible, even during a long experiment, to maintain a relatively constant electrical coupling between nerve and electrode. In two cats dorsal craniotomies were performed, and the dura were removed to expose the dorsal surface of the medulla oblongata. Bleeding was controlled with Gelfoam and bone wax.

All animals were paralyzed with gallamine triethiodide (3 mg/kg initially followed by a continuous infusion at a rate of 3 mg/kg per hr) and ventilated artificially with 100% O_2. To prevent significant changes in end-tidal and arterial CO_2 secondary to changes in metabolism, the dc voltage on the motor of the ventilator was controlled by the animal's end-tidal P_{CO_2} through an electronic circuit (21). The pumping frequency of the ventilator was thereby servo-controlled to maintain arterial P_{CO_2} constant throughout an experiment.

Reagents. Progesterone (4-pregnen-3,20-dione), 17β-estradiol [1,3,5,(10)-estratriene-3,17β-diol], testosterone (17β-hydroxy-4-androsten-3-one), and cortisol (11β,17,21-trihydroxy-4-pregnene-3,20-dione) were obtained from Steraloids (Wilton, NH). RU 486 [17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(1-propynyl)estra-4,9-dione-3-one] was provided by R. Deraedt at the Centre de Recherches Roussel-Uclaf

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(Romainville, France). The weighed hormone was dissolved in ≈0.3 ml of 95% ethyl alcohol and diluted to 1 ml with Ringer's solution.

Experimental Protocol. Hormone administration was delayed for at least 1 hr after completion of surgery. When all variables had stabilized, control values for phrenic nerve activity, arterial pressure, and end-tidal P_{CO_2} were recorded for 3 min. Repeated doses of progesterone (0.1, 0.2, 0.5, 1.0, and 2.0 μg/kg, cumulative) were then administered i.v. to 17 female and 9 male cats. The effect on phrenic activity and arterial pressure of each dose was followed for 45 min, with measurements being made at intervals of 5 min. Two male cats were pretreated with RU 486, a competitive antagonist for the progesterone receptor (22), 1 hr before receiving the first progesterone dose. Three additional male cats each received repeated doses of either 17β-estradiol, testosterone, or cortisol following the same dose regime.

A micropipette (tip diameter 15–25 μm) containing either 10 mM or 1 μM progesterone was placed stereotaxically into the region of nucleus tractus solitarii in two cats. Injections were made by applying pressure to the micropipette via flexible tubing attached to a nitrogen pressure source. The volume of fluid injected was determined by measuring the displacement of the meniscus in the micropipette using a monocular microscope fitted with a graticule.

Data Analysis. Data on phrenic nerve activity, arterial pressure, and airway P_{CO_2} were all recorded on magnetic tape. Phrenic nerve activity was rectified and integrated for each 100 msec. The highest level of activity during a phrenic burst (peak activity) is the neural equivalent of tidal volume (23). Phrenic minute activity is calculated as the product of peak phrenic activity and respiratory rate.

Phrenic activity was normalized by assigning a value of 100 units to the level of phrenic activity in each cat that corresponded to an increase in end-tidal P_{CO_2} of 40 torr (1 torr = 133 Pa) above apneic threshold (24); all other levels were scaled accordingly. Analyses of significance of differences were done using one-way analysis of variance for repeated measures. Differences from control were analyzed using Dunnett's test and were considered significant at the $P < 0.05$ level.

RESULTS

Fig. 1 shows the effect on integrated phrenic nerve activity and arterial pressure in one female cat of repeated i.v. doses of progesterone. Measurements were made at 45 min after each dose. Peak phrenic (tidal) activity increased with each dose up to a cumulative dose of 1.0 μg/kg; an additional 1.0 μg/kg of progesterone elicited no further increase. Cumulative doses of progesterone in excess of 0.5 μg/kg caused a substantial hypotension (−30 mm Hg).

The effect on minute phrenic activity in 17 female and 6 male cats of repeated i.v. doses of progesterone are shown in Fig. 2. Again, measurements (change from control) were made 45 min after each dose. End-tidal P_{CO_2} and temperature were kept constant in each animal throughout the experiment. At the highest dose (2.0 μg/kg) progesterone facilitated phrenic nerve activity in 12 of 17 female cats studied; four cats showed a slight inhibition, and in one cat minute phrenic activity was unchanged from control level (Fig. 2*A*). Most striking, however, was the high degree of variability especially evident after the highest dose. Intravenously administered progesterone facilitated respiration in all six male cats (Fig. 2*B*). Responses were noticeably more consistent than those measured in the female group.

Averaged findings for phrenic activity and arterial pressure in the female and male groups, as well as statistical results, are given in Table 1. Despite variability differences, magnitude of the stimulation of phrenic nerve activity after each progesterone dose was essentially the same for both female and male groups. Another interesting finding was that a sustained decrease in arterial pressure was measured with increasing doses of progesterone in both groups.

To rule out the possibility that the facilitatory effect of progesterone on phrenic nerve activity was mediated by a somatic or visceral afferent pathway, one male cat was studied after transection of the spinal cord at C_7/T_1. Phrenic nerve response to i.v. administered progesterone in this cat was not different than that measured in cats with intact spinal cords.

Fig. 3 shows the effect on integrated phrenic nerve activity and arterial pressure of an injection of 0.9 μl of 1.0 μM (0.9 pmol) progesterone into the region of nucleus tractus solitarii in one cat. End-tidal P_{CO_2} was maintained at 29 torr. A small increase in phrenic activity was recorded within 15 min after hormone injection. Substantial increases occurred during the subsequent 30 min (i.e., 30 and 45 min after injection). Mean arterial pressure decreased from 85 mm Hg during control to ≈65 mm Hg at 45 min after progesterone injection. These responses were similar to those measured following i.v. administration of progesterone (Fig. 1, Table 1). An almost identical respiratory response was measured in the other cat

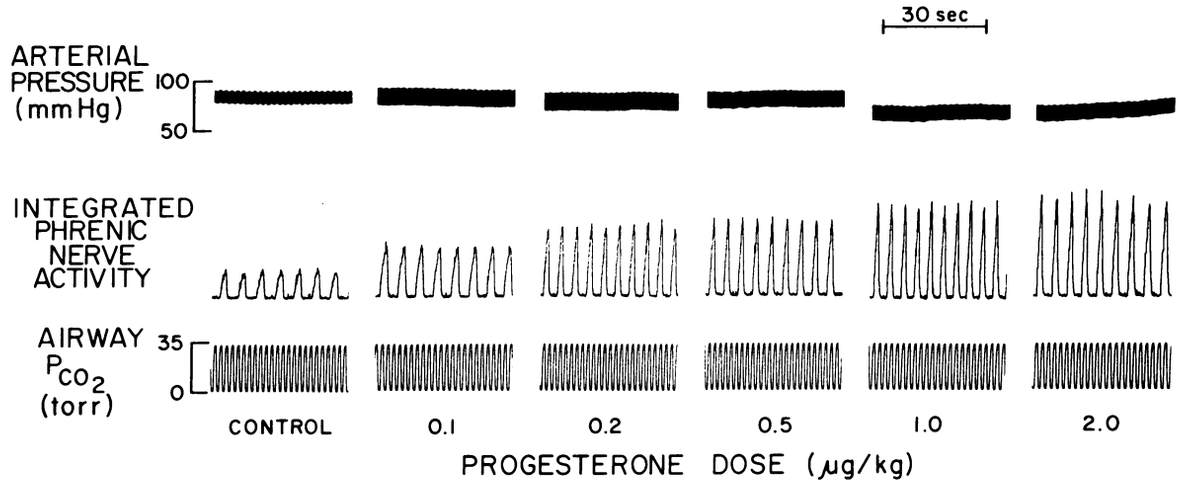

FIG. 1. Effect on arterial pressure and integrated phrenic nerve activity of progressive i.v. progesterone administration. End-tidal P_{CO_2} was held constant at 32 torr.

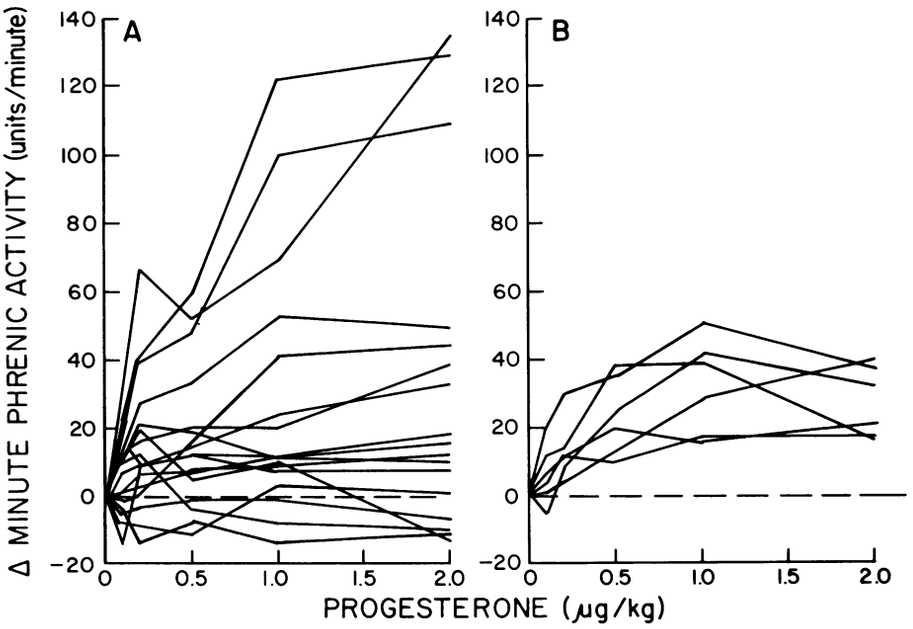

FIG. 2. Respiratory responses (change in minute phrenic activity) to progressive i.v. progesterone administration in individual female (*A*; $n = 17$) and male (*B*; $n = 6$) cats. End-tidal P_{CO_2} was held constant in all experiments.

in which a smaller volume (0.1 μl) of more concentrated progesterone (0.01 M; 1.0 nmol) was injected.

To determine whether the facilitatory effect of progesterone on respiration was mediated by a steroid receptor mechanism, two approaches were used. (*i*) Phrenic nerve response to other steroid hormones was determined. (*ii*) Respiratory response to progesterone after pretreatment with RU 486 was measured. Findings from these experiments are shown in Fig. 4, where the shaded area represents the range of respiratory responses to repeated doses of i.v. administered progesterone measured in six male cats (see Fig. 2*B*). Both cortisol and 17β-estradiol caused minor stimulation of phrenic activity by the final dose, but this increase was considerably less than the smallest response recorded during i.v. administration of progesterone (i.e., lower edge of shaded area). Testosterone substantially inhibited phrenic activity. The stimulation of respiration elicited by progesterone is evidently not due to a nonspecific steroid effect. Two additional male cats were pretreated with RU 486 at least an hour before progesterone administration. RU 486 had only a small, transient inhibitory effect on respiration that lasted for several minutes. Progesterone was not administered until all variables had become stable. Repeated doses of progesterone then caused inhibition of phrenic activity rather than the customary stimulation observed in untreated animals. In fact, both cats pretreated with RU 486 developed apnea at a cumulative dose of 1.0 to 2.0 μg/kg. That the stimulation of respiration elicited by progesterone was found to be progesterone specific and that RU 486 antagonized the response implicate involvement of a receptor mechanism.

DISCUSSION

We have studied the effect on respiration of exogenously administered progesterone; we found that progesterone administered intravenously at physiological concentrations caused prolonged (>45 min) stimulation of respiration in all male cats and in 12 of 17 female cats studied. Instability of the preparation is unlikely to account for these findings because of the long-lasting nature of the anesthesia and the stable coupling between nerve and electrode. Moreover, in a separate study we found that in the absence of intervention

Table 1. Mean data (±SEM) from male (♂) and female (♀) cats during control and at 45 min after each i.v. progesterone dose

	Control		Progesterone, μg/kg									
			0.1		0.2		0.5		1.0		2.0	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Number (*n*)	6.0	17.0	6.0	17.0	5.0*	16.0†	5.0*	17.0	6.0	17.0	6.0	17.0
Respiratory rate, breaths/min	18.3 ±1.9	17.4 ±1.7	20.1 ±0.9	19.1 ±1.7	22.9 ±0.6‡	19.8 ±1.6	25.7 ±2.3§	20.4 ±1.6	23.1 ±1.0‡	21.1 ±1.6‡	19.7 ±1.2	20.8 ±1.6
Phrenic tidal activity, units	23.2 ±1.6	23.9 ±1.4	26.5 ±2.6	27.1 ±2.5	32.2 ±3.2	32.1 ±3.5	38.8 ±6.8§	32.9 ±3.7	47.0 ±6.9§	36.7 ±4.6	50.8 ±7.2§	40.1 ±5.9‡
Phrenic minute activity, units/min	20.4 ±1.8	24.0 ±1.1	26.7 ±4.2	31.0 ±3.4	35.8 ±5.1§	39.9 ±5.7	46.2 ±6.5§	40.4 ±5.6	52.3 ±6.8§	51.4 ±9.9‡	47.8 ±5.8§	56.9 ±12.3‡
MAP, mm Hg	122.2 ±12.3	115.6 ±3.9	119.7 ±11.4	110.5 ±3.9	119.0 ±12.9	99.9 ±4.4	116.8 ±12.6	100.6 ±4.4	108.2 ±10.5‡	97.5 ±4.8‡	105.0 ±9.1‡	91.4 ±4.5§

MAP, mean arterial pressure.
*Missing data due to equipment malfunction.
†Missing data due to missed injection.
‡Significantly different from control at $P < 0.05$.
§Significantly different from control at $P < 0.01$.

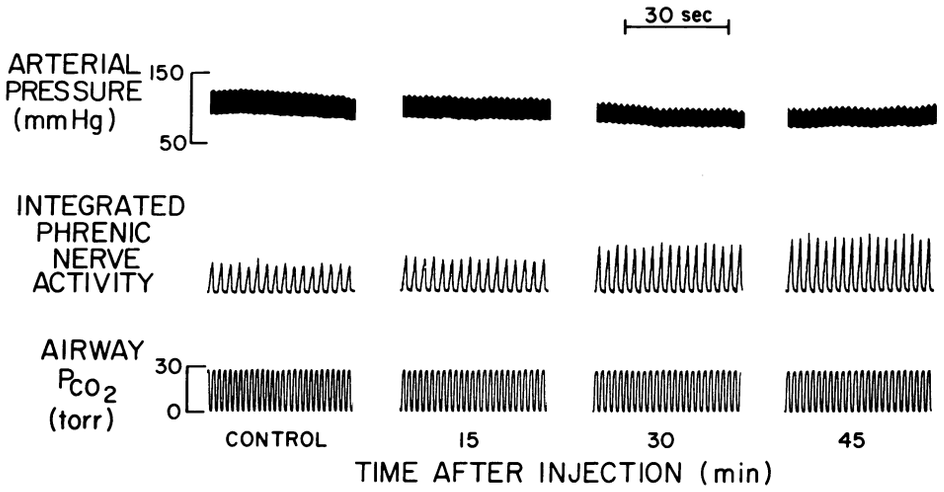

FIG. 3. Effect on arterial pressure and integrated phrenic nerve activity of a pressure injection of 0.9 μl of 1.0 μM (0.9 pmol) progesterone into the dorsal medulla. End-tidal P_{CO_2} was held constant at 28.5 torr.

all measured variables remained stable over an extended period (>6 hr, unpublished observations).

End-tidal P_{CO_2} was kept constant in these animals, and respiratory feedback from peripheral chemoreceptors and lung mechanoreceptors was eliminated. In addition, in one animal, afferent input from visceral and somatic receptors was also eliminated. Because these peripheral feedback mechanisms were not necessary for the progesterone-induced respiratory facilitation, we conclude that a central nervous system mechanism is involved in the response. [We must emphasize that this does not preclude a role for certain peripheral mechanisms (e.g., carotid body or lung receptors) in the overall response of the intact animal to progesterone.] Furthermore, microinjection of progesterone directly into the region of nucleus tractus solitarii, an area in the medulla oblongata associated with respiration (25), caused a similar enhancement of phrenic nerve activity. Thus, progesterone may act directly on medullary neurons to enhance respiration.

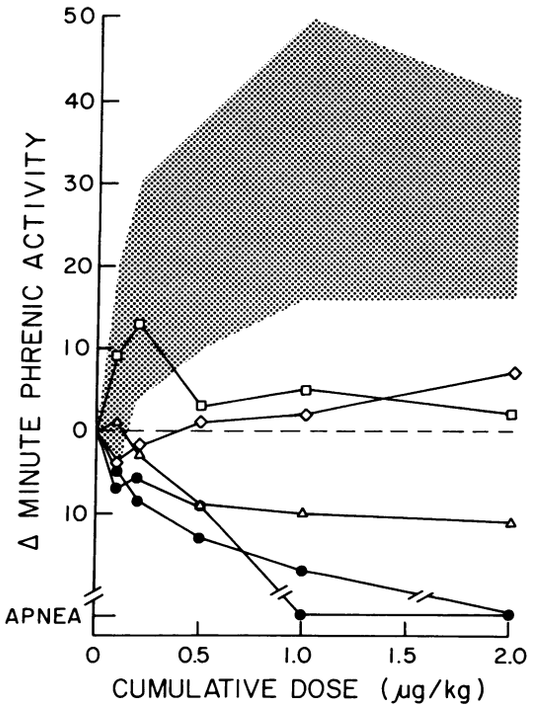

FIG. 4. Comparison of the change in minute phrenic activity with progesterone administration (shaded area is the range of findings from Fig. 2*B*) to the i.v. administration of the other steroids (◇, cortisol; □, 17β-estradiol; △, testosterone) and to the response to progesterone after pretreatment with RU 486 (●) in male cats. End-tidal P_{CO_2} was maintained constant in all experiments.

This facilitatory effect of exogenously administered progesterone on respiration could not be mimicked with other classes of steroid hormones and was blocked in animals that were pretreated with RU 486, a progesterone-receptor antagonist (22). We conclude, therefore, that this facilitation is receptor mediated. Moreover, we have shown that after pretreatment with RU 486, repeated doses of progesterone caused progressive inhibition, rather than facilitation, of phrenic nerve activity. The mechanism of this inhibition is unknown.

In our studies, 17β-estradiol and cortisol only slightly facilitated phrenic activity; testosterone substantially inhibited phrenic activity. Other workers have found that exogenously administered estradiol has no effect on respiration in either human subjects (26, 27) or rats (16). Koepchen (28) reported that exogenously administered testosterone had no acute effects on ventilation. However, because men seem more prone than women to develop apneas (29) and because elevated testosterone levels may be involved in the etiology of obesity hypoventilation syndrome (30), the inhibitory effect of testosterone that we report may be a significant finding that warrants further study.

No attempt was made to control the levels of endogenous estrogen or progesterone in the present study—i.e., castration and pretreatment with hormone. Instead we studied intact female cats that were chosen randomly from our animal holding facility. The individual female cats, therefore, may have been at different states of estrous and consequently had different levels of these hormones at the time of study. We believe these differences best account for the high degree of variability of respiratory response to progesterone. Endogenous progesterone would affect the response to exogenous progesterone by competing for the receptor. The effect of estrogen on the response to progesterone is through its ability to induce progesterone receptors in certain regions of the central nervous system (18, 19). Thus, we speculate that the greatest response to exogenous progesterone occurred in female cats at a time after induction of progesterone receptors by estrogen but while endogenous progesterone concentrations were low. Mild stimulation could have resulted when

exogenous progesterone was allowed only limited access to the receptor, either because of competition with endogenous progesterone, or because of a lower receptor density. Finally, the inhibition noticed in four female cats might have resulted from administering progesterone when most receptor sites were occupied by endogenous progesterone.

An unexpected finding was that repeated i.v. doses of progesterone caused a progressive decrease in mean arterial pressure in both female and male cats. Because injection of progesterone directly into the dorsal aspect of the medulla oblongata also caused a substantial hypotension, we believe that progesterone may have an inhibitory effect on central nervous system neurons involved in control of the circulation at high concentrations.

We present evidence that progesterone stimulates respiration centrally through a steroid receptor-mediated mechanism. We conclude that progesterone is, indeed, the stimulant that causes hyperventilation during the luteal phase of the menstrual cycle and during pregnancy. Hyperventilation during pregnancy is important in increasing the P_{O_2} in maternal arterial blood, thus allowing better oxygenation of the fetus. This mechanism illustrates how the endocrine and nervous systems can interact to regulate a vital body function.

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