Vascular Effects of Progesterone
Role of Cellular Calcium Regulation

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Abstract—Vascular actions of progesterone have been reported, independently of estrogen, affecting both blood pressure and other aspects of the cardiovascular system. To study possible mechanisms underlying these effects, we examined the effects of P in vivo in intact rats and in vitro in isolated artery and vascular smooth muscle cell preparations. In anesthetized Sprague-Dawley rats, bolus intravenous injections of P (100 µg/kg) significantly decreased pressor responses to norepinephrine (0.3 µg/kg). In vitro, progesterone (10⁻⁸ to 10⁻⁵ mmol/L) produced a significant, dose-dependent relaxation of isolated helical strips, both of rat tail artery precontracted with KCl (60 mmol/L) or arginine vasopressin (3 nmol/L), and of rat aorta precontracted with KCl (60 mmol/L) or norepinephrine (0.1 µmol/L). In isolated vascular smooth muscle cells, progesterone (5×10⁻⁷ mol/L) reversibly inhibited KCl (30 mmol/L) -induced elevation of cytosolic-free calcium by 64.1±5.5% (P<0.05), and in whole-cell patch-clamp experiments, progesterone (5×10⁻⁶ mol/L) reversibly and significantly blunted L-type calcium channel inward current, decreasing peak inward current to 65.7±4.3% of the control value (P<0.05). Our results provide evidence that progesterone is a vasoactive hormone, inhibiting agonist-induced vasconstriction. The data further suggest that progesterone effects on vascular tissue may, at least in part, be mediated by modulation of the L-type calcium channel current activity and, consequently, of cytosolic-free calcium content. (Hypertension. 2001;37:142-147.)

Key Words: progesterone ■ intracellular calcium ■ vascular smooth muscle ■ sex steroid hormones ■ L-type calcium channel ■ hypertension ■ menopause

It is well established that premenopausal women are at lower risk of developing hypertension and coronary heart disease than men of the same age and that the cardiovascular risk increases only after the cessation of ovarian function.1 Conversely, pregnancy is associated with lower blood pressure (BP), despite elevated circulating angiotensin II (Ang II) levels and sodium retention.2 Although the mechanism(s) underlying these observations are still poorly understood, a critical role for dramatic changes in the sex steroid environment is widely presumed. Previous mechanistic studies have focused on cardiovascular protective effects attributable to estrogens, but accumulating evidence suggests that progesterone independently of estrogen also exerts a protective influence on the vasculature.3 Thus progesterone receptors have been localized in the myocardium4 and in peripheral vascular tissue,5 and administration of progesterone lowers BP in humans,6,7 blunts the pressor response to Ang II in human pregnancy,7 and inhibits Ang II action in rats in some,8,9 but not all, reports.10,11

We intended to further define the vascular actions of progesterone and to study potential mechanism(s) underlying these effects. Therefore, we evaluated the short-term effects of progesterone in a variety of circumstances: on BP in anesthetized rats, on vascular contractility in endothelium-denuded isolated rat tail artery and aorta helical strips in vitro, and on cytosolic-free calcium concentrations [Ca²⁺], and L-channel Ca currents in isolated vascular smooth muscle cells (VSMC). Our results, similar to previous studies of 17-β estradiol,12-15 suggest that progesterone has modulatory effects on the contractility of blood vessels and that these vascular actions may be mediated by its effects on membrane Ca currents and thereby on [Ca²⁺], concentrations.

Materials and Methods
Experiments were performed in the research facilities of the Department of Physiology, University of Alberta, Canada. In all experiments, male Sprague-Dawley rats obtained from Charles River Canada (St. Foy, Quebec) were used. Experiments were conducted in adherence with the NIH “Principles for the utilization and care of vertebrate animals used in testing research and training.”

BP Measurements
BP was measured in anesthetized (pentobarbital sodium 65 mg/kg ip) and cannulated rats (200 to 250 g), as previously described.15 The
arterial cannula was connected to a Statham pressure transducer. Mean arterial pressure (MAP) was recorded continuously on a Grass FT03 polygraph (Grass Instruments), before and for 6 hours after a bolus injection of 100 μg/kg iv of progesterone. To determine the effect of progesterone on the pressor response to norepinephrine (NE), repeated bolus injections of NE (0.3 μg/kg) were administered before and 1, 2, 3, 4, 5, and 6 hours after the initial progesterone treatment. In the control group, solvent was injected instead of progesterone.

**Vascular Tension Studies**

Vascular tension was measured in tail artery and aorta helical strips, according to the method previously described in detail.\(^{15,16}\) The rat tail artery and aorta strips were suspended in Sawyer-Bartleson chambers containing aerated (95%O\(_2\), 5%CO\(_2\)) Krebs-Henseleit solution.\(^{16}\) The rat tail helical strips were then contracted with KCl (60 mmol/L) and arginine vasopressin (AVP, 3 mmol/L). The aorta strips were contracted with KCl (60 mmol/L) and NE (0.1 mmol/L), because it was not possible to achieve a steady tension with AVP in this preparation. When a steady tension was achieved, a cumulative dose-response for the vasodilatory effect of progesterone was obtained. Progesterone was added to the tissue bath to reach the following concentrations (mol/L): 5×10\(^{-8}\), 1.5×10\(^{-7}\), 5×10\(^{-7}\), 1.5×10\(^{-6}\), 5×10\(^{-6}\), 1.5×10\(^{-5}\), and 5×10\(^{-5}\). The entire dose response curve was obtained within 45 minutes, a period during which a steady contractile effect of the vasconstrictors was maintained. A dose-response curve with ethanol was also performed for each type of experiment.

**VSMC Studies**

All studies were performed on VSMC isolated from SD rat tail artery as previously described.\(^{15,17–19}\) For patch-clamp (pClamp) studies, primary culture cells were used within 18 to 36 hours of isolation. For [Ca\(^{2+}\)]\(_i\) measurement, cells were subcultured and used at passages 3 to 10.

**L-Current Recording**

The standard whole-cell version of the pClamp technique was used to measure whole-cell inward currents, as previously described.\(^{15,17,18}\) In brief, cells were put on the stage of an inverted phase-contrast microscope (Nikon). pClamp measurements were performed with an Axopatch-1B (Axon Instruments) pClamp amplifier. Patch micropipettes were pulled from glass tubes (OD 1.2 mm, ID 0.9 mm) with a 2-stage micropipette puller (PP83) and then fire-polished with a micro forge. The tip diameter was ~1 μm with a resistance of 2 to 8 MΩ when filled with the internal solution. The holding potential was set at ~40 mV. Barium currents (20 mmol/L Ba\(^{2+}\) was used as the inward charge carrier) through the Ca\(^{2+}\) channel were elicited by 200 ms depolarization at intervals of 5 seconds. The currents were filtered with a 4-pole Bessel filter at a cut-off frequency of 3 KHz. pClamp software and a laboratory interface (Axon Instruments) were used to generate the test pulses and to store and analyze data. In all cases, the peak current (leak current corrected) was used to construct the current versus voltage (I-V) relationship. Progesterone (5×10\(^{-8}\) mol/L) was added to the bath and inward Ba\(^{2+}\) currents were measured again (usually within 5 minutes of drug administration). L-currents were also recorded after a 2-minute washout of progesterone (obtained with a perfusion rate of ~1 mL/10s) and a 3-minute recovery period, or after the addition of Bay K 8644 (1 mmol/L) to progesterone-treated cells.

**[Ca\(^{2+}\)]\(_i\) Measurement**

VSMC [Ca\(^{2+}\)]\(_i\) studies were conducted as previously described in detail.\(^{15,19}\) Confluent cells were plated onto glass coverslips (25 mm circle) at a density of ~1×10\(^{7}\) cells/mL in DMEM and kept in culture until the cells became elongated and confluent (usually 24 to 48 hours). Cells were then incubated for 45 minutes in DMEM containing 5 μmol/L fura 2-AM (Molecular Probes, Inc.) at 37°C, in a dark compartment. The cells were then gently washed 3 times and kept in the same buffer.\(^{19}\) After about 5 minutes, the coverslip with attached cells was placed in a Sykes-Moore chamber of 1-mL volume on the stage of a Nikon (Phase Contrast-2) microscope. Fluorometric data were obtained with a dual wavelength excitation monochromater spectrofluorimeter (SPEX Industries Inc). Excitation wavelengths of 340 and 380 nm and an emission wavelength of 505 nm were used, and [Ca\(^{2+}\)]\(_i\), was calculated according to the method described by Grynkiewicz et al.\(^{20}\) with the use of the following equation: [Ca\(^{2+}\)]\(_i\) (nmol/L) = Kd×(R- R\(_{\text{min}}\))/(R\(_{\text{max}}\)-R)×b, where R is the ratio of fluorescence in the sample at 340 and 380 nm; R\(_{\text{min}}\) is the fluorescence ratio obtained by adding 5 mmol/L EGTA; b is the ratio of fluorescence of fura 2 at 380 nm at zero and saturating Ca\(^{2+}\); Kd is the dissociation constant of fura 2 for Ca\(^{2+}\), 224 nmol/L.\(^{20}\) Control [Ca\(^{2+}\)]\(_i\) elevations were induced by KCl (30 mmol/L) before the addition of the hormone. Cells showing a lack of basal responsiveness to KCl (defined as an increase of [Ca\(^{2+}\)], ≥50% of basal) were excluded from further study. No differences in KCl responsiveness were noted in cells used from passages 3 to 4 (the majority used) compared with those of later passages. Progesterone (5×10\(^{-8}\) mol/L) was added and incubated for 10 minutes. [Ca\(^{2+}\)], was measured and compared with the control. A second stimulus with KCl (30 mmol/L) was performed and the response compared with the control, in absence of the hormone. After wash-out of the hormone and a 10-minute recovery period, a third stimulus with KCl (30 mmol/L) was then performed and the response compared with the previous 2 KCl stimuli.

**Drugs**

Progesterone was purchased from Sigma Chemicals, dissolved in 95% ethanol to make a stock solution of 5×10\(^{-3}\) mol/L, and stored at 4°C. The same concentration of alcohol in a control solution had no effect in any of the in vivo or in vitro assays.

**Statistics**

Values are expressed as means±SEM. The paired t test was used for comparisons between mean values of the control and those obtained after drug administration. In the case of multiple comparisons, analysis of variance in connection with the Neuman-Keul’s multiple-range test was applied. A minimum of n=8 experiments was performed for each of the studies. A P<0.05 was considered significant.

**Results**

**In Vivo Effects on BP Responses to NE**

Bolus injection of progesterone (100 μg/kg) had no significant effect on the basal MAP during the 6-hour experimental period of observation (data not shown). However, progesterone altered the BP responsiveness to NE (0.3 μg/kg), significantly blunting the rise in MAP (Figure 1). Bolus injection of the control solvent (75 μL/kg ethanol) did not change the pressor responses to NE up to 6 hours (Figure 1). Heart rate did not significantly change during the 6-hour course of the experiment (from 284±3 bpm to 280±3 bpm, P=NS, data not shown).

**In Vitro Effects on Isolated Tissues**

The dose responses of precontracted aorta and rat tail artery strips to progesterone are shown in Figure 2. Increasing doses of progesterone produced increasing depressor responses, starting in both preparations at the dose of 5×10\(^{-6}\) mol/L (P<0.05). In the tail artery preparation, an almost complete
relaxation was obtained at 1.5×10^{-5} \text{ mol/L} (95.7\pm11.9\%) (P<0.05) for the strips precontracted with KCl and at 5×10^{-5} \text{ mol/L} (102.9\pm4.5\%) (P<0.05) for those precontracted with AVP (Figure 2, Top Panel). In the aorta helical strips, the higher concentrations tested (5×10^{-2} \text{ mol/L}) induced a relaxation of 56.5\pm8.6\% (P<0.05) in strips precontracted with KCl and of 84.5\pm4.5\% (P<0.05) in those precontracted with NE (Figure 2, Lower Panel). Concentrations of ethanol up to 2\% did not significantly change the vasoconstrictor responses of the artery strip preparation used (+2.04\pm1.1\%, P=NS at the highest concentration, data not shown).

Effects of Progesterone on \([\text{Ca}^{2+}]_{i}\) of VSMC

The effects of progesterone on \([\text{Ca}^{2+}]_{i}\), are shown in Figure 3 (original recording in the Top Panel and summary data in the Lower Panel). Basal \([\text{Ca}^{2+}]_{i}\), averaged 110\pm5.4 \text{ nmol/L}. Progesterone alone (5×10^{-7} \text{ mol/L}) did not produce any significant alteration of resting \([\text{Ca}^{2+}]_{i}\) (Figure 3). KCl (30 mmol/L) increased \([\text{Ca}^{2+}]_{i}\), by 71.6\pm9.9\% (mean \([\text{Ca}^{2+}]_{i}\), increase: +78.8\pm10.9 \text{ nmol/L}, P<0.001). However, when KCl (30 mmol/L) was added after 10 minutes’ incubation with progesterone (5×10^{-7} \text{ mol/L}), the \([\text{Ca}^{2+}]_{i}\), increase was significantly inhibited by 64.1\pm5.3\% (mean \([\text{Ca}^{2+}]_{i}\), increase: +28.2\pm4.8 \text{ nmol/L}, P<0.05) (Figure 3 Top Panel, traces A and C and Lower Panel). This effect of progesterone was reversible, because after washout of progesterone and a 5-minute recovery period, KCl responsiveness (30 mmol/L) was partially restored, \([\text{Ca}^{2+}]_{i}\), again increasing to 87.0\pm6.8\% of the original response (\([\text{Ca}^{2+}]_{i}\), +68.6\pm8.2 \text{ nmol/L}, P=NS versus control KCl effect) (Figure 3, Top Panel trace D versus A and Lower Panel). Ethanol alone did not elicit any change in basal or post-KCl \([\text{Ca}^{2+}]_{i}\), levels (data not shown).

Effects of Progesterone on L-Currents

Figure 4 (original recording in the top panel and summary data in the lower panel) shows the whole-cell pClamp data and I/V (current/voltage) relationship in the presence and absence of progesterone. With the holding potential set at −40 mV, L-currents were detected at a membrane potential of −20 mV and were maximal at +20 mV with an apparent
reversal potential beyond +60 mV. These currents were activated quickly and inactivated slowly (half-time inactivation >150 ms). The peak amplitudes of L-current could be maintained without significant deterioration for up to 20 minutes as previously reported. \(15,18,19\) Progesterone significantly blunted the L-current (Figure 4). At the dose of \(5 \times 10^{-6}\) mol/L, progesterone decreased the peak inward current to 65.7±4.3% of the control value (from 53.5±8.0 to 34.7±4.9 pA, \(P<0.05\)). This inhibition was reversible, because after a 2-minute washout of progesterone and a 3-minute recovery period, the peak amplitude of the L-current returned to 86.3±7.6% of the control value (46.2±4.1 pA, \(P=NS\) versus control). Ethanol alone did not elicit any change of L-current (data not shown). In addition, the application of the calcium ionophore Bay K 8644 (1 mmol/L) to progesterone-treated cells increased the L-current to 120.0±10.0% of the basal control value (Figure 4, Top and Lower Panels).

**Discussion**

Normal human pregnancy is characterized by profound changes in BP homeostasis and electrolyte balance, including progressive volume expansion and activation of the renin-angiotensin system. Yet the pressor response to Ang II is blunted, and BP is usually reduced in pregnancy. \(2,3\) Although the biological basis underlying these observations remains obscure, attention has been focused on estrogens, the direct vasodilatory effects of which may be mediated by their actions on L-channel Ca current and cytosolic-free calcium levels. \(15\) We wondered whether progesterone also exhibited direct vascular effects, independently of estrogen. Since the early work of Landau and Lugibihl, who demonstrated natriuretic effects of P21 more than 4 decades ago, progesterone has been reported to lower BP in hypertensive men and postmenopausal women\(^{4,5}\); to blunt the pressor response to Ang II in ovariectomized rats (which estrogen does not)\(^{6,9}\); and to alter adrenergic activity, \(22\) decreasing systemic vascular resistance in normal women\(^{23,24}\) and increasing cardiac output.

The present study demonstrates that progesterone, independently of estrogen, is a vasoactive hormone. This conclusion is reinforced by the overlapping, consistent effects observed in multiple preparations: (1) in the whole animal, progesterone blunted the pressor effects of NE. That this resulted from direct vascular actions was suggested by (2) the parallel in vitro action of progesterone to blunt agonist-induced tension both in rat aorta and tail artery strips. Because these preparations were endothelium-denuded, the effects observed here reflect direct vascular smooth muscle actions of progesterone, consistent with previous reports of endothelium-independent relaxant effects of progesterone in rabbit coronary arteries \(25\) and human placental arteries and veins. \(26\) After observing in vitro effects of progesterone with KCl, AVP, and NE-related stimuli, all of which are calcium-mediated, it seemed reasonable that the mechanism underlying the vascular effects of progesterone might involve cellular calcium homeostasis. Indeed, in VSMC, (3) progesterone directly decreased L-current, and in association with this, (4) progesterone blunted KCl-induced elevation of [Ca\(^{2+}\)]. Altogether, these data not only suggest direct vascular relaxant effects of progesterone, but also suggest that these effects are attributable, at least in part, to its actions on L-channel Ca current and concomitant [Ca\(^{2+}\)] levels.

We hypothesize that progesterone functions to modulate calcium channel activity, buffering the vasculature against excessive calcium-dependent vasoconstrictor responses to a variety of hormonal stimuli. This calcium-based mechanism may be a common denominator that helps to explain a variety of progesterone-induced effects observed in other tissues as well, such as (1) the decreased production of catecholamines by the adrenal medulla, \(27\) of PAI-1 \(28\) and of endothelin-1 by endothelial cells, \(29\) (2) the blunted pressor responses to infused Ang II, \(27\) and thus (3) the lowering of BP in normal pregnancy, in pregnancy-associated hypertension, \(30\) and in hypertensive men and postmenopausal women. \(4\) In pregnancy, progesterone also augments nifedipine-induced inhibition of uterine contractions. \(31\)

Mechanistically, under our experimental conditions, these effects of progesterone cannot easily be attributed to a genomic effect, because this presupposes a significant latency period, whereas our observations, at least in the in vitro...
experiments, were of a more immediate type. Because some latency period was observed in the in vivo experiments, the potential contribution of baroreflex and other mechanisms cannot be ruled out. As the AVP and KCl-induced vasoconstriction is independent of adrenoceptors, interaction with these receptors also cannot account for our data. Thus, these calcium-related actions of progesterone may be direct cell membrane-mediated, not involving a classic steroid/receptor mechanism. Indeed, plasma membrane binding and biochemical effects of progesterone not inhibited by classic progesterone antagonists have been reported.32,33

Certain caveats to the interpretation of our data must be considered. First, although circulating levels of progesterone in pregnancy may reach 100 to 150 ng/mL ($=10^{-6}$ mol/L), similar to concentrations having vasoactive actions in our and that decreased in vivo pressor responses to NE.35 Furthermore, 10 to 100 mmol/kg bolus injections of 10 to 100 ng/mL result from 100 mmol/kg bolus injections that decreased in vivo pressor responses to NE.35 Furthermore, as is true for many vasoactive substances, the doses required to produce an effect large enough to be measured consistently in vitro may be 1 to 2 orders of magnitude higher than those necessary for physiological effects in vivo. This may also reflect the lack of a normal extracellular ionic and hormonal milieu in which many substances and hormones, eg, estrogen, may be required for normal tissue responsiveness to progesterone.

A second caveat concerns the specificity of progesterone action on the vasculature. Although we reported similar Ca-related actions of estradiol15 and DHEAS,36 it is unlikely that the effects reported here for progesterone represent non-specific steroid action, because other steroids produce opposite effects. Thus, 1,25 (OH)$_2$D$_3$ and testosterone (unpublished data) also have vasoactive properties, but in an opposite direction, stimulating L-channel Ca currents and increasing [Ca$^{2+}$] levels in VSMC. Glucocorticoids tested at doses similar to those used in the present study do not affect calcium transients.38

In summary, we suggest that the direct calcium-dependent vascular relaxant effects of progesterone demonstrated here support a physiological role for progesterone in modulating vasoconstrictor tone in pregnancy. These direct vascular actions of progesterone, along with its natriuretic properties and effects on other ions such as magnesium,39 could also explain the lower BP and increased renin system activity during the luteal phase of the normal menstrual cycle40 if the effects observed here also occur at the lower in vivo concentrations of the nonpregnant state. Future studies are needed to explore the interactions between progesterone and estrogen and also to explore a possible link of alterations of circulating progesterone to the increased incidence of hypertension and cardiovascular disease in postmenopausal women.

References


25. Jiang CW, Sarrel PM, Lindsay DC, Poole-Wilson PA, Collins P. Proges-


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