Vascular Effects of Progesterone
Role of Cellular Calcium Regulation

Mario Barbagallo, Ligia J. Dominguez, Giuseppe Licata, Jie Shan, Li Bing, Edward Karpinski, Peter K.T. Pang, Lawrence M. Resnick

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,6,6 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 presser 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. The rat tail artery and aorta strips were suspended in Sawyer-Bartlestone bath and inward Ba3+ currents were measured with a 2-stage micropipette puller (PP83) and then performed with an Axopatch-1B (Axon Instruments) pClamp amplification.

L-Current Recording

The standard whole-cell version of the pClamp technique was used to measure whole-cell inward currents, as previously described. 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 ID 0.9 mm) with a 2-stage micropipette puller (PP83) and then fire-polished with a micro forge. The tip diameter was 2 to 8 MΩ when filled with the internal solution. The cut-off frequency of 3 KHz. pClamp software and a labmaster interface (Axon Instruments) were used to generate the test pulses described. 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 ID 0.9 mm) with a 2-stage micropipette puller (PP83) and then fire-polished with a micro forge. The tip diameter was 2 to 8 MΩ when filled with the internal solution. The cut-off frequency of 3 KHz. pClamp software and a labmaster interface (Axon Instruments) were used to generate the test pulses described. 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 2 to 8 MΩ when filled with the internal solution. The cut-off frequency of 3 KHz. pClamp software and a labmaster interface (Axon Instruments) were used to generate the test pulses described. 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 2 to 8 MΩ when filled with the internal solution. The cut-off frequency of 3 KHz. pClamp software and a labmaster interface (Axon Instruments) were used to generate the test pulses described. 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 2 to 8 MΩ when filled with the internal solution. The cut-off frequency of 3 KHz. pClamp software and a labmaster interface (Axon Instruments) were used to generate the test pulses described.
relaxation was obtained at $1.5 \times 10^{-5}$ mol/L (95.7±11.9%) ($P<0.05$) for the strips precontracted with KCl and at $5 \times 10^{-3}$ mol/L (102.9±4.5%) ($P<0.05$) for those precontracted with AVP (Figure 2, Top Panel). In the aorta helical strips, the higher concentrations tested ($5 \times 10^{-2}$ mol/L) induced a relaxation of 56.5±6.8% ($P<0.05$) in strips precontracted with KCl and of 84.5±4.5% ($P<0.05$) 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±1.1%, $P=NS$ at the highest concentration, data not shown).

**Effects of Progesterone on $[Ca^{2+}]_i$ of VSMC**

The effects of progesterone on $[Ca^{2+}]_i$ are shown in Figure 3 (original recording in the Top Panel and summary data in the Lower Panel). Basal $[Ca^{2+}]_i$ averaged 110±5.4 nmol/L. Progesterone alone ($5 \times 10^{-7}$ mol/L) did not produce any significant alteration of resting $[Ca^{2+}]_i$ (Figure 3). KCl (30 mmol/L) increased $[Ca^{2+}]_i$ by 71.6±9.9% (mean $[Ca^{2+}]_i$ increase: +78.8±10.9 nmol/L, $P<0.001$). However, when KCl (30 mmol/L) was added after 10 minutes’ incubation with progesterone ($5 \times 10^{-7}$ mol/L), the $[Ca^{2+}]_i$ increase was significantly inhibited by 64.1±5.3% (mean $[Ca^{2+}]_i$ increase: +28.2±4.8 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, $[Ca^{2+}]_i$ again increasing to 87.0±6.8% of the original response ($[Ca^{2+}]_i$: +68.6±8.2 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 $[Ca^{2+}]_i$ 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
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. 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. 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; to blunt the pressor response to Ang II in ovariectomized rats (which estrogen does not); and to alter adrenergic activity, decreasing systemic vascular resistance in normal women 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 and human placental arteries and veins. 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, progesterone directly decreased L-current, and in association with this, progesterone blunted KCl-induced elevation of [Ca2+]i. 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 [Ca2+]i 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, of PAI-1 and of endothelin-1 by endothelial cells, (2) the blunted pressor responses to infused Ang II, and thus (3) the lowering of BP in normal pregnancy, in pregnancy-associated hypertension, and in hypertensive men and postmenopausal women. In pregnancy, progesterone also augments nifedipine-induced inhibition of uterine contractions.

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 adrenoreceptors, 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 other in vitro systems,34 these levels are not observed physiologically outside pregnancy. However, non-pregnant levels 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 vasconstrictor tone in pregnancy. These direct vascular actions of progesterone, along with its natriuretic 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


Vascular Effects of Progesterone: Role of Cellular Calcium Regulation
Mario Barbagallo, Ligia J. Dominguez, Giuseppe Licata, Jie Shan, Li Bing, Edward Karpinski,
Peter K. T. Pang and Lawrence M. Resnick

Hypertension. 2001;37:142-147
doi: 10.1161/01.HYP.37.1.142
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/37/1/142