Calcium channels contribute to the decrease in blood pressure of pregnant rats

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Simaan, May, Chanterelle Cadorette, Matthieu Poterek, Jean St-Louis, and Michèle Brochu. Calcium channels contribute to the decrease in blood pressure of pregnant rats. Am J Physiol Heart Circ Physiol 282: H665–H671, 2002; 10.1152/ajpheart.01183.2001.—Pregnancy is associated with hemodynamic changes such as reduced vascular resistance and blood pressure. We reported that, during late pregnancy, the activity of voltage-dependent calcium channels (VDCC) is altered in the adrenal cortex and vascular smooth muscle. These observations suggested that the late pregnancy-induced decrease in blood pressure is linked to diminished VDCC function. We attempted to prevent pregnancy-induced reduced blood pressure with a calcium channel activator (CGP 28392) in pregnant rats and to mimic it by administration of a calcium channel blocker (nifedipine) to nonpregnant rats. Treatment was given from the 15th day of gestation for 7 days. The systolic blood pressure of CGP 28392-treated pregnant rats rose transiently for 2 days and then declined toward values of nontreated pregnant controls, although remaining higher. However, nonpregnant rats maintained their high arterial pressure throughout CGP 28392 treatment. Nifedipine lowered the blood pressure in nonpregnant rats to values of nontreated term-pregnant controls. Both agents did not affect body weight, water or food intake, plasma renin activity, and plasma aldosterone or corticosterone levels. Nifedipine did not affect body weight, water or food intake, plasma renin activity, and plasma aldosterone or corticosterone levels. Nifedipine and CGP 28392 treatment of nonpregnant and pregnant animals, respectively, did not modify the response of aortic rings to KCl. These results show that VDCC activation caused hypertension, which modified the extent of the decrease in blood pressure at the end of pregnancy.

Several mechanisms have been proposed for the decrease in blood pressure and vascular resistance as well as for the associated blunted effects of vasoressor agents observed during pregnancy: 1) augmented liberation of endogenous vasodilators (prostacyclin and nitric oxide (NO)); 2) modifications of mechanical properties and/or tissue composition (ratio of smooth muscle to connective tissue or elastin to collagen), leading to increased elasticity of blood vessel walls; and 3) reduced excitation-response coupling for vasoactive substances (angiotensin II, vasopressin, and phenylephrine) (13, 25). Accumulating evidence suggests that the latter mechanism may be significantly involved.

In earlier studies, we reported that, during late pregnancy, calcium influx through voltage-dependent calcium channels (VDCC) is functionally impaired in zona glomerulosa cells of the adrenal cortex (3, 22), aortic rings (17), and mesenteric resistance arteries (26). Recent results indicate that the functional decline of VDCC activity is linked to reduced kinetics of extracellular Ca2+ mobilization in blood vessels (18). These observations led us to believe that activation of VDCC during the last week of pregnancy would prevent the decrease of blood pressure normally seen in this period. Our objectives were to treat pregnant rats with a calcium channel activator, CGP 28392, to prevent the pregnancy-induced fall in blood pressure and to mimic it in nonpregnant rats with a calcium channel blocker, nifedipine.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats (Charles River; St. Constant, Quebec, Canada) weighing 225–250 g were mated with males. The morning when spermatozoa were found in vaginal smears was deemed to be day 1 of pregnancy. Nonpregnant rats were picked randomly during the estrous cycle. All animals were housed under controlled lighting (from 0600 to 1800 hours) and temperature (21 ± 3°C) and received a normal diet (Charles River Rodent Chow 5075). This study received approval from the institutional animal care committee, which is accredited by the Canadian Council on Animal Care.

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APPROXIMATELY 7–10% OF ALL PREGNANCIES are complicated by hypertension that is believed to result from the suppression of normal physiological responses to pregnancy. This syndrome is the major cause of maternal and perinatal morbidity and mortality. Its physiopathology is far from being understood because of our scant knowledge of the hemodynamic mechanisms responsible for reduced vascular resistance and blood pressure in normal pregnancy. Despite numerous reports on cardiovascular regulation during pregnancy (15), further investigation is needed.
Treatment protocol. On day 15 of experimentation, the rats were divided into four groups: nonpregnant and pregnant controls receiving only the vehicle (<1% ethanol in water), nonpregnant experimental animals treated with nifedipine (5 mg·kg⁻¹·day⁻¹), and pregnant experimental animals treated with CGP 28392 (10 mg·kg⁻¹·day⁻¹). Stock solutions of nifedipine (5 mg/ml) and CGP 28392 (10 mg/ml) were prepared in ethanol and diluted to the appropriate concentration (according to animal weight) in drinking water. Final ethanol concentration was <1%. Water bottles containing nifedipine and CGP 28392 were wrapped with aluminum foil to protect these agents from photodegradation. The treatments lasted for 7 days, which corresponds to the last week of gestation (i.e., the third week). Water bottles were changed every day, the drug concentration being adjusted for increasing body weight. In the course of the study, a fifth group of rats was added: nonpregnant rats receiving CGP 28392 (10 mg·kg⁻¹·day⁻¹).

Physiological measurements. Systolic blood pressure was measured by the indirect tail cuff method (model 50-0001, Rat Tail Blood Pressure Apparatus; Harvard Apparatus; St. Laurent, Quebec, Canada). The animals were trained for blood pressure measurement on days 5 and 6. Systolic blood pressure was then recorded from day 7 until day 22 (corresponding to the end of treatment). Data were compared with a two-factor analysis of variance with repeated measures. Because of the presence of interaction between days of gestation and treatment, the Student’s t-test was performed day by day.

Body weight was measured on day 1 and daily from day 6 to the end of treatment (day 22). Water intake and food consumption were recorded every day during treatment.

Sample collection and analysis. On day 22 (end of treatment), the animals were decapitated, blood samples were drawn into plain Vacutainer tubes (Becton-Dickinson; Franklin Lakes, NJ) and centrifuged at 3,000 rpm for 20 min at 4°C, and plasma was stored at −80°C until used. The left ventricle of the heart of untreated and treated rats as well as total fetoplacental units of each litter were weighed. The number of fetuses was recorded.

For plasma aldosterone measurement, plasma was extracted by a solid-phase procedure with C18 Sep-Pak cartridges (Millipore; Montréal, Quebec, Canada) and then quantitated by radioimmunoassay, as described elsewhere (2). Plasma renin activity was determined indirectly by radioimmunoassay of angiotensin I generated during a 2-h incubation period (7). The antibody used for this radioimmunoassay was purchased from Peninsula (Belmont, CA). Corticosterone was measured directly in plasma with a commercial radioimmunoassay kit (Medicorp; Montréal, Quebec, Canada). These results were compared using one-factor analysis of variance.

Plasma nifedipine or CGP 28392 was measured by HPLC according to the method of Snedden et al. (23) with modifications. HPLC was conducted with a Hewlett-Packard 1050 equipped with a spectrophotometer detector connected to a recorder. Briefly, 0.25 µg of the internal standard (isradipine) and 100 µl of NaOH (1 M) were added to 1 ml of serum. After extraction with diethyl ether, the residue was suspended in 100 µl of HPLC mobile phase (acetonitrile:H₂O, 50:50). Nifedipine, CGP 28392, and isradipine were resolved on a Nova PaK C18 column (Waters; Mississauga, Ontario, Canada) with a flow rate of 0.5 ml/min. Absorbance was monitored at 235 nm. Standard solutions of nifedipine, CGP 28392, and isradipine were dissolved in the appropriate volume of the HPLC mobile phase.

Organ bath assay. Vascular reactivity was measured in thoracic aorta rings as previously described (17, 27). In brief, after decapitation, the thoracic aorta was removed rapidly and cut into four consecutive rings (2–3 mm), which were mounted in individual jacketed tissue baths (15 ml, Radnotti Glass; Monrovia, CA) maintained at 37°C. The endothelium of each ring was gently removed by rubbing the lumen with an 18-gauge needle. In each experiment, four rings from both nonpregnant and pregnant rats were used. They were equilibrated for 60 min under 2.0 g of passive tension (27), with frequent washing and tension adjustment. The tissues were bathed in Krebs bicarbonate solution (KBS) of the following composition (in mM): 118 NaCl, 4.65 KCl, 25 NaHCO₃, 2.5 CaCl₂, 1.18 MgSO₄, 1.18 KH₂PO₄, and 5.5 dextrose. The solution was bubbled with a mixture of 95% O₂-5% CO₂; pH was 7.4. After equilibration, the tissues were challenged with 1.0 µM phenylephrine. At plateau response, carbachol (0.1 mM) was added to ensure removal of the endothelium. Tension was measured by force displacement transducers and recorded on a computerized data acquisition system using WorkBench software (Kent Scientific; Litchfield, CT).

Cumulative concentration-response curves to KCl (2–100 mM, added to normal KBS) were obtained. One of the rings of both nonpregnant and pregnant rats (control and treated) served as control while each of the other rings was preincubated with nifedipine (1, 10, or 100 nM) or BAY K 8644 (10 or 100 nM or 1 µM) added 10 min before charting the curve. BAY K 8644 was used instead of CGP 28392 in these in vitro experiments to compare with our previous results (17). After several washouts, the concentration-response curve to KCl was repeated in the presence of the dihydropyridine (nifedipine or BAY K 8644) that was not used in the first curve to KCl. The experiments were performed under sodium lamps to prevent photodegradation of these substances.

Each concentration-response curve was analyzed by computer fitting to a four-parameter logistic equation with the Prism 3.0 program (GraphPad Software; San Diego, CA) to evaluate the concentration producing 50% of the maximal response (EC50) and the maximum asymptote of the curve (max). Different curves from the same protocol were compared by two-way ANOVA on the mean negative logarithm of the EC50 (mean pD₂), on mean Emax, and on the lower asymptote of the curve (when required). Data are expressed as mean experimental points ± SE along the best curve fitted to these points.

Drugs and chemicals. All salts employed in these experiments were of analytic grade and obtained from Fisher Scientific (Montréal, Quebec, Canada). Phenylephrine hydrochloride and carbamoylcholine chloride (carbachol) were purchased from Sigma (St. Louis, MO). Nifedipine hydrochloride and BAY K 8644 (methyl ester), used in the in vitro experiments, as well as CGP 28392 were obtained from Research Biochemical International (Natick, MA). Nifedipine, used for animal treatment, was a gift from Bayer (Toronto, Ontario, Canada).

RESULTS

The systolic blood pressure of nonpregnant control rats was constant throughout treatment (Fig. 1). Nifedipine caused a decrease in blood pressure in nonpregnant animals that was manifested after 1 day and progressed gradually throughout treatment; on the last 3 days of treatment, it reached values similar in magnitude to that of vehicle-treated term-pregnant rats. In the latter group, systolic blood pressure was
constant until day 18 of gestation (128 ± 2 mmHg) and then declined gradually to reach a value of 107 ± 2 mmHg at term (Fig. 1).

In pregnant rats, CGP 28392 caused a transient increase of blood pressure to 145 ± 2 mmHg for the first 2 days of treatment and then decreased progressively on day 18 of gestation until term (Fig. 2). This decrease was parallel to that of vehicle-treated pregnant animals. However, blood pressure measurements were significantly higher in CGP 28392-treated rats than in vehicle-treated pregnant rats, at all time points, until term. These results led us to introduce a fifth group, nonpregnant rats given CGP 28392, to test the efficacy of the drug. Systolic blood pressure in these animals rose to 152 ± 2 mmHg after 2 days of CGP 28392 treatment and remained at this high level until death.

At the beginning of the study (day 1), body weight was similar in nonpregnant and pregnant rats (252 ± 4 vs. 250 ± 5 g, respectively). From day 9 until term, there was a significant increase of body weight in pregnant compared with nonpregnant animals (values at term: 287 ± 5 vs. 264 ± 5 g, respectively). Treatment of nonpregnant rats with nifedipine or CGP 28392 as well as treatment of pregnant rats with CGP 28392 did not affect this parameter. Treatments did not affect food and water intake in both groups of animals or weight of the placental unit of the pregnant rats (Table 1). However, nifedipine induced an increase in the left ventricular weight-to-total body weight ratio compared with the controls.

Treatment of nonpregnant rats with nifedipine and pregnant rats with CGP 28392 (Table 2) did not affect the renin-angiotensin system, as shown by plasma renin activity and plasma aldosterone levels. There was no significant effect of treatments on plasma corticosterone levels.

Serum levels of nifedipine and CGP 28392, measured in nonpregnant and pregnant rats after 7 days of drug administration, are shown in Table 2. Mean plasma nifedipine concentration in nonpregnant rats was 85 ± 7 ng/ml, whereas CGP 28392 reached similar levels in the plasma of pregnant (25 ± 5 ng/ml) and nonpregnant animals (19 ± 8 ng/ml).

To evaluate the vascular consequences of treatments, concentration-response curves to KCl were measured on aortic rings of control and treated rats. In a previous study (17), the in vitro effects of nifedipine and BAY K 8644 were decreased in pregnant compared with nonpregnant rats. In the present investigation, we wanted to see whether in vivo treatments with dihydropyridines could alter the in vitro vascular reactivity to KCl in the absence or presence of VDCC modulators. For clarity, only the results with BAY K 8644 (1 μM) and nifedipine (10 nM) are shown (Fig. 3). Chronic nifedipine treatment did not modify the action of KCl on aortic rings (Fig. 3, A and B, closed circles and squares). In Fig. 3A, addition of 1 μM BAY K 8644 (open symbols) to the tissue baths potentiated the response to KCl in both groups, reducing the concentration required to reach a maximum response. Upon addition of 1 μM BAY K 8644 to the tissue baths, increased tone was observed in the rings of the two groups, as reported previously (17). The potentiation was larger in nifedipine- than vehicle-treated animals.

Concentration-response curves to KCl were also measured in aortic rings of vehicle- and CGP 28392-treated pregnant rats in the absence or presence of BAY K 8644 or nifedipine (Fig. 3, C and D). Treatment with CGP 28392 did not modify the concentration-response curves to KCl in pregnant rats compared with vehicle-treated controls. The addition of BAY K 8644 (1 μM) similarly potentiated responses to KCl, again reducing the concentration required to reach maximum response. Nifedipine induced a similar decrease in the maximal response to KCl in both groups. It should be noted that, in all instances, the response to KCl in the aorta of pregnant rats was blunted, both in

![Fig. 1. Effect of nifedipine on systolic blood pressure in nonpregnant rats. The numbers in parentheses indicate the number of rats used. Data points represent the means; error bars represent SE.](http://ajpheart.org)

![Fig. 2. Effect of CGP 28392 on systolic blood pressure in pregnant and nonpregnant rats. The numbers in parentheses indicate the number of rats used. Data points represent the means; error bars represent SE.](http://ajpheart.org)
maximum responses and sensitivity (increased EC$_{50}$). This blunted response was not modified by CGP 28392 treatment of pregnant rats. Treatment of nonpregnant rats with CGP 28392 did not either affect the response to KCl, which was similarly potenti ed in both groups by addition of BAY K 8644 to tissue bath (data not shown).

**DISCUSSION**

The purpose of this study was to test the involvement of VDCC in the regulation of pregnancy-induced decrease in blood pressure. The major findings were as follows. First, nifedipine, a calcium channel blocker, elicited a reduction of blood pressure in nonpregnant rats. Second, CGP 28392, a calcium channel activator, caused a transient elevation of blood pressure in pregnant rats followed by a decrease. Despite continued treatment, blood pressure measurements remained significantly higher but parallel to that of pregnant control rats. Third, CGP 28392 evoked a maintained increase of blood pressure in nonpregnant rats throughout treatment. Fourth, body weight, the renin-angiotensin system, and plasma corticosterone levels as well as litter size and weight of the placental unit of pregnant rats were not affected by treatment in nonpregnant and pregnant rats. Finally, treatment of nonpregnant rats with nifedipine and pregnant rats with CGP 28392 did not modify the in vitro aortic response to KCl. These data suggest that, although functional alterations of VDCC have been documented during pregnancy, increasing their activity with a VDCC activator (CGP 28392) did not prevent the decline in blood pressure or the blunted response to KCl.

Pregnancy-associated decreased blood pressure is well documented, but the underlying mechanisms have not been elucidated. It has been proposed that increased endothelium-derived NO production contributes to maternal systemic vasodilatation during pregnancy. Indeed, inhibition of NO synthesis during rat pregnancy causes hypertension (28). However, even in the absence of functional endothelium, the reactivity of aorta rings of pregnant rats to vasoconstrictor is still decreased (17, 18, 27). Moreover, Buhimschi et al. (5) showed that chronic NO inhibition in Sprague-Dawley rats resulted in an initial rise in systolic blood pressure on the day after $N^\omega$-nitro-L-arginine methyl ester treatment (17 or 18 days of pregnancy) that persisted for 1 day only and rapidly returned to values in the range obtained in untreated pregnant rats. These results show that NO is, at least, not the only factor involved in the pregnancy-induced decrease in blood pressure.

Calcium channels play an important role in controlling blood vessel tone. They allow transmembrane influx of calcium from the extracellular space into the intracellular compartment, thus increasing vascular tone and resistance, which are important determinants of blood pressure regulation. We have shown that the activity of VDCC, but not the density of channel molecules, is decreased during pregnancy in zona glomerulosa cells of the adrenal cortex (3, 22), aortic rings (17), and mesenteric resistance arteries (26).

In this study, we used nifedipine as the calcium channel blocker to mimic the pregnancy-induced decrease of blood pressure. Calcium channel blockers act by inhibiting transmembrane calcium ion influx.

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**Table 1. Effects of treatments (days 16–22) on weight gain, food intake, water intake, fetoplacental unit weight, and left ventricle-to-total body weight ratio in nonpregnant and pregnant rats**

<table>
<thead>
<tr>
<th></th>
<th>Food Intake, g/day</th>
<th>Water Intake, ml/day</th>
<th>Left Ventricle/Total Body Weight, $\times 10^{-3}$</th>
<th>Weight of the Fetoplacental Unit, g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonpregnant rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18.2 ± 0.4(16)</td>
<td>29.5 ± 0.9(16)</td>
<td>2.5 ± 0.1(16)</td>
<td></td>
</tr>
<tr>
<td>Nifedipine treated</td>
<td>18.4 ± 0.7(16)</td>
<td>30.1 ± 0.5(16)</td>
<td>2.8 ± 0.1(16)*</td>
<td></td>
</tr>
<tr>
<td><strong>Pregnant rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>24.7 ± 0.4(13)</td>
<td>47.5 ± 1.5(13)</td>
<td>2.4 ± 0.0(13)</td>
<td>6.08 ± 0.09(13)</td>
</tr>
<tr>
<td>CGP28392 treated</td>
<td>26.9 ± 1.3(15)</td>
<td>51.8 ± 0.6(15)</td>
<td>2.4 ± 0.1(15)</td>
<td>5.98 ± 0.07(15)</td>
</tr>
</tbody>
</table>

Results are mean values for days 16–22 ± SE. The numbers in parentheses indicate the numbers of rats used. *P < 0.05 vs. nonpregnant control rats.

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**Table 2. Effects of treatments (days 16–22) on plasma renin, aldosterone, corticosterone, and nifedipine in nonpregnant rats and CGP 28392 in pregnant rats**

<table>
<thead>
<tr>
<th></th>
<th>Plasma Renin Activity, pmol ANG l⁻¹·h⁻¹</th>
<th>Aldosterone, nmol/ml</th>
<th>Corticosterone, nmol/ml</th>
<th>Plasma Nifedipine or CGP 28392, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonpregnant rats</strong></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>1.79 ± 0.43(16)</td>
<td>0.82 ± 0.17(16)</td>
<td>0.95 ± 0.45(16)</td>
<td></td>
</tr>
<tr>
<td>Nifedipine treated</td>
<td>1.93 ± 0.43(16)</td>
<td>0.79 ± 0.21(16)</td>
<td>0.67 ± 0.35(16)</td>
<td>85 ± 7(16)</td>
</tr>
<tr>
<td><strong>Pregnant rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.00 ± 0.93(13)</td>
<td>3.55 ± 1.01(13)</td>
<td>0.84 ± 0.38(13)</td>
<td></td>
</tr>
<tr>
<td>CGP28392</td>
<td>8.08 ± 1.62(15)</td>
<td>3.33 ± 0.66(15)</td>
<td>0.95 ± 0.39(15)</td>
<td>25 ± 5(15)</td>
</tr>
</tbody>
</table>

Results are means ± SE. The numbers in parentheses indicate the numbers of rats used.
from the extracellular space into the cytoplasm. This blocks excitation-contraction coupling in smooth muscle and results in reduced vascular tone, lowering blood pressure. With nifedipine administration to nonpregnant rats, blood pressure decreased and, after 4 days of treatment, reached values similar to those in control pregnant rats. This is in agreement with the work of Ishii et al. (8), who showed that nifedipine reduced the blood pressure of male normotensive rats by 21.8%, a level similar to that observed in the present study. In opposition, another study (14) reported that nifedipine failed to decrease blood pressure in male Sprague-Dawley rats. In their experiments, direct blood pressure was recorded from the carotid artery in anesthetized animals in which nifedipine (0.2–0.9 μmol/kg) was administered intravenously. These conflicting results are possibly related to the animal strain, anesthesia, dosage, and methods for blood pressure measurements used. Moreover, blood pressure may vary according to sex. For example, Ashton and Balment (1) reported that male New Zealand hypertensive rats had higher blood pressure than their female counterparts. Our study shows, for the first time, that nifedipine significantly decreased blood pressure in conscious female normotensive rats.

Because previous experiments have suggested that pregnancy evokes depressed function of VDCC in blood vessels, we used a VDCC activator (CGP 28392) to overcome this effect. Calcium channel activators are
effective vasoconstrictors and act by enhancing calcium influx through calcium channels. BAY K 8644 was the first derivative of this class of drugs with calcium agonist activity to be studied extensively (21). Lefer et al. (9) showed that BAY K 8644 dose dependently (1–20 μg/kg) increased arterial blood pressure in male Sprague-Dawley rats. The majority of published investigations have dealt with the actions and properties of this drug. However, CGP 28392 also activates calcium channels (4). Preuss et al. (16) demonstrated that CGP 28392, 25 to 150 μg/kg administered intravenously, increased blood pressure in a dose-dependent manner in conscious dogs. In the present study, we treated pregnant and nonpregnant rats with CGP 28392 at the same oral dosage (10 mg·kg−1·day−1). Interestingly, we found that the calcium channel activator induced a transient increase of blood pressure in pregnant rats, but this was not maintained throughout treatment. Indeed, on day 18 of gestation, blood pressure began to decline but stayed higher than that of vehicle-treated pregnant controls. However, the blood pressure of CGP 28392-treated nonpregnant rats rose and maintained a steady value until death. Our results on plasma CGP 28392 levels were similar in nonpregnant and pregnant animals, indicating that the different effects observed on blood pressure in both groups were not the consequences of different drug distribution or metabolism. These results suggest that some physiological phenomenon taking place at around day 18 of gestation are specific to this condition and elicit decreased blood pressure that cannot be overcome by activation of VDCC. The higher blood pressure measurements in CGP 28392-treated pregnant rats compared with their controls show that VDCC activation is partially responsible for the pregnancy-induced decrease in blood pressure. It is relevant to mention that blood pressure in both vehicle- and CGP 28392-treated pregnant rats began to fall on day 18 of gestation, a landmark of this phenomenon.

KCl induces smooth muscle contraction by depolarization of smooth muscle cell membranes, opening VDCC. Previous work (17) has demonstrated decreased reactivity to phenylephrine and KCl on isolated aortas from pregnant compared with nonpregnant rats. Our results of aortic rings reactivity to KCl confirm our present observations with blood pressure: that in vivo administration of CGP 28392 in pregnant rats did not modify the in vitro blunted responses to KCl. However, in nonpregnant rats treated with nifedipine, reactivity to KCl in vitro was the same as in their controls even though blood pressure was decreased in the nifedipine group. We conclude that, although nifedipine mimicked the pregnancy-induced reduction of blood pressure, it failed to blunt the responses to KCl in vitro.

BAY K 8644 by itself has been shown to cause concentration-dependent contraction of isolated vessels in nonpregnant rats that is virtually absent in pregnant animals (17). Indeed, we noted an increased tone only in nonpregnant rats after BAY K 8644 addition, which was comparable in magnitude between nifedipine- and vehicle-treated controls. Roy et al. (17) reported that the differential response to BAY K 8644 was abolished when tissues from nonpregnant and pregnant rats were preincubated with equiactive small concentrations of KCl. In this case, the contractile effects of BAY K 8644 were augmented in both groups, making the concentration-response curves to BAY K 8644 identical in the aortas of pregnant and nonpregnant animals. This observation suggests that the blunted response of blood vessels to BAY K 8644 might be dependent on altered membrane potential. The work of Meyer et al. (10) is compatible with the latter suggestion; they reported that smooth muscle cells of the mesenteric arteries in pregnant rats are hyperpolarized by 7 mV compared with nonpregnant rats. Calcium channels may exist in different conformational states: closed, open, and inactivated (24). The proportion of channel molecule in different states is regulated by membrane potential. It has been shown that membrane hyperpolarization of 2 mV decreases the opening probability and thus calcium entry by ~30% (12). If pregnancy is associated with hyperpolarization, then we could expect a greater proportion of the channels to be in a closed state. On the other hand, membrane potential is largely regulated by potassium channels. When open, they repolarize the cell membrane and cause a decrease in its depolarization. Our laboratory (6) has reported their increased activity during pregnancy.

The renin-angiotensin-aldosterone system is activated during pregnancy. In our study, we measured plasma renin activity and plasma aldosterone levels and showed that they were augmented in pregnancy and were similar to previous results reported by our laboratory (19). Although blood pressure decreased in nonpregnant nifedipine-treated animals and transiently increased in CGP 28392-treated rats, their plasma renin activity and plasma aldosterone were similar to their respective controls. In menopausal women, estrogen replacement therapy (0.2-mg patch) was associated with lower blood pressure, unchanged plasma renin activity, and plasma aldosterone levels compared with placebo-treated women (20). This indicates that in the conditions of our model, treatment of nonpregnant and pregnant rats with calcium channel modulators does not affect the active components of the renin-angiotensin-aldosterone system. Nifedipine caused cardiac hypertrophy in nonpregnant rats. This was consistent with the work of Murphy et al. (11) and could be explained by reflex activation of the sympathetic system due to a decrease in blood pressure. In addition, Zimmer (29) reported that both β- and α-adrenergic stimulation induced cardiac hypertrophy in rats. High levels of corticosteroids are usually reported during stressful conditions. Both treatments did not have an effect on plasma corticosterone levels.

In conclusion, our results show that, although calcium channels are important regulators of blood pressure, pregnancy is an antihypertensive state where these channels contribute partially to the normally reported decrease of blood pressure. Further investigation is needed to elucidate the mechanisms that evoke
REFERENCES


