Enhanced vascular effects of the Ca\(^{2+}\) channel agonist Bay K 8644 in pregnant rabbits

ROCCO VENUTO,1 GAIL BROWN,2 MARION SCHOENL,1 AND GYÖRGY LOSONCZY1,3

1Schools of Medicine and Biomedical Science and 2Nursing, University at Buffalo, State University of New York, Buffalo, New York 14215; and 3Department of Pulmonology, Semmelweis University Medical School, Budapest 114, 1536 Hungary

Received 6 August 2001; accepted in final form 7 December 2001

Venuto, Rocco, Gail Brown, Marion Schoenl, and György Losonczy. Enhanced vascular effects of the Ca\(^{2+}\) channel agonist Bay K 8644 in pregnant rabbits. Am J Physiol Regulatory Integrative Comp Physiol 282: R952–R959, 2002.—Hemodynamic studies were performed to determine if blunting of vascular pressor responsiveness to vasoconstrictors during pregnancy may be due to impaired L-type voltage-dependent calcium channels (L-VDCC). Bay K 8644 (BAY), an L-VDCC agonist, was infused in pregnant and nonpregnant anesthetized rabbits (10, 20, 40, and 60 µg/kg) and pregnant and nonpregnant conscious, chronically instrumented (conscious) rabbits (10, 25, and 50 µg/kg). BAY infusions resulted in greater elevation of mean arterial pressure in both anesthetized pregnant (n = 6) vs. nonpregnant (n = 6) (P < 0.05) and conscious pregnant (n = 10) vs. nonpregnant (n = 10) rabbits (P < 0.05). Fractional increase over baseline of total peripheral resistance index was greater in pregnant (36 ± 5 to 78 ± 14%) vs. nonpregnant rabbits (14 ± 4 to 52 ± 6%) (P < 0.02). Cardiac output index did not differ. There was a single high-affinity L-VDCC antagonist aortic binding site with similar number and affinity in pregnant (n = 7) and nonpregnant (n = 7) rabbits. In conclusion, stimulation of L-VDCC induces greater pressor responses in pregnant rabbits with heightened peripheral vasoconstriction. This does not appear to be due to a change in L-VDCC receptor parameters.

blood pressure; vascular reactivity; calcium channels; nifedipine

PHYSIOLOGICAL ALTERATIONS that occur during pregnancy in humans (7) and experimental mammals (36) include reductions in systemic vascular resistance and mean arterial blood pressure (MAP). Another hallmark of gestation is the nonspecific blunting of the hypertensive effect of pressor hormones such as ANG II, norepinephrine, and vasopressin (10, 12). On the cellular level, these pressor compounds bind with specific receptors and the subsequent activation of a signal transduction cascade ultimately leads to an increased concentration of free intracellular calcium in vascular smooth muscle cells, which is currently considered as the final mediator of vasoconstriction (33). The mechanism of the pregnancy-induced decrease of vascular pressor responsiveness to these hormones has not yet been clarified. Downregulation of specific receptors (2, 13, 18), enhanced secretion of vasodilatory substances (9), and alterations of receptor-response coupling (uncoupling) (8, 11, 28, 29, 34) have been considered as mediators of decreased vasopressor response to these hormones.

A major portion of the intracellular calcium elevation triggering contraction results from the opening of the L-type, voltage-dependent calcium channel (L-VDCC) (27). A gestational alteration of the vascular L-VDCC has already been suggested. For example, activation of the channel as induced by depolarization or Bay K 8644 (BAY), a specific L-VDCC agonist (31), was observed to be a less potent signal for vascular constriction in aortic rings and mesenteric arteries of pregnant than nonpregnant rats (11, 28, 29, 34). In addition, chronic estrogen administration was noted to decrease the number of L-VDCC (26). On the other hand, the activation of the L-VDCC in vitro by administration of BAY has been shown to induce a strong hypertensive effect in the dog (31), the male rabbit (6), and the rat (17), and also to cause constriction of the isolated rabbit aorta (31). We tested the hypothesis that the nonspecific refractoriness to pressor compounds in pregnant rabbits (10, 37) might include BAY. If so, this would indicate impaired function of the vascular L-VDCC, which, in turn, could underlie the nonspecific vascular pressor refractoriness developing during gestation. Also explored was the possibility that pregnancy might alter either the vascular receptors for BAY (the \(\alpha_1\)-subunit of the L-VDCC) (14) or the vascular release of vasoactive prostanooids, the latter of which can either attenuate (23) or potentiate (5) the effects of smooth muscle contracting substances.

METHODS

Animals

All experiments were undertaken in New Zealand White rabbits weighing 3.5–5 kg. Pregnant rabbits were studied...
within the final 8 days of the 30 ± 2 day gestation. Nonpregnant animals of similar weight and age served as controls in all protocols. The rabbits were housed individually in cages and received standard rabbit chow and water ad libitum. All protocols were reviewed and given approval by the Institutional Review Boards of the University at Buffalo and Semmelweis University, Budapest, Hungary.

Physiological Studies

Animal preparation. Acutely studied rabbits: The protocol followed was described previously in similar studies (37). Briefly, general anesthesia was achieved with intravenous pentobarbital sodium (30 mg/kg). With the use of sterile surgical technique, the femoral artery and vein were isolated and catheterized. The right jugular vein and right carotid artery were isolated. A catheter was placed in the jugular vein and advanced down the superior vena cava close to the right atrium. A thermistor (Columbus Instruments, Columbus, OH) was placed in the right carotid artery and advanced to the aortic arch. The animals, six pregnant and six nonpregnant controls, were studied after a stabilization period of at least 30 min after surgery, and anesthesia was maintained with pentobarbital sodium (10 mg/kg iv).

Conscious, chronically instrumented rabbits: The protocol followed was described previously in similar studies (10, 19, 20). After induction of general anesthesia, the left femoral artery was isolated and catheterized so that the catheter tip lay in the distal aorta. The ipsilateral femoral vein was catheterized, and the catheter was advanced to the inferior vena cava. The carotid artery was also catheterized, and the catheter was advanced to just before the aortic arch. Pregnant rabbits were instrumented between the 21st and 24th days of gestation. Experiments were performed no earlier than 72 h after instrumentation and only animals that evidenced complete recovery of normal movement, drinking, and eating habits were used. More than 95% of the rabbits who underwent the ~45-min surgery were employed in this study.

Animal studies. Acute rabbit studies: In six pregnant and six nonpregnant rabbits, MAP, heart rate (HR), and cardiac output (CO) were measured and recorded using the Cardiomax II System (Columbus Instruments). Baseline MAP and HR were determined using the mean of five consecutive readings, 1 min apart. Bolus doses of BAY (Sigma, St. Louis, MO) ranging from 10 to 50 μg/kg body wt and diluted in 1 ml saline were administered in random order, over 30 s, and the animals were allowed to recover at least 30 min between doses. In some experiments, only the saline diluent was administered. MAP and HR were recorded every 15 s for the first 2 min and every min thereafter for 18 min. We report the peak change from baseline, which consistently developed within 20–40 s after the bolus was given.

Nifedipine infusion studies: In separate experiments performed on different days and in the same manner as described above, 10 pregnant and 10 nonpregnant rabbits were given the dihydropyridine calcium channel blocker nifedipine (10–50 μg/kg body wt iv; Sigma). Also, in another series of experiments, a dose of nifedipine (25 μg/kg) was used to attempt to block the action of the calcium channel enhancer, BAY. This dose was determined empirically by testing the response to a range of doses from 10 to 50 μg/kg.

Biochemical analyses. Dihydropyridine binding in aortic membranes: Specific binding of PN 200–110, (+)-[5-methyl-3H] (specific activity 79.7 Ci/mM, DuPont, New England Nuclear, Boston, MA), a 1,4-dihydropyridine calcium channel antagonist, was assessed in aortic membranes from seven pregnant and seven nonpregnant rabbits using a radioreceptor assay similar to that previously described (18). Aortic membranes were prepared similar to that previously described for other vascular tissues (18). The final pellets were suspended in 50 mM Tris-HCl, pH 7.4, and were used immediately thereafter in the radioreceptor assay. Protein concentrations were determined by the Lowry method (21).

Steady-state binding was attained by 120 min of incubation at 24°C and specific binding was proportional with aortic protein concentrations to 125 μg/tube. The aortic protein concentrations used in the radioreceptor assays were 29.7 ± 4 and 28.0 ± 2 μg/tube for pregnant and nonpregnant rabbits, respectively. PN 200–110, (+)-[5-methyl-3H] receptor equilibrium dissociation constant (Kd) and receptor number (Bmax) were determined by Accufit Competition nonlinear analysis of binding inhibition data (Beckman Instruments, Fullerton, CA). The binding inhibition data were generated by incubating the aortic membranes with the radioligand (5 × 10–11 M) and varying quantities of unlabeled S-(+)-BAY (5.6 × 10–11 M–5.6 × 10–7 M). The incubation media was 50 mM Tris-HCl, pH 7.4, and the total incubation volume was 2.5 ml. When steady-state binding was attained, bound and free were separated by rapid Millipore filtration (Whatman GF/F glass microfiber filters) under vacuum. Filters were rinsed with 4 ml of ice-cold incubation buffer four times. The radioactivity trapped on the filter was measured by liquid scintillation counting, at an efficiency of 60%. Nonspecific binding is determined by an excess (5.6 × 10–5 M) of BAY.

In vitro vascular eicosanoid responses to BAY. Sample preparation: Production of 6-keto PGF1α, a stable metabolite of prostacyclin, and thromboxane B2 (TXB2), a stable metabolite of thromboxane A2, in response to BAY was determined similarly to that previously described (4). For each experiment, aortic tissue was obtained from one nonpregnant rabbit (3.9 ± 0.2 kg body wt, n = 6) or one pregnant rabbit (day 24–28 of gestation, 3.9 ± 0.2 kg body wt, n = 6). Rabbids were euthanized with intravenous pentobarbital sodium, 100 mg/kg, and the aortic tissue was quickly removed and rinsed in ice-cold incubation buffer (Kreb’s-Henseleit bicarbonate...
containing 25 mM HEPES and bubbled with 95% O₂-5% CO₂. On an iced glass plate, adjoining tissue was gently removed, and the vascular tissue was sliced into 1-mm rings. The rings were apportioned by weight into incubation tubes with 1 ml of incubation buffer and used immediately thereafter in experimental protocols.

EXPERIMENTAL PROTOCOLS: All incubations, except the 4°C controls, were performed in a shaking water bath at 37°C under an environment of 95% O₂-5% CO₂. The production of 6-keto PGF₁α and TXB₂ attained a maximal level between 10 and 20 min of incubation at 37°C and remained stable up to 30 min of incubation. Thereafter, the incubation time used was 20 min. Production (pg/ml) of 6-keto PGF₁α and TXB₂ was proportional to the protein content of the vascular rings up to 800 µg of aortic protein. All incubations were performed in triplicate. Aortic rings deemed basal controls were incubated at 4°C. Simultaneously, aortic rings were incubated in the absence and presence of BAY, 1.0 ng/g protein, at 37°C for 15 min. The medium was stored in aliquots at 4°C. Simultaneously, aortic rings were incubated in the absence and presence of BAY, 1.0–100.0 ng/ml, at 37°C for 20 min. Total incubation volume was 1.0 ml. After incubation, all tubes were iced and immediately centrifuged at 1,000 rpm at 5°C for 15 min. The medium was stored in aliquots at −20°C for later determination of eicosanoids by enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI). The rings were also stored at −20°C for later determination of protein content by the Lowry method. Data are reported as production and release (pg/µg protein), which is defined as the quantity in the medium after incubation of the vascular rings at 37°C minus the quantity in the medium after incubation at 4°C.

Statistical Analyses

Data are presented as means ± SE. Differences were considered significant if a value of P < 0.05 was achieved as analyzed by analysis of variance of two-factor experiments and the least-significant difference test.

RESULTS

Effects of BAY in Anesthetized, Acutely Prepared Rabbits on MAP, CO, and TPR

At baseline, pregnant rabbits had significantly lower MAP and TPR (Table 1). Although CO was significantly higher in pregnant, the COI (159 ± 5 ml·min⁻¹·kg⁻¹, pregnant vs. 142 ± 11 ml·min⁻¹·kg⁻¹, nonpregnant) was not significantly different in pregnant than nonpregnant rabbits. BAY induced a greater elevation of MAP in acutely prepared pregnant (n = 6) than nonpregnant (n = 6) rabbits (Fig. 1A). Specifically, after 40 µg/kg BAY, MAP increased by 34 ± 1 mmHg in pregnant rabbits but only by 23 ± 5 mmHg in nonpregnant rabbits. The difference between the fractional increases was even greater (e.g., after 40 µg/kg BAY, MAP increased by 47 ± 1% in pregnant and 25 ± 5% in nonpregnant rabbits, P < 0.01). An enhanced pressor response developed in pregnant rabbits despite the simultaneous fall of HR in these animals, by about 69 beats/min (P < 0.01 vs. nonpregnant rabbits; Fig. 1B). The cardiac deceleration of pregnant rabbits was associated with and partially compensated by a 20% increase in SVI. The increase of SVI in pregnant compared with nonpregnant rabbits could not be differentiated statistically (Fig. 2A). BAY reduced COI in both groups and, although the magnitude of reduction tended to be greater in pregnant rabbits (Fig. 2B), it did not achieve statistical significance. The absolute and fractional changes of TPRI are shown in Fig. 3, A and B, respectively. BAY induced a dose-dependent increase of TPRI in both groups. The absolute changes of TPRI were only numerically greater in pregnant vs.
nonpregnant rabbits, but when fractional changes were considered, the increase of TPRI was twice as great in pregnant than in nonpregnant rabbits ($P < 0.02$, Fig. 3B). Therefore, the enhanced hypertensive effect of BAY in pregnant rabbits appeared to be a consequence of the heightened peripheral vasoconstriction induced by the activation of vascular L-VDCC.

**Effects of BAY in Conscious, Chronically Instrumented Rabbits**

The baseline MAP was lower ($78 \pm 3$ vs. $89 \pm 2$ mmHg, $P < 0.01$) and the HR was higher ($241 \pm 9$ vs. $216 \pm 14$ beats/min, $P < 0.05$) in pregnant than in nonpregnant control rabbits, respectively. The intravenous administration of BAY increased MAP with the peak change developing within the first 30 s after injection. MAP increased more in pregnant than nonpregnant rabbits after the 10- and 25-µg/kg doses (Fig. 4A). The absolute increase of MAP in pregnant rabbits was remarkable, because the baseline MAP of pregnant rabbits was >10 mmHg lower than in nonpregnant controls. HR decreased comparably in both groups of rabbits (Fig. 4B).

**Effects of Nifedipine, an L-VDCC Antagonist, in Conscious, Chronically Instrumented Rabbits**

The baseline MAP was lower and the HR was higher in pregnant than in nonpregnant controls (Table 2). Nifedipine induced a dose-dependent decrease of MAP and an increase of HR and these effects reached maximum between the 2nd and 3rd min after injection. The maximal decrease of MAP and increase of HR were not different between pregnant and nonpregnant rabbits.

To confirm that the effect of BAY was mediated through specific dihydropyridine receptors, five pregnant and five nonpregnant animals received the agonist after prior administration of approximately equimolar amounts of nifedipine. The inhibition of the pressor effect of BAY by nifedipine was virtually complete. For example, in pregnant rabbits, 25 µg/kg BAY (mol wt 356.3) induced 18 ± 1 mmHg elevation of MAP when given alone; however, when given after 25 µg/kg nifedipine (mol wt 346.3), the change was 0 ± 3 mmHg ($P < 0.05$). In nonpregnant rabbits, nifedipine similarly inhibited the pressor effect of BAY.

**Dihydropyridine Binding to Aortic Membranes**

PN 200−110 (+)-(5-methyl-3H) binding parameters in aortic membranes obtained from pregnant and nonpregnant rabbits are shown in Table 3. There was a single class of specific, saturable binding sites in each group. The equilibrium dissociation constants ($K_d$, the inverse of receptor affinity) and receptor number

---

**Fig. 2. Change of stroke volume index (A; SVI, ml/kg) and cardiac output index (B; COI, ml·min$^{-1}$·kg$^{-1}$) in anesthetized, acutely prepared pregnant ($n = 6$) and nonpregnant ($n = 6$) rabbits after the intravenous infusion of various doses of Bay K 8644, the L-type voltage-dependent calcium channel activator (mean ± SE).**

**Fig. 3. Absolute (A) and fractional (B) changes of total peripheral resistance index (TPRI) in anesthetized, acutely prepared pregnant ($n = 6$) and nonpregnant ($n = 6$) rabbits after the intravenous infusion of various doses of Bay K 8644, the L-type voltage-dependent calcium channel activator (mean ± SE).**

---
Fig. 4. Change of MAP (A; mmHg) and HR (B; beats/min) in conscious, chronically instrumented pregnant (n = 10) and nonpregnant (n = 10) rabbits after the intravenous bolus injection of various doses of Bay K 8644, the L-type voltage-dependent calcium channel activator (mean ± SE). Baseline MAP was 78 ± 2 mmHg in P and 89 ± 2 mmHg in NP rabbits (P < 0.01). Baseline HR was 241 ± 8 beats/min in P and 216 ± 14 beats/min in NP rabbits (P < 0.05).

| Table 2. Effect of intravenously administered nifedipine on arterial pressure and HR in conscious, chronically instrumented nonpregnant and pregnant rabbits |
|-----------------|-----------------|-----------------|-----------------|
|                 | Baseline        | Nifedipine, µg/kg iv |                 |
|                 | MAP, mmHg       | HR, beats/min     | 10              | 25              | 50              |
|                 |                 |                  | ΔMAP            | ΔHR             | ΔMAP            | ΔHR             |
| NP (n = 10)     | 90 ± 2          | 218 ± 7          | -5 ± 2          | 7 ± 8           | -9 ± 2          | 11 ± 13         |
| P (n = 10)      | 79 ± 2†         | 239 ± 6†         | -6 ± 1          | 6 ± 6           | -9 ± 1          | 13 ± 7          |

Values are means ± SE. *P < 0.05, †P < 0.001 vs. nonpregnant rabbits. The nifedipine-induced changes in MAP and HR were not significantly different between the 2 groups.

Discussions

Attempts to determine the mechanisms of the gestational alterations of cardiovascular pressor responsiveness is relevant, because the clinical manifestation of preeclamptic hypertension is preceded by an early, definitive increase in the sensitivity to the pressor effect of ANG II (12). In contrast, physiological human pregnancy is associated with a significant decrease of arteriolar tone and blunting of the responsiveness to several pressor compounds (9, 12), including ANG II and norepinephrine. ANG II and norepinephrine are also less potent systemic vasoconstrictor in pregnant rabbits (10, 37) and other pregnant laboratory animals (36). A few studies suggested that there is a corresponding downregulation of the specific receptors (2, 13, 18), but the data are inconsistent (3, 22). Because gestationally related blunting of the vascular responsiveness is not specific for one particular compound, seeking for a common cause of the abrogated effect of vasopressors seems to be logical. Attempts have been made, using isolated vascular tissue of pregnant animals, to explore the hypothesis that there may be a unifying cause underlying the altered actions of vasoconstrictors during gestation. Conrad et al. (8) demonstrated that the inositol phosphate cycle was impaired in isolated aortas of pregnant rats. The response of isolated mesenteric arteries to vasopressin was found to be blunted in pregnant rats by St. Louis et al. (34), and the authors suggested that this may have been a consequence of impaired opening of the L-VDC.

The hypothesis that the altered vascular L-VDC is the cause of the nonspecific vascular hyporesponsiveness in pregnancy has not been directly tested in a whole animal experiment. In our study, chronically prepared conscious and anesthetized acutely instrumented pregnant and nonpregnant rabbits were given BAY, a specific agonist of the L-VDC. This agent has been shown to elevate blood pressure in various species (6, 26, 31). The hypertensive response to BAY was
enhanced, not attenuated, in pregnant rabbits. A pregnancy-induced impairment of the vascular L-VDCC was not confirmed in these in vivo studies. Why opposite changes were observed when testing the intact aorta of pregnant rats in vitro (11) and the systemic vasculature of pregnant rabbits in vivo (present study) cannot readily be explained. Similar observations, however, have been made in pregnant rabbits with U46619, a thromboxane analog. We found U46619 to be a more potent systemic arterial vasoconstrictor in pregnant rabbits (20) and rats (16) than in nonpregnant animals. More recently we compared the reactivity of the gracilis arteriole of pregnant and nonpregnant rabbits in an in vitro system in which resistance size vessels can be studied (15). In contrast to the in vivo enhancement of the vasopressor effect, the in vitro arteriolar sensitivity to U46619 was blunted (L. Ungvari, G. Brown, P. Pacher, R. Venuto, A. Koller, and G. Losonczy, unpublished observations). Thus, in rabbit pregnancy, the in vivo change of vascular response to U46619 is directionally opposite to the change observed in vitro, just as they appear to be with BAY.

The blockade of the L-VDCC by nifedipine resulted in similar reductions in MAP in pregnant and nonpregnant rabbits, which implies that the L-VDCC contributed to the maintenance of the resting vascular tone equally in the two groups. This supports the notion of unimpaired L-VDCC in intact pregnant rabbits. The same conclusion could be drawn from the biochemical studies, which indicated undiminished number and unchanged affinity of aortic L-VDCC receptors. Although the aorta is perceived as more of a conduit than a resistance vessel, it has specific binding sites for BAY (28) and it contracts in response to BAY (28, 29). Furthermore, the aorta has been shown to develop a comparable decrease of sensitivity to pressor compounds during pregnancy as observed in resistance vessels (24). The $B_{\text{max}}$ of PN 200–110 estimated by us in aortic membranes was comparable to earlier reported values in left ventricular membranes of female rabbits (26).

The number and affinity of the vascular L-VDCC do not necessarily parallel its functional response to specific ligands (28, 35). A potential explanation for observations of unaltered receptor number and affinity in the presence of altered responses to ligands (BAY and U46619) may be that there is an effect of transmembrane potentials on receptor number or affinity (1) that is dissipated in isolated membrane preparations. We observed a difference in ANG II receptor number between pregnant and nonpregnant vascular tissues when using intact glomeruli (2), but not when using isolated vascular membrane preparations (3). Roy et al. (29) also reported differences in binding of a calcium channel blocker between tissues of pregnant and nonpregnant animals when using intact aortic rings, but not when using aortic membrane preparations (28). There is evidence that vascular tissues in pregnant animals are hyperpolarized (25, 29, 34). Roy et al. (28) reported that when aortic strips from pregnant and nonpregnant rats were precontracted by KCl to the same level, but by different concentrations of KCl, the contraction-response curves to BAY were identical, suggesting an effect of membrane potential on the response to BAY. It would be expected that hyperpolarization of vascular plasma membranes would lower the sensitivity of vascular tissue to contractile ligands. In vitro arteriolar sensitivity to BAY and to U46619 is not enhanced in pregnant animals (28, 38). It could be reasoned that once threshold potential is attained in excitable tissues, the in vivo contractile response to BAY or to U46619 could be greater due to a greater store of Ca$^{2+}$ in sarcoplasmic reticulum of pregnant vs.
nonpregnant vascular tissue. This is possibly due to prolonged hyperpolarization (causing decreased sensitivity to contractile agents) of plasma membranes. In this regard, Sagawa et al. (30) showed that lipophilic dihydropyridine (DHP) agonists such as BAY exert their effect in contractile tissue by actions on both the L-type Ca\(^{2+}\) channel and on the sarcoplasmatic reticulum. Their data indicate that DHP agonists bind not only to L-VGCC receptors, but also to sarcoplasmic Ca\(^{2+}\) release channels (ryanodine receptors). In isolated adrenal glomerulosa cells, BAY potentiated KCl-induced Ca\(^{2+}\) uptake and intracellular Ca\(^{2+}\) concentration in cells from pregnant rats but not in cells from nonpregnant rats (32). The enhanced intracellular Ca\(^{2+}\) concentration in response to BAY in cells from pregnant rats was not inhibited by nifedipine. Their data support the concept that calcium may be sequestered intracellularly during pregnancy.

Another potential explanation for the enhanced blood pressure response to BAY is that this drug opens the mostly closed (or impaired (28, 29)) L-VGCC in the resistance vasculature of pregnant rabbits. This, in turn, permits the otherwise limited action of the much higher endogenous, circulating concentrations of ANG II and norepinephrine that characterize pregnant rabbits (10, 37) to fully manifest their vascular effect. Consistent with this notion, BAY has been shown to enhance the pressor effect of phenylephrine (28).

Pregnancy enhances vascular prostacyclin release in response to pressor compounds (23), but the response to BAY had not been tested. We found that intact aortic rings of pregnant rabbits released less prostacyclin in response to BAY than did the tissue of nonpregnant rabbits. It is speculated that the diminished release of prostacyclin may provide at least a partial explanation for the enhanced pressor effect of BAY in pregnant rabbits. There may be some danger, however, in attributing a systemic effect to a compound better known for its local action. This alteration may be specific for prostacyclin, because the BAY-induced release of thromboxane remained unchanged in aortic rings of pregnant rabbits.

In summary, downregulation of the L-VGCC in rabbit pregnancy does not appear to contribute to the nonspecific blunting of the pressor response to ANG II or epinephrine. The increase of the peripheral vascular resistance in response to pharmacologic activation of these calcium channels is not only unpaired but also enhanced in rabbit pregnancy, at a time when the effect of most, although not all (16, 20), pressor compounds becomes weaker. We attempted to extrapolate the results of the pharmacologic manipulations of the L-VGCC to the physiology of mammalian pregnancy, but such interpretations could be misleading.

**Perspectives**

Systemic hypotension and refractoriness to vasoconstrictive agents are considered hallmarks of physiologic mammalian pregnancy. The studies reported herein confirm that some prohypertensive agents have an enhanced rather than a blunted response in intact pregnant rabbits. Such a response could not (necessarily) have been predicted from the results of studies employing isolated tissues. These and other data emphasize the importance of investigating whole animals. These results also suggest the possibility that during pregnancy, intracellular calcium may be in greater quantity and more readily released from binding sites. Once a threshold stimulus is provided, the response of these cells may be exaggerated. Such a hypothesis is consistent with the rapid transition from hypotension to hypertension, which occurs with potentially dire consequences in pregnant women who develop preeclampsia.

This study was supported by The Renal Research Fund of the University of Buffalo Foundation (to R. Venuto), Grant 9707953A of the American Heart Association/New York State Affiliate (to R. Venuto and G. Brown), The Hungarian National Science Fund, T025422 (to G. Losonczy), and The Western New York Kidney Foundation/Upstate NY Transplant Services (to R. Venuto and G. Brown).

**REFERENCES**


