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Effects of nifedipine and Bay K 8644 on myotropic responses in aortic rings of pregnant rats

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Abstract

The hypothesis that Ca^{2+} channel function is altered during pregnancy was tested by comparing responses to potassium chloride (KCl) and phenylephrine in aortic rings of virgin and term-pregnant rats under the influence of nifedipine and Bay K 8644. Maximum response to KCl was progressively reduced by increasing nifedipine concentrations (1.0–100 nM) in both groups of tissues. Nifedipine produced a smaller inhibition of KCl-induced contraction in aortic rings of pregnant than of virgin rats. It exerted little inhibition on the concentration-response curve to phenylephrine. The Ca^{2+} channel antagonist (100 nM) reduced the maximum response to the α-adrenoceptor agonist in rings from virgin rats, but had no effect in pregnant rats. Bay K 8644, a Ca^{2+} channel activator, potentiated the responses to low concentrations of both phenylephrine and KCl in the tissues of both virgin and pregnant rats, but did not affect maximum responses. It also induced concentration-dependent contractions in rings of virgin but not of pregnant rats. The effects of Bay K 8644 were markedly potentiated by precontracting the aorta with 10 mM KCl. Nevertheless tissues from pregnant rats were still less responsive to Bay K 8644. However, when the strips were precontracted to the same level by different concentrations of KCl, the concentration-response curves to Bay K 8644 were identical in both groups. [^{3}H]Nitrendipine binding to membrane preparations of the thoracic aorta was similar in virgin and pregnant rats. These results show that the effects of both nifedipine and Bay K 8644 were decreased in aortic rings of pregnant compared to virgin rats and indicate that potential-operated Ca^{2+} channel function is altered in the aorta of pregnant rats. Furthermore, it is suggested that modulation of membrane potential with KCl can reverse this change.

Keywords: Ca^{2+} channel; Pregnancy; Vasoconstriction; Aortic ring; Nifedipine; Bay K 8644

1. Introduction

It is well recognized that pregnancy in both humans and rats is accompanied by modifications of cardiovascular homeostasis. For instance, blood pressure declines markedly (Aoi et al., 1976; MacGillivray et al., 1969), plasma volume and cardiac output increase (Barron et al., 1984; Lundgren et al., 1979), while peripheral resistance decreases correspondingly (Lundgren et al., 1979). Along with these modifications, pressor responses to several exogenous vasoconstrictors are markedly blunted (Abdul-Karim and Assali, 1961; Massicotte et al., 1986; Paller, 1984). Although there is controversy about pressor agents exerting decreased effects during pregnancy, it is generally accepted that this phenomenon is not specific to a single agent or a single class of vasopressors. It is also recognized that this decreased pressor responsiveness occurs at the time of reduced blood pressure (Massicotte et al., 1986; Paller, 1984).

This blunted responsiveness to pressor substances has even been observed in isolated vascular tissues, indicating that the mechanisms responsible are not dependent on cardiovascular reflex pathways. Indeed, many laboratories have reported that rat vascular preparations in vitro, such as the perfused tail artery (Dogterom and De Jong, 1974), perfused pulmonary bed (Fuchs et al., 1982), perfused mesenteric vasculature (Massicotte et al., 1987a), aorta and portal vein (Hart, 1982; Hart, 1984; Massicotte et al., 1987a; St-Louis et al., 1988b), show decreased responses to vasopressor agents, such as angiotensin II, vasopressin, noradrenaline, etc. Furthermore, Aalkjaer et al. (1985) have demonstrated a marked decrease in responsive-

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ness to angiotensin II in microvessels of the omentum in normal pregnant women in comparison to non-pregnant subjects. Similar findings have been made in mesenteric resistance vessels of pregnant rats (McLaughlin and Keve, 1986). These observations indicate that the blunted responses to vasoconstrictors are a direct consequence of pregnancy at all levels of the circulatory tree, from conduit-type vessels, such as the aorta, to resistance vessels.

The mechanisms of this decreased responsiveness to pressor agents are still under investigation, as are the causal factors of this phenomenon. Some putative mechanisms have been proposed (Paller, 1984; St-Louis et al., 1988a). For instance, down-regulation of membrane receptors for these ligands in vascular tissues, increased liberation of an endogenous vasodilator acting as a physiological antagonist to vasopressors, modifications of mechanical properties and/or tissue composition (ratio of smooth muscle to connective tissue, or of elastin to collagen, etc.) leading to increased compliance of vessels, and alterations (or uncoupling) of receptor-response coupling have been considered. In vivo studies have produced conflicting results. For instance, Paller (1984) demonstrated that treatment of pregnant rats with meclofenamate, an inhibitor of prostaglandin synthesis, restored the pressor response to vasopressin to the level observed in virgin animals. Opposite findings were reported in chronically instrumented pregnant rats (Conrad and Colpoys, 1986) and guinea pigs (Harrison and Grindlay-Moore, 1989). Contradictory results were published on other mechanisms, such as cardiovascular reflexes (Massicotte et al., 1987b; Hines and Barron, 1992) and endothelium-derived relaxing factor (EDRF) (Ahokas et al., 1991; Umans et al., 1990). In isolated vascular preparations, available data do not support the involvement of local endogenous prostaglandins in mediating the blunted responses to vasoconstrictors in pregnancy (Harrison and Grindlay-Moore, 1989; St-Louis and Sicotte, 1992). Studies on the proposed role of EDRF in this resistance to presssor agents gave conflicting results (St-Louis and Sicotte, 1992; Weiner et al., 1989).

In the present report, we investigated the possibility that mechanisms coupling stimulation to response in vascular smooth muscle are affected during pregnancy. It is generally accepted that the main trigger for contraction in vascular smooth muscle is an elevation of intracellular Ca^{2+} concentration (Somlyo et al., 1985), allowing the interaction of contractile myofilaments. It is also recognized that this free Ca^{2+} comes from intracellular stores and from the extracellular milieu through Ca^{2+} channels (Van Breemen et al., 1986). Therefore, we tested the hypothesis that blunted responses to vasoconstrictor agents during normal pregnancy in the rat could be linked to altered mobilization of extracellular Ca^{2+} in vascular smooth muscles by interfering with agonist-stimulated responses using dihydropyridine agents known to act on voltage-operated Ca^{2+} channels (Catterall and Striessnig, 1992). We employed isolated rings of the thoracic aorta from virgin and term-pregnant rats, since it has often been reported that the reactivity of this tissue to α-adrenoceptor agonists is reduced when obtained from pregnant animals (Hart, 1982; St-Louis et al., 1988b; Harrison and Grindlay-Moore, 1989; St-Louis and Sicotte, 1992). The decreased reactivity of the aorta of pregnant animals is equivalent to what is observed in resistance vessels during pregnancy (Aalkjaer et al., 1985; McLaughlin and Keve, 1986).

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats (Charles River Canada, St-Constant, Qué.) aged 10–11 weeks were mated with age-matched males. The morning on which vaginal smears were found to contain spermatozoa was labeled day 1 of pregnancy. The pregnant females were then placed in individual cages until used on the 22nd day of gestation. Virgin rats of the same age served as controls without considering the stage of the estrous cycle. The animals were housed in facilities of the Research Center at Hôpital Ste-Justine, which is accredited by the Canadian Council on Animal Care. The protocol was approved by the local animal care committee.

2.2. Organ bath assay

After decapitation, the thoracic aorta was rapidly removed and placed in cold Krebs bicarbonate solution (KBS). It was cleaned of fat and extraneous tissues and cut into four consecutive rings (2–3 mm) which were placed in individual jacketed tissue baths (10 ml, Radnoti Glass, Monrovia, CA) maintained at 37° C. The lumen of each ring was rubbed with a wooden stick to remove the endothelium. In each experiment, four rings of both virgin and pregnant rats were used. They were equilibrated for 60 min under 2.0 g passive tension, the optimal tension for both groups of tissues (St-Louis and Sicotte, 1992), with frequent washing and tension adjustment. The tissues were bathed in KBS of the following composition in mM: NaCl, 118; KCl, 4.65; $NaHCO_3$, 25; $CaCl_2$, 2.5; $MgSO_4$, 1.18; KH_2PO_4, 1.18; and dextrose, 5.5. The solution was bubbled with a mixture of 95% O_2-5% CO_2; pH was 7.4. After equilibration, the tissues were challenged with 1.0 μM phenylephrine. At plateau response, acetylcholine (0.1 mM) was added to verify removal of the endothelium. Tension was measured by force-displacement transducers (FT-03, Grass Instruments, Quincy, MA) and recorded on a Grass polygraph (Model 7E) or comput-

erized data acquisition system using Work Bench software (Kent Scientific, Litchfield, CT). The experiments with the dihydropyridines, nifedipine and Bay K 8644, were performed under minimal light to prevent photodegradation of these substances.

2.3. Experimental protocol

Cumulative concentration-response curves to phenylephrine (10^{-9} to 10^{-4} M) and potassium chloride (KCl, 2–100 mM, added to normal KBS) were consecutively obtained in the same tissues. A second curve was charted 2 h after completion of the first curve. One of the rings of both virgin and pregnant rats served as a control, while each of the other three was preincubated with different concentrations of nifedipine (1.0, 10, 100 nM) or Bay K 8644 (0.01, 0.1, 1 μM) added 10 min before charting the curve. The order of tissue treatment was changed each day to avoid any effect related to localisation of the ring along the aorta. In a small number of tissues, the KCl curve was obtained before phenylephrine to check if the first curve (with phenylephrine) has an effect on the second curve. Such an effect was not observed.

Concentration-response curves to Bay K 8644 (0.01–1 μM) were obtained in a cumulative fashion. In paired tissues from both virgin and pregnant rats, the curves were charted in the absence and presence of 5 or 10 mM KCl (total concentration 10.8 and 15.8 mM, respectively), added 10 min before measuring the effect of Bay K 8644, according to a protocol used previously (Storm and Webb, 1993).

2.4. Binding experiments

After decapitation of pregnant and virgin rats, the thoracic aorta was rapidly removed and prepared for assay as described earlier with some modifications (Larivière et al., 1988; Parent et al., 1991). In brief, each aorta was immersed in cold 0.25 M sucrose solution and fat and surrounding tissues were removed. Tissues from 10–12 rats were subsequently processed simultaneously to provide enough material for one binding experiment. The aortae were minced and then homogenised with a Polytron (Brinkman, Mississauga, Ont.) at setting 8 for 2×10 s. The homogenate was centrifiged at $1500 \times g$ for 10 min at 4° C. The supernatant was filtered through cheesecloth and centrifuged at $102\,000 \times g$ for 60 min at 4° C. The pellet was suspended in 0.05 M Tris-HCl buffer (pH 7.4). Protein content was measured by the coomassie blue method (Spector, 1978). The membrane preparation was diluted to a given protein concentration with the same buffer containing 0.4% (w/v) bovine serum albumin.

[^{3}H]Nitrendipine binding (specific activity 77 Ci/mmol) was studied in duplicate saturation experiments, using 0.1–6.25 nM labelled ligand and 200 μg of membrane protein per tube in a final volume of 500 μl. Incubation was conducted in 0.05 M Tris-HCl supplemented with 0.4% bovine serum albumin at 25° C for 60 min. Non-specific binding was determined for each point of the saturation binding curve by running parallel tubes containing 1 μM nifedipine.

2.5. Data analysis

Each concentration-response curve was analyzed by computer fitting to a 4-parameter logistic equation with the ALLFIT program (De Lean et al., 1978) to evaluate the concentration producing 50% of the maximum response (EC_{50}) and the maximum asymptote of the curve (E_{max}, maximum response). When Bay K 8644 was used with phenylephrine and KCl, the minimum asymptote of the curve was also evaluated. Different curves in the same protocol were compared by the partial F-test (De Lean et al., 1978) or factorial analysis of variance (GB-STAT, Dynamic Microsystems, Silver Spring, MD) on mean pD_2 (negative log of the EC_{50}), on mean E_{max} and on the lower asymptote of the curve (in the experiments with Bay K 8644). Binding data were analyzed with the LIGAND program (McPherson, 1985) to determine the density (B_{max}) and affinity (K_D) of binding sites. In the figures, the data are expressed as mean experimental points with their standard error (S.E.M.) along with the best fitted curve to these points, except for the binding experiments of which typical results are shown.

2.6. Drugs and chemicals

All salts employed in these experiments were of analytical grade obtained from Fisher Scientific (Montréal, Qué.). Phenylephrine hydrochloride and acetylcholine hydrochloride were purchased from the Sigma Chemical Co. (St. Louis, MO) and nifedipine hydrochloride and Bay K 8644 (methyl ester) from Reseach Biochemical (Natick, MA). [^{3}H]Nitrendipine (specific activity 77 Ci/mmol) was obtained from Dupont Canada (Mississauga, Ont.). The dihydropyridines were prepared in stock solution in 95% ethanol in vials protected from light. Ethanol concentrations in the tissue baths did not exceed 0.1% and were verified by adding the same concentrations of vehicle in control tissues.

3. Results

3.1. Effects of nifedipine on concentration-response curves to KCl and phenylephrine

The effects of nifedipine (1–100 nM), a dihydropyridine Ca^{2+} channel blocker, were measured on the

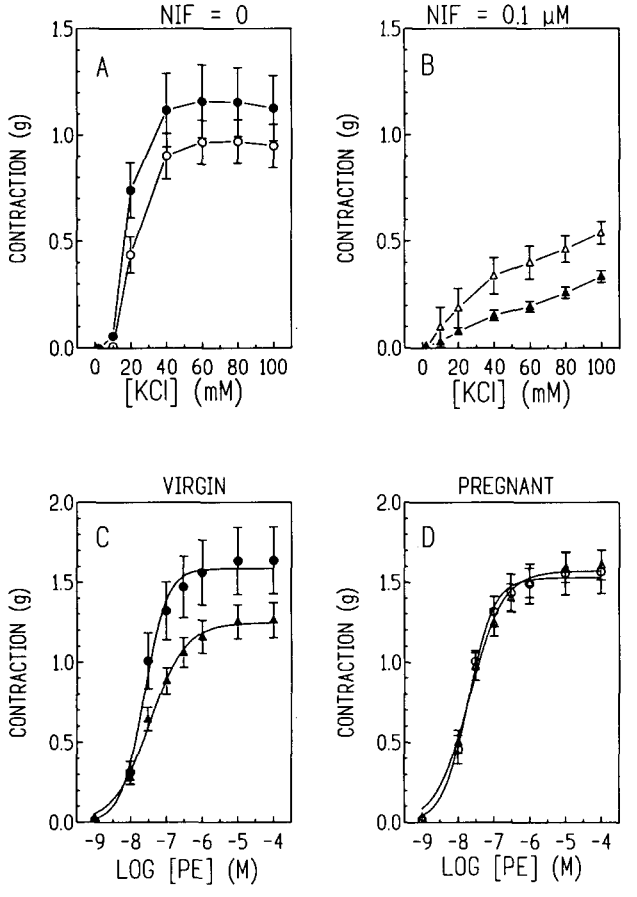

Fig. 1. Concentration-response curves to KCl (A and B) and phenylephrine (PE, C and D) in aortic rings of virgin (filled symbols) and term-pregnant (open symbols) rats in the absence (circles) and presence (triangles) of 0.1 μM nifedipine (NIF). The ordinate depicts responses to KCl or PE in g tension, while the abscissa represents KCl or PE concentrations in M. Nine experiments were performed for each curve.

concentration-response curve to KCl. The results in aortic rings of virgin and pregnant rats in the absence and presence of 100 nM nifedipine are illustrated in Fig. 1. The action of KCl was reduced in aortic rings of pregnant rats, as shown by the smaller responses to all concentrations of the stimulant in comparison to virgin animals (Fig. 1A). This produced a significant reduction of maximum responses to KCl (from 1.15 ± 0.07 to 0.96 ± 0.05, $P < 0.05$) on the fitted curve, but with a non-significant decrease in sensitivity (EC_{50}, from 18 to 21 mM). In the presence of 100 nM nifedipine, the maximum response to KCl was significantly more decreased in tissues of virgin than of pregnant rats (Fig. 1B), namely, to 0.33 ± 0.03 and 0.54 ± 0.05 g ($P < 0.001$), respectively. Sensitivity (EC_{50}) was significantly reduced in both groups to 44 and 29 mM, respectively. Intermediate results were obtained with 1 and 10 nM nifedipine (data not shown). Sensitivity to KCl was not altered by these two concentrations of the Ca^{2+} blocker, while maximum responses were reduced to 0.98 ± 0.05 ($P < 0.05$) and 0.64 ± 0.05 g ($P < 0.01$) in virgins and 0.95 ± 0.05 (N.S.) and 0.63 ± 0.04 g ($P < 0.01$) in pregnant rats in the presence of 1 and 10 nM nifedipine, respectively. These results indicate that the effects of nifedipine on the concentration-response curve to KCl in aortic rings are diminished during pregnancy.

The concentration-response curve to phenylephrine was less affected by nifedipine than that of KCl. The results with 100 nM nifedipine are shown for virgin (Fig. 1C) and pregnant (Fig. 1D) rats. In tissues of virgin rats, 1 and 10 nM nifedipine did not have any significant effect on the concentration-response curve to phenylephrine (data not shown), while 100 nM of the drug significantly depressed the maximum response (from 1.59 ± 0.09 to 1.25 ± 0.05 g, $P < 0.01$) without altering sensitivity (EC_{50}). In aortic rings of pregnant rats, at all concentrations tested, nifedipine had no impact on the concentration-response curve to phenylephrine. Indeed, all four curves were statistically identical according to the partial F-test. These results show that a fraction of the response to phenylephrine in aortic rings of virgin rats can be blocked by high concentrations of nifedipine, but this fraction of the phenylephrine response that is sensitive to nifedipine is absent in pregnancy.

3.2. Effects of Bay K 8644 on concentration-response curves to KCl and phenylephrine

Concentration-response curves to KCl and phenylephrine were also measured in aortic rings of virgin and pregnant rats in the absence and presence of Bay K 8644, a dihydropyridine Ca^{2+} channel activator. Upon its addition to the tissue bath, a concentration-dependent increase in tone was observed in rings of virgin but not of pregnant rats (Fig. 2). These sustained contractions of aortic rings in virgin rats were apparent with 0.1 and 1 μM Bay K 8644. In the concentration-response curves to KCl and phenylephrine, this level of tone was considered as the new lower asymptote.

Bay K 8644 potentiated the responses to KCl in both groups of tissues without modifying the maximum responses (Fig. 3A,B). When concentration-response curves to KCl were analyzed with the new baseline, considered to be the direct effect of Bay K 8644 in tissues of virgin rats, sensitivity (EC_{50}) to KCl was equivalent in rings of both groups, although the responses to 2, 10 and 20 mM KCl were significantly larger in tissues of virgin than of pregnant rats in the presence of the activator. This discrepancy between sensitivity and responses appeared to arise from the new baseline (lower asymptote) of the concentration-response curve to phenylephrine in rings of virgin rats, with no change in their pregnant conterparts. At 10

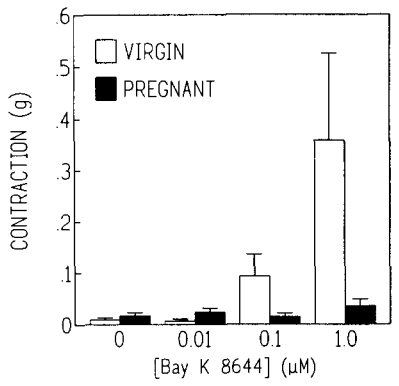

Fig. 2. Contractile effects of Bay K 8644 in aortic rings of virgin (open columns) and term-pregnant (filled columns) rats. The ordinate depicts responses to phenylephrine in g tension, while the abscissa represents concentrations of Bay K 8644 in M. Twelve experiments were performed for each response; four tissues from the same rat were used for the four Bay K 8644 concentrations.

and 100 nM, Bay K 8644 produced intermediate results compared to its absence and 1 μM concentration in both groups of tissues.

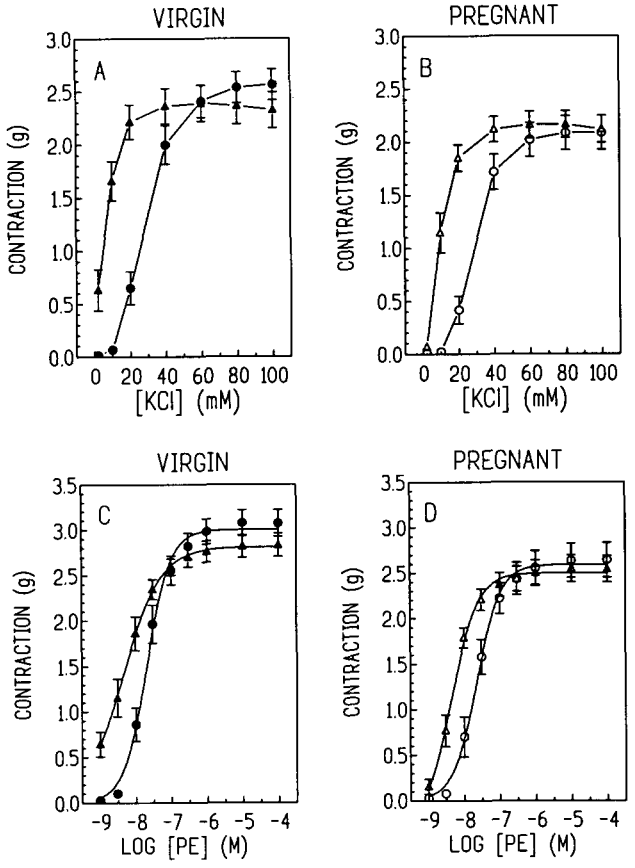

Fig. 3. Concentration-response curves to KCl (A and B) and phenylephrine (PE, C and D) in aortic rings of virgin (filled symbols) and term-pregnant (open symbols) rats in the absence (circles) and presence (triangles) of 1 μM Bay K 8644. The ordinate depicts responses in g tension, while the abscissa represents KCl or PE concentrations in M. Twelve experiments were performed for each curve.

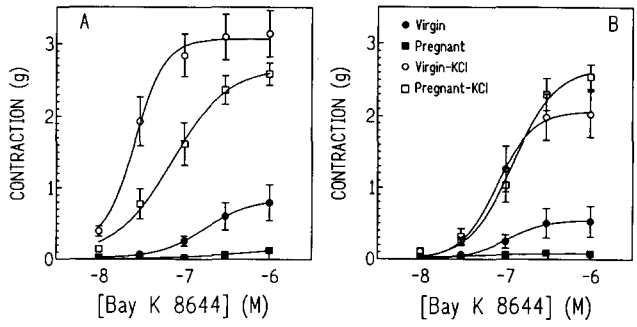

Fig. 4. Concentration-response curves to Bay K 8644 in aortic rings of virgin (circles) and pregnant (squares) rats. (A) Responses to Bay K 8644 in normal KBS (filled symbols) and in KBS supplemented with 10 mM KCl (open symbols). (B) Similar experiments except that tissues from virgin animals were precontracted with 5 mM KCl. The ordinate depicts the responses to Bay K 8644 in g tension, while the abscissa represents logarithm concentrations of the Ca^{2+} channel opener in M. Curves are the means ± S.E.M. of 10–12 experiments.

Similar results were obtained for the concentration-response curve to phenylephrine in the presence of Bay K 8644 (Fig. 3C,D). Again, the effects of lower phenylephrine concentrations appeared to be more potentiated by Bay K 8644 in the aorta of virgin than of pregnant rats, but this was a consequence of the direct action of the activator. The potentiation of phenylephrine responses was equivalent in both groups of rings when the new baseline (0.33 g with 1 μM Bay K 8644 in virgins) was considered in analysis of the curves. These results indicate that Bay K 8644 induces direct contractile effects in aortic rings of virgin but not of pregnant rats and that it can potentiate the responses to KCl and phenylephrine similarly in both groups of tissues.

3.3. Concentration-response curves to Bay K 8644

Concentration-response curves to Bay K 8644 were obtained under basal conditions and in the presence of 10 mM KCl in aortic rings of both virgin and pregnant animals. As shown in Fig. 4A, under basal conditions, Bay K 8644 produced concentration-dependent contractions in tissues of virgin rats (filled circles, E_{max} = 0.79 ± 0.25 g at 1.0 μM), but barely had an effect in rings of pregnant animals (filled squares, 0.13 ± 0.05 g). Sensitivity ($-\log EC_{50}$) to Bay K 8644 could not be evaluated in tissues of pregnant animals but was 6.76 ± 0.32 in virgins. Aortic rings were precontracted with 10 mM KCl (0.24 ± 0.04 and 0.06 ± 0.02 g in virgin and pregnant rats, respectively). Under these conditions, the responses to Bay K 8644 increased markedly in both groups. Maximum responses to Bay K 8644 reached 3.14 ± 0.32 g (Fig. 4A, open circles) in virgin and 2.58 ± 0.15 g (Fig. 4A, open squares) in pregnant rats. Sensitivity to Bay K 8644 increased to 7.58 ± 0.07 and 7.15 ± 0.12, respectively.

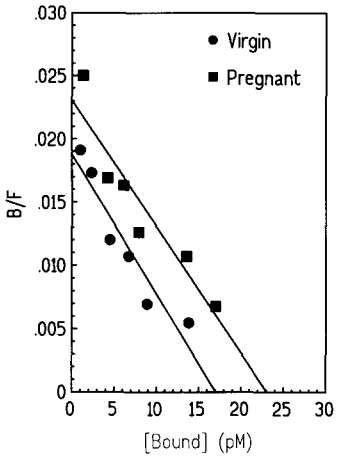

Fig. 5. Scatchard plot of specific [^{3}H]nitrendipine binding to membrane prepared from the thoracic aorta of virgin (circles) and term-pregnant (squares) rats. The results are those of a typical experiment. Regression lines represent the best fit of data points estimated with the LIGAND program. B, bound; F, free ligand.

When equieffective concentrations of KCl were used (5 mM in virgin and 10 mM in pregnant animals) to precontract their aortic rings (0.07 ± 0.02 and 0.06 ± 0.01 g, respectively), the concentration-response curves to Bay K 8644 became similar in both groups (Fig. 4B, open symbols). In tissues of virgin rats, maximum responses and sensitivity to Bay K 8644 rose from 0.55 ± 0.17 g and 6.98 ± 0.30, under basal conditions to 2.07 ± 0.22 g ($P < 0.01$) and 7.10 ± 0.11 (NS), when precontracted with 5 mM KCl, while they increased from 0.09 ± 0.05 g (pD_2 can not be evaluated) in the absence of KCl to 2.66 ± 0.22 g ($P < 0.01$) and 6.92 ± 0.08 upon precontraction with 10 mM KCl. This indicates that the contractile effects of Bay K 8644 can be modulated in aortic rings of both virgin and pregnant rats by altering membrane potential with KCl. The aortic rings of pregnant rats are postulated to be resistant to depolarization in comparison to virgin animals.

3.4. Binding of [^{3}H]nitrendipine

A typical Scatchard plot of saturation results with [^{3}H]nitrendipine in membranes prepared from the thoracic aorta of virgin and pregnant rats is shown in Fig. 5. These results demonstrate parallel regression lines, indicating that affinity for the labelled ligand was identical in both groups. The density of binding sites in the aorta of pregnant and virgin rats was not significantly different since in this experiment ($n = 6$) the density of [^{3}H]nitrendipine binding sites (B_{max}) was 36.4 ± 7.9 and 48.4 ± 13.5 fmol/mg protein, while affinity (pK_D) was 9.36 ± 0.10 and 9.23 ± 0.14 for membrane preparations from virgin and pregnant animals, respectively. These data demonstrate that the total number and affinity of [^{3}H]nitrendipine binding sites in the aorta are not modified by pregnancy.

4. Discussion

The purpose of the present study was to test the hypothesis that the blunted responses to vasoconstrictor agents in vascular tissues of pregnant rats are mediated by alterations in Ca^{2+} mobilization by smooth muscle cells within the vessel wall. The major findings were as follows: (1) nifedipine was much less effective in blocking KCl-induced contractions in aortic rings of pregnant than of virgin rats; (2) although 100 μM nifedipine reduced the maximum response to phenylephrine in tissues of virgin animals, it failed to interfere with this parameter in aortic rings of pregnant rats; (3) Bay K 8644 induced a concentration-dependent increase in tone in tissues of virgin but not of pregnant rats; (4) Bay K 8644 potentiated the responses to both phenylephrine and KCl, to a similar extent in tissues of virgin and pregnant rats; (5) in tissues precontracted with equiactive small concentrations of KCl, the contractile effects of Bay K 8644 were augmented in both groups, making the concentratrion-response curves to Bay K 8644 identical in aorta of virgin and pregnant animals; and (6) [^{3}H]nitrendipine binding was not modified by pregnancy in membrane preparations of the thoracic aorta. These results are indicative of functional changes of voltage-operated Ca^{2+} channels in the aorta of pregnant rats that could be consequent of altered membrane potential.

Experimentally, α-adrenoceptor stimulation in vascular smooth muscle can be separated into phasic and tonic components (Van Breemen et al., 1986; Villalobos-Molina et al., 1982). The phasic component is believed to be dependent on intracellularly stored Ca^{2+}, while the tonic component depends on extracellular Ca^{2+} mobilization. α-Adrenoceptors activation is linked to polyphosphoinositide hydrolysis into inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (Villalobos-Molina et al., 1982). $InsP_3$ causes the release of Ca^{2+} from intracellular stores, inducing vascular smooth muscle contraction. This is believed to be the phasic component (Rapoport, 1987). Diacylglycerol, through protein kinase C activation, opens receptor-operated Ca^{2+} channels within the cell membrane, resulting in a massive entry of Ca^{2+} from the extracellular milieu that augments and maintains smooth muscle contraction. This is said to be the tonic contraction (Van Breemen et al., 1986).

On the other hand, KCl induces smooth muscle contraction by depolarization of smooth muscle cell membranes, opening voltage-operated Ca^{2+} channels. This results in a massive influx of Ca^{2+} from the extracellular fluid, inducing sustained tonic contraction (Van Breemen et al., 1986). While voltage-operated Ca^{2+} channels have been identified in vascular smooth muscle cells with patch-clamp methods, there is no direct evidence of the existence of voltage-insensitive

receptor-operated Ca^{2+} channels in these cells. However numerous observations from functional studies suggest their existence (Hurwitz, 1986).

The data presented here show that the effects of dihydropyridine agents, that are believed to act exclusively on voltage-operated channels, were much reduced in aortic rings from pregnant compared to virgin rats. Indeed, in aortic rings of pregnant animals, the responses to KCl were much less reduced by nifedipine than in virgin animals. Similarly, Bay K 8644, a voltage-operated Ca^{2+} channel activator, induced concentration-dependent contractions in aortic rings of virgin but not of pregnant rats.

Similar results were obtained with phenylephrine. Although it is well recognized that the effects of such agonists are mediated by so-called receptor-operated Ca^{2+} channels, evidence indicates that the original Ca^{2+} movements through the cell membrane, upon stimulation of competent receptors, can activate voltage-operated channels (Boonen and De Mey, 1990). It has been reported that dihydropyridine Ca^{2+} channel blockers are much less effective in blocking responses to α-adrenoceptor agonists than to KCl (Scriabine and Van den Kerckhoff, 1988; Godfraind et al., 1982). The magnitude of this differential inhibition varies with the size of the artery (Boonen and De Mey, 1990). Indeed, the difference was reported to be around 1000-fold in large elastic arteries (Scriabine and Van den Kerckhoff, 1988) and about 10-fold in resistance arteries (Boonen and De Mey, 1990), favouring inhibition of KCl-induced contractions. These observations are in agreement with our present results and recent observations in mesenteric resistance vessels (St-Louis et al., 1995). In aortic rings of virgin rats, 100 μM nifedipine significantly reduced the responses to phenylephrine while it markedly inhibited the responses to KCl. In aortic rings of pregnant rats, nifedipine had no effect on phenylephrine-induced contractions and its action on KCl-induced contractions was much reduced in comparison to virgin rats, suggesting that the small but significant fraction of the phenylephrine response inhibited by nifedipine in tissues of virgin rats was not functional in pregnant rats.

This is in agreement with previous results indicating that, in the uterine pig artery, 4-hydroxylated estradiol, reported to increase uterine blood flow, decreased Ca^{2+} uptake through voltage-operated Ca^{2+} channels, without any effect on Ca^{2+} uptake mediated by receptor-operated channels (Stice et al., 1987). This last study showed that 4-hydroxylated estradiol and D-600, a phenylalkylamine Ca^{2+} channel blocker, act on the same channel, which is different from the receptor-operated Ca^{2+} channel.

Bay K 8644 is known to induce by itself concentration-dependent contractions of isolated vessels. This effect is reported to be much larger in the isolated femoral artery of spontaneously hypertensive than of normotensive Wistar-Kyoto rats, being over 90% and 8–26% of the action of 60 mM KCl, respectively for these two rat strains (Aoki and Asano, 1986). We observed the opposite in the present study with pregnant rats. Indeed, in aortic rings of virgin rats, we estimated the effect of Bay K 8644 (1 μmol/l) to be around 25% of that of 1 μM phenylephrine, while it was less than 4% in tissues of pregnant rats. Our results are similar in magnitude to those reported in the aortic rings of normotensive animals (Storm and Webb, 1993). While the responses to Bay K 8644 are markedly potentiated in vascular tissues of hypertensive rats (Aoki and Asano, 1986; Storm and Webb, 1993), they are strikingly obliterated in aortic rings of pregnant rats (Figs. 2 and 4), indicating that equilibrium between the activated and inactivated conformational states (Hurwitz, 1986) of voltage-operated Ca^{2+} channels in the cell membrane of smooth muscle is shifted towards the latter during pregnancy in such a way that they cannot be opened by Bay K 8644. This is supported by our results (Fig. 4A) showing that depolarization of aortic rings with 10 mM KCl produces a larger potentiation of Bay K 8644 effects in tissues of virgin than of pregnant rats.

This raises the question of mechanisms of the decreased influence of voltage-operated Ca^{2+} channels in vascular tissues of pregnant rats. A clue of a putative mechanism was obtained when equiactive small depolarizing concentrations of KCl were used (Fig. 4B): the concentration-response curves to Bay K 8644 became identical in the two groups of rats, suggesting that the smooth muscle cell membrane of pregnant rats is hyperpolarized or at least resistant to depolarization in comparison to virgin rats. Because responses to Bay K 8644 in isolated arteries can be potentiated by KCl, it was suggested that decreased resting membrane potential could account for the enhanced responsiveness to Bay K 8644 in hypertensive animals (Aoki and Asano, 1986; Storm and Webb, 1993). The opposite change, e.g., increased resting membrane potential, can be proposed for the decreased responsiveness to Bay K 8644 in aortic rings of pregnant animals. Hyperpolarization of 6 mV was recently reported in vascular smooth muscle of mesenteric resistance arteries of pregnant compared to virgin rats (Meyer et al., 1993). This observation is compatible with our present results, but hyperosmolarity can also be presented as an explanation. This last avenue remains to be studied.

The mechanism postulated is also compatible with our binding data (Fig. 5). Indeed, we did not observe any significant difference in both the affinity (K_D) and density (B_{max}) of [^{3}H]nitrendipine binding sites in membrane preparations of the aorta from virgin and pregnant rats. Our K_D and B_{max} values are similar to those found in other reports on such membrane prepa-

rations (Morel and Godfraind, 1988). Under these conditions, membrane preparations are not polarized and the total number of binding sites are measured. It is well recognized that potential-operated Ca^{2+} channels are gated by membrane potential. Specific binding of [^{3}H](+)-PN 200-110, a dihydropyridine Ca^{2+} channel antagonist, was examined over a range of KCl concentrations in the intact aorta from spontaneously hypertensive and Wistar-Kyoto rats by Godfraind et al. (1990). Their data indicate that, at physiological KCl concentrations, 30% of Ca^{2+} channels are in a high affinity conformational state in aortae of hypertensive, while only 5% exhibit this conformation in normotensive rats. These findings suggest that sensitivity to dihydropyridines under different physiological conditions, including hypertension, is related to changes in the conformational state of voltage-operated Ca^{2+} channels. Such a mechanism is compatible with the present results, but remains to be verified.

Our study demonstrates that the resistance to vasoconstrictors observed in isolated vascular preparations of pregnant animals can be attributed to the decreased sensitivity of voltage-operated Ca^{2+} channels. Decreased sensitivity of the thoracic aorta of pregnant rats can be modulated by altering membrane potential with KCl. The decreased sensitivity of voltage-operated Ca^{2+} channels may be related to the general effect of cations on membrane potential. However, our data suggest the presence of specific alterations in the functions of voltage-operated channels, the mechanisms of which remain to be identified.

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