Pregnancy-induced changes in rabbit medial collateral ligament vasoregulation

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McDougall, J, Jason J., Ryan W. Giles, Robert C. Bray, and David A. Hart. Pregnancy-induced changes in rabbit medial collateral ligament vasoregulation. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1380–R1385, 1998.—The ligaments of weight-bearing joints are known to become mechanically inferior during pregnancy, and it has been postulated that this may be due to changes in tissue perfusion. Calcitonin gene-related peptide (CGRP) and epinephrine exert a tonic influence on the vasculature of the medial collateral ligament (MCL), and the present study examined whether these vasoactive influences were altered by pregnancy. Ligament perfusion experiments were performed on primigravid New Zealand White rabbits with the use of laser Doppler perfusion imaging. In pregnant animals (day 29), MCL basal perfusion fell significantly compared with control; however, values returned to normal 5 days postpartum. In normal joints, topical application of CGRP resulted in a dose-dependent increase in MCL perfusion, whereas epinephrine administration caused a dose-dependent fall in blood flow. During pregnancy, the vasoconstrictor effect of CGRP was completely abolished, whereas adrenergic vasoconstriction was greater than normal. Both responses returned postpartum. Pregnancy in the rabbit produces hypoenzymia in the MCL, and this phenomenon may be affected by a tempering of CGRP dilator responses and an augmentation of α-adrenoceptor-mediated vasoconstriction.

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virgin animals and as such made up the normal control group. Animals were sedated with acepromazine maleate (0.2 ml iv) and then deeply anesthetized by intraperitoneal injection of urethane (1 g/kg). Animals were placed in dorsal recumbency, and their body temperature was maintained at \( \sim 37^\circ C \) by a thermostatically controlled heating pad (American Pharmaseal). All experimental interventions had prior approval by the University of Calgary Animal Care Committee and were in complete accordance with the Canadian Council for Animal Care guidelines.

Surgical procedures. The right carotid artery was isolated in the neck and cannulated with a heparinized saline-filled cannula (Clay Adams PE-90, 0.86-mm ID) which was connected to a pressure transducer (Elcomatic EM752, Neilston, UK) to allow monitoring of systemic blood pressure. Pressure readings were recorded and analyzed on a computer using Codas software (Dataq Instruments). A longitudinal incision was then made in the medial aspect of the shaved knee joint, and the overlying skin was retracted to expose an area extending from the medial collateral ligament to the distal extremity of the patellar ligament. The superficial aponeurotic and fascial tissues were excised to remove any optical barrier to the ligament. Once the tissues were exposed, 37°C physiological saline (0.9% NaCl) was regularly superfused over the joint surface to prevent desiccation of the articular tissues.

Blood flow assessment. Ligament perfusion was measured by a laser Doppler perfusion imager (LDI) (Moor Instruments, Axminster, UK) using a standardized protocol (22) which has been validated for use in ligament blood flow studies (10). Briefly, a low-power (1 mW) He-Ne laser beam (633-nm wavelength) is scanned over the exposed surface of the joint and the backscattered Doppler broadened photons are collected by a photodetector which is incident within the scanner head. This information is then centrally processed to generate a two-dimensional, color-coded image of knee joint perfusion which is represented as a flux reading and assigned arbitrary perfusion units (PU). With the scanner head mounted in a stereotaxic frame and placed 19 cm above the exposed joint, a scan region was chosen that bounded the medial knee joint and typically took 20 s to complete. For testing perfusion to other articular structures, the hindlimb was internally rotated and a scan of the patellar ligament was performed. Measurements of the medial knee were taken during various experimental interventions (test) and related to control scans which were performed before the test scan. The hemodynamic changes effected by the experimental manipulations were of much longer duration than the scan time, and thus the possibility of missing any response was avoided. At the end of the experiment, the animal was killed by an overdose of pentobarbital sodium (360 mg intracardiac) and a final perfusion measurement (the "biological zero") obtained. Because the median sampling depth of a 633-nm laser is \( \sim 250 \mu m \) through highly absorptive skin (40) and medial collateral ligament blood vessels are mainly restricted to the superficial 180 \( \mu m \) of the tissue (9, 12), it is highly likely that the imager has sufficient penetrative power to assess ligamentous blood flow.

Experimental protocol. After a suitable equilibration period, an initial basal perfusion reading was made usually in both rabbit knees. CGRP (10^{-12} \text{ to } 10^{-9} \text{ mol}) was then applied topically to the ligament in a cumulative fashion, and a scan was taken 10 min after the application of each dose. This time point was chosen because it corresponded to the maximal response of the neuropeptide. The joint was then washed with saline and allowed to recover for 1 h before progression to the epinephrine part of the experiment. Epinephrine (10^{-14} \text{ to } 10^{-7} \text{ mol}) was applied topically to the joint, and a scan was taken 2 min after administration of each of the doses, which were applied in a cumulative manner to generate dose-response curves.

Topical application was chosen as the mode of drug administration in all of these experiments because it has been shown that this procedure maximizes drug delivery to the region of interest without affecting systemic blood pressure (15, 28).

Image and data analysis. Using a standardized protocol (22), we analyzed images using Moor LDI software. An analysis region corresponding to the medial collateral ligament was chosen, and the mean flux reading for the area was noted. The biological zero was subtracted from each image before any calculations were carried out, and experimental responses were expressed as a percent change in perfusion from control.

Individual data points were presented as means \( \pm SE \) for \( n \) observations. Statistical evaluation of the data was by either Student's t-test or one- or two-way ANOVA. A P value \(< 0.05\) was considered significant.

RESULTS

In first-time pregnant rabbits, basal perfusion of the medial collateral ligament was found to be significantly lower compared with age-matched virgin control animals (\( P < 0.03\), unpaired 2-tailed Student's t-test; \( n = 13 \) and 15 for control and pregnant animals, respectively), with blood flow falling from 358.1 \pm 41.3 PU in control rabbits to 233.3 \pm 35.2 PU during gravidity (Fig. 1). At 5 days postpartum, ligamentous perfusion returned to control levels. During pregnancy, mean arterial pressure appeared to fall slightly and then recover postpartum (Fig. 2), although this effect was not found to be statistically significant (\( P = 0.40\), 1-way ANOVA; \( n = 6 \) for pregnant and control groups and \( n = 3 \) for postpartum group). It
Fig. 2. Effect of pregnancy on systemic mean arterial blood pressure (MAP). Pregnancy was found to have no significant effect on blood pressure. NS, not significantly different. Data shown as means ± SE; n = 6 control, 6 pregnant and 3 postpartum.

is unlikely that the pregnancy-induced hypoeemia in the medial collateral ligament was due to the animal being hypotensive because perfusion to surrounding articular structures was unaltered during pregnancy. LDI-derived perfusion values derived from the patellar ligament of each group were not found to be significantly different from each other (P = 0.34; Fig. 1).

CGRP responses in normal and pregnant rabbits. Topical application of CGRP (10⁻¹³ to 10⁻⁹ mol) onto medial collateral ligaments of virgin animals caused an increase in tissue perfusion (Fig. 3). The vasodilatation was found to be dose dependent (P < 0.0001, 1-way ANOVA; n = 7), with the 10⁻⁹ mol dose producing the maximum increase in perfusion by 117.3 ± 26.0%. In primigravid animals, this dilator effect of CGRP was completely abolished (P = 0.2, 1-way ANOVA; n = 7) and in some instances a constrictor response to CGRP occurred. However, in postpartum animals the vasoactive effects of CGRP returned (P < 0.0001; n = 8) although the magnitude of the response was not as pronounced as in normal rabbits (P < 0.01, 2-way ANOVA).

Epinephrine responses in normal and pregnant rabbits. Epinephrine, when applied topically to the exposed surface of virgin rabbit medial collateral ligaments, caused a dose-dependent (P < 0.0001 1-way ANOVA) vasoconstriction of ligamentous blood vessels, culminating in a peak reduction in perfusion of 54.8 ± 4.8% compared with control with the 10⁻⁷ mol dose. Pregnancy caused a leftward shift in the dose-response curve to epinephrine (Fig. 4), indicating an augmentation of the constrictor response. Two-way ANOVA revealed a highly significant difference between control responses and those found in the primigravid animal (P < 0.0001). Five days postpartum, adrenergic vasoconstriction had returned toward control levels; however, the responsiveness was not normal at this early postpartum time point.

DISCUSSION

Pregnancy is known to affect the metabolism and structural integrity of ligaments in the peripheral joints of humans (1, 6, 11, 14, 32), rats (18, 37), and rabbits (16, 19). The putative correlation between connective tissue function and blood flow prompted this investigation into whether the increased laxity of knee joint ligaments during pregnancy could be related to a rise in ligament perfusion. It was found that perfusion to the medial collateral ligament of preterm primigravid rabbits fell compared with age-matched virgin controls. Thus there is no evidence to suggest that pregnancy-related ligament laxity is due to an upregulation of ligament perfusion in this model. The hypoeemic response to parity was pregnancy dependent because 5 days postpartum, ligament perfusion returned back toward control levels. It follows, therefore, that pregnancy affects the normal vasomotor control mechanisms of the medial collateral ligament only transiently. It could be argued, however, that because the rabbit knee is excluded from baroreflex vasomodulation (28), the observed changes in ligament basal blood flow were merely a consequence of pregnancy-related hypotension. Although systemic blood pressure did appear to show a slight reduction in the pregnant animal, it was not found to be statistically different from control rabbits and could not therefore be responsible for the conspicuous fall in ligament perfusion. Moreover, basal perfusion to the patellar ligament of the knee was unaffected by pregnancy, reaffirming the
position that pregnancy-induced hypoemia of the medial collateral ligament was not the result of a fall in systemic blood pressure.

Previous studies have shown that rabbit knee ligament blood vessels are richly innervated by CGRP containing primary afferent nerves (27) and that this neuropeptide is released tonically to oppose sympathetic vascular tone in this joint (15). The present study showed that, during pregnancy, the dilator effects of CGRP were completely abolished even at $10^{-9}$ mol, the highest dose used. Postpartum, CGRP caused a dose-dependent increase in ligamentous perfusion, although the magnitude of the response was not quite as pronounced as normal. This finding suggests that during pregnancy there may be a downregulation of CGRP receptor function or a decreased expression of the receptors on medial collateral ligament blood vessels. The fact that the response recovered almost completely postpartum indicates that the loss of CGRP vasoactivity is a pregnancy-dependent phenomenon. This suggests that during pregnancy the CGRP receptors are merely latent and have the potential to be "reactivated" once the inhibitory effects associated with pregnancy have subsided. Stevenson et al. (39) found that systemic levels of CGRP were significantly elevated during pregnancy but returned to control levels postpartum. It is possible, therefore, that an increased concentration of plasma CGRP during pregnancy could cause downregulation of CGRP receptors in certain tissues. In another study, pregnancy was shown to have a differential effect on CGRP-induced smooth muscle relaxation in the rat. At day 22 gestation, the normal relaxant effect of CGRP was abolished in the myometrium but maintained in the aorta, implying that gestational loss of CGRP efficacy is tissue specific (3). This result is very interesting because it could explain why the medial collateral ligament becomes hypoemic during pregnancy whereas perfusion to the patellar ligament is unaltered.

In addition to altered neuropeptide activity, topical administration of epinephrine to pregnant rabbit medial collateral ligaments caused a profound dose-dependent fall in tissue perfusion, the extent of which was greater than that of control animals. Again, this effect only lasted as long as the animal was pregnant, and constrictor responses subsequently returned toward control levels in the postpartum period. Possible reasons for increased epinephrine sensitivity include upregulation and/or increased expression of $\alpha$-adrenoceptors on the ligament vasculature. Alternatively, there could be a reduction in $\beta$-adrenoceptor population or function which would allow the constrictor effects of the $\alpha$-adrenoceptors to prevail. Previous studies on rat, human, and rabbit smooth muscle have shown that the number of $\alpha$-adrenoceptors is greater in pregnant than in nonpregnant animals (7, 33). This increase in potential $\alpha$-adrenergic binding sites is thought to be mediated by elevated estrogen levels during pregnancy because exogenously administered estradiol caused a similar rise in the number of smooth muscle $\alpha$-adrenoceptors (33). In vitro vascular reactivity studies have shown that pregnancy-induced changes in adrenergic contractility may be variable depending on the vascular bed being studied. $\alpha$-Adrenergic vasoconstriction is either attenuated or unaffected by pregnancy in carotid, renal, and mesenteric arteries (2, 13), whereas blood vessels of the uterus and hindlimb are more sensitive to sympathetic adrenergic activity (2, 13, 21, 30).

One possible limitation of this study is that pregnancy alters the pharmacokinetics of various anesthetics (17), and the resultant diverse sensitivity to these agents in the intrapartuant makes interpretation of perfusion changes problematic. This shortcoming aside, the combination of a decreased sensitivity to CGRP and upregulation of constrictor adrenergic responses during pregnancy could be contributing to the basal hypoemia observed in the collateral ligament at this time. This finding is in contrast to results from a host of other major tissues which clearly show an increase in blood flow during pregnancy and which have been postulated to be mediated by sex hormones such as relaxin (4, 5) and estrogen (23, 34). In light of estrogen receptors having been identified in human (24, 31) and rabbit (36) ligaments, the expected result of this study would have been a rise in ligament perfusion. However, estrogen is thought to play only a minor role in tissue vasoregulation, and therefore any effects of the hor-
more on ligament blood flow may only be secondary to the more potent effects of altered neuropeptidergic/adrenergic mechanisms outlined here. One possible purpose for the reduction in blood flow to the medial collateral ligament may be local enhancement of a shunting system whereby blood is redirected away from certain peripheral organs toward reproductive tissues such as the placenta. Because most articular tissues require a constant blood supply to maintain their integrity (29), this beneficial physiological response to pregnancy may occur at the expense of ligament homeostasis.

In summary, the results of the present study demonstrate that, during pregnancy, perfusion of the medial collateral ligament is reduced and this may be implemented by altered vasoregulatory mechanisms occurring at the tissue level. In addition to a hormonal component of ligament instability, pregnancy-induced hypoxia of the medial collateral ligament may also lead to a loss of tissue function and these effects may begin to explain the higher incidence of ligament injuries and joint degeneration in women.

Perspectives

It appears that intraparturient ligament laxity is not a direct result of vascular changes occurring in gravid tissues but is likely due to a complex combination of factors, including relaxin, which are upregulated in the pregnant animal. This principle is in contrast to what has previously been described in a ligament injury model in which tissue laxity occurred in conjunction with an increase in ligament perfusion (8). This apparent blood flow/biomechanical paradox may be allayed somewhat by recognizing that pregnancy and joint injury are two very disparate models, each with their own unique set of physiological parameters. In gravid animals, for example, connective tissues are subjected to heightened levels of pregnancy-associated hormones which may act directly on the tissue to induce laxity, whereas adaptive responses to ligament injury involve overt inflammatory mediators and proangiogenic events which may conspire to bring about changes in ligament function (26). Furthermore, joint injury tends to involve irreversible chronic disturbances to ligament homeostasis, whereas pregnancy is a temporary process, although it has yet to be ascertained whether these tissues return to prepregnancy conditions. Therefore, a variety of physiological mechanisms may bring about alterations in ligament function in a model and time-specific manner.

We thank Dr. W. Giles for helpful comments and Carol Reno for technical assistance.

Financial support for this work was provided by the Medical Research Council of Canada (MRC) and the Alberta Heritage Foundation for Medical Research (AHFMR). J. J. McDougall is an MRC and AHFMR Postdoctoral Fellow. D. A. Hart is the Calgary Foundation–Grace Glaucoma Professor, and R. C. Bray is an AHFMR Scholar.

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Received 10 February 1998; accepted in final form 10 July 1998.

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