Abrogation of α-adrenergic vasoactivity in chronically inflamed rat knee joints

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McDougall, Jason J. Abrogation of α-adrenergic vasoactivity in chronically inflamed rat knee joints. Am J Physiol Regulatory Integrative Comp Physiol 281: R821–R827, 2001.—It has previously been shown that chronic inflammation causes a reduction in sympathetic nerve-mediated vasoconstriction in rat knees. To determine whether this phenomenon is due to an alteration in smooth muscle adrenoceptor function, the present study compared the α-adrenoceptor profile of blood vessels supplying the anteromedial capsule of normal and chronically inflamed rat knee joints. While the rats were under urethan anesthesia, the α1-adrenoceptor agonists methoxamine and phenylephrine and the α2-adrenoceptor agonist clonidine (0.1-ml bolus; dose range 10^−12–10^−7 mol) were applied to exposed normal rat knees, resulting in a dose-dependent fall in capsular perfusion. Comparison of drug potencies indicated that α2-adrenergic effects > α1-vasoactivity. One week after intra-articular injection of Freund’s complete adjuvant to induce chronic joint inflammation, the vasoconstrictor effects of methoxamine, phenylephrine, and clonidine were all significantly attenuated compared with normal controls. These findings show that the preponderance of sympathetic adrenergic vasoconstriction in the anteromedial capsule of the rat is carried out by postjunctional α2-adrenoceptors. Chronic joint inflammation compromises α1- and α2-adrenoceptor function, and this change in α-adrenergic responsiveness may help explain the perfusion changes commonly associated with inflammatory arthritis.

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THE NEURAL CONTROL OF KNEE JOINT blood flow is central to the preservation of healthy articular tissues and is a principal factor in the maintenance of normal joint homeostasis (33). Joints are known to be richly innervated by both myelinated and unmyelinated nerve fibers (37, 38) with the unmyelinated group constituting the majority of all knee joint afferents (19, 20). About two-thirds of the unmyelinated fibers are sympathetic efferents whose number recedes following surgical or chemical sympathectomy with guanethidine (10). The autonomic nervous system is integral to vasoregulatory mechanisms with sympathetic adrenergic vasoconstriction having been described in knee joints of the rat (12, 18, 31), rabbit (29, 35, 36), cat (13), and dog (5). The adrenergic system is responsible for a resting vasoconstrictor tone in articular blood vessels that is offset, in part, by cholinergic vasodilator effects (4, 28) such that the relative contribution of these two opponent systems permits a regulated and constant blood supply to the tissues. The constrictor influence of the adrenergic neurotransmitter noradrenaline is known to be mediated by α-adrenoceptors resident on vascular smooth muscle (1), and these receptors have been shown to be functionally active in synovial tissue (6). By using selective adrenergic agonists and antagonists, Najafipour and Ferrell (35, 36) showed that sympathetic vasomotor control in the posterior capsule of the rabbit knee joint was mainly carried out by α2- and β1-adrenoceptors with the constrictor influence of the α-adrenoceptors predominating. Whether this ar-ticular adrenoceptor profile is consistent in other species and other regions of the knee requires further verification.

When a joint becomes inflamed, the normal vaso-regulatory mechanisms are altered, and articular per-fusion is affected. Intra-articular injection of Freund’s complete adjuvant, for example, produces an immunologically driven synovitis that is restricted to the treated joint. Blood flow studies have revealed that adjuvant monoarthritic joints are hypoaemic compared with control at 1 wk posttreatment but then recover back toward control levels by the third week (28, 30, 31). Sympathetic vasoconstrictor and cholinergic vasodilator responses have also been found to be attenuated at these time intervals, indicating a deterioration in autonomic neurovascular control in chronically inflamed knees (28, 30, 31). It was suggested at the time that the loss of nerve-mediated vasoconstriction may have been due to a reduction in the number of sympathetic nerve fibers innervating the joint, because adjuvant inflammation has been shown to deplete nerve terminals in the superficial lining of the capsule (11, 16), although it should be pointed out that these experiments were performed in the adjuvant polyarthritis model. Nevertheless, this rationale is not consistent with the commonly accepted belief that the sympa-
thetic nervous system contributes to inflammatory joint disease (3, 21, 23). An alternative explanation for abrogated constrictor responses in joints is a potential alteration in smooth muscle adrenoceptor function such that although noradrenaline is still being released from articular sympathetic efferents, its vasoactive effects are diminished. The aim of the present study was to assess α-adrenoceptor vasoactivity in the anteromedial aspect of normal rat knee joints and to ascertain whether adjuvant monoarthritis alters these vasoregulatory responses.

MATERIALS AND METHODS

A total of 16 male Wistar rats (371–630 g) was used in the present investigation of which eight underwent chronic, unilateral knee joint inflammation, whereas the remaining eight rats served as normal control animals. The inflamed group of rats was deeply anesthetized (2% halothane, 1 l/min O2), the right knee joint was shaved, and the diameter was measured with a digital micrometer (Mitutoyo Instruments, Tokyo, Japan). To provide comparable diameter readings, the anatomic placement of the micrometer was standardized with the callipers oriented along the joint line in a mediolateral plane between the femoral condyles and the tibial plateaus. Under aseptic conditions, inflammation was induced by injecting 0.2 ml of Freund’s complete adjuvant into the knee joint space (0.1 ml into the posterior region and 0.1 ml into the anterior compartment). Animals were allowed to recover without restricted cage activity for 1 wk. All surgical and experimental interventions were preapproved by the University of Calgary Animal Care Committee, which is in complete accordance with the guidelines set out by the Canadian Council for Animal Care.

Blood flow assessment. For terminal blood flow experiments, animals were deeply anesthetized by an intraperitoneal injection of urethan (25% stock solution, 2 g/kg), and the knee diameter was again measured to confirm an inflammatory reaction in the adjuvant-treated rats. The carotid artery was cannulated (PE-60 tubing, 0.76-mm internal diameter; Clay Adams, Sparks, MD) and attached to a pressure transducer (Elcomatic EM475, Nielston, Scotland) to allow continuous measurement of systemic blood pressure. Pressure readings were captured by a computer-based data-acquisition system (Dataq Instruments, Akron, OH) and stored for later analysis. Rat core body temperature was maintained at 37°C by placing the animal supine on a heating blanket (American Pharmaseal, Valencia, CA), and the medial region of the knee joint was exposed by removal of overlying skin and fascia. The joint was kept moist by regular superfusion of warmed physiological saline (0.9% NaCl, 37°C), which itself has no discernible effect on knee joint blood flow (26). A laser Doppler perfusion imager (Moor Instruments, Axminster, England) was used to measure articular blood flow using standardized protocols that have been validated for rat knee joint-perfusion studies (12, 25). The technique allows the tissue of interest to be scanned with a low-power He-Ne laser (633 nm) to provide a two-dimensional representation of articular perfusion that is based on both the concentration of circulating erythrocytes as well as the velocity at which the red corpuscles are moving through the tissue. With the scanner head positioned 20 cm above the rat knees, scans typically took 40 s to complete. Scans were taken before (control) and at 0, 2, and 5 min following administration of various adrenergic drugs (test). At the end of the experiment, the animal was killed by an overdose of pentobarbital sodium (240 mg intracardiac), and a dead scan was obtained. This “biological zero,” which was typically 5–10% of control, was subtracted from each image before any data calculation.

Adrenergic vasomodulation protocol. After knee joint exposure, warmed (37°C) physiological saline (0.9% NaCl) was applied topically to the joint for 10 min to ensure perfusion stability. Before drug administration, an initial control scan of the joint was taken to obtain a basal perfusion reading. Adrenergic agents were then applied topically to the joint as a 100-μl bolus. The α1-adreno ergic agonists used in this study were methoxamine and phenylephrine, whereas clonidine was used to assess α2-adrenergic vasoactivity. The reason for using two α1-adrenoceptor agonists was that although phenylephrine is commonly used as a selective α1-agonist, it does show a degree of α2-adrenoceptor agonism at higher doses (32). The use of an alternative α1-agonist (i.e., methoxamine) allowed us to verify the phenylephrine results. Prazosin and rauwolscine were used to determine α1- and α2-adrenoceptor antagonistic behavior, respectively. The dose range for the agonists was 10–12–10–7 mol, whereas a single dose of 10–7 mol was employed for the antagonists. These doses were chosen because when applied topically to the joint, they produced dynamic changes in articular perfusion without altering systemic blood pressure (see Table 1). On completion of the vasoreactive effects of each dose of methoxamine or phenylephrine, the knee was washed with warmed physiological saline until control perfusion levels resumed. Because saline washing was unable to reverse the vasoreactive effects of clonidine, it was necessary to generate a cumulative dose-response curve for this drug instead.

Image and statistical analysis. Images were analyzed using a standardized protocol (12) with customized software (Image Processor V.2.0, Moor Instruments). A region of interest corresponding to the anteromedial aspect of the knee was enclosed by a region of interest (ROI) from which background noise was subtracted. Imaging and statistical analysis.

<table>
<thead>
<tr>
<th>Table 1. Mean arterial pressure before and after topical administration of the various α-adrenergic agonists</th>
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<td>Control</td>
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<tr>
<td>Methoxamine</td>
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<td>Normal</td>
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<td>Inflamed</td>
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<td>Phenylephrine</td>
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<td>Inflamed</td>
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<tr>
<td>Clonidine</td>
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<tr>
<td>Normal</td>
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<tr>
<td>Inflamed</td>
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Values are means ± SE. Topical administration of the drugs had no significant (NS) effect on mean arterial blood pressure.
was selected, and the mean flux for this segment of the joint was calculated and recorded in arbitrary perfusion units (PU). Blood flow changes in response to drug activity were expressed as percent change in PU between control and test images. All data conformed to a Gaussian distribution and were therefore analyzed with parametric statistical tests (Student’s t-test, one- and two-way ANOVA) using GraphPad Prism software (GraphPad software, San Diego, CA). Data sets were considered significantly different when \( P < 0.05 \), and all data points were presented as means \( \pm SE \). Adrenergic agonist potencies were evaluated for both animal groups by comparing the relevant ED50, i.e., the dose of agonist that provokes a response halfway between the baseline and maximum response. The ED50s were derived from linear regression analyses of the dose-response curves using the GraphPad Prism software.

**Drugs.** Clonidine hydrochloride, methoxamine hydrochloride, phenylephrine hydrochloride, prazosin hydrochloride, and urethan were all obtained from Sigma Chemical. Pentobarbital sodium (Euthanyl) was supplied by MTC Pharmaceuticals (Cambridge, Ontario, Canada) and rauwolscine hydrochloride from Research Biochemicals International (Natick, MA). All adrenergic drugs were dissolved in 0.9% saline to attain the relevant concentrations and stored as 0.15-ml aliquots in the dark at 4°C until required.

**RESULTS**

As reported in other studies (28, 31), intra-articular injection of Freund’s complete adjuvant into the rat knee produced a conspicuous increase in knee joint diameter, confirming an inflammatory reaction localized to the treated joint.

**\( \alpha_1 \)-Adrenergic vasoactivity.** Administration of phenylephrine across the dose range of \( 10^{-12} \text{–} 10^{-7} \) mol resulted in a dose-dependent \(( P = 0.0025 \); repeated-measures one-factor ANOVA; \( n = 7 \text{–} 8 \)) fall in normal knee joint perfusion (Fig. 1). The maximal effect of the drug occurred with the \( 10^{-7} \)-mol dose, which caused blood flow to decrease by 86.26 \( \pm \) 15.5%. Methoxamine also produced a conspicuous reduction in articular perfusion (Fig. 2); however, the activity of this drug was found to be not dose dependent \(( P = 0.2134 \)). The \( 10^{-7} \)-mol dose of methoxamine also produced the greatest constrictor effect with normal knee joint perfusion falling by 68.17 \( \pm \) 4.7%. The ED50 for phenylephrine is shown in Table 2. Unfortunately, the ED50 for methoxamine could not be calculated due to the lack of dose dependency with this drug. Finally, topical

![Fig. 1. Vasoconstrictor effects of the \( \alpha_1 \)-adrenoceptor agonist phenylephrine in normal (●) and adjuvant-inflamed (○) rat knee joints. Phenylephrine caused a dose-dependent reduction in articular perfusion in normal joints that was significantly attenuated by chronic inflammation \(( P < 0.0001 \); 2-factor ANOVA). Data shown as means \( \pm SE \); \( n = 7 \text{ or } 8 \) for each group.](image)

![Fig. 2. Dose-response curves to the \( \alpha_1 \)-adrenoceptor agonist methoxamine on knee joint perfusion in normal (●) and chronically inflamed (○) rat knees. Joint inflammation causes a significant reduction in the constrictor effect of the drug compared with normal control \(( P < 0.0001 \); 2-factor ANOVA). Values are means \( \pm SE \); \( n = 7 \text{ or } 8 \) for each group.](image)
application of the $\alpha_1$-adrenoceptor antagonist prazosin ($10^{-7}$ mol) to normal knees caused basal blood flow to increase by $20.17 \pm 10.3\%$ (Fig. 3), indicating tonic activation of this receptor subtype under normal conditions.

One week after intra-articular injection of Freund’s complete adjuvant, the constrictor effects of both phenylephrine and methoxamine were significantly attenuated compared with normal control ($P < 0.0001$; two-factor ANOVA; $n = 7–8$; Figs. 1 and 2). Comparison of phenylephrine ED$_{50}$s for each group of animals (Table 2) showed that chronic inflammation of the rat knee caused a rightward shift in the dose-response curve of the drug by a factor of 10. Interestingly, administration of the antagonist prazosin to inflamed knees resulted in about a 15% decrease in articular perfusion, suggesting that prazosin may be acting as a partial agonist in these joints (Fig. 3). A two-tailed, unpaired Student’s $t$-test confirmed that the vasoactive effect of prazosin was significantly different between normal and adjuvant monoarthritic knees ($P = 0.0153$).

$\alpha_2$-Adrenergic vasoactivity. Topical application of the $\alpha_2$-adrenergic agonist clonidine to normal joints caused a dose-dependent ($P < 0.0005$; one-factor ANOVA; $n = 7–8$) fall in perfusion (Fig. 4). The most conspicuous constrictor effect of clonidine occurred with the $10^{-9}$-mol dose with perfusion falling by $62.37 \pm 5.8\%$ from control. The ED$_{50}$ for clonidine is shown in Table 2. The selective $\alpha_2$-adrenoceptor antagonist rauwolscine ($10^{-7}$ mol) caused a 30% increase in joint perfusion that was not significantly different from normal control ($P = 0.7025$).

Table 2. Comparison of $\alpha$-adrenergic agonist drug potencies as determined by ED$_{50}$ calculation in normal and chronically inflamed rat knees

<table>
<thead>
<tr>
<th>Drug</th>
<th>Normal</th>
<th>Inflamed</th>
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<tr>
<td>Phenylephrine</td>
<td>$6.0 \times 10^{-9}$</td>
<td>$5.9 \times 10^{-8}$</td>
</tr>
<tr>
<td>Clonidine</td>
<td>$1.2 \times 10^{-10}$</td>
<td>$4.7 \times 10^{-9}$</td>
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The selective $\alpha_2$-adrenoceptor agonist clonidine is more potent in normal knees than the selective $\alpha_1$-adrenoceptor agonist phenylephrine. In the inflamed joints, the ED$_{50}$s for each drug increase, indicating a rightward shift in the corresponding dose-response curves.
DISCUSSION

A constant and controlled blood supply in the joint capsule is essential to maintain the integrity of various articular structures such as the joint cartilage and cruciate ligaments. The present study investigated the relative contribution of α-adrenoceptor subtypes in regulating perfusion in the anteromedial capsule of the rat knee joint and demonstrated the alteration of these responses by an experimentally induced chronic inflammation. The increase in joint perfusion observed following topical application of the α-adrenergic antagonists prazosin and rauwolscine in normal joints suggests tonic release of noradrenaline from postganglionic sympathetic nerve terminals to maintain a constrictor tone in rat knee joint blood vessels. The occurrence of this phenomenon in the rat is consistent with what has been previously reported in the knee joint of the dog, cat, and rabbit (5, 7, 8). Comparison of the relative potencies of the α-agonists used here (Table 2) indicates that in the anteromedial aspect of the rat knee joint, postjunctial α2-adrenoceptors are the most abundant or are the most efficacious of the basic α-adrenoceptor subtypes. This finding extends our understanding of the distribution of different subclasses of α-adrenoceptors throughout the various regions of the knee. For instance, α1-adrenoceptors predominate in the anterior capsule and the medial collateral ligament of the knee (14, 27), whereas α2-adrenoceptors are responsible for the preponderance of sympathetic adrenergic vasoconstriction in the posterior region of the joint (35). This spatial heterogeneity in α-adrenoceptor activity may have an hereto unknown functional significance related to distinct metabolic requirements within disparate regions of the joint. It could be argued that the α-adrenoceptor profile on joint vascular smooth muscle described here may not be entirely accurate because phenylephrine is known to possess a mild affinity for other adrenoceptor subtypes at high concentrations (32). However, by using an alternative α1-agonist (i.e., methoxamine), we were able to confirm the validity of the phenylephrine results. Furthermore, by using a host of highly selective α-adrenoceptor antagonists, other investigators have shown that α1-autonomic selectivity is conserved within the joint, and there is no cross-reactivity between the various adrenoceptor subtypes provided the agonist dose range is limited to that described here (2, 34, 35).

Earlier studies found that 1 wk after intra-articular injection of Freund’s complete adjuvant into the rat knee, nerve-mediated sympathetic vasoconstriction was abrogated compared with control (30, 31). It was not known whether this was due to a decline in sympathetic neurotransmission, a degeneration of sympathetic nerve fibers, or an alteration in postsynaptic receptor sites. The latter alternative was recently supported by the fact that phenylephrine vasoactivity is diminished in adjuvant monoarthritic knees (2). The present study extended this finding by showing that in addition to phenylephrine attenuation, the vasoconstrictor effects of the α1-adrenoceptor agonist methoxamine as well as the α2-agonist clonidine were also significantly diminished compared with control. This observation implies either a downregulation and/or desensitization of postjunctional α-adrenoceptors situated on articular blood vessels supplying the anteromedial capsule of a chronically inflamed knee. The precise mechanism responsible for this alteration in α-adrenoceptor responsiveness is uncertain but may be due to sympathetic hyperactivity in the affected knee. There is considerable clinical and experimental evidence to suggest that peripheral sympathetic efferents contribute to the severity of synovial joint inflammation and that blockade of the sympathetic chain by either chemical or surgical means can alleviate some of the structural changes associated with arthritis (17, 21–24). Moreover, sympathetic hyperactivity is known to exacerbate joint inflammation in rats (21), implying that this division of the peripheral nervous system may be involved in the pathogenesis of inflammatory arthritis. If this is the case, then an increase in articular sympathetic nerve activity would lead to a substantial release of noradrenaline in the knee, resulting in overt synovial vasocstriction. Because noradrenaline acts on both α1- and α2-adrenoceptors, then the continuous activation of these receptors through chronic sympathetic hyperactivity would likely lead to a downregulation and/or desensitization of all α-adrenergic binding sites in the joint. This process would explain the reduced constrictor response of exogenously applied α1-agonists as demonstrated in this study. Evidence that at least some α1-adrenoceptors are still functionally active in the inflamed knee came from the fact that the α1-antagonist prazosin appeared to act as a partial agonist in the monoarthritic joint causing blood flow to decrease by ~15%. This finding also confirms that capsular blood vessels supplying adjuvant monoarthritic joints are not maximally vasoconstricted but have the capacity for further smooth muscle contraction. Thus we may be certain that the attenuated sympathetic vasoconstrictor responses described here and elsewhere are not merely a consequence of the inability of inflamed joint blood vessels to undergo smooth muscle contraction. The conspicuous rise in articular perfusion following topical application of rauwolscine onto adjuvant-treated knees indicates that this drug was able to block postsynaptic α2-adrenoceptor activity and hence offset a proportion of sympathetic adrenergic vasocstriction in inflamed joints. Rauwolscine, therefore, may have the potential to be a valuable therapeutic agent in the amelioration of synovial hypoaemia associated with chronic inflammatory joint disease.

To summarize, rat knee joint blood vessels possess vasoconstrictor tone brought about by basal activation of α1- and α2-adrenoceptors. This study also shows for the first time that α2-adrenoceptors are responsible for the preponderance of sympathetic adrenergic vasoconstriction in the anteromedial aspect of the normal rat knee joint. At 1 wk following adjuvant monoarthritis induction, there is a reduction in the density or affinity of postjunctional α1- and α2-adrenoceptors occupying...
the capsular blood vessels in this region of the joint. Although many other factors are undoubtedly involved, these findings begin to elucidate some of the mechanisms responsible for altered blood flow patterns in chronically inflamed joints.

Perspectives

Clinical and experimental evidence indicates that chronically inflamed joints tend to be hypoxic, and this is partly due to abnormally low articular blood flow. intermittently, these diseased joints exhibit episodic “flare-ups” in which the joint becomes acutely hyperaemic and intensely painful. These transitory increases in joint blood flow are thought to be the result of locally released vasodilators acting on joint blood vessels while these same mediators also sensitize articular primary afferent nerve endings leading to the heightened pain response. The more persistent hypoaemic phase of joint inflammation is probably due to sympathetic overdrive causing a profound vasoconstriction of joint blood flow. Prolonged activation of constrictor α-adrenoceptors ultimately leads to them being either downregulated or desensitized as evidenced in this study. With the vasoconstrictor capacity of the joint now compromised, it is vulnerable to the actions of proinflammatory vasodilators that gradually accumulate in the joint and the flare response ensues. Interestingly, during this acute hyperaemic phase of joint inflammation, α-adrenoceptor sensitivity is suddenly augmented (9, 15) leaving the joint susceptible to adrenergic vasoconstriction. This cyclical alteration in articular α-adrenoceptor function is probably a physiological response to the inflammatory process whereby the joint attempts to correct perfusion irregularities. Further investigation into the fluctuation in perfusion associated with joint inflammation and greater understanding of the mechanisms responsible for these changes may assist in the discovery of novel therapeutic strategies aimed at normalizing joint blood flow and alleviating the destructive effects of joint hypoxia.

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REFERENCES


