Norepinephrine-induced calcium signaling and expression of adrenoceptors in avian tendon cells

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Norepinephrine-induced calcium signaling and expression of adrenoceptors in avian tendon cells. *Am J Physiol Cell Physiol* 287: C912–C918, 2004.—Sympathetic efferent nerves are present in tendons, but their function within tendon is unknown. α1-Adrenoceptors are expressed by a variety of cell types. In the presence of norepinephrine (NE), adrenoceptors activate Gq/11 signaling pathways that subsequently increase intracellular Ca2+ concentration ([Ca2+]i). It was hypothesized that avian tendon cells express functional adrenoceptors that respond to NE by increasing [Ca2+]i. Avian tendon cells were analyzed for mRNA expression of α1-adrenoceptors by RT-PCR. Avian tendons expressed the α1A- and α1B-adrenoceptor subtypes. Furthermore, both tendon surface epitenon cells and internal fibroblasts infused with a Ca2+-sensitive dye, fura 2, and stimulated with NE responded by increasing [Ca2+]i. KMD-3213, an α1A-adrenoceptor antagonist, significantly reduced the Ca2+ response. Other adrenoceptor antagonists had no effect on the Ca2+ response. The absence of extracellular Ca2+ also significantly reduced the response to NE, indicating that Ca2+ influx contributed to the rise in [Ca2+]i. This study provides the first evidence that tendon cells express adrenoceptors and that the NE-induced Ca2+ response is coupled to the α1A-adrenoceptor subtype.

α-adrenoceptors; fibroblasts; catecholamines; tenocytes

GROWTH FACTORS, HORMONES, the mechanical environment, and catecholamines help mediate tissue growth, development, and repair. Norepinephrine (NE) stimulation of vascular α-adrenoceptors induces mitosis (14, 35, 52), vascular wall growth, contraction, and an increase in blood pressure as well as actions in other cell types (see Refs. 17 and 43 for review). Despite the presence of sympathetic efferent nerves in tendon, ligament, and other connective tissues (2, 9, 19, 46), little is known about the expression and function of adrenoceptors in these tissues.

Adrenoceptors are G protein-linked cell membrane receptors that are divided into three main families: α1, α2, and β. Stimulation of α1-adrenoceptors can liberate inositol triphosphate, diacylglycerol, arachidonic acid, and phosphatidic acid and produce changes in cAMP and intracellular Ca2+ concentration ([Ca2+]i) (12, 17, 22, 43, 51). Stimulation of α2-adrenoceptors can inhibit (block or close) Ca2+ channels, open potassium channels, and inhibit adenyl cyclase (12, 17, 43). However, data also indicate that α2-adrenoceptors are linked to Ca2+ influx (43). Stimulation of β-adrenoceptors activates adenyl cyclase and thus increases cAMP (12).

There are three α1-adrenoceptor subtypes: α1A, α1B, and α1D, the functions of which are widespread and the actions of which can depend on which cell type expresses them and the anatomical location (see Refs. 15, 17, 32, and 49 for reviews). For instance, stimulation of α1B-adrenoceptors on rat aorta adventitial fibroblasts induces proliferation, whereas α1A-adrenoceptors mediate this action on medial smooth muscle cells. In contrast, α1D-adrenoceptors mediate constriction of this vessel (52). With the vascular system, all α1-adrenoceptor subtypes are important for controlling contraction, vessel tone, and blood pressure.

Adrenergic nerves (i.e., containing tyrosine hydroxylase and neuropeptide Y immunoreactivity) are associated with the microvasculature within and around tendons and in the paratenon (2). Sympathetic, hemodynamic, and biomechanical factors modulate blood flow in tendon and ligament as in most other tissues (39, 48). In several cell types, NE or epinephrine induces an increase in [Ca2+]i through both Ca2+ influx and/or release from intracellular stores (11, 29, 31, 33, 38, 41, 44).

Evidence of adrenoceptors in connective tissues is found in UMR-106 cells, a rat osteosarcoma cell line, wherein cAMP and intracellular Ca2+ increased in response to NE (10, 33). The Ca2+ response was linked to both α1- and β-adrenoceptors. Ca2+ release from bone is also modulated by α- and β-adrenoceptors (47). Furthermore, adrenoceptors were unexpectedly found in relatively high abundance in vascular adventitial fibroblasts (20) that migrated and proliferated in response to α1-adrenoceptor stimulation (20, 52, 53). Mouse embryonic fibroblasts (3) as well as rat cardiac fibroblasts (35) also proliferated in response to β-adrenoceptor stimulation. However, no published data implicate a particular adrenoceptor subtype expressed in tendon cells.

Within tendons, an endotenon is continuous with the epitenon that encases the whole tendon. The endotenon, a sheet of loose connective tissue, binds together the blood vessels, nerves, and collagen fascicles. Thus the surface cells of the epitenon are more likely to be exposed to NE under normal physiological conditions than the internal fibroblasts that reside amidst the collagen fibrils. NE may have a greater effect on these epitenon cells than the internal fibroblasts. Therefore, it was hypothesized that avian tendon surface epitenon cells and internal fibroblasts infused with a Ca2+-sensitive dye, fura 2, and stimulated with NE responded by increasing [Ca2+]i. KMD-3213, an α1A-adrenoceptor antagonist, significantly reduced the Ca2+ response. Other adrenoceptor antagonists had no effect on the Ca2+ response. The absence of extracellular Ca2+ also significantly reduced the response to NE, indicating that Ca2+ influx contributed to the rise in [Ca2+]i. This study provides the first evidence that tendon cells express adrenoceptors and that the NE-induced Ca2+ response is coupled to the α1A-adrenoceptor subtype.

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METHODS

Cell isolation and culture. Surface epitenon cells and internal fibroblasts were isolated from 6-8 pooled digital flexor digitum profundus (DFP) tendons of 42-day-old Cornish chickens (Perdue Farms, Robbins, NC) by sequential collagenase and trypsin digestions modified from Banes et al. (5). The tendon was severed proximal to the osteotendinous junction and at the level of the third metatarsal bone. Cells were cultured in DMEM with 5% fetal bovine serum, 100 μg streptomycin, and 100 units/ml penicillin. To evaluate the in vitro Ca^{2+} response, both ATSC and ATIF between passages 1 and 6 were plated in micromass, spot cultures at 2,000 cells/10 mm^2. RNA were mixed with the following in chloroform, and vortexed. After sedimentation, the supernatant fluid was extracted with RNA Stat-60 and chloroform, mixed, and sedimented. Pellets were reconstituted in 1 ml of diethylpyrocarbonate-treated deionized water.

Total RNA isolation from tissue. Total RNA was isolated from tissue samples (1-5 g wet wt) of 3 to 6 wk-old White Leghorn chickens (Cackle Hatchery, Lebanon, MO). Each DFP was cut into three sections, labeled with fura 2-acetoxy methyl ester (fura 2-AM; Molecular Probes, Eugene, OR), and then potted in 1% agarose gel (to stabilize the tendon during incubation at room temperature for 5 min, and then treated with 1% agarose gel (to stabilize the tendon during incubation). Tendon explants were incubated at room temperature for 5 min followed by 3 cycles of denaturing, annealing, and extension at 94°C, 62°C, and 72°C, respectively, for 30 s each and a final extension at 72°C for 5 min in a Perkin-Elmer 2400 thermocycler (Wellesley, MA). Amplification products were separated in 1.8% agarose gels for 22 min at 93 V (20 mA). Gels were stained with ethidium bromide (Fisher Scientific) and photographed with a Polaroid camera (Waltham, MA). The relative band intensity was quantified by image analysis.

Cloning. Primers for rat α1-adrenoceptors were used to amplify mRNA in the bone tissue from 52-day-old White Leghorn chickens. The cDNAs were extracted from the gel using a QIAquick gel extraction kit and purified with a QIAquick PCR purification kit (Qiagen, Valencia, CA). The cDNAs were ligated using an Original TA cloning kit and transformed into TOP10 Escherichia coli cells using a One Shot kit (Invitrogen). Plasmid DNA was isolated from bacteria using a QIAwells Ultra Plasmid kit (Qiagen) and then sequenced. Sequences were submitted to GenBank.

Intracellular Ca^{2+} measurements. On day 6 after plating, ATSC and ATIF were rinsed twice with EBSS. The cells were then incubated in the dark at room temperature in 2.5 or 5 μM fura 2-AM with 0.1% Pluronic-127 (Molecular Probes) for 45-60 min. After incubation, the cells were rinsed twice in EBSS to remove any unincorporated fura 2-AM. The six-well dish was mounted on the stage of an Olympus BX-51 upright fluorescence microscope (Melville, NY) equipped with a 40× water immersion ultraviolet objective lens, a Sutter Lambda DG4 wavelength switcher and light guide (Novato, CA), and a CoolSnap digital camera (Roper Scientific, Trenton, NJ). Image analysis software (ImageScape Imaging Systems, Raleigh, NC) was used to quantify the changes in Ca^{2+} concentration. The ratio method, with 340/380 (bound) to 380 (free) nm excitation and 510-nm and above emission, was used to convert the fluorescence emission intensity of the labeled tendon cells to Ca^{2+} ion concentration by comparison to known Ca^{2+} standards (23). All cells in a field of view [9-77 cells in 2-dimensional (2-D) cultures, 12-151 cells in explants] were selected and outlined, and the pixel intensity relating to Ca^{2+} concentration was quantified for each cell. Background images obtained from an area without cells at the start of each experiment. Background pixel values were subtracted from each image to correct for nonuniformity due to variation in the fluorescence excitation beam across the field.
Treatment with NE and differentiation of adrenoceptor subtypes. Avian tendon cells were exposed to 0.01, 0.03, 0.1, 0.3, 1, or 10 μM NE (n = 2–8 isolations with 2–12 replicates). Cells in any one specimen were exposed to only one NE concentration. Basal Ca\(^{2+}\) levels were determined by averaging the [Ca\(^{2+}\)]\(_{ic}\) over 30–60 s before NE treatment. For 2-D cultures, a cellular response was mathematically represented as an increase in [Ca\(^{2+}\)]\(_{ic}\) that averaged three standard deviations above the basal level of the cell. Avian FDP explant sections were treated with 1 or 10 μM NE, and an FDP section from the opposing leg was treated with 1, 10, or 100 μM ATP as a positive control. Each section received only one drug concentration. For explants, a cellular response was mathematically represented as an increase in [Ca\(^{2+}\)]\(_{ic}\) that averaged two standard deviations above the basal level, since there was a large change in the background across the data set from the autofluorescence of collagen. The response was averaged from the initial increase in [Ca\(^{2+}\)]\(_{ic}\) after treatment until the Ca\(^{2+}\) signal output returned to a steady level.

ATSC and ATIF were also exposed to 1 μM NE in EBSS that contained no exogenous Ca\(^{2+}\) to differentiate between extracellular Ca\(^{2+}\) influx vs. release of Ca\(^{2+}\) from intracellular stores (n = 4 isolations with 2–6 replicates). Cells were incubated in EBSS without Ca\(^{2+}\) for at least 30 min before NE treatment. To differentiate among the possible adrenoceptors activated by NE, ATIF (n = 3–4 isolations with 3–4 replicates) were incubated in 100 nM of α1A-adrenoceptor antagonist KMD-3213 (kindly proved by Dr. Y. Kurashina and Kissie Pharmaceutical, Matsumoto City, Japan), α1B-adrenoceptor antagonist AH-11110A, or α2-adrenoceptor antagonist RX-821002 or 1 μM β-adrenoceptor antagonist propranolol for 30 min before exposure to NE. The concentrations of the antagonists were within the selective concentration for these compounds (52, 53). All pharmacological agents were purchased from Calbiochem (San Diego, CA), Tocris (Ballwin, MO), or Sigma unless otherwise noted.

Statistical analysis. Data were analyzed using JMP (SAS Institute, Cary, NC) or SigmaStat (SPSS, Chicago, IL) and subjected to a Student’s t-test, nonparametric test, or one-way ANOVA with a post hoc test, where appropriate, to determine significance between and among groups (P < 0.05). Data are presented as means ± SE. About 1.1% of all cells analyzed in 2-D cultures had a slow gradual increase in [Ca\(^{2+}\)]\(_{ic}\) over the entire period of data collection. Because these cells did not satisfy the defining criteria of a responding cell, they were considered to be nonresponders.

RESULTS

α-Adrenoceptor expression. The partial avian α1A-adrenoceptor sequence is aagagaaaa gcgggcggct catgtagcgc ctcaa-gacc acaagtacgtagcggct ccaccagc ctaaaatgtc ctcctaga gcgggcggat ccagcctgcc aagaatatgtc ctcacttctc actgtgcttgctggctgcc tctagtaatgtc (GenBank AF548388). This sequence is 100% homologous with rat α1A-adrenoceptor. The partial avian α1B-adrenoceptor sequence is cctgggtgg ctctagctgcc actgtctgcc aaattatgac ctcctacttctc ctcacttctc actgatcgtgct gaggctg ctcaagtttt ctcgag (GenBank AF548388). This sequence is 85% homologous for rat α1B-adrenoceptor. All avian tissues analyzed expressed both the α1A- and α1B-adrenoceptor subtype mRNA (Fig. 1). The relative amounts of each adrenoceptor subtype within the tissues were not determined, only presence and absence of adrenoceptors. The avian α1D-adrenoceptor sequence lacked homology to the known rat sequence. Furthermore, the α1D-adrenoceptor antagonist did not affect the Ca\(^{2+}\) response to NE (see below); therefore, α1D-adrenoceptor expression was not further analyzed.

Response to NE. NE treatment increased [Ca\(^{2+}\)]\(_{ic}\) in both ATSC and ATIF cultured from the FDP of 42-day-old Cornish chickens to NE concentrations >0.1 μM (Fig. 2). About 1% of ATSC and 6% of ATIF spontaneously responded before NE treatment. Neither the overall percentage of responding cells to NE nor the pattern of the response was different between the two cell types except at high concentrations of NE (Fig. 2), which is surprising because ATSC are more closely associated with the blood vessels and nerves in tendon. NE induced an immediate Ca\(^{2+}\) transient in ATSC and ATIF (Fig. 2). Ca\(^{2+}\) oscillations also were observed in a small percentage of the cells in response to NE (Fig. 3). An oscillatory response was characterized by an initial Ca\(^{2+}\) transient that returned to a baseline followed by one or more secondary transients that usually had a smaller [Ca\(^{2+}\)]\(_{ic}\) peak (Fig. 3). The Ca\(^{2+}\) response to NE of tenocytes in FDP explants was relatively low compared with an ATP positive control (Fig. 4).

Source of rise in intracellular Ca\(^{2+}\). To determine whether the rise in Ca\(^{2+}\) originated from intracellular stores, Ca\(^{2+}\) influx, or both, ATSC and ATIF were treated with 1 μM NE in the absence of exogenous Ca\(^{2+}\). For both cell types, the NE-induced Ca\(^{2+}\) response was moderately attenuated with 20–30% of the cells responding (Fig. 5). The overall increase in [Ca\(^{2+}\)]\(_{ic}\) over baseline was unaffected by the removal of
extracellular Ca\(^{2+}\) (Fig. 5). In addition, \(<1\%\) of the cells had an oscillatory response (data not shown).

**Adrenoceptor subtype dependency.** To determine the adrenoceptor subtype activated by NE, ATIF were treated with one of the following: KMD-3213, AH-11110A, BMY-7378, or RX-821002 (all at 0.1 \(\mu M\)) or 1 \(\mu M\) propranolol, for 30 min before exposure to 0.01, 0.03, 0.1, 0.3, or 1 \(\mu M\) NE. Only KMD-3213 significantly reduced the percentage of cells responding and the increase in \([Ca^{2+}]_{ic}\) over baseline (Fig. 6, Table 1). In the presence of RX-821002, there was a reduced response to 0.03 \(\mu M\) NE (Table 1).

**DISCUSSION**

Tendons are sympathetically innervated (2). Therefore, the purpose of this study was to determine whether avian tendon cells express adrenoceptors and respond to NE stimulation through a Ca\(^{2+}\) signal. Avian tendon cells expressed mRNA for both \(\alpha_{1A}\)- and \(\alpha_{1B}\)-adrenoceptors. Furthermore, ATSC and ATIF, as well as tenocytes in whole tendon explants, responded to NE by increasing \([Ca^{2+}]_{ic}\), demonstrating that these cells have functional adrenoceptors.
Tendon cells significantly responded to NE concentrations >0.1 μM. Physiological NE concentration can depend on nearness to adrenergic nerves and level of activity where concentrations are in the micromolar range (8). If the tissue is innervated, then the NE concentration depends on plasma concentrations. Basal plasma NE is ~2–3 nmol/l and can increase 10-fold during exercise (25, 30). Injury and inflammation can increase local NE release by locally activating nerves (13). In addition, ischemia can increase NE efflux and thus raise tissue concentrations to micromolar levels (45). Thus the response to NE within tendon cells in vitro is within physiological and/or pathophysiological ranges.

NE induced either an immediate or a biphasic Ca²⁺ signal. The majority of tenocytes had an immediate Ca²⁺ transient upon stimulation by NE that rapidly returned to basal levels. In a small percentage of avian tendon cells as well as in astrocytes (41) and hepatocytes (50), a biphasic Ca²⁺ response was also observed. This response is characterized by an initial Ca²⁺ transient followed by secondary Ca²⁺ oscillations. In Chinese hamster ovary cells transfected with α₁A-adrenoceptor (29) and rat afferent arterioles (44), the Ca²⁺ response is characterized by an initial Ca²⁺ peak followed by a secondary sustained plateau increase in [Ca²⁺]ic. In UMR-106 cells (33), the secondary phase was characterized by a slow rise in [Ca²⁺]ic. This secondary oscillatory response in the avian cells is dependent on Ca²⁺ influx as illustrated by the loss of oscillations in the absence of exogenous Ca²⁺. Similar losses of the biphasic response were also seen in Chinese hamster ovary cells, afferent arterioles, and UMR-106 cells.

Because the source of Ca²⁺ can originate from intracellular stores or from extracellular Ca²⁺, intracellular Ca²⁺ was removed from the bathing solution and cells were then stimulated with NE. The Ca²⁺ response in the absence of exogenous Ca²⁺ was attenuated but not completely abolished, indicating that NE induced the release of Ca²⁺ from both intracellular stores and influx of Ca²⁺ through ion channels in the plasma membrane. In addition, the NE-induced Ca²⁺ rise in tendon cells appeared to be mediated by α₁A-adrenoceptor because a specific antagonist for this adrenoceptor reduced the overall percentage of cells responding to NE as well as the increase in [Ca²⁺]ic. The reduced response to 0.03 μM NE in the presence of RX-821002 indicated that there may be a small contribution from α₂-adrenoceptors. There was a trend toward reduction in the percentage of cells responding to NE (Fig. 6) and in the rise in [Ca²⁺]ic (Table 1) in the presence of the α₁B-, α₁D-, and α₂-adrenoceptor antagonists (not significant by 1-way ANOVA). This may reflect, in the case of AH-11110A and BMY-7378, partial inhibition of α₁A-adrenoceptor population by these compounds. Although these agents are the most selective α₁B- and α₁D-adrenoceptor antagonists available, they are not fully “specific.” BMY-7378 has a 267-fold selectivity for α₁D-adrenoceptors over the α₁A- and α₁B-adrenoceptors (see Ref. 53). AH-11110A has only a 32- and 26-fold selectivity for α₁B-adrenoceptors over the α₁A- and α₁B-adrenoceptors, respectively (see Ref. 53). KMD-3213 is 56- and 583-fold selective for α₁A- over α₁D- and α₁B-adrenoceptors, respectively (see Ref. 53). RX-821002 and propranolol have 1,000-fold or more selectivity for α₂- and β-adrenoceptors, respectively, over α₁-adrenoceptors. Thus, given the limitations of available antagonists, our data suggest that α₁A-adrenoceptors are primarily responsible for NE activation of avian tendon fibroblasts, with a potential small contribution by α₂-adrenoceptors.

In addition to stimulation of tenocytes in vitro, cells within whole tendon explants responded to NE. However, only a low percentage of the cells responded. This low responsiveness could be due to the anatomical location of the tenocytes within the FDP. Cells in closer proximity to blood vessels or nerves could have a greater response than those cells more isolated

Table 1. Normalized increase in intracellular Ca²⁺ in the presence of α-adrenoceptor antagonist

<table>
<thead>
<tr>
<th>NE Concentration</th>
<th>0.01, μM</th>
<th>0.03, μM</th>
<th>0.1, μM</th>
<th>0.3, μM</th>
<th>1, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>71 ± 12</td>
<td>102 ± 12</td>
<td>117 ± 10</td>
<td>100 ± 10</td>
<td>144 ± 11</td>
</tr>
<tr>
<td>KMD-3213</td>
<td>68 ± 11</td>
<td>59 ± 11*</td>
<td>67 ± 6*</td>
<td>79 ± 11</td>
<td>79 ± 7*</td>
</tr>
<tr>
<td>AH-11110A</td>
<td>70 ± 16</td>
<td>91 ± 18</td>
<td>85 ± 13</td>
<td>93 ± 11</td>
<td>103 ± 11</td>
</tr>
<tr>
<td>BMY-7378</td>
<td>61 ± 14</td>
<td>80 ± 8</td>
<td>96 ± 12</td>
<td>102 ± 12</td>
<td>103 ± 17</td>
</tr>
<tr>
<td>RX-821002</td>
<td>65 ± 8</td>
<td>51 ± 13</td>
<td>93 ± 7</td>
<td>103 ± 11</td>
<td>94 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SE (in nM) and represent the normalized (%) increase in intracellular Ca²⁺ in response to each treatment. NE, norepinephrine. *P < 0.05 vs. no drug for same NE concentration.
from the sources of NE. However, we found that epitenon surface cells, which are closely associated with blood vessels and nerves, did not respond differently from internal fibroblasts from the tendon midsubstance. The age of the tissue (5–6 wk old) could also account for the lower response. Cells in avian aortic smooth muscle respond to NE with the greatest Ca\(^{2+}\) response seen in 12- to 18-wk-old chickens and less in older (20–37 wk old) animals (42). Muscle from younger (5–8 wk old) chickens did not respond to NE with a Ca\(^{2+}\) transient. In addition, rat myocyte adrenoceptor coupling to L-type Ca\(^{2+}\) channels changes during development (37). Explant tissue may present fewer adrenoceptor-expressing cells. Alternatively, the explant model itself may decrease responsiveness to NE by reducing NE diffusion or increasing degradation.

The importance of adrenergic activation in tenocytes is unknown. Within most connective tissues, it is believed that sympathetic nerves control blood flow through the tissues, since most of the nerves are located in or around blood vessels (2, 9, 39). Adrenoceptors also may be involved in endocrine regulation of connective tissues. Both cartilage and meniscus have little vasculature and nerves but express the mRNA for \(\alpha_{1A}\) and \(\alpha_{1B}\)-adrenoceptors (see RESULTS). Only about one-third of the meniscus is innervated and vascularized (16). Cartilage is aneural and avascular, relying on diffusion from the synovial fluid for nutrition, metabolites, and waste removal. However, the synovium is sympathetically innervated (27), NE is also secreted from cells in synovial tissue taken from arthritic knees (40). Thus NE or circulating adrenaline could diffuse from blood vessels into the synovial fluid and stimulate adrenoceptors in meniscus or cartilage cells. Finally, adrenoceptors may be involved in the response to healing after injury. In rheumatoid arthritis, tyrosine hydroxylase-positive nerve fibers were reduced, but the density of tyrosine hydroxylase-negative cells, which spontaneously secrete NE, was increased. Furthermore, NE (10\(^{-5}\) M) reduced the secretion of inflammatory cytokines in synoviocytes from patients with arthritis (40). Thus NE may play a role in modulating inflammation.

Sympathetic efferent nerves corelease neuropeptides and neurotransmitters. Neuropeptide Y is released with NE. In tendon, recent data have indicated that neuropeptides may be important in healing. Ackermann and colleagues (1) reported that in Achilles tendons, autonomic reinnervation was low up to 4 wk postrupture while the greatest increase occurred around 6–8 wk postrupture. Neuropeptide Y-positive nerve fibers were observed in the surrounding connective tissue and tendon proper, mainly in association with the vasculature. In addition, within the synovial fluid of arthritic joints, both sensory and autonomic neuropeptides were elevated (4, 26, 34). Neuropeptide Y also promotes wound healing and angiogenesis (18). Thus autonomic neuropeptides may act locally alone or with NE to modulate the response to healing and perhaps promote the growth of new vasculature within the healing tendon.

During flexor tendon healing, the epitelen surface cells and even synovial sheath cells, where present, initiate the repair process (21, 24, 28, 36). In addition, blood vessels, and possibly nerves, may aid in healing (36). In avian tendons subjected to trauma, collagen synthesis was decreased in partially devascularized tendons compared with those tendons allowed to heal with the blood supply intact (6). It has also been shown that vascular adventitial fibroblast proliferation was induced by prolonged stimulation of \(\alpha_{1}\)-adrenoceptors by NE in the intact vascular wall, and this growth was further augmented after injury (52). In addition, NE and growth factors synergized to promote migration of adventitial fibroblasts (53). Thus, in a similar fashion to adventitial fibroblasts, the migration of tendon cells into a wound and the growth of new tissue during repair could be augmented by adrenergic stimulation. Indeed, stimulation of the mitotic pathway in most cells by growth factors and certain G protein-coupled agonists involves a rise in intracellular Ca\(^{2+}\) early in the signaling pathway. Therefore, activation of adrenoceptors, mainly \(\alpha_{1}\), with a concomitant rise in [Ca\(^{2+}\)]\(_{ic}\) may be involved in a maintenance function as well as a healing response in tendon.

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GRANTS

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