Annexin I Is a Local Mediator in Neural-Endocrine Feedback Control of Inflammation

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Green, Paul G., Holly J. Strausbaugh, and Jon D. Levine. Annexin I is a local mediator in neural-endocrine feedback control of inflammation. J. Neurophysiol. 80: 3120–3126, 1998. Activation of primary afferent nociceptors induces a neural endocrine–mediated inhibition of the inflammatory response via a circuit that includes ascending spinal pathways and activation of the hypothalamic-pituitary-adrenal (HPA) axis. This circuit inhibits sympathetic neuron-dependent plasma extravasation (PE) in the rat knee joint produced by bradykinin (BK), but not sympathetic neuron–independent PE produced by platelet activating factor (PAF). Nocuous (25 mA) but not nonnocuous (2.5 mA) electrical stimulation significantly increased plasma corticosterone concentrations, and intravenous infusion of corticosterone (5 µg/min) mimicked inhibition of BK-induced PE produced by noxious stimulation. However, perfusion of corticosterone locally through the knee joint, at doses that do not have a systemic action (i.e., ≤1 µM), did not inhibit BK-induced PE. Annexin I (lipocortin-1), a 37-kDa member of a family of phospholipid and calcium binding proteins, can mediate local anti-inflammatory effects of glucocorticoids via a mechanism that is partially dependent on inhibition of phospholipase A₂ activity and adhesion and transmigration of polymorphonuclear leukocytes. Because BK-induced PE is dependent on both polymorphonuclear leukocytes and phospholipase A₂ activity, we tested the hypothesis that the action of corticosterone to inhibit BK-induced PE is mediated by stimulating the production and release of annexin I. Perfusion of BK (150 nM) through the rat knee joint induces a rapid and sustained increase in PE. Co-perfusion of BK with annexin I (100 ng/ml) through the knee joint mimics the inhibition of BK-induced PE produced by noxious electrical stimulation or by intravenous corticosterone. Co-perfusion of BK with annexin I antibody (LCPS1, 1:60 dilution) prevented the inhibition of BK-induced PE produced by noxious electrical stimulation or intravenous corticosterone administration. PAF-induced PE, which is not dependent on polymorphonuclear leukocytes, was not inhibited by local perfusion of annexin I. These data suggest that the inhibitory effect of C-fiber activity on BK-induced PE, acting via an HPA circuit, is mediated by annexin I in the knee joint.

I N T R O D U C T I O N

Activation of primary afferent nociceptors induces a neural endocrine–mediated inhibition of the inflammatory response via a circuit that includes ascending spinal pathways and activation of the hypothalamic-pituitary-adrenal (HPA) axis (Green et al. 1995). During chronic inflammation, produced by injection of complete Freund’s adjuvant in the rat hind paw, or during continuous C-fiber intensity electrical stimulation in the hind paw, there is a marked inhibition of plasma extravasation (PE; a component of the inflammatory response), induced by the potent inflammatory mediator, bradykinin (BK). We have provided evidence that activation of this negative feedback control of PE, induced by noxious stimulation, is mediated by release of corticosterone (Green et al. 1995), the end product of the HPA axis. Corticosterone inhibits the component of BK-induced PE that is dependent on the sympathetic postganglionic neuron (SPGN) terminal (Green et al. 1997), but not the SPGN-independent component of BK-induced PE or the SPGN-independent PE produced by platelet activating factor (PAF). Although it is not known at what target corticosterone acts to produce an inhibition of BK-induced PE, we report here that corticosterone itself does not appear to act locally in the knee joint. This suggests that there is a mediator released by corticosterone that acts locally to inhibit BK-induced PE. There is a large body of literature describing the role of annexin I (lipocortin-1) in local anti-inflammatory effects of glucocorticoids (Flower and Rothwell 1994; Goulding and Guyre 1993). Annexin I is a 37-kDa member of a family of phospholipid and calcium binding proteins, known to inhibit phospholipase A₂ and influence membrane-cytoskeletal linkage (Burgoyne and Geisow 1989), and has been reported to contribute anti-inflammatory effects of glucocorticoids (Flower and Rothwell 1994; Goulding and Guyre 1992, 1993; Moreno 1997; Perretti and Flower 1994, 1995; Solito et al. 1993). The anti-inflammatory effects of annexin I appear to be at least partially dependent on inhibition of phospholipase A₂, a proximal enzyme in the pathway that metabolizes membrane lipids to prostaglandins and leukotrienes, but also on inhibition of adhesion and transmigration of polymorphonuclear leukocytes and their generation of superoxide (Maridonneau-Parini et al. 1989; Perretti et al. 1995, 1996b). Because of these properties of annexin I and because BK-induced PE is dependent on both polymorphonuclear leukocytes and prostaglandins, we tested the hypothesis that the effect of corticosterone to inhibit SPGN-dependent BK-induced PE is mediated via stimulation of the production and release of annexin I.

M E T H O D S

A n i m a l s

The experiments were performed on 300- to 400-g male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA). The rats were housed two to a cage in a temperature (72 ± 2°C) and...
humidity (45 ± 5%) controlled environment and were maintained on a 12-h light/dark cycle (lights on at 06:00). Food and water were available ad libitum. To minimize stress, animals were only handled during cage cleaning (bi-weekly), and one investigator administered the pentobarbital sodium anesthesia. Experiments were performed between 0900 and 1500 h. All procedures involving animals in our experimental protocols have been reviewed and approved by the Committee on Animal Research at the University of California, San Francisco.

PE

Rats were anesthetized with pentobarbital sodium (Anthony Products, Arcadia, CA; 65 mg/kg). Skin overlying both knees was excised to expose the joint capsules, and rats were then given an intravenous injection of Evans blue dye (50 mg/kg, in a volume of 2.5 ml/kg). A 30-gauge hypodermic needle was then inserted for the inflow of perfusion fluid (250 µl/min; controlled by a syringe pump, Sage Instruments model 341B); after perfusion of 100–200 µl of fluid, a second needle (25 gauge) was inserted into the joint cavity for outflow of the perfusion fluid (250 µl/min; syringe pump, Sage Instruments model 351). Both knees were perfused; samples of perfusion fluid were collected over 5-min intervals for a period of 70 min, and samples were analyzed for Evans blue dye concentration by spectrophotometric measurement of absorbance at 620 nm, which is linearly related to dye concentration (Carr and Wilhelm 1964). After establishment of baseline levels of PE in the first three samples, BK (150 nM) was added to the perfusing fluid and remained present in the fluid for the duration of the experiment. Other drugs added to the perfusion fluid, as indicated in individual experiments, remained in the perfusion fluid for the duration of the experiment.

Noxious electrical stimulation

Two stainless steel electrodes (23-gauge hypodermic needles) were placed transversely through the plantar side of one hind paw (~10 mm apart); the stimulus intensity necessary to excite C fibers has been previously determined electrophysiologically (Green et al. 1995). Noxious (C-fiber strength) electrical stimulation was applied to the hind paw during the assessment of BK–induced PE starting 25 min after the initiation of BK perfusion in the knee joint, rats received noxious electrical stimulation (25 mA, 0.25 ms duration pulses, 3 Hz) via the two stimulating electrodes, which continued for the duration of the experiment.

Corticosterone administration

Corticosterone was administered intra-articularly (ia) or intravenously (iv). When administered intra-articularly, corticosterone was co-perfused with BK at either 1- or 10-µM concentrations. When administered intravenously, corticosterone was administered via a 25-gauge infusion catheter inserted into the lateral tail vein, at a rate of 1 ml/h, to deliver a dose of 0.05, 0.5, or 5 µg/min (rate controlled by a Sage 351 syringe pump).

Annexin administration

To determine the effect of exogenously administered annexin I in BK-induced PE, 25 min after beginning BK perfusion, annexin I (100 ng/ml) was added to the perfusion fluid, in one knee joint, and remained in the perfusion fluid for the remainder of the experiment.

Immunoneutralization of annexin with anti-annexin antibody

An antibody, LCPS1, raised in sheep against a 25 amino acid N-terminal region of annexin I (annexin I, 3-26) has previously been shown to exacerbate the inflammatory response when administered subcutaneously before the injection of zymosan to induce inflammation (Perretti et al. 1996a). This antibody has been shown to have no cross-reactivity with other members of the annexin family (Perretti et al. 1996a). We co-perfused LCPS1, or normal sheep serum, with BK through the knee joint, immediately after saline baseline samples, to immunoneutralize endogenous annexin. To verify that the anti-annexin I antibody could immunoneutralize the effect of annexin I in this system, 25 min after beginning BK + anti-annexin I antibody perfusion, annexin I (100 ng/ml) was added to the perfusion fluid, in one knee joint, as described above.

Anti-annexin antibody administration interaction with corticosterone- or noxious electrical stimulation-induced inhibition of BK-induced PE

Anti-annexin antibody (1:60 dilution) (M. Perretti, personal communication) was administered with BK after the baseline perfusion period (i.e., 15 min after the start of the perfusion period). Corticosterone infusion (5 µg/min iv) or noxious electrical stimulation was initiated 40 min after the start of the perfusion period and continued for the remainder of the experiment.

Corticosterone assay

Blood samples (50–100 µl) were collected from pentobarbital-anesthetized animals by venipuncture (tail vein) immediately before and during noxious (25 mA) and nonnoxious (2.5 mA) electrical stimulation. Samples were immediately centrifuged and plasma taken and stored at −20°C until assayed. Total plasma corticosterone was assayed with a double antibody 125I radio-immunoassay kit (ICN Biomedicals, Costa Mesa, CA) as previously described (Akana et al. 1985). The assay has a sensitivity of detection of 0.5 µg/ml and has a cross-reactivity reported by ICN Biomedicals as 0.34% for desoxytocorticosterone and 0.1% for testosterone.

Materials

Evans blue dye, BK triacetate, corticosterone (11,21-dihydroxy-4-pregnene-3,20-dione), PAF (l-o-phosphatidylcholine, b-acetylketo-7-o-alkyl), normal sheep serum, and dimethyl sulfoxide were obtained from Sigma Chemical Company (St. Louis, MO). Annexin b was obtained from Zymed Laboratories (South San Francisco, CA). Anti-annexin I antibody (LCPS1) was a generous gift of Dr. M. Perretti (The William Harvey Research Institute, St. Bartholomew’s Hospital, London, UK). Corticosterone was dissolved in ethanol and diluted in 0.2% bovine serum albumin (in 0.9% saline) to give a final concentration of 2% ethanol. Infusion of the vehicle did not affect BK-induced PE (Green et al. 1997); all other drugs were dissolved in 0.9% saline.

Statistics

Data were analyzed using repeated measures analysis of variance (ANOVA) with one between subjects factor, treatment, with 2 levels (e.g., control and electrical stimulation, or control and corticosterone), and one within subjects factor, time, with 10 levels (45–90 min, 5-min intervals). We present the results of the analysis of the main effect, treatment; differences were considered significant when P < 0.05. When the result of ANOVA was significant (P < 0.05), a post hoc Fisher’s test (Fisher 1949) was performed to determine statistical significance (P < 0.05) between treatment and control groups.
At several time points thereafter. Noxious electrical (BK) levels were determined 10 min before onset of electrical stimulation and BK (BK) ± induced plasma extravasation (PE) and increases plasma corticosterone (BK-induced PE) by releasing a second mediator, annexin I, significantly increases plasma corticosterone compared with unstimulated control (control ± BK ± induced PE). Intra-articular corticosterone inhibits BK-induced PE only at a dose that inhibits BK-induced PE in the contralateral knee.

A two-way repeated ANOVA showed a significant inhibitory effect of intra-articular corticosterone on BK-induced PE \[ F(3,19) = 16.075, P < 0.05 \]. Post hoc Fisher comparisons revealed that BK-induced PE was not significantly attenuated by intra-articular corticosterone at 1 \( \mu \)M, but intra-articular perfusion of corticosterone 10 \( \mu \)M through one knee joint did significantly attenuate BK-induced PE \( P < 0.05 \), and this inhibition was not significantly different from that seen in the ipsilateral knee (BK + corticosterone 10 \( \mu \)M intra-articular vs. BK + corticosterone 10 \( \mu \)M intra-articular, \( P > 0.05 \)). At lower intra-articular concentrations, corticosterone (i.e., \( = 1 \mu \)M ia) did not inhibit BK-induced PE either ipsi- or contralateral to the corticosterone perfused knee (data not shown). These data suggest that corticosterone does not act in the knee joint to inhibit BK-induced PE. Subsequent experiments were designed to test the hypothesis that corticosterone exerts its inhibitory effect on BK-induced PE by releasing a second mediator, annexin I, which does act in the knee joint.

Annexin I perfusion mimics inhibition of BK-induced PE produced by noxious electrical stimulation and corticosterone (iv) administration

Co-perfusion of BK with annexin I (100 ng/ml) through one knee joint inhibited the magnitude of BK-induced PE.

\[ FIG. 2. \] Dose response effect of intravenous corticosterone inhibition of BK –induced PE in the rat knee joint. After establishment of baseline PE in the 1st 3 samples, BK (150 nM; \( \bigcirc, n = 8 \)) was added to the perfusion fluid and, for the remainder of the experiment, was the only substance in the perfusion fluid. In a 2nd group of rats (\( \bullet, n = 6 \)), C fibers were stimulated electrically (25 mA, 3 Hz), via electrodes in one hindpaw, starting 40 min after the onset of the perfusion of BK. In this and subsequent figures, data are presented as means ± SE of \( n \) values, and ordinate is absorbance of light at 620 nm, Co-perfusion of BK with annexin I (100 ng/ml) through which is linearly proportional to the concentration of Evans blue dye (Carr and Wilhelm 1964). Once a drug is added to the perfusate, it continues to be present for the duration of the experiment. \( Inset: \) plasma corticosterone levels were determined 10 min before onset of electrical stimulation and at several time points thereafter. Noxious electrical (\( \bullet, n = 7 \) ) stimulation significantly increases plasma corticosterone compared with unstimulated control (\( \bullet, n = 8 \)).

FIG. 1. Noxious electrical stimulation to rat hind paw inhibits bradykinin (BK) –induced plasma extravasation (PE) and increases plasma corticosterone levels. After establishment of baseline PE, in the 1st 3 samples, BK (150 nM; \( \bigcirc, n = 8 \)) was added to the perfusion fluid and, for the remainder of the experiment, was the only substance in the perfusion fluid. In a 2nd group of rats (\( \bullet, n = 6 \)), C fibers were stimulated electrically (25 mA, 3 Hz), via electrodes in one hindpaw, starting 40 min after the onset of the perfusion of BK. In this and subsequent figures, data are presented as means ± SE of \( n \) values, and ordinate is absorbance of light at 620 nm, Co-perfusion of BK with annexin I (100 ng/ml) through which is linearly proportional to the concentration of Evans blue dye (Carr and Wilhelm 1964). Once a drug is added to the perfusate, it continues to be present for the duration of the experiment. \( Inset: \) plasma corticosterone levels were determined 10 min before onset of electrical stimulation and at several time points thereafter. Noxious electrical (\( \bullet, n = 7 \) ) stimulation significantly increases plasma corticosterone compared with unstimulated control (\( \bullet, n = 8 \)).

 RESULTS

Electrical stimulation and systemic (intravenous) corticosterone inhibit BK-induced PE

Continuous perfusion of BK (150 nM) through the knee joint produced a sustained increase in PE for the duration of the 90-min perfusion period (Fig. 1). Noxious electrical stimulation inhibited BK-induced PE in both knee joints [BK vs. BK + electrical stimulation, \( F(1,11) = 10.543; P < 0.05 \)]. Plasma corticosterone concentrations were significantly \( F(1,16) = 8.517, P < 0.05 \) increased after noxious electrical stimulation (which activates both A and C fibers) compared with nonstimulated controls (Fig. 1, \( Inset \)). A two-way repeated ANOVA showed a significant inhibitory effect of intravenous corticosterone on BK-induced PE \( F(3,31) = 5.77, P < 0.05 \). Post hoc Fisher comparisons revealed that BK-induced PE was significantly attenuated by intravenous corticosterone at 5 \( \mu \)g/min, but not 0.05 or 0.5 \( \mu \)g/min \( P < 0.05 \), Fig. 2). The magnitude of inhibition produced by either noxious electrical stimulation or intravenous corticosterone on BK-induced PE was the same in both knee joints (data not shown).

\[ FIG. 2. \] Dose response effect of intravenous corticosterone inhibition of BK –induced PE in the rat knee joint. After establishment of baseline PE in the 1st 3 samples, BK (150 nM; \( \bigcirc, n = 8 \)) was added to the perfusion fluid and, for the remainder of the experiment, was the only substance in the perfusion fluid. In 3 other groups of rats, corticosterone was infused intravenously, starting 40 min after the onset of perfusion of BK, at a rate of 0.05 \( \mu \)g/min (\( \bullet, n = 5 \)) , 0.5 \( \mu \)g/min (\( \bigcirc, n = 12 \)) , or 5 \( \mu \)g/min (\( \bullet, n = 12 \)).
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FIG. 3. Intra-articular corticosterone inhibits BK-induced PE both ipsi- and contralateral to corticosterone in the rat knee joint. After establishment of baseline PE in the 1st 3 samples, BK (150 nM; ○, n = 8) was added to the perfusion fluid and, for the remainder of the experiment, was the only substance in the perfusion fluid. In a 2nd group of rats (●, n = 7), corticosterone (1 μM) was co-perfused with BK intra-articularly. In a 3rd group of rats (▲, n = 6), corticosterone (10 μM) was co-perfused through one knee joint, while the contralateral knee joint (△, n = 6) was perfused with BK alone.

in that knee joint [BK vs. BK + annexin I ipsilateral, F(1,11) = 8.986 P < 0.05; Fig. 4], but not in the contralateral knee, which only received BK (BK vs. BK + annexin I contralateral, P > 0.05). Annexin I inhibition was not statistically different (P > 0.05) from that produced by either corticosterone (iv) or electrical stimulation.

FIG. 4. Intra-articular annexin inhibits BK-induced PE in the rat knee joint, similar to the inhibition produced by noxious electrical stimulation or intravenous corticosterone. After establishment of baseline PE in the 1st 3 samples, BK (150 nM; ○, n = 8) was added to the perfusion fluid and, for the remainder of the experiment, was the only substance in the perfusion fluid. In a 2nd group of rats (●, n = 6), annexin I (100 ng/ml) was co-perfused through one knee joint, while the contralateral knee joint (▲, n = 6) was perfused with BK alone. Inhibition of BK-induced PE produced by intravenous corticosterone (△, n = 6) or noxious electrical stimulation (△, n = 6) are shown for comparison.

FIG. 5. Neither intravenous corticosterone nor intra-articular annexin inhibits platelet activating factor (PAF)–induced PE in the rat knee joint. After establishment of baseline PE in the 1st 3 samples, PAF (35 nM; ○, n = 8) was added to the perfusion fluid and, for the remainder of the experiment, was the only substance in the perfusion fluid. In a 2nd group of rats (●, n = 7) corticosterone (5 μg/min) was infused intravenously, starting 40 min after the onset of the perfusion of PAF, while in a 3rd group of rats (▲, n = 9) annexin I (100 ng/ml) was co-perfused with PAF through the knee joint.

Annexin I perfusion does not inhibit PAF-induced PE

Similar to the effect of intravenous corticosterone administration on PAF-induced PE (Green et al. 1997), intra-articular co-perfusion of PAF with annexin I (100 ng/ml) did not inhibit the magnitude of PAF-induced PE (PAF vs. PAF + annexin I, P > 0.05; Fig. 5).

Anti-annexin antibody antagonizes inhibition of BK-induced PE by annexin I, electrical stimulation or corticosterone (iv)

Inhibition of BK-induced PE by noxious electrical stimulation was blocked by perfusion of anti-annexin I antibody (LCPS1, 1:60 dilution) through the knee joint [BK + electrical stimulation + LCPS1 vs. BK + electrical stimulation vs. BK + control sheep serum F(2,17) = 5.801 P < 0.05, with Fishers post hoc analysis revealing significant effect of LCPS1; Fig. 6A]. LCPS1 completely blocked the inhibition of BK-induced PE produced by corticosterone administration [5 μg/min iv; BK + LCPS1 vs. BK + corticosterone (iv) + LCPS1 vs. BK + corticosterone (iv), F(2,19) = 6.801 P < 0.05, with Fishers post hoc analysis revealing significant effect of LCPS1; Fig. 6B]. In control experiments, to demonstrate the ability of anti-annexin I antibodies to block effects of annexin I in the knee joint of the rat, immunoneutralization of annexin I with LCPS1 completely blocked the inhibitory effect of annexin I on BK-induced PE (BK vs. BK + annexin I + anti-annexin I antibody, P > 0.05; Fig. 6B). Perfusion of normal sheep serum (1:60 dilution) had no effect on BK-induced PE or its inhibition by noxious electrical stimulation.

DISCUSSION

In this study, we have shown that in the knee joint of the rat, inhibition of BK-induced PE by noxious electrical...
FIG. 6. Anti-annexin antibody attenuates inhibition of BK-induced PE produced by either electrical stimulation or by corticosterone (iv).

A: after establishment of baseline PE in the 1st 3 samples, BK (150 nM) and normal sheep serum (1:60 dilution; □, n = 8) were added to the perfusion fluid and, for the remainder of the experiment, were the only substances in the perfusion fluid. In a 2nd group of rats (●, n = 6), C fibers were stimulated electrically (25 mA, 3 Hz), via electrodes in one hind paw, starting 40 min after the onset of perfusion of BK, while in a 3rd group (●, n = 7) noxious electrical stimulation was started 40 min after the onset of anti-annexin I antibody (1:60 dilution) co-perfused with BK through the knee joint.

B: after establishment of baseline PE in the 1st 3 samples, BK (150 nM; ○, n = 8) was added to the perfusion fluid and, for the remainder of the experiment, was the only substance in the perfusion fluid. The inhibition of BK-induced PE produced by corticosterone (5 μg/min iv; ●, n = 12) is attenuated when co-perfused with anti-annexin I antibody (△, n = 6). Similarly, inhibition of BK-induced PE produced by annexin I (100 ng/ml ia; ▪, n = 6) is also attenuated when co-perfused with anti-annexin I antibody (△, n = 6).

stimulation, which is HPA axis dependent (Green et al. 1997) and increases plasma corticosterone levels, or by intravenous corticosterone infusion cannot be explained by a local action of corticosterone in the knee joint. Corticosterone infused locally into the knee joint affected BK-induced PE only at concentrations high enough to have a systemic action (i.e., the contralateral knee joint was also inhibited), similar to that seen with intravenously administered corticosterone. However, we have found that local perfusion of annexin I through the knee joint mimics, and anti-annexin I antibody antagonizes, the inhibition of BK-induced PE produced by both noxious electrical stimulation and intravenous corticosterone. Thus we have provided evidence that the inhibitory effect of corticosterone on BK-induced PE in the knee joint as well as inhibition of BK-induced PE produced by C-fiber activity is mediated by annexin I.

Annexin I is widely distributed in mammalian tissues; in particular it is produced in cell types associated with the inflammatory response [e.g., leukocytes and synoviocytes in inflamed joints (Goulding et al. 1995)]. Annexin I inhibits cytosolic phospholipase A2 activity (Croxtall et al. 1996; Kim et al. 1994) and production of arachidonic acid metabolites [e.g., prostaglandin E2 and leukotriene B4 (Errasfa and Russo 1989)] and other pro-inflammatory mediators, such as tumor necrosis factor α, from mononuclear cells (Sudlow et al. 1996). Annexin I also inhibits recruitment of polymorphonuclear leukocytes to sites of inflammation (Errasfa and Russo 1989), transendothelial passage of polymorphonuclear leukocytes (Perretti et al. 1996b), and stimulated degranulation of primary granules (Perretti et al. 1995). Of note, inhibition of arachidonic acid synthesis with indomethacin inhibits BK-induced PE (Green et al. 1993), and migration of leukocytes is necessary for the production of PE by BK (Bjerknes et al. 1991; Green et al. 1993). Polymorphonuclear leukocytes have binding sites for annexin I; PAF-induced PE is not inhibited by intravenous corticosterone (Green et al. 1995) or by annexin I. Thus annexin I would be expected to affect BK-induced PE, which is polymorphonuclear leukocyte dependent, but not PAF-induced PE, which is polymorphonuclear leukocyte independent (Green et al. 1993); PAF-induced PE is not inhibited by intravenous corticosterone (Green et al. 1995) or by annexin I.

Taken together, our data suggest that after activation of the feedback inhibitory pathway by either an inflammatory process or activation of nociceptors (Green et al. 1995, 1997), the increased level of circulating corticosterone acts on polymorphonuclear leukocytes to release annexin I. Release of annexin I from polymorphonuclear leukocytes acts on polymorphonuclear leukocytes to release annexin I. Release of annexin I from polymorphonuclear leukocytes is a very rapid (<15 min) process (Comera and Russo Marie 1995), which correlates well with the observed time course of inhibition of BK-induced PE after activation of the feed-
back pathway. We would then expect that released annexin I inhibits the transmigration of polymorphonuclear leukocytes into the knee joint synovium, by causing the polymorphonuclear leukocytes to detach from the synovial endothelium and return to the circulation (Mancuso et al. 1995). Further support for this hypothesis comes from our recent observation that noxious electrical stimulation attenuates BK-stimulated accumulation of polymorphonuclear leukocytes into the microcirculation of the synovium (E. Lo, P. G. Green, H. J. Strausbaugh, and J. D. Levine, unpublished observations).

Our data suggest that, at least with respect to the control of BK-induced PE over the period studied (<90 min), glucocorticoid exerts its anti-inflammatory effect by a systemic action (possibly on circulating polymorphonuclear leukocytes), and not by a local action in the knee joint synovium. This is consistent with the observation that locally (intradermal) administered glucocorticoids have no effect on polymorphonuclear leukocyte accumulation while systemically administered glucocorticoids inhibit polymorphonuclear leukocyte accumulation (Yarwood et al. 1993). Interestingly, glucocorticoids given intra-articularly over weeks have been shown to reduce arthritic severity (suppression of inflammation and pannus measured using magnetic resonance imaging) (Huppertz et al. 1995), and although glucocorticoids may have anti-inflammatory effects at sites of administration, e.g., by virtue of having a direct action on endothelial cells (Cronstein et al. 1992; Yarwood et al. 1993) and/or by their ability to be vasoconstrictors (Marks and Sawyer 1986), our data suggest that a primary anti-inflammatory effect of local corticosteroid injection may occur only after systemic absorption. Importantly, Perretti and Flower (1993) observed that although both systemic and local administration of the glucocorticoid dexamethasone inhibited polymorphonuclear leukocyte accumulation into mouse air pouch, anti-annexin I antibodies attenuated this effect when given intravenously but not locally into the air pouch. This suggests that to have an annexin I–mediated action, glucocorticoids must act on leukocytes before they migrate into tissues.

Annexin I could affect PE by a mechanism that is independent of polymorphonuclear leukocytes. Specifically, annexin I interacts with actin to regulate actin microfilament dynamics (Alvarez-Martinez et al. 1996), and because inflammatory mediators, such as BK, are thought to increase microvascular permeability, in part by an action on actin cytoskeleton integrity (Wang et al. 1997), annexin I may have a direct effect on PE at the level of the endothelial cell (where intercellular fenestrae allow for the extravasation of plasma proteins). However, our observation that annexin I did not inhibit PAF-induced PE, which also may affect endothelial cell cytoskeleton integrity to induce an increase in PE (Bussoolin et al. 1994; Northover 1989), suggests that a contribution of such a direct effect of annexin I on endothelial cell cytostructure, to inhibit either noxious stimulation- or corticosteroid-induced inhibition of PE, is unlikely. Because annexin I inhibits SPGN-dependent (BK) but not -independent (PAF) PE, annexin I may be acting directly on SPGNs; annexin I inhibits both prostaglandin (Croxtall et al. 1996; Kim et al. 1994) and nitric oxide (Bryant et al. 1998; Yang et al. 1998) synthesis and release, and inhibition of either inhibits BK- but not PAF-induced PE (Green et al. 1993).

In addition to releasing annexin, glucocorticoids stimulate the production and/or release of other mediators e.g., somatostatin and angiotensin converting enzyme, which may influence the magnitude of the inflammatory response. For example, somatostatin synthesis is stimulated by glucocorticoids and somatostatin analogues potent inhibit the inflammatory response, through actions on leukocytes and cytokines (Karalis et al. 1995). Angiotensin converting enzyme has been shown to be markedly up-regulated by corticosterone; this enzyme cleaves BK (with a $K_m$ lower than for cleavage of angiotensin I), and this results in a decrease of BK levels at sites of inflammation following glucocorticoid administration. However, although corticosterone has several potential modes of action to inhibit the inflammatory response, our observation that feedback inhibition of the inflammatory response can be attenuated with immunoneutralizing doses of anti-annexin I antibody suggests that action by annexin I accounts for much of the inhibitory effect of noxious stimulus–induced inhibition of BK-induced PE. It should be noted, however, that there is likely to be an annexin I–independent component of noxious stimulation of the hind paw, because we have recently shown that noxious stimulation (produced by capsaicin injected intradermally) is partially mediated by the sympathetic adrenal pathway (Miao et al. 1998).

In summary, we have provided evidence that the feedback inhibition produced by inflammation or by C-fiber stimulation results in release of corticosterone, which induces the synthesis and/or release of annexin I, possibly from circulating cells, which then acts to inhibit BK-induced PE.

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