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Negative Feedback Neuroendocrine Control of Inflammatory Response in the Rat is Dependent on the Sympathetic Postganglionic Neuron

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Negative feedback control of inflammation is mediated by activation of nociceptive afferents that in turn activates the hypothalamic-pituitary-adrenal axis to release corticosteroids. Plasma extravasation (PE) produced by the potent inflammatory mediator, bradykinin (BK), but not that induced by another potent inflammatory mediator, platelet-activating factor (PAF), is inhibited by released corticosterone. Because bradykinin, but not PAF, produces PE by a mechanism that is, in part, dependent on the sympathetic postganglionic neuron (SPGN) terminal, we tested the hypothesis that the negative feedback control of inflammation is dependent on the SPGN terminal in the inflamed tissue. In sympathectomized rats, the residual (i.e., SPGN-independent) PE in the knee joint produced by BK was not inhibited by noxious electrical stimulation. Furthermore, intravenous administration of corticosterone potently inhibited, with a similar time-course, the SPGN-dependent, but not the SPGN-independent, component of BK-induced PE. Neither electrical stimulation nor corticosterone inhibited PAF-induced PE. Finally, corticosterone's actions do not appear to be mediated by release of norepinephrine from the SPGN terminal, because neither the α-adrenergic receptor antagonist phentolamine nor the β_2-adrenergic receptor antagonist ICI 118,551 antagonized the inhibition of BK-induced PE by corticosterone. We conclude that in the rat knee joint, negative feedback control of the inflammatory response is dependent on the presence of the SPGN terminal. Further, our data suggest that a significant component of corticosteroid-induced inhibition of PE produced by inflammatory mediators is SPGN-dependent.

Key words: neurogenic inflammation; sympathetic postganglionic neuron; sympathectomy; noxious electrical stimulation; hypothalamic-pituitary-adrenal axis; bradykinin; platelet activating factor; corticosterone; inflammation; negative feedback control

Inflammation can induce negative feedback control of plasma extravasation (PE), a component of inflammation, at a second site. This negative feedback control of inflammation is mediated by a pathway involving activation of C-fiber afferents, ascending tracts in the spinal cord, and the hypothalamic-pituitary-adrenal (HPA) axis (Green et al., 1995). This feedback circuit inhibits PE produced by one potent inflammatory mediator, bradykinin (BK), but not that produced by another, platelet-activating factor (PAF). Because a component (60–70%) of the PE response produced by BK, unlike that produced by PAF, requires the presence of the sympathetic terminal (Coderre et al., 1989; Green et al., 1993b; Miao et al., 1996a,b), we tested the hypothesis that feedback inhibition is dependent on the sympathetic postganglionic neuron (SPGN) terminal at the site of PE. We have tested this hypothesis by showing that the negative feedback circuit acts only on the BK-induced PE that is dependent on the sympathetic terminals and not on BK-induced PE that is independent of the sympathetic terminals, and that corticosterone, the final common mediator of the HPA-axis, acts similarly.

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MATERIALS AND METHODS

Animals. The experiments were performed on 90 male (300–400 gm) Sprague Dawley rats (Bantin and Kingman, Fremont, CA). The rats were housed in a temperature- and humidity-controlled environment and were maintained on a 12 hr light/dark cycle (lights on at 06 A.M.). Food and water were available *ad libitum*.

Plasma extravasation. Rats were anesthetized with sodium pentobarbital (Anthony Products, Arcadia, CA; 65 mg/kg). Skin overlying the knee was excised to expose the joint capsule, 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), and 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). Samples of perfusion fluid were collected over 5 min intervals for 70 min. Samples were analyzed for Evans blue dye concentration by spectrophotometric measurement of absorbance at 620 nm. Absorbance is linearly related to dye concentration (Carr and Wilhelm, 1964). After collection of the first three samples (to establish baseline PE levels), BK (150 nM; 160 ng/ml) was added to the perfusing fluid and remained present in the fluid for the duration of the experiment. Other drugs were added to the perfusion fluid as indicated in Results, and they remained in the perfusion fluid for the duration of the experiment.

Surgical removal of the lumbar sympathetic chain (sympathectomy). The lumbar sympathetic chains were removed using a lateral retroperitoneal approach, as described previously (Baron et al., 1988; Miao et al., 1995). Briefly, after cutting the skin and abdominal muscles on the left side of the abdomen, the lumbar sympathetic chains were fully exposed bilaterally by excising part of the left erector spinae and transversospinalis muscles of lumbar vertebrae L2–5, the left quadratus lumborum, the psoas minor and major, as well as the upper part of the iliacus muscles. The lumbar sympathetic chain was then exposed from paravertebral

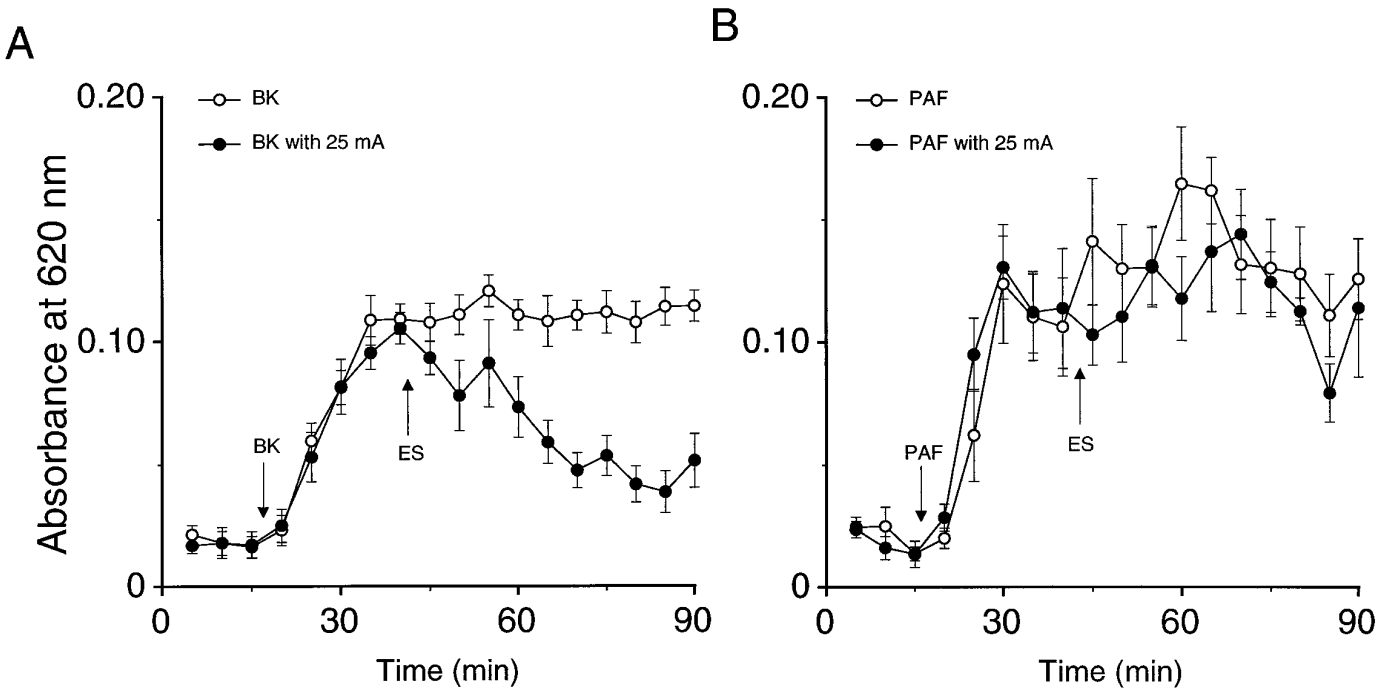

Figure 1. A, Effect of electrical stimulation on BK-induced PE in the rat knee joint. In both groups of rats, baseline PE was established in the first three samples. In one group, BK (150 nM; ○, $n = 8$) was then added to the perfusion fluid (0.9% sodium chloride) and, for the remainder of the experiment, was the only substance in the perfusion fluid. In the second group (●, $n = 6$), BK was added after the first three baseline samples, and then 40 min after beginning knee perfusion, a noxious electrical stimulation of the hindpaw (ES, 25 mA, 0.5 msec. pulse duration, 1 Hz) was applied continuously for the remainder of the experiment. *B,* Effect of electrical stimulation on PAF-induced PE in the rat knee joint. In both groups of rats, baseline PE was established in the first three samples. In one group, PAF (35 nM; ○, $n = 6$) was then added to the perfusion fluid (0.9% sodium chloride) and, for the remainder of the experiment, was the only substance in the perfusion fluid. In the second group (●, $n = 8$), PAF was added after the first three baseline samples, and then 40 min after beginning knee perfusion, a noxious electrical stimulation (25 mA) was applied to the hindpaw continuously for the remainder of the experiment. In this and subsequent figures, data are presented as mean ± SEM of n values, and ordinate is absorbance of light at 620 nm. After a drug is added to the perfusate, it is present throughout the experiment.

ganglia L2 to L4, and the ganglia were excised (Miao et al., 1996b). The wound was closed separately in layers. Rats recovered 1 week postsurgery before being used in the PE experiments. The paravertebral ganglia L2–L4 contain the cell bodies of the postganglionic neurons projecting to the rat hindlimb, only a small number of which are in the more rostral paravertebral ganglia and in the L5 ganglion (Baron et al., 1988). Neurons in the L5 ganglion are unlikely to innervate the knee joint, because the sympathetic supply to the knee joint has a more rostral representation. In a parallel study, we found that the temperature on the plantar paw had increased by 2°–3°C 4 min after sympathectomy, indicating vasodilation caused by removal of sympathetic control (Miao et al., 1996b). The residual 30–40% PE produced by BK in sympathectomized rats is presumably caused by an action of BK on other target(s), such as endothelial cells and primary afferent neurons (Majno et al., 1969; Northover, 1989; Morel et al., 1990; Khalil and Helme, 1992; Geppetti, 1993; Cambridge and Brain, 1995). Whereas surgical procedures presumably activate C-fibers and therefore might be expected to engage the negative feedback system, this does not appear to be the case, because we have shown previously that acute interruption of the sympathetic chain (during perfusion) and decentralization of the lumbar sympathetic chain (preganglionic sympathectomy) performed 1 week before perfusion did not affect the feedback inhibitory circuit (Green et al., 1995).

Electrical stimulation of the hindpaw. Two stainless steel electrodes were placed transversely in the plantar area of the hindpaw contralateral to the perfused knee joint (~10 mm apart). Stimulus intensities necessary to excite C-fibers have been determined previously electrophysiologically (Green et al., 1995). Noxious (C-fiber strength) electrical stimulation was applied to the hindpaw during the assessment of BK-induced PE. Twenty-five minutes after the initiation of BK perfusion in the knee joint, rats received noxious electrical stimulation (25 mA, 0.25 msec duration pulses, 3 Hz) via the two stimulating electrodes, which continued throughout the experiment.

Materials. Evans blue dye, BK triacetate, and PAF were obtained from Sigma Chemical (St. Louis, MO); phentolamine mesylate was obtained from Ciba-Geigy (Summit, NJ); and ICI 118,551 was obtained from Research Biochemicals International (Natick, MA). PAF was dissolved in 0.2% BSA (in 0.9% saline), and all other drugs were dissolved in 0.9% saline.

Statistical analysis. Data were analyzed using repeated-measures ANOVA with one between-subjects factor, treatment, with two levels (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 $p < 0.05$.

RESULTS

BK induces synovial PE, in part, via a mechanism that is dependent on the SPGN terminals, whereas PAF-induced PE is independent of the SPGN terminals. This difference allows us to test the hypothesis that the target of noxious stimulation-induced feedback inhibition of PE is the SPGN terminals in the knee joint. Noxious electrical stimulation (25 mA) was applied to the hindpaw of normal and sympathectomized rats. This stimulation significantly inhibited BK-induced PE to a level produced by BK in sympathectomized animals that did not receive electrical stimulation ($p < 0.001$) (Fig. 1*A*), but did not inhibit PAF-induced PE [p = not significant (NS)] (Fig. 1*B*). To control for the possibility that feedback inhibition was only effective at the magnitude of PE induced by 150 nM BK, the 35 nM dose of PAF, which produced the same magnitude of PE as that produced by 150 nM BK, was chosen (p = NS).

In sympathectomized rats, maximal BK-induced PE is ~30–40% of that produced in normal animals. Noxious electrical stimulation of the skin did not significantly change the magnitude

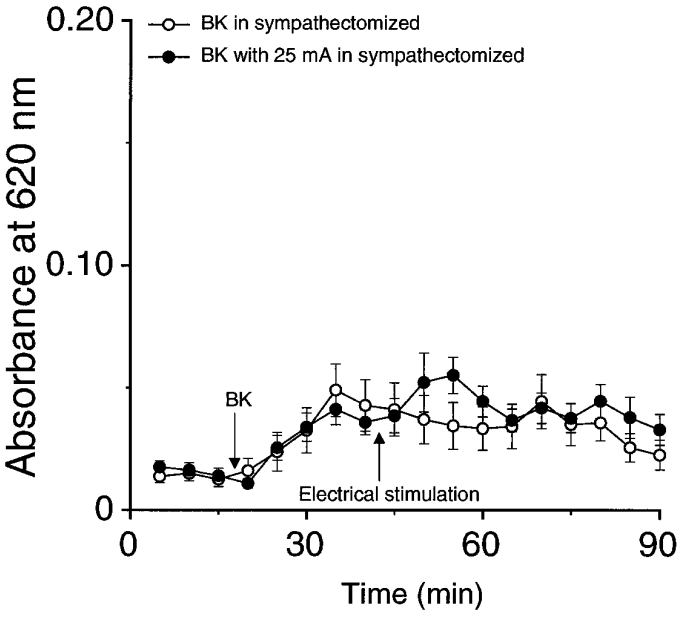

Figure 2. Effect of sympathectomy on noxious stimulation-induced inhibition of BK-induced PE. For both groups in this figure, BK (150 nM) was added to the perfusion fluid after the first three baseline samples and remained in the perfusion fluid for the duration of the experiment. Both groups had undergone sympathectomy 1 week before the perfusion experiment. The first group (○, $n = 15$) received BK only, whereas the second group (●, $n = 14$) also received continuous noxious electrical stimulation of the hindpaw (ES, 25 mA, 0.5 msec, 1 Hz) 40 min after beginning knee perfusion.

of BK-induced PE in these sympathectomized animals (p = NS) (Fig. 2).

Because noxious stimulation-induced inhibition of PE is mediated via activation of the HPA axis, we tested the corollary hypothesis that electrical stimulation-induced inhibition of BK-induced PE is mimicked by intravenously administering corticosterone. Intravenous infusion of corticosterone at a rate of 5 μg/min inhibited BK-induced PE ($p < 0.005$) over a similar time course and with the same magnitude of inhibition as that produced by electrical stimulation of the skin (p = NS) (Fig. 3*A*). Also, the SPGN-independent component of BK-induced PE (i.e., that remaining after sympathectomy) was not inhibited by corticosterone administration (p = NS) (Fig. 4). In addition, intravenous corticosterone had no effect on the SPGN-independent PE induced by PAF (p = NS) (Fig. 3*B*), contrasting with the effect on BK-induced PE in normal animals.

To test for the possibility that corticosterone is acting directly on the SPGN to release norepinephrine, which has been shown to inhibit BK-induced PE (Green et al., 1993a), we coperfused BK with a receptor antagonist to block the inhibitory effects of norepinephrine. However, coperfusion of BK with either the α-adrenergic receptor antagonist phentolamine (1 μM) or the β_2 receptor antagonist ICI 118,551 (100 nM) did not affect the inhibition of BK-induced PE by intravenous corticosterone electrical stimulation (both p = NS) (Fig. 5).

DISCUSSION

The data presented here show that (1) noxious electrical stimulation markedly inhibits BK-induced PE in the knee joint of the rat but has no significant effect on BK-induced PE in sympathectomized animals; (2) noxious electrical stimulation reduces BK-induced PE to a level produced by BK in sympathectomized animals that did not receive electrical stimulation; (3) PAF-induced PE, which is sympathetic neuron terminal-independent (Green et al., 1993b), is not affected by electrical stimulation of skin; (4) administration of the final mediator of the HPA axis, intravenous corticosterone, inhibits BK-induced PE in normal but not in sympathectomized rats; and (5) PAF-induced PE is not affected by intravenous corticosterone.

These data taken together provide strong support for the hypothesis that the sympathetic postganglionic terminal is necessary for the noxious stimulus-induced feedback inhibitory system. We have shown that because acute decentralization of the postganglionic neuron terminal during perfusion of the knee joint cavity with BK does not affect the attenuation of BK-induced PE by activation of the feedback system (Green et al., 1995), the effect of neither noxious electrical stimulation nor corticosterone is a result of inhibition of activity in the SPGN originating from the CNS and propagated via preganglionic neuron activity. Instead, we found that the electrical stimulation induced inhibition of PE that is mediated by corticosteroids (Green et al., 1995) is also dependent on the SPGN terminals at the site of inflammation. Of note, the SPGN terminal is also known to be able to function independently of central input in other tissues; for example, several neurochemicals such as BK, angiotensin II, purines, and cholinergics produce cardiac arrhythmias by a direct action on intrinsic cardiac SPGN terminals even after decentralization (Huang et al., 1994). Although we have not shown that corticosteroids act directly on SPGN terminals in the knee joint, immunocytochemical and electrophysiological studies have demonstrated the presence of glucocorticoid receptors on sympathetic neurons (Bohn et al., 1984; Hua and Chen, 1989).

Although glucocorticoids are normally considered to produce long-term changes in cell function by acting on cytosolic genomic glucocorticoid receptors, glucocorticoids also act on membrane receptors to produce more rapid effects. For example, administration of glucocorticoids, acting in the CNS or the periphery, has been shown to acutely inhibit sympathetic nerve activity in both human (Golczynska et al., 1995; Lenders et al., 1995) and animal (Brown and Fisher, 1986) studies. Corticosterone also potently inhibits prostaglandin synthesis (Nusing and Ullrich, 1992; Masferrer and Seibert, 1994), and prostaglandins are mediators of inflammatory responses (Robinson, 1989; Hedqvist et al., 1990), including SPGN-dependent PE (Coderre et al., 1989). But this effect of corticosterone is believed to be mediated via cytosolic receptors, and so the time course would be too slow for the inhibition we see in our study. Another acute effect of corticosterone is inhibition of norepinephrine reuptake (uptake-2) such that the local extracellular concentration of norepinephrine from sympathetic neurons is increased (Parker et al., 1994). However, it is unlikely that corticosterone inhibits uptake-2 in our system, because we show that neither intra-articular phentolamine nor intra-articular ICI 118,551 administration (both of which would be expected to block the effects of norepinephrine) affected corticosterone inhibition. Moreover, a corticosterone concentration of ~30 μM is required to inhibit uptake-2 (Stjarne et al., 1994), and our protocol produces plasma levels of ~2.5–3.5 μM (data not shown).

In summary, our present study supports the hypothesis that activation of the negative feedback control of PE by noxious stimulation is mediated by release of corticosterone (Green et al., 1995), and provides data to suggest that corticosterone, the final common mediator of the HPA axis, requires the presence of the

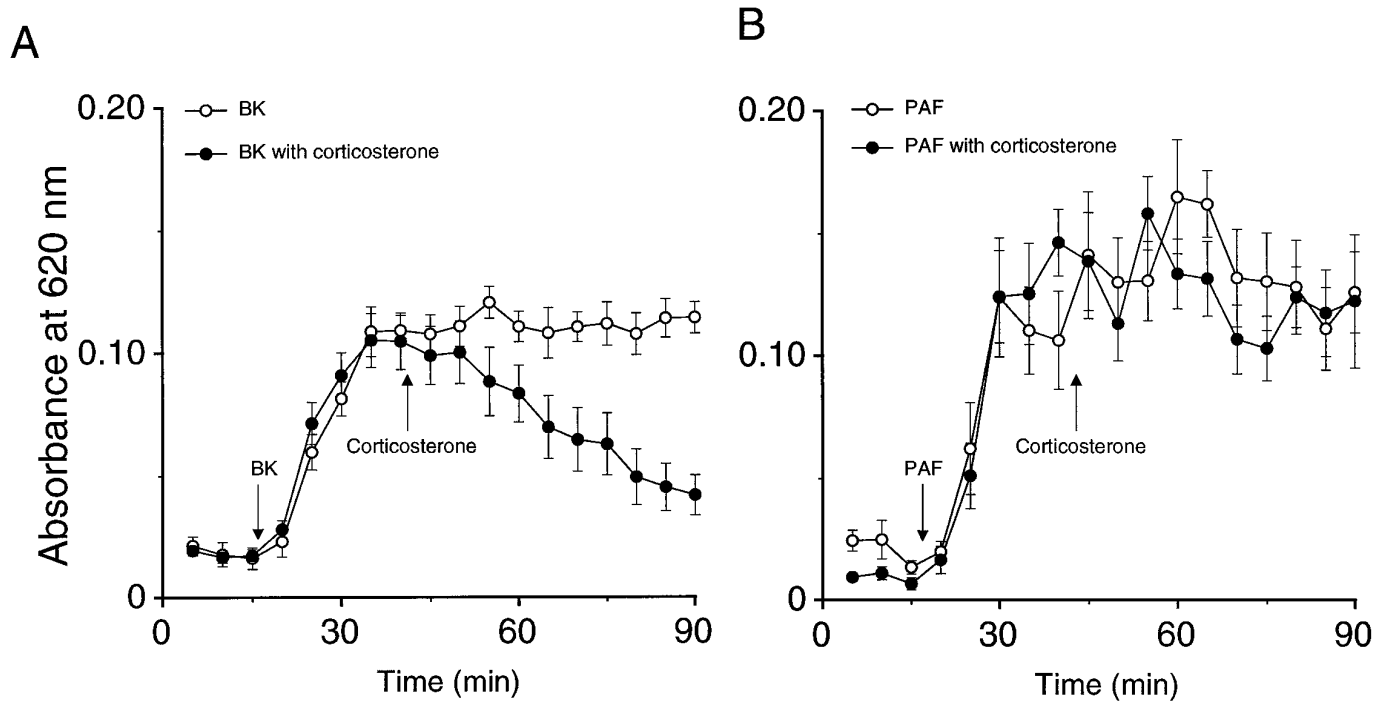

Figure 3. A, Effect of intravenous corticosterone on BK-induced PE in the rat knee joint. In both groups of rats, baseline PE was established in the first three samples. In one group, BK (150 nM; ○, $n = 8$) was then added to the perfusion fluid (0.9% sodium chloride) and, for the remainder of the experiment, was the only substance in the perfusion fluid. In the second group (●, $n = 12$), BK was added after the first three baseline samples, and then 40 min after knee perfusion corticosterone was infused intravenously at a rate of 5 μg/min for the remainder of the experiment. *B,* Effect of intravenous corticosterone on PAF-induced PE. For both groups in *B*, PAF (35 nM) was added to the perfusion fluid after the first three baseline samples and remained in the perfusion fluid for the duration of the experiment. The first group (○, $n = 6$) were normal animals, and the second group (●, $n = 7$) received corticosterone (5 μg/min, i.v.) 40 min after beginning knee perfusion.

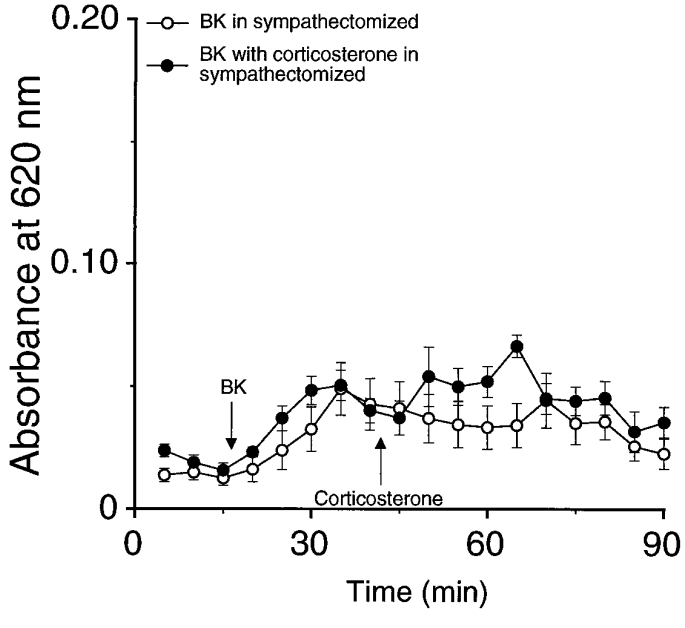

Figure 4. Effect of sympathectomy on corticosterone-induced inhibition of BK-induced PE. For both groups in this figure, BK (150 nM) was added to the perfusion fluid after the first three baseline samples and remained in the perfusion fluid for the duration of the experiment. Both groups had undergone sympathectomy 1 week before perfusion experiment. The first group (○, $n = 15$) received BK only, whereas the second group (●, $n = 9$) also received corticosterone (5 μg/min, i.v.) 40 min after beginning knee perfusion.

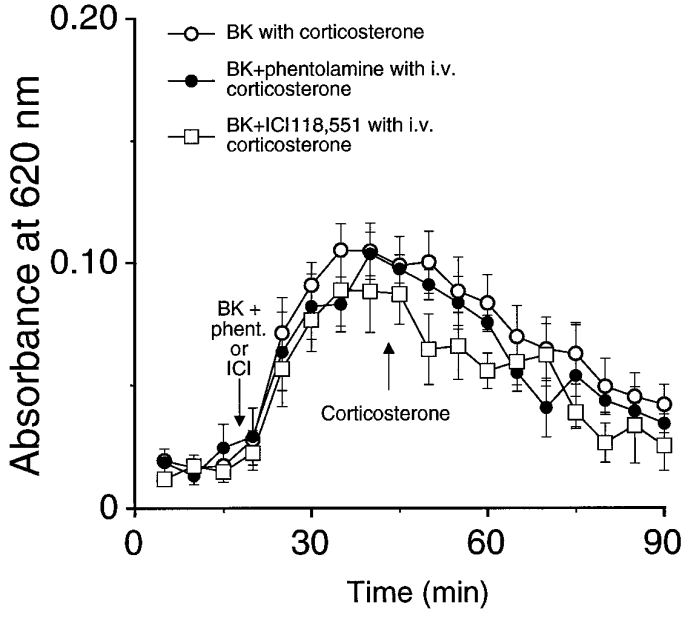

Figure 5. Effect of intra-articular phentolamine or ICI 118,551 on corticosterone-induced inhibition of BK-induced PE. For all groups in this figure, bradykinin (150 nM) was added to the perfusion fluid after the first three baseline samples and remained in the perfusion fluid for the duration of the experiment. The first group (○, $n = 8$) received BK only, whereas the second group (●, $n = 5$) also received phentolamine (1 μM) co-perfused with BK in the knee joint. The third group (■, $n = 5$) received BK with ICI 118,551 (100 nM). Corticosterone infusion (5 μg/min, i.v.) was started 40 min after beginning knee perfusion.

SPGN terminal to attenuate the neurogenic (SPGN terminal-dependent) component of PE. Because both the SPGN-independent component of BK-induced PE and PAF-induced PE are not inhibited by corticosterone, we suggest that this mechanism (i.e., corticosterone-induced inhibition of SPGN-terminal-dependent PE) may play an important role in the anti-inflammatory effect of corticosterone.

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