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β_2-Adrenergic mechanisms in experimental arthritis

(neurogenic inflammation/sympathetic nervous system/rat/catecholamines)

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ABSTRACT **We have studied (*i*) the contribution of specific adrenergic receptors to the proinflammatory effects of the sympathetic nervous system in experimental arthritis and (*ii*) the phases of the disease during which the sympathetic nervous system influences joint injury. Severity of joint injury was measured radiographically 28 days after induction of adjuvant arthritis in control rats and in rats treated with a variety of sympatholytic agents at various times during the course of the disease. Rats treated with a nonspecific catecholamine depletor (reserpine) or a β-adrenergic receptor antagonist (propranolol) had a delayed onset and significantly less severe joint injury than saline-treated controls when treatment began prior to injection of the adjuvant and continued to day 28 after the injection. When administered over the same treatment period, neither nonselective (phenoxybenzamine) nor selective [prazosin (α_1) and yohimbine (α_2)] α-adrenergic receptor antagonists affected the onset or severity of joint injury. Metoprolol, a β_1 antagonist, was also without effect. In contrast, two β_2 antagonists (butoxamine and ICI 118,551) significantly retarded disease onset and reduced the severity of joint injury. When reserpine or butoxamine treatment was initiated after the onset of clinically apparent arthritis, it was still possible to favorably influence the course of the disease. These data indicate an important contribution of the β_2-adrenergic receptor to joint injury in experimental arthritis.**

A contribution of the sympathetic nervous system to experimental arthritis in the rat, as well as to rheumatoid arthritis in patients, has been demonstrated. Specifically sympathectomy markedly prevents both the signs of inflammation and the severity of joint injury in rats with experimentally induced arthritis (1), as well as a reflex neurogenic inflammation that is generated at sites remote from an injury (2). The severity of joint injury in arthritis is increased in spontaneously hypertensive rats, which have increased sympathetic tone; and intracerebroventricular administration of morphine, which decreases sympathetic tone, decreases arthritic severity (1). It has been reported that dogs chronically maintained on β-adrenergic agonists develop a rheumatoid arthritis-like syndrome (3). Propranolol, a β-adrenergic receptor antagonist, has been shown to decrease signs and symptoms of inflammation in patients with active rheumatoid arthritis (4), and we have demonstrated that regional sympathetic block with guanethidine reduces pain and increases pinch strength in patients with active rheumatoid arthritis (5).

In the present studies we examined the adrenergic receptor subclass at which catecholamines exert proinflammatory effects in experimental arthritis, and we addressed the phases of the disease during which the sympathetic nervous system is most influential. Specifically, we assessed the effects of nonselective and selective α- and β-adrenergic antagonists on the joint injury that characterizes adjuvant arthritis.

METHODS

The experiments were performed with 250- to 350-g male Sprague–Dawley rats (Bantin and Kingman, Fremont CA). Arthritis was induced by intradermal injection of 0.1 ml of a 10-mg/ml suspension of *Mycobacterium butyricum* in mineral oil (6). Arthritic rats were bedded on soft wood shavings. Food and water were placed within easy reach inside the cages so that the rats were able to eat and drink normally.

Twenty-eight days after injection of the adjuvant, we anesthetized and x-rayed the rats to assess the severity of arthritis radiologically (1, 7). Immediately after radiography, rats were killed. An observer (C.H.) who was unaware of the experimental protocol evaluated and scored the radiographs for each hindpaw according to the 0–3 grading scale of Ackerman *et al.* (7), which assesses the following signs of injury: soft-tissue swelling, decreased bone density (osteoporosis), narrowing of the joint space (loss of cartilage), destruction of bone (erosions), and formation of periosteal new bone. On this scale, a score of 0 is normal and 3 is maximal joint injury. Radiographic scores derived with this scale correlate well with scores from histological sections of arthritic joints and periarticular tissues (7).

The contribution of the sympathetic nervous system in general was first investigated by administering reserpine (Eli Lilly), 0.25 mg/kg, once daily starting 2 days before administration of adjuvant (1). Next we addressed the specific contribution of α- and β-adrenergic receptors. Nonselective α-adrenergic blockade was produced with the antagonist phenoxybenzamine [30 mg/kg, once daily (8, 9); Smith Kline & French], and nonselective β-adrenergic blockade, with propranolol [20 mg/kg, three times each day (9–11); Ayerst Laboratories, New York)]. The following subclass-selective receptor antagonists were also used: for α_1 receptors, prazosin [2 mg/kg, five times each day (9, 12, 13); Pfizer]; for α_2 receptors, yohimbine [3 mg/kg, once daily (14); Sigma]; for β_1 receptors, metoprolol [50 mg/kg, three times each day (9, 15, 16); CIBA-Geigy]); and for β_2 receptors, butoxamine [10, 25, or 50 mg/kg, three times daily (17); Burroughs Wellcome, Research Triangle Park, NC] or ICI 118,551 [25 mg/kg, three times each day (18); Imperial Chemical Industries, Macclesfield, U.K.]. Reserpine (Sandril, for injection, U.S.P.) was dissolved at 2.5 mg/ml in 30% (wt/vol) polyethylene glycol/1% (vol/vol) monothioglycerol/1% (wt/vol) ascorbic acid/2% (vol/vol) benzyl alcohol. All of the other sympatholytic agents were dissolved or suspended in saline (0.9% NaCl) and administered subcutaneously or intraperitoneally. The control group of arthritic rats received injections of saline three times daily. All dosages were based on levels used in previous studies with Sprague–Dawley rats (1, 8–17).

Since the pathophysiology of experimental arthritis involves physiological, immunological, and clinical events that can be temporally divided into at least two distinct periods,

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we also examined the effects of catecholamine depletion/antagonism during different phases of the disease. The first, preonset, phase is the period prior to the development of clinically apparent disease. It begins at the time of injection of mycobacteria into the tail and ends with the onset of the second, postonset, phase of the disease. The latter is signaled by the abrupt appearance of tenderness and swelling.

Three groups of rats were used in this study that assessed the importance of the time of drug administration. In the first group, catecholamines were depleted, or antagonists were administered, starting 2 days before injection of adjuvant and continuing until day 28 after injection (i.e., both pre- and postonset), at which time all animals were examined radiologically. In the second group of rats, catecholamines were depleted, or antagonists were administered, only during the preonset phase of arthritis. Reserpine treatment was started 2 days before injection of adjuvant and continued to day 3 postinjection. This protocol allows time for recovery of catecholamines prior to the onset of clinically apparent arthritis (19). Butoxamine was administered from 2 days prior to adjuvant injection and continued to day 8 postinjection. In these two groups of rats, the effect of sympatholytic agents on the time course of the arthritis was also assessed by measuring the time to onset of clinically apparent disease after the injection of mycobacteria. The onset of clinically apparent arthritis was defined by the first occurrence of tenderness and swelling on daily examination. In the third group of rats, reserpine or butoxamine was started on the day when clinically apparent arthritis was first observed (i.e., postonset) and continued to day 28. The control group of arthritic rats, for the study of the effect phase of drug administration on severity of joint injury, did not receive any injections (i.e., were untreated).

RESULTS

α- vs. β-Adrenergic Blockade. The first studies were designed to confirm our initial report (1) on the effects of chronic reserpine treatment. We again found that reserpine, 0.25 mg/kg, given once daily starting 2 days before injection of adjuvant and continuing to day 28, significantly attenuated the severity of joint injury ($P < 0.05$) (Table 1).

Chronic administration of the nonselective α-adrenergic receptor antagonist phenoxybezamine, 30 mg/kg, once daily starting 2 days before injection of adjuvant and continuing to day 28, did not significantly affect the severity of joint injury. In contrast, the nonselective β-adrenergic receptor antagonist propranolol (20 mg/kg, three times daily) markedly attenuated the severity of joint injury ($P < 0.01$) (Table 1). For statistical analysis of radiographic scores in this and the following section, experimental groups were compared with the saline control group by using a χ^2 statistic on a 2 × 2 table produced by combining the frequencies of 0 and 1 scores and of 2 and 3 scores. Fisher's exact test was used in cases where cell frequencies were of inadequate size for χ^2.

In a control group of rats that were treated with saline, arthritis first appeared 13.5 ± 1.5 days (mean ± SEM, n = 24 paws) after injection of the adjuvant. Consistent with the protective effect of reserpine or propranolol on joint injury, we found that the onset of clinically apparent arthritis after chronic administration of reserpine (21.8 ± 3.0 days, $P < 0.01$) or propranolol (19.8 ± 1.7 days, $P < 0.05$), but not phenoxybenzamine (13.0 ± 2.1 days, $P > 0.05$), was significantly delayed with respect to saline-treated rats. Comparisons of mean time of onset in this and the following section were based on Dunnett t contrasts following a significant analysis of variance [$F(8,52) = 3.26$, $P < 0.01$].

Receptor Subclass. Consistent with the lack of effect of the nonselective α-adrenergic receptor antagonist, neither the α_1-selective antagonist prazosin, 2 mg/kg, given five times each day, nor the α_2-selective antagonist yohimbine, 3 mg/kg, given once daily, significantly affected the severity of joint injury in arthritis compared to saline-treated controls (Table 1). The β_1-adrenergic specific receptor antagonist metoprolol, at the high dose of 50 mg/kg three times each day, also failed to significantly affect the severity of joint injury. In contrast, administration of a selective β_2-adrenergic antagonist, butoxamine or ICI 118,551, at 25 mg/kg, three times each day, produced very significant ($P < 0.01$) inhibition of joint injury (Table 1).

As was the case for reserpine and propranolol, the onset of clinically apparent arthritis was significantly delayed in the groups of rats treated with either butoxamine (22.9 ± 1.9 days, $P < 0.012$) or ICI 118,551 (20.0 ± 3.0 days, $P < 0.05$). On the other hand, animals treated with prazosin (16.2 ± 2.9 days), yohimbine (13.5 ± 0.6 days), or metoprolol (14.0 ± 1.8 days) had onset latencies that did not differ from that of saline-treated rats (13.5 ± 1.5 days).

Effect of Treatment in Different Phases of the Disease. *Severity of arthritis.* An ameliorative effect of catecholamine depletion was found when reserpine administration (0.25 mg/kg each day) was started 2 days before injection of adjuvant and continued either throughout the duration of the 28-day experiment (i.e., pre- and postonset) or only until day 3 (i.e., preonset). In both cases there was significant attenuation of the severity of joint injury measured radiographi-

Table 1. Degree of joint injury in the hindlimbs of arthritic rats administered selective sympatholytic drugs from day −2 to 28

Treatment	n	% with radiographic score*				Mean score	$P^\dagger$
		0	1	2	3		
Saline	24	8.5	25.0	37.5	29.0	1.9 ± 0.2	
Reserpine	12	66.0	17.0	17.0	0.0	0.5 ± 0.2	<0.05
Phenoxybenzamine	6	0.0	0.0	50.0	50.0	2.5 ± 0.2	NS
Prazosin	12	17.0	49.0	17.0	17.0	1.3 ± 0.3	NS
Yohimbine	12	17.0	17.0	58.0	8.0	1.5 ± 0.3	NS
Propranolol	18	61.0	39.0	0.0	0.0	0.4 ± 0.1	<0.01
Metoprolol	12	16.0	0.0	42.0	42.0	2.0 ± 0.3	NS
Butoxamine‡	14	57.0	43.0	0.0	0.0	0.4 ± 0.1	<0.01
ICI 118,551	12	67.0	33.0	0.0	0.0	0.3 ± 0.1	<0.01

*Radiographic scoring of individual hindpaws was based on the scale of Ackerman *et al.* (7): 0 = no effect, 1 = mild effect, 2 = moderate effect, and 3 = severe effect. Values indicate the percentage of joints in a treatment group with that score.

†Comparisons with saline (control) group based on χ^2 or Fisher's exact test on a 2 × 2 table produced by combining the frequencies of 0 and 1 scores and of 2 and 3 scores. NS, not significant.

‡Dose, 25 mg/kg.

cally on day 28 compared to untreated controls, whose severity score was 2.5 ± 1.5 (Fig. 1; both $P < 0.01$). When reserpine treatment was started at the first clinical sign of inflammation and continued to day 28 (i.e., postonset), there was also a significant reduction in the severity of joint injury measured on day 28 (Fig. 1; $P < 0.01$).

Because of the potent effects of β_2-adrenergic antagonists when administered throughout the entire course of the disease, we also examined the relative contribution of β_2-adrenergic effects in experimental arthritis during the two phases of the disease. The contribution of the β_2-adrenergic receptor to the phase prior to onset of arthritis was evaluated by treating rats with butoxamine (25 mg/kg) from 2 days before until 8 days after injection of the adjuvant. Similar to the effect of administering butoxamine from −2 to 28 days, the severity of arthritis in this group of rats was significantly less than in the control group (Fig. 1; $P < 0.01$). When the butoxamine treatment was started on the first day that clinical arthritis was detected and continued to day 28 (i.e., throughout the postonset phase), the severity of arthritis was also significantly less than in the control group (Fig. 1; $P < 0.05$).

Onset of arthritis. The latency to onset of clinically apparent arthritis in untreated controls was 13.5 ± 0.8 days. In the group of rats given reserpine from day −2 to day 3, there was a significant increase in the latency to onset (19.3 ± 2.7 days, $P < 0.01$). There was no difference in onset in rats treated with butoxamine (25 mg/kg) from day −2 to day 8 (15.2 ± 1.3 days). As expected, the onset latencies did not differ significantly from the control group when the reserpine (13.0 ± 0.8 days) or butoxamine (12.0 ± 0.7 days) treatment started on the first day that clinical arthritis was detected and continued to day 28. Comparisons of mean onset of arthritis were based on Dunnett t contrasts following a significant analysis of variance [$F(6,55) = 8.75$, $P < 0.01$].

Dose–Response Effects of Butoxamine. Fig. 2 illustrates the effects of various doses of butoxamine on the radiographic scores of arthritic rats treated either from day −2 to day 28 or from the first day that clinical arthritis was detected to day 28. Although both the 10-mg/kg and the 25-mg/kg doses of butoxamine administered from day −2 to day 28 produced significant reductions in joint injury compared to an untreated control group, only a 50-mg/kg dose significantly affected joint injury when the treatment started the first day of clinical arthritis. Although not statistically significant, the reductions in the radiographic scores for the 10- and 25-mg/kg doses of butoxamine, administered starting on the first day of clinical arthritis, fit the general pattern of the dose–response curve (Fig. 2).

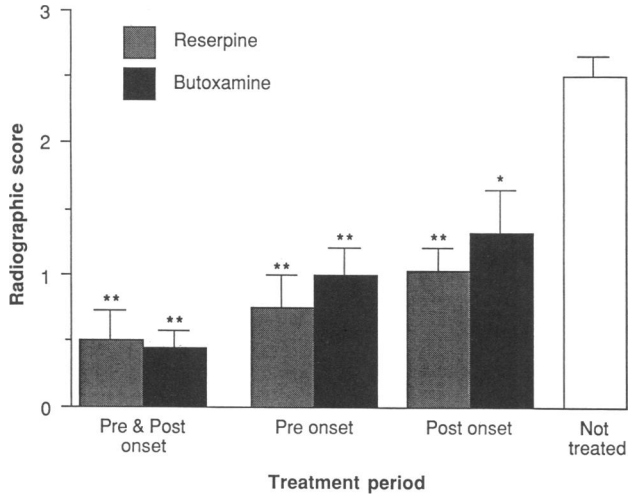

FIG. 1. Mean day-28 radiographic severity scores (+ SEM) of adjuvant arthritic rats that were either untreated (open bar) or treated with reserpine (gray bars) or butoxamine (black bars) over treatment periods extending from (*i*) days −2 to 28 (pre- and postonset), (*ii*) either days −2 to 3 (reserpine) or days −2 to 8 (butoxamine) (preonset), or (*iii*) the first day clinical arthritis was detected to day 28 (postonset). χ^2 or Fisher's exact test comparisons were performed following a significant Kruskal–Wallis test statistic [$H(6) = 44.2$, $P < 0.01$]. Significant differences from the untreated group are indicated: *, $P < 0.05$; **, $P < 0.01$.

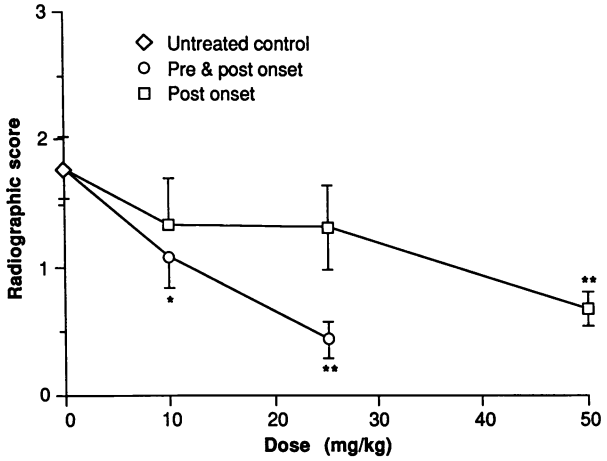

FIG. 2. Dose–response curve for the effects of butoxamine on the radiographic scores (mean ± SEM) of adjuvant arthritic rats when administered either from day −2 to day 28 (pre- and postonset) or from the first day that clinical arthritis was detected to day 28 (postonset). χ^2 on Fisher's exact test comparisons were performed following a significant Kruskal–Wallis test statistic [$H(5) = 17.5$, $P < 0.01$]. Significant differences from the untreated (zero-dose) level are indicated: *, $P < 0.05$; **, $P < 0.01$.

DISCUSSION

Previously, we reported (1) that sympathectomy, by reserpine or guanethidine treatment, reduced inflammation and joint injury in rats with experimentally induced arthritis. The present study confirmed those observations, but more importantly, it addressed the contribution of the different adrenergic receptors. Selective blockade of β-adrenergic, but not α-adrenergic, receptors attenuated the severity of joint injury when treatment began 2 days before induction of arthritis (i.e., approximately 2 weeks before the onset of clinically apparent disease) and continued throughout the duration of the experiment. Since the relatively selective β_2-antagonists butoxamine and ICI 118,551, but not the selective β_1-antagonist metoprolol, significantly attenuated joint injury in experimental arthritis, the therapeutic effect of the nonspecific β-adrenergic antagonist was likely mediated by its blockade of β_2- rather than β_1-adrenergic receptors.

The first studies established that pharmacological intervention before clinical signs of disease appeared could retard the onset and overall severity of disease. Since patients with rheumatoid arthritis do not present for treatment until the disease is manifested clinically, we also evaluated the effect of reserpine and butoxamine on severity of joint injury in experimental arthritis when each therapy was initiated after the onset of clinically apparent disease. In fact, reduction in sympathetic activity (depletion or β_2 blockade) produced a highly significant attenuation of joint injury even when treatment began after the onset of this rapidly destructive form of arthritis. We conclude that events mediated through the β_2-adrenergic receptor influence both the onset (initiation) and the progression of the joint injury in arthritis.

Two effects mediated by activation of β_2-adrenergic receptors are of potential interest in regard to a contribution of those receptors to arthritis. In most tissues (20), there are presynaptic β_2-adrenergic receptors located on the peripheral terminals of the sympathetic postganglionic neuron (20–22). At this site, β_2-agonist binding facilitates norepinephrine release when the terminal is depolarized (22) and presumably modulates the release of other factors from the sympathetic postganglionic neuron terminals. In addition to norepinephrine, adenosine triphosphate and neuropeptide Y, both of which have been implicated as key factors in the control of vascular function (23, 24) and which may influence the function of the immune system (25), are also contained within sympathetic nerves. Thus, β_2-antagonists may attenuate the severity of joint injury in arthritis by reducing release of some compounds from sympathetic postganglionic nerve terminals that would otherwise contribute to injury. By reducing the release of these compounds, the effect of β_2-antagonism would be somewhat comparable to sympatholytic therapy. β_2-Adrenergic receptors have also been found on a number of cells of the immune system, including lymphocytes (26, 27), macrophages (28), and polymorphonuclear leukocytes (29). All of these have been implicated in rheumatoid arthritis and/or experimental arthritis in the rat. Interactions through the receptor presumably modulate the inflammatory response. In this manner β_2-agonists would act directly on the cells of the immune system that contribute to joint injury in arthritis.

The two major sources of the endogenous agonist for the β_2-adrenergic receptor are the adrenal medulla and the sympathetic postganglionic neuron. Epinephrine is a potent agonist at the β_2 receptor; its release from the adrenal medulla could significantly influence arthritic severity. Norepinephrine is generally believed to have minimal β_2-agonist activity under physiological conditions. However, its actions in inflammatory states are unknown. Preliminary experiments have demonstrated that adrenal medullectomy reduces arthritis (T.J.C., Mary Dallman, A.I.B., and J.D.L., unpublished data), which suggests that the β_2-adrenergic effect may be produced by epinephrine released from the adrenal medulla and acting on presynaptic receptors on the sympathetic postganglionic nerve terminal.

In summary, we have found that β_2-adrenergic receptors make an important contribution to the severity of joint injury in experimental arthritis in the rat. Since it was possible to attenuate the progression of joint injury when treatment was started after, as well as before, the onset of clinically apparent disease, we believe that this class of agents may have significant clinical value.

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