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Ankle Joint Urate Arthritis in Rats Provides a Useful Tool for the Evaluation of Analgesic and Anti-Arthritic Agents

TERENCE J. CODERRE AND PATRICK D WALL[1]

Cerebral Functions Research Group, Department of Anatomy and Embryology
University College London, Gower St , London, England U.K. WC1E 6BT

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CODERRE, T J AND P D WALL *Ankle joint urate arthritis in rats provides a useful tool for the evaluation of analgesic and anti-arthritic agents* PHARMACOL BIOCHEM BEHAV 29(3) 461–466, 1988 —Arthritis was induced in ether anesthetised rats by injecting 1 25 mg of sodium urate crystals into the ankle joint Twenty-four hr after the injection the ankle is swollen and the animal does not place full weight on the affected foot The ankle is more sensitive than normal to movement and pressure Responses to stimulation of the foot and toes on the arthritic limb are reduced due to a reluctance to move the affected limb These measures, which reflect ongoing pain, hyperalgesia or tenderness and guarding, are attenuated in animals treated with dexamethasone, phenylbutazone, and morphine, as well as in animals whose nerves to the ankle had been pretreated with capsaicin Guanethidine and colchicine failed to influence the behavioural responses to the urate injection Ankle joint urate arthritis has advantages over other models of arthritis for therapeutic testing in that in a short time it affects a single joint in rats, and it produces responses which can be assessed by simple, sensitive measures

Sodium urate Arthritis Hyperalgesia Inflammation Steroidal anti-inflammatory drugs NSAID
Capsaicin Guanethidine Colchicine

THE development and screening of analgesic and anti-arthritic agents depends on the effective use of animal models in the evaluation of the pain reducing as well as anti-inflammatory actions of new compounds Animal models for analgesic and anti-arthritic drug testing typically fall into two classes (1) nociceptive tests which involve non-injurious exposure to acute pain produced by thermal, mechanical and electrical stimuli, and (2) inflammatory tests which involve the injury of tissue by injection of noxious chemicals Although very reliable, simple to perform, and sensitive to narcotic agents, withdrawal-type acute pain models such as the tail-flick, hot-plate and flinch-jump tests are inappropriate for the evaluation of anti-arthritic agents since they are insensitive to the effects of non-narcotic, anti-inflammatory drugs [24] Furthermore, these stimuli do not produce conditions similar to those seen in arthritis, and there is no way to measure the anti-inflammatory properties of the drugs with such tests On the other hand, many of the chemical stimuli which produce inflammation do not allow for adequate evaluation of the analgesic properties of drugs either because they do not produce reliable indices of inflammatory pain (including both spontaneous pain and hyperalgesia), or because the mechanisms by which they produce their effects do not resemble those present in arthritic or inflammatory diseases

The present study assesses the usefulness of ankle joint urate arthritis (AJUA) in rats for the evaluation of analgesic and anti-arthritic agents The urate model of arthritis is promising because in addition to an inflammatory response it produces directly measurable, spontaneous painful responses which are attributed to a mechanism common to that involved in the arthritic condition gout, the phagocytosis of urate crystals by polymorphonuclear leukocytes, and the subsequent production of hyperalgesic mediators [17,20] Painful responses to articular injections of monosodium urate crystals have been reported in rats [15], cats [14], dogs [7, 19, 26] and man [7, 20, 22] In human volunteers and gout patients, sodium urate produces inflammation and pain which is described as mimicking an attack of gout [11,20] In animals the injection produces limping and a reluctance to place weight on the injected limb [7, 14, 15, 26]

We have recently [4] described the time course and dose response characteristics of the behavioural responses to AJUA in rats Unlike arthritis produced by Freund's adjuvant, which develops slowly over weeks following innoculation [12,16], AJUA develops starting 2–3 hr after injection

[1]Requests for reprints should be addressed to Patrick D Wall

and reaches a maximal level by 24 hr In addition to alterations in spontaneous behaviour including limping and a reduction in the weight placed on the injected limb, there are a series of behavioural responses to sensory tests indicating the presence of hyperalgesia, and a reluctance to move the affected limb While rats vocalize on passive movement of the injected ankle, and have reduced foot-withdrawal thresholds to pressure on the ankle, their foot-withdrawal thresholds to stimulation of other parts of the injected limb are increased This is likely due to an avoidance of active movement of the affected limb to prevent noxious inputs from the ankle, an effect which is also demonstrated by a reduction in placing reflexes of the injected limb

In addition to examining the sensitivity of AJUA to the analgesic effects of a narcotic (morphine), steroidal (dexamethasone), and non-steroidal (phenylbutazone) anti-inflammatory drugs, the present study assessed the influence on AJUA of drugs which specifically affect the neurogenic mechanisms of inflammation by reducing C-fiber transmission (capsaicin) and the action of the sympathetic nervous system (guanethidine) The effects of colchicine will also be assessed, since it is particularly useful in the treatment of gout [25], the arthritis which this model most closely resembles The present study also stresses the usefulness of AJUA over previous models of urate arthritis, given the reduced expenses involved using rats as subjects, as well as the advantages of having multiple behavioural measures of pain sensitivity which are extremely reliable, and simple to perform

METHOD

Injection of Urate Crystals

Sodium urate crystals were prepared based on the method of Seegmiller *et al* [20] as described previously [4] After mixing in a solution of 10% Tween 80 in 0 9% saline, the urate crystals were injected into the medial side of the tibiotarsal joint (ankle) of ether anesthetized rats After making a small skin incision, 1 25 mg of sodium urate (0 05 ml volume) was injected into the ankle joint through a 21 gauge needle inserted just medial to the tendon of the tibialis anterior Behavioural tests were performed 24 hr after the injection, after which all animals were sacrificed

Drugs

Dexamethasone sodium phosphate (Decadron, MSD) was diluted in 0 9% saline, and 0 1 mg/kg was injected intraperitoneally both 1 hr before and 6 hr after the sodium urate treatment Colchicine (Sigma) was dissolved in normal saline, and 2 mg/kg was injected intraperitoneally approximately 20 hr after the sodium urate treatment or 4 hr before testing Capsaicin (Sigma) was mixed in 10% Tween 80, 10% ethanol in normal saline Under general anesthesia (pentobarbital, 50 mg/kg IP) cotton pledgets soaked in 1 5% capsaicin were wrapped around the sciatic and saphenous nerve in the thigh of the subsequently urate-treated hindlimb [8] The pledgets were removed after 15 min, the muscle and skin were sutured, and animals were left for 1 week before sodium urate injection to the ankle Guanethidine sulphate (Ciba) in normal saline was injected intraperitoneally (30 mg/kg) once daily for 4 days with the last injection 4 hr before the urate injection Phenylbutazone (Sigma) was dissolved in 0 01 M HCl, with the pH titrated to 6 5–7 0 using NaOH A 100 mg/kg dose was injected in a 5 ml/kg volume intraperitoneally 3 hr before testing Morphine (5 mg/kg) was injected subcutaneously 30 min before testing A 2 5 mg/kg dose of naloxone hydrochloride (Dupont) was given to rats treated with morphine 45 min earlier, 15 min before testing

TABLE 1

Rating scale for standing (A) and walking (B) paw pressure scores

(A) 0 normal paw pressure, equal weight on both hindpaws
1 slightly reduced paw pressure, paw is completely on the floor but toes are not spread
2 moderately reduced paw pressure, foot curled with only some parts of the foot lightly touching the floor
3 severely reduced paw pressure, foot elevated completely

(B) 0 normal gait
1 slight limp, visible over-flexion of injected limb
2 moderate limp, paw of injected hindlimb only briefly touches the floor
3 severe limp, 3-legged gait

Behavioural Assessments

Paw pressure Male Wistar rats (250–300 g) were taken from their home group cages and placed in a 12″×12″ by 9″ high Plexiglas chamber and were observed for a standard period of 5 min Under the chamber a mirror was set at a 45° angle to allow a clear view of the rats' feet The amount of weight (standing paw pressure) the rat was willing to put on the hindpaw of the injected limb was evaluated and categorized according to the scale given in Table 1A The alteration of gait or limping produced by the injection of sodium urate was assessed and categorized according to the walking paw pressure scale given in Table 1B

Foot-withdrawal to ankle pressure Foot-withdrawal thresholds to mechanical pressure on the ankle were measured using Von Frey hairs This method is similar to that used to obtain withdrawal thresholds following stimulation of the dorsal and plantar surfaces of the rat hindpaw [31] Rats were hand-held and the ankles of the right and left hindlimbs were exposed to an ascending series of tests with Von Frey hairs which exerted force in the range between 1 and 440 g Threshold was defined as when a hair in contact with the ankle produced a flexor reflex or vocalization as it just began to bend The hairs were calibrated in grams of force at the first sign of bending

Foot-withdrawal to heating of foot Foot-withdrawal latencies were measured following immersion of the hindpaw in water at 50°C Rats were hand-held and the right and left hindpaws were alternately lowered between the experimenter's fingers into a beaker of water Time was measured until the rat flicked its paw out of the water, up to a 12 sec cutoff Foot-withdrawal latency scores were based on an average of two tests, with a 5 min interval between tests

Foot-manipulation Responses to passive ankle movements were examined following gentle manipulations of the foot Manipulations included foot flexions and extensions in the normal working range of the ankle joint Responses were classified as noxious or non-noxious based on the presence or absence of vocalization on manipulation

Placing reflex Active ankle movements were assessed by examining the integrity of the placing reflex Rats were slowly moved toward a table so that the dorsal surface of the

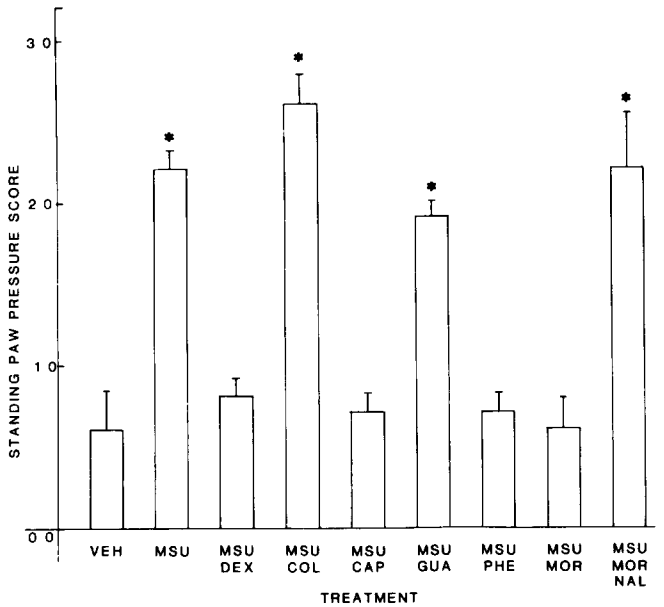

FIG 1 Standing paw pressure scores of injected limbs for articular vehicle-injected rats and urate-injected rats either untreated or treated with various therapeutic agents This and subsequent graphs depict the mean and standard error of each score n=5 for each group in all figures A Kruskal-Wallis test reveals a significant effect of the drug treatment, H(8)=35 44 $p<0$ 01 Significant differences from the scores for the vehicle-injected rats are indicated by asterisks (*$p<0$ 05, **$p<0$ 01) (Abbreviations for treatments in this and subsequent figures VEH—vehicle, MSU—monosodium urate, DEX—dexamethasone, COL—colchicine CAP—capsaicin, GUA—guanethidine, PHE—phenylbutazone, MOR—morphine, NAL—naloxone)

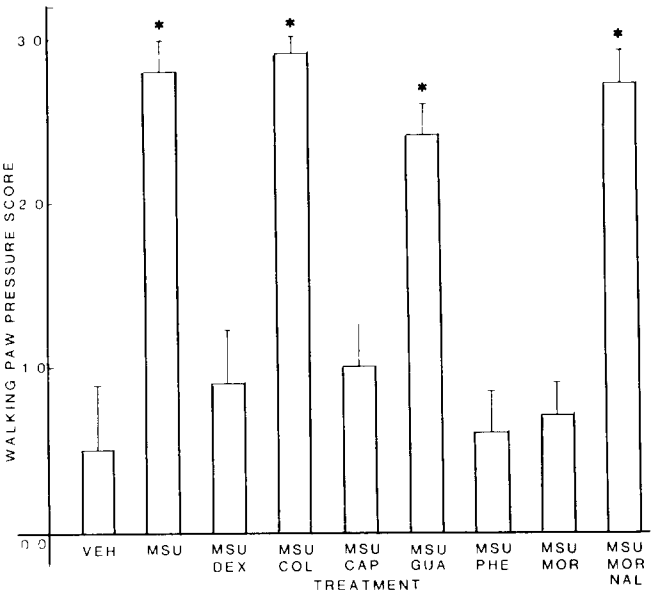

FIG 2 Walking paw pressure scores of injected limbs for articular vehicle-injected rats and urate-injected rats either untreated or treated with various therapeutic agents A Kruskal-Wallis test reveals a significant effect of the drug treatment, H(8)=34 35, $p<0$ 01 Significant difference from the scores for the vehicle-injected rats are indicated by asterisks (*$p<0$ 05, **$p<0$ 01)

right or left hindpaw just touched the edge of the table The response was classified as a placing reflex if the rat lifted its paw in such a way as to prepare for supporting the weight of the body on the surface The test was repeated 5 times for each hindpaw, and scores were based on the number of clear reflexes displayed out of the 5 trials

RESULTS

Since the results are very consistent across the separate measures they can be summarised quite briefly As compared with articular vehicle injections, sodium urate injections produced significant elevations in standing (Fig 1) and walking (Fig 2) paw pressure scores, and noxious responses to foot-manipulation (Fig 5) Compared to the uninjected contralateral limb, the sodium urate injected limb was found to have significantly reduced foot-withdrawal thresholds to ankle pressure (Fig 3), significantly reduced placing reflex scores (Fig 6) and significantly elevated foot-withdrawal responses to heating the foot (Fig 4) Dexamethasone, capsaicin, phenylbutazone and morphine significantly reduced the abnormal behaviour in all behavioural tests Colchicine and morphine with naloxone had no effect Guanethidine had no effect on five of the tests but did influence foot withdrawal from hot water The effect of capsaicin on the AJUA induced prolongation of foot withdrawal from hot water could not be tested since capsaicin alone produces a marked prolongation [8]

DISCUSSION

The results confirm earlier findings that AJUA in rats produces conditions comparable to gouty arthritis in humans [4] Urate-treated rats showed a lowered threshold to manipulation of or pressure on the ankle, and demonstrated guarding with a reluctance to flex the ankle if the foot distal to the ankle was stimulated with touch (placing reflex), pressure or heat Treatment with sodium urate also produced dramatic inflammation in the injected ankle Inflammation was reflected by increased ankle diameter, marked tissue oedema, and a high degree of infiltration of the joint tissue with polymorphonuclear leukocytes

The AJUA model clearly demonstrates the analgesic properties of dexamethasone, phenylbutazone, morphine and capsaicin The pain reduction produced by dexamethasone supports a similar effect of this drug on adjuvant arthritis [29], as well as of the steroid hydrocortisone on urate arthritis [2] This analgesia likely depends on the potent anti-inflammatory properties of corticosteroids which result from effective membrane stabilization The analgesia produced by phenylbutazone agrees with a number of studies where pain associated with urate arthritis was reduced by NSAIDs [3, 11, 13, 18, 24, 26] This treatment likely reduced hyperalgesia by inhibiting the biosynthesis of prostaglandins, which contribute to both oedema and inflammatory pain [27] That AJUA is sensitive to analgesia produced by morphine, as well as its naloxone reversibility, supports earlier reports that urate arthritis is capable of detecting the specific receptor-mediated analgesic effects of morphine [14,15] The decreased pain responses following local nerve capsaicin also support recent results of Otsuki *et al* [15], and suggest that the urate model of arthritis, like adjuvant arthritis [5,10], is extremely sensitive to treatments which influence C fiber afferents Capsaicin applied to an

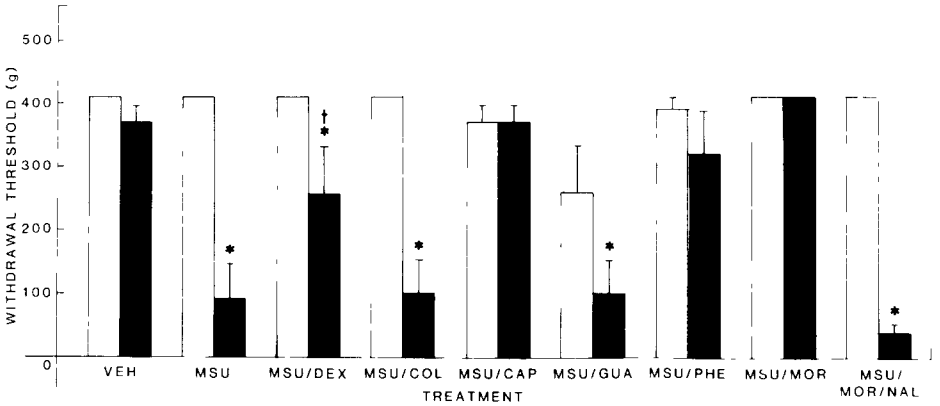

FIG 3 Foot-withdrawal thresholds to ankle pressure with von Frey hairs on each ankle for articular vehicle-injected rats and urate-injected rats either untreated or treated with various therapeutic agents Two-way ANOVA reveals significant main effects of drug treatment, $F(8,36)=7\ 01$, $p<0\ 01$, foot (treated vs untreated), $F(1,36)=102\ 1$, $p<0\ 01$, as well as the drug treatment × foot interaction, $F(8,36)=9\ 42$, $p<0\ 01$ Significant differences between the injected (shaded bars) and uninjected (open bars) feet in each group are indicated by asterisks (*$p<0\ 05$, **$p<0\ 01$) Although the urate-injected feet of the dexamethasone-treated rats have significantly lower thresholds than their uninjected control feet, the thresholds are still significantly higher than that of the urate-injected feet in urate-only-treated rats ($p<0\ 01$)

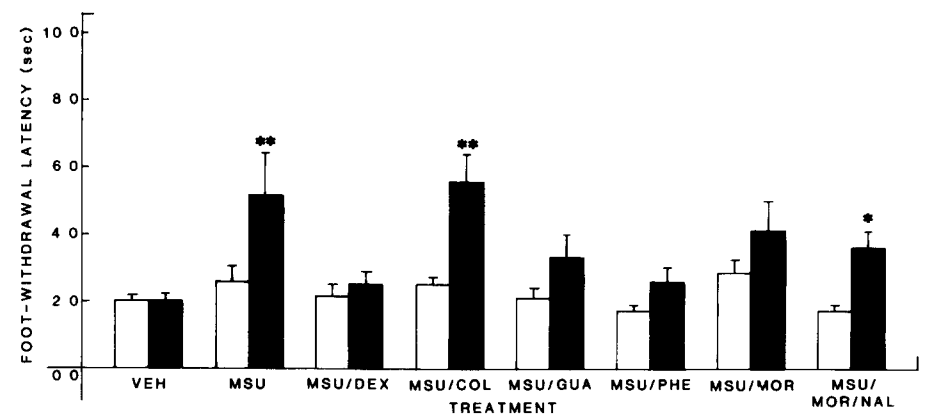

FIG 4 Foot-withdrawal latencies of each paw when exposed to water at 50°C for articular vehicle-injected rats and urate-injected rats either treated or untreated with various therapeutic agents Two-way ANOVA reveals significant main effects of drug treatment, $F(7,32)=2\ 79$, $p<0\ 05$, and foot, $F(1,32)=45\ 97$, $p<0\ 01$, as well as significant drug treatment × foot interaction, $F(7,32)=3\ 32$, $p<0\ 01$ Significant differences between injected (shaded bars) and uninjected (open bars) feet within each group are indicated by asterisks (*$p<0\ 05$, **$p<0\ 01$)

adult nerve reduces the central effect of C afferents [28], blocks transport in C fibers [9] and depletes most C-fiber peptides [1], while leaving unmyelinated A fibers unaffected

No attempt was made in this initial test of this model to vary dose, timing or route of drug administration This may well explain the failure of the single post-urate treatment with colchicine to affect pain responses When administered as a pretreatment in previous studies, colchicine has effectively reduced urate arthritis in both humans [11,22] and animals [21] It might also explain the failure to confirm the anti-arthritic effects of guanethidine reported by Levine *et al* [10], since they examined the effect of prolonged pretreatment with guanethidine on adjuvant arthritis

Although clearly a painful stimulus in man [7,20], intra-articular sodium urate has only recently been investigated in the rat [4,15] Knee joint injections of sodium urate produce limping and a reduction in the weight placed on the injected limb These behaviours can be measured subjectively, as in the present study, as well as objectively by recording the difference in standing weight between the paws of the injected and uninjected limbs, using a pressure transducer [15] We have recently demonstrated further advantages of injecting sodium urate into a single ankle joint of rats [4] In addition to paw pressure changes which indicate spontaneous painful responses to AJUA, the injection produces a series of alterations in the behavioural responses to noxious and non-noxious stimuli Together, these fast, simple measures provide an index of ongoing pain, hyperalgesia or tenderness, and a reluctance to move the affected joint or guarding, which are extremely sensitive to the analgesic effects of anti-arthritic agents

AJUA offers advantages over other methods of producing

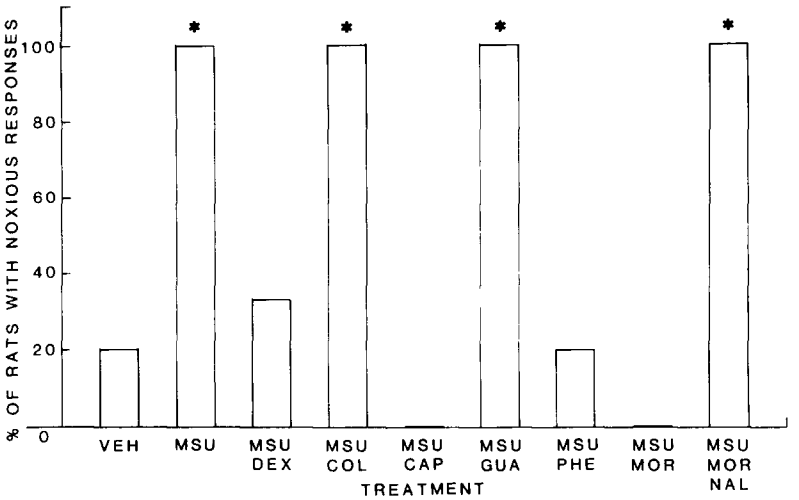

FIG 5 Percentage of rats with noxious responses to foot-manipulation of the injected limb for articular vehicle-injected rats and urate-injected rats either untreated or treated with various therapeutic agents Significant differences from the scores for vehicle-injected rats are indicated by asterisks statistic (**$p<0$ 01)

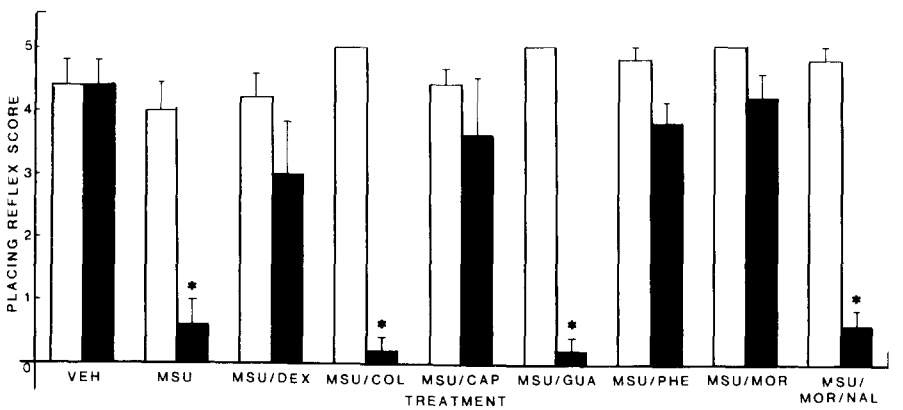

FIG 6 Placing reflex scores of each limb for articular vehicle-injected rats and urate-injected rats either untreated or treated with various therapeutic agents Kruskal-Wallis tests indicate a significant drug effect for the treated, H(8)=29 56, $p<0$ 01, but not the untreated foot, H(8)=13 84, $p>0$ 05 Significant differences between the injected (shaded bars) and uninjected (open bars) feet within each group are indicated by asterisks (**$p<0$ 01)

arthritis or inflammatory pain. With respect to carrageenan [30], formalin [6], and other noxious chemicals, AJUA is more advantageous because, unlike these other treatments, it produces its effects through mechanisms which mimic a known cause of arthritis (i e., that which produces gout) With respect to adjuvant arthritis, the AJUA model is more desirable for analgesic testing since its effects reach a peak within 1 day of injection, compared to the 2–3 week period of development in adjuvant arthritis This is not only more convenient for the experimenter, but also means that the animal is not subjected to prolonged suffering Furthermore, it is possible (unlike in adjuvant arthritis) to compare the responses of normal and abnormal joints in the same animal

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