Effects of paracetamol and aspirin on neural activity of joint mechanonociceptors in adjuvant arthritis

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1 The effects of paracetamol and lysine acetylsalicylate (L-AS) on high-threshold mechanonociceptors have been investigated by recording neural activity from the inflamed ankle joint in anaesthetized rats with mild adjuvant-induced monoarthritis.

2 Paracetamol (50 mg kg⁻¹, i.v.) and L-AS (100 mg kg⁻¹, i.v., equivalent to 50 mg kg⁻¹ aspirin) both caused a maximal reduction of about 40% in mechanically-evoked discharge and of 30% in ongoing (spontaneous) activity by about 15 min after the injection: a second dose of either drug did not have any significant additional effect on discharge.

3 The prostanoid IP receptor agonist, cicaprost (0.1–0.5 µg), increased both mechanically-evoked and ongoing discharge to pre-paracetamol levels when injected close-arterially 30–50 min after paracetamol, whereas prostaglandin E₂ (PGE₂) was relatively ineffective at restoring activity.

4 The results suggest that prostacyclin (PGI₂) contributes to the sensitization of high-threshold joint mechanonociceptors in adjuvant-induced monoarthritis, and that paracetamol and L-AS both act to reduce discharge by inhibiting the synthesis of prostacyclin in the joint capsule.

5 Paracetamol has a direct peripheral action affecting joint capsule mechanonociceptors in rat adjuvant-induced arthritis which is very similar to that of the soluble aspirin preparation, L-AS. These findings, together with the existing literature concerning the anti-arthritic effects of paracetamol, are relevant to the treatment of chronic inflammatory disorders such as rheumatoid arthritis.

Keywords: Adjuvant arthritis; paracetamol; aspirin; high threshold joint mechanonociceptors

Introduction

The rat ankle joint contains high-threshold mechanoreceptors that are located in the joint capsule (Guilbaud *et al.*, 1985) and are classified as type IV articular receptors having a nociceptive function (Wyke, 1981). In the normal joint these sensory receptors only respond to intense mechanical stimuli and the afferent fibres rarely show spontaneous activity. In adjuvant-induced poly- or mono-arthritis the sensors become hypersensitive to mechanical stimuli and spontaneous activity can readily be recorded from the afferent nerve fibres (Guilbaud *et al.*, 1985; Grubb *et al.*, 1991). Prostanoids appear to be involved in this sensitization because the soluble aspirin preparation lysine acetylsalicylate (L-AS) substantially reduced both 'spontaneous' and mechanically-evoked discharge from high-threshold mechanonociceptors, possibly as a result of cyclo-oxygenase inhibition reducing prostacyclin levels within the inflamed joint (Guilbaud & Iggo, 1985).

We have developed a model of arthritis in which the lesion induced by local administration of Freund's complete adjuvant in a low dose is mild and confined to one ankle joint i.e. monoarthritis (Grubb *et al.*, 1988; Birrell *et al.*, 1990). This preparation has now been used to compare the effects of paracetamol and aspirin on afferent activity recorded from high threshold mechanonociceptors in the arthritic ankle joints of anaesthetized rats. The aim was to determine whether or not paracetamol has peripheral actions comparable with those of aspirin; a common view is that aspirin, by virtue of its anti-inflammatory action, reduces sensitization of nociceptors in chronic inflammatory states such as arthritis, whereas paracetamol has no or only weak anti-inflammatory actions at peripheral sites and is, therefore, ineffective (e.g. Rang & Dale, 1988; Bradley, 1989).

Preliminary accounts of some of this work have been published (Birrell *et al.*, 1990; McQueen *et al.*, 1990).

Methods

Experiments were performed on male Wistar rats weighing between 252 and 397 g, mean ($\pm$ s.e.mean) 310 ± 12 g. Arthritis was induced between 12 and 32 days (mean 20 ± 2) before the acute experiment. Freund's complete adjuvant (1 mg ml⁻¹ heat killed and dried *Mycobacterium tuberculosis*, suspended in paraffin oil; Sigma) was injected subdermally in a total volume of 0.15 ml (0.15 mg bacteria) at three separate sites around the left ankle (tibio-tarsal) joint in rats which were anaesthetized with halothane (2% in oxygen). The left ankle joint showed signs of mild inflammation within a day and the peak increase ($\sim 50\%$) in circumference of the joint occurred after approximately 10 days. Thereafter the inflammation remained stable for a further 25 days. The animals continued to move, behave and gain weight normally and did not differ significantly from uninjected controls or from vehicle-injected rats which had no inflammation and only a slight swelling (lasting 2–7 days) of the left ankle. The contralateral (un-injected) limb was, in terms of circumference and appearance, no different from that of uninjected controls at any time during the post-injection period ($P > 0.05$, Wilcoxon test).

Neuropharmacological experiments

The arthritic rats were anaesthetized with urethane (0.6 ml 100 g⁻¹ body weight of 25% w/v aqueous ethylcarbamate solution injected i.p.) and body temperature was maintained at 37°C by an automated heating blanket connected to a thermistor probe inserted in the rectum. The trachea, a carotid artery (for B.P. monitoring), and the right femoral vein were cannulated, and a catheter was introduced into the abdominal aorta via the right femoral artery and used for intra-arterial administration of substances.

Neural recordings were obtained from fine filaments dissected from the left primary articular cutaneous ramus, a

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branch of the tibial nerve which innervates the ankle joint capsule, see Guilbaud *et al.* (1985) for details. Bipolar platinum-iridium wire electrodes were used to provide an extracellular signal that was amplified (Neurolog), digitized (Sony PCM 701-ES) and recorded on video tape (Sony Betamax, SLF 25 UB), and subsequently analysed by playing back through a voltage discriminator (WPI, 120) which enabled individual action potentials to be selected for quantification, in conjunction with a CED 1401 (Cambridge Electronics) interface, by a PC compatible microcomputer (DCS.XT).

High threshold mechanoreceptors were identified by probing the left ankle joint manually with a fine-tipped rod and, once a receptive field had been found, calibrated Von Frey hairs were used to determine the threshold force needed to activate the receptor. A mechanical stimulator (Somedic) was then positioned over the site with the 1 mm diameter tip of its probe resting on the tissues above the joint. A timer was used to trigger a ramp and plateau indentation of constant amplitude (100–500 μm , usually 400 μm) and duration (2 s) repeated once every 2 min. The displacement of the probe was digitised and recorded on video tape together with the associated neural discharge.

The conduction velocity of the units recorded was measured by passing current from an electrical stimulator (Neurolog) through a pair of electrodes in the tip of the probe attached to the mechanical stimulator. This allowed small localised currents to be passed within the receptive field with the probe pressing firmly into the tissue and the conduction velocity was calculated from the time taken to reach the recording electrodes (17–35 mm away).

Estimation of salicylate and paracetamol in plasma samples

Samples of arterial blood were taken, heparin added (50 units), and the plasma separated following centrifugation. Total salicylate was estimated by colorimetry (see McQueen *et al.*, 1989) and paracetamol was measured by high performance liquid chromatography (h.p.l.c.) (Adriaenssens & Prescott, 1978).

Data analysis

Ongoing (spontaneous) neural activity was measured by averaging discharge in successive 60 s periods. Mechanically-evoked activity was determined by counting the total number of action potentials generated by each 2 s indent. Data were normalized with respect to pre-drug values (= 100%) in individual experiments.

Mean values are shown $\pm$ s.e.mean. The Wilcoxon two sample test (Colquhoun, 1971) was used to test for statistically significant differences between groups, and the null hypothesis rejected at $P \leq 0.05$. The paired *t* test was used for small samples.

Drugs

The following drugs were used, dissolved in 0.9% w/v aqueous NaCl: lysine acetylsalicylate (L-AS), (Aspegic, Synthelabo, France; the dose used was 100 mg, equivalent to 50 mg acetylsalicylic acid-aspirin), paracetamol (Aldrich), prostaglandin E_2 (Sigma), cicaprost (Searle), and ethyl carbamate (B.D.H.).

The L-AS preparation and the paracetamol were injected i.v. in a volume of 1 ml delivered over 10 s. Cicaprost was injected over 2 s into the abdominal aorta in volumes of 0.1 ml washed in with 0.2 ml saline.

Results

The high threshold mechanoreceptors recorded from arthritic ankle joints during this study were more sensitive to mechanical stimulation (mean threshold 53 ± 3 mN) than those from control joints (mean 81 ± 7 mN; $P < 0.01$). The conduction

velocities of the fibres ranged from 0.3 to 2.0 ms^{-1} (mean $0.9 \pm 0.1 \text{ ms}^{-1}$; $n = 21$ units) which means they were fine unmyelinated C fibre afferents (conduction velocity $< 2.5 \text{ ms}^{-1}$, Schaible & Schmidt, 1983).

Responses to mechanical stimulation – effects of paracetamol and lysine acetylsalicylate

Pooled data from experiments in which the activity of high threshold mechanoreceptors recorded from the left (arthritic) ankle was evoked by mechanical stimulation of the joint are shown as absolute values in Figure 1. Paracetamol (50 mg kg^{-1} , i.v.) and L-AS (100 mg kg^{-1} , i.v.) both caused a reduction in the discharge which varied somewhat between experiments as reflected in the high s.e.mean. The variability is a consequence of pooling data that have a wide range of basal values (i.e. impulses per standard indent), coupled with the finding that individual mechanoreceptors differed in their responsiveness to paracetamol or L-AS; all showed depression of the response, but the size of the reduction and the time course was variable. There was no correlation between the circumference of the joint or the conduction velocity of the afferent fibre with the magnitude of the response to the drugs.

Normalizing the data as a % of the pre-injection control values eliminates inter-experiment variability attributable to differing basal values, and plotting the result in this way shows that paracetamol and L-AS caused similar reductions in mechanically-evoked discharge (Figure 2). The only significant difference between the effects of paracetamol and L-AS lay in the rapid transient reduction observed 6 min after paracetamol ($P < 0.05$ in comparison with L-AS). Otherwise there were no significant differences between the drugs: both reduced discharge to about half of control levels, reaching the nadir 36–46 min after the single injection. Mechanoreceptor responsiveness returned to pre-injection levels 60–80 min post-injection, but this was not investigated systematically as the preparations were generally used for further studies 46 min after the original dose, which explains the decrease to $n = 3$ in n after this time; this small sample size precluded meaningful statistical analysis for measurements at 60 min.

Effects of paracetamol and lysine acetylsalicylate on spontaneous mechanoreceptor discharge

In most, but not all, recordings of neural activity from single high threshold mechanoreceptors the units displayed spontaneous ongoing discharge, in addition to mechanically-evoked activity. Paracetamol and L-AS both reduced the spontaneous

Figure 1 Effects of injecting paracetamol (50 mg kg^{-1} i.v., ●, $n = 7$), lysine acetylsalicylate (100 mg kg^{-1} i.v., △, $n = 8$), or saline (1 ml i.v., ○, $n = 9$) on the discharge evoked by mechanical stimulation of high-threshold joint mechanoreceptors in arthritic ankle joints. The mean responses are shown; s.e.mean indicated by vertical bars. From 25 min onwards n decreased, but was never less than 3.

Figure 2 Normalized data showing the decrease in discharge evoked from mechanoreceptors following i.v. injection of 100 mg kg^{-1} lysine acetylsalicylate (L-AS, Δ , $n = 3-8$) or 50 mg kg^{-1} paracetamol ($\bullet$, $n = 3-7$). The dashed line represents the pre-injection response (100%), which, in absolute terms, was 34 ± 4 impulses for paracetamol and 27 ± 3 impulses for L-AS in response to the standard indent. * $P < 0.05$, paired t test, versus pre-injection control discharge.

discharge, in comparison with the saline controls, and the overall pattern was similar (see Figure 3). L-AS transiently reduced discharge at 5 min post-injection, whereas paracetamol did not, but apart from this the effects of the drugs were similar in that both reduced spontaneous mechanoreceptor discharge to 60–70% of preinjection levels by 15 min post-injection, with some inter-experiment variability.

Additional doses of paracetamol or lysine acetylsalicylate

A second i.v. injection (same dose) was made 45 min after the first in order to determine whether any additional effect could be observed on either spontaneous or mechanically-evoked activity of the joint sensors. In three experiments a second dose of paracetamol (50 mg kg^{-1} i.v.) had no additional effect, and nor did a second dose of L-AS (100 mg kg^{-1} i.v.) in three experiments when it was given 45 min after the original dose of salicylate. In a further three experiments L-AS (100 mg kg^{-1} i.v.) was injected 45 min after paracetamol had been administered. The salicylate caused a further slight

Figure 3 Effects of injecting paracetamol (50 mg kg^{-1} i.v., $\bullet$, $n = 3-8$), lysine acetylsalicylate (L-AS, 100 mg kg^{-1} i.v., Δ , $n = 3-5$) or 1 ml saline ($\circ$, $n = 3-9$) on background (spontaneous) discharge of high-threshold joint mechanoreceptors in arthritic ankle joints. Mean discharge was averaged over 60 s before (0) and 5, 10, 15 and 30 min after the i.v. injection and expressed as a percentage of the preinjection value (dashed line = 100%). Preinjection discharge averaged $1.2 \text{ counts per second (ct s}^{-1})$ pre-paracetamol, 2.5 ct s^{-1} pre-L-AS, and 1.9 ct s^{-1} pre-saline. * $P < 0.05$, Wilcoxon test versus saline.

reduction in mechanically-evoked activity as well as in spontaneous discharge, but this reduction was not statistically significant ($P > 0.05$; time zero versus 30 min post-L-AS, paired t test).

Effects of cicaprost injected after paracetamol

The prostanoid IP receptor agonist (see Coleman *et al.*, 1984, for a review on prostanoid receptor classification) cicaprost was injected 30–50 min after discharge had been reduced by paracetamol. Both spontaneous and mechanically-evoked discharge were enhanced after cicaprost ($0.1-0.5 \mu\text{g}$, i.a.), with discharge being restored to pre-paracetamol levels; similar results were obtained in the three experiments performed, and representative examples from two of them are shown in Figure 4. In a separate experiment prostaglandin E_2 (PGE_2 , $3 \mu\text{g}$, i.a.) was injected 45 min after paracetamol had reduced spontaneous and mechanically evoked mechanoreceptor discharge. There was no additional effect on evoked activity, and although spontaneous discharge increased slightly after PGE_2 , the effect was transient and lasted for only 3–4 min.

Plasma concentrations of aspirin and paracetamol

Samples of blood were taken 5, 15, 30 and 45 min after a single i.v. injection of lysine acetylsalicylate (100 mg kg^{-1} , equivalent to 50 mg kg^{-1} aspirin). A second injection of the same drug was made 50–60 min after the original dose, and samples taken 5, 15 and 30 min thereafter. In a separate series

Figure 4 Data from separate experiments illustrating the ability of cicaprost ($0.1-0.5 \mu\text{g}$, i.a.) to restore (a) mechanically-evoked and (b) spontaneous discharge of high-threshold mechanoreceptors in arthritic-joints to levels comparable with those obtained before the injection of paracetamol (50 mg kg^{-1} i.v.) given approximately 45 min earlier. Basal (100%) values were 42 impulses in (a), 0.5 ct s^{-1} in (b); cicaprost was injected at time 0.

of experiments estimations of paracetamol in plasma were made 45 min after injecting paracetamol at a dose of 50 mg kg^{-1} , i.v. The highest salicylate level was $75.9 \pm 1.0 \text{ mg l}^{-1}$ 10 min after the first dose of L-AS, declining to $50.1 \pm 12.9 \text{ mg l}^{-1}$ at 30 min. Following the second dose, corresponding levels at 5 and 30 min were 127.5 ± 23.2 and $108.2 \pm 17.1 \text{ mg l}^{-1}$ respectively of salicylate ($n = 3$). Paracetamol values were $16.3 \pm 1.4 \text{ mg l}^{-1}$ 45 min after the first dose, $43.9 \pm 5.7 \text{ mg l}^{-1}$ 30 min after the second ($n = 3$).

Discussion

Our results show that neural discharge from high threshold mechanoreceptors (presumed nociceptors) in the ankle joint of anaesthetized rats with adjuvant-induced monoarthritis is reduced by paracetamol as well as by the soluble aspirin preparation L-AS; both ongoing and mechanically-evoked discharge were affected. The effect of L-AS on these ankle joint sensors confirms previous findings in rats with either adjuvant-induced polyarthritis (Guilbaud & Iggo, 1985) or monoarthritis (Grubb *et al.*, 1991). L-AS has no effect on the activity of these sensory receptors in normal joints (Guilbaud & Iggo, 1985), so the reduction in activity seen in arthritis suggests sensitization of the receptors by a mediator which is susceptible to the action(s) of salicylate and paracetamol.

We found that PGE_2 , given 30 min after paracetamol had reduced mechanoreceptor discharge, was ineffective in restoring discharge to pre-paracetamol levels. This result is similar to that obtained during earlier studies with L-AS on this preparation (Birrell *et al.*, 1990; Grubb *et al.*, 1991) and suggests that inhibition of PGE_2 production by paracetamol is not responsible for the decreased responsiveness of the sensors following administration of the drug in arthritis. In contrast, the stable and highly selective IP receptor agonist cicaprost (Skuballa *et al.*, 1986) did restore discharge rapidly, and this action was sustained. We have already shown that PGI_2 , in contrast to the feeble action of PGE_2 , can activate these sensory receptors in normal rat ankle joints, although the effect evoked is shorter-lasting than that of cicaprost (Birrell *et al.*, 1991). It seems likely, therefore, that PGI_2 acting at IP receptors is involved in the sensitization of high threshold mechanonociceptors observed in this model of arthritis; this is supported by the relatively rapid effect of paracetamol and of L-AS in reducing discharge, which points to involvement of a prostanoid having a short half-life in the tissues. PGI_2 meets this requirement, whereas PGE_2 does not, as discussed in detail by Guilbaud & Iggo (1985). Additional studies involving measurement of eicosanoid levels in the joint capsule would provide more direct evidence regarding the relative role of PGI_2 and PGE_2 in the sensitization of joint mechanonociceptors in this model of arthritis.

The doses of L-AS (equivalent to 50 mg kg^{-1} aspirin) and paracetamol (50 mg kg^{-1}) evidently caused maximal reductions in discharge since a second dose given 45 min after

the first produced no additional effect. Paracetamol given after L-AS also failed to cause any further decrease in activity, whereas L-AS given after paracetamol did cause a further slight reduction, although this was not statistically significant. The two non-steroidal anti-inflammatory drugs appear to act via a common mechanism but the possibility that part of their action may involve separate mechanisms cannot be excluded. Further studies will be needed to investigate this aspect and also to establish whether PGE_2 , or another eicosanoid with a long half-life in the tissues, contributes to the 'residual' nociceptor discharge remaining after doses of L-AS or paracetamol. Our results demonstrating that paracetamol is effective in reducing discharge from presumed nociceptors in this model of arthritis is very strong evidence for a peripheral action of the drug in or near the joint. L-AS was established as having a local rather than systemic action in desensitizing joint capsule mechanonociceptors by application to the joint (Guilbaud & Iggo, 1985), and Ferreira *et al.* (1978) proposed that the peripheral analgesic effect of paracetamol involves action of the drug at nociceptors.

There is a consensus that non-steroidal anti-inflammatory drugs act mainly in the periphery to reduce sensitization of nociceptors (e.g. Ferreira, 1972; Moncada *et al.*, 1975; Handwerker, 1976; Ferreira *et al.*, 1978; Tyers & Haywood, 1979; Martin *et al.*, 1987), and that they achieve this by inhibiting the cyclo-oxygenase enzyme involved in synthesizing prostanoids. However, a commonly held view is that paracetamol lacks anti-inflammatory properties because it is a very weak cyclo-oxygenase inhibitor, at least as far as the periphery is concerned (e.g. Rang & Dale, 1988). In fact paracetamol is quite capable of inhibiting prostaglandin biosynthesis in the periphery, although its potency varies depending on the tissue studied (see Higgs *et al.*, 1974; Flower & Vane, 1974), and it is also anti-arthritis and anti-inflammatory in a variety of acute and chronic inflammatory tests in rats, including adjuvant-induced arthritis (Vinegar *et al.*, 1976; Wong & Gardocki, 1983). Phenacetin was used as an effective treatment for rheumatoid arthritis in man, and it is generally considered that its metabolite, paracetamol, was the active agent (Smith, 1958). Paracetamol is an anti-inflammatory drug in man (McQueen, 1973; Skjelbred *et al.*, 1977) and it has been shown to reduce prostanoid levels in inflammatory exudates and in synovial fluid aspirated from the knee joints of patients with rheumatoid arthritis (Higgs *et al.*, 1974; 1976).

In conclusion, our results provide neuropharmacological evidence that paracetamol, like aspirin, has a direct action in the periphery to reduce the sensitization of presumed nociceptors in a rat model of arthritis and, given that paracetamol has fewer 'side effects' than aspirin, may be relevant to the treatment of pain and inflammation in rheumatoid arthritis.

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