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The effects of 5-HT on articular sensory receptors in normal and arthritic rats

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1 The effects of intra arterial (i.a.) injections of 5-hydroxytryptamine (5-HT, 1–100 μg) on the discharge of (a) identified articular high threshold mechanoreceptors and (b) unidentified chemosensitive receptors in the ankle joint have been studied electrophysiologically in anaesthetized normal and arthritic rats. Recordings were made from a fine branch of the medial plantar nerve.

2 5-HT increased the mechanical responsiveness of high threshold nociceptive mechanoreceptors with C and Aδ fibre afferents in both normal and adjuvant-arthritic rats. Receptors in arthritic joints were more sensitive to 5-HT than were those from normal joints.

3 5-HT produced a complex response from both types of articular receptors following i.a. injection. Two separate components were identified: (a) a fast transient burst of activity was obtained within 10 s of this injection in 66% of units from normal animals and 45% from arthritics, followed by (b) a delayed slow longer-lasting excitation seen in 62% of the units examined from normals and 77% of units from arthritic rats.

4 Increased mechanoreceptor resonsiveness produced by 5-HT was reduced or abolished by the 5-HT_3 receptor antagonists studied (MDL 72222, ICS 205-930, or GR 38032F, in single doses of 100 $\mu g\,kg^{-1}$, i.a.).

5 Fast excitation showed marked tachyphylaxis and was antagonized by MDL 72222, ICS 205-930 or GR 38032F. It was unaffected by ketanserin (100 $\mu g\,kg^{-1}$, i.a.). Delayed excitation was reduced or abolished by ketanserin but was unaffected by the 5-HT_3-receptor antagonists.

6 Administration of MDL 72222, ICS 205-930 or GR 38032F caused short lasting (< 5 min) reductions in background activity from both types of unit recorded in arthritic rats, as well as in normal rats in which activity had increased following administration of 5-HT. Ketanserin caused similar reductions in background activity in chemosensitive units, but had no effect on mechanoreceptors.

7 At least two types of receptor are involved in the actions of 5-HT on articular sensory receptors with fine afferent fibres. Increased mechano-responsiveness involves a 5-HT_3-receptor as does fast excitation. Delayed excitation probably involves a 5-HT_2-receptor. Endogenous 5-HT appears not to play a crucial role in sensitization of high threshold mechanoreceptors in this model of chronic inflammation and arthritis, although its local release may potentiate the actions of other inflammatory mediators on sensory receptors in the ankle joint.

Introduction

Adjuvant-induced polyarthritis in rats has been used extensively as a model for the study of chronic inflammatory pain (see Colpaert, 1987). Electrophysiological studies with this model have shown that high threshold C-fibre mechanoreceptors (putative nociceptors) have lower thresholds in the ankle joints of these animals in comparison with normal rats (Guilbaud *et al.*, 1985). These results suggest that the behavioural changes seen in adjuvant polyarthritis can partly be accounted for in terms of altered properties of articular sensory receptors. The enhanced receptor sensitivity can be reduced by lysine acetylsalicylate, suggesting that locally produced cyclo-oxygenase metabolites may be responsible for at least part of the sensitization (Guilbaud & Iggo, 1985). It is still uncertain, however, the extent to which other inflammatory mediators found in tissue exudates may contribute to the sensitization of peripheral sensory receptor mechanisms.

Keele & Armstrong (1964) demonstrated that 5-hydroxytryptamine (5-HT) has the ability to cause pain when applied to a blister base, and 5-HT was later shown to lower thresholds for chemically-induced pain in man (Sicuteri *et al.*, 1965) and to enhance pseudoaffective responses to bradykinin in animals (Nakano & Taira, 1976). Sensory nerve endings associated with small myelinated and non-myelinated axons have been found to be activated and sensitized by 5-HT in skin (Fjallbrant & Iggo, 1961; Beck & Handwerker, 1974) and muscle (Mense, 1981), as are cutaneous SAII mechanoreceptors with rapidly conducting afferent fibres (Fjallbrant & Iggo, 1961).

Although the pain evoked by application of 5-HT to a blister base has been demonstrated to be antagonized by ICS 205-930, and therefore probably involves a 5-HT_3-receptor (Donatsch *et al.*, 1984; Richardson *et al.*, 1985), in most cases the pharmacological identity of the 5-HT-receptor associated with sensory endings has not been established. The present study was undertaken to examine the effects of 5-HT on sensory receptors in the rat ankle joint, and to characterize the 5-HT receptors mediating these effects by the use of selective antagonists (Fozard, 1984; Bradley *et al.*, 1986; Brittain *et al.*, 1987). We also investigated whether 5-HT plays a role in the sensitization of high threshold mechanoreceptors in arthritic joints by using a rat model of adjuvant-induced mono-arthritis in which the arthritis is mild and confined to one ankle (Grubb *et al.*, 1988).

Methods

Induction of arthritis

Male Wistar rats weighing 200–250 g were anaesthetized with ether during subdermal injection of 0.15 ml of Freund's complete adjuvant (1.0 mg ml^{-1} heat killed *Myobacterium tuberculosis* in paraffin oil, Sigma) around the left ankle joint.

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Experiments were performed on anaesthetized animals following a period of two to nine weeks, during which time a localized arthritis consisting of swelling (approx. 50% increase in circumference of the left ankle joint) and redness of the injected ankle had developed and was maintained.

Surgical procedures

A total of 10 arthritic and 12 normal male Wistar rats weighing between 200 and 300 g was used in these experiments. Animals were anaesthetized with urethane (25% w/v, 0.6 ml $100\,g^{-1}$ body wt. i.p.). The trachea was cannulated and arterial blood pressure monitored via a cannula in the left carotid artery. A cannula was also inserted into the right femoral artery for the injection of drugs into the abdominal aorta at the level of the iliac bifurcation. Drugs were dissolved in 0.9% w/v aqueous NaCl solution (saline) and injected in volumes of 0.1 ml followed by a 0.2 ml saline wash. Accessibility to articular receptors via the vasculature was tested by use of a single low dose of capsaicin (1 μM i.a.) which caused a transient increase in neural discharge of all the afferents studied.

Electrophysiological recording

Neural recordings were made from the primary articulocutaneous ramus (PACR) of the left tibial nerve with platinum-iridium wire electrodes, by employing techniques described in detail elsewhere (Guilbaud *et al.*, 1985). Spontaneously active units for which no mechano-sensitive receptor fields could be found (termed 'chemosensitive' because of their excitation by 5-HT and capsaicin) and high threshold slowly-adapting mechanoreceptors with axons in the PACR were examined. Neural recordings were stored on videotape for subsequent analysis of individual afferent units by use of a pulse height voltage discriminator linked to a microcomputer.

Mechanoreceptors were identified by their response to mechanical stimulation, by use of smooth tipped glass probes of 0.5–1.0 mm diameter. Thresholds of individual units were determined with a series of calibrated Von Frey hairs; these high threshold mechanoreceptors probably function as nociceptors (Wyke, 1981; Guilbaud *et al.*, 1985). Mechanical stimuli in any given trial were delivered at fixed intervals by an electromechanical indentation generator; ramp and plateau waveforms were used routinely, with indentations of 200–600 μm and 2 s duration repeated at 60–120 s intervals. The indentation probe consisted of a sealed metal tube (1 mm diameter) smoothed over at the tip with epoxy-resin, containing a silver wire core, insulated except at the tip, used as the cathode for localized electrical stimulation when measuring conduction velocities. Afferent fibre conduction velocity was measured by localized electrical stimulation, via the probe at the level of the receptor, and determining the time take for the action potential to reach the recording electrodes.

Data analysis

Neural discharge (counts per second) was plotted against time for each test, and the change in frequency from the pre-injection control period calculated. In order to standardize results from experiments with different absolute discharge frequencies, mean values for blocks of 10 s duration were used to calculate peak response as a percentage of the mean discharge in the pre-injection 10 s control period. Mechanoreceptor responses were quantified in counts per mechanical stimulus and expressed as a percentage of the pre-injection response.

Statistics

Mean values are given $\pm$ s.e.mean. Statistical analysis of differences between means was carried out by the Wilcoxon two-sample test (two-tailed) and the null hypothesis rejected if $P < 0.05$.

Drugs

The following compounds were used in this study, with concentrations being expressed in terms of the salt: 5-hydroxytryptamine creatinine sulphate complex, dopamine hydrochloride (Sigma); MDL 72222 ((1αH, 3α, 5αH-tropan-3-yl) 3,5-dichlorobenzoate methanosulphonate salt, kindly donated by Merrell Dow Research Institute Strasbourg); ICS 205-930 ((3α-tropanyl)-3yl)-1H-indole-3-carboxylic acid ester, kindly donated by Sandoz, Basel); GR 38032F (1,2,3,9-tetrahydro-9-methyl-3-[(2methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one hydrochloride dihydrate, kindly donated by Glaxo Group Research, Ware); ketanserin, tartaric acid salt (kindly donated by Janssen Pharmaceuticals, Beerse). Capsaicin (8-methyl-n-vanylyl-nonenamide, Sigma) was prepared by diluting a stock solution (1 mg ml^{-1} in 10% ethanol: 10% Tween 80: 80% saline) in saline.

Results

Two types of sensory activity were investigated: mechanoreceptors for which receptive fields were found in the joint capsule (Guilbaud *et al.*, 1985), and 'chemosensitive' units, previously described by Grubb *et al.* (1988), which were excited by 5-HT and capsaicin but for which no receptor fields for mechanical stimuli were found (Table 1). Both mechanosensitive and chemosensitive units were excited by capsaicin (1–10 μg, i.a.) in all experiments. Low threshold, rapidly adapting mechanoreceptors with receptive fields in the tissues adjacent to the joint capsule were not considered in these studies.

Normal rats – mechanoreceptors

Mechanoreceptors with afferent fibres in the PACR and with receptive fields in the ankle joint tissues of normal rats had high mechanical threshold, were slowly adapting with punctate receptive fields of approximately 1 mm diameter and were therefore similar to those described by Guilband *et al.*, (1985). Units had mean von Frey thresholds of 81 $\pm$ 6.8 mN, and the conduction velocities of their afferents (2.1–10.5 ms^{-1}) indicated that they were C or Aδ fine afferent fibres. Only one mechanosensitive unit showed any background (spontaneous) discharge (0.2 i.p.s.) before the addition of 5-HT – the number of these high threshold units found in individual animals was small, and their lack of resting discharge and high mechanical

Table 1 Summary of the number of mechanosensitive and chemosensitive units responding to 5-hydroxytryptamine (5-HT) and the minimal effective doses for these effects in normal and arthritic rats

			No. of units displaying each type of response		
	Units	n	*Rapid excitation*	*Delayed response*	*Minimal effective dose* (μg)
Normals	Mechanosensitive	6	4	3	100
	Chemosensitive	16	12	12	1
Arthritic	Mechanosensitive	10	3	5	1
	Chemosensitive	12	7	12	1

thresholds made them difficult to find. A summary of all units responding to 5-HT is given in Table 1.

Effects of 5-HT on responsiveness to mechanical stimulation In the six units examined 5-HT (1–100 μg) evoked a dose-dependent increase in responsiveness to the standard mechanical stimulus (illustrated for 100 μg in Figure 1). The minimal effective dose which gave reproducible responses in all four of the units tested in this way was found to be 5 μg. As illustrated in Figure 2 a mean peak increase of 56% ($n = 4$) in response to subsequent mechanical stimuli was observed following injection of 5 μg 5-HT, and this effect lasted for 38 $\pm$ 34 s. Larger doses of 5-HT had a more prolonged action, as can be seen in Figure 1 where the response to a mechanical stimulus was still elevated three minutes after the injection of 100 μg 5-HT. Repeated injections of 5-HT produced a sensitization to the drug in four mechanosensitive units.

Excitatory effects of 5-HT on spontaneous activity of mechanosensitive units Close arterial injection of 1–100 μg 5-HT evoked a discharge in three previously silent mechanoreceptors and enhanced the discharge of one unit from a very low initial level of discharge. The effect was reproducible in two of these units at the highest dose used (100 μg 5-HT). A biphasic response observed following injection of 5-HT consisted of a transient burst of activity (hereafter called a 'fast' response) with rapid onset (<10 s), followed by a delayed (>20 s), longer-lasting increase in discharge, hereafter called a 'slow' response (see Figure 3).

Normal rats – chemosensitive afferents

In the 12 normal animals examined in this study 16 recordings consisting of between one and three different action potential spikes were obtained from units with a low level of activity before the addition of 5-HT. Their action potential spike characteristics were similar to those of identified C-fibre afferents, and their mean rate of discharge was 1.4 $\pm$ 0.3 i.p.s.

Excitatory effects of 5-HT on chemosensitive units All the units with an ongoing discharge were excited by an initial or subsequent injection of 5-HT (1–100 μg, i.a.); a further three units became spontaneously active following the drug administration.

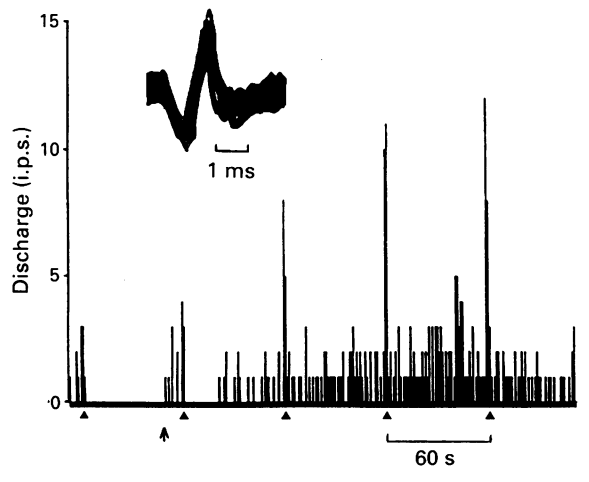

Figure 1 Effects of 100 μg 5-hydroxytryptamine (5-HT) on the activity of a high threshold mechanoreceptor with afferent fibre conduction velocity of 4 ms^{-1} from a normal animal. The graph (bin width 1 s) illustrates afferent discharge and shows fast and slow excitation. Mechanical stimuli (arrowheads) were repeated once every minute and neural responsiveness was increased following an injection of 5-HT (arrow). The inset shows 100 superimposed oscilloscope sweeps of the single afferent unit whose discharge was counted.

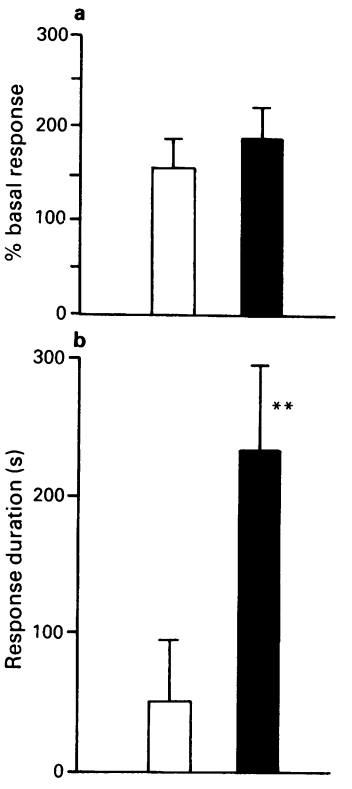

Figure 2 Effects of 5-hydroxytryptamine (5-HT) on mechanoreceptor responsiveness in both normal and arthritic rats. (a) The mean peak increase in mechanoreceptor responsiveness is shown as a percentage of preinjected control produced by 5 μg 5-HT (i.a.) in four normal rats (open column) and by 1 μg 5-HT (i.a.) in six arthritic rats (solid column). (b) Illustrates mean duration of increased responsiveness produced by the same injections of 5-HT (normal rats, open column; arthritics, solid column). Bars represent s.e. mean. ** $P < 0.01$ (Wilcoxon, two-tailed).

Two main components could be recognised in the response to 5-HT. An early, brief burst of activity, which was seen in 75% of active units, followed by a slow sustained increase in discharge in 75% of units. Responses in individual units were either monophasic or biphasic; fast responses occurred within 10 s following injection of 5-HT and lasted for a maximum of 30 s. Tachyphylaxis developed to repeated injections of 5-HT (20–100 μg at 10 min intervals; data not illustrated). However, with lower doses (1–10 μg) and a 15 min interval between injections, a relatively consistent response was obtained in four recordings. The slow response generally took longer than 15 s to develop and lasted for over 4 min in some cases. Depression of activity following the initial excitation was also seen in a small number of units when background activity was elevated.

Arthritic rats – mechanoreceptors

A characteristic feature of arthritic preparations, as previously described (Guilbaud *et al.*, 1985), was the large number of mechanoreceptors found in the joint. On average approximately three times as many mechanoreceptors were found in the ankle joint tissues of arthritic rats in comparison with control animals. These units had lower thresholds than normal for activation with von Frey hairs (52.7 $\pm$ 4.6 mN, $P < 0.05$) and generally had overlapping receptive fields; their conduction velocities (0.5–7.8 ms^{-1}) indicated that they were C/Aδ fibres. In contrast to the lack of spontaneous mechanoreceptor activity in normal rats, nine of the 10 mechanosensitive units studied showed spontaneous discharge, averaging 1.2 $\pm$ 0.4 i.p.s., before the administration of 5-HT.

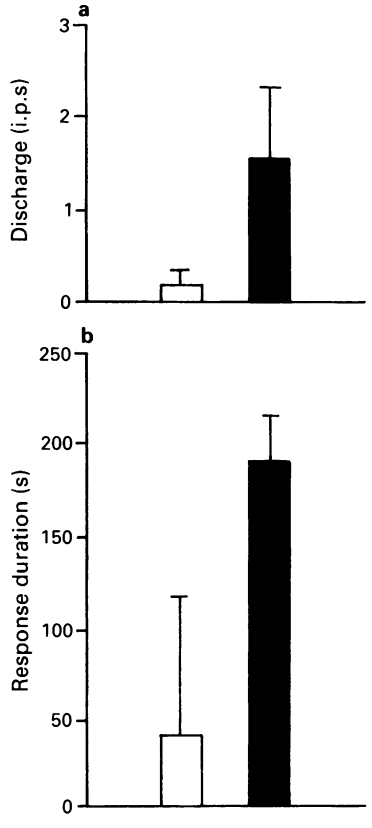

Figure 3 Comparison of 5-hydroxytryptamine (5-HT)-induced slow excitation of mechanosensitive units in normal and arthritic rats. (a) Shows the peak increase in discharge produced by 100 μg 5-HT (i.a.) in two responding units of six tested from normal rats (open column) and the mean peak increase produced by 1 μg 5-HT in four out of five units from arthritics (solid column). The mean basal rate of discharge in arthritic rats was 1.1 $\pm$ 0.8 i.p.s., whereas in control rats no activity was present before the injection of 5-HT. (b) Shows the duration of effects for the same injections as in (a). Open column, represents data from the two individual units which were excited by 5-HT in normal rats. Solid column represents mean of four units from arthritic rats. Bars show s.e.mean.

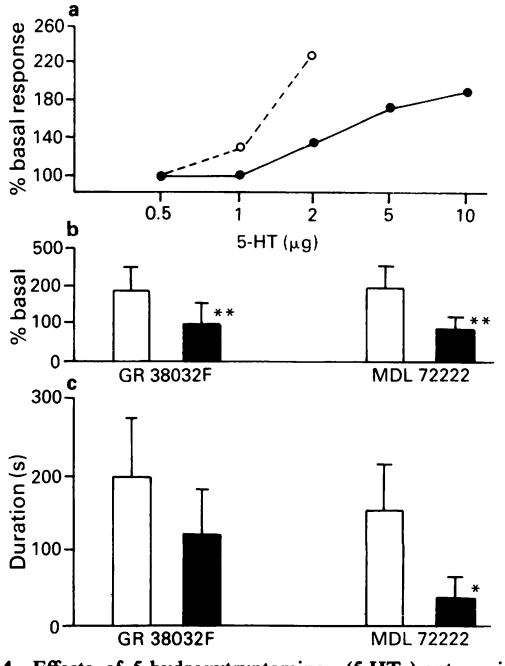

Figure 4 Effects of 5-hydroxytryptamine$_3$ (5-HT$_3$)-antagonists GR 38032F (100 μg kg^{-1}, i.a.), MDL 72222 (100 μg kg^{-1}, i.a.) and ICS 205-930 (100 μg kg^{-1} i.a.) on 5-HT-induced enhancement of mechanoreceptor responsiveness in both normal and arthritic rats. (a) Shows a shift to the right of the log dose-response curve caused by ICS 205-930 (100 μg kg^{-1} i.a.) in a high threshold mechanoreceptor with afferent fibre conduction velocity of 2.7 ms^{-1} from an arthritic rat. (○) and (●) responses before and after addition of antagonist, respectively. The dose of 5-HT is shown in μg with the peak response obtained given as a percentage of the preinjection control. (b) Illustrates the effect of GR 38032F (n = 5) and MDL 72222 (n = 5) on the mean peak increase in mechanoreceptor responsiveness produced by an effective standard dose of 5-HT (1–100 μg i.a.). Columns represent peak responses before (open) and after (solid) injection of antagonist. (c) The duration of response obtained for the same injections as in (a). Bars represent s.e.mean. Significantly different mean values are shown as: * $P < 0.05$ and ** $P < 0.01$.

Effects of 5-HT on responsiveness to mechanical stimulation In all 10 units examined a dose-dependent increase in responsiveness to the standard mechanical stimulus was obtained following the injection of 5-HT (1–100 μg). In seven units the effective threshold dose for production of consistent responses was found to be 1 μg. A mean increase of 75% (n = 6) in response to subsequent mechanical stimuli was produced following injection of the threshold dose. This effect lasted for 240 $\pm$ 48 s, a duration which is significantly greater ($P < 0.01$) than that produced by 5 μg 5-HT in normal rats (Figure 2). Sensitization of mechanoreceptor responses to 5-HT was observed in two units.

Excitatory effects of 5-HT on activity of mechanosensitive units Increases in spontaneous activity of six mechanosensitive units was seen following injection of 5-HT (1–100 μg). The biphasic response seen in normal animals was much less conspicuous, being obtained in only one recording. One unit gave a fast and slow response, and another unit displayed only the fast response. Three units responded with only a slow increase in spontaneous activity. In four of the units the slow response consisted of a mean peak increase of 1.6 $\pm$ 0.6 i.p.s. above basal discharge (1.1 $\pm$ 0.7 i.p.s.) which lasted for 185 $\pm$ 34 s. This effect of 5-HT contrasts markedly with the small response obtained following injection of 100 μg 5-HT in the normal rat (Figure 3). Spontaneous activity in a seventh unit was depressed following 5-HT administration.

Arthritic rats – chemosensitive units

Chemosensitive units Spontaneously active units lacking any identifiable mechanosensitive receptive fields were more numerous in arthritic rats than in controls. In experiments on 10 animals the effects of 5-HT on spontaneous discharge were examined in 12 recordings consisting of between one and three different units with action potentials characteristic of identified C-fibre afferents. Their mean rate of spontaneous discharge before the administration of 5-HT was 1.4 $\pm$ 0.2 i.p.s.

Excitatory effects of 5-HT on chemosensitive units All the units examined were responsive to injections of 5-HT (1–100 μg). A biphasic response was seen, as in normal rats. A fast excitatory response was seen in 58% of units, followed in all the units studied by a slow long lasting increase in discharge.

Effects of 5-HT-receptor antagonists in normal and in arthritic rats

The 5-HT receptor antagonists, MDL 72222, ICS 205-930 and ketanserin, were administered at 100 μg kg^{-1} i.a., doses previously found to be active in abolishing chemoreceptor responses to 5-HT in the cat (Kirby & McQueen, 1984). In the present experiments the 5-HT$_3$-receptor antagonists selectively blocked the 5-HT$_3$-receptor-mediated Bezold-Jarisch-like reflex evoked by 5-HT, and ketanserin selectively antagonized 5-HT-induced hypotension.

Table 2 Summary of the effects of 5-hydroxytryptamine (5-HT)-receptor antagonists on spontaneous discharge of chemosensitive units

	MDL 72222 ($100\,\mu g\,kg^{-1}$)		*ICS 205-930* ($100\,\mu g\,kg^{-1}$)		*GR 38032F* ($100\,\mu g\,kg^{-1}$)		*Ketanserin* ($100\,\mu g\,kg^{-1}$	
	n	% *reduction in discharge*	n	% *reduction in discharge*	n	% *reduction in discharge*	n	% *reduction in discharge*
Normals	4/4	53 (43–68)	3/3	62 (33–100)	4/4	53 (64–99)	5/9	63 (43–86)
Arthritic	1/4	100			4/4	36.5 (13–50)	6/9	58 (76–25)

Mean values are given – figures in parentheses show range of effect.
n = number of units.

Mechanoreceptor responsiveness

The 5-HT_3-receptor antagonists, MDL 72222, ICS 205-930 and GR 38032F, administered intra-arterially, each antagonized the 5-HT-induced sensitization of mechanoreceptors to mechanical stimuli in both normal and arthritic rats. In five units, treatment with MDL 72222 ($100\,\mu g\,kg^{-1}$) markedly reduced the increased responsiveness produced by 5-HT; injection of ICS 205-930 ($100\,\mu g\,kg^{-1}$) produced a clear rightward shift in the 5-HT dose-response curve in one unit. In studies on five units, GR 38032F ($100\,\mu g\,kg^{-1}$) abolished the response in one unit and produced a marked reduction in the response in the other four units (see Figure 4).

The 5-HT_2-receptor antagonist, ketanserin ($100\,\mu g\,kg^{-1}$), did not affect the 5-HT-evoked increase in mechanoreceptor responsiveness, either in normal or in arthritic rats when tested in six units.

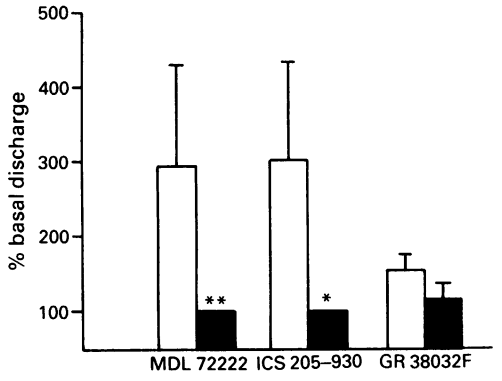

Figure 5 Effects of MDL 72222 ($100\,\mu g\,kg^{-1}$ i.a., $n = 6$), GR 38032F ($100\,\mu g\,kg^{-1}$ i.a., $n = 4$) and ICS 205-930) ($100\,\mu g\,kg^{-1}$ i.a., $n = 4$) on 5-hydroxytryptamine (5-HT)-induced fast excitation in chemosensitive units from both normal and arthritic rats. Each column shows the mean peak response as a percentage of basal discharge to a standard effective dose of 5-HT (5–$100\,\mu g$ i.a.) before (open) and after (solid) injection of antagonist. Bars represent s.e.mean. * $P < 0.05$ and ** $P < 0.01$.

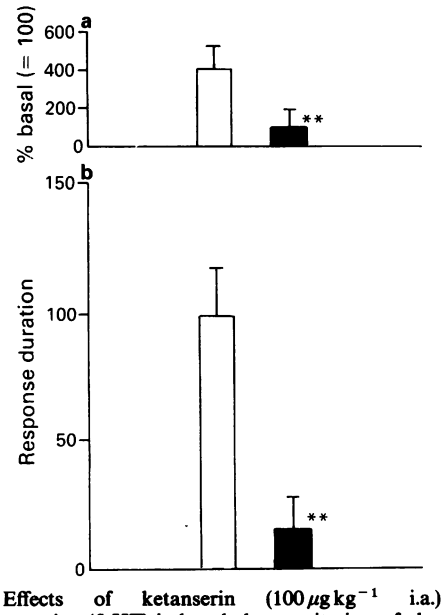

Figure 6 Effects of ketanserin ($100\,\mu g\,kg^{-1}$ i.a.) on 5-hydroxytryptamine (5-HT)-induced slow excitation of chemosensitive afferent units from normal and arthritic rats. (a) The mean peak discharge expressed as a percentage of preinjection control produced in response to a standard dose of 5-HT (1–$100\,\mu g$ i.a.) before (open column) and after (solid column) injection of ketanserin ($100\,\mu g\,kg^{-1}$, i.a., $n = 14$). (b) The mean duration of the effects shown in (a). Bars represent s.e.mean. ** $P < 0.01$.

Spontaneous activity of mechanosensitive units

In spontaneously active mechanosensitive units from arthritic rats, MDL 72222 ($100\,\mu g\,kg^{-1}$, $n = 1$) or GR 38032F ($100\,\mu g\,kg^{-1}$, $n = 2$) caused reductions in ongoing activity of 70% and 65% respectively when injected on their own. Reductions of activity lasted for no longer than 5 min in each case. In two mechanosensitive units, ketanserin ($100\,\mu g\,kg^{-1}$) had no effect on spontaneous activity.

An examination of the effects of the various 5-HT receptor antagonists on the fast and slow components of 5-HT-induced increases in spontaneous activity was complicated by the inconsistent nature of the fast response and its marked susceptibility to tachyphylaxis. The slow response, however, was consistently observed in all cases, and in arthritic animals neither MDL 72222 ($100\,\mu g\,kg^{-1}$, $n = 1$), ICS 205-903 ($100\,\mu g\,kg^{-1}$, $n = 1$) nor GR 38032F ($100\,\mu g\,kg^{-1}$, $n = 1$) had any affect on it. In only one out of two units did ketanserin cause a shift to the right of the 5-HT dose-response curve.

Chemosensitive units

In the case of spontaneously active chemosensitive units, MDL 7222, ICS 205-930 and GR 38032F all reduced ongoing discharge in arthritic rats as well as in normal animals previously exposed to 5-HT (see Table 2). Reductions in activity produced by antagonists lasted for 1–3 min. Ketanserin also markedly reduced ongoing discharge in arthritic and normal rats (Table 2).

Analysis of the effects of 5-HT-receptor antagonists on the fast response to injection of 5-HT were complicated by inconsistency of the response and its susceptibility to tachyphylaxis. However, it was markedly reduced or abolished following injections of the 5-HT_3-receptor antagonists MDL 72222 ($n = 6$), ICS 205-930 ($n = 4$) and GR 38032F ($n = 4$) (see Figure 5).

The slow response to injection of 5-HT was unaffected by the 5-HT_3-receptor antagonists, but in 14 out of 15 recordings was blocked or markedly reduced by ketanserin ($100\,\mu g\,kg^{-1}$) (see Figure 6).

Discussion

This investigation has shown that exogenous 5-HT can both excite and increase the responsiveness of sensory receptors

with fine (C, possibly some Aδ) afferents located in the ankle joint tissues of normal and arthritic rats. High threshold nociceptive mechanoreceptors were affected by 5-HT in both normal and arthritic animals, the effect of the amine on arthritic joints being more marked. The units recorded from arthritic joints had spontaneous activity, in contrast with the general lack of activity in units from normal joints. These findings are in good agreement with those obtained previously for the inflamed ankle joint (Guilbaud et al., 1985). Furthermore, background discharge originating from chemosensitive units for which no mechanosensitive receptive fields could be found was greater in arthritic rats. These units were activated by 5-HT or capsaicin and responded to 5-HT with two components – fast brief excitation followed by a slow prolonged increase in activity. The effects of exogenous 5-HT on joint sensory receptors are quire similar to those on cat carotid chemosensors, where fast and slow excitatory effects involving 5-HT_3- and 5-HT_2-receptors, respectively, have been obtained (Kirby & McQueen, 1984).

Action of 5-HT on mechanoreceptors

Single close-arterial bolus injections of 5-HT increased the responses of high threshold mechanoreceptors to a standard mechanical stimulus for as long as six minutes. This duration of action is similar to that obtained for 5-HT-induced sensitization of high threshold mechanoreceptors in muscle to excitation induced by bradykinin (Mense, 1981), as well as for the action of 5-HT on SAII cutaneous mechanoreceptors (Fjallbrant & Iggo, 1961). MDL 72222, ICS 205-930 or GR 38032F prevented this action, but did not otherwise affect the response to mechanical stimuli. Ketanserin was without effect. These results suggest that the sensitization demonstrated may involve the action of 5-HT at a 5-HT_3-receptor located on the mechanoreceptor terminals within the joint tissues.

Fast excitation

Brief excitation of mechanosensitive units and chemosensitive units occurred within 10s of the injection of 5-HT in both normal and arthritic joints. This action was blocked or reduced by the 5-HT_3-receptor antagonists. Fast depolarization evoked by 5-HT has been observed in several isolated neuronal preparations. For example, in cat and rabbit superior cervical ganglion (Haefely, 1974; Wallis & North, 1978), rabbit nodose ganglion (Higashi & Nishi, 1982) and guinea-pig coeliac ganglion (Wallis & Dun, 1988) 5-HT produced a rapid depolarization which was prone to tachyphylaxis and was sensitive to MDL 72222 or ICS 205-950 (Azami et al., 1985; Round & Wallis, 1986; 1987; Wallis & Dun, 1988).

Slow excitation

The most consistent response to 5-HT was a slow dose-dependent long-lasting increase in discharge that was seen in the majority of the chemosensitive units examined, as well as in mechanosensitive units from normal and arthritic rats. The 5-HT_3-receptor antagonists had no effect on this slow excitation, whereas in the case of chemosensitive units ketanserin reduced or abolished it. The delayed nature of this effect could mean that 5-HT is acting indirectly to increase afferent activity. In our preparation, slow excitation was dose-dependent and outlasted the hypotensive effect of 5-HT, thus making it unlikely to be secondary to changes in blood pressure. Alternative mechanisms could include the involvement of a second messenger system in the afferent nerve terminal or the release of other algogenic substances from surrounding tissues by 5-HT. Evidence for a direct effect is suggested from studies on isolated neuronal preparations where a slow response produced by 5-HT has also been described (Kiraly et al., 1983; Dun et al., 1984).

The finding that long-lasting mechanoreceptor sensitization involves a 5-HT_3-receptor, whereas delayed excitation does not, suggests that separate mechanisms may be involved in receptor sensitization and 5-HT-induced excitation. This may relate to differing transduction pathways for mechanically- or chemically-evoked activation of sensory nerve endings.

Involvement of 5-HT in sensitization of sensory receptors during inflammation

The ability of 5-HT to sensitize high threshold articular mechanoreceptors suggests that endogenous 5-HT could play a role in the increased responsiveness of these receptors in chronically inflamed joints. Our results indicate that a 5-HT_3-receptor may be involved in this process. However, in arthritic rats the administration of antagonists selective for 5-HT_3- and 5-HT_2-receptors did not reduce mechanoreceptor sensitivity significantly, which they should have done if endogenous 5-HT acting at these receptors was a significant cause of sensitization. Low levels of spontaneous activity in mechanosensitive units recorded from arthritic joints were, on the other hand, reduced markedly by the addition of 5-HT_3-receptor antagonists. Similar results were obtained for chemosensitive units following administration of both 5-HT_3- and 5-HT_2-receptor antagonists. These observations suggest that while endogenous 5-HT may contribute to ongoing neural activity seen in inflamed joints, it is not a major factor in the sensitization of afferents.

In mechanoreceptors recorded from arthritic joints, with already enhanced mechanosensitivity, responsiveness to 5-HT was much greater than in normal joints, providing clear evidence that sensitivity of these sensors to 5-HT is increased in inflamed joints, and showing that the acute release of endogenous 5-HT could further boost sensitivity. The induction of high threshold mechanoreceptor sensitization and fast excitation by 5-HT, via a 5-HT_3-receptor, is consistent with the observation that pain produced by application to 5-HT to a blister base is mediated through a 5-HT_3-receptor (Donatsch et al., 1984; Richardson et al., 1985), assuming that the discharge recorded from the ankle joint afferents is involved in nociception. A role for 5-HT_2-receptors in 5-HT-induced pain has not previously been described, and further studies may be warranted in view of our results showing that 5-HT_2- and 5-HT_3-antagonists reduced afferent discharge in arthritic rats.

Finally, a role for 5-HT in the development of acute inflammatory pain has been suggested by Eschalier et al. (1989), who have shown that the administration of ICS 205-930 inhibits and reverses carrageenan-induced hyperalgesia in rats. It may be that endogenous 5-HT, released from platelets (Page, 1988), mast cells (Johnson & Erdos, 1973) or nerve fibres (Williams, 1967; Verhofstad et al., 1981) is responsible for development of sensitization during the acute inflammatory response and become less important for chronic sensitization. However, further (acute) release of 5-HT may cause additional short-lasting sensitization of sensory receptors in chronic arthritis.

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