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Contribution of peripheral α_{1A}-adrenoceptors to pain induced by formalin or by α-methyl-5-hydroxytryptamine plus noradrenaline

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Abstract

We examined the peripheral adrenergic mechanisms involved in pain induced by α-methyl-5-hydroxytryptamine (α-methyl-5-HT) plus (±)-noradrenaline or prostaglandin E_2 and by intraplantar formalin. Agents were injected s.c. into the plantar surface of rats' paws, and the paw lifting and licking response scored. Pain produced by α-methyl-5-HT (10 μg) plus noradrenaline (10 μg) was blocked by pretreatment with the α-adrenoceptor antagonists, phentolamine (10 μg) and prazocin HCl (α_1; 40 μg), but not by timolol (β; 10 μg) or idazoxan (α_2; 40 μg). Phenylepherine, but not clonidine, substituted for noradrenaline to induce pain when combined with α-methyl-5-HT. The α_{1A}-adrenoceptor antagonist, WB-4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane HCl), but not the α_{1B}-adrenoceptor antagonist, chloroethylclonidine, also blocked the pain response produced by α-methyl-5-HT plus noradrenaline. Neither of these agents altered pain produced by α-methyl-5-HT plus prostaglandin E_2 (0.1 μg). Formalin-induced pain (1%, 50 μl) was biphasic, and timolol increased the first phase response. The second phase was attenuated by 40% by phentolamine (10 μg) injected 10 min before formalin or at the beginning of the second phase; 30 μg did not produce a larger effect. Prazosin and WP-4101, but not idazoxan or chloroethylclonidine, also attenuated the second phase. Thus, activation of α_{1A}-adrenoceptors can contribute to pain, but pain induced by α-methyl-5-HT plus prostaglandin E_2 is independent of adrenergic function, indicating that adrenergic function is not necessary for induction of pain by inflammatory mediators. α_{1A}-Adrenoceptor blockade attenuates pain when administered after development of pain, implying that peripheral adrenergic mechanisms contribute to ongoing maintenance of pain.

Keywords: Pain; Inflammation; Adrenoceptor agonist; Adrenoceptor antagonist; Formalin test

1. Introduction

The formalin test is an animal model of pain associated with tissue injury in which the behavioural response to an injection of dilute formalin into the paw of a rat is quantified (Tjølsen et al., 1992; Abbott et al., 1995). Recently, we explored the ability of a series of inflammatory mediators to induce a behavioural response similar to that produced by formalin when injected into the plantar surface of rats' paws. 5-Hydroxytryptamine (5-HT), prostaglandin E_2 and bradykinin produced transient paw elevation and paw licking at high doses, while substance P, histamine and noradrenaline were inactive. Combination of any one of these agents, including noradrenaline, with low doses of 5-HT produced a synergistic increase in the pain response (Hong and Abbott, 1994). This action of 5-HT is mediated by activation of the 5-HT_{2A} receptor subtype, and local injection of 5-HT_{2A} receptor antagonists produce analgesia in the formalin test (Abbott et al., 1996a). The data suggest that 5-HT, probably released during platelet degranulation, plays a critical role in pain-associated injury and inflammation.

The finding that noradrenaline plus a 5-HT_2 receptor agonist is sufficient to induce a behavioural pain response is consistent with a considerable body of literature implicating noradrenaline in pain. Activation of α_2-adrenoceptors in the central nervous system produces analgesia and/or antinociception in a number of animal models of pain (Petrovaara, 1993) including the formalin test (Mastrianni et al., 1989). There is also evidence that these mechanisms may produce analgesia or hypoalgesia in humans (Kunos et al., 1987; Petrovaara, 1993). However, in the periphery, adrenergic mechanisms appear to be largely pronociceptive, although there are some conflicting re-

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ports. Sympatholytic treatments have been shown to produce relief of pain and/or hyperalgesia in a number of animal models of pain (Levine et al., 1986; Coderre et al., 1984a; Coderre et al., 1984b; Kinnman and Levine, 1995b). In humans, the most prominent effects of antisympathtic treatments are observed in chronic pain states involving hyperpathia and exacerbation by stress (Raja et al., 1991; McMahon, 1991; Ghostine et al., 1984; Hannington-Kiff, 1974; Treede et al., 1992). However, administration of noradrenaline into the normal tissue does not produce significant pain or mechanical hyperalgesia. Instead, local administration of adrenoceptor agonists produces pain or mechanical hyperalgesia where there is a preexisting inflammation or injury, including long-standing nerve injury (Abbott et al., 1996a; Levine et al., 1986; Davis et al., 1991). Increases in the pain index with α_1-adrenoceptor agonists and/or decreases with α_1-adrenoceptor antagonists consistently suggest that activation of α_1-adrenoceptors is pronociceptive (Davis et al., 1991; Ghostine et al., 1984; Chabal et al., 1992; Kinnman and Levine, 1995a). The data for α_2-adrenoceptor involvement in pain in the periphery are mixed: in various clinical conditions and animal pain models, there are a number of studies reporting decreases (Davis et al., 1991; Nakamura and Ferreira, 1988; Sato and Perl, 1991) as well as increases (Khasar et al., 1995; Levine et al., 1986) in the index of pain and/or nociception following local application of the α_2-adrenoceptor agonist, clonidine. β-Adrenoceptor agonists inhibit bradykinin-induced extravasation (Coderre et al., 1991), and thus may also alter pain secondary to attenuating inflammation.

The present experiments explored the adrenoceptor subtype mediating the synergistic pain response produced by intraplantar injection of the combination of a 5-HT receptor agonist plus noradrenaline. Because the serotonergic component of this phenomenon is mediated by 5-HT_{2A} receptors (Abbott et al., 1996a), the 5-HT_2 receptor agonist, α-methyl-5-HT was used instead of 5-HT itself to induce pain. We also examined the role of adrenoceptor subtypes in pain induced by formalin. A preliminary report has been presented (Hong and Abbott, 1995a).

2. Materials and methods

2.1. Subjects and behavioural testing

Male Long-Evans rats weighing 180–240 g, were obtained from Charles River, Quebec (Canada). Rats were housed in groups in shoe box cages in the colony room with food and water available ad libitum. A 12:12 h light-dark cycle with lights on at 7:00 h was maintained and testing was done between 9:00 and 17:00 h. Rats were acclimatized to the laboratory and habituated to the test boxes for 1 h per day for 5 days prior to testing, and for a minimum of 30 min immediately prior to administration of the agents to minimize activation of stress-mediated pain suppression mechanisms (Franklin and Abbott, 1989; Terman et al., 1984; Devor, 1984) which can modulate the behavioural response in the formalin test (Abbott et al., 1986; Harris and Westbrook, 1994). Rats were tested once on each hind paw. After testing on the first paw, they were randomly reassigned to another condition, given a 2 day rest period and then 5 further days of habituation.

Behavioural testing took place in clear plastic chambers ($32 \times 32 \times 30$ cm) with a mirror placed at a 45° angle beneath the floor to allow an unobstructed view of the paws. The test agents were injected subcutaneously into the plantar surface of the hind paw using a 30 g 1/2 needle connected to a microsyringe with PE-10 tubing. Pain was induced by either 20 μl of α-methyl-5-HT plus noradrenaline or prostaglandin E_2, or by 50 μl 1% formalin. The behavioural response was rated using the following criteria: *lifting* – the injected paw is elevated and not in contact with the floor; *licking* – the injected paw is licked or bitten. The time spent on the lifting and licking was recorded for 30 min after α-methyl-5-HT plus another inflammatory mediator, or for 40 min after formalin, using a computer program that allowed simultaneous rating of two rats. The response to formalin is biphasic. The initial response lasts 5–10 min, and is followed a period of normal behaviour, and reemergence of the pain response at 15–20 min, after a period of normal exploratory behaviour. For the formalin concentration used here, using well habituated rats, pain behaviours are observed for a *total* of 1–3 min during the first phase (from 0 to 10 min after injection), and for 10–12 min during the second phase (11–40 min). Thus, pain behaviours were below asymptotic levels that preclude observing exacerbation of the pain response. The pain response produced by α-methyl-5-HT plus noradrenaline or prostaglandin E_2 was similar to the second phase of the formalin response. Adrenoceptor antagonists (20 μl) were injected 10 min before the algogens except in one group of experiments, where they were injected 15 min after formalin.

2.2. Agents

Agents used in this study were: α-methyl-5-hydroxytryptamine maleate (α-methyl-5-HT), clonidine HCl, phentolamine mesylate, timolol maleate, prazosin HCl, idazoxan HCl, 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane HCl (WB-4101), chloroethylclonidine dihydrochloride (Research Biochemical Int., Natick, MA), *l*-phenylephrine HCl, [±]-noradrenaline bitartrate and prostaglandin E2 (Sigma, St. Louis, MO). Stock solutions were made as follows: prostaglandin E_2 was dissolved in 10% ethanol in saline (10 mg/ml); prazosin in 20% methanol in saline. Physiological saline was used to dilute the stock solutions and to dissolve all other agents. One percent formalin was prepared by mixing one part satu-

rated solution of formaldehyde (38%; Fischer Chemicals) with 99 parts normal saline.

2.3. Data analysis

The number of minutes lifting and licking the injected paw was summed over 30 min after injection of α-methyl-5-HT plus prostaglandin E_2, or for 0–10 and 11–40 min after injection of formalin, and used as the index of pain. Together, these behaviours explain more than 60% of the variance in the behavioural response when formalin concentration is varied, or when centrally acting analgesic agents are administered. Adding other behaviours does not increase the proportion of variance explained (Abbott et al., 1995). Data for the first 10 and the last 30 min of the formalin test period were analysed separately. There were six to eight rats per group. Analysis of variance followed by Turkey's protected *t*-test was used to determine the reliability of differences between drug and control groups.

3. Results

3.1. Effects of adrenoceptor antagonists on pain induced by α-methyl-5-HT plus noradrenaline or prostaglandin E_2

Fig. 1, shows the pain response produced by 10 μg α-methyl-5-HT plus 10 μg noradrenaline or 0.1 μg prostaglandin E_2. As we have reported previously (Hong and Abbott, 1994; Abbott et al., 1995), the lifting and licking response to these combinations builds over 8–10 min and then wanes. The time course and magnitude of the response to the doses used is similar to the second phase of the behavioural response to 1% formalin (Abbott et al., 1995; see below). Injection of the non-specific α-adrenoceptor antagonist, phentolamine (10 μg), markedly attenuated the response to α-methyl-5-HT plus noradrenaline (3.09 ± 0.39 vs. 8.63 ± 0.53 min; $P < 0.01$), while 10 μg timolol, a non-specific β-adrenoceptor antagonist had no effect. For the combination of α-methyl-5-HT and prostaglandin E_2, neither of the adrenoceptor antagonists altered the pain response. These data indicate that activation of peripheral α-adrenoceptors is implicated in pain when noradrenaline is used to evoke pain, but that peripheral adrenoceptors are unlikely to be involved in pain when prostaglandin E_2 was used.

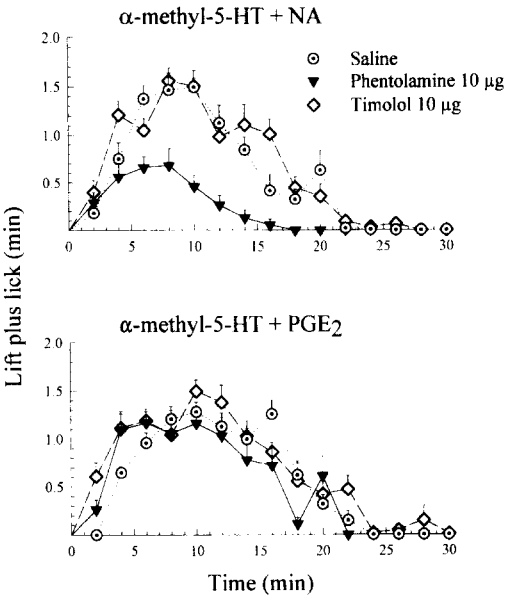

Fig. 1. Time course of the lifting and licking response evoked by α-methyl-5-HT plus noradrenaline (NA; upper panel) or prostaglindin E_2 (PGE_2; lower panel) after local pretreatment with phentolamine, timolol or the saline vehicle.

3.2. Effects of α-adrenoceptor antagonists on pain induced by combinations of α-methyl-5-HT plus noradrenaline

The specific α_1- and α_2-adrenoceptor antagonists, prazosin and idazoxan, respectively, were used to explore the subtype of α-adrenoceptors involved in the pain produced by α-methyl-5-HT plus noradrenaline. As Table 1 indicates, idazoxan (40 μg), injected 10 min before the algogens did not alter the lifting and licking response. To examine the effects of prazosin, it was necessary to compare the data with controls treated with the vehicle, 20% methanol. Pretreatment with 20% methanol significantly increased the response produced by α-methyl-5-HT plus noradrenaline. Pretreatment with 40 μg prazosin attenuated the response, such that it was not different from that produced by α-methyl-5-HT alone preceded by 20% methanol. Thus, prazosin completely blocked the adrenergic component of the potentiated pain response produced by the combination of α-methyl-5-HT plus noradrenaline, strongly implicating α_1-adrenoceptors in the pain response.

Table 1
Time spent lifting and licking injected paw after pretreatment with α-adrenoceptor antagonists or their respective vehicle solutions

Pretreatment	Treatment	Lifting and licking (min) ± S.E.
Saline	α-Methyl-5-HT + noradrenaline	8.02 ± 0.38
Idazoxan (α_2)	α-Methyl-5-HT + noradrenaline	8.08 ± 0.50
20% methanol in saline	α-Methyl-5-HT + noradrenaline	11.52 ± 1.71 [a]
Prazosin (α_1)	α-Methyl-5-HT + noradrenaline	3.24 ± 0.41 [b,c]
20% methanol in saline	α-Methyl-5-HT	4.02 ± 0.62 [b,c]

[a] $P < 0.05$ (one-tailed) compared to saline group; [b] $P < 0.01$ compared to saline group; [c] not different from each other.

Table 2
Time spent lifting and licking injected paw in response to treatment with saline or α-methyl-5-HT plus the indicated adrenoceptor agonist or the saline vehicle

Adrenoceptor agonist	Injected with	Lifting and licking (min) ± S.E.
Noradrenaline	α-Methyl-5-HT	10.12 ± 0.54 [a,b]
Phenylepherine (α_1)	α-Methyl-5-HT	8.58 ± 2.77 [a,b]
Clonidine (α_2)	α-Methyl-5-HT	2.23 ± 0.74 [a]
Saline	α-Methyl-5-HT	4.16 ± 0.61
Phenylepherine	Saline	0.23 ± 0.12 [a]
Clonidine	Saline	0 [a]

[a] $P < 0.01$ compared to saline group; [b] not different from each other.

3.3. Responses to combinations of α-methyl-5-HT with α-adrenoceptor agonists

To confirm the role of α_1-adrenoceptors, the effects of combining α-methyl-5-HT with specific α-adrenoceptor agonists were tested. As indicated in Table 2, the combination of α-methyl-5-HT plus noradrenaline induced about 10 min of lifting and licking behaviour. Injection of α-methyl-5-HT with phenylepherine, an α_1-adrenoceptor agonist, produced a similar response. The effect of α-methyl-5-HT plus clonidine, an α_2-adrenoceptor agonist, was lower than that produced by α-methyl-5-HT alone ($P < 0.05$). Phenylepherine by itself produced a transient response that was only marginally greater than 0 ($0.1 > P > 0.05$), consistent with the very brief pain observed in human subjects in normal tissue (Davis et al., 1991), and clonidine was inactive.

3.4. Effects of specific α_1-adrenoceptor antagonists on pain induced by α-methyl-5-HT plus noradrenaline

WB 4101 and chloroethylclonidine were used to distinguish between the α_{1A}- and α_{1B}-adrenoceptors, respectively (Testa et al., 1993; Minneman, 1988). They were administered 10 min before α-methyl-5-HT plus noradrenaline. As indicated in Table 3, the α_{1A}-adrenoceptor antagonist, WB 4101, 10 μg, produced a complete blockade of the augmented pain response. Chloroethylclonidine did not alter the response.

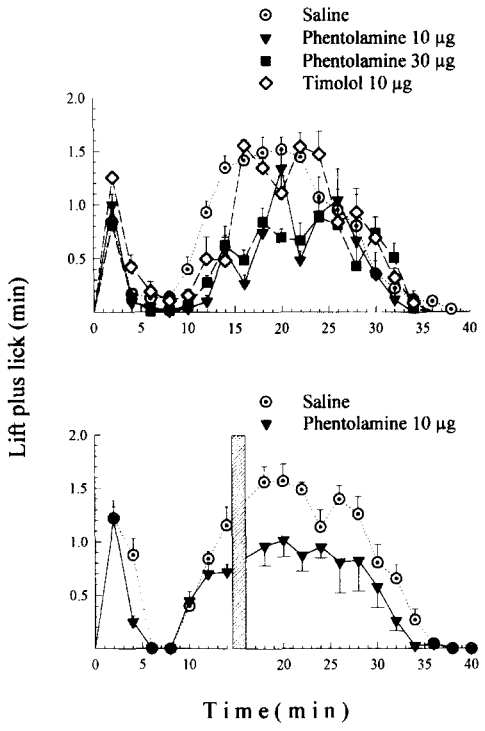

Fig. 2. Time course of the lifting and licking response evoked by 1% formalin. The upper panel shows the effects of phentolamine, timolol or the saline vehicle injected into the paw 10 min before the adrenoceptor antagonists. The lower panel shows the effects injecting phentolamine into the paw 15 min after formalin. The vertical bar indicates the 2 min time out period when injections were made.

3.5. Effects of adrenoceptor antagonists on pain induced by formalin

Fig. 2, upper panel, shows the effects of α- and β-adrenoceptor antagonists, phentolamine and timolol, respectively, injected 10 min before 1% formalin. The control group, which received an injection of normal saline into the paw 10 min before formalin, showed a typical biphasic response, and for the concentration of formalin used, the response during the peak of the second phase remained below the ceiling of continuous pain behaviour indicating sensitivity to increases in pain. Injection of the β-adrenoceptor antagonist, timolol, 10 min before formalin produced a 64% increase in the first phase response ($P <$

Table 3
Minutes of lifting and licking induced by α-methyl-5-HT plus noradrenaline or its vehicle after pretreatment with α_{1A}- and α_{1B}-adrenoceptor antagonists or their vehicles

Pretreatment	Treatment	Lifting and licking (min) ± S.E.
Saline	α-Methyl-5-HT + noradrenaline	7.45 ± 0.75 [c]
WB 4101 (α_{1A})	α-Methyl-5-HT + noradrenaline	3.85 ± 0.60 [a,b]
Chloroethylclonidine (α_{1B})	α-Methyl-5-HT + noradrenaline	8.69 ± 1.20 [c]
Saline	α-Methyl-5-HT	4.06 ± 0.61 [a,b]

[a] $P < 0.01$ compared to saline group; [b,c] not different from each other.

Table 4
The second phase lifting and licking response produced by 1% formalin after injection of α-adrenergic antagonists or their respective vehicles a the beginning of the second phase

α-Antagonist	Lifting and licking (min) ± S.E.
Saline	8.67 ± 0.97 [b]
Idazoxan (α_2)	7.75 ± 0.05 [b]
20% methanol	8.75 ± 0.86 [b]
Prazosin (α_1)	5.43 ± 0.67 [a]
Saline	9.40 ± 1.09 [b]
WB 4101 (α_{1A})	6.32 ± 0.51 [a]
Chloroethylclonidine (α_{1B})	9.42 ± 0.70 [b]

[a] $P < 0.01$ compared to the respective control group; [b] not different from each other.

0.01), but had no effect on the second phase. Pretreatment with 10 μg phentolamine had no effect on the first phase of the formalin response, but reduced the second phase by 44%. Increasing the dose of phentolamine to 30 μg did not produce a further decrement in the response (41% decrease). The lower panel of Fig. 2 shows the effects of injecting 10 μg phentolamine 15 min after formalin, at a time when the second phase of the formalin response is rising. Compared to the group that received an injection of saline at this time, phentolamine decreased the response by 38%, very similar to the reduction when phentolamine was administered before formalin.

3.6. Effects of specific α-adrenoceptor antagonists on the second phase of the response to formalin

Because the effects of phentolamine on formalin-induced pain were similar regardless of whether it was injected before or after the beginning of the second phase the pain response to formalin response, specific α-adrenoceptor antagonists were injected at the beginning of the second phase. As indicated in Table 4, the α_2-adrenoceptor antagonist, idazoxan, did not alter the second phase response induced by formalin. The 20% methanol vehicle used for the α_1-adrenoceptor antagonist, prazosin, injected after the beginning of the second phase did not alter the second phase pain response. This is in contrast to its effects when injected 10 min before α-methyl-5-HT plus noradrenaline (Table 1). We have previously found that an ethanol vehicle does increase the second phase of the formalin response when injected 10 min prior to formalin (Abbott et al., 1996a), suggesting that the irritant effects are most prominent 20–30 min after injection, and that any initial stinging may have been masked by the presence of formalin pain. Prazosin produced a 38% decrease relative to the vehicle group. The α_{1A}-adrenoceptor antagonist, WB 4101, produced a decrease in the response similar to that of prazosin (33%). Chloroethylclonidine, an α_{1B}-adrenoceptor antagonist, was inactive on the second phase of the formalin test.

4. Discussion

The present data replicate our finding that intraplantar injection of the 5-HT_2 receptor agonist, α-methyl-5-HT, combined with either noradrenaline or prostaglandin E_2, induces a behavioural pain response similar to the second phase of the response to formalin. Local pretreatment with α- and β-adrenoceptor antagonists did not alter the pain response produced by α-methyl-5-HT plus prostaglandin E_2, indicating that this response is independent of adrenergic mechanisms. The pain response produced by α-methyl-5-HT plus noradrenaline, on the other hand, was blocked completely by pretreatment with α_{1A}-adrenoceptor antagonists, and these agents also partially blocked the second phase of the response to formalin. Thus, although α-methyl-5-HT synergizes with both prostaglandin E_2 and noradrenaline, only in the latter does activation of adrenoceptors contribute to the pain response.

Before taking up the issues that arise from these findings, the question of the doses of agents used needs to be addressed. Except in the case of phentolamine in formalin-induced pain, where the effect was small and a larger dose was tested to determine if a larger effect could be produced, all agents were tested at single doses. This raises the question of whether the agents that produced negative results, timolol, clonidine, chloroethylclonidine and idazoxan, were used at high enough doses. Doses were determined on the basis of the following studies, all of which reported a positive effect of the agent in question. Intradermal injection of clonidine has been shown to attenuate mechanical hyperalgesia produced by noradrenaline plus a calcium ionophore maximally at a dose of 10 ng (Khasar et al., 1995). In the present case, injections were subcutaneous, so that more spread of the agent would be expected. The dose used, 10 μg, is 3 orders of magnitude higher, and as high as one can go without producing systemic antinociceptive effects (Tchakarov et al., 1985; Green et al., 1993a). Timolol is used to treat glaucoma, and the dose used here, 10 μg, is the same as that instilled into the human eye (Vuori and Kaila, 1995). Chloroethylclonidine has been injected into the rat kidney by close arterial injection at a dose of 5 μg/kg (Abdul Sattar and Johns, 1994), a dose substantially lower than the 10 μg dose used in the present study. Idazoxan has been administered intravenously at 400 μg/kg to the rat (Thomas, 1995), and if it is assumed that the rats weigh 250 g, the dose is only 10-fold higher the dose used here. On the basis of these data, it is unlikely that negative effects were due to the dose being too low.

In the formalin test, the non-specific β-adrenoceptor antagonist, timolol produced an increase in the first phase of pain response. β-Adrenoceptor blockade has been re-

ported to increase sympathetically mediated extravasation (Coderre et al., 1991), so that the effect observed here may relate to an increase in the immediate inflammatory reaction to formalin. However, must be remembered that extravasation and behavioural pain induced by formalin are not necessarily correlated (Hong and Abbott, 1995b; Coderre et al., 1984b). The data support extensive evidence that the first and second phases of the formalin test reflect different physiological processes (Tjølsen et al., 1992; Porro and Cavazzuti, 1993).

The second phase response, which is thought to be a model of acute pain associated with tissue injury and inflammation (Tjølsen et al., 1992; Franklin and Abbott, 1989), was attenuated by a maximum of about 50% by α_{1A}-adrenoceptor antagonists, regardless of whether the antagonist was injected before formalin, or at the beginning of the second phase. These data raise several issues. The first concerns the fact that α_{1A}-adrenoceptor antagonists attenuated the second phase of the formalin pain response to the same degree regardless of the time of injection. The present data are consistent with earlier studies indicating that chemical sympathectomy attenuates formalin-induced pain (Coderre et al., 1984a, 1984b), and the implication is that noradrenaline, released from peripheral sympathetic terminals, plays a role in formalin pain. The data indicate, furthermore, that the adrenergic contribution to pain injury-related pain is ongoing, and similar to the contribution that peripheral opioid mechanisms make to pain (Hong and Abbott, 1995b). In contrast, local treatment with 5-HT_{2A} receptor antagonists after the beginning of the pain response resulted in considerable loss of efficacy (Abbott et al., 1996b), but even this loss of efficacy was not correlated with the amount of pain that preceded drug administration, as would be expected if spinal sensitization was involved. Thus, there is no evidence in the present study to support the notion that pain produces sensitization in spinal pain transmission neurons so that pain becomes resistant to peripheral treatments (cf. (Coderre, 1993)). To the extent that it is possible to take advantage of adrenergic blockade to produce clinical analgesia, the effects would be expected to be immediate, regardless of whether administered before or after the beginning of pain. This is consistent with the immediate effects of sympathetic blockade in sympathetically maintained chronic pain syndromes (Ghostine et al., 1984; Raja et al., 1991).

The adrenergic contribution to formalin pain was blocked by the same α_{1A}-adrenoceptor antagonist as was pain induced by α-methyl-5-HT plus noradrenaline, suggesting that this combination mimics one component of the second phase of the formalin response. However, the response to α-methyl-5-HT plus noradrenaline was almost completely blocked by α_{1A}-adrenoceptor blockade. In the case of the formalin test, increasing the dose of phentolamine by a factor of 3 did not produce more analgesia than the lower dose. Clearly, formalin-induced pain involves other mechanisms in addition to the adrenergic component, one of which may be release of prostaglandin E_2 (Hunskaar and Hole, 1987). The present data are inconsistent with finding that systemic injection of α_1-adrenoceptor agonists, that would be expected to act peripherally, attenuates formalin-induced pain (Tasker et al., 1992). The most likely reason for the inconsistency is that systemic injection of α-adrenoceptor agonists would be expected to produce a pressor response that would activate brainstem antinociceptive mechanisms (Randich and Gebhart, 1992).

The involvement of α_{1A}-adrenoceptors in the behavioural pain response produced both by α-methyl-5-HT plus noradrenaline and by formalin is different from the adrenergic mechanisms involved in extravasation and in hyperalgesia. Injection of 6-hydroxydopamine (noradrenaline-releasing agent) or of bradykinin produces extravasation of plasma proteins that is dependent on an intact sympathetic nervous system (Green et al., 1993b; Bjerknes et al., 1991). In the case of bradykinin-induced extravasation, co-injection of the α_2-adrenoceptor antagonist, yohimbine, or of the β_2-adrenoceptor agonist, salbutamol, attenuates extravasation, while the α_2-adrenoceptor agonist, clonidine, or the β_2-adrenoceptor antagonist, ICI-118,551, exacerbates it (Coderre et al., 1991). Because the adrenergic modulation of extravasation is abolished by sympathectomy, Coderre et al. (1991) interpret their findings as indicating that catecholamines have acute pro- and anti-inflammatory actions through presynaptic activation of α_2- and β_2-adrenoceptors, respectively. In the present studies the non-specific β-adrenoceptor antagonist, timolol, had no effect on the pain response to α-methyl-5-HT plus noradrenaline or prostaglandin E_2, but it did increase the first phase of the response to formalin. However, as noted above, there is no reason to expect a perfect correlation between extravasation and pain.

There are three different sets of findings regarding the adrenoceptor subtype involved in mechanical hyperalgesia in inflamed tissue. In tissue inflamed by repeated application of chloroform, intradermal injection of noradrenaline produces hyperalgesia that is attenuated by yohimbine, implying that blockade of α_2-adrenoceptors inhibits a pro-inflammatory or algogenic action of noradrenaline (Levine et al., 1986). In the same model an antinociceptive effect of clonidine was observed when noradrenaline was applied with a calcium ionophore instead of inducing inflammation with chloroform (Khasar et al., 1995). Clonidine also had a peripheral analgesic action in inflammation induced by carrageenin or prostaglandin E_2 (Nakamura and Ferreira, 1988). In the present study, there was no evidence for involvement of peripheral α_2-adrenoceptors except a marginal *decrease* in the response to α-methyl-5-HT alone. Thus, there is as best a minor role for α_2-adrenoceptors that is opposite to that observed in the chloroform/noradrenaline hyperalgesia model of hyperalgesia, and consistent with the prostaglandin E_2/carrageenin model. In a third model, in which hyperalgesia is assessed

at a distance from the site of a capsaicin injection (i.e., 'secondary hyperalgesia'), local injection of the α_1-adrenoceptor antagonist, prazosin, attenuated the response (Kinnman and Levine, 1995a). These data are consistent with the adrenergic mechanism observed in the present study.

Despite the inconsistencies between the present data and studies using extravasation or hyperalgesia, the finding of a major role for α_{1A}-adrenoceptors in the present study are generally consistent with the role that catecholamines play in sympathetically maintained chronic pain (Hannington-Kiff, 1974; Loh and Nathan, 1978; McMahon, 1991). The non-specific adrenoceptor antagonist, phentolamine (Raja et al., 1991), and the α_1-adrenoceptor antagonist, phenoxybenzamine (Ghostine et al., 1984), both produce analgesia, and the β-adrenoceptor antagonist, propranolol, was ineffective in a well controlled study (Scadding et al., 1982). A characteristic feature of pain syndromes that are relieved by α-adrenoceptor antagonists and sympatholitic treatments is that the pain is frequently exacerbated by stress (Steranka et al., 1988). One apparently inconsistent study found that application of a clonidine patch to the affected region in patients with sympathetically maintained pain relieved ongoing pain and hyperalgesia (Davis et al., 1991). However, injection of the α_1-adrenoceptor agonist, phenylepherine evoked intense pain at the patch site, indicating that activation of α_1-adrenoceptors played a role in pain, and the effect of high local concentrations of clonidine may have been to inhibit release of noradrenaline. In addition, the clonidine patches used in this study may have produced effects mediated in the central nervous as a consequence of systemic absorption. In the present experiments, exogenous noradrenaline was applied in the case of pain induced by α-methyl-5-HT plus noradrenaline, so that an agent that inhibits release would not be expected to have effects. In formalin-induced pain, idazoxan might have been expected to increase the pain response if α_2-adrenoceptors are involved. However, formalin produces an acute injury, and it is possible that the control over noradrenaline release is different from that in chronic pathological states that develops in sympathetically maintained pain.

Electrophysiological recordings of activity in afferent nerves are inconsistent with the present data. Noradrenaline has virtually no effect on nociceptive afferents in normal rats. However, after induction of arthritis (Sato et al., 1993) or damage to the peripheral nerve (Sato and Perl, 1991), application noradrenaline or stimulation of the sympathetic chain activated unmyelinated neurons. This action of noradrenaline was blocked by the α_2-adrenoceptor antagonist, yohimbine. In another study, sympathectomy prior to harvesting 'skin-nerve' tissue for in vitro electrophysiology did not alter the sensitization produced by application of bradykinin (Koltzenberg et al., 1992). These data suggest that nerve damage-induced responsiveness to noradrenaline is different from sympathetically maintained pain.

In summary, the present study shows that activation of α_{1A}-adrenoceptors contributes to pain induced by α-methyl-5-HT plus noradrenaline and by formalin. This action of noradrenaline is distinct from the role played by adrenergic mechanisms in extravasation of plasma proteins, hyperalgesia in inflamed tissue and electrophysiological studies in damaged tissues. It is, however, consistent with the data implicating α_1-adrenergic mechanisms in chronic sympathetically maintained pain. The data emphasize the differences among various animal models of pain and inflammation, and the need to establish the clinical significance of pain-related phenomena in animals. The behaviours scored in the present study, paw lifting and licking, are log-linearly related to formalin concentration and, holding formalin concentration constant, to morphine or amphetamine dose (Abbott et al., 1995). These behaviours are also critically dependent on activation of 5-HT_{2A} receptors, regardless of whether pain is produced by formalin or by combinations of inflammatory mediators (Hong and Abbott, 1994; Abbott et al., 1996a), whereas hyperalgesia is mediated by the 5-HT_{1A} receptor subtype (Taiwo and Levine, 1992). We have suggested (Abbott et al., 1996a) that they reflect pain generated through activation of nociceptive afferent elements by inflammatory mediators ('it hurts'), and that these mechanisms are different from those that are involved in sensitization of nociceptors to mechanical stimuli ('it hurts when prodded').

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