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Influence of selective α_2-adrenergic agents on mustard oil-induced central hyperalgesia in rats

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Abstract

The effects of systemically administered medetomidine, an α_2-adrenoceptor agonist, and atipamezole, an α_2-adrenoceptor antagonist, on mustard oil-induced central hyperalgesia were determined in unanesthetized rats. The mechanical threshold for eliciting a hindlimb flexion reflex (a nocifensive response) was determined with a series of calibrated monofilaments. Under control conditions mustard oil produced a significant decrease of the hindlimb withdrawal threshold for mechanical stimuli applied to a distal site in the hindlimb, whereas the corresponding threshold in the (untreated) contralateral side was not changed. Medetomidine administered 12 min prior to mustard oil treatment produced a significant dose-dependent (3–30 μg/kg s.c.) attenuation of the mustard oil-induced threshold decrease whereas the withdrawal threshold of the contralateral (untreated) hindlimb was not changed at these low doses. The antinociceptive effect of medetomidine (10 μg/kg) administered 12 min prior to the mustard oil treatment was not significantly stronger than the effect of medetomidine administered immediately after the mustard oil treatment. Atipamezole at a high (1000 μg/kg) or a low (10 μg/kg) dose did not influence the mustard oil-induced threshold decrease, whereas at an intermediate dose (100 μg/kg) atipamezole alone had a significant antinociceptive effect on mustard oil-induced hyperalgesia. The results indicate that medetomidine produces a selective attenuation of central hyperalgesia at doses which are sub-antinociceptive in intact rats. A pre-emptive treatment with medetomidine did not produce stronger antinociception than medetomidine treatment after the development of hyperalgesia. An α_2-adrenoceptor antagonist, atipamezole, attenuated central hyperalgesia in a non-monotonic fashion.

Keywords: Neuropathy; Hyperalgesia; Medetomidine; Atipamezole; Mustard oil

1. Introduction

Topical application of capsaicin or mustard oil to the skin of humans or animals produces a selective tonic activation of nociceptive fibers (Reeh et al., 1986; Szolcsanyi et al., 1988). This is accompanied by spontaneous pain and hyperalgesia in response to mechanical and thermal stimuli in the treated region. These phenomena can be explained by a change in the response properties of the primary afferent nociceptors (= primary or peripheral hyperalgesia; Culp et al., 1989; LaMotte et al., 1992). Additionally, stimulation of the skin adjacent to the treated region produces unpleasant sensations and nocifensive reflex responses to low intensity mechanical and electrical stimuli (Grönroos and Pertovaara, 1993; Koltzenburg et al., 1992). Since outside the treated skin area the response properties of primary afferent fibers are not changed (Torebjörk et al., 1992), the unpleasant and nocifensive responses elicited by non-noxious stimuli can be explained by central mechanisms (= central/secondary hyperalgesia/allodynia; Woolf and Wall, 1986). Thus, application of capsaicin or mustard oil to the skin and the determination of sensory or nocifensive responses to mechanical stimulation surrounding the treated area provide the means to study quantitatively central hyperalgesia in humans and animals.

In the current investigation we studied the effects of a selective α_2-adrenoceptor agonist, medetomidine (Virtanen et al., 1988), and an α_2-adrenoceptor antagonist, atipamezole (Scheinin et al., 1988), on the cen-

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trally induced facilitation of a nocifensive limb flexion reflex induced by mechanical stimulation of the skin in awake rats. We especially wished to determine if central hyperalgesia can be attenuated by a systemically administered α_2-adrenoceptor agonist at low doses which do not yet influence the nocifensive response in an intact limb. Secondly, we addressed the question of whether pre-emptive administration of an α_2-adrenoceptor agonist is more effective to attenuate the central hyperalgesia than administration after the development of the central hyperalgesia. Thirdly, we determined if a selective α_2-adrenoceptor antagonist alone modulates hyperalgesia.

2. Materials and methods

The experiments were performed with adult male Hannover-Wistar rats (The Finnish National Laboratory Animal Center; weight range: 250–375 g). The experiments were approved by the Institutional Ethics Committee of the University of Helsinki.

To produce central hyperalgesia, mustard oil (50% in ethanol, Merck, Darmstadt, Germany) was applied for 2 min on a piece of filter paper (2 cm^2) on the skin of the ankle of the rat. During testing the rat was standing or walking on a metal grid and the paw of the hindpaw ipsilateral or contralateral (= control site) was stimulated with a series of calibrated monofilaments (Stoelting, Wood Dale, IL; Chaplan et al., 1994). The hairs used in this experiment produced forces ranging from 0.445 to 84.96 g. The stimulus site in the ipsilateral hindpaw was at least 2 cm distal from the border of the site to which mustard oil was applied. At each time point monofilaments were applied to the foot pad in a series of increasing forces until the rat withdrew its hindlimb. The lowest force producing a withdrawal response was considered the threshold. The left and right hindpaws were tested consecutively, and at each time point the threshold for each hindpaw was based on two separate measurements. The withdrawal thresholds were determined before the administration of mustard oil, and at various time points following mustard oil application. Medetomidine or atipamezole, or both, were applied 12 min before the application of mustard oil. In one group, medetomidine was applied immediately following the mustard oil application.

There were nine experimental groups. A control group without α_2-adrenergic agents (= saline control), four medetomidine alone groups (3–30 μg/kg before mustard oil, and 10 μg/kg following mustard oil), three atipamezole alone groups (10–1000 μg before mustard oil), and medetomidine (10 μg/kg before mustard oil) with atipamezole (100 μg/kg before mustard oil) group. In each group the number of rats varied from four to six. The thresholds were determined for both hindpaws before drug applications, and at 5- to 10-min intervals for 40 min following the mustard oil application. Some rats were tested twice at a 1-week interval. When the same rat were tested a second time, the mustard oil was applied to the hindpaw which was the control hindpaw in the preceding session. Medetomidine and atipamezole were generously provided by the Farmos Group, Orion, Turku, Finland. The drugs were administered s.c. When medetomidine and atipamezole were administered simultaneously, they were injected into contralateral body parts to avoid peripheral interactions.

Statistical evaluation was done with a three-way maximum likelihood analysis of variance (ANOVA) and t-test. In the ANOVA, the thresholds of both hindlimbs ($n = 2$) measured at all time points before and after the mustard oil treatment ($n = 8$) at all drug doses ($n = 3$ + control) were taken into account. $P < 0.05$ was considered to represent a significant difference.

3. Results

Mustard oil produced hyperalgesia in the treated hindlimb but not contralaterally, as indicated by the decrease of the mechanically induced withdrawal threshold in the treated hindlimb but not contralaterally (Fig. 1A). This hyperalgesia lasted about 30 min, after which the threshold recovered to the control (pre-mustard oil) level. Medetomidine dose dependently (3–30 μg/kg s.c.) attenuated the mustard oil-induced threshold decrease at doses which did not influence the withdrawal threshold in the contralateral control limb (Fig., 1 B-D). According to three-way maximum likelihood ANOVA the effect of medetomidine treatment on mustard oil-induced hyperalgesia was highly significant ($P = 0.0001$)

A submaximal dose of medetomidine (10 μg/kg) applied 12 min prior to mustard oil treatment (= pre-emptively) did not produce stronger attenuation of the mustard oil-induced hyperalgesia than application of medetomidine following the mustard oil treatment (= after the induction of the injury discharge producing hyperalgesia; $P = 0.324$, t-test; Fig. 2).

Atipamezole alone had a significant effect on the mustard oil-induced hyperalgesia ($P = 0.0221$, ANOVA). Atipamezole attenuated the mustard oil-induced hyperalgesia in a non-monotonic fashion. At a very low (10 μg/kg) or high (1000 μg/kg) dose atipamezole was without effect on mustard oil-induced hyperalgesia whereas at an intermediate dose (100 μg/kg) atipamezole attenuated the mustard oil-induced hyperalgesia (Fig. 3).

When an antinociceptive dose of medetomidine (10 μg/kg) was combined with an antinociceptive dose of

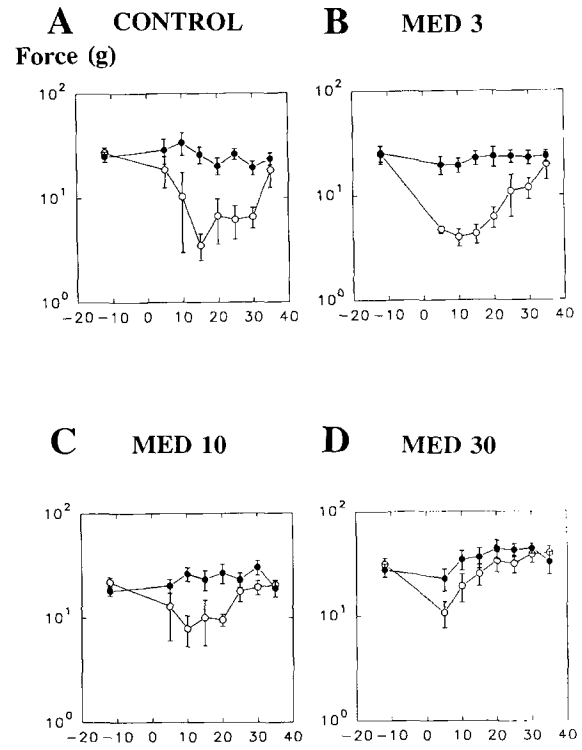

Fig. 1. Effect of medetomidine on the threshold of the mechanically induced hindlimb withdrawal response. In B and C the dose of medetomidine (μg/kg, s.c.) is given above each graph. In all cases, mustard oil was applied at time point 0, and the medetomidine/saline control was administered 12 min prior to mustard oil. The first control measurement was taken immediately before drug administration. The open symbols indicate the threshold in the mustard oil-treated hindlimb, and the filled symbols indicate the threshold in the contralateral untreated hindlimb. The error bars indicate ±S.E.M. (n = 4–6 in each group).

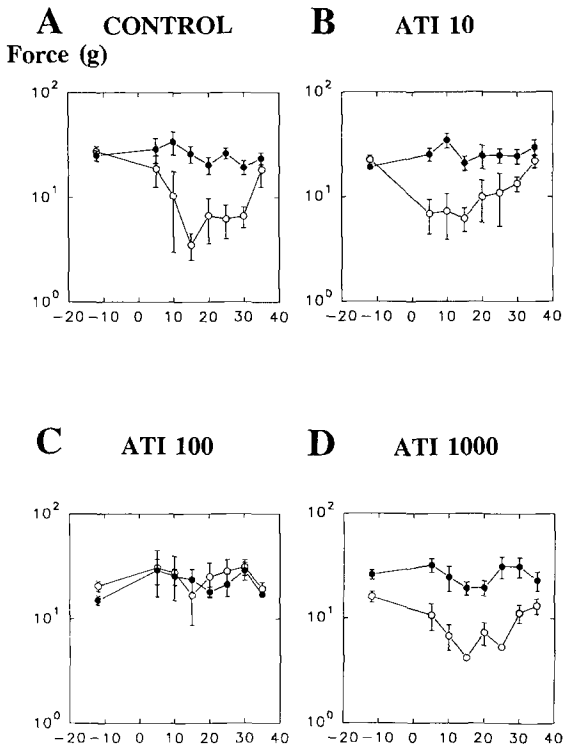

Fig. 3. Effect of atipamezole alone on the threshold of the hindlimb withdrawal response. In B and C the dose of atipamezole (μg/kg, s.c.) is given above each graph. In all cases atipamezole was administered 12 min prior to mustard oil treatment (= time point 0). For other details, see the legend for Fig. 1.

atipamezole (100 μg/kg) the antinociceptive effect was not enhanced, but if anything, was reduced from that produced by either compound alone (Fig. 4).

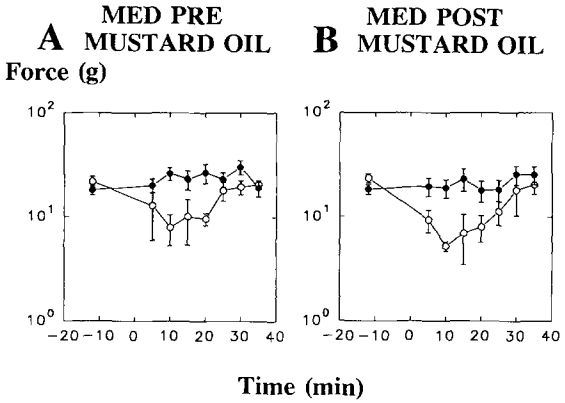

Fig. 2. Effect of a submaximal dose of medetomidine (10 μg/kg, s.c.) on hindlimb withdrawal threshold when administered pre-emptively (A, 12 min prior to medetomidine) versus immediately following the mustard oil treatment (B). For other details, see the legend for Fig. 1.

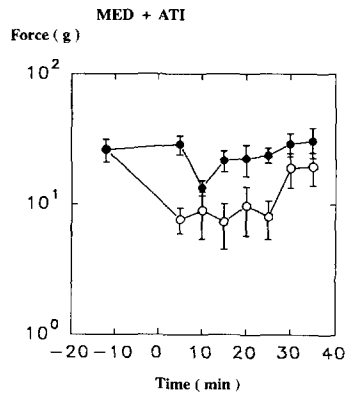

Fig. 4. Effect of an antinociceptive dose of medetomidine (10 μg/kg) together with an antinociceptive dose of atipamezole (100 μg/kg) on hindlimb withdrawal response. Medetomidine and atipamezole were administered 12 min prior to mustard oil treatment (= time point 0). For other details, see the legend for Fig. 1.

4. Discussion

The results indicate that medetomidine, an α_2-adrenoceptor agonist, can significantly attenuate central hyperalgesia at doses which are sub-antinociceptive in an intact limb as verified in this study. From earlier experiments it is known, that at doses higher than used in the present study (> 30 μg/kg), medetomidine produces dose-dependent sedation and attenuation of nociceptive signals from non-hyperalgesic skin (Pertovaara et al., 1994). Interestingly, also atipamezole alone, a selective α_2-adrenoceptor antagonist, produced attenuation of mustard oil-induced central hyperalgesia at an intermediate dose (100 μg/kg) but no effects at a very low (10 μg/kg) or a high (1000 μg/kg) dose. When the antinociceptive doses of medetomidine and atipamezole were given together, they attenuated each other's antinociceptive effects, suggesting that these effects were mediated by α_2-adrenoceptors. Finally, in this study, pre-emptive treatment with medetomidine did not produce significantly stronger attenuation of central hyperalgesia than did admistration following the development of hyperalgesia.

4.1. Enhanced antinociceptive effect of medetomidine

The currently found increased antinociceptive effect of medetomidine in an experimental model of central neurogenic pain is in line with recent findings demonstrating increased antinociceptive effects of α_2-adrenergic agents in various pathophysiological models of primary and secondary hyperalgesia. For example, systemically administered clonidine, a prototype α_2-adrenoceptor agonist, had a highly potent antinociceptive effect in experimental arthritis (Kayser et al., 1992). Following carrageenan-induced inflammation, the inflamed paws were sensitized to the antinociceptive effects of intrathecally or systemically administered clonidine (Hylden et al., 1991). The central hyperalgesia induced by experimental inflammation was also highly sensitive to intrathecally administered dexmedetomidine (Idänpään-Heikkilä et al., 1994). Intrathecally administered clonidine and dexmedetomidine were highly effective to reduce autotomy, an index of deafferentation pain, following sciatic nerve section (Puke and Wiesenfeld-Hallin, 1993). Systemically administered clonidine significantly attenuated the hyperalgesia induced by a chronic constriction mononeuropathy in rats (Smith et al., 1993). Collectively, these experimental findings indicate that α_2-adrenergic antinociceptive mechanisms may be sensitized in various pathophysiological conditions with increased pain sensitivity. Interestingly, in the presence of some pathophysiological conditions, increased levels and turnover of noradrenaline in the spinal cord (Weil-Fugazza et al., 1986) and an increased number of spinal α_2-adrenoceptors (Brandt and Livingston, 1990) have been described. These findings may be connected with the increased potency of α_2-adrenergic drugs, at least in pain conditions which take a longer time to develop than the mustard oil-induced central hyperalgesia. The present experimental findings suggest that, in some clinical conditions, a selective analgesic effect on central hyperalgesia might be obtained with an α_2-adrenoceptor agonist at very low doses producing very little sedative or hypotensive side-effects and no effects on the intact pain-signaling system.

4.2. Antinociception by atipamezole

The reversal of hyperalgesia by an intermediate dose of atipamezole, a selective α_2-adrenoceptor antagonist, was unexpected, although paradoxical antinociceptive effects of high doses of yohimbine and idazoxan, other α_2-adrenoceptor antagonists, have been described earlier in some pain models (Dennis et al., 1980; Hayes et al., 1986; Kanui et al., 1993; Kayser et al., 1992; Paalzow and Paalzow, 1983). Since in the present study, atipamezole had no effects on the withdrawal threshold of the intact limb, the paradoxical analgesic effect of atipamezole was due to a mustard oil-induced change in the function of the α_2-adrenergic antinociceptive system. For the same reason, the atipamezole-induced antinociceptive effect in the present study could not be explained by the atipamezole-induced increase in anxiety (Kauppila et al., 1991) and by a concomitant freezing response, which might artefactually produce a delayed motor response to a noxious stimulus. Interestingly, a paradoxical analgesic effect has also been described in arthritic rats with naloxone, an opioid antagonist (Kayser and Guilbaud, 1981). Further studies are needed to reveal the location and the mechanism of the atipamezole-induced paradoxical antinociception.

4.3. Pre-emptive treatment with medetomidine

In a recent study, medetomidine attenuated the immediate-early gene response of the spinal dorsal horn neurons to a noxious stimulus more effectively when administered pre-emptively than after the induction of the injury discharge (Pertovaara et al., 1993). Furthermore, the antinociceptive effect of clonidine on postoperative hyperalgesia induced by a constriction injury of the sciatic nerve was more effective when administered prior to than after the injury (Smith et al., 1993). In contrast to these findings, in the present study, pre-emptive administration of medetomidine did not produce stronger antinociceptive effects than medetomidine administered after the development of hyperalgesia. This is in line with the results obtained with a model of deafferentation pain showing that

pre-injury treatment with clonidine or (dex)medetomidine does not prevent pain-related behavior postoperatively (Puke and Wiesenfeld-Hallin, 1993; Taira et al., 1995). Clonidine and dexmedetomidine proved effective to alleviate pain-related behavior induced by deafferentation only when administered continuously after the surgery (Puke and Wiesenfeld-Hallin, 1993). These differences in results with various pain models are in line with the hypothesis that there are fundamental differences in pain mechanisms between the models. Mustard oil as well as capsaicin induce a tonic discharge in nociceptive primary afferent fibers (Reeh et al., 1986; Szolcsanyi et al., 1988). The central hyperalgesia induced by these chemicals is obviously more dependent on ongoing afferent barrage in nociceptive primary afferent fibers than on injury-triggered changes in the excitability of central neurons as also indicated by results of recent human studies (Koltzenburg et al., 1992; Grönroos and Pertovaara, 1993). This mechanism would be consistent with the lack of a stronger effect by a pre-emptive drug treatment in the present study. Hyperalgesia induced by a constriction injury may be more dependent on the central changes caused at the time of surgery, an underlying mechanism which might explain the beneficial effect of pre-emptive drug treatment in this model with clonidine (Smith et al., 1993) as well as with lidocaine (Dougherty et al., 1992; Luukko et al., 1994).

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