α_{2A} Adrenoceptors Contribute to Feedback Inhibition of Capsaicin-induced Hyperalgesia

Heikki Mansikka, M.D., Ph.D.,* Janne Lähdesmäki, M.D., Ph.D.,† Mika Scheinin, M.D., Ph.D.,‡ Antti Pertovaara, M.D., Ph.D.§

Background: Studies on receptor knockout mice have so far shown that of the three α_2-adrenoceptor subtypes, the α_{2A} adrenoceptor has a major role in mediating the powerful central analgesia induced by synthetic α_2-adrenoceptor agonists. However, because a knockout of the gene for the α_{2A} adrenoceptor has produced only little if any change in the pain sensitivity of control, nerve-injured, or inflamed animals, it has not been clear whether activation of α_{2A}-adrenoceptors by endogenous ligands has a significant pain regulatory role.

Methods: The authors assessed spontaneous pain behavior and mechanical hypersensitivity induced by administration of capsaicin in the colon or paw of α_{2A}-adrenoceptor knockout mice *versus* their wild-type controls.

Results: Enhanced pain hypersensitivity was observed in α_{2A}-adrenoceptor knockout mice 20 min or more after administration of capsaicin, but before, hypersensitivity and spontaneous pain were of equal magnitude in α_{2A}-adrenoceptor knockout and wild-type mice. When wild-type mice were pretreated with an α_2-adrenoceptor antagonist, capsaicin-induced pain hypersensitivity increased to a level equal to that in α_{2A}-adrenoceptor knockout mice. Capsaicin-induced hypersensitivity was suppressed in wild-type but not α_{2A}-adrenoceptor knockout mice by a centrally acting α_2-adrenoceptor agonist, whereas a peripherally acting α_2-adrenoceptor agonist was without effect on hypersensitivity, although it attenuated capsaicin-induced spontaneous pain behavior in wild-type mice.

Conclusions: This study shows that central α_{2A}-adrenoceptors contribute to feedback inhibition of pain hypersensitivity. Also, α_{2A}-adrenoceptors are critical for not only somatic but also visceral antinociceptive effects induced by synthetic α_2-adrenoceptor agonists.

α_2 ADRENOCEPTORS mediate analgesia,[1] but the α_2-adrenoceptor subtypes (α_{2A}, α_{2B}, and α_{2C} adrenoceptors) involved in different aspects of pain regulation have not been identified in detail. Because of lack of subtype-selective drugs, it was difficult to study the functional role of various α_2-adrenoceptor subtypes before development of subtype-selective α_2-adrenoceptor knockout animals.[2–5] Previous investigations with subtype selective α_2-adrenoceptor knockout mice revealed that the powerful analgesia induced by α_2-adrenergic agonists is dependent on the expression of α_{2A} adrenoceptors.[6–8] Therefore, it might be expected that endogenous ligands activating α_{2A} adrenoceptors have a significant role in physiologic pain regulation, and that this would be reflected as marked differences in pain behavior between α_{2A}-adrenoceptor knockout mice and their wild-type controls. However, the baseline responses to short-term painful stimulation in α_{2A}-adrenoceptor knockout mice have not differed from those in their wild-type controls,[6–8] nor did the development of hypersensitivity after carrageenan-induced inflammation[9] or nerve injury[10] depend on α_{2A} adrenoceptors, with the exception of a sympathetically maintained component of heat hyperalgesia that is presumably controlled by peripheral α_{2A} adrenoceptors.[11] These negative findings on the involvement of the α_{2A} adrenoceptor in pain behavior contrast with the almost complete abolition of α_2-adrenoceptor agonist–induced analgesia in the same studies and raise the question on the role of α_{2A} adrenoceptors and their endogenous ligands (catecholamines) in physiologic pain regulation.

Because spinal α_2-adrenoceptors, the main target of parenteral α_2-adrenoceptor agonists, are endogenously activated by noradrenergic brainstem–spinal pathways,[1] we hypothesized that pain models inducing strong descending noradrenergic inhibition from the brainstem might be sensitive to knockout of the gene for the α_{2A} adrenoceptor. Pain induced by capsaicin was considered a promising candidate in this respect because α_2-adrenoceptor agonists with a preference for $\alpha_{2A/D}$ adrenoceptors have been shown to reduce capsaicin-induced glutamate release in spinal synaptosomes.[12]

Materials and Methods

Experimental Animals

The experiments were performed using male α_{2A}-adrenoceptor knockout and wild-type control mice of aged 15–21 weeks. Mice deficient in α_{2A} adrenoceptors were generated by gene targeting, which has been described previously.[13] The α_{2A}-adrenoceptor knockout mice were backcrossed for five generations to C57B1/6J mice to form a congenic strain. Age-matched wild-type C57B1/6J mice of the same genetic background (Jackson Laboratories, Bar Harbor, ME) were used as control animals. When comparing the wild-type and α_{2A}-adrenoceptor knockout mice populations used in our laboratory, the binding of the α_2-adrenoceptor specific but subtype nonselective antagonist radioligand [^{3}H]RS-79948-197 is al-

most totally abolished (*e.g.*, 83% reduction in receptor density in cortex) in the brains of α_{2A}-adrenoceptor knockout mice.[14] The remaining binding is most prominent in brain regions known to contain the α_{2C} adrenoceptor.[14] The animals were maintained on a 12-h light: dark cycle and were provided with food and water *ad libitum*. The α_{2A}-adrenoceptor knockout mice could not be distinguished from the control mice by their appearance. For all behavioral experiments, the animals were allowed to habituate for 5 days to the testing environment and for 15 min before the actual testing was started. The research protocol was approved by the Institutional Animal Care Committee of the University of Turku and by the Regional Government of Western Finland. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. Each animal was used only once, and on completion of testing, the animal was killed by an overdose of pentobarbitone. Mice were studied with the investigator blinded to the genotype and the drug treatments.

Assessment of Pain and Hyperalgesia

Intracolonic capsaicin–induced spontaneous pain and referred hyperalgesia were assessed using the method developed by Laird *et al.*[15,16] Capsaicin (0.1%/50 μl) was administered into the colon by introducing a fine cannula with a rounded tip *via* the anus. The spontaneous behavior was observed directly during 20 min after intracolonic capsaicin. The latency of the first pain-related behavior was recorded, as were the number and type of behaviors displayed. To assess the development of referred hyperalgesia, the frequency of withdrawal responses to the application of von Frey hairs to the abdomen and the hind paws was examined before and 20 min after the administration of capsaicin. Six hairs with forces of 0.06–3.6 g were applied five times each in ascending order of force, and the frequency of responses at each force was calculated. The data from the left and right hind paw were pooled. For statistical comparisons, the frequencies of responses to all stimulus forces were pooled in each time point of each condition.

When assessing the effects of intracolonic capsaicin, there were six experimental conditions in the wild-type group: (1) intracolonic vehicle (control); (2) intracolonic capsaicin alone; and (3–6) effects of dexmedetomidine (3 and 10 μg/kg subcutaneously), atipamezole (1 mg/kg), and fadolmidine (100 μg/kg) on capsaicin-induced pain and referred hyperalgesia. α_2-Adrenergic compounds were administered 15 min before capsaicin. In the knockout group, only the effects by capsaicin alone and its modulation by dexmedetomidine at the higher dose (10 μg/kg) or by atipamezole were assessed.

Because of a long-lasting spontaneous pain behavior induced by intracolonic capsaicin,[15] hyperalgesia to mechanical stimulation cannot be reliably studied in this model until approximately 20 min after instillation of capsaicin. To assess capsaicin-induced mechanical hyperalgesia at an earlier time point, we used plantar injections of capsaicin because in this model, spontaneous pain behavior is considerably shorter.[17] The effects of intraplantar administration of capsaicin (10 μg/20 μl) on paw licking and mechanical sensitivity in the treated side and in the contralateral hind limb were assessed as described in detail elsewhere.[17] Because the plantar capsaicin model was only used to assess capsaicin-induced mechanical hyperalgesia at an earlier time point, the effects of α_2-adrenergic compounds were not studied in this model.

Drugs

Dexmedetomidine and fadolmidine are α_2-adrenoceptor agonists, and atipamezole is an α_2-adrenoceptor antagonist (all provided by OrionPharma, Turku, Finland). None of these compounds are α_2-adrenoceptor subtype selective. All these drugs were administered subcutaneously. When given centrally, fadolmidine and dexmedetomidine are equipotent.[18] Unlike atipamezole and dexmedetomidine, fadolmidine only poorly spreads to the central nervous system after peripheral administration.[18] Fadolmidine was used only to determine whether dexmedetomidine-induced effects were due to action on peripheral α_2 adrenoceptors. The dose of fadolmidine used (100 μg/kg) was the highest not producing central effects after peripheral injection.[18,19] Fadolmidine was not used in animal groups that were insensitive to dexmedetomidine-induced effects. Also, to minimize the number of animals exposed to pain studies, the low dose of dexmedetomidine (3 μg/kg) was not used in animal groups that were insensitive to the higher dose (10 μg/kg).

Statistics

Spontaneous pain behavior and the data on hyperalgesia were analyzed using one-way or two-way analysis of variance, respectively, followed by the Tukey test. The Student *t* test was used to compare differences between two groups. $P < 0.05$ was considered to represent a significant difference.

Results

Effects of Intracolonic Capsaicin

After intracolonic administration of capsaicin, the latency to the first pain-related spontaneous behavioral response was significantly shorter in the wild-type mice than in the knockout animals. Administration of dexmedetomidine, an α_2-adrenoceptor agonist, prolonged in a dose-related fashion the onset latency of visceral pain in wild-type animals, whereas it had no effect in knockout animals (fig. 1A). During the first 20 min after intracolonic administration of capsaicin, the number of spontaneous
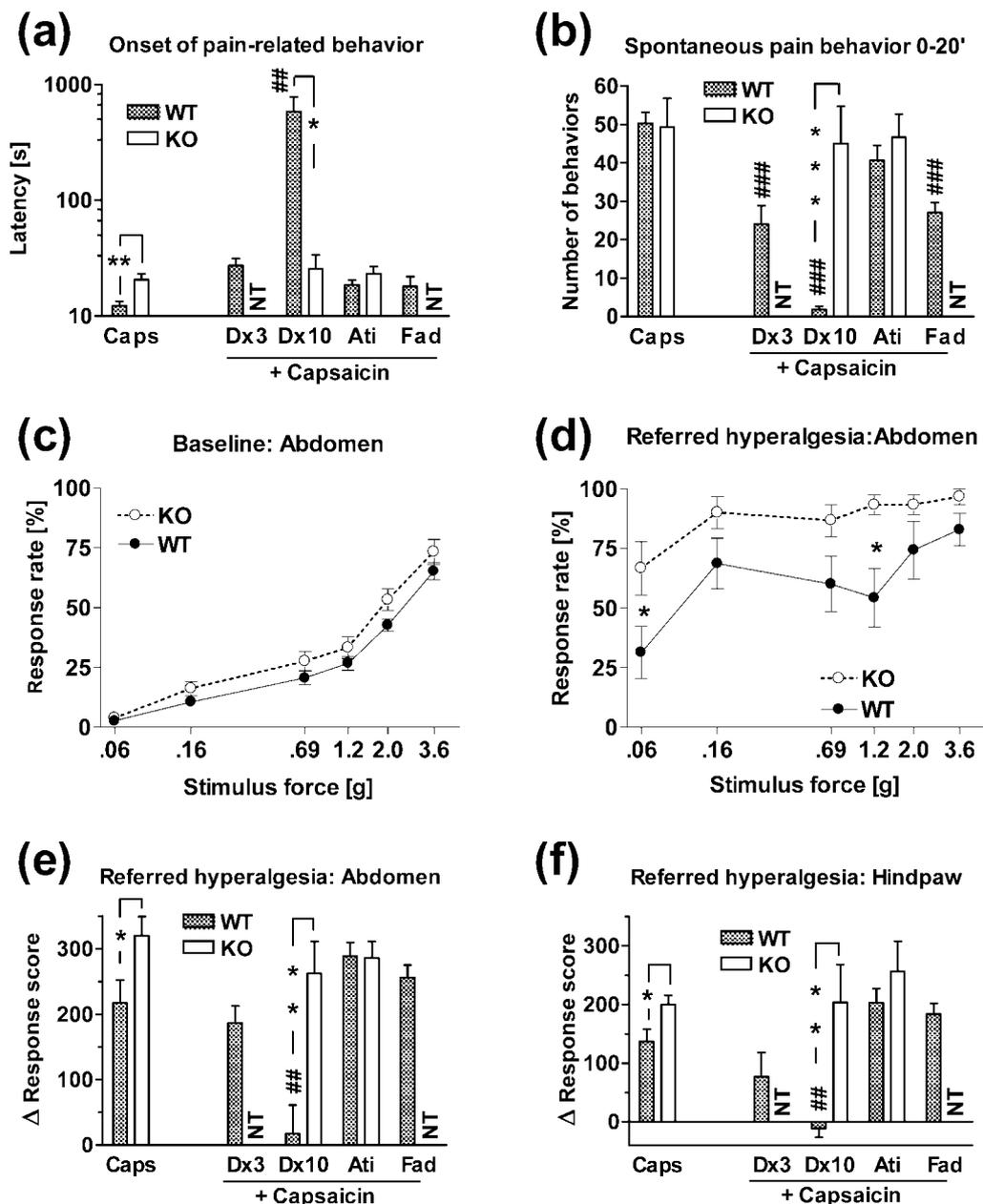
Fig. 1. Pain behavior and referred hyperalgesia induced by intracolonic capsaicin in wild-type (WT) and α_{2A}-adrenoceptor knockout (KO) mice. (*A*) Latency to first behavior. (*B*) Behavioral reactions in the first 20 min after administration. (*C*) Baseline responses to mechanical stimulation of the abdomen with von Frey hairs. (*D*) Responses to mechanical stimulation of the abdomen 20–30 min after instillation of capsaicin. (*E* and *F*) Responses to mechanical stimulation of the abdomen and the hind paw, respectively, in different drug-treatment conditions. In *C* and *D*, data are shown as mean percent response frequency at each stimulus intensity. In *E* and *F*, response frequencies at all stimulus intensities in each drug treatment condition are pooled, and the differences between the pooled data obtained after and before treatments are shown (scores > 0 indicate a hyperalgesic effect). n = 6–8/group. The *error bars* represent SEMs. Ati = 1 mg/kg atipamezole; Caps = capsaicin; Dx3 = 3 μg/kg dexmedetomidine; Dx10 = 10 μg/kg dexmedetomidine; Fad = 100 μg/kg fadolmidine; NT = not tested (missing groups). * Significant difference between wild-type and knockout groups; # significant difference from the corresponding capsaicin group (* *t* test, # Tukey test: *# *P* < 0.05, **## *P* < 0.01, ***### *P* < 0.005).

pain behaviors was not significantly different between the wild-type and knockout groups. Dexmedetomidine induced a dose-related reduction in the number of pain behaviors in the wild-type group but not in the knockout group (fig. 1B). Fadolmidine, an α_2-adrenoceptor agonist penetrating poorly the blood–brain barrier, did not in-

fluence the onset latency of pain behavior, but it attenuated the number of spontaneous pain behaviors during the first 20 min after intracolonic administration of capsaicin in the wild-type group.

Baseline responses to von Frey hair stimulation of the skin of the abdomen (fig. 1C) or the hind paw (not

shown) were not different between the wild-type and knockout groups. Assessment of referred hyperalgesia started after cessation of spontaneous pain behavior, 20 min after intracolonic instillation of capsaicin. The von Frey hair–induced responses were markedly increased after capsaicin in both groups (fig. 1D), but the increase (an index of referred hyperalgesia) was significantly larger in knockout mice than in wild-type mice (in the hind paw: $F_{1,66} = 5.99$, $P < 0.02$; in the abdomen: $F_{1,66} = 22.84$, $P < 0.0001$). The referred hyperalgesia was suppressed in a dose-related fashion by dexmedetomidine in the wild-type group but not in the knockout group (figs. 1E and F). Fadolmidine did not suppress referred hyperalgesia. After atipamezole, an α_2-adrenoceptor antagonist, the difference in the magnitude of referred hyperalgesia between the wild-type group and the knockout group disappeared. Intracolonic vehicle had no effects (n = 5; not shown).

Effects of Intraplantar Capsaicin

Spontaneous pain behavior (licking) during the first 10 min after intraplantar administration of capsaicin was of equal magnitude in the wild-type and knockout groups (fig. 2A).

Application of von Frey hairs to the hind paw resulted in equal baseline responses in the wild-type and knockout groups (fig. 2B). Ten minutes after intraplantar capsaicin, the responses to von Frey hairs were markedly increased in both groups, indicating development of hyperalgesia. Plantar hyperalgesia at the 10-min time point was of equal magnitude in the wild-type and knockout groups (fig. 2B). Thirty minutes after application of capsaicin, the hyperalgesia had completely recovered to the baseline level in the wild-type group but not in the knockout group (fig. 2C).

Discussion

α_{2A} *Adrenoceptors Are Involved in Physiologic Regulation of Pain*

This is the first study to show that the α_{2A} adrenoceptor has a major role, not only in mediating analgesic actions of synthetic α_2-adrenergic agonists,[6-8] but also in physiologic regulation of pain hypersensitivity by endogenous ligands (catecholamines). The referred hyperalgesia induced by capsaicin was significantly enhanced in the α_{2A}-adrenoceptor knockout group of animals, indicating lack of feedback inhibition. When the α_2-adrenergic feedback inhibition was blocked by atipamezole, referred hyperalgesia in wild-type animals increased to a level equal to that in the α_{2A}-adrenoceptor knockout group. This feedback inhibition was not observed in the α_{2A}-adrenoceptor knockout group until 20 min or more after intracolonic administration of capsaicin, and a block of the α_2-adrenergic feedback inhibition by atipa-
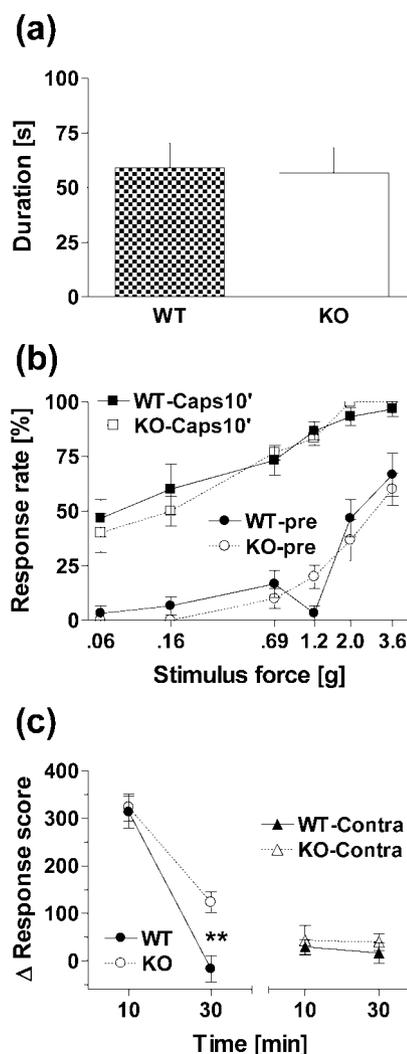

Fig. 2. Pain behavior and hyperalgesia induced by intraplantar capsaicin in wild-type (WT) and α_{2A}-adrenoceptor knockout (KO) mice. (*A*) Duration of licking behavior in the first 10 min after administration. (*B*) Responses to mechanical stimulation of the hind paw with von Frey hairs in the baseline condition (pre) and 10 min after injection of capsaicin (Caps). (*C*) Responses to mechanical stimulation of the hind paw 10 and 30 min after capsaicin ipsilateral (left) and contralateral (right) to the injection. In *B*, data are shown as mean percent response frequency at each stimulus intensity. In *C*, response frequencies at all stimulus intensities in each condition are pooled, and the differences between the pooled data obtained after and before capsaicin treatment are shown (score > 0 represents hyperalgesia). n = 6/group. The *error bars* represent SEMs. ** $P < 0.01$ (*t* test).

mezole did not influence pain responses within the first 20 min after capsaicin instillation in wild-type animals. The enhanced hyperalgesia induced by intraplantar capsaicin was not observed in the α_{2A}-adrenoceptor knockout group until the recovery phase, when the thresholds in the wild-type group were already back to baseline levels. The feedback inhibition involving α_{2A} adrenoceptors is not tonically active in nonpainful conditions, as shown by the baseline responses to short-term painful stimulation in this and earlier studies performed with

α_{2A}-adrenoceptor knockout mice,[6-11] nor are carrageenan-induced inflammation[9] or chronic nerve injury[10] sufficient stimuli to activate the feedback inhibition involving α_{2A} adrenoceptors. The findings that, in the wild-type animals, a peripherally acting α_2 adrenoceptor agonist had no antihyperalgesic effect, whereas a centrally acting α_2-adrenoceptor agonist induced strong antihyperalgesia indicate that the α_2-adrenergic feedback inhibition of hypersensitivity was a central phenomenon, presumably due to activation of descending axons from noradrenergic nuclei of the brainstem.[20,21] Interestingly, although a peripherally acting α_2 adrenoceptor agonist did not attenuate capsaicin-induced hypersensitivity, it attenuated spontaneous pain behaviors, suggesting that peripheral α_2 adrenoceptors may have a selective role in contributing to the antinociceptive actions of α_2-adrenoceptor agonists on capsaicin-induced spontaneous pain. Noradrenaline released from terminals of descending axons may have produced a central antihyperalgesic effect due to action on α_{2A} adrenoceptors in central terminals of primary afferent nociceptors, spinal neurons, or both.[1] The role of supraspinal α_2 adrenoceptors in attenuation of nociceptive reflex responses has not been as well established as that of spinal α_2 adrenoceptors. More variable effects produced by supraspinal[22-24] than spinal[1] administration of α_2-adrenoceptor agonists on spinal nociception may reflect the higher complexity of supraspinal pain control circuitries. Therefore, although α_{2A} adrenoceptors at the spinal cord level may explain the α_2-adrenergic antihyperalgesia in the current study, we cannot exclude the possibility that also supraspinal α_{2A} adrenoceptors are involved in the capsaicin-induced central feedback inhibition of spinal hypersensitivity.

Paradoxically, the α_{2A}-adrenoceptor knockout group had a prolonged latency to the onset of spontaneous pain behavior after intracolonic capsaicin. This reduction in sensitivity at the start of painful stimulation might be explained by increased release of noradrenaline due to lack of release-inhibiting α_{2A} adrenoceptors[14,25] and subsequent activation of spinal α_{2C} adrenoceptors that may also contribute to regulation of pain.[26] Alternatively, the delayed response onset might be due to compensatory up-regulation of other pain inhibitory mechanisms. However, such adaptations have minor effects as indicated by normal baseline responses in other pain tests.[9]

α_{2A} Adrenoceptors Mediate Visceral Antinociception Induced by Synthetic α_2-Adrenoceptor Agonists

Earlier studies demonstrating the importance of α_{2A} adrenoceptors for the α_2-adrenoceptor agonist–induced analgesia used experimental models of somatic pain.[6-8] The current results extend these findings by showing that α_{2A} adrenoceptors are critical not only for somatic but also for visceral antinociceptive effects induced by synthetic α_2-adrenoceptor agonists. Moreover, because

visceral antinociceptive efficacy was strongest after peripheral drug administration of an α_2-adrenoceptor agonist spreading centrally, attenuation of visceral pain behavior was predominantly due to action on α_{2A} adrenoceptors within the central nervous system. This is in accord with previous evidence indicating that spinal administration of α_2-adrenoceptor agonists produces visceral antinociception.[27-29]

Delayed Suppression of Pain Hypersensitivity: Possible Explanations

The α_{2A}-adrenoceptor–dependent feedback inhibition was not observed until 20 min after administration of capsaicin, independent of whether hyperalgesia was induced by intracolonic or intraplantar capsaicin and independent of whether the contribution of this feedback inhibition was revealed by a knockout of the α_{2A} adrenoceptor or by administering a synthetic α_2-adrenoceptor antagonist in wild-type animals. These findings suggest that the α_{2A}-adrenoceptor–dependent feedback inhibition had a slow onset. The earlier finding that α_2-adrenoceptor antagonists predominantly enhanced late responses of nociceptive spinal dorsal horn neurons to formalin[30] supports the hypothesis that the α_2-adrenergic feedback inhibition suppresses pain after a delay. A slow onset of feedback inhibition could be due to a delayed activation of pain inhibitory mechanisms. One possible mechanism contributing to a delay in the feedback inhibition would be if a significant amount of noradrenaline released from descending axon terminals did not act on spinal α_{2A} adrenoceptors through classic synaptic mechanisms, but interactions of noradrenaline with spinal α_{2A} adrenoceptors occurred predominantly *via* volume transmission.

It might be argued that the α_{2A}-adrenoceptor–dependent feedback inhibition did not have a slow onset, but it selectively attenuated hypersensitivity without influencing spontaneous pain behavior induced by intracolonic capsaicin. Because of long-lasting spontaneous pain behavior induced by intracolonic capsaicin, mechanical hypersensitivity could not be assessed earlier in this model than at a time point of 20 min. However, in animals treated with intraplantar capsaicin, it was possible to assess hypersensitivity in an earlier time point, because in this model, the duration of spontaneous pain behavior was shorter. Importantly, hypersensitivity induced by intraplantar capsaicin was not different between the wild-type and α_{2A}-adrenoceptor knockout mice in an earlier time point of 10 min but only in a late time point of 30 min. This result is in accord with the hypothesis that the noradrenergic feedback inhibition had a slow onset in producing antihyperalgesia. However, further studies are still needed to exclude the possibility that the early response to capsaicin as well as formalin may be too strong to be suppressed by noradrenergic feedback inhibition, and consequently, the α_{2A}-

adrenoceptor–dependent pain suppressive effect is observed only after a delay when the nociceptive afferent barrage is weaker.

After blockade of α_{2A} adrenoceptors by a synthetic antagonist atipamezole or genetic engineering, a prominent enhancement of pain-related responses was observed in a capsaicin-induced pain model producing a significant amount of spontaneous pain behavior. In line with this, pain-related responses evoked by formalin, another chemical producing spontaneous pain behavior, were enhanced after administration of α_2-adrenoceptor antagonists.[30] In contrast, a knockout of α_{2A} adrenoceptors has not caused marked changes in baseline pain sensitivity in models that do not produce significant amounts of spontaneous pain behavior.[6–11] These findings indicate that the intensity of painful stimulation may be an important parameter determining the activation of α_{2A}-adrenergic feedback inhibition. However, a decrease of formalin-induced pain behavior has also been reported after administration of yohimbine, a synthetic α_2-adrenoceptor antagonist,[31] and a lesion of descending noradrenergic pathways with toxins.[32,33] Yohimbine and atipamezole have a marked difference in their selectivity for the α_2 adrenoceptor,[34] which might contribute to the difference in their pain modulatory effects. It remains to be determined whether denervation-induced plasticity might explain why a lesion of descending noradrenergic pathways by toxins produced a suppression of sustained pain behavior,[32,33] whereas the opposite effect was observed after a blockade of α_2-adrenergic feedback inhibition by a highly selective antagonist and a knockout of α_{2A} adrenoceptors.

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