Metabotropic Glutamate and Cannabinoid Receptor Crosstalk in Periaqueductal Grey Pain Processing

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Abstract: Metabotropic glutamate (mGlu) and cannabinoid receptors are G-protein coupled receptors which have shown synaptic co-operation through small lipid messengers in the central nervous system (CNS). A functional interaction between these two receptor families could have a relevant potential in the treatment of CNS disorders, including chronic pain. Indeed, both mGlu and cannabinoid receptors play a crucial role in the neurobiology of pain and their simultaneous manipulation could lead to novel strategies in pain management. In particular, as both mGlu and cannabinoid receptors have been found in the periaqueductal grey (PAG), a crucial station in the pain modulatory system, these receptors could be a substrate for producing analgesia at this level. In this review we aim to briefly illustrate the role of mGlu and cannabinoid receptors in controlling nociceptive processes, some points of convergence, and their functional interaction in pain processing. Further insights into this functional linkage between the mGlu and cannabinoid receptors could pave the way to a new strategy for pain relief, such as a drug cocktail acting on cannabinoid/metabotropic glutamate receptors.

Key Words: Periaqueductal grey, metabotropic glutamate receptors; cannabinoid receptors, pain.

METABOTROPIC GLUTAMATE RECEPTORS AND PAIN

Metabotropic glutamate (mGlu) receptors play an important role in the modulation of nociceptive processing and central sensitisation associated with persistent and chronic pain at several levels in the CNS [29,42,67,68].

Several studies have shown that mGlu receptors modulate nociception from peripheral terminals [5,10,67,98,104]. This may stimulate the search for peripherally active mGlu receptor ligands, which are devoid of central adverse effects [30]. A role for mGlu receptors in spinal nociceptive processing has been reported, with particular emphasis on group I mGlu receptors [13,20,69,70,100,101,102,103]. Stimulation of group I mGlu receptors at spinal level generally produces pro-nociceptive effects [31,100], although functional differences between mGlu₁ and mGlu₅ receptors in modulating pain are recently emerging at the same rate as new selective mGlu receptor ligands are developed [69,90,101]. Spinal group I mGlu receptors also play an important role in nociceptive hypersensitivity associated with inflammation [8, 69,101]. Intrathecal application of group II and group III agonists inhibit nociceptive responses [20,70,91], which may reflect the inhibition of glutamate release from nerve endings. Upregulation of mGlu₃ mRNA in the spinal cord following hind paw inflammation has also been reported [6]. A role for supraspinal mGlu receptors in the modulation of pain is also emerging, mGlu receptors modulate nociceptive responses at the level of ventrobasal thalamus [83-85], dorsal raphe [72] and amygdala [32,33,48]. This review will

focus on the role of mGlu receptors within the midbrain periaqueductal grey (PAG), which is the processing center for nociceptive information controlling the pain descending pathway [3].

PERIAQUEDUCTAL GREY METABOTROPIC GLUTAMATE RECEPTORS IN PAIN PROCESSING

The discovery of an endogenous analgesic system dates back to Reynolds's observation [78] that analgesia could be produced in rats by electrical stimulation of PAG. PAG-induced analgesia is produced through the modulation of neurons within the nucleus raphe magnus, the adjacent reticular formation and the nucleus gigantocellularis, which together form the rostral ventromedial medulla (RVM) [77]. RVM in turn projects through the dorsolateral fasciculus to the dorsal horns of the spinal cord [4], negatively modulating pain transmission.

mGlu receptors exert a multiple modulation of pain at the level of PAG, due to their localization on pre- and postsynaptic elements of glutamate, GABA and non-GABA containing neurons [18].

The presence of mGlu receptors in the PAG has been demonstrated by autoradiographic [12] and pharmacological studies [46]. Glutamate-produced analgesia within the PAG, through stimulation of mGlu receptors, was first reported by Behbehani and Fields [4]. Besides that, glutamate can also induce the development and maintenance of central sensitisation and secondary hyperalgesia in the PAG [44, 66,74].

Clear evidence for the contribution of PAG mGlu receptors in modulating nociception has been shown in the mouse by local microinjections of selective mGlu receptor ligands [53]. In particular, activation of group I mGlu receptors stimulates PAG-mediated activation of descending pain system, while activation of groups II and III mGlu

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receptors produces opposite effects. Thus, glutamate may produce antinociception or hyperalgesia, by stimulating specific mGlu receptor subtypes.

The role of supraspinal mGlu receptors in the modulation of persistent pain induced by formalin has subsequently been investigated by intra-PAG microinjections of selective mGlu receptor ligands in mice [54]. PAG mGlu receptors play a role in modulating the second hyperalgesic phase induced by formalin. In particular, group I and group II mGlu receptors, and group III mGlu receptors positively and negatively modulate, respectively, the PAG antinociceptive descending pathway following persistent noxious stimulation. Stimulation of group III mGlu receptors may selectively decrease the release of glutamate in the PAG, which might in turn be responsible for a negative modulation at the antinociceptive pathway *via* RVM nuclei. Thus, mGlu receptors in the PAG and spinal cord seem to modulate inflammatory nociceptive responses in an opposite way [20,70,91].

Recently, we have shown that mGlu₈ receptors modulate the release of GABA and glutamate "bidirectionally" at PAG level. In particular, intra-PAG perfusion with the selective mGlu₈ receptor agonist (S)-3,4-DCPG, by reverse microdialysis, led to a facilitation of glutamate and inhibition of GABA release. These effects are mediated by a positive and negative coupling to the adenylate cyclase/protein kinase A (PKA) pathway, respectively [58]. This evidence is in contrast with the "classical" function of group III mGlu receptors, which are expected to decrease both GABA and glutamate release [11], although a facilitation of glutamate release mediated by mGlu₄ receptors in the entorhinal cortex has been reported [22]. However, an alternative explanation for the facilitatory or inhibitory effect of mGlu₈ receptor on glutamate and GABA release is that receptor activation primarily decreases GABA release, thus promoting a secondary increase in glutamate release. This implicates a role for mGlu₈ receptors as presynaptic hetero-receptors localized on GABAergic terminals within the PAG. Whatever the explanation, the opposite control of glutamate and GABA release is consistent with the activation of the descending pathway and PAG-induced analgesia.

We therefore examined the effect of the (S)-3,4-DCPG in models of inflammatory and neuropathic pain [59]. Systemic administration of (S)-3,4-DCPG decreased nociceptive responses in the formalin test. Similarly, systemic (S)-3,4-DCPG reduced carrageenan-induced thermal hyperalgesia and mechanical allodynia when administered before carrageenan, but was inactive when administered after the development of carrageenan-induced inflammatory pain. When microinjected into the lateral PAG, the group III mGlu receptor antagonist, MSOP, antagonised the analgesic effect induced by (S)-3,4-DCPG in both inflammatory pain models. The involvement of PAG mGlu₈ receptor stimulation in analgesia has been confirmed by studies in which local injection of (S)-3,4-DCPG was able to relieve a nocifensive response induced by formalin. In neuropathic rats, (S)-3,4-DCPG proved ineffective in alleviating thermal hyperalgesia and mechanical allodynia 7 days after the chronic constriction injury of the sciatic nerve, whereas it was effective 3 days after surgery. Taken together, these results suggest that mGlu₈ receptors

relieve formalin and carrageenan-induced hyperalgesia without affecting an established inflammatory or neuropathic pain. It therefore appears that a key contributor to the antinociceptive activity of (S)-3,4-DCPG is the duration and the intensity of the pain state: (S)-3,4-DCPG was more effective in the short-lasting formalin test, was effective only as a pre-treatment in the carrageenan model, and was ineffective 7 days after chronic constriction injury of the sciatic nerve.

Our data with (S)-3,4-DCPG are divergent from previous studies performed with the prototypic group III mGlu receptor agonist, L-serine-O-phosphate (L-SOP). L-SOP reduces the latency of nociceptive reaction in the hot plate test [53], and produces a late pro-nociceptive effect at high doses in the formalin test in the mouse [54]. These effects are blocked by intra-PAG administration of the selective group III mGlu receptor antagonist, MSOP. L-SOP might lower pain threshold by activating mGlu₇ receptors in the PAG. Accordingly, we have found that intra-PAG injection of the selective mGlu₇ receptor agonist, AMN082, produces a dose-dependent hyperalgesic effect, as monitored by behavioural [59] and *in vivo* electrophysiological studies (manuscript in preparation). Thus, mGlu₇ and mGlu₈ receptors may subserve opposite function within the PAG, producing hyperalgesic and analgesic effects, respectively. This might be due to their different location within GABAergic or glutamatergic synapses in the PAG circuitry, with the mGlu₇ receptor being presumably the main pre-synaptic glutamatergic auto-receptor in the PAG.

CANNABINOIDS AND PAIN

Basic research supports a role for endocannabinoids in modulating behavioural responses to acute, inflammatory and neuropathic pain. In recent years a large body of investigations addressing this issue have been carried out and the antinociceptive role of cannabinoids is now well-established [41,75,89,97]. Cannabinoid subtype 1 (CB₁) receptors have been found in the main stations of pain pathways. They densely populate the PAG and the RVM within the descending pain pathway, the superficial layers of spinal dorsal horns, the dorsal root ganglia and the peripheral terminals of primary afferent neurons. Cannabinoid subtype 2 (CB₂) receptors are mainly located in the immune system, thus representing a target in inflammatory pain processing. However, their presence within the brainstem, cortex and cerebellum has recently been reported [94]. Cannabinoids have been shown to reduce nociception in many pain models. In acute pain in particular, cannabinoids inhibit responses to noxious thermal and mechanical stimuli, as well as nociceptive behaviour in the formalin test [50,62,81,88,99]. In more persistent nociceptive states, cannabinoids reduce thermal and mechanical hyperalgesia and mechanical allodynia following peripheral inflammation [61,80], capsaicin injection [47] or peripheral nerve injury [35]. Both the analgesic and hyperalgesic effects of cannabinoids are mediated by CB₁ receptors [9,80], because they are blocked by the selective CB₁ receptor antagonist, SR141716A [82]. More recently, an inhibition of pain responses mediated by CB₂ receptors has been reported [14,56]; this is relevant from a therapeutical standpoint because selective CB₂ receptor agonists should not produce the typical psychoactive effects associated with CB₁

receptor activation. The pain-suppressive activity of cannabinoids is mediated by supraspinal, spinal and peripheral mechanisms [36,41,73,79,96].

Cannabinoids are similar to opioids in alleviating pain and, under some conditions such as neuropathic pain, they show even greater potency and efficacy than opioids [7,15,28,35,43,51,87]. Because neuropathic pain is only partially sensitive to conventional analgesic drugs, such as opiates and non-steroidal anti-inflammatory drugs, this evidence represents an important finding for clinical settings.

PAG CANNABINOID RECEPTORS IN PAIN PROCESSING

PAG-produced analgesia shows a dichotomy between the dorsal and ventral region. Antinociception mediated by the ventral PAG is attenuated by naloxone, shows tolerance with continuous stimulation and cross tolerance to opioids, and involves stimulation of the RVM. In contrast, antinociception produced by the stimulation of dorsal PAG is not opioid-dependent, is tolerance-resistant, and involves RVM stimulation. Neurons in the RVM project directly to the dorsal horns and show reciprocal connections with PAG neurons [23]. Activation of this pathway is required for the production of morphine antinociception [57]. Dorsal PAG-induced analgesia has long been considered to be mediated by an unidentified non-opiate transmitter. In an attempt to discover this unknown pain-modulating transmitter, the prime candidates were endogenous cannabinoids, which can produce pain suppression through supraspinal, spinal and peripheral mechanisms. The first direct evidence for the presence of CB₁ receptors in the PAG dates back to 1991 when Herkenham *et al.* [34] found a higher density of these receptors in the PAG than in other brainstem structures. Cannabinoid receptors [55,92] and the anandamide-degrading-enzyme, fatty acid amide hydrolase (FAAH) [21] were found to be located within the PAG. It was later observed that direct stimulation of intra-PAG cannabinoid receptors by CP-55,940 microinjection produces antinociception in tail flick responses to radiant heat [49]. Similarly, cannabinoid receptor stimulation within the RVM increases tail flick latencies [63,64]. The effects of intra-PAG administrations of the CB₁ receptor agonists, WIN 55,212-2 and HU210, have also been investigated. The antinociceptive effects of these agonists are fully reversed by SR141716A, which highlights the role of CB₁ receptor in dorsal-PAG cannabinoid-mediated analgesia [26,27,73]. Evidence that cannabinoid-induced antinociception involves the activation of supraspinal sites of action [50] and that disruption of the descending inhibitory circuit suppresses the antinociceptive effects of systemically administered cannabinoids [38], supports the finding that cannabinoid-induced antinociception involves the activation of descending inhibitory pain pathways. Accordingly, spinal transection in rats reduces systemic cannabinoid-induced antinociception in the tail flick test [50]. Electrical stimulation of the PAG produces analgesia through the release of anandamide, which activates CB₁ receptors. Pain also causes the release of anandamide in the PAG, promoting the activation of the cannabinergic pain suppressive system [97]. Taken together, these data support the possibility that endogenous cannabinoids play a crucial role in the dorsal and lateral PAG cir-

cuitries that promote analgesia. However, the cellular mechanisms of cannabinoid-induced antinociception within the PAG are still unknown.

A later study by Vaughan *et al.* [95] demonstrates that cannabinoids act *via* CB₁ receptors to inhibit GABAergic synaptic transmission within the PAG, thus decreasing the inhibitory GABAergic tone on PAG and RVM output projection neurons. Similarly to μ -opioid receptors, the ability of CB₁ receptors to inhibit GABAergic synaptic transmission is mediated by a presynaptic Ca²⁺-independent mechanism. However, unlike μ -opioid receptors, CB₁ receptors do not modulate post-synaptic transmission through a direct action on Ca²⁺ and K⁺ conductances. Taken together these findings suggest that cannabinoids and opioids produce analgesia within the PAG by distinct, but partially overlapping mechanisms. More recently, it has been proposed that cannabinoids are implicated in the adaptive responses activated by a painful stress, a mechanism referred to as stress-induced analgesia. It was already well-documented that endogenous opioid peptides have a central function in this process, which involves the recruitment of the PAG-RVM-dorsal horn spinal cord circuits [1,54]. Evidence that stress activates an endogenous cannabinoid mechanism that suppresses sensitivity to pain has been reported [37]. In this work, further evidence confirms that stress induces the rapid concerted release of the two main endogenous cannabinoids, anandamide and 2-arachidonoylglycerol (2-AG), within the PAG. The two endocannabinoids acting on PAG CB₁ receptors regulate glutamate and GABA transmission with an ensuing disinhibition of the pain descending pathway. These findings support the hypothesis that stress activates endocannabinoid release at the level of dorsolateral PAG and RVM to suppress nociceptive processing. Pharmacological enhancement of the endocannabinoid system induced by selective and reversible FAAH inhibitors might represent an alternative therapeutic strategy which limited central adverse effects.

We recently studied the effect of intra-PAG injections of the selective FAAH inhibitor, URB597, on (i) nocifensive responses to heat in the plantar test; (ii) PAG levels of anandamide and 2-AG; and (iii) the spontaneous activity of "OFF" and "ON" neurons in the RVM [52].

Endocannabinoids such as anandamide, noladin, N-arachidonoyl-glycine (NAGly) and N-arachidonoyl-dopamine (NADA) may also activate a vanilloid type 1 (TRPV1, also known as VR1) receptor [19]. This cation channel receptor is activated not only by vanilloids (such as capsaicin, which is responsible for piquancy in hot chili peppers, or resiniferatoxin, which is isolated from cactus-like plants), but also by noxious heat and low pH, representing a molecular integrator of noxious stimuli. We have shown that URB597 either suppresses or increases thermal nociception *via* indirect activation of either TRPV1 or CB₁ receptors, respectively, depending on the dose and time schedule of administration [52]. Accordingly, TRPV1 and CB₁ receptors are co-expressed in several PAG neurons [52,16]. The TRPV1 and cannabinoid receptor agonists, capsaicin and WIN 55,212-2, suppress and induce nociception, respectively. URB597 dose-dependently enhances PAG levels of anandamide and/or 2-AG, which may activate TRPV1 and CB₁ receptors, respec-

tively. The TRPV1-mediated antinociception and CB₁-mediated nociception caused by URB597 correlates with an enhanced or reduced activity of RVM "OFF" neurons, suggesting that they occur through the stimulation or inhibition of output PAG neurons [52]. Finally, at the highest doses tested, URB597 and WIN 55,212-2 also cause CB₁-mediated analgesia, which correlates with a dis-inhibition of RVM "OFF" neurons. Thus, endocannabinoids affect the descending pathways of pain control by acting at either CB₁ or TRPV1 receptors in the PAG. Indeed, it appeared for the first time that low doses of both "direct" and "indirect" CB₁ agonists exert a pro-nociceptive effect after injection into the ventrolateral PAG, in agreement with the view that brain CB₁ receptors are presynaptically located not only in GABAergic interneurons, but also in glutamatergic neurons. Our data add further depth and value to the pharmacological profile of FAAH inhibitors, and suggest multiple pathways and molecular targets for endocannabinoid control of descending pain pathways in rodents [52].

FUNCTIONAL INTERACTION BETWEEN CANNABINOID AND METABOTROPIC GLUTAMATE RECEPTORS IN PERIAQUEDUCTAL GREY PAIN PROCESSING

Several studies have shown that activation of group I mGlu receptors promotes the formation of endocannabinoids. In particular, postsynaptic activation of mGlu₁ or mGlu₅ receptors causes the production of endocannabinoids, which in turn diffuse out of postsynaptic neurons in a retrograde fashion to presynaptic terminals. Endocannabinoids bind to CB₁ receptors and reduce neurotransmitter release. The mechanism involves activation of PLC and an increased intracellular Ca²⁺ release. Two biosynthetic pathways have been proposed: the first is the production of 2-arachidonyl glycerol (2-AG) *via* PLC-mediated hydrolysis of membrane lipids; the second is the activation of phospholipase A with an ensuing production of lysophospholipids converted to 2-AG by lysophospholipase C [17,60].

An interaction between mGlu and cannabinoid receptors in the control of acute nociception at PAG level has been observed in our laboratory [73]. mGlu receptors are able to alter the antinociceptive effect of PAG microinjections of WIN 55,212-2, which increases the latency of the nociceptive response in the plantar test. This effect is prevented by pretreatment with SR141716A. Selective mGlu₁ receptor blockade with CPCCOEt is unable to prevent analgesia produced by WIN 55,212-2. In contrast, the selective mGlu₅ receptor antagonist, MPEP, prevents the effect of WIN 55,212-2. However, analgesia induced by (RS)-2-chloro-5-hydroxy-phenylglycine (CHPG), a selective mGlu₅ receptor agonist, is blocked by MPEP, but not by SR141716A. When injected alone, CPCCOEt produces no effect, whereas MPEP produces a significant reduction in the latency of nociceptive response at the highest doses used. These data indicate that mGlu₅ but not mGlu₁ receptors, exert a tonic modulation of nociception in the PAG. Similarly, pretreatment with either EGlu or MSOP, which are selective antagonists of group II and III mGlu receptors, respectively, prevents WIN 55,212-2-induced analgesia. This suggests that physiological stimulation of mGlu receptors is required for the cannabinoid-induced analgesia in the PAG. In particu-

lar, pharmacological blockade of postsynaptic mGlu₅ receptors might reduce the tonic excitatory control of glutamate on antinociceptive pathways originating from the PAG. The effect of group II and III mGlu receptor antagonists might be mediated by an increased GABAergic activity. In both cases, the cannabinoid-induced antinociception in the PAG may be considerably depressed.

A functional interaction has been shown between PAG mGlu₅ and cannabinoid receptors in modulating the activity of RVM nociceptive ON- and OFF-cells in response to subcutaneous injection of formalin. ON- and OFF-cells, have opposite behaviours in nociceptive modulation [17]. ON-cells show a burst of activity while OFF-cells become silent just prior to withdrawal reflexes in the tail flick test [65]. These cells respond in an opposite way to pharmacological stimulation with opioid receptors agonists: systemic or local injections of opioid receptor agonists sufficient to inhibit nociceptive reflexes inhibit ON-cell and increase OFF-cell activities [24,25]. Intra-PAG injections of WIN 55,212-2 or group I mGlu receptor agonist or antagonists affect the formalin-induced changes in the activity of RVM neurons. Subcutaneous injection of formalin into the hind paw produces a facilitation of tail flick reflexes associated with an increase in the basal activity in RVM ON-cells and a decrease in the activity of OFF-cells. Intra-PAG microinjection of WIN 55,212-2 prevents formalin-induced changes in RVM cell activities. Higher doses of WIN 55,212-2 increase the tail flick latencies, reduce the tail flick-related onset to ON-cell burst, and decreased the duration of the OFF-cell pause. Furthermore, WIN 55,212-2 at higher doses is able to reduce RVM ON-cell and increase OFF-cell ongoing activities. Such effects are prevented by either CB₁ receptor antagonist, SR141716A, or the selective mGlu₅ receptor antagonist, MPEP. In contrast, the selective mGlu₁ receptor antagonists, CPCCOEt and LY367385, do not prevent WIN 55,212-2-induced effects. This study suggests that subcutaneous injection of formalin modifies RVM neuronal activities and this effect is prevented by PAG cannabinoid receptor stimulation. In addition, the physiological stimulation of PAG mGlu₅ but not mGlu₁ receptors is required for the cannabinoid-mediated effect.

A presynaptic interaction between CB₁ and group II mGlu receptors has also been detected in the prefrontal cortex [2], an area projecting to the PAG within the descending pathway that inhibits pain. In particular, group II mGlu receptor-induced long-lasting depression at proximal excitatory synapses is occluded by CB₁ receptor stimulation.

mGlu receptors can also modulate the function of TRPV1 receptors. It has been shown that group I mGlu receptors expressed in the peripheral terminals, increase thermal sensitivity by enhancing TRPV1 receptor function *via* the PLC pathway. Such an effect is mediated by the metabolism of diacylglycerol and the production of prostaglandins *via* the cyclooxygenase pathway. Prostaglandin production in turn leads to the activation of cAMP-dependent protein kinase, which enhances TRPV1 receptor function [39].

The earliest evidence for the presence of TRPV1 receptor in the PAG came from the observation that intra-PAG microinjections of capsaicin increased the latency of NR in a

significant and dose-dependent fashion in the plantar test [71]. This effect was prevented by capsazepine, a selective TRPV1 antagonist. CPCCOEt and MPEP, which respectively antagonize mGlu₁ and mGlu₅ receptors, completely blocked the effect of capsaicin. In contrast, pharmacological blockade of group II and group III mGlu receptors had no effect on capsaicin-induced analgesia. This study demonstrates the first pharmacological interactions between mGlu₁ receptors and mGlu₅ receptors with capsaicin-induced antinociception at PAG level. Stimulation of PAG TRPV1 receptors, possibly expressed on glutamatergic terminals or dendrites [86,93], may generate antinociception by increasing the release of glutamate, which in turn activates mGlu₁ and mGlu₅ receptors, as well as NMDA receptors. Group I mGlu receptor blockade prevents such capsaicin-induced antinociception. Interactions between mGlu₁/mGlu₅ and TRPV1 receptors via PKA activation have also been reported under chronic pain conditions at the spinal dorsal horn [42], and peripheral A-fiber level [40].

CONCLUSION

The finding of a reciprocal interaction between cannabinoid and mGlu receptors has important implications for the control of synaptic transmission and for novel therapeutic strategies. Of particular relevance is the interaction between mGlu receptors and endocannabinoids in drug development. A key area of convergence is the midbrain PAG where CB₁ receptor activation inhibits the release of both glutamate and GABA. Reciprocally, a tonic stimulation of mGlu₅ receptors is necessary for the cannabinoid-mediated antinociception in the PAG. The interaction of these two systems in the modulation of chronic pain could have important therapeutic implications in terms of clinical settings. Since an interaction between cannabinoid and mGlu_{1/5} receptors, such as vanilloid and mGlu_{1/5} receptors, has been identified, their dual stimulation at PAG level may prove effective for the treatment of chronic pain. In our opinion a combined activation of cannabinoid CB₁/vanilloid TRPV1 and mGlu receptors may cause analgesic effects that are stronger than those produced by the activation of individual classes of receptors. Drug cocktails acting on both systems may be effective under conditions in which "classic" analgesics have failed. A greater understanding of the mechanisms underlying these reciprocal interactions may break the ground for the development of novel treatments for pathological pain.

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