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Cannabinoid CB_1 receptor upregulation in a rat model of chronic neuropathic pain

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Abstract

Although cannabinoids are known to be more effective analgesics against chronic rather than acute pain, the mechanism underlying this phenomenon is still unclear. We report now that contralateral thalamic cannabinoid CB_1 receptors are upregulated after unilateral axotomy of the tibial branch of the sciatic nerve, a rat model of chronic neuropathic pain, and hypothesize that cannabinoid CB_1 receptor upregulation contributes to the increased analgesic efficacy of cannabinoids in chronic pain conditions.

Keywords: Analgesia; Cannabinoid CB_1 receptor; Tibial nerve injury

Accumulating evidence suggests that cannabinoids are effective analgesics, particularly in chronic pain conditions (for recent review, see Pertwee, 2000). The synthetic cannabinoid WIN-55,212 and the endocannabinoid anandamide were found to attenuate thermal hyperalgesia and tactile allodynia in rat models of chronic neuropathic or inflammatory pain (e.g., Herzberg et al., 1997; Richardson et al., 1998). Interestingly, the antihyperalgesic and antiallodynic effects of cannabinoids were obtained at doses, which did not affect pain-related behavior in non-pathological conditions, suggesting that the endogenous cannabinoid system becomes more sensitive to the analgesic effects of cannabinoids in chronic pain conditions.

Although it is generally accepted that the antihyperalgesic/antiallodynic activity of cannabinoids is predominantly mediated through activation of cannabinoid CB_1 receptors located in central and peripheral nervous structures involved in the processing of pain (such as the thalamus and the brain stem, the spinal cord, and the peripheral sensory neurons), the relative contribution of these anatomical substrates, as well as, the possible contribution of peripheral cannabinoid CB_2 or CB_2-like receptors remain unclear (Pertwee, 2000; Piomelli et al., 2000). In addition, the neurobiological mechanism(s) underlying the increased sensitivity of the endogenous cannabinoid system in chronic pain still needs to be identified. As discussed by Piomelli et al. (2000), Drew et al. (2000) and Richardson et al. (1998), possible mechanisms for such increased sensitivity include, (1) increased expression of cannabinoid CB_1 and/or CB_2 (-like) receptors, (2) changes in cannabinoid CB_1 and/or CB_2 (-like) receptor function and/or G-protein coupling, (3) altered formation/release of endocannabinoids such as anandamide and 2-arachidonylglycerol (reduced endocannabinoid tone), or (4) synergism with other endogenous ligands that are active only during chronic pain conditions.

In order to test the first hypothesis, we investigated cannabinoid CB_1 receptor expression in the thalamus in a new rat model of chronic neuropathic pain. The thalamus was selected as it is a crucial relay in the pathways of nociception and because it has previously been shown that thalamic cannabinoid CB_1 receptor activation contributes to the antinociceptive efficacy of cannabinoids (e.g., Martin et al., 1996). The model of chronic neuropathic pain consisted of unilateral transsection of the tibial branch of the sciatic nerve (tibial nerve injury; Hofmann et al., 2000). In this model, thermal hyperalgesia and tactile allodynia develop readily after nerve lesion, and these symptoms coincide with altered expression of pain-rele-

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vant genes such as vasointestinal peptide and galanin in the spinal ganglia, and Trk B and c-*Jun* in the dorsal horn (Hofmann et al., 2000). Moreover, in accordance with the reported increased sensitivity to cannabinoids in chronic pain models, cannabinoids were found to potently inhibit thermal hyperalgesia and tactile allodynia in the tibial nerve injury model (Denzer et al., unpublished). In a preliminary study on the time-course of thalamic cannabinoid CB_1 receptor expression in the tibial nerve injury model, we found that cannabinoid CB_1 receptor expression peaked within the first 2 days and returned to control levels after 14 days. Therefore, the present study concentrated on thalamic cannabinoid CB_1 receptor expression on day 1 after tibial nerve injury.

Male Wistar rats (180–200 g; strain HsdCpb:WU) were housed in groups of six under standardized conditions and a normal 12-h:12-h light/dark regime. The animals were randomly assigned to a control, sham-operated or transsected group (control, sham and tibial nerve injury group, respectively; $n = 5$ per group). Transsection of the tibial branch of the sciatic nerve, as well as, sham surgery were carried out unilaterally on the left side under pentobarbital anesthesia, and rats were sacrificed the day after surgery. Thalamus tissue was harvested and snap frozen with liquid nitrogen. Isolation of total RNA and cDNA synthesis was performed as previously described (Siegling et al., 1994). Gene expression was quantified using the 7700 and the 5700 Sequence Detector (Taqman) and SYBR Green PCR Core Reagent-Kit, as described in the manufacturers manual (Applied Biosystems, Foster City, CA, USA). Cyclophilin served as an intrinsic control for variations in cDNA amounts. The cannabinoid CB_1 receptor primers were designed according to the rat cannabinoid CB_1 receptor mRNA sequence (accession number U40395) and were sense 5′-CGT CGT TCA AGG AGA ATG AGG and antisense 5′-TGC CGA TGA AGT GGT AGG AAG, yielding a 213-bp product. Primers for rat cyclophilin were designed as reported by Costigan et al. (1998). Taqman–PCR reactions were performed in 25 μl volumes with a final concentration of 300 nmol for each primer, with 95°C for 30 s and 60°C for 60 s, for 40 cycles.

As shown in Fig. 1, unilateral transsection of the tibial branch of the sciatic nerve induced a selective contralateral upregulation of thalamic cannabinoid CB_1 receptor mRNA, as assessed on day 1 after surgery [contralateral: $F(2,12) = 6.49$, $P < 0.02$; ipsilateral: $F(2,12) = 3.71$, $P > 0.05$; one-way ANOVA; outcome of Tukey post hoc *t*-test presented in Fig. 1]. Therefore, it is hypothesized that thalamic cannabinoid CB_1 receptor upregulation may at least partly underlie the increased antinociceptive efficacy of cannabinoids in chronic pain conditions. We are currently investigating to what extent chronic neuropathic pain affects cannabinoid CB_1 receptor expression in dorsal root ganglia and dorsal horn of the spinal cord as these structures have also been suggested to play a role in cannabinoid-induced antinociception and attenuation of central sensitization, a characteristic feature of chronic pain (e.g., Drew et al., 2000; Richardson et al., 1998).

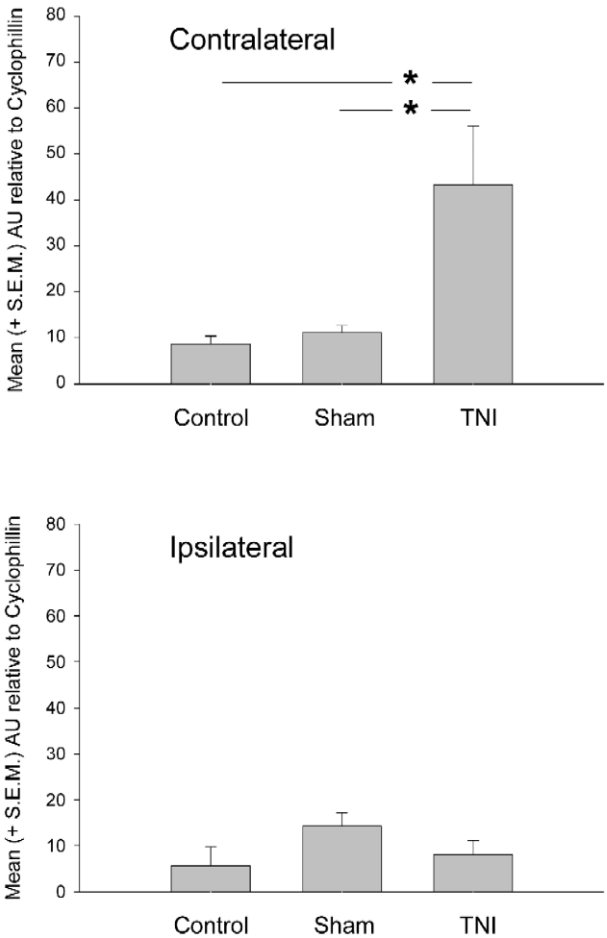

Fig. 1. Cannabinoid CB_1 receptor mRNA expression in the contralateral and ipsilateral thalamus after transsection of the tibial branch of the sciatic nerve (TNI) of rats. Tissues were harvested from control, sham-operated and axotomized animals ($n = 5$ per group), 1 day after surgery. Gene expression of cannabinoid CB_1 receptors and cyclophilin was quantified by real time PCR. Values for cannabinoid CB_1 receptor mRNA expression are shown as the mean (+1 S.E.M.) of arbitrary units (AU) relative to cyclophilin mRNA expression. $^{*}P < 0.05$ versus control groups.

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References

Costigan, M., Mannion, R.J., Kendall, G., Lewis, S.E., Campagna, J.A., Coggeshall, R.E., Meridith-Middleton, J., Tate, S., Woolf, C.J., 1998. Heat shock protein 27: developmental regulation and expression after peripheral nerve injury. J. Neurosci., 18, 5891–5900.

Drew, L.J., Harris, J., Millns, P.J., Kendall, D.A., Chapman, V., 2000.

Activation of spinal cannabinoid$_1$ receptors inhibits C-fibre-driven hyperexcitable neuronal responses and increases [^{35}S]GTPγS binding in the dorsal horn of the spinal cord of noninflamed and inflamed rats. Eur. J. Neurosci., 12, 2079–2086.

Herzberg, U., Eliav, E., Bennett, G.J., Kopin, I.J., 1997. The analgesic effects of R(+)-WIN 55,212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. Neurosci. Lett., 221, 157–160.

Hofmann, H.A., Denzer, D., Boeß, F.G., Siegling, A., 2000. Altered gene expression in a new rat model of neuropathic pain. Soc. Neurosci. Abstr., 26, 1214.

Martin, W.J., Hohmann, A.G., Walker, J.M., 1996. Suppression of noxious stimulus-evoked activity in the ventral posterolateral nucleus of the thalamus by a cannabinoid agonist: correlation between electrophysiological and antinociceptive effects. J. Neurosci., 16, 6601–6611.

Pertwee, R.G., 2000. Cannabinoid receptors and pain. Prog. Neurobiol., in press.

Piomelli, D., Giuffrida, A., Calignano, A., Rodríguez de Fonseca, F., 2000. The endocannabinoid system as a target for therapeutic drugs. TIPS, 21, 218–224.

Richardson, J.D., Aanonsen, L., Hargreaves, K.M., 1998. Antihyperalgesic effects of spinal cannabinoids. Eur. J. Pharmacol., 345, 145–157.

Siegling, A., Lehmann, M., Platzer, C., Emmrich, F., Volk, H.D., 1994. A novel multispecific competitor fragment for quantitative PCR analysis of cytokine gene expression in rats. J. Immunol. Methods, 177, 23–28.