nature medicine

Nonopioid placebo analgesia is mediated by CB1 cannabinoid receptors

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Placebo analgesia is mediated by both opioid and nonopioid mechanisms, but so far nothing is known about the nonopioid component. Here we show that the specific CB1 cannabinoid receptor antagonist 5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (rimonabant or SR141716) blocks nonopioid placebo analgesic responses but has no effect on opioid placebo responses. These findings suggest that the endocannabinoid system has a pivotal role in placebo analgesia in some circumstances when the opioid system is not involved.

Most of our knowledge about the neurobiological mechanisms of the placebo response comes from the field of pain[1–4], where placebos have been found to activate endogenous opioids[5–7] and pain-modulating networks[8–10]. However, the activation of endogenous opioids by placebos has been found to occur only in some circumstances, such as pharmacological preconditioning. If placebo analgesia is induced after repeated exposure to opioid drugs, such as morphine, the placebo response is blocked by the opioid antagonist naloxone, whereas repeated exposure to nonopioid agents, such as nonsteroidal anti-inflammatory drugs (NSAIDs), induces placebo responses that are naloxone insensitive, both in humans[5] and mice[11].

There is accumulating evidence that the effects of NSAIDs go well beyond the inhibition of cyclooxygenase and prostaglandin synthesis. In fact, NSAIDs have been found to interact with endocannabinoids, a class of lipid mediators, both *in vivo* and *in vitro*[12,13], and cyclooxygenase-2 has been shown to utilize endocannabinoids as substrates[14]. Therefore, the endocannabinoid system may have a key role in both the therapeutic and adverse effects of NSAIDs[15], as well as in NSAIDs-induced placebo responses[5].

On the basis of these considerations, we induced opioid or nonopioid placebo analgesic responses and assessed the effects of the CB1 cannabinoid receptor antagonist 5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide monohydrochloride (rimonabant or SR141716) (**Fig. 1a**). To do this, healthy volunteers underwent a pain challenge with the tourniquet technique when rimonabant maximum plasma concentration was reached at 90 min after oral administration (**Fig. 1b**), as determined by liquid chromatography mass spectrometry (**Supplementary Methods**).

A first group (natural history, $n = 12$) represented the no-treatment group and underwent a pain tolerance test for four nonconsecutive days (**Table 1**, **Supplementary Fig. 1** and **Supplementary Tables 1–4**) to assess the natural course of this kind of pain. A second group (hidden rimonabant, $n = 12$) underwent the same procedure, but we administered rimonabant on days 2 and 4 unbeknownst to these subjects. We used this group to see whether rimonabant affected this type of pain. We tested the third group (opioid conditioning, $n = 14$) over a period of five nonconsecutive days. On days 1 and 5, we administered no treatment (controls), whereas on days 2 and 3, we administered morphine as a conditioning drug. On day 4, we replaced morphine with a placebo, unbeknownst to the subjects. We used this group to elicit a placebo analgesic response after opioid preconditioning[5,7]. The fourth group (opioid conditioning plus rimonabant, $n = 15$) underwent the same procedure as the opioid conditioning group, but we added rimonabant to the placebo on day 4. We used this group to see the effects of rimonabant on the placebo analgesic response induced by opioid preconditioning.

The fifth group (nonopioid conditioning, $n = 15$) underwent the same procedure as the opioid conditioning group, but the preconditioning drug we used was the nonopioid ketorolac. We used this group to elicit placebo analgesia after nonopioid preconditioning. The sixth group (nonopioid conditioning plus rimonabant, $n = 14$) underwent the same procedure as the nonopioid conditioning group, but we added rimonabant to the placebo on day 4. We used this group to observe the effects of rimonabant on placebo analgesia induced by nonopioid preconditioning.

The natural history group showed no significant variation in pain tolerance when the tourniquet was repeated for four nonconsecutive days, indicating that pain tolerances remained constant for several days ($F_{(3,33)} = 0.19$, $P = 0.90$). The hidden administrations of rimonabant in the hidden rimonabant group on days 2 and 4 did not produce significant variations in pain tolerance compared to days 1 and 3, which indicates that this pain is not affected by rimonabant ($F_{(3,33)} = 0.33$, $P = 0.80$). As shown in **Table 1**, in the opioid conditioning group, when we administered morphine on days 2 and 3, its analgesic effect was indicated by a substantial increase in pain tolerance. Placebo on day 4, which the subjects believed to be morphine, mimicked the morphine responses, and pain tolerance was significantly different from the controls of days 1 and 5. Rimonabant in the opioid conditioning plus rimonabant group had no effect on this placebo analgesic response, and the effect of placebo on day 4 was significantly different from the baseline of days 1 and 5. When we induced placebo analgesia after

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Received 9 November 2010; accepted 7 July 2011; published online 2 October 2011; doi:10.1038/nm.2435

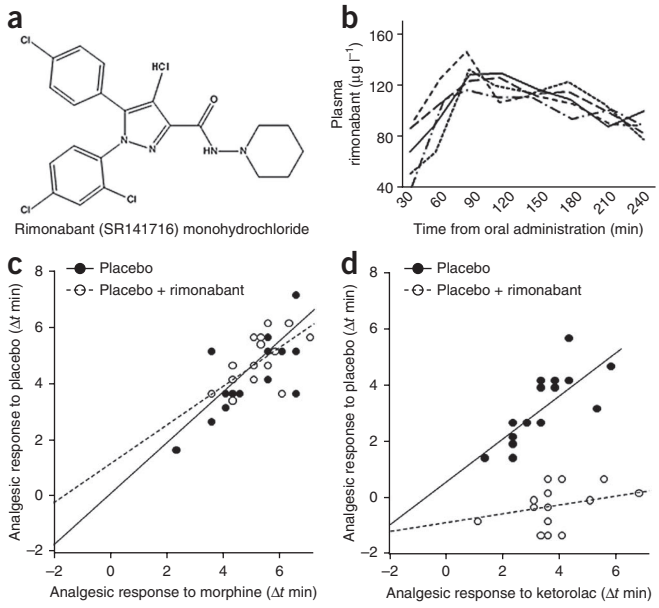

Figure 1 The CB1 cannabinoid receptor antagonist rimonabant blocks nonopioid placebo analgesia. (**a**,**b**) The chemical structure of rimonabant monohydrochloride used in the present study (**a**) and its pharmacokinetic profile in five subjects with peak plasma concentrations at 90 min after an oral dose of 0.6 mg kg^{-1} (**b**). (**c**) The relationship between the analgesic response to morphine on day 3 and the analgesic response to placebo on day 4. Each circle represents the response of a single subject. The responses are expressed as Δt, or the difference of pain tolerance between days 3 and 1 for morphine and between days 4 and 1 for placebo. Rimonabant had no effect on the correlation between morphine and placebo. (**d**) The relationship between the analgesic response to ketorolac on day 3 and the analgesic response to placebo on day 4. The correlation between ketorolac and placebo is completely disrupted by rimonabant.

nonopioid preconditioning with ketorolac, rimonabant blocked this placebo response completely. In fact, the means differences in day 4–day 1 and day 4–day 5 were not statistically significant (−0.43 min, 95% confidence interval −0.86 to 0.01 and 0.14 min, 95% confidence interval −0.54 to 0.83, respectively) (**Table 1**).

A between-subjects one-way analysis of variance revealed a significant main effect of the experimental group on differences between days 1 and 4 ($F_{(5,76)} = 48.72$, $P < 0.001$), with a significant difference between the nonopioid conditioning compared to the nonopioid conditioning plus rimonabant groups ($P < 0.001$). A linear regression analysis showed a high correlation between the response to morphine on day 3 and the response to placebo on day 4 ($r = 0.71$, $t_{(12)} = 3.5$, $P < 0.005$) (**Fig. 1c**) and between the response to ketorolac on day 3 and the response to placebo on day 4 ($r = 0.74$, $t_{(13)} = 4.0$, $P < 0.002$) (**Fig. 1d**) according to the rule 'the larger the morphine or ketorolac responses, the larger the placebo response'. Rimonabant disrupted this correlation completely in the ketorolac group ($r = 0.38$, $t_{(12)} = 1.45$, $P = 0.17$) (**Fig. 1d**) but not in the morphine group (**Fig. 1c**). A global coincidence test showed a significant difference between the two regression lines in the ketorolac group (**Fig. 1d**) ($F_{(2,25)} = 82.42$, $P < 0.001$).

In previous studies in humans[5,7] and mice[11], naloxone blocked opioid-induced placebo analgesia but had no effect on nonopioid-induced placebo analgesia. In the present study, the opposite effect occurred: rimonabant had no effect on opioid-induced placebo analgesia but it completely blocked placebo analgesia induced by nonopioid preconditioning. These findings suggest that those placebo analgesic responses that are elicited by nonopioid pharmacological conditioning with NSAIDs are mediated by CB1 cannabinoid receptors. Although this study cannot establish the site of action of rimonabant, recent *in vivo* studies in baboons[16] and humans[17] indicated that CB1 receptors are abundant in the basal ganglia, for example, in the striatum, which has been found to have a key role in the placebo response[18,19]. It is also worth noting that neurotransmitters other than endocannabinoids, such as endogenous opioids[5–8], dopamine[18,19] and cholecystokinin[20], take part in placebo responses. These neurotransmitters are involved in different conditions[1–4], and the high interindividual variability in placebo responsiveness may be attributable, among other factors, to variation in their activity.

Table 1 Pain tolerances (min) across different days in all groups

Group	D1	D2	D3	D4	D5	Means Δ D4–D1	Means Δ D4–D5
NH	11.58 (9.73–13.43)	11.83 (10.24–13.43)	12.00 (10.21–13.79)	12.00 (10.45–13.55)	–	0.42 (−0.76–1.60)	–
HR	11.63 (10.08–13.17)	Hidden rimonabant 11.29 (10.07–12.51)	11.67 (10.10–13.23)	Hidden rimonabant 11.38 (9.76–12.99)	–	−0.25 (−0.93–0.43)	–
OC	12.32 (10.79–13.85)	Morphine 16.61 (15.34–17.87)	Morphine 17.18 (15.93–18.43)	Placebo 16.39 (14.86–17.92)	12.32 (10.91–13.73)	**4.07 (3.27–4.86)**	**4.07 (3.15–4.99)**
OC + R	12.00 (10.53–13.47)	Morphine 16.43 (15.04–17.83)	Morphine 17.30 (16.03–18.57)	Placebo + rimonabant 16.77 (15.64–17.89)	11.87 (10.31–13.42)	**4.77 (4.25–5.28)**	**4.90 (4.09–5.70)**
NOC	12.03 (10.56–13.51)	Ketorolac 15.37 (14.13–16.60)	Ketorolac 15.60 (14.33–16.87)	Placebo 15.20 (14.05–16.35)	11.77 (10.47–13.06)	**3.17 (2.50–3.83)**	**3.43 (2.95–3.91)**
NOC + R	12.29 (10.68–13.89)	Ketorolac 16.00 (14.71–17.29)	Ketorolac 16.11 (14.69–17.52)	Placebo + rimonabant 11.86 (10.40–13.31)	11.71 (10.42–13.01)	−0.43 (−0.86–0.01)	0.14 (−0.54–0.83)

Daily means (95% confidence intervals) of pain tolerance, expressed in minutes, and means difference Δ (95% confidence intervals) between experimental day 4 of placebo administration (D4) and baseline days (D1 and D5) are shown for all groups. NH, natural history; HR, hidden rimonabant; OC, opioid conditioning; OC + R, opioid conditioning plus rimonabant; NOC, nonopioid conditioning; NOC + R, nonopioid conditioning plus rimonabant. The means Δ that show placebo responses are in bold.

Note: Supplementary information is available on the Nature Medicine website.

ACKNOWLEDGMENTS

This work was supported by grants from Istituto San Paolo di Torino and Regione Piemonte (Turin, Italy) and from the Volkswagen Foundation (Hannover, Germany).

AUTHOR CONTRIBUTIONS

F.B. planned and conducted the experiments, analyzed the data and wrote the manuscript. M.A. and R.R. analyzed the data and contributed to the writing of the manuscript. C.B. conducted the experiments and contributed to the writing of the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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