Nonopioid placebo analgesia is mediated by CB1 cannabinoid receptors.

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Nonopioid placebo analgesia is mediated by CB1 cannabinoid receptors

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Summary

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.

Main

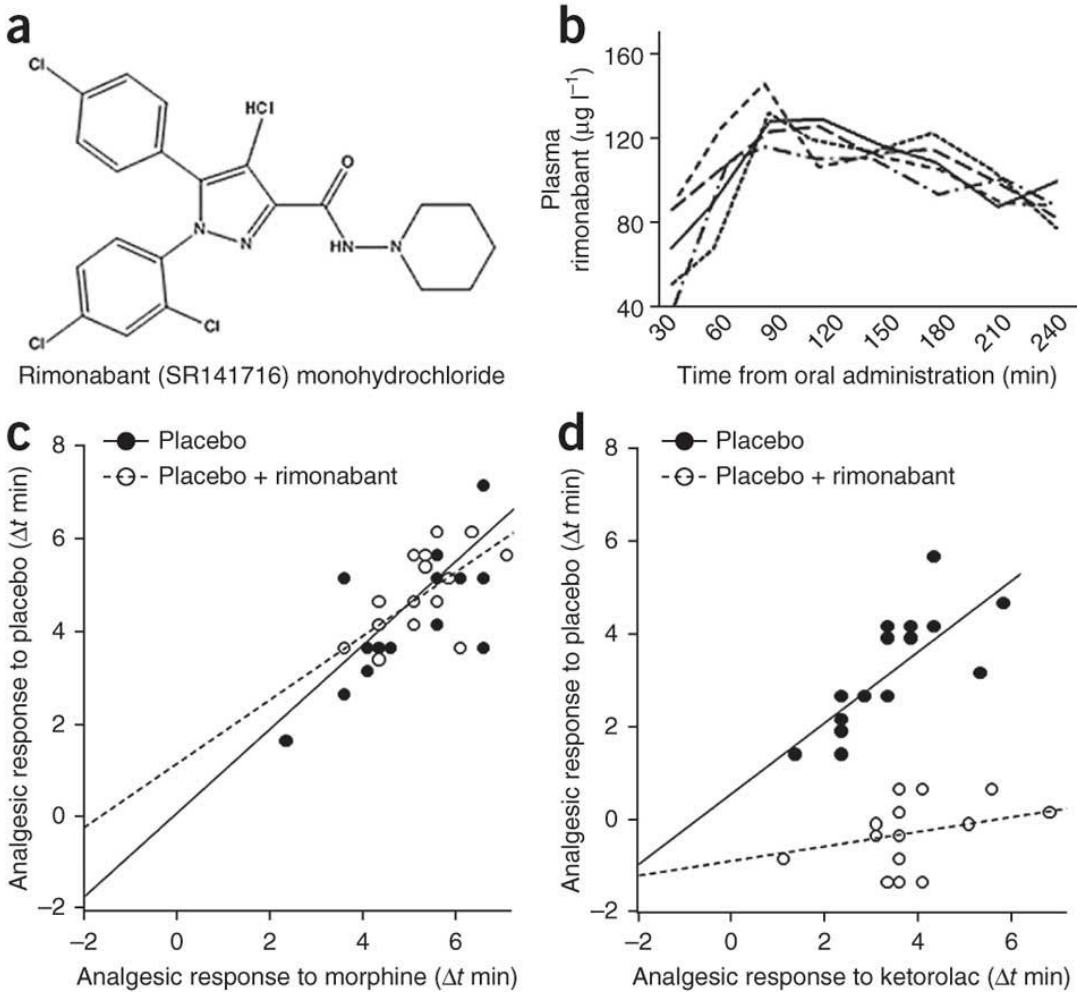
Most of our knowledge about the neurobiological mechanisms of the placebo response comes from the field of pain[1, 2, 3, 4], where placebos have been found to activate endogenous opioids[5, 6, 7] and pain-modulating networks[8, 9, 10]. However, the activation of endogenous opioids by placebos has been 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 antiinflammatory 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).

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 Δ*t*, 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.

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.

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.

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 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**, **6**, **7**, **8**], dopamine[18, 19] and cholecystokinin[**20**], take part in placebo responses. These neurotransmitters are involved in different conditions[1, 2, 3, 4], and the high interindividual variability in placebo responsiveness may be attributable, among other factors, to variation in their activity.

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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.

COMPETINGFINANCIALINTERESTS

The authors declare no competing financial interests.

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Supplementary Material for

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Materials and Methods

Subjects

The subjects were healthy males and females who agreed to participate in one of the experimental groups after they signed an informed consent form in which the details of the experiment, including the drugs to be administered, were explained. In particular, the subjects were told that either morphine or ketorolac or rimonabant would be administered at a given time, depending on the experimental group. All the experiments were approved and performed according to the rules of our ethics committee (Center for Endocrine and Metabolic Disorders) and to the Declaration of Helsinki. We randomly assigned 12 subjects to the natural history group (Group NH) (males/females = 6/6; mean age = 24.5$\pm$5.2 years; mean weight = 66.1$\pm$8.1 kg), 12 to the hidden rimonabant group (Group HR) (males/females = 6/6; mean age = 24.9$\pm$4.8 years; mean weight = 68.1$\pm$7.3 kg), 14 to the "opioid conditioning" group (Group OC) (males/females = 7/7; mean age = 25.2$\pm$5 years; mean weight = 65.5$\pm$9.2 kg), 15 to the "opioid conditioning + rimonabant" group (Group OC+R) (males/females = 7/8; mean age = 24.1$\pm$6.8 years; mean weight = 67.5$\pm$8.1 kg), 15 to the "non-opioid conditioning" group (Group NOC) (males/females = 8/7; mean age = 24.8$\pm$4.4 years; mean weight = 64.5$\pm$9 kg), 14 to the "non-opioid conditioning + rimonabant" group (Group NOC+R) (males/females = 7/7; mean age = 23.7$\pm$6.5 years; mean weight = 66.6$\pm$7.6 kg).

One week before the beginning of the experiments, the subjects underwent a clinical examination, including an electrocardiogram, in order to ascertain their physical conditions and to rule out main diseases. All the subjects were informed that they had to abstain from consuming coffee, tea and caffeine-containing drinks for 48 hours before each session, as well as alcohol and any medication.

Drugs and double-blind procedure

Oral morphine (Oramorph Molteni) was given to groups OC and OC+R during the conditioning phase on days 2 and 3 at a dose of 0.2 mg kg^{-1}, and was dissolved in 100 ml of strawberry milk (**Fig. S1**).

Oral ketorolac (Ketorolac Brunifarma) was given to groups NOC and NOC+R during the conditioning phase on days 2 and 3 at a dose of 0.6 mg kg^{-1}, and dissolved in 100 ml of strawberry milk (Fig. S1). Oral administration 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) (Acomplia, generic pharmaceutical preparation), was given to group HR on day 2 and 4 and to groups OC+R and NOC+R on day 4 at a dose of 0.6 mg kg^{-1} dissolved in 100 ml of strawberry milk (**Fig. S1**). Drugs were administered 90 minutes before the induction of pain. Rimonabant was given according to a randomized double-blind design in groups OC+R and NOC+R in which neither the subject nor the experimenter knew what drug was being administered. To do this, either the active drug or strawberry milk alone was given. To avoid a large number of subjects, two or three additional subjects per group received strawberry milk alone in place of the active drug. These subjects were not included in the study because they were used only to allow the double-blind design, as already described by Benedetti et al. (2007). By contrast, rimonabant was administered unbeknownst to the subject in group HR. In fact, in this group the subjects had to drink the strawberry milk in each session but rimonabant was added to the drink only on days 2 and 4 unbeknownst to them (**Fig. S1**).
At the oral dose we used (0.6 mg kg^{-1}) we did not find any particular effect of rimonabant that was reported by the subjects. Therefore, rimonabant can be used with a hidden administration because the subjects do not realize that any drug is being administered. As for naloxone in previous studies (e.g., Amanzio and Benedetti 1999), this makes rimonabant an interesting drug that can be used in placebo research to explore the endocannabinoid system by means of hidden administrations.

Experimental pain induction

Pain was induced experimentally by means of the sub-maximal effort tourniquet technique, according to the procedures described by Amanzio and Benedetti (1999). The subject reclined on a bed, his or her nondominant forearm was extended vertically, and venous blood was drained by means of an Esmarch bandage. A sphygmomanometer was placed around the upper arm and inflated to a pressure of 300 mmHg. The Esmarch bandage was maintained around the forearm, which was lowered on the subject's side. After this, the subject started squeezing a hand spring exerciser 12 times while his or her arm rested on the bed. Each squeeze was timed to last 2 s, followed by a 2 s rest. The force necessary to bring the handles together was 7.2 kg. This type of ischemic pain increases over time very quickly, and the pain becomes unbearable after about 14 min. All the subjects were told that they had to tolerate the tourniquet test as long as possible. In order to make the subjects tolerate the pain as long as possible, the tolerance times were taken with steps of 30 seconds (15, 15.5, 16, 16.5, 17,

17.5……minutes), and the subjects were told that they had to complete a full step in order to increase their scores. In other words, if a subject resisted 16 minutes and 29 seconds, his tolerance time was 16, whereas if he resisted 16 minutes and 31 seconds, his tolerance time was 16.5 (Benedetti et al. 2007). In order to avoid response biases, no clock was shown. In this way, the subjects did not receive any feedback that could bias subsequent tests. This method was shown to be reliable and with low inter-individual variability in previous studies (e.g., Benedetti et al. 2007).

Experimental procedure (Fig. S1 and Tables S1, S2, S3)

The natural history (NH) group (n=12) underwent the tourniquet test and pain tolerance assessment for four non-consecutive days. The inter-day interval was 3-4 days. This group represented the no-treatment, or natural history group, and was used to assess the natural course of this kind of pain over the testing period.

The hidden rimonabant (HR) group (n=12) underwent the same procedure, but rimonabant was administered on day 2 and 4 unbeknownst to the subjects (see above). This group was used to see whether rimonabant per se affected this type of pain.

The "opioid conditioning" (OC) group (n=14) was tested over a period of five non-consecutive days. In this case also, the inter-day interval was 3-4 days. On day 1, no treatment was carried out (control), whereas on day 2 and 3 morphine dissolved in strawberry milk was given as a conditioning drug. On day 4, a placebo (strawberry milk alone) was administered along with verbal suggestions that it was the same morphine of the previous days. Day 5 was the same as day 1 and used as a control. This group was used to elicit a placebo analgesic response after opioid pre-conditioning, according to the procedure used by Amanzio and Benedetti (1999) and Benedetti et al. (2007) in humans and by Guo et al. (2010) in mice. However, differently from these previous studies, in the present study morphine was given orally. The "opioid conditioning + rimonabant" (OC+R) group (n=15) underwent the very same procedure as the OC group, but rimonabant was added to the placebo on day 4 unbeknownst to the subjects. This group was used to see the effects of rimonabant on the placebo analgesic response induced by opioid conditioning.

The "non-opioid conditioning" (NOC) group (n=15) was tested over a period of five non-consecutive days. In this case also, the inter-day interval was 3-4 days. On day 1, no treatment was carried out (control), whereas on day 2 and 3 ketorolac dissolved in strawberry milk was given as a conditioning drug. On day 4, a placebo (strawberry milk alone) was administered along with verbal suggestions that it was the same ketorolac of the previous days. Day 5 was the same as day 1 and used as a control. This group was used to elicit a placebo analgesic response after non-opioid pre-conditioning,

according to the procedure used by Amanzio and Benedetti (1999) in humans and by Guo et al. (2010) in mice. However, differently from this previous study, in the present study ketorolac was given orally. The "non-opioid conditioning + rimonabant" (NOC+R) group (n=14) underwent the very same procedure as the NOC group, but rimonabant was added to the placebo on day 4 unbeknownst to the subjects. This group was used to see the effects of rimonabant on the placebo analgesic response induced by non-opioid conditioning.

Rimonabant pharmacokinetics (Table S4)

In a different group of five subjects (males/females = 2/3; mean age = 24±3.3 years; mean weight = 64.4±7.7 kg) we assessed the pharmacokinetics of rimonabant in order to optimize the experimental design in relation to its plasma concentration peaks following the 0.6 mg kg^{-1} oral dose administration. Venous blood samples were collected at 30, 60, 90, 120, 150, 180, 210 and 240 minutes after rimonabant administration. The blood samples were heparinezed and stored at –20°C before analysis. This was performed by means of liquid chromatography mass spectrometry (HPLC-MS) with a 1.0 μg l^{-1} limit of quantification (McCulloch et al. 2008). Rimonabant maximum concentrations (Cmax) were obtained from concentration time profiles.

Statistical analysis

Results are expressed as means and 95% confidence intervals (CI) unless otherwise stated. Paired Student's t-test was used to compare means differences between baseline (day 1 and day 5) and placebo and/or rimonabant treatment (day 4) within each experimental group. Statistical analyses of pain tolerance during the experimental days were conducted using a repeated-measures ANOVA. A one way ANOVA of differences between baseline (day1) and placebo and/or rimonabant treatment (day 4) was applied, and in order to clarify the nature of any such differences, planned orthogonal contrasts comparing the effect of OC vs OC+R group and NOC vs NOC+R were performed. Relationships between pain scores in different days were analyzed using linear regression, and correlation coefficients are presented to quantify the strength of these relationships. Comparisons between regression lines were performed by means of the global coincidence test and a slope comparison t-test.

Figure S1. Experimental design.

	Day 1 *Tourniquet*	Day 2 *Tourniquet*	Day 3 *Tourniquet*	Day 4 *Tourniquet*	Day 5 *Tourniquet*
Group 1 (NH)	No treatment	No treatment	No treatment	No treatment	
Group 2 (HR)	No treatment	Hidden rimonabant	No treatment	Hidden rimonabant	
Group 3 (OC)	No treatment	Morphine	Morphine	Placebo	No treatment
Group 4 (OC+R)	No treatment	Morphine	Morphine	Placebo + rimonabant	No treatment
Group 5 (NOC)	No treatment	Ketorolac	Ketorolac	Placebo	No treatment
Group 6 (NOC+R)	No treatment	Ketorolac	Ketorolac	Placebo + rimonabant	No treatment

Results

Table S1. Tolerance times (min) for each subject in groups NH and HR.

Group NH (natural history)

Subject	Day 1	Day 2	Day 3	Day 4
1	8	12	10.5	11
2	13.5	9.5	11	10
3	9.5	14	8	11
4	8	8.5	9	8.5
5	7.5	10.5	11	9
6	15	15	17	15
7	12	9.5	11.5	14
8	16.5	14	15	17
9	10.5	8.5	9.5	12
10	16	17	17	15.5
11	13.5	13.5	15	11
12	9	10	9.5	10
Mean±SD	11.6±3.3	11.8±2.8	12±3.2	12±2.7

Group HR (hidden rimonabant)

Subject	Day 1	Rimonabant Day 2	Day 3	Rimonabant Day 4
1	12	12	13.5	9
2	10.5	11.5	9.5	10
3	16	15.5	17	16
4	14	13	14	13
5	17	14	15.5	17.5
6	11	11	11	11.5
7	8	8.5	9.5	9
8	10	8.5	9.5	10.5
9	9	10	9	8.5
10	11	12	12.5	11
11	10	10	9	9
12	11	9.5	10	11.5
Mean±SD	11.6±2.7	11.3±2.2	11.7±2.8	11.4±2.9

Table S2. Tolerance times (min) for each subject in groups OC and OC+R.

Group OC (opioid conditioning)

Subject	Day 1	Morphine Day 2	Morphine Day 3	Placebo Day 4	Day 5
1	11	15.5	16.5	16	10.5
2	13	18	17.5	16.5	13.5
3	8	14	14.5	11.5	9.5
4	10	15	15.5	15	9
5	17	20.5	20.5	22	16
6	14.5	19.5	16.5	16	12
7	16	20	22	21	15.5
8	12	16	18.5	17	13
9	9.5	14.5	16	16.5	10
10	10.5	17	15	14	12
11	16	18.5	19.5	18.5	16
12	9	13	14.5	13	10
13	11	14	15	14	9.5
14	15	17	19	18.5	16
Mean±SD	12.3±2.9	16.6±2.4	17.2±2.4	16.4±2.9	12.3±2.7

Group OC+R (opioid conditioning + rimonabant)

Subject	Day 1	Morphine Day 2	Morphine Day 3	Placebo+ Rimonabant Day 4	Day 5
1	10	14.5	16	15.5	9.5
2	11	17	16	16.5	10.5
3	13	17	17.5	17.5	10.5
4	9	13	14.5	13.5	8
5	15.5	19.5	21.5	19	17
6	10.5	14.5	17.5	16	9
7	14	18	19	18	15
8	8	13	13.5	13.5	9.5
9	16.5	20.5	20	20	16
10	10.5	16	17	16.5	11
11	10	15.5	15.5	16	11
12	17	22	21.5	21	16
13	10.5	14	15.5	15	9.5
14	15	18	19.5	18.5	15.5
15	9.5	14	15	15	10
Mean±SD	12±2.9	16.4±2.8	17.3±2.5	16.8±2.3	11.9±3.1

Table S3. Tolerance times (min) for each subject in groups NOC and NOC+R.

Group NOC (non-opioid conditioning)

Subject	Day 1	Ketorolac Day 2	Ketorolac Day 3	Placebo Day 4	Day 5
1	15	16.5	17.5	17	14.5
2	11	14	15	15	10.5
3	11.5	14	14.5	14	11
4	10	15	15.5	13	9.5
5	9.5	14.5	13.5	13.5	9
6	16.5	18.5	19	19	17
7	8.5	12	12	12.5	9.5
8	17	20	19.5	19	15.5
9	11.5	14.5	16	15.5	11
10	9.5	16	14	15	10
11	12.5	15	15	14	11
12	13	14.5	14.5	14.5	12
13	11	16	17	15.5	12
14	16	19	19.5	18.5	15
15	8	11	11.5	12	9
Mean±SD	12±2.9	15.4±2.4	15.6±2.5	15.2±2.2	11.7±2.5

Group NOC+R (non-opioid conditioning + rimonabant)

Subject	Day 1	Ketorolac Day 2	Ketorolac Day 3	Placebo+ Rimonabant Day 4	Day 5
1	16.5	19.5	20	16.5	15
2	10	15	17	10	11
3	13	16	16.5	12.5	11
4	10	13	13.5	9	8.5
5	8	13	11.5	8.5	8.5
6	17	20.5	20.5	15.5	16
7	9.5	14.5	13.5	10	9.5
8	16	19	17	15	15
9	12.5	15	15.5	12	10
10	11	15.5	16.5	11.5	11.5
11	9	13	14	9	10
12	10	15	13.5	8.5	11
13	14	17	17	14	13
14	15.5	18	19.5	14	14
Mean±SD	12.3±3.0	16±2.5	16.2±2.7	11.8±2.5	11.7±2.4

Table S4. Pharmacokinetic profile of rimonabant for each subject in the first 4 hours after oral administration of a 0.6 mg kg^{-1} dose, expressed as plasma concentrations ($\mu g\ l^{-1}$).

Subject	30min	60min	90min	120min	150min	180min	210min	240min
1	52	74	135	121	114	120	106	81
2	73	91	130	132	120	115	93	100
3	40	112	118	110	110	95	102	88
4	90	126	145	107	118	110	95	95
5	86	104	122	125	118	120	103	85

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