dopaminergic neurons in the ventral tegmental area (VTA), respectively. THC, administered intravenously at escalating cumulative doses (0.15, 0.3, 0.6, and 1.2 mg/kg) infused at 1-min intervals (*9*), induced a significant increase in extracellular NAc dopamine levels (Fig. 4G) and in the firing activity of VTA neurons (Fig. 4F). Both effects were blunted by pretreatment with pregnenolone (2 m/kg) (Fig. 4, F and G).

We then analyzed the impact of pregnenolone on the reinforcing effects of cannabinoid drugs, using the intravenous self-administration model (*9*). In this model, CD1 mice were used, because this strain readily learns to produce an operant response (nose-poking into a hole) to obtain an intravenous infusion of CB_1 agonists. Mice readily learned to self-administer the CB_1 agonist WIN55,212-2, showing a clear preference for the device that triggered the infusion of the drug (active hole) in comparison to the inactive device, in which responding had no scheduled consequences (inactive hole) (Fig. 4H). Injections of pregnenolone (2 and 4 mg/kg) before each self-administration session reduced the intake of WIN 55,212-2 (Fig. 4I) and reduced the break point in a progressive ratio schedule (Fig. 4J), which is considered a reliable measure of the motivation for the drug (*9*).

To provide first insights about the mechanism of action through which pregnenolone can modify the behavioral and neurobiological effects of THC, we studied the effects of pregnenolone in cell lines expressing the human CB_1 (hCB_1) receptor (fig. S2). Briefly (*9*), pregnenolone (up to 100 μM) did not modify the equilibrium binding of the radiolabeled CB_1 receptor agonists [^{3}H]CP55,940 and [^{3}H]WIN 55,212-2 (fig. S2A). In contrast, pregnenolone (between 10 nM and 1 μM, depending on the cellular model) inhibited the increase in P-Erk1/2^{MAPK} and the decrease in cellular and mitochondrial respiration induced by THC (*27*) (fig. S2, B to F). This range of pregnenolone concentrations is compatible with the ones (between 10 and 80 ng/g, approximately 30 and 250 nM, respectively) that are observed after THC injections (Fig. 1 and fig. S1) or pregnenolone injections at behaviorally active doses (fig. S3). Pregnenolone up to 1 μM did not decrease the THC-induced reduction of adenosine 3′,5′-monophosphate (cAMP).

These effects suggest that pregnenolone acts as a signaling-specific negative allosteric modulator. Synthetic negative allosteric modulators of CB_1 receptors have been described to display signaling pathway specificity (*28*, *29*). However, these drugs increase agonist binding affinity to the CB_1 receptor, increase agonist-induced Erk1/2^{MAPK} phosphorylation, and inhibit CB_1 agonist–induced inhibition of adenylyl cyclase (*28*, *29*). One possible explanation of these differences is that synthetic antagonists bind to a structural pocket that is devoid of a physiological binding function. In contrast, the endogenous negative allosteric modulator pregnenolone probably binds to a different, evolution-selected, physiologic binding pocket. By using the Forced-Biased Metropolis Monte Carlo (MMC) simulated annealing program (*9*, *30*), we found a potential binding pocket for pregnenolone in the lipid facing the TMH1/TMH7/Hx8 region of the CB_1 receptor (fig. S4A). This binding pocket was validated using a mutant hCB1 receptor (*9*) that contained a point mutation that should forbid the binding of the ketone end of pregnenolone to the CB_1 receptor (fig. S4B). Pregnenolone lost its inhibitory effects on THC-induced decrease in cellular respiration in cells transfected with the mutant hCB1 receptor (fig. S4E).

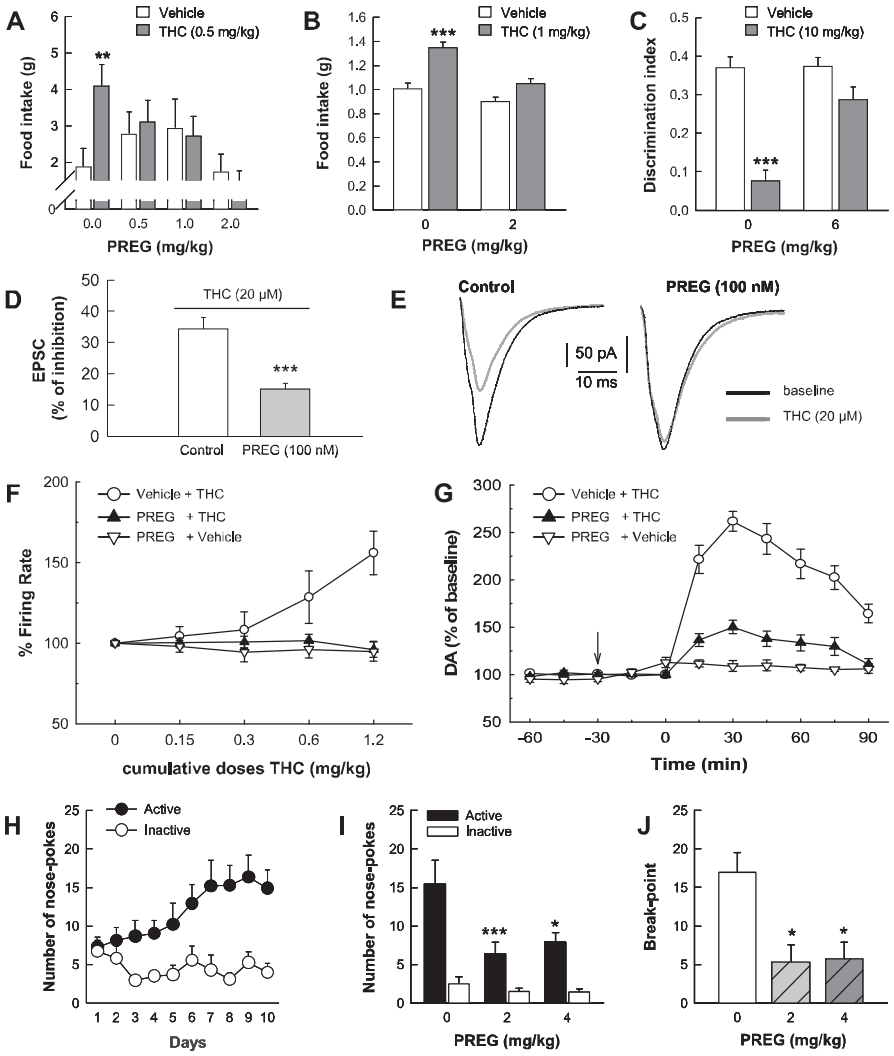

Fig. 4. Pregnenolone inhibits behavioral and neurobiological effects of cannabinoid drugs. Pregnenolone injections inhibited the increase in food intake in (**A**) ad libitum fed Wistar rats [$F(3,94) = 3.65$, $P < 0.02$] and (**B**) 24-hour food-deprived C57Bl/6N mice, as well as (**C**) the memory impairment [$F(3,23) = 24.6$, $P < 0.001$] induced by THC in C57Bl/6N mice. (**D**) Bath application of THC (20 μM) inhibited glutamatergic synaptic transmission in NAc principal neurons in brain slices obtained from adult Sprague-Dawley rats (controls, $n = 8$). This effect was reduced when brain slices were preincubated with pregnenolone 100 nM ($n = 9$). (**E**) Synaptic current traces from representative experiments averaged during baseline and after 40 min of THC exposure. Pregnenolone injections (2 mg/kg, sc, 30 min before THC) in Sprague-Dawley rats decreased the THC-induced increase in (**F**) the firing rate of VTA dopaminergic neurons [$F(4,48) = 8.33$, $P < 0.001$] and in (**G**) the dopamine outflow in the NAc [$F(10,120) = 20.28$, $P < 0.001$]. THC was administered intravenously at escalating cumulative doses (0.15, 0.3, 0.6, and 1.2 mg/kg) infused at 1-min intervals. (**H**) CD1 mice acquired intravenous self-administration of the cannabinoid agonists WIN 55,512-2 (0.0125 mg/kg per infusion) as shown by the higher number of nose pokes in the active device (hole) than in the inactive one [$F(1,18) = 38.3$, $P < 0.001$]. (**I**) After acquisition, the injection of pregnenolone (2 or 4 mg/kg, sc) decreased the number of responses in the active device. (**J**) Pregnenolone also decreased the motivation for WIN 55,512-2, as measured by the reduction in the break point in a progressive ratio schedule. Data are expressed as mean ± SEM. (A) to (C) ($n = 6$ to 12 animals per group), (F) and (G) ($n = 6$ or 7 animals per group), (H) to (J) ($n = 8$ animals per group). The arrow indicates the time of pregnenolone injection, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle-treated controls.

The results presented here provide an example of an unforeseen paracrine/autocrine loop, through which brain steroids can control the activity of a G protein–coupled receptor (GPCR). Thus, CB_1 receptor stimulation increases brain pregnenolone levels, which in turn exerts a negative feedback on the activity of the CB_1 receptor, antagonizing most of the known behavioral and somatic effects of THC. Pregnenolone probably acts as a signaling-specific negative allosteric modulator binding to a site distinct from that occupied by orthosteric ligands. Pregnenolone, similarly to some of the other previously described allosteric modulators (*31*, *32*), does not modify agonist binding but only agonist efficacy; these effects are compatible with the allosteric two-state model (*31*).

Other drugs of abuse also increased pregnenolone levels, but such an increase was in a much lower range of concentrations than the ones induced by THC, which suggests a different mechanism of action. However, most drugs of abuse also modify the activity of the endocannabinoid system (*33*) and could increase pregnenolone through an indirect activation of the CB_1 receptor. This seemed to be the case for cocaine, whose effects on pregnenolone were blocked by pretreatment with a CB_1 antagonist (fig. S5).

Although pregnenolone has been considered an inactive precursor, our data indicate that pregnenolone, and not its downstream-derived neurosteroids, inhibits the effects of THC that are mediated by the CB_1 receptors. Thus, in mice, the administration of THC or of pregnenolone, in the range of behaviorally active doses (2 to 8 mg/kg), did not modify pregnenolone downstream-active steroids such as allopregnanolone (figs. S1 and S3). In addition, the administration of allopregnanolone did not modify behavioral responses to THC, such as THC-induced food intake (fig. S6).

An increasing number of synthetic allosteric modulators of GPCRs have been described (*28*, *29*, *31*, *32*, *34*, *35*). However, whether endogenous allosteric modulators physiologically regulate the activity of GPCRs has been questioned (*31*). Recently, the lipid lipoxin A4 has been proposed as a positive allosteric modulator of CB_1 receptors, suggesting that endogenous modulation of endocannabinoid signaling is a physiological process (*36*). Our findings confirm and extend this hypothesis, uncovering an endogenous negative allosteric modulator of the CB_1 receptor and revealing one of the possible functions of endogenous negative allosterism: the control of GPCR overactivation.

Allosteric modulators may offer several advantages as therapeutic drugs (*31*, *32*, *34*, *35*). Allosteric modulators do not modify the activity of the receptors per se but enhance or attenuate the effects of endogenous or exogenous ligands. Allosteric drugs can also be signaling-specific, thereby regulating only some of the functions of the receptor. As such, they respect the physiology of the target system, can modify only the signaling pathway involved in the disease, and have a more targeted action than orthosteric compounds (*31*, *32*, *34*, *35*).

In comparison with orthosteric antagonists, drugs with the pharmacological profile of pregnenolone could have supplementary advantages for the treatment of drug dependence. When used at high doses, which effectively block the activity of the target receptor, orthosteric antagonists often induce a profound discomfort that is not well tolerated by patients. Lower doses of orthosteric antagonists are also not practical, because their reversible antagonism can be overcome by taking higher doses of the drug. Signaling pathway–specific allosteric inhibitors, such as pregnenolone, should be better tolerated because they do not produce an inhibition of all CB_1 receptor activities, and their effects cannot be overcome by increasing drug intake. This new understanding of the role of pregnenolone has the potential to generate new therapies for the treatment of cannabis dependence.

References and Notes

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Supplementary Materials

www.sciencemag.org/content/343/6166/94/suppl/DC1
Materials and Methods
Figs. S1 to S10
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References

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