How might cannabinoids influence sexual behavior?

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Marijuana and hashish are widespread drugs of abuse that contain Δ^9-tetrahydrocannabinol (THC), a bioactive ingredient best known for its psychotropic effects. Remarkably, THC also produces multiple nonpsychotropic effects: for example, analgesia, hypotension, modulation of bronchospasm, and reduction of inflammation (1–6). That THC also influences sexual behavior was clearly demonstrated for the first time in the early 1980s; yet the precise molecular mechanism of this effect has remained unsolved. In this issue of PNAS, Mani *et al.* (7) revisit these seminal experiments. They identify the molecular target by which THC affects sexual behavior, and unveil a remarkable operative cross talk mechanism between THC and the progesterone and dopamine signaling pathways, which were already known to play a central role in reproductive behavior.

The chemical synthesis of THC was first described in 1964 (8). In the following years, researchers used synthetic THC to study its diverse biological effects. Whether these effects occurred through receptors or simply by changing plasma membrane fluidity was unknown. One early hypothesis, which has now been abandoned, was that THC could directly bind to the estrogen receptor, thus competing for the sequence of events initiated by estrogen (9, 10). Although controversial, this hypothesis had the advantage of stimulating research to ascertain a link between cannabinoids and sexual behavior.

An important step forward in understanding the biological effects of cannabinoids was made in 1988 when Howlett and colleagues (11) used a high-affinity radioactive cannabinoid ligand, [^{3}H]CP-55,940, to demonstrate the existence of a specific cannabinoid binding site in cell membranes of rat brain. This discovery was shortly followed by the molecular cloning and sequencing of the cannabinoid CB1 and CB2 receptors (12, 13). Both receptors are seven transmembrane $G_{i/o}$-coupled receptors that display distinct patterns of tissue expression. CB1 receptors are abundantly expressed in the CNS and to a lesser extent in the periphery, whereas CB2 receptors seem to be exclusively expressed by immune cells (14). It is well known that activation of CB1 receptors modulates the release of various neurotransmitters, which could account for the effects produced by THC on higher cognitive functions (15, 16). Engagement of CB2 receptors expressed by circulating macrophages reduces their immune response, which might account for the anti-inflammatory effect produced by THC intake (6, 17, 18).

An influence of THC on reproductive behavior has been suspected for at least 30 years. Studies carried out in the 1960s reported that chronic oral administration of marijuana resin somehow "significantly reduces fertility" in rats (19). THC can influence reproductive behavior by acting at multiple levels. In males, THC suppresses spermatogenesis, reduces the weight of reproductive organs, decreases the concentration of circulating hormones (such as testosterone) in plasma, and affects some components of sexual behavior. In females, THC prolongs the estrous cycle and decreases the proestrous surge of luteinizing hormone inhibiting ovulation. On the other hand, if THC facilitates sexual behavior: Where does it act? Mani *et al.* (7) address these questions by using ovariectomized rats and quantifying lordosis quotient, one of the well characterized components of sexual receptivity. The inhibitory effect of ovariectomy on lordosis is quite dramatic; however, complete receptive behavior can be restored by exogenous hormone administration, such as estradiol benzoate, an estrogen receptor agonist. Ovariectomized animals treated with high doses of THC alone fail to show lordosis, but relatively low doses of THC significantly increase the lordosis primed by estradiol benzoate. By using antagonists against either CB1 or CB2 receptors, namely SR141617A and SR14528, Mani *et al.* (7) demonstrate that the effect produced by THC on sexual behavior occurs through engagement of CB1 receptors. This finding is consistent with the fact that CB1 receptors are expressed in the hypothalamus, in particular at the level of the ventromedial nucleus (20, 21). Future studies using CB1 knockout mice could unambiguously demonstrate the involvement of CB1 receptors in the THC-induced lordosis (22, 23). At this point, however, it was necessary to address the noninvolvement of CB2 receptors, as at least one study suggested their presence in the CNS (24). Indeed, Mani *et al.* show that SR14528 does not antagonize the effect produced by THC on lordosis.

Mani *et al.* identify the molecular target by which THC affects sexual behavior, and unveil a remarkable operative cross talk mechanism between THC and the progesterone and dopamine signaling pathways.

A bell-shaped curve response is often observed with *in vivo* administration of THC. In this study, Mani *et al.* (7) give an interesting rationale for this phenomenon observed on lordosis quotient. At high concentrations, such as 400 ng injected i.c.v., THC reduces motor activity. Thus, the smaller lordosis quotient observed at high concentrations of THC might be attributed to the fact that the females are less mobile, which is obviously important for this behavior.

It has been shown that the estradiol benzoate-induced lordosis is also increased by progesterone and dopamine (25). The temporal pattern of hormone levels, as well as the behavioral receptivity in intact or ovariectomized estradiol benzoate- and progesterone-primed rats is consistent with an important role for both progesterone and estrogen in the control of sexual receptivity. Interestingly, a molecular mechanism has been proposed for this cross talk between progesterone and estrogen, in which progestin receptors could directly interact with estrogen receptors to activate MAP kinase (26). Dopamine is also a crucial part of this cross talk mechanism (25), acting through D1B (also known as dopamine D5 receptors) (27, 28). Mani *et al.* (7) push this idea one step further and explore the possibility that CB1 receptors

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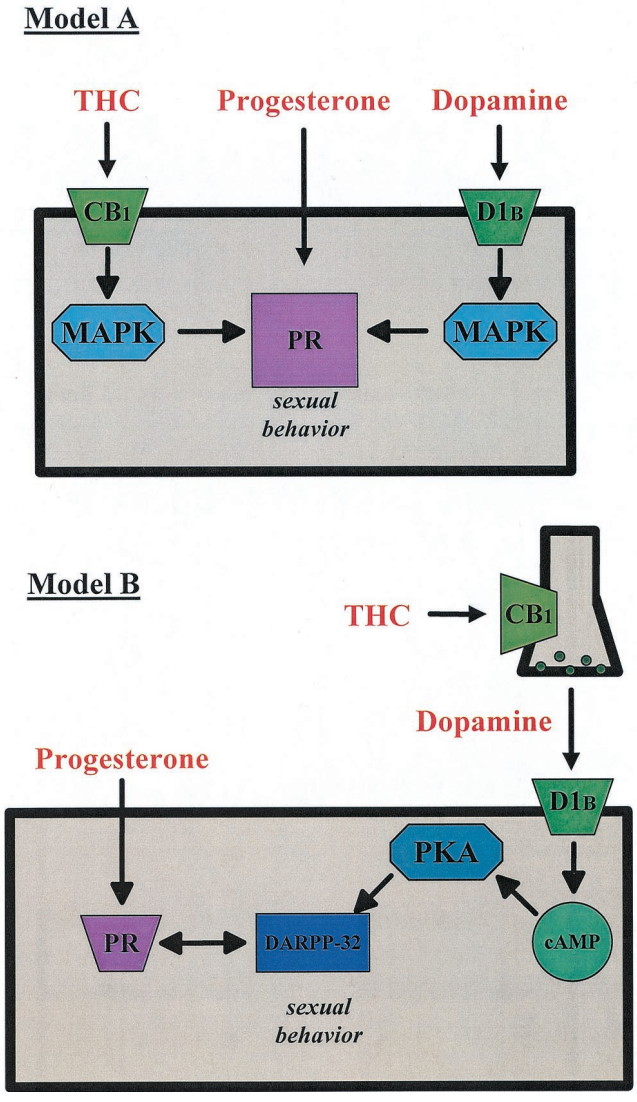

Fig. 1. Two models that could account for the molecular mechanism underlying the cross talk between Δ^9-tetrahydrocannabinol (THC), progesterone, and dopamine. (*A*) THC, progesterone, and dopamine act through CB1, progestin (PR), and dopamine D1B receptors, respectively. If the same target cell expresses these three receptors, a cross talk that involves mitogen-activated protein kinase (MAPK) could take place. (*B*) THC acts through CB1 receptors expressed on dopaminergic terminals, increasing the release of dopamine. Dopamine would then activate D1B receptors, which in turn stimulate the cAMP–protein kinase A, a pathway leading to the phosphorylation of dopamine and cAMP regulated phosphoprotein 32 (DARPP-32) (29).

are also part of this operative cross talk mechanism modulating sexual behavior. These results are schematized in Fig. 1. Progesterone acts through progestin receptors. By using receptor antagonists and antisense oligonucleotides, they demonstrate that the increased lordosis induced by THC requires functional progestin and D1B receptors. Furthermore, progesterone's effect requires operative CB1 and D1B receptors. Finally, increased lordosis quotient induced by the D1/D5 agonist, which had previously been shown to require progestin receptors (25), also requires operative CB1 receptors.

Having identified the receptor subtype by which THC, progesterone, and the D1 agonist SKF 38393 increases the lordosis quotient, Mani *et al.* (7) propose two models that could account for the molecular mechanism underlying this cross talk. In Fig. 1*A*, the MAP kinase signaling pathway is activated by CB1 and D1B receptors, which could then phosphorylate coactivators of progestin receptors. In Fig. 1*B*, activation of CB1 receptors modulates the release of dopamine, which in turn activates D1B receptors. The cross talk between D1B and the progestin receptor could occur within the same target cell; possibly through the dopamine and cAMP regulated phosphoprotein 32 (DARPP-32) as a common target protein.

The presence of cannabinoid receptors in different tissues, and the diversity of the biological effects produced by THC suggest the presence of distinct endogenous cannabinoid signaling systems. Two cannabinoid receptors have been identified at the molecular level, with a possible third cannabinoid receptor that has been pharmacologically pinpointed (30). Two endogenous cannabinoid ligands, namely anandamide and 2-arachidonoylglycerol, have been identified, with at least two additional candidates (31–35). One of the most exciting question in the field of cannabinoid research is to understand the functional role of the endogenous cannabinoid signaling system (36). How, where, and under which circumstances are these endogenous cannabinoid ligands produced? Are there different endogenous cannabinoid signaling systems for different biological functions? Since THC influences sexual behavior, which endogenous cannabinoid ligand is involved in modulating this biological function through CB1 receptors? It has recently been shown that levels of anandamide fluctuate during the ovarian cycle in both the hypothalamus and the pituitary gland (37). Could anandamide be involved in regulating sexual behavior? These are only some of the questions opened by Mani *et al.* (7).

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