Ventral Tegmental Area Cannabinoid Type-1 Receptors Control Voluntary Exercise Performance

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Background: We have shown that the endogenous stimulation of cannabinoid type-1 (CB_1) receptors is a prerequisite for voluntary running in mice, but the precise mechanisms through which the endocannabinoid system exerts a tonic control on running performance remain unknown.

Methods: We analyzed the respective impacts of constitutive/conditional CB_1 receptor mutations and of CB_1 receptor blockade on wheel-running performance. We then assessed the consequences of ventral tegmental area (VTA) CB_1 receptor blockade on the wheel-running performances of wildtype (gamma-aminobutyric acid [GABA]-$CB_1^{+/+}$) and mutant (GABA-$CB_1^{-/-}$) mice for CB_1 receptors in brain GABA neurons. Using in vivo electrophysiology, the consequences of wheel running on VTA dopamine (DA) neuronal activity were examined in GABA-$CB_1^{+/+}$ and GABA-$CB_1^{-/-}$ mice.

Results: Conditional deletion of CB_1 receptors from brain GABA neurons, but not from several other neuronal populations or from astrocytes, decreased wheel-running performance in mice. The inhibitory consequences of either the systemic or the intra-VTA administration of CB_1 receptor antagonists on running behavior were abolished in GABA-$CB_1^{-/-}$ mice. The absence of CB_1 receptors from GABAergic neurons led to a depression of VTA DA neuronal activity after acute/repeated wheel running.

Conclusions: This study provides evidence that CB_1 receptors on VTA GABAergic terminals exert a permissive control on rodent voluntary running performance. Furthermore, it is shown that CB_1 receptors located on GABAergic neurons impede negative consequences of voluntary exercise on VTA DA neuronal activity. These results position the endocannabinoid control of inhibitory transmission as a prerequisite for wheel-running performance in mice.

Key Words: CB_1 receptors, dopamine, GABA, physical exercise, ventral tegmental area, wheel running

Regular physical activity is beneficial to health, including mental health (1–3). Although this finding is repeatedly publicized, physical inactivity remains a major issue (4,5). Physical inactivity is multifactorial, and the inability to experience pleasure with exercise is considered one cause for the lack of adherence to, or dropout from, exercise programs (6). Systems governing reward-based motivation, such as the mesocorticolimbic dopamine (DA) system (7–9), might thus have an impact on voluntary exercise performance. Although voluntary wheel running in laboratory rodents models only certain aspects of human adherence to exercise (10), it is worthy of mention that wheel running has rewarding properties (10,11). This observation fits with the ability of wheel running to elicit temporary changes in DA neuronal activity in the ventral tegmental area (VTA) (12), the origin of the mesocorticolimbic DA system.

The endocannabinoid system (ECS), formed by cannabinoid type-1 (CB_1) and type-2 (CB_2) receptors, their endogenous ligands (endocannabinoids), and the machinery for endocannabinoid synthesis/degradation (13), controls wheel-running behavior. Thus, compared with wildtype littermates ($CB_1^{+/+}$), mutant mice lacking CB_1 receptors ($CB_1^{-/-}$) display a 30% to 50% decrease in wheel running (14,15), but not in locomotor (16), activity. The quest for the mechanisms underlying this observation is complex given the plethora of ECS-regulated functions. CB_1 receptors, which are mainly located on neuronal presynaptic terminals and on glial cells in numerous brain regions, are retrogradely stimulated by endocannabinoids released "on demand" from postsynaptic cells (13,17). This stimulation reduces the presynaptic release of several neurotransmitters (e.g., gamma-aminobutyric acid [GABA], glutamate), providing to the ECS a key position in the regulation of central nervous system functions (13,17–19). Several of these, including motor control (20), metabolism (19,21), pain (22), mood (23), and reward seeking (24,25), might be involved in running performance. As for reward seeking, the ECS, through CB_1 receptors located on VTA GABAergic and glutamatergic terminals that synapse onto DA neurons (26,27), modulates mesocorticolimbic dopaminergic activity (27–31). Hence, the ECS controls the motivation to seek rewards (24,25,31), but whether this rule includes voluntary running is unknown.

This study investigated the mechanisms underlying the involvement of CB_1 receptors in wheel-running behavior. We show that CB_1 receptors located on VTA GABAergic terminals exert a permissive control on running performance. Moreover, the absence of CB_1 receptors from GABAergic neurons is associated with inhibitory consequences of running on VTA DA neuronal activity, suggesting that the ECS might play a pivotal role in voluntary exercise.

Methods and Materials

Animals

All experiments complied with the French and European rules on animal experimentation. This study involved 2-month-old

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male C57BL/6N mice and 2- to 3-month-old male CB_1 receptor mutant and wildtype animals, including constitutive CB_1 receptor mutant mice ($CB_1^{-/-}$), conditional mutant mice lacking CB_1 receptors from principal neurons (CaMK-$CB_1^{-/-}$), brain GABAergic neurons (GABA-$CB_1^{-/-}$), cortical glutamatergic neurons (Glu-$CB_1^{-/-}$), serotonergic neurons (TPH2-$CB_1^{-/-}$), dopamine D1 receptor-expressing neurons (D1-$CB_1^{-/-}$), glial fibrillary acidic protein-expressing astrocytes (GFAP-$CB_1^{-/-}$), and their respective wildtype littermates (32–37) (see Methods in Supplement 1 for additional details). The influence of Cre expression in the wheel-running performance of GABA-$CB_1^{-/-}$ mice was assessed by using Dlx5/6-Cre-positive and Dlx5/6-Cre-negative (wildtype) mice (33,35). Although Dlx5/6-Cre is thought to confer a forebrain-specific deletion of CB_1 receptors from GABAergic neurons (38) (Figure S1 in Supplement 1), the possibility that CB_1 receptors are deleted in other GABAergic neuronal populations cannot be excluded (see Discussion).

Wheel-Running Protocols

On arrival, all mice were housed with 25-cm diameter running wheels (IntelliBio, Nomeny, France) (14) set free for limited/unlimited periods or blocked permanently (controls). The wheels were connected to a computer that recorded all running variables (distance covered, running duration and maximal speed; ActiviWheel software, IntelliBio). These wheels were unlocked 3 hours per day, except in one series of experiments (unlimited access to the wheels). The 3-hour daily running episodes always started at the onset of the dark phase (7:00 PM) of the 12-hour light–dark cycle, excepted from several experiments in which wheels were set free from 12:00 AM to 3:00 AM or from 4:00 AM to 7:00 AM (see below and Methods in Supplement 1).

Injection and Infusion Protocols

Systemic injections and surgery for bilateral cannulae implantation always occurred when mice reached their plateau level of performance (sixth–seventh day). Intracerebral infusions were performed at least 1 week after surgery when mice displayed their presurgery body weight and wheel-running performances. Mice were injected/infused with the drug (or the vehicle) and 2 to 3 days later with the vehicle (or the drug). In all cases, the effects of drugs and vehicles on wheel-running performance were gone by the following day.

Drug Infusion in the VTA

Mice were anaesthetized by the intraperitoneal injection of a mixture of ketamine/xylazine, and placed into a stereotaxic apparatus (David Kopf Instruments, Tujunga, California). Mice were bilaterally implanted with 2.7-mm stainless cannulae targeting the VTA with the following coordinates: anteroposterior −3.0; lateral ± .5; dorsoventral −4.7 (39). The cannulae were secured with dental cement, and the mice were allowed to recover for at least another week. Drugs were bilaterally infused over 2 min and the injectors left in place for 1 min to allow further diffusion (see Methods in Supplement 1).

Locomotor Activity

Locomotor activity was assessed in soundproof cubicles hosting individual cages equipped with food, water, and infrared sensors allowing to detect horizontal crossings (Imetronic, Pessac, France) (35). All animals were housed therein 5 to 7 days before testing (see Methods in Supplement 1).

In Vivo Electrophysiology

Mice were housed 1 day (acute running) or 1 to 3 weeks (repeated running) with wheels that were either permanently locked (controls) or set free 3 hours per day (4:00–7:00 AM; runners). Electrophysiologic experiments, which started 3 to 4 hours or 24 hours after the end of the running session, were performed as described previously (40,41) (see also Methods in Supplement 1).

Drugs

Rimonabant and JZL195 were from Caiman Chemical (Interchim, Montluçon, France). AM251, O-2050, JWH133, and AM630 were from Tocris Bioscience (Bristol, United Kingdom). Δ^9-tetrahydrocannabinol (THC) was from Sigma-Aldrich (Saint Quentin Fallavier, France). All drugs and their respective vehicles were prepared and administered according to standard methods (see Methods in Supplement 1).

Statistics

All analyses were performed with GB-Stat software (version 10; Dynamic Microsystems, Silver Spring, Maryland). Comparisons were achieved by means of Student *t* tests (two-group comparisons) and analyses of variance with/without repeated factors (multiple-group comparisons). In the latter case, post hoc group comparisons (Tukey's test) were performed only if interactions between main variables were found significant. When necessary, data were log-transformed to reach homogeneity of the variances.

Results

CB_1 Receptors on Brain GABAergic Neurons Control Voluntary Running

Mutant mice lacking CB_1 receptors from the whole body ($CB_1^{-/-}$) and their wildtype littermates ($CB_1^{+/+}$), bred as in Dubreucq *et al.* (14), were offered 3-hour daily access to running wheels for 2 weeks. This paradigm allowed observation of plateau levels of performance in both genotypes (Figure 1A). $CB_1^{-/-}$ mice displayed low running activity, compared with $CB_1^{+/+}$ mice [$F(1,35) = 8.22$, $p = .007$], the amplitude of this difference increasing with the number of running sessions [$F(13,455) = 3.23$, $p = .0001$ for the genotype × time interaction] (Figure 1A). Repeated intraperitoneal injections (30 min before the onset of wheel-running sessions on Days 1–8) with 3 mg/kg of the CB_1 receptor antagonist rimonabant (SR141716) to C57BL/6N mice mimicked the negative impact of the CB_1 receptor mutation [$F(13,130) = 27.49$, $p < .0001$; Figure 1B]. The inhibitory effect of rimonabant was rapidly reversible and was not accounted for by an interaction with wheel-running acquisition. Thus, rimonabant also decreased wheel running when acutely administered to mice that had reached their maximal daily performance (Figure 1B). Conversely, rimonabant administration to mice housed in cages equipped with activity detectors did not affect locomotor activity (71.8 ± 13.7 beam brakes/3 hours; $n = 5$), compared with vehicle treatment (69.2 ± 12.2 beam brakes/3 hours; $n = 5$). To ensure that CB_1 receptors mediated rimonabant effects on running, rimonabant was administered to $CB_1^{+/+}$ and $CB_1^{-/-}$ mice. Besides genotype differences [$F(1,12) = 15.17$, $p = .0021$], this experiment revealed that acute rimonabant reduced running performances in $CB_1^{+/+}$ mice but not in $CB_1^{-/-}$ mice [$F(1,12) = 12.47$, $p = .0041$ for the genotype × rimonabant interaction; Figure 1C].

These results indicated that CB_1 receptors exert a tonic stimulatory influence on voluntary running. To identify the