European Journal of Pharmacology 721 (2013) 64–69

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

Diuretic effects of cannabinoid agonists in mice

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ARTICLE INFO

Article history:
Received 27 April 2013
Received in revised form
17 September 2013
Accepted 26 September 2013
Available online 5 October 2013

Keywords:
Cannabinoid
Diuresis
Δ^9-Tetrahydrocannabinol
THC
Mice
Bladder dysfunction

ABSTRACT

Cannabinoids both increase urine output and decrease urinary frequency in human subjects. However, these effects have not been systematically evaluated in intact mice, a species commonly used to evaluate the effects of novel cannabinoids. The present studies investigated whether cannabinoid agonists reliably produce diuresis in mice at doses comparable to those that produce other cannabinoid effects and, further, identified the receptors that may mediate these effects. Diuretic effects were measured in male mice over 6 h. In some studies, urine was collected and analyzed for electrolyte measurements. In other studies, agonist injections were preceded by pretreatment with cannabinoid CB_1 or CB_2 selective antagonists, including a peripherally constrained CB_1 antagonist. Companion studies evaluated the antinociceptive effects of the cannabinoid agonists in a warm-water tail-withdrawal assay. Direct-acting cannabinoid CB_1 agonists Δ^9-tetrahydrocannabinol (THC), WIN 55,212, AM7418 and AM4054, had biphasic effects on diuresis, with peak diuretic effects occurring at lower doses than peak antinociceptive effects. Cannabinoid diuresis was similar to κ-opioid agonist-induced diuresis in terms of maximum effects with only moderate loss of Na^+. Antagonism studies indicate that the diuretic effects of cannabinoids are CB_1-receptor mediated, with both central and peripheral components. These findings suggest that mice may provide a model for understanding the mixed effects of marijuana on urine output, as described in clinical studies, and aid in the development of targeted cannabinoid based therapies for bladder dysfunction.

1. Introduction

The principal psychoactive constituent of marijuana, Δ^9-tetrahydrocannabinol (THC), was identified and synthesized in the early 1960s (Mechoulam, 1970). Since then, many synthetic analogues of THC have been discovered and their behavioral and physiological effects have been characterized in laboratory animals using various in vivo procedures. Among these different effects, diuretic responses to THC have been anecdotally reported but rarely been systematically evaluated (Pryor et al., 1977). One early clinical study by Ames (1958) reported a 3-fold increase in the magnitude of urine output after THC administration and a more thorough investigation in rats suggested that THC-evoked diuretic responses were greater than those produced by thiazide diuretics (Sofia et al., 1977). A recent report from our laboratory confirmed these early findings in rats, and further, demonstrated that cannabinoid-induced diuresis in rats is mediated by cannabinoid CB_1 receptors (Paronis et al., 2013).

Although our previous results indicate that cannabinoid agonists produce their diuretic effects in rats primarily by actions at cannabinoid CB_1 receptors, more specific roles for centrally or peripherally located CB_1 receptors were not explored (Paronis et al., 2013). Cannabinoid CB_1 receptors are found throughout the body, including within the central nervous system (CNS) as well as in the lower urinary tract of humans, mice and other commonly used laboratory animals (Pertwee and Fernando, 1996; Walczak et al., 2009) and early studies suggested that THC increased urine output by actions both in the CNS as well as in peripheral systems (Barry et al., 1973; Sofia et al., 1977). In contrast to the increase in urine output observed in awake, un-instrumented animals, more recent cystometry studies in anesthetized rats have reported that WIN 55,212 increased micturition thresholds and decreased bladder motility, suggesting a role for peripheral cannabinoid CB_1 receptors in potentially decreasing diuresis (Dmitrieva and Berkley, 2002). These studies have identified a potential role for peripheral cannabinoid CB_1 receptors in the urinary tract of unconscious rodents or in isolated bladder tissue (Walczak et al., 2009) yet their function in either micturition or diuresis in intact mice has, to the best of our knowledge, not been evaluated

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http://dx.doi.org/10.1016/j.ejphar.2013.09.053

previously. Indeed, diuresis has not been identified as a quantitative or qualitative measure of cannabinoid effect in mice. Hence, the studies described here characterize the diuretic effects of cannabinoid agonists in mice and, further, identify roles for both central and peripheral cannabinoid CB_1 receptors in mediating these effects.

Our results extend previous reports in rats and humans by showing that cannabinoid agonists produce diuresis in intact mice. Our results further uniquely demonstrate that these effects are biphasic for all cannabinoid agonists tested, and suggest that the increases in urine output produced after administration of low to moderate cannabinoid doses occur by actions at cannabinoid CB_1 receptors within the CNS while decreases in urine output produced at higher doses may also involve actions at peripheral cannabinoid CB_1 receptors. Finally, we show here that the increased urine output after cannabinoids is weakly naturetic without affecting excretion of Cl^- or K^+. Further studies addressing the mechanisms of cannabinoid induced diuresis may reveal new insights into the role of cannabinoid receptors in maintaining water homeostasis.

2. Material and methods

2.1. Animals

Male CD-1 mice, weighing 20–25 g at the start of the study (Charles River Laboratories, Wilmington MA), were housed 4/cage in a climate controlled vivarium with food and water available ad libitum. Mice were acclimatized to the animal facility for 7 days, and to study procedures twice, prior to testing. Mice were re-used with a minimum 7 day interval between drug testing. Each group or data point in the paper represents $n=6$–8 mice. All experiments were performed during the light portion of the light/dark cycle. All studies were approved by the Northeastern University Animal Care and Use Committee, in accordance with guidelines established by the National Research Council.

2.2. Diuresis

Urine output was measured over 6 h during which mice did not have access to food and water. Mice were placed on an elevated grid floor and isolated under a plastic cup (10 cm × 5 cm; d × ht); weigh boats were placed underneath each mouse to collect the voided urine. Voided urine was measured by determining the change in weight of the boats every 2 h to minimize volume loss due to evaporation. Mice were used for 4–8 weeks; doses of drugs and vehicle were always randomized to minimize time dependent bias. Except where noted, injections were delivered s.c. in volumes of 10 ml kg^{-1}. When drugs were studied in combination, doses were delivered in half volumes, e.g., for antagonism studies 30 min pretreatment with 5 ml kg^{-1} vehicle or antagonist was followed by 5 ml kg^{-1} injection of the agonist.

2.3. Measurement of urine pH, Na^+, K^+ and Cl^-

The total urine voided by individual mice over 6 h was collected, weighed, transferred to eppendorf tubes, and stored at −4 °C until analysis. The samples were diluted (1:5 in deionized water) and urine pH and Na^+, K^+, and Cl^- concentrations were measured using ion selective microelectrodes according to manufacturer's protocol (Lazar Research Laboratory, Inc, Los Angeles, CA, USA). Total amounts of each electrolyte were quantified for each 6 h sample using the formula: 5 × diluted sample concentration (μEq/ml) × total volume (ml) of sample.

2.4. Antinociception

Antinociceptive responses were determined using a warm water tail-withdrawal assay. A water bath maintained water temperature at 52.0 ± 0.5 °C. Each mouse was gently hand held and the distal 2–3 cm of its tail immersed in the water; latency to tail-withdrawal was measured using a stopwatch and a cut-off time of 8 s was established to avoid tissue damage. Baseline latencies were determined twice on each test day with a 10 min interval; only mice with baseline latencies of 1–3 s were used in drug studies. Complete dose response curves were generated in each mouse using cumulative dosing procedures similar to those described previously (Paronis and Woods, 1997). Briefly, 30 min (morphine, WIN 55,212-2 and pentobarbital) or 60 min (vehicle, THC, AM7418 and AM4054) after an injection, tail-withdrawal latencies were determined and mice were then injected with the next dose, such that the total cumulative dose was increased by 0.25 or 0.5 log units. This procedure was repeated until the tail-withdrawal latency reached the cut-off or no longer increased with subsequent increase in dose of the test drug.

2.5. Drugs

Δ^9-THC and rimonabant were obtained from the National Institute on Drug Abuse [(NIDA), Rockville, MD]; WIN-55-212 [((*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo [1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate], U50,488 [*trans*-(±)3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl]-cyclohexyl)-benzeneacetamide methane sulfate] and furosemide were purchased from Sigma-Aldrich (St. Louis, MO). AM7418 [Butyl-2-[(6a*R*, 10a*R*)-6a, 7, 10, 10a-tetrahydro-1-hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6*H*-benzo[c]chromen-3-yl]-2-methylpropanoate], AM-4054 [9β-(hydroxymethyl)-3-(1-adamantyl)-hexahydrocannabinol], AM6545 [5-(4-(4-cyanobut-1-yn-1-yl)phenyl-1-(2,4-dichlorophenyl)-*N*-(1,1-ioxothiomorpholino)-4-methyl-1*H*-pyrazole-3-carboxamide] and AM630 [6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl) methanone] were synthesized at the Center for Drug Discovery, Northeastern University. Morphine and U50,488 were dissolved in saline; furosemide was dissolved in 1% 1 N NaOH and sterile water; all other compounds were prepared in 5% ethanol, 5% emulphor-620 (Rhodia, Cranbury, NJ) and 90% saline, and further diluted with saline. Drug doses are expressed in terms of the weight of free base.

2.6. Statistical analysis

Tail withdrawal latencies are expressed as a percentage of maximum possible effect (%MPE), calculated using the formula: %MPE = [(test latency − baseline latency)/(8 − baseline latency)] × 100. To determine ED_{50} values for diuresis, 50% of the maximum effect was defined using the formula: [((maximum urine output with the drug − urine output with vehicle)/2) + urine output with vehicle]. ED_{50} values were calculated using linear regression when more than two data points were available, and otherwise were calculated by interpolation. All drug data were plotted and analyzed using log transformed values of doses. Data were analyzed using one way ANOVA followed by Dunnett's or Bonferroni's multiple comparison tests; significance for all tests was set at $P \leq 0.05$.

3. Results

3.1. Quantifying diuresis

Mice that received sham injections voided, on average, 4 ml kg^{-1} urine whereas mice that were injected with 10 or 30 ml kg^{-1} saline