

Diuretic Effects of Cannabinoids

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ABSTRACT

In vivo effects of cannabinoid (CB) agonists are often assessed using four well-established measures: locomotor activity, hypothermia, cataleptic-like effects, and analgesia. The present studies demonstrate that doses of CB agonists that produce these effects also reliably increase diuresis. Diuretic effects of several CB agonists were measured in female rats over 2 hours immediately after drug injection, and results were compared with hypothermic effects. Direct-acting CB1 agonists, including Δ^9 -tetrahydrocannabinol, WIN 55,212 [R-(1)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate], AM2389 [9 β -hydroxy-3-(1-hexyl-cyclobut-1-yl)-hexahydrocannabinol], and AM4054 [9 β -(hydroxymethyl)-3-(1-adamantyl)-hexahydrocannabinol], produced dose-dependent increases in diuresis and decreases in colonic temperature, with slightly lower ED₅₀ values for diuresis than for hypothermia. The highest doses of cannabinoid drugs yielded, on average,

26–32 g/kg urine; comparable effects were obtained with 10 mg/kg furosemide and 3.0 mg/kg *trans*-(-)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (U50-488). Methanandamide (10.0 mg/kg) had lesser effect than other CB agonists, and the CB2 agonist AM1241 [1-(methylpiperidin-2-ylmethyl)-3-(2-iodo-5-nitrobenzoyl)indole], the anandamide transport inhibitor AM404, and the CB antagonist rimonabant did not have diuretic effects. In further studies, the diuretic effects of the CB1 agonist AM4054 were similar in male and female rats, displayed a relatively rapid onset to action, and were dose-dependently antagonized by 30 minutes pretreatment with rimonabant, but not by the vanilloid receptor type I antagonist capsazepine, nor were the effects of WIN 55,212 antagonized by the CB2 antagonist AM630 [(6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl) methanone]. These data indicate that cannabinoids have robust diuretic effects in rats that are mediated via CB1 receptor mechanisms.

Introduction

Cannabinoid CB1 receptor ligands often are studied for their ability to concomitantly produce four effects in mice; decreases in locomotor activity, hypothermia, antinociception, and immobility (Martin et al., 1991; Wiley et al., 2007; Wiley et al., 2012). Δ^9 -Tetrahydrocannabinol (THC) and related compounds also induce diuresis, but this has received scant attention, even though early clinical reports of the effects of cannabis in humans included anecdotal observations of increases in diuresis (Allentuck and Bowman, 1942; Parker and Wrigley, 1947; Stockings, 1947). In one such study, voiding rates in human subjects were specifically measured, with urine volumes averaging 320% of control values following cannabis ingestion (Ames, 1958). In a subsequent study in hydrated rats, oral Δ^9 -THC was found to produce diuretic effects equivalent to or greater than those of

hydrochlorothiazide (Sofia et al., 1977). Surprisingly, the diuretic effects of cannabinoids were not studied further until reports began to appear that suggested symptoms of urinary urgency and incontinence in patients with multiple sclerosis were attenuated after cannabis use (Consroe et al., 1997). A more recent pilot study with cannabis extracts confirmed the earlier findings of decreased urinary frequency and urine volume, and led to speculation that cannabinoids may be useful in treating cystitis or other lower urinary tract dysfunction (Brady et al., 2004; Apostolidis, 2012). These results contrast with the earlier reports and suggest that cannabinoids may have mixed effects on diuresis or that the observed effect—diuretic or anti-diuretic—may vary in different patient populations.

Despite the paucity of data regarding exogenous cannabinoids and diuresis, a role for endocannabinoids, particularly anandamide, in urinary function has been identified. As with the effects of cannabis, both increases and decreases in urine loss have been reported with anandamide, and mechanisms through which these different effects are mediated appear to be complex. Endocannabinoids influence the release of urine through multiple mechanisms that may include vanilloid receptor type I (TRPV1) or cannabinoid CB1 and CB2 receptors,

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ABBREVIATIONS: AM4054, 9 β -(hydroxymethyl)-3-(1-adamantyl)-hexahydrocannabinol; AM2389, 9 β -hydroxy-3-(1-hexyl-cyclobut-1-yl)-hexahydrocannabinol; AM1241, 1-(methylpiperidin-2-ylmethyl)-3-(2-iodo-5-nitrobenzoyl)indole; AM630, (6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl)(4-methoxyphenyl) methanone; Δ^9 -THC, Δ^9 -tetrahydrocannabinol; TRPV1, vanilloid receptor type I; WIN 55,212, R-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate; U50-488, *trans*-(-)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide.

which are all found throughout the lower urinary tract (Avelino and Cruz, 2006; Tyagi et al., 2009; Strittmatter et al., 2012). For example, anandamide may increase bladder contractility, especially in inflamed tissues; these effects are blocked by coadministration of capsazepine, indicating involvement of TRPV1 receptors (Dinis et al., 2004; Avelino and Cruz, 2006). On the other hand, increasing levels of anandamide after local administration of a fatty acid amide hydrolase inhibitor also lead to decreased contractility of normal bladder tissue—effects that are antagonized by CB1 and CB2 selective antagonists (Strittmatter et al., 2012). Such opposing actions have led to the suggestion that the net regulatory effects of anandamide on bladder function result from a balance of CB1, CB2, and TRPV1 effects (Dinis et al., 2004; Avelino and Cruz, 2006).

In addition to their complex actions on urine storage and release, endocannabinoids may increase urine production by both peripheral and central actions. Thus, direct infusion of methanandamide into the renal medulla increases urine flow rate, suggesting that endocannabinoids alter urine production at the level of the kidney (Li and Wang, 2006). Work from other laboratories suggests that both anandamide and 2-arachidonylglycerol, released from the hypothalamus, may modulate the release of hypophysial hormones, including vasopressin (Di et al., 2005; Soya et al., 2005). In keeping with data from these separate studies, it is worth noting that Sofia and colleagues (Sofia et al., 1971) similarly suggested that the diuretic actions of oral Δ^9 -THC were mediated both peripherally and within the central nervous system. In summary, exogenous and endogenous cannabinoids clearly modulate urinary function, although their effects and the mechanisms mediating these effects may differ depending on the site of action and the experimental conditions.

Many synthetic cannabinoid ligands have been developed in the past 20 years, including compounds such as CP55,940 and WIN55,212 (Devane et al., 1988; Compton et al., 1992); however, potential diuretic effects of these drugs have not been previously evaluated. The present studies were undertaken to compare the net diuretic effects of systemic administration of cannabinoid agonists in conscious rats. Results show that CB1 agonists dose-dependently increase diuresis concomitantly with hypothermic effects, and further studies with the nonselective agonist AM4054 (Thakur et al., 2012) reveal that these effects are antagonized by rimonabant. Overall, our data indicate that diuresis is a CB1-mediated effect that may serve as a reliable and objective physiologic measure of cannabinoid action in rats; the circumstances under which these results represent a potential therapeutic benefit or potential liability of cannabinoids remain to be determined.

Materials and Methods

Subjects. Adult female Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 200–350 g and adult male Sprague-Dawley rats weighing 530–770 g were used. Animals were group-housed in a climate-controlled vivarium with a 12-hour light/dark cycle (lights on at 7 AM). Subjects had unrestricted access to food and water outside experimental sessions. All studies were approved by the McLean Hospital and Northeastern University Institutional Animal Care and Use Committees.

Diuresis Studies. After initial exposure to handling procedures, rats were placed in customized restraint devices made of polyvinyl chloride tubing. Individual absorbent pads, placed in each restraint device, were weighed before and after the experiment; the difference

in pad weight was recorded as the weight (in grams) of voided urine. Unless otherwise noted, sessions lasted 2 hours.

Temperature Measurements. After initial exposure to handling procedures, thermal probes (YSI, No. 401) were inserted rectally to a depth of 7 cm, 30 minutes before drug administration. The probes were secured to the tails with porous tape, and the animals were placed in individual chambers measuring $30 \times 12 \times 12$ cm. Two baseline temperature readings were recorded before drug injection, and temperature was recorded every 30–60 minutes for 6 hours after injection. The change in temperature was determined for each rat by subtracting temperature readings from the mean of the 2 baseline measures.

Drugs. Δ^9 -THC and rimonabant were obtained from the National Institute on Drug Abuse (Rockville, MD); sodium pentobarbital and furosemide were purchased from Sigma-Aldrich (St. Louis, MO), capsazepine and WIN-55-212 were purchased from Tocris Bioscience (Ellisville, MO), and U50,488 was obtained from the Upjohn Company (Kalamazoo, MI; now Pfizer Inc.). AM4054, AM2389, AM404, AM630, and methanandamide were synthesized at Northeastern University, as previously described (Abadji et al., 1994; Beltramo et al., 1997; Nikas et al., 2010; Thakur et al., 2012), and AM1241 was a generous gift of MAK Scientific (West Mystic, CT). Pentobarbital and U50,488 were dissolved in saline; furosemide was dissolved in 1% 1N NaOH and sterile water. All other compounds were prepared in 20% ethanol, 20% alkamuls-620 (Rhone-Poulenc, Princeton, NJ), and 60% saline and were further diluted with saline. Except where noted, injections were delivered subcutaneously in volumes of 1 ml/kg body weight; drug doses are expressed in terms of the weight of the free base. At least three days separated each drug test.

Data Analysis. Group means and S.E.M. were calculated for each treatment condition, and statistical analysis was completed using GraphPad Prism, version 5.03 (GraphPad Software, San Diego California). Drug effects were compared with the effects of saline with use of one-way analysis of variance procedures with $P < 0.05$, followed by Dunnett's multiple comparison t test. To facilitate comparisons of drug potency across different effects, dose-effect functions were constructed with the dependent variable expressed as a percentage of a particular effect (i.e., loss of 35 g/kg urine or decreases in body temperature of -6°C were designated as 100% effect). Slopes of dose-effect functions were calculated using linear regression of the ascending portion of the curves when more than two data points were available and, otherwise, were calculated by interpolation. Dose effect functions were tested for parallelism, and ED₅₀ values, 95% confidence limits, and dose ratio values were calculated from the log-transformed values.

Results

Effects of Water-Loading and Noncannabinoid Diuretics. Rats that received no treatment or that were subcutaneously injected with 1.0 ml/kg saline or vehicle voided a mean of 0.9 ± 0.2 g urine over 2 hours. Hydrating the rats with 10.0–30.0 ml/kg, p.o. water immediately before the session significantly increased diuresis, resulting in 4–9 g of voided urine or 12.5–27.5 g urine per kg body weight (Fig. 1A). In nonhydrated rats, both the loop diuretic furosemide and the κ -opioid agonist U50,488 produced dose-related increases in diuresis and, at the highest doses, resulted in urine volumes similar to those obtained in rats that had received 30.0 ml/kg water (Fig. 1B).

Diuretic and Hypothermic Effects of Cannabinoids. The cannabinoid agonists Δ^9 -THC; WIN 55,212; AM4054; and AM2389 increased urine output in a dose-dependent manner, with maxima of 26–32 g/kg urine after the highest doses (Fig. 2). Likewise, each of the cannabinoid agonists significantly and dose-dependently decreased temperature. When the data are expressed as a percentage of a maximum effect (35 g/kg urine

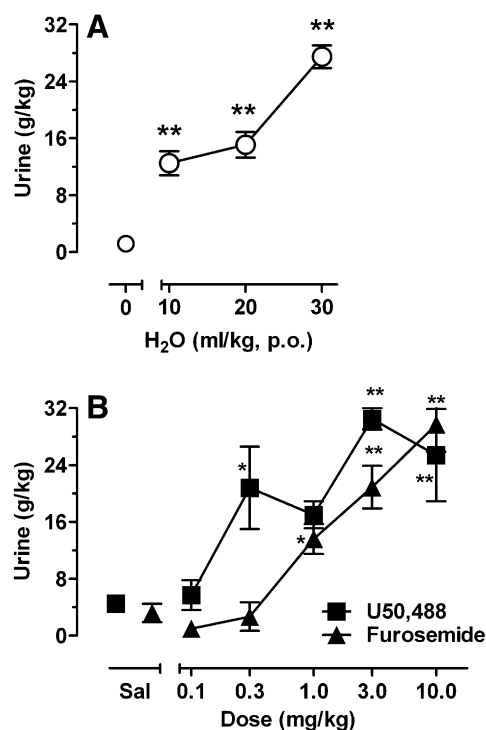


Fig. 1. (A) Diuretic effects produced by p.o. injection of water ($n = 12$). (B) Diuretic effects produced by furosemide or U50-488 ($n = 6$). Abscissae: Volumes of water (A) or doses of drug (B) in milligrams per kilogram bodyweight; points to the left, above 0 or sal represent effects obtained after s.c. saline injection. Ordinates: Urine volume expressed as grams per kilogram bodyweight. Symbols and associated vertical lines represent the mean and S.E.M.; asterisks indicate values significantly different from saline control; * $P < 0.05$, ** $P < 0.01$.

or -6°C ; not shown), the dose-effect functions for each of the four agonists across the two procedures are parallel. ED_{50} values calculated from these functions reveal an order of potency of $\text{AM2389} \geq \text{AM4054} > \text{Win55,212} \geq \Delta^9\text{-THC}$ for diuretic and hypothermic effects. Differences in potency across the two effects were small; however, for all four drugs, diuretic effects occurred at slightly lower doses than did hypothermic effects (Table 1). To evaluate potential sex differences in the diuretic effects of cannabinoids, the effects of AM4054 were compared in male and female rats; AM4054 significantly increased diuresis in both male and female rats, with no evidence of significant sex differences (Table 2).

In contrast to the effects of the cannabinoid agonists, the cannabinoid antagonist rimonabant had neither diuretic nor hypothermic effects (Fig. 2). Rimonabant also did not have antidiuretic effects in rats that had been preloaded with 10.0–30.0 ml/kg water (Table 3). Although diuresis was slightly increased after 0.3 mg/kg rimonabant in rats prehydrated with 30.0 ml/kg water, this effect was not dose-related and further increases in dose to 1.0 and 3.0 mg/kg did not reveal any significant increase in diuresis. Among other cannabinergic drugs that were evaluated for diuretic effects, only methanandamide increased voided urine and, these effects were observed only with a single dose, 10 mg/kg. In contrast to other CB1 agonists displaying intermediate effects, a 3-fold increase in dose, to 30 mg/kg of methanandamide, did not correspondingly increase urine output but, instead, reduced diuresis to control levels. The remaining drugs, including the CB2 selective cannabinoid agonist AM1241 and

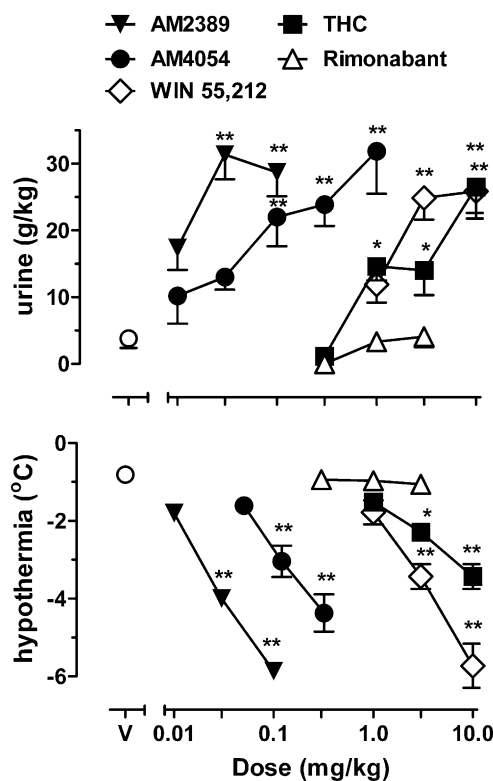


Fig. 2. Effects of AM4054; $\Delta^9\text{-THC}$; AM2389; WIN 55,212; and rimonabant on diuresis (top) and hypothermia (bottom) ($n=6$ per drug); points above V represent the effects of vehicle, averaged from all groups. Bottom ordinate represents peak hypothermic effects obtained within 6 hours after drug injections and are expressed as a change from baseline values (mean, 38.55°C ; range, $37.60\text{--}39.01^{\circ}\text{C}$); other details as in Fig. 2.

the endocannabinoid transport inhibitor AM404, were without effect on urine output (Table 4).

Diuresis and hypothermia after 0.1 or 0.3 mg/kg AM4054 were compared with saline and 10 mg/kg furosemide at different times over a 6-hour period. AM4054 had a very quick onset to action, and relative to saline, AM4054 significantly increased urine loss within 30 minutes after injection, with maximum values obtained 60–120 minutes after injection. In contrast, significant decreases in body temperature occurred later, with peak effects occurring more than 2 hours after injection (Fig. 3). To determine whether fluid loss contributes, at least in part, to the later onset of hypothermic effects, the diuretic and hypothermic effects of pentobarbital given alone and in combination with 10.0 mg/kg furosemide were determined. Pentobarbital, at 10.0–30.0 mg/kg, did not produce diuresis (Table 4) but did decrease temperature ($F_{3,20} = 9.13$; $P < 0.05$); peak hypothermic effects of 30.0 mg/kg pentobarbital, a decrease in temperature of $5.2 \pm 1.0^{\circ}\text{C}$, occurred 2.5–3 hours after injection with a recovery toward baseline

TABLE 1

ED_{50} values (in mg/kg) and 95% confidence interval for diuretic and hypothermic effects in female rats ($n = 6$)

Variable	AM4054	$\Delta^9\text{-THC}$	WIN55,212	AM2389
Diuresis	0.06 (0.02–0.12)	3.0 (1.9–5.8)	1.6 (0.8–2.8)	0.010 (0.009–0.016)
Hypothermia	0.10 (0.07–0.15)	6.4 (4.4–11.8)	2.2 (1.5–5.8)	0.019 (0.014–0.024)

TABLE 2

Voided urine in female and male rats ($n = 6$) after injection of saline or AM4054

Values given are the mean (\pm S.E.M.) in grams per kilogram bodyweight.

Sex	Saline	AM4054		
		0.03 mg/kg	0.1 mg/kg	0.3 mg/kg
Female	4.1 (\pm 1.0)	13.0 (\pm 1.9)	22.0 (\pm 4.4)	23.9 (\pm 6.4)
Male	4.6 (\pm 3.1)	9.2 (\pm 3.6)	23.0 (\pm 2.3)	21.7 (\pm 4.1)

temperatures within 6 hours. The dose of 10.0 mg/kg furosemide alone had no effects on body temperature but did produce significant diuresis within 30 minutes after injection (see Fig. 3). Coadministration of 30.0 mg/kg pentobarbital and 10.0 mg/kg furosemide did not have additive effect in either measure. Thus, rats that received both pentobarbital and furosemide voided 27.2 ± 5.1 g/kg urine and had peak hypothermic effects of $5.3 \pm 0.6^\circ\text{C}$ that recovered toward baseline values within 6 hours after injection.

Antagonism Studies. Rimonabant antagonized the diuretic effects of AM4054; 0.1, 1.0, and 3.0 mg/kg rimonabant produced 5–15-fold rightward shifts of the AM4054 dose-effect function, suggesting that competitive interactions occur between the drugs (Fig. 4 and Table 5). However, the effects of AM4054 were not diminished by 0.3 mg/kg rimonabant, suggesting that a more complex relationship than simple competitive antagonism underlies the diuretic effects of these drugs given in combination. The effects of 0.1–0.3 mg/kg AM4054 after pretreatment with 0.3 mg/kg rimonabant were redetermined in another groups of rats, and again, there was no attenuation of the diuretic effects of AM4054. To further evaluate contributions of other receptor systems, the effects of \sim ED₅₀ doses AM4054 and WIN 55,212 were re-determined in the presence of the TRPV1 antagonist capsazepine or the CB2 antagonist AM630, respectively. Results show that a 15-minute pretreatment with 10.0 mg/kg capsazepine did not enhance or reduce the effects of 0.1 mg/kg AM4054 (Fig. 5A) and a 30-minute pretreatment with 10.0 mg/kg AM630 did not alter the effects of 3.0 mg/kg WIN 55,212 (Fig. 5B).

Discussion

Products of the cannabis plant were used as diuretics in ancient India, and early laboratory studies on the effects of cannabis or Δ^9 -THC reported that phytocannabinoids increase amounts of voided urine in both rats and humans (Ames, 1958; Sofia et al., 1977; Touw, 1981). However, recent advances in cannabinoid pharmacology, including the development of synthetic cannabinoid agonists, have neglected

TABLE 3

Effects of rimonabant on urine loss in female rats ($n = 6$ –11) after preloading with 10 or 30 ml/kg water

Values given are the mean \pm S.E.M. (in grams per kilogram bodyweight).

Preload	Saline	Rimonabant		
		0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
10 ml/kg H ₂ O	6.2 \pm 1.3	8.3 \pm 2.5	5.2 \pm 1.2	10.9 \pm 1.3
30 ml/kg H ₂ O	28.4 \pm 2.7	39.6 \pm 5.8*	21.8 \pm 1.5	29.8 \pm 1.9

* $P < 0.05$

further study of the diuretic effects of cannabinoids. The present studies demonstrate that synthetic cannabinoid agonists can dose-dependently increase diuresis in rats. Similar effects were obtained with the cannabinoids Δ^9 -THC, AM2389, and AM4054 and with an aminoalkylindole, WIN55,212; thus, the effects are not unique to a particular chemical class (Compton et al., 1992; Nikas et al., 2010; Thakur et al., 2012). The diuretic effects produced by cannabinoids are comparable to those of the loop diuretic furosemide and the κ -opioid agonist U50,488. The effects of all four cannabinoid agonists were dose-related for all drugs, and moderate diuresis appeared at lower doses. However, the maximum diuretic effects of the four compounds were rather high and corresponded to the effect of preloading rats with 30.0 ml/kg water. It remains to be determined whether diuresis represents a potential new therapeutic use for cannabinoids or, instead, an additional clinical liability. Nevertheless, the present studies clearly show that urine output provides an easily obtained physiologic measure of the effects of CB1 agonists and, consequently, a robust and objective metric with which to assess the effects of novel cannabinoid agonists.

Hypothermia is one of four measures commonly used to describe the effects of novel cannabinoid agonists in vivo (Martin et al., 1991; Wiley et al., 2007) and was included in the present study as an objective comparative measure of cannabinoid effects. Hypothermia has been characterized previously as a CB1 receptor-mediated effect, based on evidence that decreases in body temperature produced by Δ^9 -THC; CP55,940; and WIN 55,212 are blocked by the CB1-selective antagonist rimonabant and, furthermore, that CB1 receptor knockout mice do not exhibit cannabinoid-produced hypothermia (Rinaldi-Carmona et al., 1994; Compton et al., 1996; Zimmer et al., 1999; De Vry et al., 2004; McMahon and Koek, 2007). Of the drugs studied here, Δ^9 -THC and AM4054 have equal affinity for CB1 and CB2 receptors; AM2389 is CB1 receptor-preferring, with \sim 25-fold higher affinity at CB1, compared with CB2 receptors; and WIN 55,212 is CB2 receptor-preferring, with \sim 20-fold higher affinity at CB2, compared with CB1 receptors (Felder et al., 1995; Nikas et al.,

TABLE 4

Voided urine after injection with listed drugs in female rats ($n = 6$ except saline, $n = 6$ –12)

Values given are the mean \pm S.E.M. (in grams per kilogram bodyweight).

Drug	Saline	0.1 mg/kg	1.0 mg/kg	3.0 mg/kg	10.0 mg/kg	30 mg/kg
Methanandamide	3.3 \pm 0.8	ND	ND	1.3 \pm 0.4	11.4 \pm 2.9*	1.6 \pm 1.1
AM404	5.8 \pm 1.1	ND	5.3 \pm 1.6	7.5 \pm 1.5	3.8 \pm 1.4	ND
AM1241	3.9 \pm 0.8	8.8 \pm 1.1	6.1 \pm 2.2	ND	4.1 \pm 2.1	ND
Pentobarbital	4.1 \pm 1.1	ND	ND	ND	1.6 \pm 0.8	0.0 \pm 0.0

ND, not determined.

* $P < 0.01$

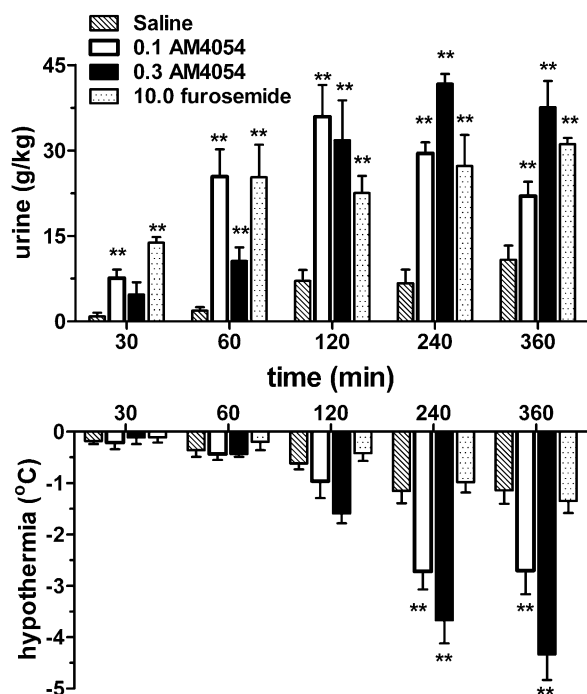


Fig. 3. Time course of diuretic and hypothermic effects of saline, 0.1–0.3 mg/kg AM4054, and 10 mg/kg furosemide ($n=6$ per drug). Abscissae: Time since injection (in minutes); other details as in Fig 2.

2010; Thakur et al., 2012). Despite their different selectivities for CB1 and CB2 receptors, the four drugs produced diuresis and hypothermia with the same order of potency, suggesting that the effects are mediated by the same receptor. Given the evidence that hypothermia is mediated by CB1 receptors, it is therefore likely that diuresis is also a CB1 receptor-mediated effect. Antagonism studies with rimonabant provide more evidence that the diuretic effects of cannabinoids in rats are mediated primarily by CB1 receptors. Thus, AM4054-induced diuresis was antagonized by doses of rimonabant similar to those used to antagonize numerous behavioral effects of

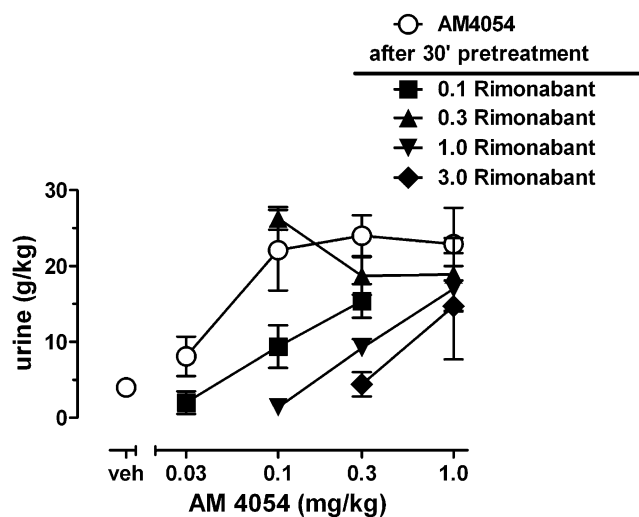


Fig. 4. Effects of AM4054 alone (open symbols) or after 30-minute pretreatment with rimonabant; other details as in Fig 2.

TABLE 5

ED₅₀ values with 95% confidence intervals (CI; in mg/kg) and potency ratios for AM4054-induced diuresis after 30 minutes pretreatment with rimonabant

Treatment	ED	95% CI	Potency Ratio
AM4054 alone	0.09	0.05–0.17	–
+0.1 mg/kg rimonabant	0.44	0.24–2.00	4.7
+0.3 mg/kg rimonabant	ND	ND	ND
+1.0 mg/kg rimonabant	1.07	0.68–2.66	11.6
+3.0 mg/kg rimonabant	1.36	ND	14.7

ND, not determined.

Δ^9 -THC and other cannabinoid ligands, including suppression of locomotor activity, antinociception, catalepsy, hypothermia, discriminative stimulus effects, and self-administration (Wiley et al., 2001; Järbe et al., 2005; Justinova et al., 2005; McMahon, 2006; McMahon and Koek, 2007).

Although it is likely that the mechanisms underlying cannabinoid-induced diuresis primarily involve CB1 receptor activation, a role for other pharmacological mechanisms cannot be completely dismissed. Antagonism of diuresis by rimonabant was generally dose-related, with the exception that 0.3 mg/kg rimonabant did not alter the position of the AM4054 dose-response function. These results were surprising because this dose of rimonabant has previously been shown to attenuate the analgesic, cataleptic, and hypothermic effects of cannabinoid agonists in mice and rats (De Vry et al., 2004; McMahon and Koek, 2007). Rimonabant has been described as an inverse agonist producing effects opposite to those of cannabinoid agonists (e.g., potentiating smooth muscle contractions, stimulating adenyl cyclase, and decreasing GTP γ S binding) (Meschler et al., 2000; Sim-Selley et al., 2001; Makwana et al., 2010). Inverse agonist activity might explain a lack of a stepwise, competitive interaction between AM4054 and rimonabant, although there is little evidence of inverse agonist effects of rimonabant *in vivo*. For example, in the present studies, one might predict that an inverse agonist would have antidiuretic effects; however, in rats preloaded with either 10 or 30 ml/kg water, there was no

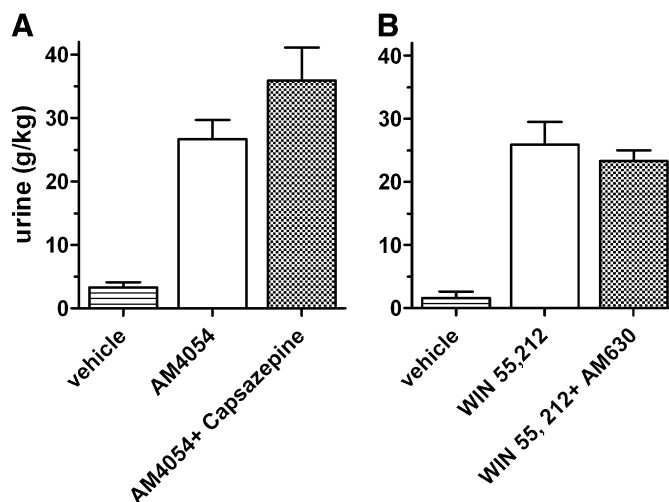


Fig. 5. (A) Diuretic effects after injection of vehicle, 0.1 mg/kg AM4054, or 0.1 mg/kg AM4054 after a 15-minute pretreatment with 10 mg/kg capsaizepine. (B) Diuretic effects after injection of vehicle; 3.0 mg/kg WIN 55,212; or 3.0 mg/kg WIN 55,212 after a 30-minute pretreatment with 10 mg/kg AM630; other details as in Fig 2.

evidence of any antidiuretic activity of rimonabant. Indeed, in one instance, rimonabant—of note, with the same dose of 0.3 mg/kg—unexpectedly increased the amount of voided urine. When coupled with the biphasic effects obtained with methanandamide, our results suggest that the diuretic effects of cannabinoids involve more than simple direct agonist effects at one receptor type. Other potential targets for cannabinoid activity include direct effects on CB2 receptors or alteration of endocannabinoid activity. A role for both CB1 and CB2 receptors and TRPV1 receptors has been implicated in mediating the effects of endocannabinoids on bladder contractility (Dinis et al., 2004; Walczak et al., 2009; Strittmatter et al., 2012); furthermore, at least one report has suggested that endocannabinoids alter urine production through a noncannabinoid, nonvanilloid mechanism in rats (Li and Wang, 2006). At present, we have no explanation for these results, although our data with the selective CB2 ligands AM1241 and AM630, the TRPV1 antagonist capsazepine, and the endocannabinoid transport inhibitor AM404 do not support a major role for any of these systems in exocannabinoid-induced diuresis.

In the present report, the loss of urine is referred to as diuresis, as opposed to micturition; however, it is unclear whether cannabinoids increase urine production, stimulate the release of urine from the bladder, or both. Nonetheless, the present data suggest that the effects reported here represent more than simple micturition, as the highest doses of cannabinoids resulted in urine loss within 2 hours that is similar to 24-hour urine loss in untreated female Sprague-Dawley rats [unpublished observations and (Powers, 2001)]. The rapidity with which cannabinoids produce their full diuretic effects was surprising. Although many effects of cannabinoids appear within minutes and are measured within the first hour after drug injection (Martin et al., 1991; Wiley et al., 2007), it often takes several hours for cannabis-related drugs to reach their peak effects (Davis et al., 1973; Schlosburg et al., 2009). The present findings indicate that maximum diuresis preceded the peak hypothermic effects of cannabinoids, which occurred more than 2 hours after injection. The fast onset of diuresis, coupled with the marginally lower doses required for diuretic than hypothermic effects, suggest that fluid loss may contribute to the hypothermic effects of cannabinoid drugs. However, the finding that pentobarbital-induced hypothermia is not altered by coadministration of furosemide and the lack of hypothermia after furosemide alone demonstrates that hypothermic and diuretic effects can occur separately. The clear separation of diuresis and hypothermia after noncannabinoid treatment may distinguish diuresis from other behavioral measures of cannabinoid activity. Thus, cannabinoid effects commonly measured in previous studies—catalepsy, decreased locomotion, antinociception, and hypothermia—are difficult to view as completely separate phenomena. Three of the four measures involve decreased movement, and consequently, the different assays are often treated as one basic measure, referred to as the tetrad test or cannabinoid tetrad (Bosier et al., 2010; Brents et al., 2012). The finding that cannabinoids also dose-dependently increase diuresis importantly provides a separate and independent physiologic response that can serve as a robust and reliable measure of CB1-mediated actions.

In conclusion, the present results demonstrate that behaviorally active doses of exogenous cannabinoids also produce

profound diuretic effects in female and male rats. These results suggest that diuresis may occur separately, yet simultaneously, with other measures of cannabinoid activity in laboratory animals and, perhaps, in humans. Antagonism of cannabinoid diuretic effects by rimonabant, coupled with a lack of effect of non-CB1 ligands, indicates that these effects are mediated primarily by CB1 receptors; however, other mechanisms may contribute to these physiologic effects. The implications of these findings currently are poorly understood, although a better understanding of mechanisms and sites of action by which cannabinoids increase urine loss may lead to the rational development of novel cannabinergic medications.

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Authorship Contributions

Participated in research design: Paronis.

Conducted experiments: Paronis.

Contributed new reagents: Makriyannis, Vemuri, Thakur, Bajaj, Nikas.

Performed data analysis: Paronis.

Wrote or contributed to the writing of the manuscript: Paronis, Bergman.

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