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Role of TRPV1 and cannabinoid CB_1 receptors in AM 404-evoked hypothermia in rats

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Abstract

AM 404 inhibits endocannabinoid uptake and enhances the cannabinoid CB_1-mediated effects of endogenous cannabinoids. Accumulating evidence also suggests that AM 404 acts at sites other than the endocannabinoid system. One site is the transient receptor potential vanilloid 1 cation channel (TRPV1). A useful endpoint for discriminating between TRPV1- or CB_1-mediated effects of AM 404 is hypothermia. This is because TRPV1 or CB_1 receptor activation produces a significant hypothermia in rats. The present study investigated the effects of AM 404 (1, 5, 10 and 20 mg/kg, i.p.) on body temperature in rats and the involvement of TRPV1 and CB_1 receptors in the effects of AM 404. Doses of 10 and 20 mg/kg of AM 404 produced significant hypothermia. Pre-treatment with capsazepine (30 mg/kg, i.p.) blocked the hypothermia caused by 10 and 20 mg/kg of AM 404. Pre-treatment with SB 366791 (2 mg/kg, i.p.), a new TRPV1 antagonist, also abolished the hypothermia evoked by AM 404 (20 mg/kg, i.p.). In contrast, pre-treatment with SR 141716A (Rimonabant), a CB_1 antagonist, or AA-5-HT, a fatty acid amide hydrolase (FAAH) blocker, did not affect AM 404-evoked hypothermia. The present data demonstrate that AM 404 evokes a significant hypothermia in rats that is dependent on TRPV1 receptor activation.

Keywords: AM 404; SB 366791; Capsazepine; Vanilloid; Rimonabant; SR 141716A; Hypothermia; Cannabinoid

1. Introduction

Drugs which alter the uptake and hydrolysis of endogenous cannabinoids are attractive alternatives to marijuana-based therapeutics (Devane et al., 1992; Di Marzo, 1999; Dinh et al., 2002; Freund et al., 2003). One such drug is AM-404 [*N*-(4-hydroxyphenyl)-arachidonylethanolamide]. AM 404 blocks endogenous cannabinoid transport across cell membranes and enhances the cannabinoid CB_1 receptor-mediated effects of endogenous cannabinoids (Beltramo et al., 1997, 2000; Calignano et al., 1997). Despite these effects on endocannabinoid transport, numerous examples in the literature suggest that AM-404 acts at sites other than the endocannabinoid system. These sites include transient receptor potential vanilloid 1 cation channels (TRPV1), calcium channels, sodium channels, cannabinoid receptors, and a novel *N*-acyl fatty acid-sensitive receptor (Zygmunt et al., 2000; Kelley and Thayer, 2004; Nicholson et al., 2003; Hajos et al., 2004; Chen et al., 2001; Jonsson et al., 2003; van der Stelt and Di Marzo, 2003). Moreover, AM 404 is formed from acetaminophen in vivo (Hogestatt et al., 2005). This evidence raises the possibility that AM 404 contributes to the analgesic and antipyretic actions of acetaminophen.

Prior work indicates that AM 404 is a full agonist at rat and human recombinant TRPV1 receptors (De Petrocellis et al., 2000; Ross et al., 2001). TRPV1 receptors mediate a number of biological effects caused by AM 404 in vitro and in vivo (Jennings et al., 2003; Rodella et al., 2005; Cabranes et al., 2005). For example, when tested in a rat model of Huntington's disease, AM 404 reduces the increased ambulation displayed in the open-field test by rats with 3-nitropropionic acid lesions (Lastres-Becker et al., 2002, 2003). Pre-treatment with capsazepine, but not SR141716A, a cannabinoid CB_1 antagonist, prevented the antihyperkinetic effect of AM 404 (Lastres-Becker et al., 2003). Another study demonstrates that AM 404 increases glutamate release from rat spinal cord slices by activating TRPV1 receptors (Yue et al., 2004). At the cellular level, AM 404 activates TRPV1 receptors to cause a

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concentration-dependent increase in intracellular calcium concentration (Jerman et al., 2002). AM 404 also causes concentration-dependent relaxations in segments of rat isolated hepatic artery contracted with phenylephrine, and the effects of AM 404 are abolished by capsazepine (Zygmunt et al., 2000). Those data support a role for TRPV1 receptors in the pharmacological actions of AM 404.

The goal of the present study is to determine the effect of AM 404 on body temperature in rats and to determine if TRPV1 or CB_1 receptors mediate the effects of AM 404. We hypothesized that AM 404 would produce a significant hypothermia that would be antagonized by TRPV1 receptor antagonists. We report findings that confirm these hypotheses, indicating that TRPV1 receptor activation is necessary for AM 404 to cause hypothermia in rats.

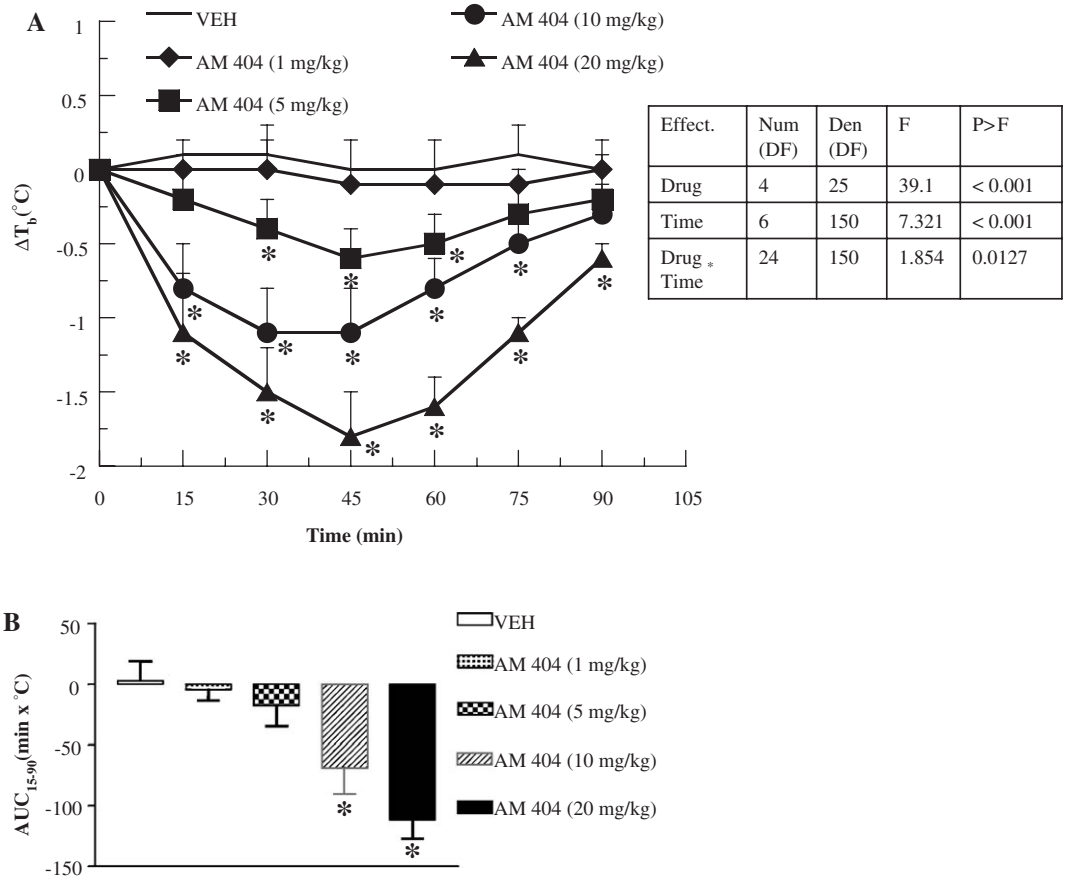
2. Materials and Methods

2.1. Animals

All animal use procedures were conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Temple University Animal Care and Use Committee. Male Sprague–Dawley rats (Zivic-Miller, Pittsburgh, PA, USA) weighing 150–200 g were housed 1 per cage for a minimum of 5 days before experimental use. Rats were maintained on a 12-h light/dark cycle and fed rat chow and water ad libitum.

2.2. Drugs

AM 404, anandamide, capsazepine, and SB 366791 were purchased from Tocris-Cookson (St. Louis, MO, USA). SR 141716A was obtained from the National Institutes on Drug Abuse. Arachidonoylserotonin (AA-5-HT), an inhibitor of fatty acid amide hydrolase (FAAH), was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). *N*-(4-Hydroxyphenyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide (AM 404), anandamide, and AA-5-HT were suspended in a 30% cremophor/saline solution and administered intraperitoneally (i.p.) in a volume of 2 ml/kg. Capsazepine, *N*-(3-methoxyphenyl)-4-chlorocinnamide (SB 366791) and SR 141716A (Rimonabant) were dissolved in a vehicle of 20% ethanol, 20% cremophor and 60% saline and administered i.p. in a volume of 1 ml/kg.



Effect.	Num (DF)	Den (DF)	F	P>F
Drug	4	25	39.1	< 0.001
Time	6	150	7.321	< 0.001
Drug * Time	24	150	1.854	0.0127

Fig. 1. AM 404 produces hypothermia in rats. (A) Time course: AM 404 (1, 5, 10 or 20 mg/kg, i.p.) or vehicle (VEH) (i.p.) was injected at 0 min. Data are expressed as the mean ± S.E.M. of the change in body temperature (ΔT_b) from baseline (time 0) and determined from at least 5 animals. Data were analyzed using a two-way (drug, time) mixed-model analysis of variance (ANOVA) with repeated measures on time followed by pair-wise multiple comparisons incorporating the Bonferroni correction at the different time points (15–90 min). The ANOVA table illustrates the main effects and interaction (drug, time, and drug × time). *$P<0.05$, compared to VEH. (B) AUC_{15-90} profile: Area under the body temperature time curve (AUC) was calculated from 15 to 90 min using the difference score from 0 min (trapezoidal rule). *$P<0.05$, compared to VEH.

2.3. Experimental protocol

Between 8 and 9 AM on the morning of the experiment, rats were removed from the Animal Facility and placed randomly, one per cage, into an environmental room. The environmental room was maintained at a constant temperature of 21±0.3 °C and relative humidity of 52±2% throughout our experiments to ensure controlled conditions. Rats were allowed to acclimate to their environment for 60 min prior to measuring the first body temperature value, according to standard procedures in our laboratory (Rawls et al., 2005). Baseline temperature measurements were taken for 90 min at 30 min intervals (−90, −60, −30, and 0 min) using a thermistor probe (YSI series 400, Yellow Springs Instrument Co., Yellow Springs, OH), which was lubricated and inserted approximately 7 cm into the colon. A digital thermometer (Model 49 TA, YSI) was used to record body temperature. Rats were unrestrained during the temperature readings.

2.4. Effect of AM 404 on body temperature in rats

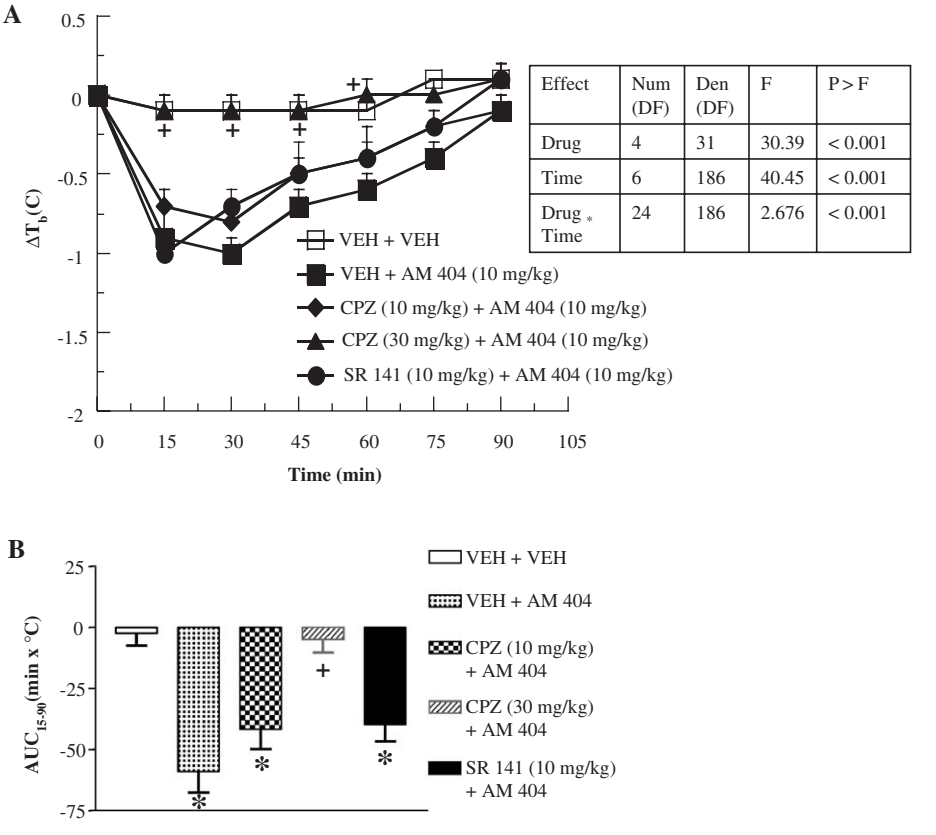
Following a 90-min baseline interval, AM 404 (1, 5, 10 or 20 mg/kg, i.p.) or an equivalent volume of vehicle (i.p.) was injected. Body temperatures were taken 15, 30, 45, 60 and 90 min following the injection of AM 404. Doses of AM 404 were based on previous studies using conscious rats (Lastres-Becker et al., 2002, 2003; Giuffrida et al., 2000).

2.5. Effect of capsazepine and SR 141716A on AM 404-evoked hypothermia in rats

Following a 90-min baseline interval, a hypothermic dose of AM 404 (10 mg/kg, i.p.) or an equivalent volume of vehicle was given. Sixty min prior to AM 404 or vehicle, rats received a pre-treatment of capsazepine (10 or 30 mg/kg, i.p.), SR 141716A (10 mg/kg, i.p.) or vehicle. The dose range and pre-treatment time of capsazepine were based on a previous study which demonstrated that a 90 min pre-treatment with capsazepine (60 mg/kg, i.p.) prevents capsaicin-induced hypothermia in rats (Dogan et al., 2004; Ding et al., 2005). The dose of SR 141716A was based on a previous study in our laboratory (Rawls et al., 2002).

2.6. Effect of capsazepine and SB 366791 on AM 404-evoked hypothermia in rats

Following a 90-min baseline interval, AM 404 (20 mg/kg, i.p.) or vehicle was given. Sixty min before AM 404 or



Effect	Num (DF)	Den (DF)	F	P > F
Drug	4	31	30.39	< 0.001
Time	6	186	40.45	< 0.001
Drug * Time	24	186	2.676	< 0.001

Fig. 2. Capsazepine (CPZ) blocks the hypothermia caused by 10 mg/kg of AM-404 in rats. (A) Time course: AM 404 (10 mg/kg, i.p.) or vehicle (VEH) (i.p.) was injected at 0 min. CPZ (10 or 30 mg/kg, i.p.), SR 141716A (SR 141) (10 mg/kg, i.p.), or VEH (i.p.) was administered 60 min before AM 404. Data are expressed as the mean±S.E.M. of the change in body temperature (ΔT_b) from baseline (time 0) and determined from at least 6 animals. Data were analyzed using a two-way (drug, time) mixed-model analysis of variance (ANOVA) with repeated measures on time followed by pair-wise multiple comparisons incorporating the Bonferroni correction at the different time points (15–90 min). The ANOVA table illustrates the main effects and interaction (drug, time, and drug × time). $^{+}P<0.05$, compared to VEH+AM 404. (B) AUC_{15-90} profile: Area under the body temperature time curve (AUC) was calculated from 15 to 90 min using the difference score from 0 min (trapezoidal rule). $^{*}P<0.05$, compared to VEH+VEH and $^{+}P<0.05$, compared to VEH+AM 404.

vehicle, rats received a pre-treatment of capsazepine (10 or 30 mg/kg, i.p.), SB 366791 (0.5 or 2 mg/kg, i.p.) or vehicle. The doses and pre-treatment time of SB 366791 were based on prior work demonstrating that a 60 min pre-treatment with SB 366791 attenuates capsaicin-evoked hypothermia in rats (Varga et al., 2005; Ding et al., 2005).

2.7. Effect of SR 141716A and SB 366791 on anandamide-evoked hypothermia in rats

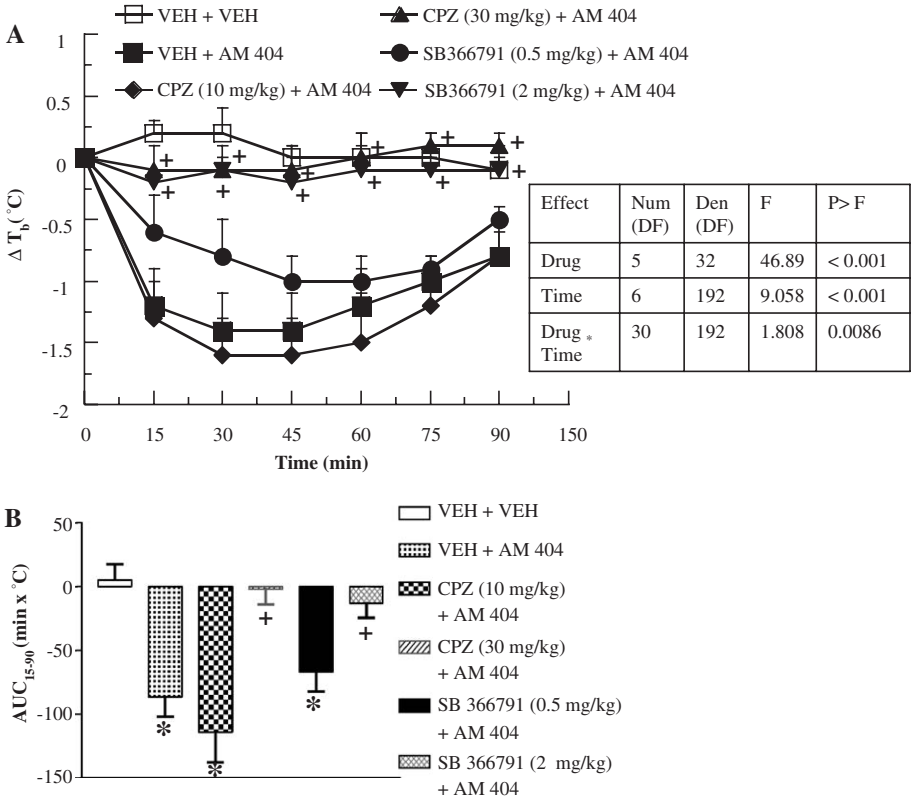
Following a 90-min baseline interval, anandamide (20 mg/kg, i.p.) or vehicle was given. Sixty min before anandamide or vehicle, rats received a pre-treatment of SR 141716A (5 mg/kg, i.p.), SB 366791 (2 mg/kg, i.p.) or vehicle. The dose of anandamide was based on prior work demonstrating that 20 mg/kg causes a significant hypothermia in rats (Costa et al., 1999).

2.8. Effect of AA-5-HT on AM 404-evoked hypothermia in rats

To determine a role for FAAH in AM 404-evoked hypothermia, we injected AM 404 with AA-5-HT. Following a 90-min baseline interval, AM 404 (20 mg/kg, i.p.) or vehicle was given. Sixty min before AM 404 or vehicle, rats received a pre-treatment of AA-5-HT (10 mg/kg, i.p.), or vehicle. The dose of AA-5-HT was based on two previous in vivo studies (de Lago et al., 2005; Suplita et al., 2005).

2.9. Data analysis

Three consecutive temperature readings were then recorded and averaged to establish a baseline temperature prior to drug injection. Data were calculated as the mean ± S.E.M. of the change in body temperature. Prior to analysis, all data were transformed into 'normalized ranks' to address non-normality. Transformed data were analyzed using a two-way (drug, time) mixed-model analysis of variance (ANOVA) with repeated measures on time followed by pair-wise multiple comparisons incorporating the Bonferroni correction at the different time points (15–90 min). Area under the body temperature time curve (AUC) was calculated for each individual rat from 15 to 90 min using the difference score from 0 min (trapezoidal rule). The AUC_{15-90} data were analyzed by a one-way analysis of variance (ANOVA) followed by a Dunnett's or Tukey's post hoc analysis. Values of $P<0.05$ were considered to be statistically significant.



Effect	Num (DF)	Den (DF)	F	P> F
Drug	5	32	46.89	< 0.001
Time	6	192	9.058	< 0.001
Drug * Time	30	192	1.808	0.0086

Fig. 3. SB 366791 and capsazepine (CPZ) block the hypothermia caused by 20 mg/kg of AM-404 in rats. (A) Time course: AM 404 (20 mg/kg, i.p.) or vehicle (VEH) (i.p.) was injected at 0 min. CPZ (10 or 30 mg/kg, i.p.), SB 366791 (0.5 or 2 mg/kg, i.p.), or VEH (i.p.) was administered 60 min before AM 404. Data are expressed as the mean ± S.E.M. of the change in body temperature (ΔT_b) from baseline (time 0) and determined from at least 6 animals. Data were analyzed using a two-way (drug, time) mixed-model analysis of variance (ANOVA) with repeated measures on time followed by pair-wise multiple comparisons incorporating the Bonferroni correction at the different time points (15–90 min). The ANOVA table illustrates the main effects and interaction (drug, time, and drug × time). $^{+}P<0.05$, compared to VEH + AM 404. (B) AUC_{15-90} profile: Area under the body temperature time curve (AUC) was calculated from 15 to 90 min using the difference score from 0 min (trapezoidal rule). $*P<0.05$, compared to VEH + VEH and $^{+}P<0.05$, compared to VEH + AM 404.

3. Results

3.1. AM 404 evokes hypothermia

Fig. 1 illustrates the effects of graded doses of AM-404 (1, 5, 10 and −20 mg/kg, i.p.) on body temperature in rats. The peak hypothermic response to AM 404 was 45 min post-injection (Fig. 1A). The highest dose, 20 mg/kg, of AM 404 caused a maximal hypothermia of 1.8±0.3 °C 45 min post-injection (Fig. 1A). A one-way ANOVA on $AUC_{15–90}$ means showed a significant main effect ($F_{4, 25}=8.832$, $P<0.0001$) (Fig. 1B). Dunnett's post-hoc analysis revealed that doses of 10 and 20 mg/kg of AM 404 produced a significant hypothermia compared to vehicle-treated rats ($P<0.05$).

3.2. Capsazepine, but not SR 141176A, blocks the hypothermic response to AM 404 (10 mg/kg)

Fig. 2 shows the effects of capsazepine (10 and 30 mg/kg, i.p.) and SR 141716A (10 mg/kg, i.p.) on the hypothermia caused by AM 404 (10 mg/kg, i.p.). A one-way ANOVA on $AUC_{15–90}$ means showed a significant main effect ($F_{4, 31}=12.41$, $P<0.0001$) (Fig. 2B). Tukey's post-hoc analysis revealed that AM 404 (10 mg/kg, i.p.) evoked a significant hypothermia compared to vehicle-treated rats ($P<0.05$) (Fig. 2B). Pre-treatment of rats with 30 mg/kg of capsazepine abolished the hypothermic response to AM 404 (10 mg/kg, i.p.) ($P<0.05$). A lower dose, 10 mg/kg, of capsazepine did not alter AM 404-induced hypothermia ($P>0.05$). Pre-treatment of rats with SR 141716A (10 mg/kg, i.p.) did not significantly alter the hypothermia caused by 10 mg/kg of AM 404 ($P>0.05$).

3.3. SB 366791 and capsazepine block the hypothermic response to AM 404 (20 mg/kg)

Fig. 3 illustrates the effects of capsazepine (10 and 30 mg/kg, i.p.) and a new VR1 antagonist, SB 366791 (0.5 and 2 mg/kg, i.p.), on the hypothermia generated by AM 404 (20 mg/kg, i.p.). A one-way ANOVA on $AUC_{15–90}$ means showed a significant main effect ($F_{5, 32}=10.00$, $P<0.0001$) (Fig. 3B). Tukey's post-hoc analysis revealed that AM 404 (20 mg/kg, i.p.) evoked a significant hypothermia compared to vehicle-treated rats ($P<0.05$) (Fig. 3B). Pre-treatment of rats with capsazepine (30 mg/kg, i.p.) blocked the hypothermia caused by 20 mg/kg of

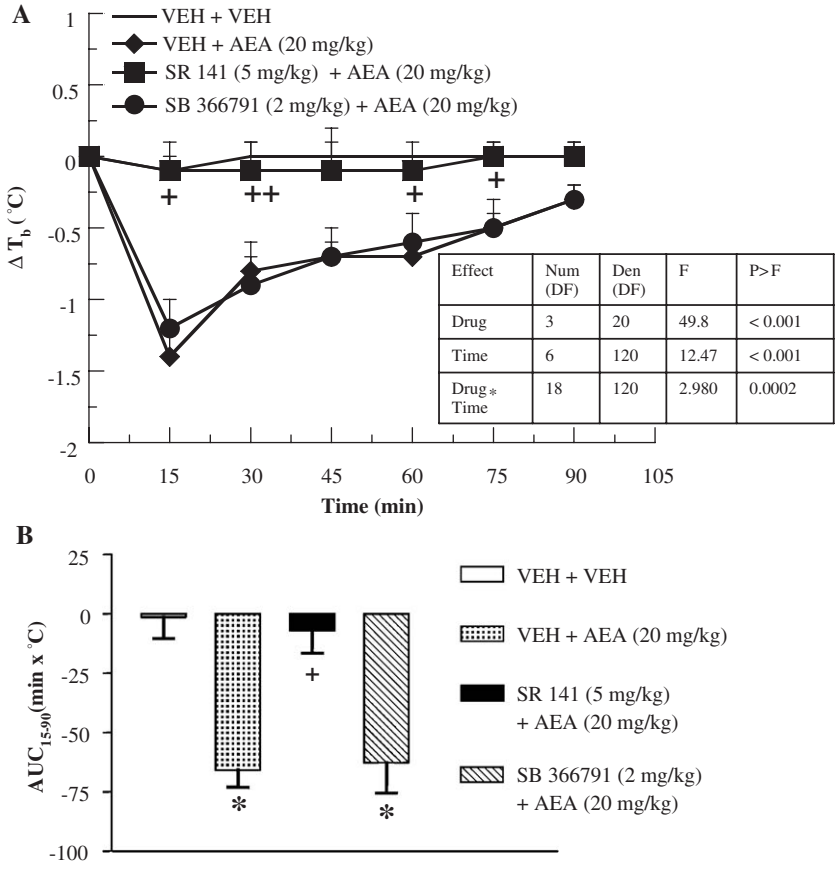


Fig. 4. Anandamide (AEA) causes hypothermia that is blocked by SR 141716A (SR 141). (A) Time course: AEA (20 mg/kg, i.p.) or vehicle (VEH) (i.p.) was injected at 0 min. SR 141 (5 mg/kg, i.p.), SB 366791 (2 mg/kg, i.p.), or VEH (i.p.) was administered 60 min before AEA. Data are expressed as the mean±S.E.M. of the change in body temperature (ΔT_b) from baseline (time 0) and determined from at least 6 animals. Data were analyzed using a two-way (drug, time) mixed-model analysis of variance (ANOVA) with repeated measures on time followed by pair-wise multiple comparisons incorporating the Bonferroni correction at the different time points (15–90 min). The ANOVA table illustrates the main effects and interaction (drug, time, and drug × time). $^{+}P<0.05$, compared to VEH+AEA. (B) $AUC_{15–90}$ profile: Area under the body temperature time curve (AUC) was calculated from 15 to 90 min using the difference score from 0 min. $^{*}P<0.05$, compared to VEH+VEH and $^{+}P<0.05$, compared to VEH+AEA.

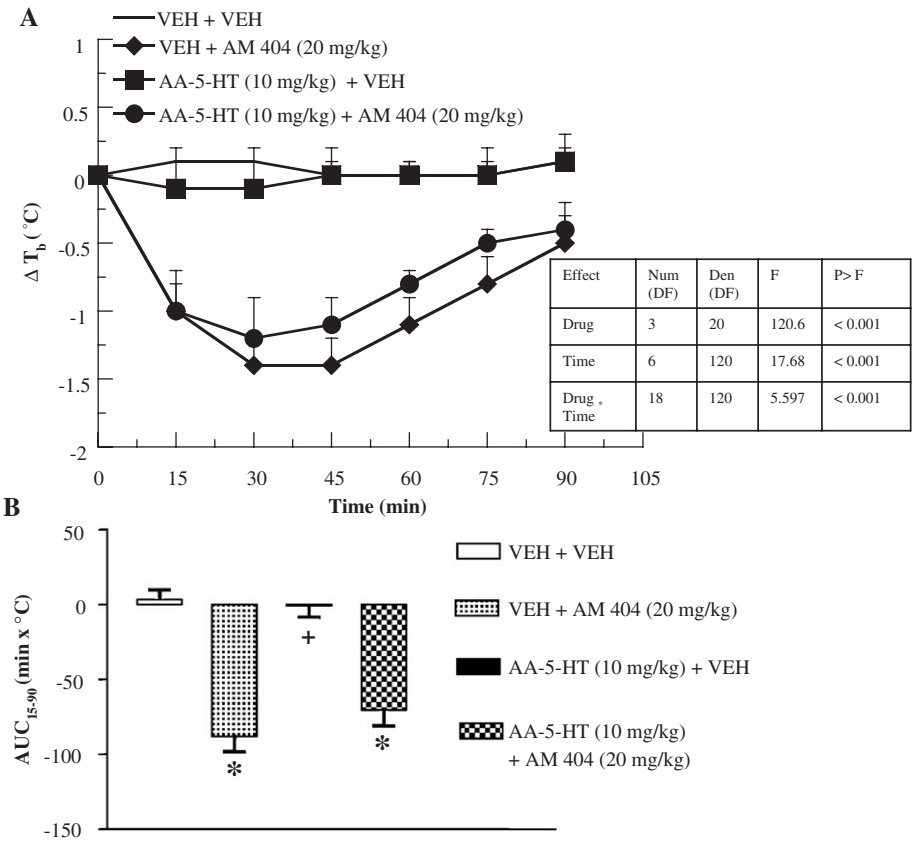


Fig. 5. AM 404 causes hypothermia that is not altered by a FAAH inhibitor, AA-5-HT. (A) Time course: AM 404 (20 mg/kg, i.p.) or vehicle (VEH) (i.p.) was injected at 0 min. AA-5-HT (10 mg/kg, i.p.) or VEH (i.p.) was administered 60 min before Am 404. Data are expressed as the mean ± S.E.M. of the change in body temperature (ΔT_b) from baseline (time 0) and determined from at least 6 animals. Data were analyzed using a two-way (drug, time) mixed-model analysis of variance (ANOVA) with repeated measures on time followed by pair-wise multiple comparisons incorporating the Bonferroni correction at the different time points (15–90 min). The ANOVA table illustrates the main effects and interaction (drug, time, and drug × time). $^{+}P<0.05$, compared to VEH + AM 404. (B) AUC_{15-90} profile: Area under the body temperature time curve (AUC) was calculated from 15 to 90 min using the difference score from 0 min. $*P<0.05$, compared to VEH + VEH and $^{+}P<0.05$, compared to VEH + AM 404.

AM 404 ($P<0.05$). Pre-treatment of rats with 2 mg/kg of SB 366791 prevented the hypothermic effect of AM 404 (20 mg/kg, i.p.) ($P<0.05$). None of the antagonists–capsazepine (30 mg/kg, i.p.), SR 141716A (10 mg/kg, i.p.) or SB 366791 (2 mg/kg, i.p.)–caused a change in body temperature compared to vehicle-treated rats ($P>0.05$, data not shown).

3.4. Anandamide causes hypothermia that is blocked by SR 141716A

To investigate a role for CB_1 and TRPV1 receptors in anandamide-induced hypothermia, we injected anandamide (20 mg/kg, i.p.) by itself and with SR 141716A (5 mg/kg, i.p.) or SB 366791 (2 mg/kg, i.p.) (Fig. 4). A one-way ANOVA on AUC_{15-90} means showed a significant main effect ($F_{3,\ 20}=12.08$, $P<0.0001$) (Fig. 4B). Tukey's post-hoc analysis revealed that anandamide (20 mg/kg, i.p.) evoked a significant hypothermia compared to vehicle-treated rats ($P<0.05$) (Fig. 4B). Pre-treatment with SR 141716A (5 mg/kg, i.p.) blocked anandamide-evoked hypothermia ($P<0.05$). In contrast, pre-treatment with of SB 366791 (2 mg/kg, i.p.) was ineffective ($P>0.05$). Analysis of the time-course data revealed similar results, with SR 141716A blocking anandamide-induced hypothermia ($P<0.05$) (Fig. 4A).

3.5. AA-5-HT does not alter AM 404-induced hypothermia

Fig. 5 shows the effect of AA-5-HT (10 mg/kg, i.p.) on the hypothermia caused by AM 404 (20 mg/kg, i.p.). A one-way ANOVA on AUC_{15-90} means showed a significant main effect ($F_{3,\ 20}=27.97$, $P<0.0001$) (Fig. 5B). Tukey's post-hoc analysis showed that AM 404 (20 mg/kg, i.p.) evoked a significant hypothermia compared to vehicle-treated rats ($P<0.05$) (Fig. 5B). Pre-treatment with AA-5-HT (10 mg/kg, i.p.) did not affect the hypothermic response to AM 404 (20 mg/kg, i.p.) ($P>0.05$). The time-course data confirmed that a dose of 10 mg/kg of AA-5-HT does not alter AM 404-evoked hypothermia ($P>0.05$) (Fig. 5A).

4. Discussion

The present study investigated the effects of graded doses of AM 404 on body temperature in rats. In addition, receptor antagonists were used to investigate a potential role for TRPV1

and cannabinoid CB_1 receptors in the hypothermia caused by AM 404. Experiments revealed that systemic AM 404 administration causes a significant hypothermia in rats. The AM 404-induced hypothermia was blocked by the TRPV1 antagonists, capsazepine and SB 366791. Conversely, AM 404-evoked hypothermia was not affected by a CB_1 antagonist, SR 141716A, or a FAAH inhibitor, AA-5-HT. These results suggest that the hypothermic response to AM 404 is dependent on TRPV1 receptor activation.

Hypothermia is an endpoint with a rich and productive history for evaluating cannabinoid and vanilloid drugs in conscious animals. Drugs that activate TRPV1 or cannabinoid CB_1 receptors cause marked hypothermia in conscious animals (Miller et al., 1982; Dogan et al., 2004; Rawls et al., 2002; Compton et al., 1992, 1996; Fox et al., 2001; Varga et al., 2005). Because more selective and definitive antagonists that block TRPV1 or cannabinoid CB_1 receptors are becoming available, hypothermia can be used as a valuable endpoint for discriminating a TRPV1 or cannabinoid CB_1 site of action for new drugs. Significant advantages of hypothermia over other endpoints are that it is a convenient, reproducible and sensitive measure of cannabinoid and vanilloid response. Furthermore, hypothermia is a quantifiable metric which can be confirmed statistically using a single assay. The fact that people who inhabit warmer climates use capsaicin, the prototypical TRPV1 receptor agonist and active ingredient of hot peppers, to cool themselves underscores the therapeutic significance of hypothermia, especially as related to TRPV1 receptors.

AM 404 administration produced a significant hypothermia in rats. An effect of AM 404 on body temperature has not been shown previously, but effects of AM 404 on other endpoints have been demonstrated in conscious animals. The analgesic action of AM 404 is not entirely clear yet. One study demonstrated that the spinal perfusion of AM 404 by reverse microdialysis blocks the pain induced by the formalin test in mice, thus indicating a potent analgesic action of AM 404 in mice (Guhring et al., 2002). However, another study demonstrated that AM 404 was not analgesic in rats (Beltramo et al., 2000). Other significant effects of AM 404 in conscious rats include a(n) (1) reduction of hyperkinesias in a rat model of Huntington's disease; (2) prevention of apomorphine-evoked yawning; (3) blockade of quinpirole-evoked motor activity; (4) inhibition of hyperactivity in juvenile hypertensive rats; (5) reduction of ambulatory and exploratory activities in the open-field test; (6) increase in plasma prolactin levels; and (7) elevation in tyrosine hydroxylase activity in the hypothalamus (Lastres-Becker et al., 2002, 2003; Beltramo et al., 2000; Gonzalez et al., 1999).

SB 366791 and capsazepine blocked the hypothermic effects of AM-404. This suggests that vanilloid TRPV1 receptor activation is required for AM-404 to produce hypothermia. Indeed, it has been shown that AM 404 is a full agonist at both rat and human recombinant TRPV1 receptors (De Petrocellis et al., 2000; Ross et al., 2001). The mechanism of AM 404-evoked hypothermia is unclear, but it is likely that AM-404, in a process similar to that proposed for capsaicin, induces hypothermia by directly activating TRPV1 receptors. Support for our interpretation is that capsazepine (Dogan et al., 2004) or SB 366791 (Varga et al., 2005) blocks a significant proportion of capsaicin-evoked hypothermia in rats. The possibility that AM-404 caused hypothermia by increasing extracellular levels of anandamide, an endogenous ligand with affinity for CB_1 receptors (Devane et al., 1992) and TRPV1 (Zygmunt et al., 1999), by causing an uptake block is unlikely for three reasons. First, the binding site for TRPV1 ligands is intracellular (De Petrocellis et al., 2001). Second, inhibition of the anandamide plasma membrane transporter prevents, rather than enhances, the effects of anandamide on TRPV1 receptors (De Petrocellis et al., 2001). Third, SR 141716A prevents the hypothermic effect of anandamide in rats but is without effect on anandamide-induced hypothermia in mice (Costa et al., 1999; Adams et al., 1998). The data from Costa and associates (1999) suggest that that cannabinoid CB_1 receptor activation is required for anandamide to induce hypothermia in rats. Because anandamide causes hypothermia by activating CB_1 receptors in rats (Costa et al., 1999) and AM 404 causes hypothermia by activating TRVP1 receptors, it is unlikely that AM 404 causes hypothermia by increasing endogenous anandamide levels. This is true despite the fact that a hypothermic dose of AM 404 increases plasma anandamide levels (Giuffrida et al., 2000).

Consistent with previous studies, anandamide produced a significant hypothermia in rats (Costa et al., 1999). The anandamide-evoked hypothermia was abolished by SR 141716A, thus confirming that CB_1 receptor activation is necessary for anandamide to cause hypothermia in rats (Costa et al., 1999). In contrast, TRVP1 receptor blockade with SB 366791 did not affect anandamide-evoked hypothermia. This indicates that TRPV1 receptor activation is not necessary for anandamide to produce hypothermia in rats. It is important to note that a TRPV1 antagonist abolishes the hypothermic effect of AM 404, but not of anandamide. The demonstration that TRPV1 receptors mediate AM 404-induced hypothermia and CB_1 receptors mediate anandamide-evoked hypothermia suggests that anandamide does not play a major role in the hypothermic effects of AM 404. Collectively, these data show that AM 404-evoked hypothermia is TRPV1 receptor-dependent and CB_1 receptor-independent.

We investigated a potential role for fatty acid amide hydrolase (FAAH) in the hypothermic effect of AM 404. Prior work shows that AM 404 is metabolized by FAAH (Fegley et al., 2004; Glaser et al., 2003). Because at least one of the primary metabolites, arachidonic acid, has been shown to cause hypothermia (Doggett and Jawaharlal, 1977), we determined if the inhibition of FAAH altered the hypothermic response to AM 404. Experiments revealed that a FAAH inhibitor, AA-5-HT, does not affect AM 404-induced hypothermia. The lack of effect of AA-5-HT suggests that the metabolism of AM 404 by FAAH is not a major factor in the hypothermic effects of AM 404.

Capsazepine or SB 366791 by itself did not affect body temperature. Consistent with the present data, prior work from our laboratory also demonstrates that body temperature is not affected by the systemic injection of capsazepine or SB

366791 (Ding et al., 2005). The lack of effect by capsazepine or SB 366791 suggests that body temperature is not controlled by an endogenous tone exerted at TRPV1 receptors. Likewise, the systemic administration of SR141716A does not affect body temperature, thus indicating that an endogenous tone at CB_1 receptors is not critical in maintaining body temperature (Rawls et al., 2002). In contrast to the lack of effect of TRPV1 and CB_1 antagonists on body temperature, antagonists of other receptors, including opioid, GABA, and dopamine, cause significant changes in body temperature (Baker and Meert, 2002; Ishiwata et al., 2005; Ghosh and Poddar, 1995; Adler et al., 1991).

In summary, we have shown that AM 404 causes significant hypothermia in rats. For combined administration, capsazepine and SB 36679 blocked AM 404-evoked hypothermia. The hypothermia was not affected by a dose of SR 141716A that blocks cannabinoid-evoked hypothermia in rats (Rawls et al., 2002). The present data suggest that TRPV1, but not cannabinoid CB_1, receptor activation is required for AM 404 to cause hypothermia in rats.

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