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Effect of calcium channel modulators on temperature regulation in ovariectomized rats

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Abstract

Clinical studies evaluating a calcium channel modulator, gabapentin, for the treatment of vasomotor symptoms have been reported. The present studies evaluated three calcium channel modulators in ovariectomized (OVX) rodent models of temperature regulation. Gabapentin, reported to interact with the $\alpha_2\delta$ subunit of voltage-sensitive calcium channels and the L-type voltage-gated calcium channel blockers, verapamil and nifedipine, were examined. These series of experiments demonstrated that orally administered gabapentin, verapamil and nifedipine all acutely and dose-dependently lower tail skin temperature in both models of OVX-induced thermoregulatory dysfunction. These compounds all had a rapid onset of action, however, the efficacy of all three calcium channel modulators is less than that observed following chronic estrogen treatment. Additionally, these compounds were also tested in a telemetric rat model measuring core body temperature to evaluate any temperature effects on internal core temperature. The present data suggests that gabapentin, verapamil and nifedipine all act to globally alter temperature regulation in steroid-dependent models of thermoregulatory function.

Keywords: Thermoregulation; Vasomotor symptoms; Hot flush; Menopause; Estrogen; Circadian rhythm

1. Introduction

Vasomotor symptoms (VMS), referred to as hot flushes and night sweats, are the most common symptoms associated with menopause, occurring in 60–80% of all women following natural or surgically-induced menopause (McKinlay and Jefferys, 1974). They are also the principal reason why menopausal women seek medical treatment. Hot flushes are characterized by a warming sensation in the chest and face accompanied by sweating, vasodilatation (flushing) and in some instances, feelings of nausea and illness. Vasomotor symptoms are thought to result from declining sex steroids during the menopause transition phase. While the cause of menopause-related VMS is not precisely known, the symptoms are not strictly predicted by fluctuating hormone levels (Erlik et al., 1982). Since hormone-containing therapies provide dose-related relief, the majority of therapeutic strategies to date have focused primarily on restoring declining hormone levels.

The most effective therapies for VMS are hormone-based treatments, including estrogens and/or some progestins (Lomax and Schonbaum, 1993). Although hormonal treatments are a very effective means of alleviating VMS, these therapies may not be desired or appropriate for all women. Thus, non-hormonal therapies including clonidine (Laufer et al., 1982; Loprinzi et al., 1994; Pandya et al., 2000), gabapentin (Guttuso, 2000; Loprinzi et al., 2002a; Guttuso et al., 2003) and the antidepressants venlafaxine (Loprinzi et al., 1998, 2000), fluoxetine (Loprinzi et al., 2002a) and paroxetine (Stearns et al., 2000, 2003; Weitzner et al., 2002) are currently being evaluated clinically. Clinical studies have reported efficacy with the calcium channel modulator gabapentin in both post-menopausal women (Guttuso, 2000; Guttuso et al., 2003) and in breast cancer survivors (Guttuso, 2000; Loprinzi et al., 2002b; Guttuso et al., 2003). Although some of these compounds have shown

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some promise in decreasing the frequency and intensity of hot flushes, very little is known on whether these agents act directly to restore thermoregulatory dysfunction due to sex steroid depletion. Gabapentin has been reported to interact directly with the $\alpha_2\delta$ subunit of voltage-sensitive calcium channels (Gee et al., 1996; Brown et al., 1998; Gong et al., 2001; Maneuf et al., 2003), whereas verapamil and nifedipine are both L-type voltage-gated calcium channel blockers (Catterall and Striessnig, 1992). Therefore, the present studies used OVX rodent models, which specifically measure sex steroid-dependent alterations in temperature regulation, to determine if the calcium channel modulators gabapentin, verapamil and nifedipine could restore or effect thermoregulatory function.

The present series of studies use rodent models of thermoregulatory dysfunction based on measuring changes in tail skin temperature (TST) in ovariectomized (OVX) rats. Although these preclinical models may not directly mimic menopausal hot flushes, previous studies have demonstrated that both models exhibit predictive validity of efficacy in the clinic (Simpkins et al., 1983; Berendsen et al., 2001). The first is a well characterized pharmacologically-based model that relies on a transient increase in TST in a morphine-dependent rat following naloxone-induced withdrawal (Simpkins et al., 1983; Katovich et al., 1986; Sipe et al., 2004). This model has been validated using various standard estrogens and has similar neuroendocrine and cardiovascular responses observed during menopausal hot flushes (Simpkins et al., 1983; Katovich et al., 1986; Merchenthaler et al., 1998). Gabapentin, verapamil and nifedipine's efficacy in the morphine-dependent model was evaluated following acute oral administration. In contrast to standard estrogen treatments, acute administration was chosen since all three of the test compounds have previously been shown to act acutely in various preclinical rodent models.

In order to verify that a compound's efficacy in the morphine-dependent model is affecting thermoregulatory responses and not morphine-dependence, a second physiological based model that relies on estrogen's regulation of diurnal TST patterns was also evaluated (Berendsen et al., 2001; Sipe et al., 2004). This model relies on the observation that over a 24-h period, intact cycling rats decrease TST during the active (dark) phase and TST remains elevated during the inactive (light) phase. In OVX rats, TST is elevated over the entire 24-h period, thus the normal decrease in TST during the active phase is lost (Berendsen et al., 2001). Since estrogens have been reported to restore the normal diurnal temperature rhythms (Berendsen et al., 2001), it has been suggested that this model may represent the thermodysregulation that occurs in menopausal women experiencing vasomotor symptoms. Therefore, the ability of gabapentin, verapamil and nifedipine to restore this lowering of TST during the active phase was also examined in OVX rats following acute oral administration. An additional measure of temperature regulation was utilized to determine whether any of these agents affected core body temperature in OVX rats. Specifically, a telemetric device was used to continually monitor changes in internal core temperature. Finally, the activity of calcium channel modulators was compared with the activity of chronic estrogen treatment.

2. Materials and methods

2.1. Animals

All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by Wyeth's Institutional Animal Care and Use Committee. Ovariectomized female (Sprague–Dawley rats (180–220 g, Taconic, Germantown, NY) were individually housed on a 12 h light/dark cycle with standard rat chow and water available ad libitum. For the morphine-dependent rat model, rats were housed in a room maintained at 25 °C and experimental procedures were performed in a room maintained at 21 °C. For the thermoregulatory dysfunction telemetry model (telemetry model) rats were housed and tested in a room maintained at 21 °C. In all experiments 8–10 rats per group were used.

2.2. Test compounds

Gabapentin (30–300 mg/kg, Sigma-Aldrich, St. Louis, MO) and verapamil (0.3–30 mg/kg, Sigma-Aldrich, St. Louis, MO) were dissolved in 0.25% Tween in 0.5%/methylcellulose and nifedipine (0.1–10 mg/kg, Sigma-Aldrich, St. Louis, MO) and 17α-ethinyl estradiol, 1.0 mg/kg, Wyeth Research, Princeton, NJ) were dissolved 10% ethanol/oil. All compounds were administered orally (po) on test day, except for 17α-ethinyl estradiol that was chronically administered (for 8 days in the morphine-dependent model and 5 days in the telemetry model). For all compounds except 17α-ethinyl estradiol, rats were food deprived for 16 h prior to testing in the morphine-dependent model and for 4 h prior to testing in the telemetry model.

2.3. Morphine-dependent rat model

2.3.1. Experimental paradigm

This model is based upon an established morphine-dependent naloxone-induced flush paradigm (Simpkins et al., 1983; Katovich et al., 1986; Merchenthaler et al., 1998) (Fig. 1; panel A). For all studies using acute dosing paradigms rats were injected subcutaneously (sc) once daily for 8–9 days with vehicle. Estrogen was administered chronically (po) since previous studies have determined that repeated dosing is required for abating the naloxone-induced flush (Simpkins et al., 1983; Katovich et al., 1986; Merchenthaler et al., 1998). Morphine

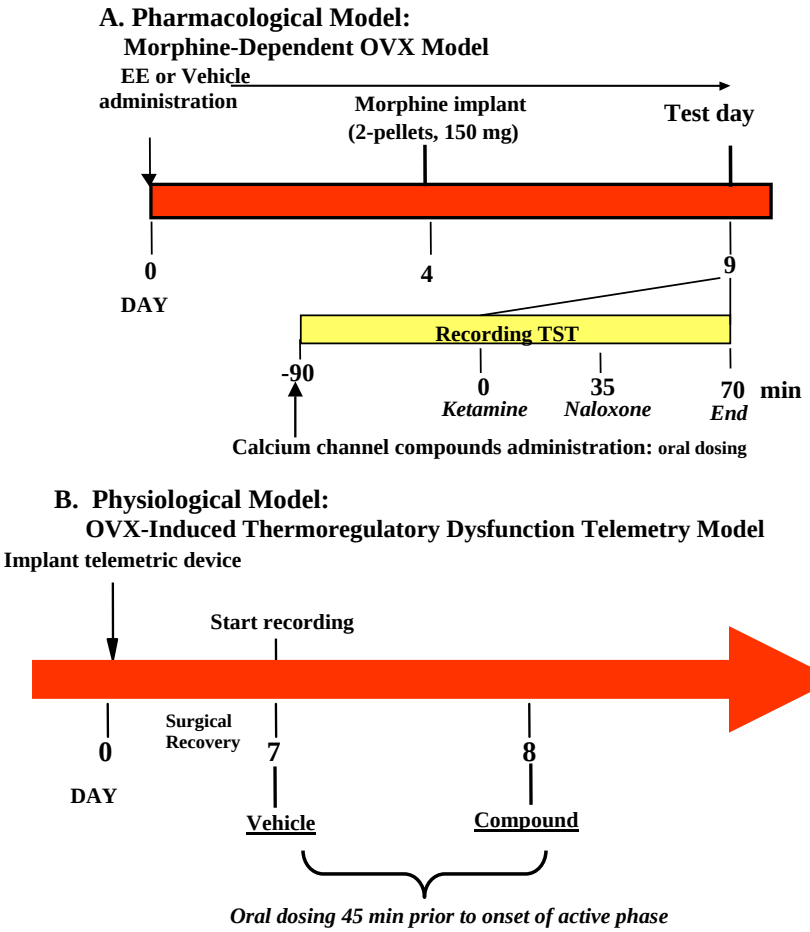


Fig. 1. Experimental testing paradigms. Panel A: Morphine-dependent rat model. Panel B: OVX-induced thermoregulatory dysfunction telemetry model.

dependence was induced by sc implantation of two slow-release morphine pellets (75 mg/pellet, Murty Pharmaceuticals, Lexington, KY) in the dorsal scapular region on day 4 of dosing. Five to six days after implantation, morphine withdrawal was induced with the general opioid antagonist naloxone (1.0 mg/kg, sc, Research Biochemicals International, St. Louis, MO). On test day rats were orally administered compound 90 min prior to naloxone injection. Rats were then injected with ketamine (Ketaject, Phoenix Pharmaceuticals, Belmont, CA) at a dosage (40 mg/kg, intramusclar) previously determined to be mildly sedative without causing a change in TST (data not shown) (Merchenthaler et al., 1998). Following ketamine administration, a thermistor connected to a MacLab data acquisition system was taped to the base of the rat's tail (CB Sciences, Dover, NH). Tail skin temperature was then monitored continuously for 35 min to establish baseline temperatures. Naloxone was subsequently administered and TST was measured for an additional 35 min (total recording time 70 min).

2.3.2. Statistical analysis

To analyze changes in TST induced by naloxone in the morphine-dependent model, data were analyzed using a two factor repeated measure ANOVA for "treatment" and "time". The model was fit to test whether there were significant differences in the responses between treatment groups. Naloxone administration was designated as time zero and data are then analyzed at 5 min intervals. The first three readings (−35, −30, and −25 min prior to naloxone administration) were averaged and used as baseline TST scores. All data were analyzed as ΔTST (TST for each time point–baseline). Multiple comparisons (LSD p-values) among the treatment groups at each time point were used for the analysis. Hot flush abatement was determined by evaluating statistical differences at the peak response time of 15 min post-naloxone treatment when the maximal change in TST is observed. A customized SAS-excel (SAS Institute, Cary, NC) application was used applying a four parameter logistic model to determine ED_{50} values. A logistic dose transformation was performed on ΔTST. Maximum flush (ΔTST at 15 min post-naloxone treatment) was used in the analysis and the minimum was locked at zero. The ED_{50} value is reported as the dose of test compound that abates 50% of the naloxone-induced flush. Statisticians in the Biometrics Department (Wyeth Research, Collegeville, PA) developed a customized JMP statistical software application.

2.4. OVX-induced telemetry rat model (telemetry model)

2.4.1. Experimental paradigm

Compounds were evaluated for their ability to restore normal lowering of TST during the active (dark) phase in OVX rats by telemetry. This model has been modified from a previous literature report based on estrogen regulation of diurnal TST patterns (Berendsen et al., 2001). Over a 24 h-period, intact cycling rats decrease TST during the active (dark) phase and TST remains elevated during the inactive (light) phase. In OVX rats, TST is elevated over the entire 24-h period, thus the usual decrease in TST during the active phase is lost. Therefore, a compound's ability to restore this lowering of TST during the active phase was examined in OVX rats. A telemetric transmitter (PhysioTel TA10TA-F40, Data Sciences International) was implanted sc in the dorsal scapular region and the tip of the temperature probe tunneled 2.5 cm beyond the base of the tail. After a 7-day recovery period, rats were orally administered a vehicle and TST was monitored continuously for 12 h (Day 1). Twenty-four hours later rats were orally administered with either vehicle or test compound and TST was monitored continuously for 12 h (Day 2). All vehicle and test compounds were administered 45 min prior to the onset of the active (dark) phase. Estrogen was administered chronically (po) for 5 days since previous studies have determined that repeated dosing is required for efficacy (Berendsen et al., 2001). An identical telemetry protocol was used to measure changes in core temperature (ΔCT) in OVX rats during the active phase except for the following modification. A 3–4 cm long skin incision was made in the midline of the abdomen and extended through the abdominal musculature and than the transmitter (PhysioTel TA-F20, Data Sciences International) was placed in the abdominal cavity. For evaluation of compound effect on core temperature the dose that showed approximately the mid-point effect in the TST telemetric model was tested.

2.4.2. Statistical analysis

Evaluation of test compound's ability to restore normal lowering of TST in the telemetry model was analyzed using a 2-day paradigm. For both vehicle (baseline) and compound testing, TST was recorded at 5 min intervals and an average TST was calculated for every 30 min time point. On Day 1, an overall average baseline TST was established for each animal by taking the mean over the 12 h observation period. On Day 2, calcium channel modulators were administered and TST readings were recorded every 30 min as described above. For the estrogen study all procedures were identical except Day 5 of treatment was used for analysis. All data were analyzed as ΔTST (TST for each time point Day 2–average baseline TST Day 1). A one-way ANOVA was performed to obtain the average within-group standard deviation for that compound. For each 30 min interval, a *t*-test was performed and evaluated to determine if the average ΔTST was statistically different ($p \leq 0.05$) from zero. Data will be presented as onset of treatment, duration (h) of treatment, mean ΔTST, duration (°C) and overall TST activity index (mean ΔTST *X* duration) during the active phase. The onset of treatment effect will be the first half-hour interval of two consecutive significant half-hour intervals following any number of non-significant half-hour intervals. The treatment effect will be considered to have ended when two consecutive non-significant half-hour intervals follow any number of significant half-hour intervals (duration). Mean temperature change will be calculated from half-hour TST averages obtained over the treatment duration. The activity index can be used to profile and compare compounds that are tested in this model as a measure for overall TST activity including both ΔTST and duration of effect. An identical method of statistical analysis was employed for measuring ΔCT during the dark cycle.

3. Results

3.1. Gabapentin administration in morphine-dependent model

Significant differences in ΔTST were observed over time and among treatment groups [[$F(33,374)=3.46$, $p<0.0001$] (Fig. 2; panel A). Gabapentin pretreatment prior to naloxone administration did not cause any significant alterations of TST compared to vehicle-treated animals. At maximal flush time (15 min post-naloxone injection), gabapentin significantly and dose-dependently abated the naloxone-induced flush at 100 and 300 mg/kg doses (Fig. 2; panel A and B). In contrast, vehicle and the 30 mg/kg dose of gabapentin failed to decrease the naloxone-induced flush. The estimated ED_{50} value is 83.8±34.1 mg/kg. At the highest dose tested (300 mg/kg) gabapentin did not demonstrate full efficacy, but produced a 75.1% abatement of the naloxone-induced flush relative to vehicle treatment.

3.2. Gabapentin administration in the OVX-induced TST telemetry model

Gabapentin (30–300 mg/kg) resulted in significant changes in TST over time and among treatment groups during the active phase (Fig. 3; panel A, $p<0.05$). At the 30 and 100 mg/kg doses gabapentin significantly decreased TST (mean −1.76 and −1.59 and maximum −2.03 and −1.73 °C, respectively) relative to vehicle (Fig. 3; panel B) with an onset of action at 1 h (Fig. 3; panel A) and a duration of 2 and 1.5 h, respectively (Fig. 3, panel B). At the highest dose (300 mg/kg) tested gabapentin significantly decreased TST (mean −2.21 and −3.37 maximum °C) relative to vehicle with an immediate onset of action and duration of 4.5 h (Fig. 3; panel A and B). The maximal

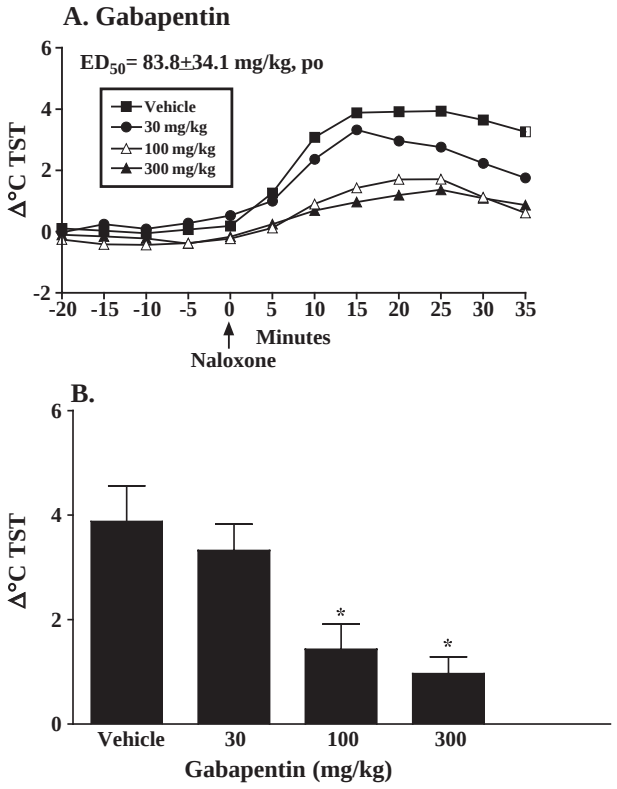
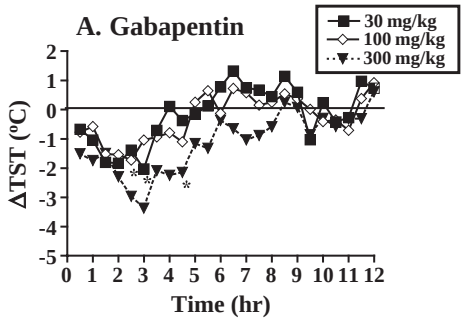


Fig. 2. Oral administration of gabapentin dose-dependently abates a naloxone-induced flush in the morphine-dependent rat model of vasomotor symptoms. Panel A: Changes in TST (Δ°C) over time in the morphine-dependent rat model depict significant differences between groups. Panel B: At maximal flush (15 min post-naloxone; Δ°C±SEM), gabapentin significantly and dose-dependently abated the naloxone-induced flush at 100 and 300 mg/kg, but had no effect at 30 mg/kg. (*Indicates $p<0.01$ compared to vehicle control).



B.

Gabapentin (mg/kg)	Onset (hr)	Duration (hrs)	Mean Δ Temperature (°C)	Maximum Δ Temperature (°C)
30	1	2	-1.76	-2.03
100	1	1.5	-1.59	-1.73
300	0	4.5	-2.21	-3.37

Fig. 3. Oral administration of gabapentin dose-dependently restores tail skin temperature in a rat telemetry model of thermoregulatory dysfunction. Panel A: Changes in TST (Δ°C) over time in a telemetry model of thermoregulatory dysfunction. At all doses tested gabapentin resulted in a decrease in TST relative to vehicle treatment. (*Indicates the last 30 min time point where there is a significant decrease in TST) Panel B: Table summarizing onset and duration of action, mean ΔTST and maximum ΔTST for each dose of gabapentin tested.

Table 1
Summary of activity in MD and telemetry model

Compound	ED_{50} MD model (mg/kg)	Maximal percent abatement MD model	Maximal overall activity index telemetry model
Gabapentin	83.8	75	−9.9
Verapamil	7.8	69	−21.5
Nifedipine	2.2	77	−8.0
Ethinyl Estradiol	N/A	90	−44.9

overall TST activity index (mean ΔTST *X* duration) for gabapentin was −9.9 (Table 1).

3.3. Verapamil administration in the morphine-dependent model

Significant differences in ΔTST were observed over time and among treatment groups [[$F(33,396)=3.84$, $p<0.0001$] (Fig. 4; panel A). Verapamil pretreatment prior to naloxone administration did not cause any significant alterations of TST compared to vehicle-treated animals. At maximal flush time (15 min post-naloxone injection), verapamil signifi-

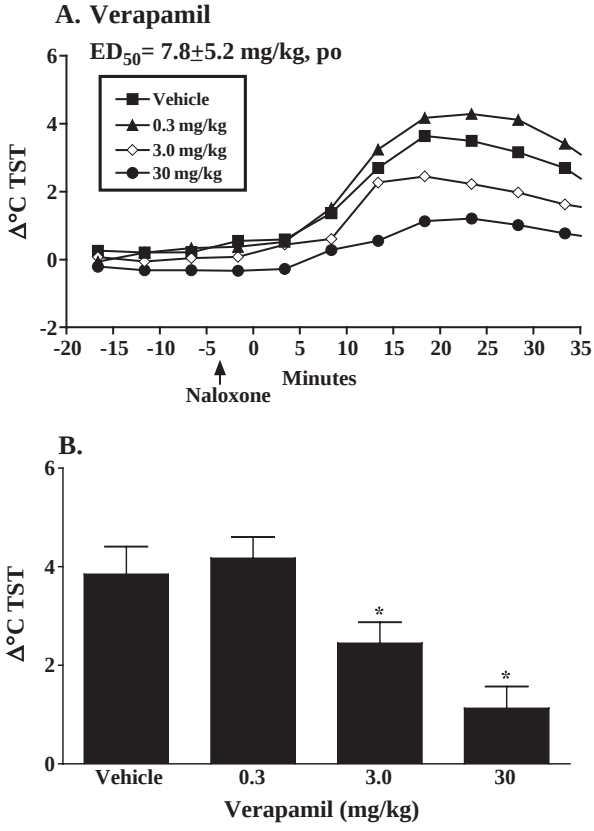


Fig. 4. Oral administration of verapamil dose-dependently abates a naloxone-induced flush in the morphine-dependent rat model. Panel A: Changes in TST (Δ°C) over time in the morphine-dependent rat model depict significant differences between groups. Panel B: At maximal flush (15 min post-naloxone; Δ°C±SEM), verapamil significantly and dose-dependently abated the naloxone-induced flush at 3.0 and 30 mg/kg, but had no effect at 0.3 mg/kg. (*Indicates $p<0.01$ compared to vehicle control).

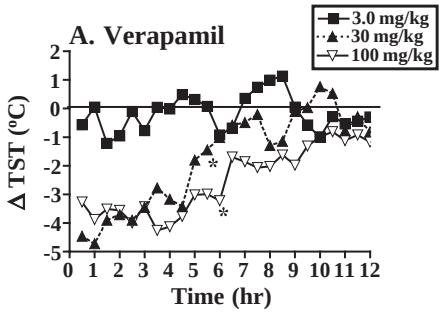
cantly and dose-dependently abated the naloxone-induced flush at the 3.0 and 30 mg/kg doses (Fig. 4; panel A and B). In contrast, vehicle and the 0.3 mg/kg dose of verapamil failed to decrease the naloxone-induced flush. The estimated ED_{50} value for verapamil is 7.8±5.2 mg/kg. At the highest dose tested (30 mg/kg) verapamil did not demonstrate full efficacy, but produced a 69.1% abatement of the naloxone-induced flush relative to vehicle treatment.

3.4. Verapamil administration in the TST telemetry model

Verapamil (3.0–100 mg/kg) resulted in significant changes in TST over time and among treatment groups during the active phase (Fig. 5; panel A, $p<0.05$). At the 30 and 100 mg/kg doses verapamil significantly decreased TST (mean −3.34 and −3.58 and maximum 4.73 and −4.26 °C, respectively) relative to vehicle (Fig. 5; panel B) with an immediate onset (Fig. 5; panel A) and a duration of 5.5 and 6.0 h, respectively (Fig. 5; panel B). In contrast, the lowest dose of verapamil (3.0 mg/kg) was inactive. The maximal overall TST activity index (mean ΔTST *X* duration) for verapamil was −21.5 (Table 1).

3.5. Nifedipine administration in the morphine-dependent model

Significant differences in ΔTST were observed over time and among treatment groups [[$F(33,396)=3.01$, $p<0.0001$] (Fig. 6; panel A). Nifedipine pretreatment prior to naloxone administration did not cause any significant alterations of TST compared to vehicle-treated animals. At maximal flush time (15 min post-naloxone injection),



B.

Verapamil (mg/kg)	Onset (hr)	Duration (hrs)	Mean Δ Temperature (°C)	Maximum Δ Temperature (°C)
3.0	-	0	0	0
30	0	5.5	-3.34	-4.73
100	0	6.0	-3.58	-4.26

Fig. 5. Oral administration of verapamil dose-dependently restores tail skin temperature in a rat telemetry model of thermoregulatory dysfunction. Panel A: Changes in TST (Δ°C) over time in a telemetry model of thermoregulatory dysfunction. Verapamil at 30 and 100 mg/kg resulted in a decrease in TST relative to vehicle treatment. (*Indicates the last 30 min time point where there is a significant decrease in TST) Panel B: Table summarizing onset and duration of action, mean ΔTST and maximum ΔTST for each dose of verapamil tested.

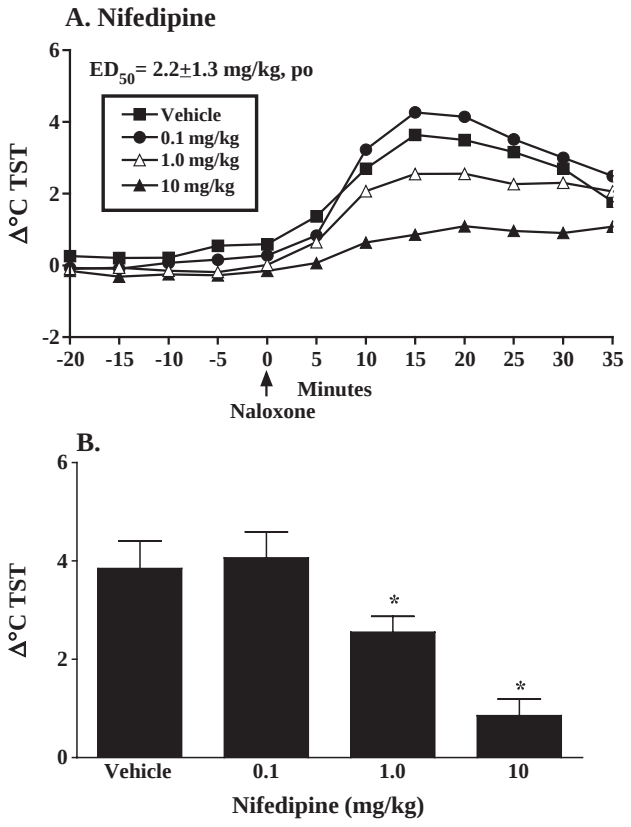
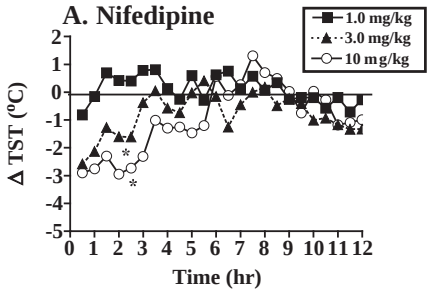


Fig. 6. Oral administration of nifedipine dose-dependently abates a naloxone-induced flush in the morphine-dependent rat model of vasomotor symptoms. Panel A: Changes in TST (Δ°C) over time in the morphine-dependent rat model depict significant differences between groups. Panel B: At maximal flush (15 min post-naloxone; Δ°C±SEM), nifedipine significantly and dose-dependently abated the naloxone-induced flush at 1.0 and 10 mg/kg, but had no effect at 0.1 mg/kg. (*Indicates $p<0.01$ compared to vehicle control).

nifedipine significantly and dose-dependently abated the naloxone-induced flush at the 1.0 and 10 mg/kg doses (Fig. 6; panel A and B). In contrast, vehicle and the 0.1 mg/kg dose of nifedipine failed to decrease the naloxone-induced flush. The estimated ED_{50} value for nifedipine is 2.2±1.3 mg/kg. At the highest dose tested (10 mg/kg) nifedipine did not demonstrate full efficacy, but produced a 76.6% abatement of the naloxone-induced flush relative to vehicle treatment.

3.6. Nifedipine administration in the TST telemetry model

Nifedipine (1.0–10 mg/kg) resulted in significant changes in TST over time and among treatment groups during the active phase (Fig. 7; panel A, $p<0.05$). The 3.0 mg/kg dose of nifedipine significantly decreased TST (mean −1.84 and maximum −2.58 °C) relative to vehicle (Fig. 7; panel B) with an immediate onset (Fig. 7; panel A) and a duration of 2.5 h (Fig. 7, panel B). At the highest dose tested nifedipine (10 mg/kg) significantly decreased TST (mean −2.65 and maximum 2.94 °C) relative to vehicle with an immediate onset of action and duration of 3.0 h (Fig. 7;



B.

Nifedipine (mg/kg)	Onset (hr)	Duration (hrs)	Mean Δ Temperature (°C)	Maximum Δ Temperature (°C)
1.0	-	0	0	0
3.0	0	2.5	-1.84	-2.58
10	0	3.0	-2.65	-2.94

Fig. 7. Oral administration of nifedipine dose-dependently restores tail skin temperature in a rat telemetry model of thermoregulatory dysfunction. Panel A: Changes in TST (Δ°C) over time in a telemetry model of thermoregulatory dysfunction. Nifedipine at 3.0 and 10 mg/kg resulted in a decrease in TST relative to vehicle treatment. (*Indicates the last 30 min time point where there is a significant decrease in TST) Panel B: Table summarizing onset and duration of action, mean ΔTST and maximum ΔTST for each dose of nifedipine tested.

panel A and B). In contrast, the lowest dose of nifedipine (1.0 mg/kg) was inactive. The maximal overall TST activity index (mean ΔTST *X* duration) for nifedipine was −7.9 (Table 1).

3.7. Evaluation of core body temperature following administration of calcium channel modulators

Gabapentin (100 mg/kg) resulted in a minor but significant change in CT over time during the active phase, significantly decreasing CT (mean −0.28 and maximum −0.47 °C) relative to vehicle with onset of action at 1.5 h, duration of 1 h and an overall CT activity index (mean ΔCT *X* duration) of −0.28 (Table 2).

Verapamil (10 mg/kg) failed to significantly alter CT during the active phase (Table 2). Although when tested a 30 mg/kg dose of verapamil caused a minor but significant change in CT over time (data not shown) as noted with gabapentin and nifedipine.

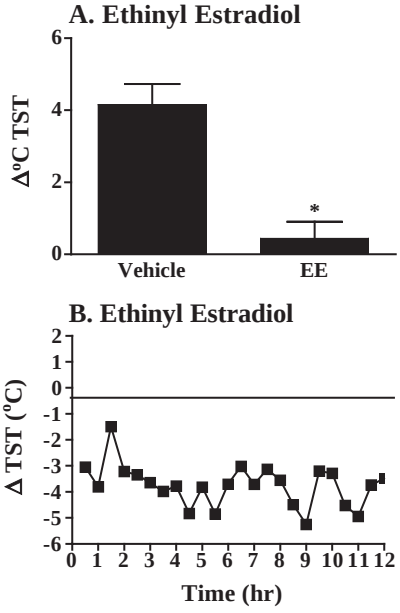
Nifedipine (3.0 mg/kg) resulted in a minor but significant change in CT over time during the active phase, significantly decreasing CT (mean −0.48 and maximum −0.53 °C) relative to vehicle with an onset of action at 1 h, duration of 1.5 h and an overall CT activity index (mean ΔCT *X* duration) of −0.72 (Table 2).

Table 2
Summary of alterations in CT in telemetry model

Compound	Onset (h)	Duration (h)	Mean Δ temperature (°C)	Maximum Δ temperature (°C)
Gabapentin	1.5	1.0	−0.28	−0.47
Verapamil	–	0	0	0
Nifedipine	1.0	1.5	−0.48	−0.53

3.8. 17α-ethinyl estradiol administration in the morphine-dependent and telemetry models

These rodent models have been validated by demonstrating alterations in TST can be achieved by pretreatment (>3 days) with estrogens (Simpkins et al., 1983; Katovich et al., 1986; Merchenthaler et al., 1998; Berendsen et al., 2001). Following 8 days of daily dosing significant differences in ΔTST were observed over time and among treatment groups [[$F(1,12)=13.36$, $p<0.0001$] in the morphine-dependent model. At maximal flush time (15 min post-naloxone injection), 17α-ethinyl estradiol (1.0 mg/kg) significantly abated the naloxone-induced flush (Fig. 8; panel A). In contrast, vehicle failed to decrease the naloxone-induced flush. Near full efficacy was achieved following 17α-ethinyl estradiol treatment with an 89.9% abatement of the naloxone-induced flush relative to vehicle treatment. Further, following 5 days of daily administration 17α-ethinyl estradiol treatment resulted in significant changes in TST over time and among treatment groups during the active phase (Fig. 8; panel B, $p<0.05$) in the telemetry model. 17α-



C.

Ethynil Estradion (mg/kg)	Onset (hr)	Duration (hrs)	Mean Δ Temperature (°C)	Maximum Δ Temperature (°C)
1.0	0	12	-3.74	-5.25

Fig. 8. Oral administration of 17α-ethinyl estradiol abates a naloxone-induced flush in the morphine-dependent rat model of vasomotor symptoms and restores tail skin temperature in a rat telemetry model of thermoregulatory dysfunction. Panel A: At maximal flush (15 min post-naloxone; Δ°C±SEM), 17α-ethinyl estradiol (1.0 mg/kg) significantly abated the naloxone-induced flush with near full efficacy. (*Indicates $p<0.01$ compared to vehicle control) Panel B: Changes in TST (Δ°C) over time in a telemetry model of thermoregulatory dysfunction. 17α-Ethinyl estradiol (1.0 mg/kg) resulted in a decrease in TST relative to vehicle treatment. Panel C: Table summarizing onset and duration of action, mean ΔTST and maximum ΔTST for 17α-ethinyl estradiol treatment.

Ethinyl estradiol significantly decreased TST for the entire active phase (mean −3.74 and maximum 5.25 °C) relative to vehicle with an immediate onset of action and duration of 12.0 h (Fig. 8; panel B and C). The overall TST activity index for 17α-ethinyl estradiol was −44.9 (Table 1).

4. Discussion

The present series of experiments demonstrate that gabapentin, verapamil and nifedipine are all active in both a pharmacological and a physiological model of sex steroid-dependent temperature regulation. Specifically, acute orally administered gabapentin, verapamil and nifedipine rapidly and dose-dependently abated the naloxone-induced flush in the morphine-dependent model. Additionally, in the TST telemetry model gabapentin, verapamil and nifedipine transiently lowered TST with a rapid (i.e., verapamil and nifedipine) or delayed (i.e., gabapentin) onset of action. The rank order potency of these compounds based on estimated ED_{50} values from the morphine-dependent model is as follows: nifedipine > verapamil > gabapentin (see Table 1). The rank order for overall activity in the telemetry model based on the activity index (overall TST activity index = duration *X* mean ΔTST) is as follows: verapamil > gabapentin > nifedipine. The profiling differs between models since the morphine-dependent model is more sensitive to potency while the telemetry model also evaluates duration of action (see Table 1). However, when compared to 17α-ethinyl estradiol treatment, nifedipine and verapamil, in an acute dosing paradigm, are less active (potent) both in the morphine-dependent and TST telemetry models. It is noteworthy that the dose used for 17α-ethinyl estradiol does exceed the physiological range but such doses are required in these preclinical rat models even though such doses are not required clinically to treat vasomotor symptoms. While the results suggest that these agents may have clinical efficacy, these models have not been proven to predict the doses required for clinical efficacy. Additionally, these compounds were evaluated for alterations in core temperature in the telemetry model. Only gabapentin and nifedipine induced minor changes in core body temperature at moderate doses. Thus, these series of studies support observations that calcium channel modulators modify steroid-dependent alterations in temperature regulation in vivo.

The similarity of morphine withdrawal and menopausal hot flushes has been previously been described (Simpkins et al., 1983). However, this dual model approach (i.e., pharmacological and physiological model) was used since skepticism has been raised regarding the "classic" morphine-dependent model (Simpkins et al., 1983; Katovich et al., 1986) and its relevance to vasomotor instability (Berendsen et al., 2001). Moreover, numerous rodent studies have reported that L-type calcium channel blockers including verapamil and nifedipine are effective at reducing the symptoms of morphine withdrawal (e.g., diarrhea, jumping and ptosis) (Bongianni et al., 1986; Baeyens et al., 1987; Schnur et al., 1992; Michaluk et al., 1998; Piepponen et al., 1999; Blackburn-Munro et al., 2000). No preclinical data exists regarding the ability of gabapentin to attenuate the symptoms of morphine withdrawal. It is noteworthy that none of these previous studies were specifically evaluating sex steroid-depleted rats and that L-type calcium channel modulators have never been clinically evaluated for the treatment of menopausal hot flushes. Given that all of the compounds evaluated lowered TST to some degree during the active (dark) phase and partially restored normal thermoregulation in OVX rats, their activity is relevant to sex steroid-dependent regulation of temperature. The data from the TST telemetry model corroborates that gabapentin, verapamil and nifedipine alleviates vasomotor instability, suggesting that the activity noted in the morphine-dependent model is not likely due to interaction(s) with morphine or alteration of morphine-dependence.

The calcium channel modulators evaluated in the present studies had two different purported mechanisms of action. Gabapentin's primary mode of action has been reported to interact with the $\alpha_2\delta$ subunit of voltage-sensitive calcium channels, whereas verapamil and nifedipine are both L-type voltage-gated calcium channel blockers. The most distinct difference observed between these two classes of compounds was the greater potency and rapid onset of action of the L-type calcium channel blockers compared to gabapentin. This may partially be mechanism based, but is more likely to be compound specific since a pharmacokinetic analysis of gabapentin reports that peak plasma level are not achieved until 3–3.2 h after ingestion (Rose and Kam, 2002). There are no preclinical studies evaluating gabapentin in thermoregulatory endpoints. However, both verapamil and nifedipine have been reported to have varying effects on core body temperature and tail skin temperature (personal communication, M. Katovich). Specifically, verapamil administered directly into the anterior hypothalamic preoptic area of a cat dose-dependently reduced core body temperature (Beleslin et al., 1985; Rezvani et al., 1986) in contrast, intracerebroventricular administration of either verapamil or nifedipine dose-dependently induced increased core body temperature with accompanied vasoconstriction of the ear vascular bed in rabbits (Palmi and Sgaragli, 1989). Whereas, data from the current study showed small transient decreases in core body temperature following compound administration. It is also possible that these compounds may have peripheral vasoactive (i.e., constrictive) properties that contributed to lowering tail skin temperature. However, peripheral vasoconstriction alone cannot account for the observed results in the morphine-dependent model. Prior to naloxone administration, compounds are evaluated to determine whether significant alterations (i.e., lowering) of TST are observed compared to vehicle-treated animals. None of the compounds tested showed significant changes

in TST during this evaluation period. Although, the activity of these drugs on the peripheral and autonomic nervous system cannot be ruled out, these data support a centrally mediated role for L-type voltage-gated calcium channel in temperature regulation. The means by which $\alpha_2\delta$ subunit of voltage-sensitive calcium channel affects temperature regulation remains to be elucidated.

The rapid effect of the present compounds suggests a direct mechanism of action compared to classical hormone therapy. Ultimately all these calcium channel modulators may be affecting the route of entry for calcium ions across plasma membrane, and consequentially altering the regulation of neurotransmitter release and changing thermoregulation states. It should be noted that the activity of this series of compounds, in both rodent models, is distinct from estrogen (Merchenthaler et al., 1998; Berendsen et al., 2001). Previous studies evaluating estrogens in rodent models of vasomotor instability have determined that chronic dosing is required in order to restore OVX-induced changes in TST (Katovich et al., 1986; Berendsen et al., 2001). This delay in efficacy following estrogen treatment suggests its effects are mediated through an indirect action possibly via alteration of neurotransmitter levels (Berendsen, 2000; Berendsen et al., 2001).

The present findings demonstrate a rapid onset of action with gabapentin, verapamil or nifedipine and may suggest a more rapid onset of action clinically. Preliminary clinical data from post-menopausal women and breast cancer survivors treated with gabapentin is mixed. One study reports beneficial effects within the first week of treatment (Guttuso et al., 2003) while a separate study reported onset of efficacy following 4 weeks of treatment (Loprinzi et al., 2002b). This is similar to classical hormonal therapies that require 3–4 weeks of treatment in order to see a clinically relevant reduction in the frequency and severity of vasomotor symptoms (Loprinzi et al., 1998, 2000). However, the longer onset of efficacy for gabapentin may be attributable to titration of the compound and not mechanism specific. The present results also suggest the level of efficacy observed with these calcium channel modulators may not dramatically differ from 17α-ethinyl estradiol treatment. However, clinical studies to date using non-hormonal therapies have not reported equal efficacy compared to hormone-based therapies (Loprinzi et al., 1998, 2000). Also it is noteworthy that unlike 17α-ethinyl estradiol treatment all the calcium channel modulators were unable to fully abate the naloxone-induced flush or lower TST for the duration of the dark cycle at relatively high doses of each respective compound (Berendsen et al., 2001). The effect of chronic treatment with calcium channel modulators remains to be determined. It is conceivable that chronic administration may restore levels of thermoregulatory function to those comparable to 17α-ethinyl estradiol treatment. However, the current studies evaluated acute dosing paradigms since all three compounds have previously be shown to have acute actions in various preclinical rat models.

In summary, the present studies utilizing calcium channel modulators in sex steroid-dependent rodent models of thermoregulatory dysfunction support previously reported clinical efficacy noted for gabapentin (see review: (Loprinzi et al., 2001)). Further, the pharmacological activity of the calcium channel modulators in the present studies are likely modifying the physiological events contributing to thermoregulatory dysfunction.

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