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Estrogen facilitates and the kappa and mu opioid receptors mediate antinociception produced by intrathecal (−)-pentazocine in female rats

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Abstract

Pentazocine, a mixed-action kappa opioid receptor (KOR) agonist, has high affinity for both KOR and the mu opioid receptor (MOR), and has been shown clinically to alleviate pain with a pronounced effect in women. However, whether local application of pentazocine in the spinal cord produces antinociception and the contribution of spinal KOR and MOR in mediating the effect of pentazocine in female rats remain unknown. Also, it is not known whether pentazocine-induced antinociception in females is estrogen-dependent. Hence, we investigated whether intrathecal (i.t.) (−)-pentazocine produces thermal antinociception and whether estrogen modulates the drug effect in female rats. Only the highest dose of pentazocine (500 nmol) was effective in producing antinociception in ovariectomized (OVX) rats. In contrast, pentazocine produced antinociception in estradiol-treated ovariectomized females (OVX+E) rats with the lowest effective dose being 250 nmol. KOR or MOR mediated the effect of the lowest effective dose in OVX+E rats; however, MOR blockade extended the KOR-mediated effect of 500 nmol pentazocine in both groups. In normally cycling females, the 250 nmol dose was effective in producing antinociception at the proestrous, but not at the diestrous stage of the estrous cycle. Thus, estrogen facilitates and KOR or MOR mediate the antinociceptive effect of i.t. (−)-pentazocine in female rats. Selective doses of (−)-pentazocine, with or without MOR blockade, may have a therapeutic benefit.

Keywords

kappa opioid receptor (KOR); mu opioid receptor (MOR); mixed-action kappa opioid agonist; analgesia; spinal cord; estrogen

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Conflict of interest

The authors do not have a conflict of interest involving this work.

1. Introduction

Women experience a higher prevalence of numerous chronic pain conditions, such as fibromyalgia, migraines, temporomandibular joint pain, and irritable bowel syndrome [1–5]. We [6–8] and others [9–11] have demonstrated previously that activation of opioid receptors produces antinociception that is modulated by estradiol and testosterone.

Mixed-action kappa opioid agonists, such as pentazocine, nalbuphine, and butorphanol, given systemically, have been shown to produce significant analgesia in women following removal of the third molar tooth [12–14] or when pain was induced experimentally [15]. Experimental data collected from animal studies have demonstrated that mixed-action kappa opioid agonists, administered systemically, produce antinociception [16–18]. Pentazocine displays binding affinity to both KOR and MOR [19, 20]. Using MOR knockout mice and MOR antagonist, the antinociceptive effect of pentazocine has been shown to be mediated by MOR [21, 22]. In this study, we used (−)-pentazocine since it has been shown to produce opioid receptor-mediated antinociception [17, 18, 21, 23]. (+)-pentazocine binds to the sigma 1 receptor and inhibits antinociception [23], but racemic (±)-pentazocine (Talwin) produces analgesia in humans and rodents when administered systemically [12, 23, 24]. However, (±)-pentazocine administered intrathecally in rats did not produce antinociception using the tail flick nociceptive assay [25]. Because previous research did not delineate whether spinal KOR or MOR mediated the antinociceptive effect of (−)-pentazocine, we examined if pentazocine delivered intrathecally in females produces antinociception and the contribution of spinal KOR and MOR in mediating its antinociceptive effect. In addition, previous research from our laboratory [7] demonstrated that activation of KOR by a selective kappa opioid agonist, U50-488H, produced estrogen-dependent antinociception and antihyperalgesia. Hence, we also sought to determine whether estrogen is required for the antinociceptive effect of pentazocine.

The design of the study is as follows: first, we investigated whether (−)-pentazocine produces antinociception and the role of exogenous estradiol in modulating its effect in OVX animals using an acute thermal nociceptive tail flick assay. Subsequently, by using normally cycling females at proestrous (high levels of estrogen) and diestrous (low levels of estrogen) stages of the estrous cycle, we assessed whether the fluctuation in the endogenous levels of estrogen play a role in modulating the antinociceptive effect of pentazocine. Finally, we evaluated the contribution of spinal KOR and/or MOR in mediating the antinociceptive effect of various doses of pentazocine.

2. Methods

2.1. Animals

Sprague-Dawley female (normally cycling) and ovariectomized (OVX) rats (250–274g; 3–4 months of age) were purchased from Harlan, Inc., (Envigo) (Indianapolis, IN, USA). The total number of rats used was 122. The rodents were housed in an animal care facility approved by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) at Meharry Medical College with a 12-hour light/12-hour dark cycle, temperature range between 68–70°F, and provided with ample water and food (Lab Diet

Prolab RMH 1000, Prolab Laboratory Animal Diet, Brentwood, MO, USA) ad libitum. Rats were housed three per cage with bedding material of Diamond Soft and Soft Cob (Harlan Teklab, Madison, WI, USA). All experiments were conducted during the light cycle. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Meharry Medical College and conformed to the guidelines established by the National Research Council Guide for the Care and Use of Laboratory Animals and the International Association for the Study of Pain (IASP). All efforts were made to minimize stress to the animals and the number of animals used.

2.2. Intrathecal implantation of cannulae in rats

Using a modified version of the Yaksh and Rudy chronic catheterization protocol [26], a mixture of ketamine (80 mg/kg) and xylazine (4 mg/kg) was employed to deeply anesthetize rats before positioning their shaved heads in a stereotaxic frame. Subsequent to removing the muscle dorsal to the atlanto-occipital membrane, the dura was penetrated to permit insertion of a stretched PE-10 cannula (Intramedics, Clay Adams, Sparks, MD, USA) caudally 8.8–9.0 cm in the subarachnoid space of the lumbosacral segment of the spinal cord. We determined this length to be the most appropriate to achieve the optimal drug effect with the tip of the cannula located in the lumbosacral region. Lastly, the cannula was fastened with dental cement. The dead volume of the cannula was 10 μl. The length of the surgery was 20 minutes. This surgery was performed during the diestrous phase of normally cycling female animals. In OVX rats, this surgery was performed 2 weeks after the ovariectomy procedure. Pain medication was not administered following surgery to avoid any residual effect that may confound our results. Seven to fourteen days after implanting a cannula, the rats were subjected to behavioral testing. Subsequent to testing, the position of the cannula in the subarachnoid space of the spinal cord was determined by injecting 2% lidocaine, which induced short-lasting hind limb paralysis. Further, after euthanasia, 1% Chicago Sky Blue dye was injected to determine the patency of the cannula, location of the tip, and dye spread along the lumbosacral spinal cord.

2.3. Estrogen replacement

To determine the role of estrogen in females, estradiol benzoate (Sigma Aldrich, St. Louis, MO, USA) (10 μg/ 100 μl sesame oil; 48 hours prior to nociceptive testing) was administered subcutaneously in OVX rats as described previously [6, 7, 27, 28]. The rationale for using this dose of estradiol was that it produced a proestrous-like vaginal cytology as described below.

2.4. Vaginal cytology

Vaginal smears were taken from OVX and OVX+E animals immediately after behavioral testing, stained using hematoxylin and eosin, and examined microscopically to determine diestrous-like (OVX) and proestrous-like (OVX+E) cytology [6, 29]. The presence of at least 75% nucleated epithelial cells was indicative of the proestrous-like stage. In normally cycling female rats, two regular estrous cycles were established prior to conducting experiments at proestrous and diestrous stages.

2.5. Tail flick nociceptive testing

This nocifensive assay was conducted as described previously [6, 7, 30, 31]. Rats were restrained in a tail access rodent restrainer (Stoelting, Wood Dale, IL, USA) and allowed thirty minutes to habituate prior to testing. Using an analgesia meter (IITC Model 33T, Woodland Hills, CA, USA), a noxious heat stimulus was applied consecutively at three different positions along the dorsal surface of the tail. Three baseline readings were recorded. The heat intensity was set to generate a baseline tail flick latency (TFL) of 2–5 seconds. Over an eighty-five minute time period, the TFL was recorded automatically every five minutes. The trigger temperature was set at 32°C and a commonly used cut-off time at 15 seconds was employed to prevent tissue damage. A cutoff latency of 15 seconds or higher is commonly used in the field in rats [6, 7, 32, 33]. During the time course of the experiment, we did not observe any reduction in TFLs below the baseline that could be indicative of hyperalgesia caused by tissue damage in vehicle-treated animals.

2.6. Drugs

(−)-pentazocine-succinate (125–500 nmol/10 μl), nor-binaltorphimine (nor-BNI) (26 nmol/ 10 μl) [34], and D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (CTAP) (34 nmol/ 10 μl) [35, 36] were administered intrathecally (i.t.) and flushed with 0.9% sterile saline (10 μl). CTAP [10] and nor-BNI [11] were administered five minutes and 18 hours, respectively, before pentazocine. Using this time interval (18 hours), 26 nmol nor-BNI has been shown to selectively antagonize KOR [11]. Pentazocine was administered at time point zero. The vehicle for pentazocine was sterile water, and for nor-BNI and CTAP, 0.9% sterile saline. The rate at which the drug or vehicle was delivered was 1.5–2 μl/ second. Following drug injection, a TFL reading was taken immediately (under 1 minute), and plotted as time point zero in the figures. Each rat was tested only once and subsequently euthanized with an intraperitoneal injection of Beuthanasia (Schering-Plough Animal Health Corporation, Union, NJ, USA) (150 mg/kg). Pentazocine was generously provided by the National Institute on Drug Abuse (NIDA, National Institutes of Health (NIH); Bethesda, Maryland, USA). nor-BNI and CTAP were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.7. Statistical analysis

Data were subjected to ANOVA, corrected for repeated measures using between (group) and within (time) subject factors and the dependent variable (tail flick latency) using SPSS (SPSS Inc., Chicago, IL, USA). The Bonferroni post-hoc test was used for intergroup comparison only where ANOVA yielded a significant main effect or interaction. A (p) value of <0.05 was considered significant. Data are plotted as mean± standard error of mean (SEM) using SigmaPlot (Systat Software, Inc., San Jose, CA, USA). The post hoc power analysis was conducted using G*Power 3.1.9.2 (Germany) to estimate the power in the data presented in each figure. A minimal power value of 0.8 was obtained for each figure.

3. Results

3.1. Pentazocine produces antinociception in OVX rats and estradiol facilitates its effect

To investigate whether pentazocine produces antinociception in a dose-dependent manner, effect of i.t. application of (−)-pentazocine was determined on the tail flick test in OVX and OVX+E rats (Fig. 1–2). In both groups, pentazocine significantly increased the TFL, but at different doses. In OVX rats, ANOVA yielded significant main effects of time [$F_{(17,476)}$ = 34.86; $p < 0.0001$], dose [$F_{(4,28)}$ = 36.49; $p < 0.0001$], and time×dose interaction [$F_{(68,476)}$ = 10.69; $p < 0.0001$] (Fig. 1). Only the highest dose of pentazocine (500 nmol) produced a significant increase in TFL in OVX animals with the peak effect at 10–15 minutes post injection and lasting for 50 minutes ($p < 0.05$). In order to determine whether estradiol modulates the antinociceptive effect of pentazocine, various doses of pentazocine were tested in OVX+E rats. In OVX+E rats, ANOVA yielded significant main effects of time [$F_{(17,544)}$ = 16.57; $p < 0.0001$], dose [$F_{(4,32)}$ = 17.96; $p < 0.0001$], and time×dose interaction [$F_{(68,544)}$ = 6.21; $p < 0.0001$] (Fig. 2). Post hoc test revealed that two doses of pentazocine (250 and 500 nmol) produced a significant increase in TFL with the peak effect at 10–15 minutes post injection and lasting for 35 minutes ($p < 0.05$). Hence, estradiol enabled the expression of antinociception at the 250 nmol dose of pentazocine in OVX animals. ANOVA conducted on baseline TFL did not yield a significant main effect and baseline TFL values were comparable between various groups throughout this study. Because there appeared to be an all-or-none effect with 250 and/or 500 nmol of pentazocine, an intermediate dose (187.5 nmol) was added; however, it failed to produce antinociception in either group (Fig. 1–2).

3.2. Endogenous estrogen facilitates pentazocine antinociception in normal females at proestrous stage of the estrous cycle

To determine whether fluctuations in endogenous levels of estrogen modulate the antinociceptive effect of 250 nmol pentazocine, effect of i.t. application of (−)-pentazocine was determined in normally cycling female rats at proestrous (with a high blood serum level of estrogen) and diestrous (with a low blood serum level of estrogen) stages (Fig. 3). In only the proestrous group, 250 nmol pentazocine significantly increased the TFL. ANOVA yielded significant main effects of time [$F_{(17,170)}$ = 14.77; $p < 0.0001$], group [$F_{(3,10)}$ = 56.93; $p < 0.0001$], and time×group interaction [$F_{(51,170)}$ = 12.19; $p < 0.0001$]. Post hoc test revealed that the peak drug effect in proestrous rats was at 5 minutes post injection and lasted for 45 minutes (all $p < 0.05$). Hence, at the 250 nmol dose of pentazocine, estrogen facilitates the expression of antinociception in both OVX+E and normally cycling females.

3.3. The antinociceptive effect of the lowest effective dose of pentazocine was mediated by KOR or MOR in OVX+E rats

Intrathecal application of nor-BNI or CTAP, antagonists of KOR and MOR, respectively, blocked the antinociceptive effect produced by 250 nmol pentazocine (Fig. 4). ANOVA yielded significant main effects of time [$F_{(17,289)}$ = 4.78; $p < 0.0001$], group [$F_{(2,17)}$ = 17.95; $p < 0.0001$], and time×group interaction [$F_{(34,289)}$ = 5.34; $p < 0.0001$]. Post hoc test revealed that nor-BNI or CTAP blocked the antinociceptive effect of the lowest effective dose of

pentazocine throughout the duration of the experiment (all $p < 0.05$). Hence, KOR or MOR is required for antinociception produced by 250 nmol pentazocine in OVX+E rats.

3.4. MOR blockade extended the KOR-mediated antinociceptive effect of 500 nmol pentazocine

To investigate whether KOR and/or MOR mediated the antinociceptive effect of 500 nmol pentazocine, the effects of i.t. pretreatment with nor-BNI or CTAP were determined (Fig 5–6). While nor-BNI blocked the antinociceptive effect of 500 nmol pentazocine, the duration of the drug effect was significantly extended by CTAP in OVX and OVX+E groups. CTAP-induced enhancement became apparent shortly after pentazocine injection and persisted for the duration of the experiment. In OVX rats, ANOVA yielded significant main effects of time [$F_{(17,221)} = 12.81$; $p < 0.0001$], group [$F_{(4,13)} = 35.86$; $p < 0.0001$], and time×group interaction [$F_{(68,221)} = 7.8$; $p < 0.0001$] (Fig. 5). In OVX+E rats, ANOVA yielded significant main effects of time [$F_{(17,323)} = 12.85$; $p < 0.0001$], group [$F_{(4,19)} = 44.88$; $p < 0.0001$], and time×group interaction [$F_{(68,323)} = 6.62$; $p < 0.0001$] (Fig. 6). Post hoc test revealed that nor-BNI blocked the antinociceptive effect of pentazocine; however, CTAP extended the drug effect for the duration of the experiment in OVX and OVX+E rats (all $p < 0.05$).

4. Discussion

We demonstrate for the first time that intrathecally administered (−)-pentazocine produced antinociception in female rats, which was (a) mediated by spinal KOR or MOR and (b) facilitated by estrogen. First, the lowest effective dose of pentazocine was lower in OVX+E as compared to OVX rats. Second, KOR or MOR mediated the effect of the lowest effective dose of pentazocine in OVX+E rats. Third, the lowest effective dose, 250 nmol pentazocine, induced antinociception in proestrous animals, but not in diestrous rats, implicating the role of sex-steroid hormones, like estrogen, in modulating the effect of pentazocine. Observations reported here demonstrate that intrathecal pentazocine produces antinociception in female (OVX, OVX+E, and proestrous) rats, which is consistent with findings of previous studies that determined the effects of systemically administered pentazocine, nalbuphine, and butorphanol on acute thermal nociception [17, 18, 37–39]. Overall, (−)-pentazocine produces antinociception in female rats, and estrogen facilitates the drug effect.

The present study sought to determine whether estradiol played a role in modulating pentazocine-induced antinociception. A lower dose of pentazocine (250 nmol) produced the maximal drug effect in OVX+E rats in comparison to OVX animals. Further support for this notion comes from another study that reported a greater thermal antinociceptive effect of butorphanol in OVX+E as compared to OVX rhesus monkeys [9]. Also, the lower dose of pentazocine produced a significant increase in the TFL in proestrous animals but not in diestrous rats. Thus, in the context of acute thermal nociception in an animal model, estrogen facilitates antinociception produced by pentazocine. This critical observation substantiates our previous findings that show estrogen dependence of antinociception and antihyperalgesia produced by a selective KOR agonist, U50-488H [7]. However, in a human study, fluctuations in the level of estrogen during the menstrual cycle did not influence pentazocine-induced analgesia; the analgesia produced by pentazocine in humans did not

vary between the follicular and luteal phase amongst females or females on oral contraceptives [40]. The incongruent findings between the animal and human studies support the notion that experimental subject, hormonal status, time of testing after drug administration, mode of drug administration, and testing parameters play a crucial role in whether an estrogen-dependent drug effect will be observed [41, 42].

We have shown that intrathecal administration of (−)-pentazocine can effectively produce thermal antinociception in female rats. The maximal drug effect is obtained in various animal groups at differential, lowest doses. This finding supports the notion that pentazocine could be a therapeutic drug for both pre- and post-menopausal women depending on route of administration, i.e. systemic vs. epidural. The current data does not demonstrate a linear dose response curve for (−)-pentazocine. However, our findings are not unique in this regard considering that nalbuphine, administered systemically in women, did not produce a linear dose response; neither 5 or 20 mg nalbuphine produced analgesia, but 10 mg was effective [14]. Since mixed-action kappa opioid agonists simultaneously target two opioid receptors (KOR and MOR), the results may not be similar to that obtained traditionally using a selective agonist. A bidirectional effect of MOR activation, as observed in our study, may further complicate the dose response curve. Because KOR mediated the antinociceptive effect of the highest dose of pentazocine, and CTAP, a selective antagonist for MOR, prolonged the drug effect of pentazocine in rats, combining the two drugs (pentazocine and CTAP) may yield the best analgesic effect for patients. Previous research has demonstrated that coadministering nalbuphine and naloxone increased the analgesic effect in women [43]. In the context of using 500 nmol pentazocine, we speculate that MOR couples to the stimulatory G protein (G_s), which would facilitate pain transmission (hyperalgesia). However, when MOR is antagonized with CTAP, this pathway is blocked and the antinociceptive effect of pentazocine is prolonged.

In addition, since KOR or MOR mediated the antinociceptive effect of the lowest effective dose of pentazocine in OVX+E rats, our current study supports previous literature demonstrating that pentazocine has binding affinity for both KOR and MOR [19–21]. The effect of pentazocine at the lowest effective dose in OVX+E rats was presumably mediated by a common intracellular pathway activated by both KOR and MOR. Thus, if one of the spinal opioid receptors (KOR or MOR) is antagonized, the common intracellular messenger would not be activated at threshold level to produce antinociception. We speculate that estrogen may facilitate KOR-induced antinociception by increasing the gene [7] and protein [44] expression of KOR in the lumbosacral spinal cord. Although estrogen has been shown to sensitive nociceptors [5], according to our current data, when estradiol was administered to OVX animals, their baseline tail flick latencies did not differ in comparison to OVX rats. Thus, in the context of using the tail flick assay, estrogen neither enhanced nociception (hyperalgesia) nor produced antinociception by itself. However, its facilitory effect on pentazocine-induced analgesia was evident.

5. Conclusions

We conclude that spinal KOR or MOR mediate and estrogen facilitates antinociception produced by intrathecal administration of (−)-pentazocine in female rats. This new

knowledge may promote the development of a suitable dose strategy of pentazocine in women with or without MOR blockade. Considering the involvement of opioid receptors in various traits, such as learning and memory, addiction, stress and depression, antipruritic effect, diuresis, and reproductive function, it is possible that estrogen can modulate opioid-mediated effects on these traits as well [16, 45, 46].

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Highlights

- Intrathecal (−)-pentazocine produces thermal antinociception in female rats
- Estrogen facilitates the antinociceptive effect of pentazocine
- KOR or MOR mediates the antinociceptive effect of pentazocine
- Blockade of MOR prolongs the antinociceptive effect of the highest dose of pentazocine

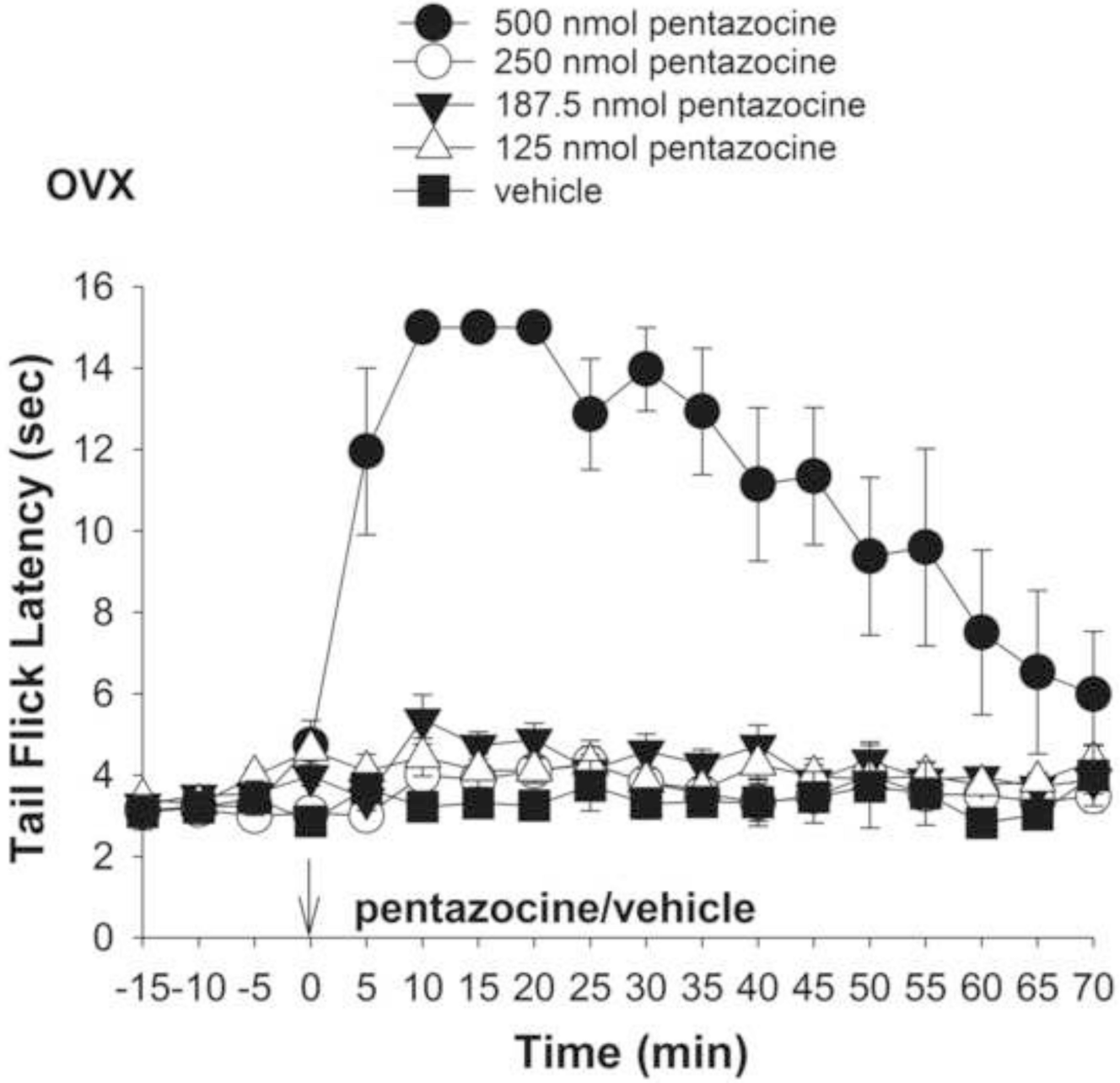

Fig. 1.

In OVX rats, intrathecally administered pentazocine produced thermal antinociception. Only the 500 nmol dose was effective in increasing TFL between 5–60 minutes relative to the vehicle-treated group. Number of animals per group: 500 nmol=6, 250 nmol=9, 187.5 nmol=6, 125 nmol=7, vehicle=5.

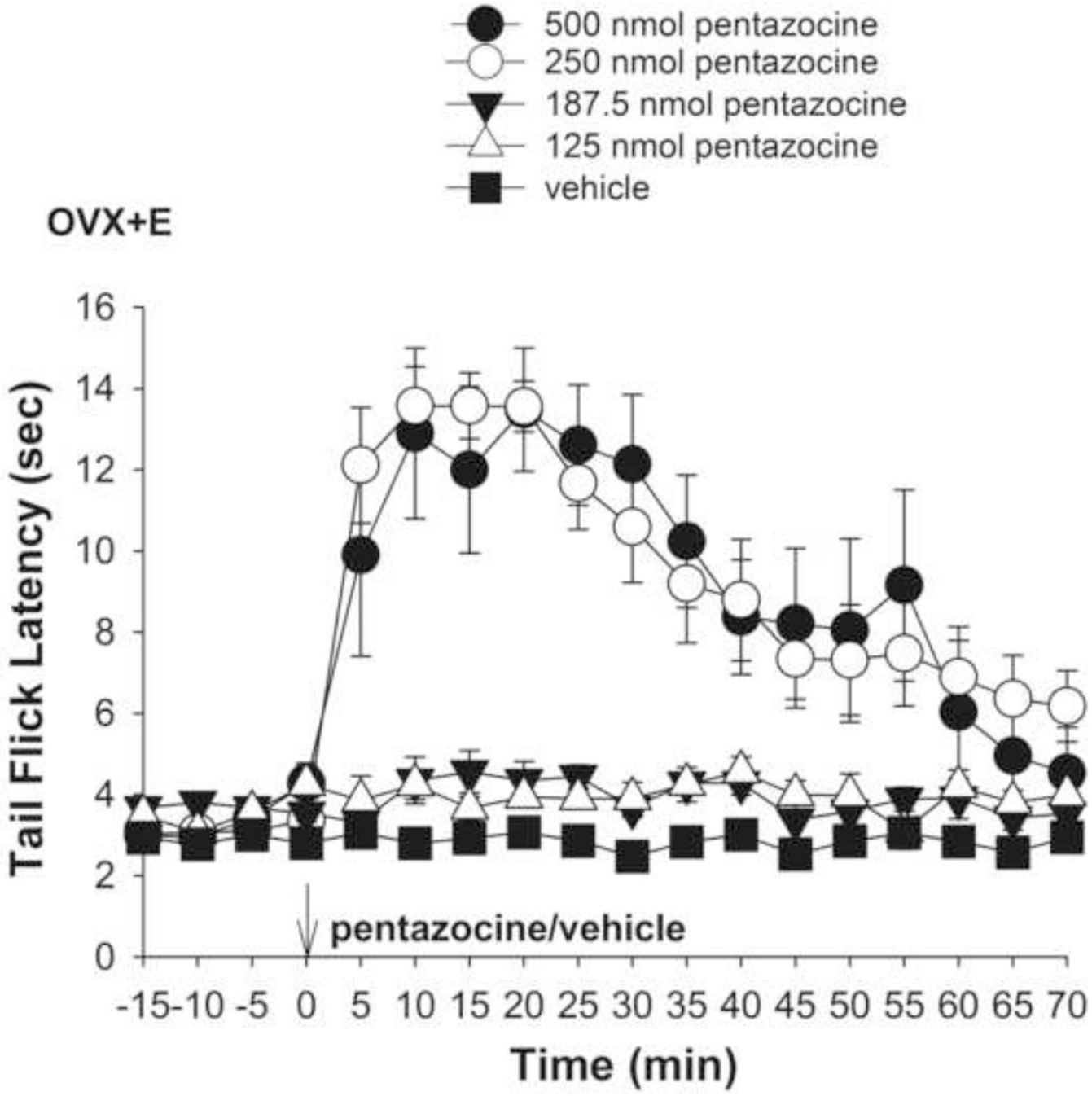

Fig. 2.

Pentazocine produced antinociception in estradiol-treated ovariectomized (OVX+E) rats. 250 and 500 nmol doses significantly increased TFL from 5–45 minutes in comparison to the vehicle-treated group. Number of animals per group: 500 nmol=6, 250 nmol=12, 187.5 nmol=7, 125 nmol=6, vehicle=6.

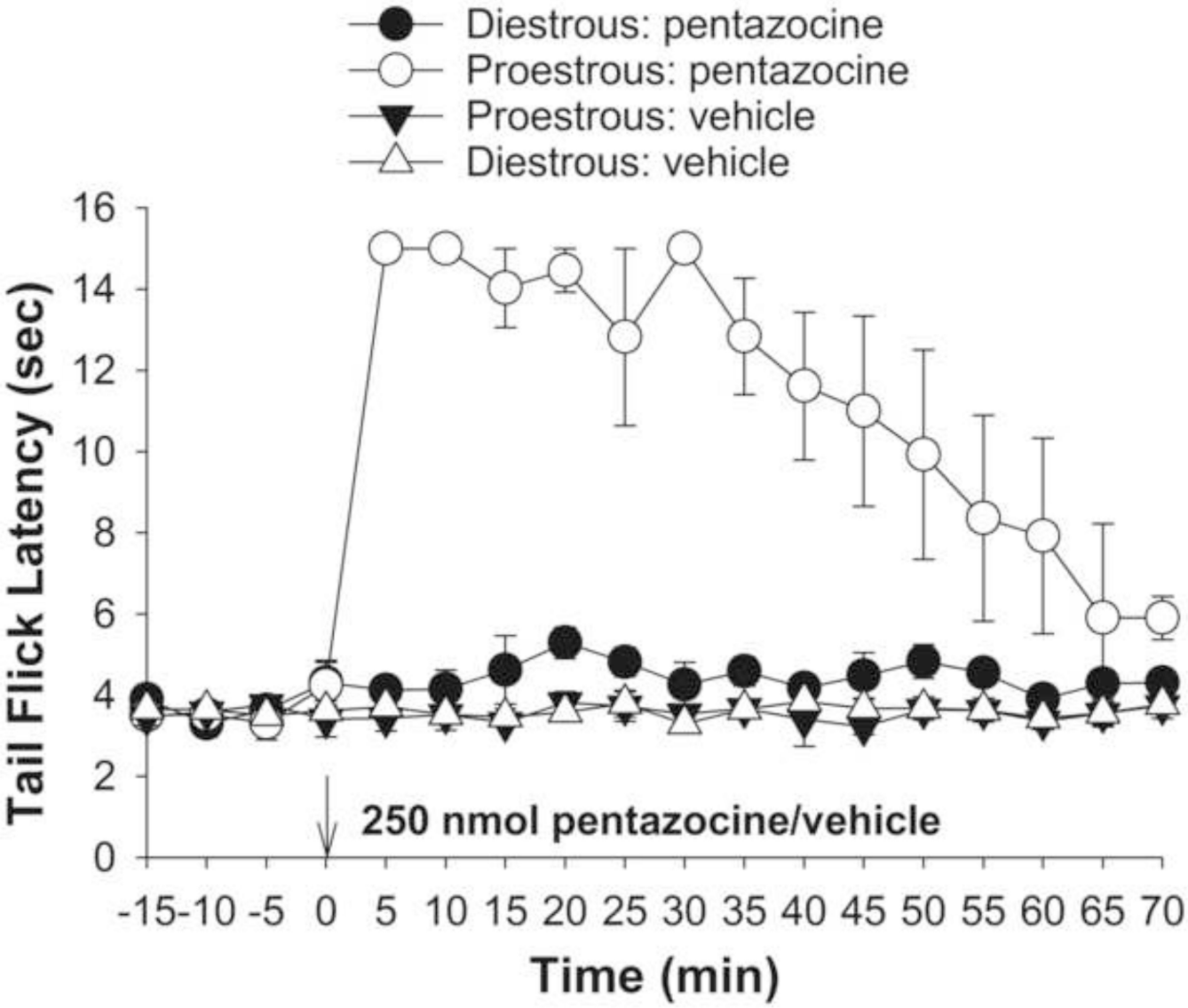

Fig. 3.

Estrogen facilitates the antinociceptive effect produced by 250 nmol pentazocine in normally cycling females. Pentazocine significantly increased the TFL from 5 to 50 minutes in only proestrous females, but not in diestrous animals. Number of animals per group: proestrous-pentazocine=3, diestrous-pentazocine=3, proestrous-vehicle=3, diestrous-vehicle=5.

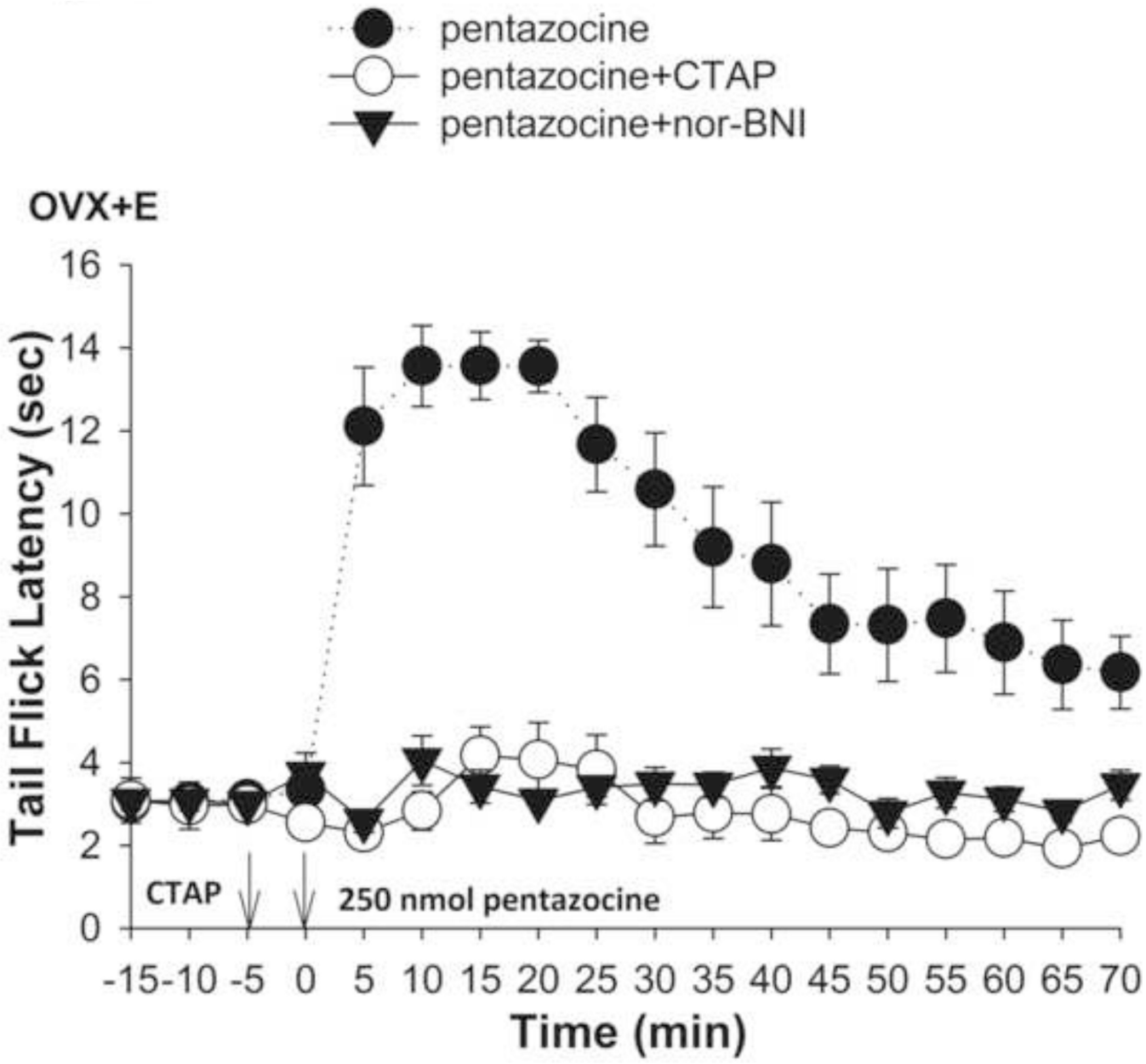

Fig. 4.

The thermal antinociceptive effect of the lowest effective dose of pentazocine is mediated by KOR or MOR in OVX+E rats. The significant increase in TFL by 250 nmol pentazocine in OVX+E rats was blocked by either nor-BNI or CTAP pretreatment. Dotted lines indicate replotted data from Fig. 1 for clarity. Number of animals per group: pentazocine=12, pentazocine+CTAP=3, pentazocine+nor-BNI=5.

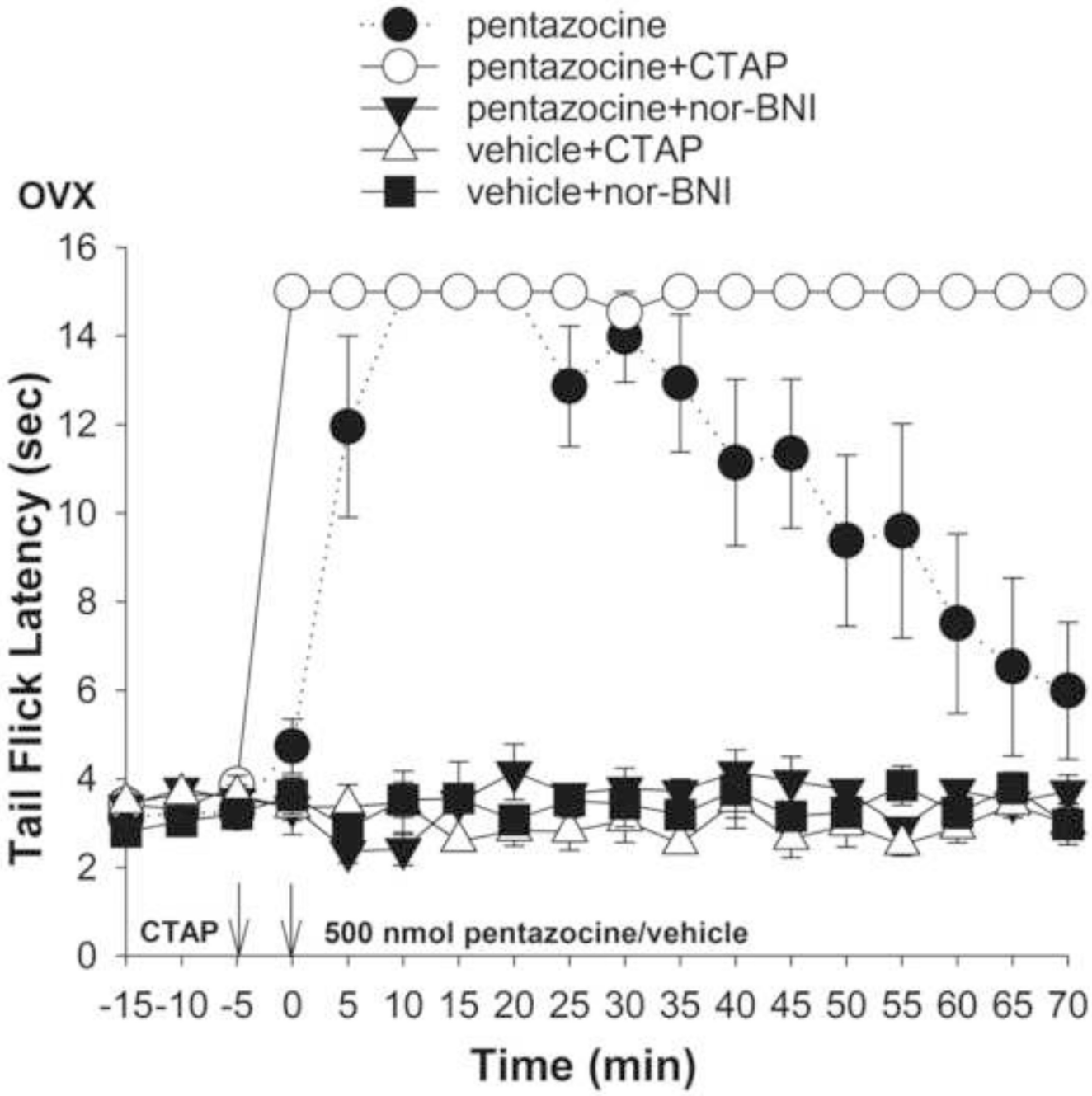

Fig. 5.

The KOR-mediated thermal antinociceptive effect of 500 nmol pentazocine was prolonged following MOR blockade in OVX rats. Antinociception produced by 500 nmol pentazocine was blocked by a KOR antagonist, nor-BNI; however, this effect was significantly prolonged by a MOR antagonist, CTAP. Number of animals per group: pentazocine=6, pentazocine +CTAP=3, pentazocine+nor-BNI=3, vehicle+CTAP=3, vehicle+nor-BNI=3.

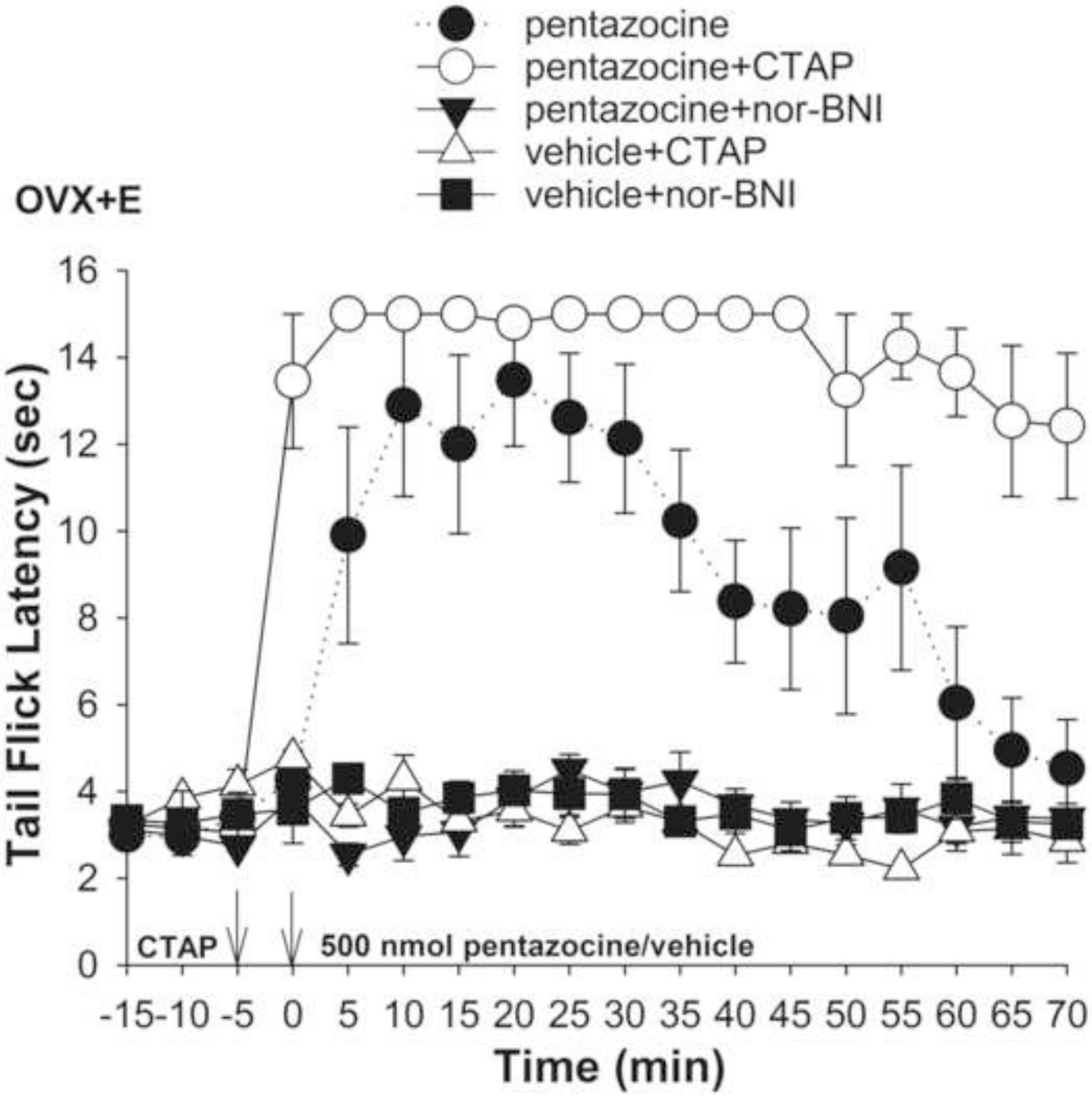

Fig. 6.

Pretreatment with CTAP increased the duration of the antinociceptive effect of 500 nmol pentazocine in OVX+E rats. The antinociceptive effect of 500 nmol pentazocine was mediated by KOR. Number of animals per group: pentazocine=6, pentazocine+CTAP=6, pentazocine+nor-BNI=6, vehicle+CTAP=3, vehicle+nor-BNI=3.