

A synergistic analgesic effect of morphine in combination with the CB1 receptor agonist, ACPA, in normal, hypothyroid, and hyperthyroid male rats

Mohammad-Reza Zarrindast^{1,2}, Fatemeh Khakpai^{3*}

¹ Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran,

² Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran, Iran,

³ Department of Physiology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran,

*Email: khakpai@iautmu.ac.ir

Both cannabinoid and opioid receptors are involved in pain behavior. The administration of morphine and cannabis in rats has been shown to decrease thyroid weight and thyroid-stimulating hormone (TSH) levels. We hypothesized that the third ventricle, due to its adjacency to the hypothalamus, is involved in the modulation of hypothalamic-pituitary-thyroid axis activity and descending pain pathways. The present study examined the effect of intra-third ventricle administration of morphine and cannabis agents on the modulation of pain behavior in normal, hypothyroid (increased serum TSH), and hyperthyroid (decreased serum TSH) rats using the tail-flick test. The results indicated that intra-third ventricle injection of AM251 (CB1 receptor antagonist) caused hyperalgesia, while intra-third ventricle administration of ACPA (CB1 receptor agonist) and morphine produced analgesia in normal, hypothyroid, and hyperthyroid rats. A non-effective dose of morphine (0.5 µg/rat) did not attenuate hyperalgesia induced by an effective dose of AM251. Co-injection of ACPA and morphine into the third ventricle induced anti-nociceptive effect in normal, hypothyroid, and hyperthyroid rats. An isobolographic analysis demonstrated a synergistic effect between ACPA and morphine in the production of the anti-nociceptive effect. Consequently, the third ventricle may modulate pain behavior induced by cannabinoid and opioid receptors *via* descending pain pathways in normal, hypothyroid, and hyperthyroid rats.

Key words: hypothyroid, hyperthyroid, third ventricle, cannabinoid, opioid, pain

INTRODUCTION

Pain is a complex process that includes an unpleasant sensory and emotional experience correlated to actual or potential tissue damage (Loeser and Treede, 2008), which serves a vital protective evolutionary function (Woodhams et al., 2017). The arrival of nociceptive stimuli to the central nervous system (CNS) stimulates many neurochemical systems that inhibit pain sensation (Fields, 2004). Among the many neurochemical systems involved in the inhibitory control of pain, the opioid and cannabinoid systems are of spe-

cific interest, in terms of physiological relevance, and are the target of currently effective therapeutic methods (Nadal et al., 2013).

Three diverse subtypes of opioid receptors, μ , δ , and κ receptors, have been recognized and studied at the pharmacological level (Kieffer and Evans, 2009). Opioid receptors are G-protein-coupled receptors distributed in the CNS and peripheral tissues (Armario, 2010; Nadal et al., 2013). The μ receptor is distributed in the brain, mainly in the areas related to nociceptive control. The endogenous opioid system controls nociceptive responses at both peripheral and central levels (Nadal et al., 2013; Rittner et al., 2008). In the CNS, the endog-

enous opioid system modulates the nociceptive pathways at the spinal and supraspinal levels (Langerman et al., 1995; Nadal et al., 2013).

Cannabinoids induce pharmacological effects by activating two distinct cannabinoid receptors (CB), CB1 and CB2. These receptors are a member of the G-protein-coupled receptor superfamily. The distribution of these receptors in the CNS and peripheral tissues differs (Armario, 2010; Nadal et al., 2013; Ueda et al., 2014; Starowicz and Finn, 2017). The CB1 receptor is extensively expressed in the CNS. The endocannabinoid system modulates nociceptive responses by acting at numerous central and peripheral levels (Nadal et al., 2013). At the level of the CNS, the endocannabinoid system controls nociception mainly through the CB1 receptor (Nadal et al., 2013; Woodhams et al., 2017).

Anatomical, biochemical, and pharmacological studies support the existence of bidirectional interactions between cannabinoid and opioid systems. Cannabinoid and opioid agonists induce anti-nociception by acting on similar structures within the CNS, and a peripheral mechanism has also been detected for both compounds (Maldonado and Valverde, 2003; Scavone et al., 2013). μ -opioid and CB1 receptors have been demonstrated to co-localize in similar neurons in the superficial dorsal horn of the spinal cord, the first location of synaptic contact for peripheral nociceptive afferents, increasing the probability of direct interactions between these receptor types within the same cells (Salio et al., 2001; Zubrzycki et al., 2019). Studies have revealed that the administration of morphine and cannabis to adult male rats decreased body weight, thyroid weight, and thyroid-stimulating hormone (TSH) level (Lomax and George, 1966; Lomax, 1970; Bakke et al., 1974; Muraki et al., 1980; Hillard et al., 1984; Malhotra et al., 2017).

Thyroid hormone, which regulates metabolic processes essential for growth and development (Cheng et al., 2010; Brent, 2012; Park et al., 2012; Salazar et al., 2017), affects many neurotransmitter systems, including the opioid system (Edmondson et al., 1990). The effect of thyroid hormone on opiate receptor function was assessed in rats with excess thyroid hormone, hyperthyroid, and rats with reduced thyroid hormone levels, hypothyroid (Edmondson et al., 1990; Mullur et al., 2014; Yu et al., 2015). Hypothyroidism, enhanced serum TSH, and hyperthyroidism, reduced serum TSH, disorders significantly influence the hypothalamic-pituitary-thyroid (HPT) axis in both animal models and humans (Yu et al., 2015). The third ventricle is one of four connected fluid-filled cavities of the mammalian brain. The floor of the third ventricle is formed by the hypothalamic structures (Hendrie and Pickles, 2010), so it can influence HPT axis

activity (Yu et al., 2015) and descending pain pathways (Puopolo, 2019).

We attempted to determine the effect of exogenous administration of morphine and cannabis agents into the third ventricle on the modulation of pain behavior in normal, hypothyroid, and hyperthyroid male rats using the tail-flick test; the study took into consideration the distribution of μ receptors and CB1 receptors in the areas related to pain control (Nadal et al., 2013), cerebral ventricles (Mansour et al., 1994; Suárez et al., 2010) and the involvement of the HPT axis (Aloisi et al., 2013), opioid, and cannabis systems in the modulation of pain behavior (Maldonado and Valverde, 2003; Scavone et al., 2013), as well as their roles in affecting thyroid hormone levels (Lomax and George, 1966; Lomax, 1970; Bakke et al., 1974; Muraki et al., 1980; Hillard et al., 1984; Malhotra et al., 2017).

METHODS

Subjects

Adult male Wistar rats (obtained from the University of Tehran, Tehran, Iran) weighing 200–220 g were used in the experiments. The subject room was kept on a 12:12 light/dark cycle (lights on 07:00 h) at a temperature of $22 \pm 2^\circ\text{C}$. All subjects had free access to food and water. Eight rats were tested in each experiment only once and were handled about 5 min per day for one week before behavioral testing. The experiments were carried out between 8:00 a.m. and 1:00 p.m. All procedures for the treatment of rats were confirmed by the Research and Ethics Committee of the School of Medicine, Tehran University of Medical Sciences, and were performed under the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH publications no. 80-23; revised 1996).

Induction of the hypothyroid and hyperthyroid groups

The rats in the hypothyroid group received lithium carbonate at a dose of 2 $\mu\text{g}/\text{ml}$, diluted in drinking water. Rats in the hyperthyroid group received L-thyroxine daily at a dose of 2 $\mu\text{g}/\text{ml}$, diluted in drinking water, similar to the hypothyroid group. The control group received distilled water. The drugs were administered for one month in an attempt to obtain a clinical-physiological status of thyroid dysfunction in the rats. The body weight and core body temperature of rats were measured at the beginning of each week during the one-month treatment to record the rats' development by drug ingestion.

Surgery and microinjection procedures

After one month of treatment, each rat was anesthetized with ketamine/xylazine (100 mg/kg ketamine and 5 mg/kg xylazine mixture, i.p.), and a 15 mm-long, 22-gauge cannula was implanted into the third ventricle and fixed with acrylic dental cement to the skull. The stereotaxic coordinates for the third ventricle were: anteroposterior about the interaural line, Anterior-Posterior=-1 mm; lateral about the sagittal line, Lateral=0 mm; and vertically from the top of the skull, Ventral=-7 mm (Paxinos and Watson, 2007). Drug microinjections were carried out by a 27-gauge stainless steel needle attached to a Hamilton micro-syringe by polyethylene tubing. The rats were held by hand, with the drug microinjection lasting for 60 s, and the cannula was left in place for an additional 60 s to evade backflow of the solution. Before the experiments, the rats had at least a five-day recovery period.

Drugs

The drugs used in the experiments were L-thyroxin (Merck, UK), lithium carbonate (Tocris, UK), morphine sulfate (Temad, Tehran, Iran), ACPA (arachidonylecyclopropylamide; N-(2-cyclopropyl)-5Z,8Z,11Z,14Z-eicosatetraenamide; Tocris, UK), and AM251 (N-(piperidine-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; Tocris, UK). The drugs were dissolved in saline. ACPA was received in Tocrisolve™ (a soya oil and water emulsion from Tocris, UK) and diluted directly into sterile 0.9% saline. In experiments where ACPA was injected, the control solution contained Tocrisolve at a similar concentration as the microinjection solution. AM251 was dissolved in dimethyl sulphoxide (DMSO; up to 10% v/v), 0.9% saline, and a drop of Tween 80. Injections of the drugs into the third ventricle were in a volume of 0.5 µl.

Behavioral tests

Rectal thermistor probe

Body temperature was recorded at the beginning of the drug (L-thyroxin and lithium carbonate) treatments as a baseline temperature. Other than the baseline temperatures, temperatures were recorded at time point zero (before the drug treatment) and one, two, three, and four weeks after drug treatment. For temperature recording, the rats were placed individually in experimental cages, and body temperature was

recorded three times in 10 min intervals. The mean was calculated. This was to exclude any confounding influence of handling on the subject's temperature. Just before the drug treatment, body temperature was recorded using a rectal thermistor probe (Light Lab, Brighton, UK, sensitivity 0.1°C). The probe was lubricated with petroleum jelly, then inserted into the rectum to a depth of 2 cm. The data are presented as changes in rectal temperature from the baseline values. Baseline values were those taken just before the drug treatment.

Tail-flick apparatus

A tail-flick apparatus was used to determine the nociceptive response to thermal stimulation (Borj Sanat Company, Iran). The reaction time between the onset of the heat stimulus and the removal of the tail from the heat source was recorded through a sensor as tail-flick latency. Because tail-flick latency time (s) normally depends on the proximal-distal place of heating on the tail (Ness et al., 1987), the tail was marked with a line in 1 cm increments beginning at the tip for a total of 5 increments. Each subject was wrapped in a soft towel and the dorsal surface of the subject's tail from its distal end was immediately located in the apparatus every 15 min (for 60 min) after the drug/saline or vehicle administration. The heat source and a timer were activated simultaneously through a pedal. Both were finished automatically through a tail movement, which exposed a photocell beneath the tail and through the experimenter at the end of a ten second-cut-off time. This cut-off time was set to avoid skin damage. To assess the sensitivity of each rat to a nociceptive stimulus, we considered the rat's tail-flick latency before drug treatment as a baseline pain threshold. The subjects were tested twice within a 15 min interval, and the mean of these measurements was considered the baseline latency. Light intensity was adjusted to obtain a baseline tail-flick latency of 2–4 s. All data were normalized to the pre-administration baseline. Individual tail withdrawal latencies were converted to a percentage of maximum possible effect (MPE%) through the formula: $MPE\% = [(test\ latency - baseline\ latency) / (cut-off\ latency - baseline\ latency)] \times 100$. There were no significant differences in baseline tail-flick latencies between the experimental groups before the application of the drug/saline or vehicle.

Experimental design

Each experimental group included eight male rats, and each male rat was used only once. A series of sev-

en experiments were designed to determine the effect of cannabis and morphine drugs on pain behavior in normal, hypothyroid, and hyperthyroid male rats. The protocol is described below and presented in Table 1. After one month of the hypothyroid and hyperthyroid group induction, implantation of the cannula was done into the third ventricle, and after a five-day recovery period, experimental tests were carried out. Each experiment began with intra-third ventricle microinjection of AM251, ACPA, and morphine five minutes before the tail-flick test. Each subject received a volume of 0.5 µg/rat of drugs. In the experiments, the rats received two injections, first cannabis and then morphine. The interval time between the two injections was five minutes. All procedures were carried out between 8:00 a.m. and 1:00 p.m. The doses and duration of the drug infusions were chosen based on previous research and our pilot studies. One week was allowed between testing the different doses (Kosarmadar et al., 2015; Khakpai et al., 2019).

Experiment 1

In this experiment, the effects of L-thyroxine and lithium carbonate treatment on body weight and core body temperature were assessed. The body weight and core body temperature of rats were measured at the experiment's beginning and one, two, three, and four weeks after drug treatment in normal, hypothyroid, and hyperthyroid male rats.

Experiment 2

Experiment 2 evaluated the effect of intra-third ventricle administration of the CB1 receptor antagonist AM251 on tail-flick latency in normal, hypothyroid, and hyperthyroid male rats. The subjects of the normal, hypothyroid, and hyperthyroid groups were injected with different doses of AM251 (0.125, 0.25, and 0.5 µg/rat; intra-third ventricle). The tail-flick latency of each rat was measured on the tail-flick apparatus.

Table 1. The table clarifies experimental design.

Figure	Panel	L-thyroxine and lithium carbonate treatment	Intra-third ventricle treatment	Weight	Temperature	Pain response
2	A	1 month	–	L-thyroxine → Decrease	L-thyroxine → Increase	–
	B	1 month	–	Lithium → Increase	Lithium → Decrease	–
3	A	–	AM251 (0.125, 0.25 and 0.5 µg/rat)	–	–	Hyperalgesia
	B	1 month	AM251 (0.125, 0.25 and 0.5 µg/rat)	–	–	Hyperalgesia
	C	1 month	AM251 (0.125, 0.25 and 0.5 µg/rat)	–	–	Hyperalgesia
4	A	–	ACPA (0.125, 0.25 and 0.5 µg/rat)	–	–	Analgesia
	B	1 month	ACPA (0.125, 0.25 and 0.5 µg/rat)	–	–	Analgesia
	C	1 month	ACPA (0.125, 0.25 and 0.5 µg/rat)	–	–	Analgesia
5	A	–	Morphine (0.5, 1 and 2 µg/rat)	–	–	Analgesia
	B	1 month	Morphine (0.5, 1 and 2 µg/rat)	–	–	Analgesia
	C	1 month	Morphine (0.5, 1 and 2 µg/rat)	–	–	Analgesia
6	A	–	AM251 (0.125, 0.25 and 0.5 µg/rat) + morphine (0.5 µg/rat)	–	–	Hyperalgesia
	B	1 month	AM251 (0.125, 0.25 and 0.5 µg/rat) + morphine (0.5 µg/rat)	–	–	Hyperalgesia
	C	1 month	AM251 (0.125, 0.25 and 0.5 µg/rat) + morphine (0.5 µg/rat)	–	–	Hyperalgesia
7	A	–	ACPA (0.125, 0.25 and 0.5 µg/rat) + morphine (0.5 µg/rat)	–	–	Analgesia
	B	1 month	ACPA (0.125, 0.25 and 0.5 µg/rat) + morphine (0.5 µg/rat)	–	–	Analgesia
	C	1 month	ACPA (0.125, 0.25 and 0.5 µg/rat) + morphine (0.5 µg/rat)	–	–	Analgesia
8	A	–	AM251 (0.125 µg/rat) + morphine (0.5 µg/rat)	–	–	Synergistic effect
	B	1 month	AM251 (0.0625 µg/rat) + morphine (0.25 µg/rat)	–	–	Synergistic effect
	C	1 month	AM251 (0.03125 µg/rat) + morphine (0.125 µg/rat)	–	–	Synergistic effect

Experiment 3

This experiment examined the effect of intra-third ventricle injection of the CB1 receptor agonist ACPA on tail-flick latency in normal, hypothyroid, and hyperthyroid male rats. The rats of the normal, hypothyroid, and hyperthyroid groups were injected with different doses of ACPA (0.125, 0.25, and 0.5 µg/rat; intra-third ventricle).

Experiment 4

In experiment 4, the effect of intra-third ventricle injection of morphine on tail-flick latency was evaluated in normal, hypothyroid, and hyperthyroid male rats. The subjects of the normal, hypothyroid, and hyperthyroid groups were injected with different doses of morphine (0.5, 1, and 2 µg/rat; intra-third ventricle).

Experiment 5

In this experiment, the effect of intra-third ventricle co-injection of AM251 and morphine on tail-flick latency was assessed in normal, hypothyroid, and hyperthyroid male rats. The subjects of the normal, hypothyroid, and hyperthyroid groups were injected with different doses of AM251 (0.125, 0.25, and 0.5 µg/rat; intra-third ventricle) and received morphine (0.5 µg/rat; intra-third ventricle) five minutes later.

Experiment 6

Experiment 6 studied the effect of intra-third ventricle co-injection of ACPA and morphine on tail-flick latency in normal, hypothyroid, and hyperthyroid male rats. The subjects of the normal, hypothyroid, and hyperthyroid groups were injected with various doses of ACPA (0.125, 0.25, and 0.5 µg/rat; intra-third ventricle) plus an ineffective dose of morphine (0.5 µg/rat; intra-third ventricle) with a five-minute interval.

Experiment 7

To confirm whether ACPA and morphine administration would exert additive or synergistic effects on their induced anti-nociception response, an isobolographic analysis was performed to compare the theoretical and experimental ED50 of the drugs when administered together. According to the dose-response curve of ACPA and morphine, subjects of the normal, hypothyroid, and hyperthyroid male rat groups received the following doses of drugs: ACPA 0.125 µg/rat + morphine 0.5 µg/rat, ACPA 0.0625 µg/rat + morphine 0.25 µg/rat, and ACPA 0.03125 µg/rat + morphine 0.125 µg/rat (Nasehi et al., 2016; 2017).

Data analysis

The results are presented as the mean of the percentage of maximal possible effect (MPE%) ± standard error of the mean (S.E.M.). The mean MPE% in all groups was subjected to repeated measures and two-way ANOVA followed by the Tukey *post hoc* test to detect differences among the treatments. $P < 0.05$ was considered statistically significant.

Histology

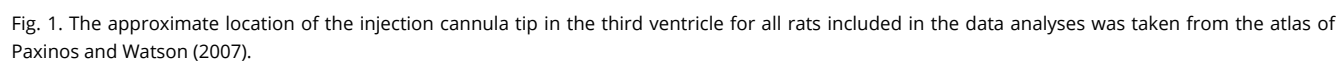
At the completion of all of the experiments, the rats were anesthetized, and methylene blue solution (1%, 0.5 µl/rat) was injected into the guide cannula for the verification of the cannula site; the brain of each rat was then removed and placed in formaldehyde (10%). After seven days, the brain sections were assessed according to a rat brain atlas (Paxinos and Watson, 2007) (Fig. 1). Only data from rats with a correctly located microinjection were included for statistical analyses. We added subject rats to replace those whose cannula was badly placed.

Furthermore, thyroid glands were inspected and processed to confirm the L-thyroxine and lithium carbonate effects on gland morphology. Thyroid glands were fixed in neuter formol buffered with 10% phosphate, and then processed according to a routine method of paraffin inclusion. Histological sections of 4 µm were stained using the hematoxylin-eosin method for morphological evaluation (Titford, 2005; Llewellyn, 2009), where the height of follicular epithelium, the size of follicles, the intensity and density of colloid were evaluated.

RESULTS

The effects of L-thyroxine and lithium carbonate treatment on body weight and core body temperature

Fig. 2 indicates the effect of lithium carbonate and L-thyroxine treatment on body weight and core body temperature. Two-way ANOVA followed by Tukey's test for repeated measures over time revealed that lithium carbonate treatment increased body weight [time effect: $F_{(4,120)}=30.646$, $P < 0.001$; treatment effect: $F_{(3,120)}=144.998$, $P < 0.001$; and treatment × time interaction: $F_{(12,120)}=9.267$, $P < 0.01$], whereas L-thyroxine treatment decreased body weight [time effect: $F_{(4,120)}=16.069$, $P < 0.001$; treatment effect: $F_{(3,120)}=72.956$, $P < 0.001$; and treatment × time interaction: $F_{(12,120)}=15.601$, $P < 0.001$] (Fig. 2A).



Also, as shown in Fig. 2B, two-way ANOVA followed by Tukey's test for repeated measures over time demonstrated that lithium carbonate treatment decreased core body temperature [time effect: $F_{(4,120)}=3.710$, $P<0.05$; treatment effect: $F_{(3,120)}=9.662$, $P<0.01$; and treatment \times time interaction: $F_{(12,120)}=12.620$, $P<0.01$], but L-thyroxin treatment increased core body temperature [time effect: $F_{(4,120)}=4.537$, $P<0.01$; treatment effect: $F_{(3,120)}=19.290$, $P<0.001$; and treatment \times time interaction: $F_{(12,120)}=15.443$, $P<0.001$].

The effects of AM251 administration on tail-flick latency in normal, hypothyroid, and hyperthyroid male rats

Fig. 3 shows the effect of intra-third ventricle administration of AM251 on tail-flick latency at set intervals (15, 30, 45, and 60 min) in normal, hypothyroid, and hyperthyroid male rats. As shown in Fig. 3A, two-way ANOVA revealed no significant interaction between AM251 dose and time interval on MPE% in normal rats [time effect: $F_{(3,84)}=64.134$, $P<0.001$; AM251 effect: $F_{(3,28)}=2.185$, $P>0.05$; time interval \times AM251 interaction: $F_{(9,84)}=0.329$, $P>0.05$].

Regarding the time Intervals effect and AM251 effect, Tukey's multiple comparisons indicated that AM251 (0.5 $\mu\text{g}/\text{rat}$), at the time intervals of 15, 30, 45, and 60 min after administration, decreased MPE%, suggesting a hyperalgesic effect in normal male rats.

As shown in Fig. 3B, there was no significant interaction between AM251 dose and time interval on MPE% in hypothyroid rats [time effect: $F_{(3,84)}=148.007$, $P<0.001$; AM251 effect: $F_{(3,28)}=3.325$, $P<0.05$; time interval \times AM251 interaction: $F_{(9,84)}=0.559$, $P>0.05$]. Tukey's multiple comparisons revealed that AM251 (0.5 and 0.25 $\mu\text{g}/\text{rat}$), at the time intervals of 15, 30, 45, and 60 min after injection, reduced MPE%, suggesting a hyperalgesic effect in hypothyroid male rats.

Additionally, two-way ANOVA revealed a significant interaction between AM251 doses and time interval on MPE% in hyperthyroid rats [time effect: $F_{(3,84)}=219.082$, $P<0.001$; AM251 effect: $F_{(3,28)}=28.918$, $P<0.001$; time interval \times AM251 interaction: $F_{(9,84)}=2.608$, $P<0.01$] (Fig. 3C). Multiple comparisons indicated that AM251 (0.5 and 0.25 $\mu\text{g}/\text{rat}$), at a time interval of 15, 30, 45, and 60 min after administration, decreased MPE%, suggesting a hyperalgesic effect in hyperthyroid male rats.

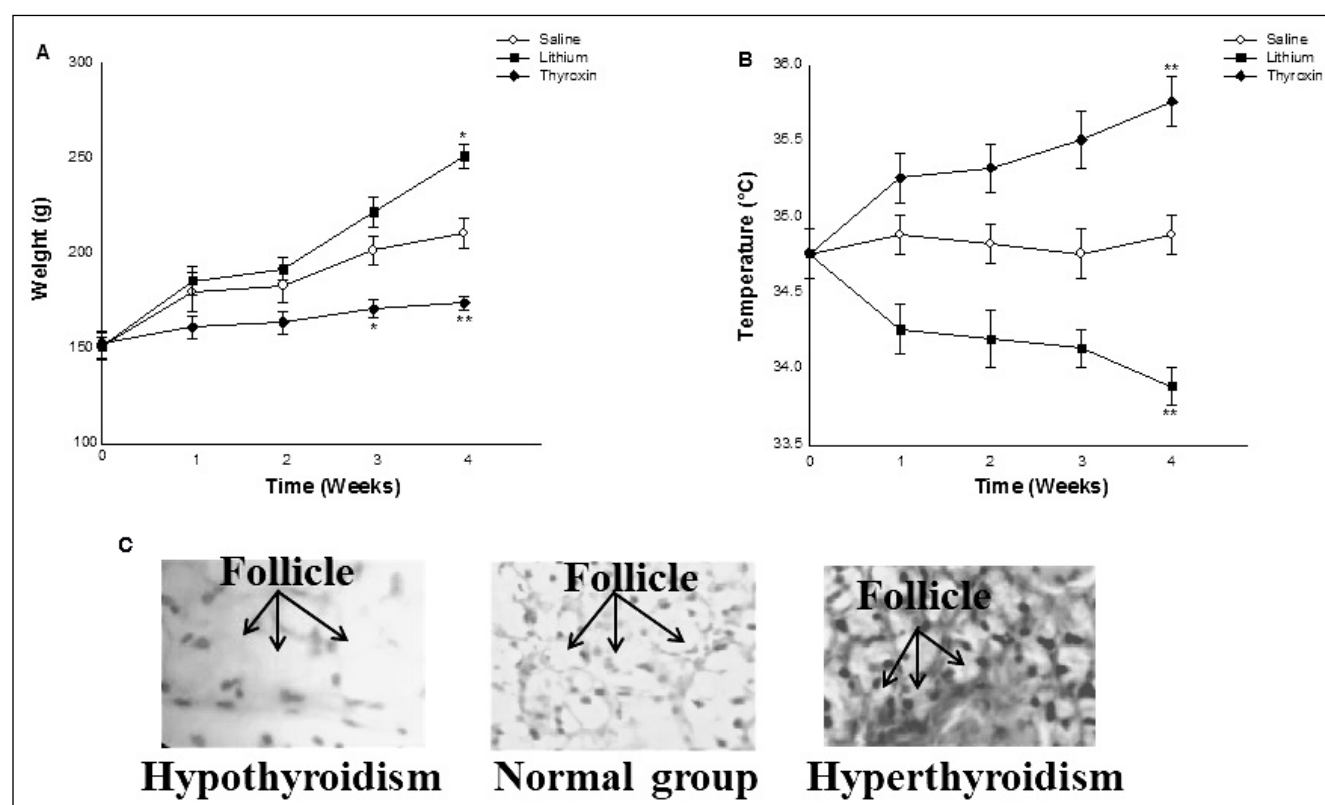


Fig. 2. (A) shows the effects of L-thyroxin and lithium carbonate on body weight. (B) shows the effects of these drugs on core body temperature. The data bars represent mean \pm S.E.M. (C) presents histological sections of thyroid tissue in normal, hypothyroid, and hyperthyroid male rats. These histological sections differ in terms of follicle sizes and quantity of colloid.

The effects of ACPA administration on tail-flick latency in normal, hypothyroid, and hyperthyroid male rats

Fig. 4 shows the effect of intra-third ventricle injection of ACPA on tail-flick latency at set intervals (15, 30, 45, and 60 min) in normal, hypothyroid, and hyperthyroid male rats. As shown in Fig. 4A, two-way ANOVA revealed no significant interaction between ACPA dose and time interval on MPE% in normal rats [time effect: $F_{(3,84)}=118.706$, $P<0.001$; ACPA effect: $F_{(3,28)}=5.754$, $P<0.05$; time interval \times ACPA interaction: $F_{(9,84)}=0.585$, $P>0.05$]. Regarding the time interval effect and ACPA

effect, Tukey's multiple comparisons revealed that ACPA (0.5 $\mu\text{g}/\text{rat}$), at the time intervals of 15, 30, 45, and 60 min after injection, increased MPE%, suggesting an anti-nociceptive effect in normal rats.

As shown in Fig. 4B, there was no significant interaction between ACPA dose and time interval on MPE% in hypothyroid rats [time effect: $F_{(3,84)}=70.395$, $P<0.001$; ACPA effect: $F_{(3,28)}=9.980$, $P<0.001$; time interval \times ACPA interaction: $F_{(9,84)}=0.439$, $P>0.05$]. Tukey's multiple comparisons showed that ACPA (0.5 and 0.25 $\mu\text{g}/\text{rat}$), at the time intervals of 15, 30, 45, and 60 min after application, enhanced MPE%, suggesting an analgesic effect in hypothyroid rats.

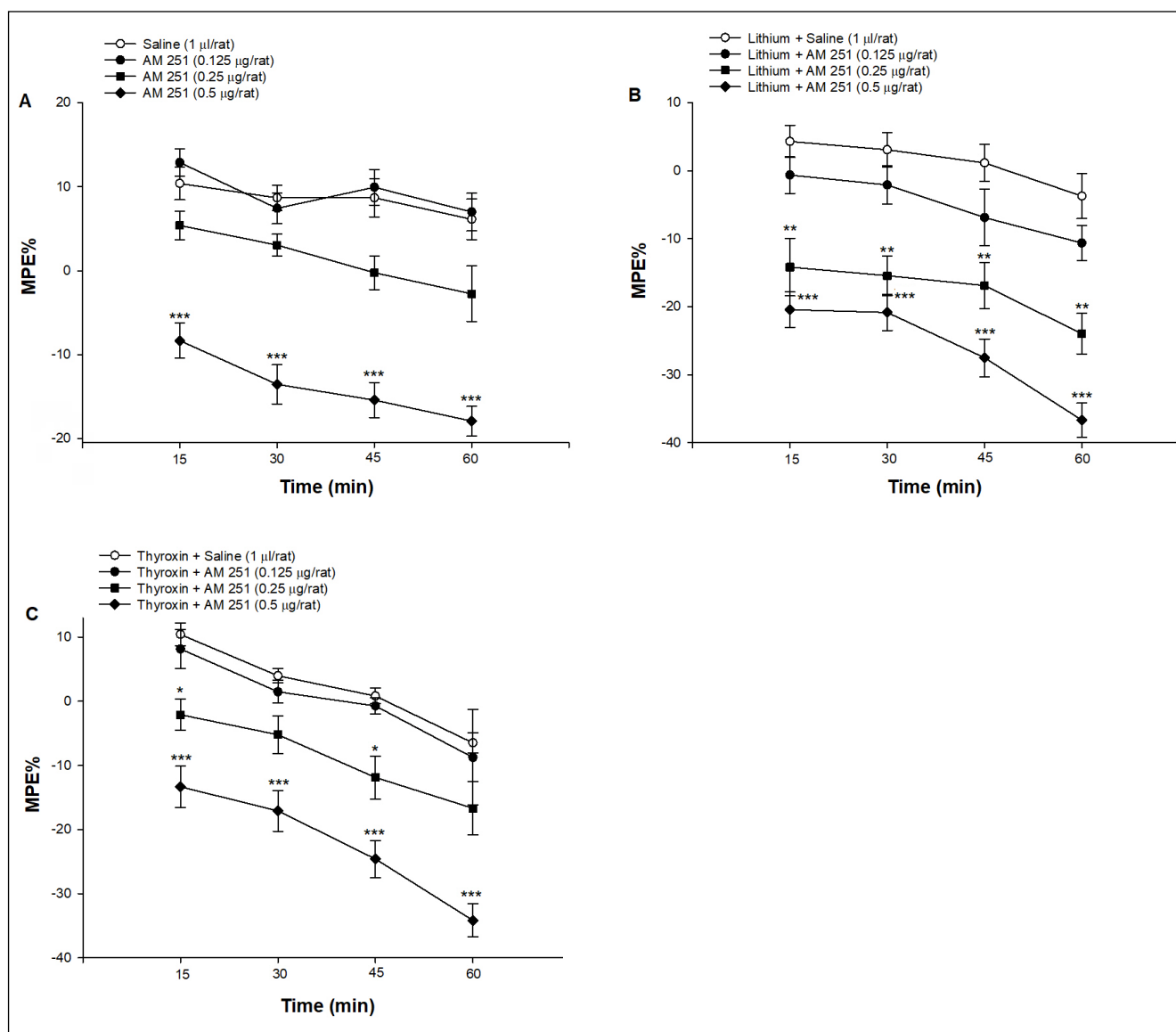


Fig. 3. The effect of intra-third ventricle injection of AM251 alone or in combination with L-thyroxine and lithium carbonate on pain behavior. Data is presented as mean \pm S.E.M.

Furthermore, as shown in Fig. 4C, there was no significant interaction between ACPA dose and time interval on MPE% in hyperthyroid rats [time effect: $F_{(3,84)}=58.837$, $P<0.001$; ACPA effect: $F_{(3,28)}=15.801$, $P<0.001$; time interval \times ACPA interaction: $F_{(9,84)}=0.319$, $P>0.05$]. Multiple comparisons displayed that ACPA (0.5 and 0.25 $\mu\text{g}/\text{rat}$), at a time interval of 15, 30, 45, and 60 min after injection, increased MPE%, suggesting an anti-nociceptive effect in hyperthyroid rats.

The effects of morphine administration on tail-flick latency in normal, hypothyroid, and hyperthyroid male rats

Fig. 5 shows the effect of intra-third ventricle injection of morphine on tail-flick latency at set time intervals (15, 30, 45, and 60 min) in normal, hypothyroid, and hyperthyroid male rats. As shown in Fig. 5A, two-way ANOVA indicated no significant interaction between morphine dose and time interval on MPE% in normal rats [time effect: $F_{(3,84)}=414.843$, $P<0.001$; morphine effect: $F_{(3,28)}=1.640$, $P>0.05$; time interval \times morphine interaction: $F_{(9,84)}=1.772$, $P>0.05$]. Regarding the time interval

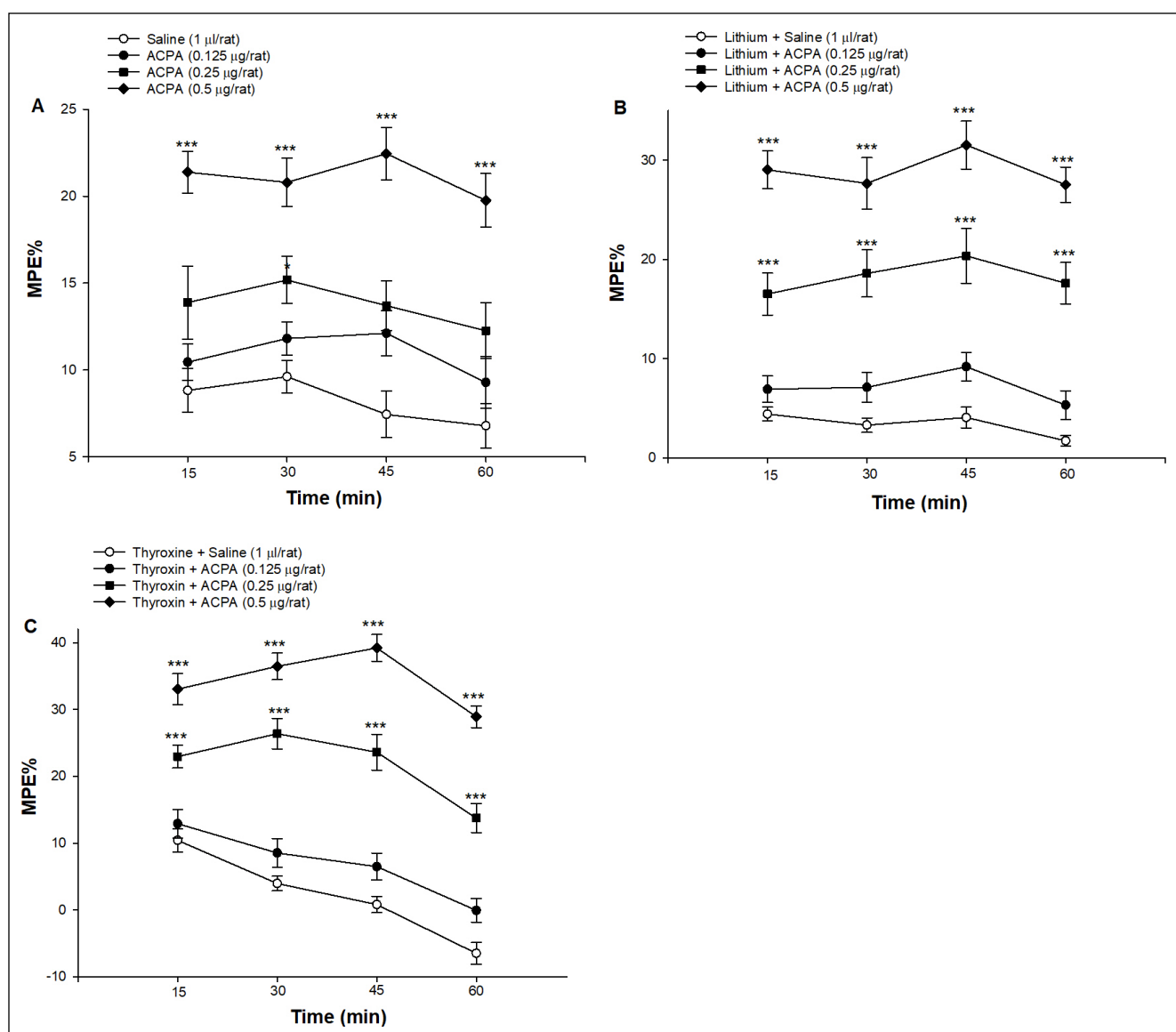


Fig. 4. The effect of intra-third ventricle administration of ACPA alone or in combination with L-thyroxine and lithium carbonate on the pain process. Data are presented as mean \pm S.E.M.

effect and morphine effect, Tukey's multiple comparisons showed that morphine (1 and 2 $\mu\text{g}/\text{rat}$) at the time intervals of 15, 30, 45, and 60 min after injection increased MPE%, suggesting an anti-nociceptive effect in normal rats.

As shown in Fig. 5B, there was no significant interaction between morphine dose and time interval on MPE% in hypothyroid rats [time effect: $F_{(3,84)}=139.843$, $P<0.001$; morphine effect: $F_{(3,28)}=1.595$, $P>0.05$; time interval \times morphine interaction: $F_{(9,84)}=0.893$, $P>0.05$]. Tukey's multiple comparisons revealed that morphine (2 $\mu\text{g}/\text{rat}$) at a time interval of 15, 30, 45, and 60 min af-

ter injection enhanced MPE%, suggesting an analgesic effect in hypothyroid rats.

Moreover, two-way ANOVA indicated a significant interaction between morphine dose and time interval on MPE% in hyperthyroid rats [time effect: $F_{(3,84)}=143.486$, $P<0.001$; morphine effect: $F_{(3,28)}=2.337$, $P<0.05$; time interval \times morphine interaction: $F_{(9,84)}=4.925$, $P<0.001$] (Fig. 5C). Multiple comparisons showed that morphine (2 $\mu\text{g}/\text{rat}$), at a time interval of 15, 30, 45, and 60 min after injection, increased MPE%, suggesting an anti-nociceptive effect in hyperthyroid rats.

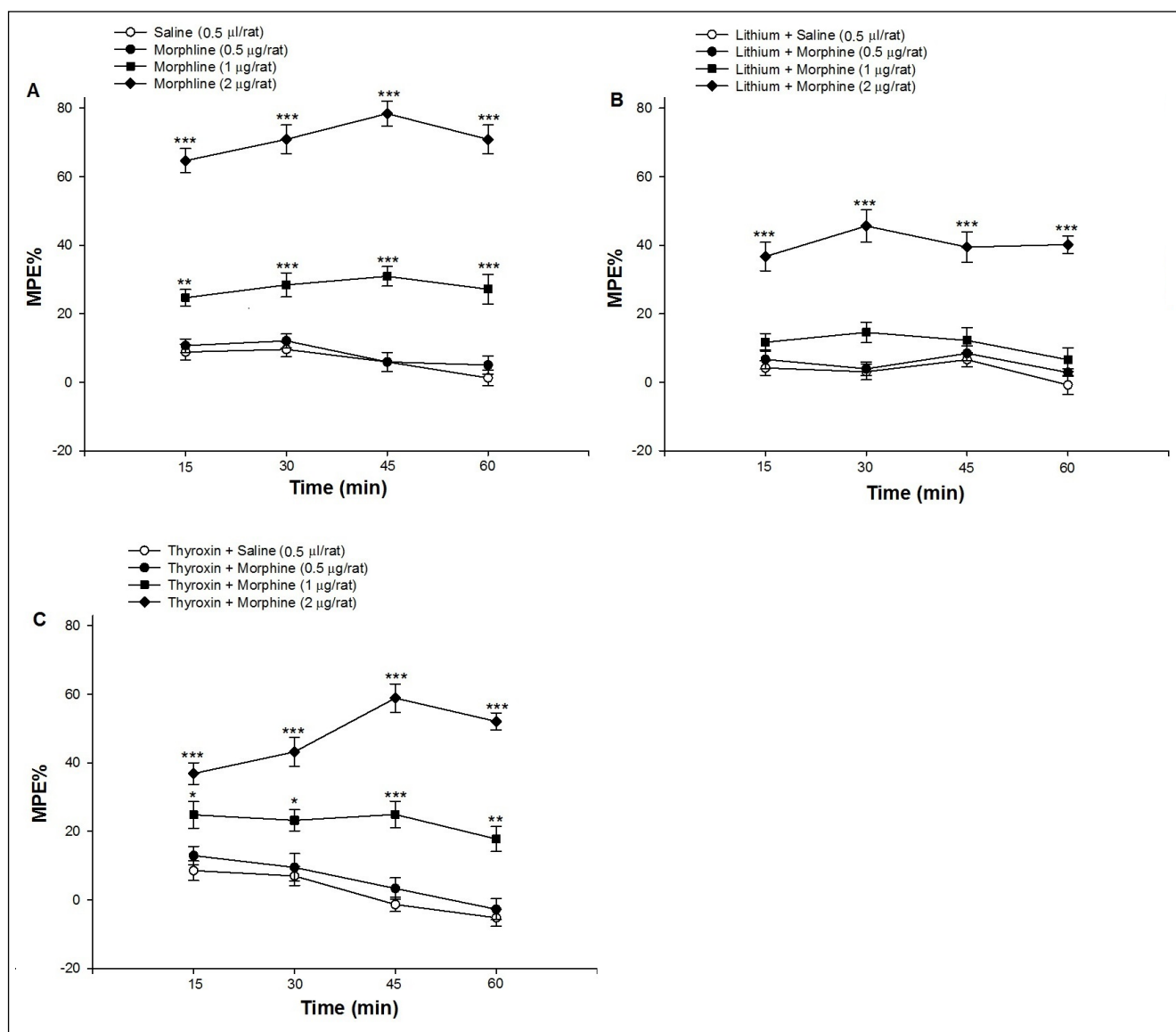


Fig. 5. The effect of intra-third ventricle injection of morphine alone or in combination with L-thyroxine and lithium carbonate on pain response. Data are shown as mean \pm S.E.M.

The effects of intra-third ventricle injection of morphine on pain response induced by AM251

The data in Fig. 6 show the effect of intra-third ventricle co-administration of AM251 and morphine on tail-flick latency at set time intervals (15, 30, 45, and 60 min) in normal, hypothyroid, and hyperthyroid male rats. Two-way ANOVA followed by Tukey's test for repeated measures over time (Fig. 6A) revealed a significant effect of time [$F_{(3,84)}=363.183$, $P<0.001$], but no significant effect of treatment [$F_{(3,28)}=1.093$, $P>0.05$] and treatment \times time interaction [$F_{(9,84)}=1.581$, $P>0.05$] in normal male rats. Multiple comparisons showed that morphine (0.5 $\mu\text{g}/\text{rat}$) + AM251 (0.5 and/or 0.25 $\mu\text{g}/\text{rat}$)

at the time interval of 15, 30, 45, and 60 min after injection decreased MPE%, suggesting a hyperalgesic effect in normal male rats.

Also, two-way ANOVA for repeated measures over time for the data (Fig. 6B) revealed a significant effect of time [$F_{(3,84)}=197.676$, $P<0.001$] but no significant effect of treatment [$F_{(3,28)}=1.221$, $P>0.05$] or treatment \times time interaction [$F_{(9,84)}=0.738$, $P>0.05$] in hypothyroid rats. Multiple comparisons revealed that morphine (0.5 $\mu\text{g}/\text{rat}$) along with AM251 (0.5 $\mu\text{g}/\text{rat}$) at the time interval of 15, 30, 45, and 60 min after injection reduced MPE%, suggesting a hyperalgesic effect in hypothyroid male rats.

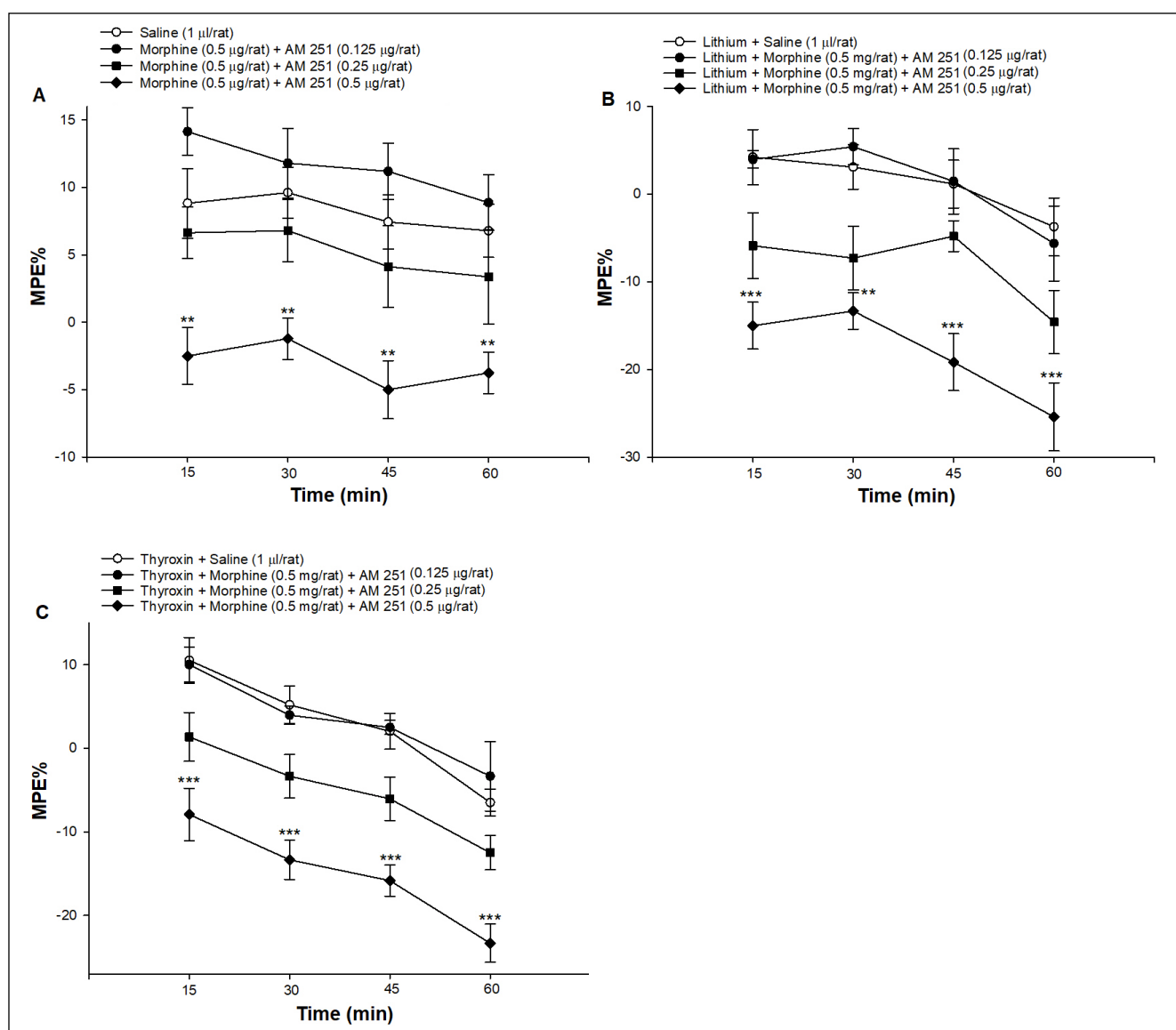


Fig. 6. The effect of intra-third ventricle co-injection of morphine and AM251 on pain response in normal, hypothyroid, and hyperthyroid male rats. Data are shown as mean \pm S.E.M.

As shown in Fig. 6C, two-way ANOVA revealed no significant interaction between morphine along with AM251 dose and time interval on MPE% in hyperthyroid rats [time effect: $F_{(3,84)}=259.726$, $P<0.001$; treatment effect: $F_{(3,28)}=9.418$, $P<0.001$ and treatment \times time interaction: $F_{(9,84)}=1.063$, $P>0.05$]. Multiple comparisons revealed that morphine (0.5 $\mu\text{g}/\text{rat}$) + AM251 (0.5 and/or 0.25 $\mu\text{g}/\text{rat}$) at the time interval of 15, 30, 45, and 60 min after injections decreased MPE%, proposing a hyperalgesic effect in hyperthyroid male rats.

The effects of intra-third ventricle injection of morphine on pain response induced by ACPA

Fig. 7 shows the effects of intra-third ventricle co-injection of ACPA and morphine on tail-flick latency at set time intervals (15, 30, 45, and 60 min) in normal, hypothyroid, and hyperthyroid male rats. Two-way ANOVA followed by Tukey's test for repeated measures over time (Fig. 7A) indicated a significant effect of time [$F_{(3,84)}=34.257$, $P<0.001$], but no significant effect of treatment [$F_{(3,28)}=1.464$, $P>0.05$] or treatment \times time interaction [$F_{(9,84)}=0.185$, $P>0.05$] in normal male rats. Multiple comparisons revealed that morphine (0.5 $\mu\text{g}/\text{rat}$) + ACPA (0.5 $\mu\text{g}/\text{rat}$), at the time interval of 15, 30, 45,

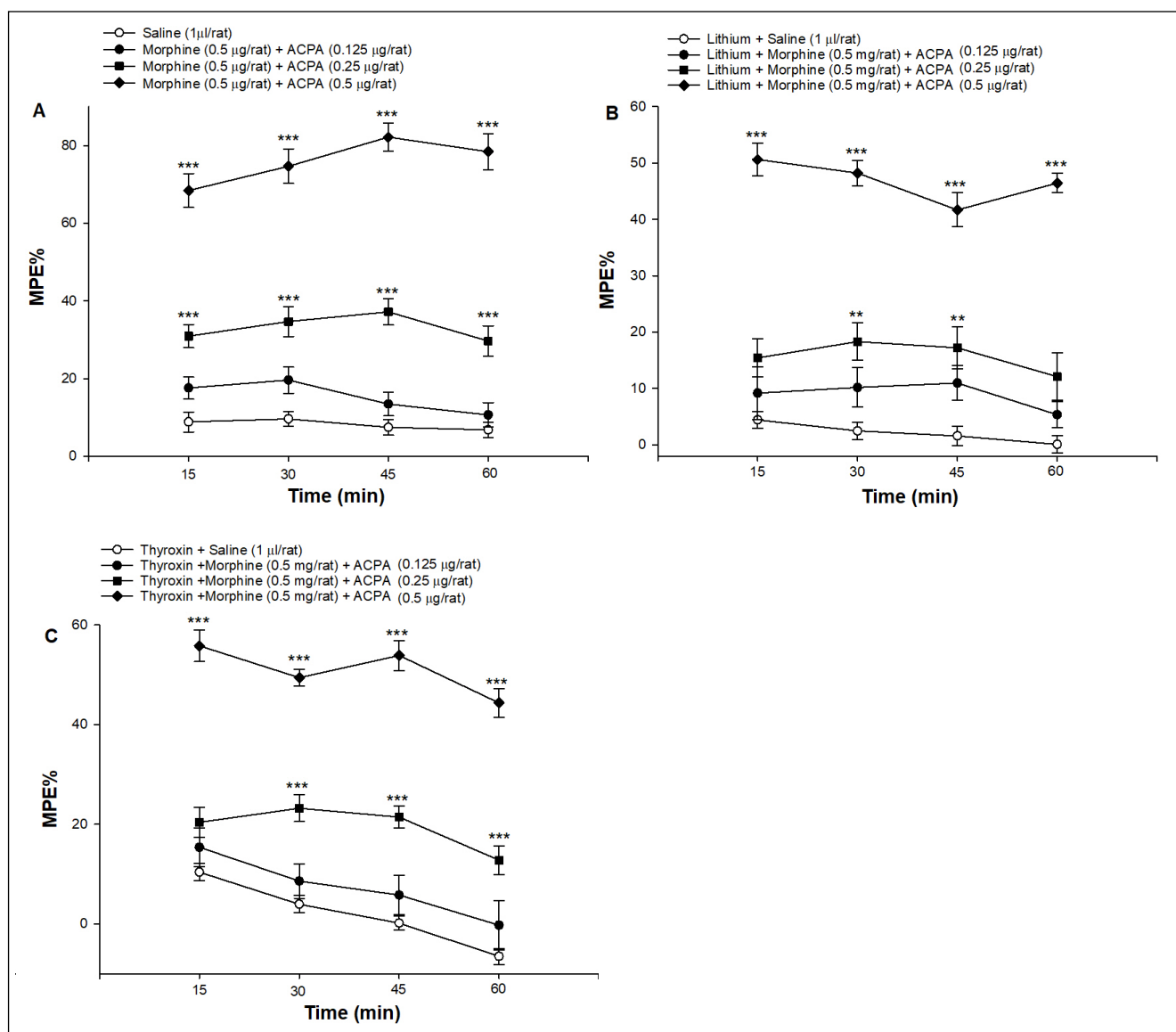


Fig. 7. The effect of intra-third ventricle co-administration of morphine and ACPA on pain response in normal, hypothyroid, and hyperthyroid male subjects. Data is displayed as mean \pm S.E.M.

and 60 min after injection, increased MPE%, suggesting an analgesic effect in normal rats.

Moreover, two-way ANOVA for repeated measures over time (Fig. 7B) indicated a significant effect of time [$F_{(3,84)}=42.843$, $P<0.001$] but no significant effect of treatment [$F_{(3,28)}=4.892$, $P<0.01$] or treatment \times time interaction [$F_{(9,84)}=0.342$, $P>0.05$] in hypothyroid rats. Multiple comparisons revealed that morphine (0.5 $\mu\text{g}/\text{rat}$) + ACPA (0.5 $\mu\text{g}/\text{rat}$), at a time interval of 15, 30, 45, and 60 min after injection, increased MPE%, suggesting an anti-nociceptive effect in hypothyroid rats.

As shown in Fig. 7C, two-way ANOVA demonstrated that there were no significant interactions between morphine in combination with ACPA doses and time interval on MPE% in hyperthyroid rats [time effect: $F_{(3,84)}=50.523$, $P<0.001$; treatment effect: $F_{(3,28)}=26.989$, $P<0.001$ and treatment \times time interaction: $F_{(9,84)}=0.121$, $P>0.05$]. Multiple comparisons showed that morphine (0.5 $\mu\text{g}/\text{rat}$) + ACPA (0.5 $\mu\text{g}/\text{rat}$), at time intervals of 15, 30, 45, and 60 min after injection, enhanced MPE%, suggesting an analgesic effect in hyperthyroid rats.

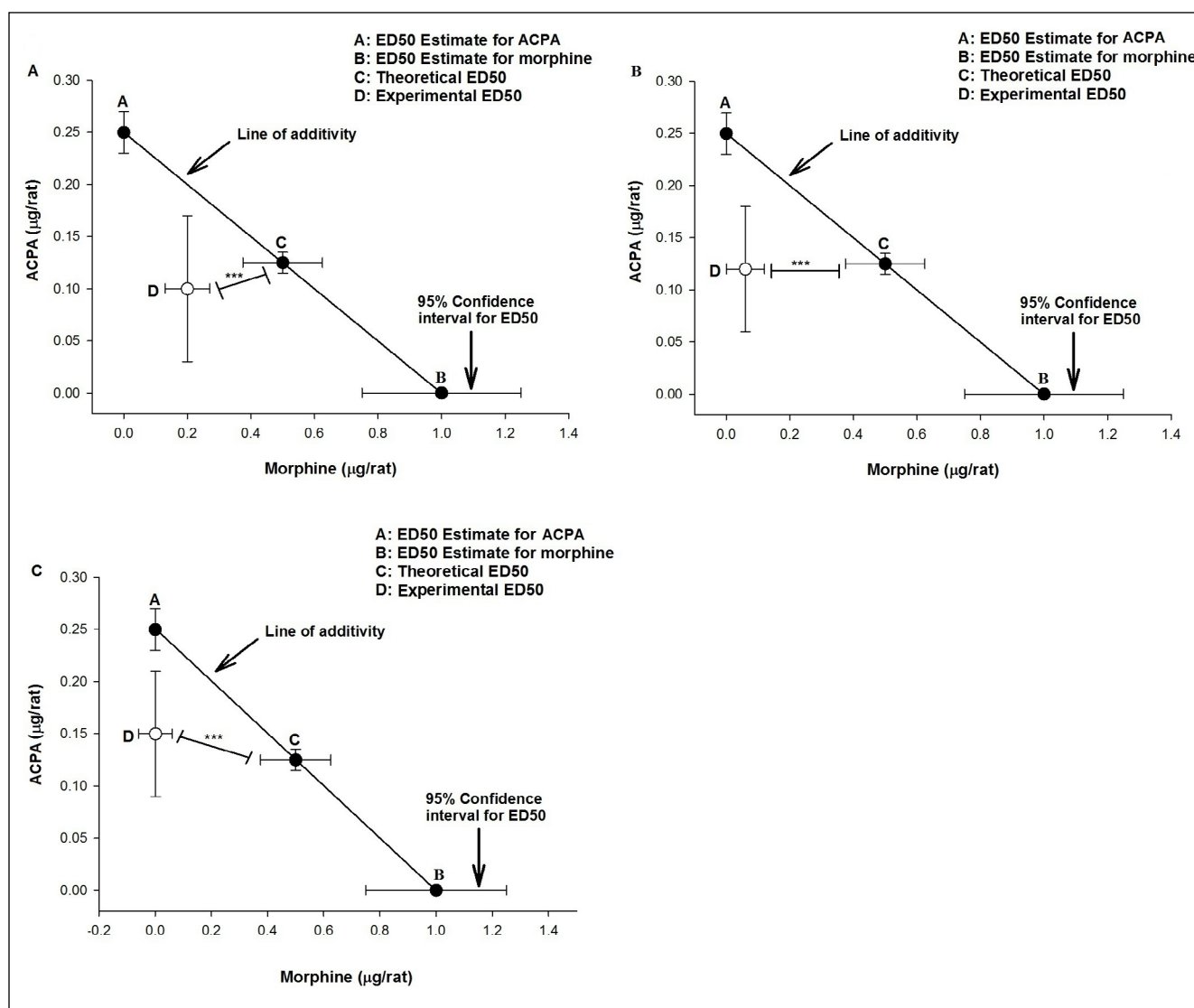


Fig. 8. The isobologram analysis of the effects of drug administration revealed a synergistic effect of intra-third ventricle injection of ACPA and morphine in the induction of analgesic effect. The foci A and B mean represent the ED50 of ACPA and morphine, respectively. In brief, the ED50 of each drug was calculated by linear regression analysis. The oblique line between A and B is the theoretical additive effect line of ACPA and morphine administrations. Focus C, in the middle of the line, is the theoretical ED50 of the drug combination, which is measured from the individual drug ED50. Focus D, far below the line, is the experimental ED50 of the drug combination, which is determined after drug injection. Statistical analysis demonstrated that there was a significant difference between the experimental ED50 and theoretical ED50 points, suggesting a synergistic effect of the administration of the drugs (A) normal, (B) hypothyroid, and (C) hyperthyroid male rats. ED50, effective dose 50.

The synergistic effect between ACPA and morphine on pain behavior

To confirm whether ACPA and morphine injection would exert additive or synergistic effects on their induced analgesic effect, an isobolographic analysis was carried out to compare the theoretical and experimental ED₅₀ of the drugs when injected together. The theoretical ED₅₀ for an additive interaction between ACPA and morphine can be estimated from the isobologram (Fig. 8).

One sample t-test revealed that there was a significant difference between experimental ED₅₀ and theoretical ED₅₀. Our data suggest a synergistic effect occurred for ACPA and morphine injection upon induction of an analgesic effect (Fig. 8).

DISCUSSION

This study presents evidence of negative effects triggered by lithium carbonate- and L-thyroxine-induced hypothyroid and hyperthyroid male rats. Our findings indicated a clear impact on core body temperature and body weight. Thyroid hormones play a strong role in metabolism, expenditure of energy, and body weight as they regulate basal and total energy consumption (Hwang et al., 2018). Weight gain and loss have been related to apparent hypothyroid and hyperthyroid conditions, respectively (Roef et al., 2012). As demonstrated in various experimental models of hypothyroidism and hyperthyroidism, both in animal models and humans, thyroid hormones were shown to possibly act directly on important metabolically active organs and therefore change obligatory thermogenesis (Silva, 2006; Iwen et al., 2018).

Furthermore, our results demonstrated that an intra-third ventricle injection of AM251 caused a decrease in the pain threshold of normal, hypothyroid, and hyperthyroid subjects in the tail-flick test, while ACPA caused an increase. Although there were no differences in the tail-flick response of rats with disrupted thyroid function and normal rats in response to a CB1 receptor agonist or antagonist, the effect of the CB1 receptor agonist and antagonist was extremely effective in modulating pain behavior in hypothyroid and hyperthyroid rats. There are examples of possible interactions between the HPT axis and pain (Aloisi et al., 2013). For example, thyrotropin-releasing hormone (TRH), TSH, and thyroid hormones play a role in the regulation of pain perception (Hennessey, 2000; Frohlich and Wahl, 2019). Boschi et al. (1983) reported that intracerebroventricular (i.c.v.)

administration of TRH produced a short, strong anti-nociceptive effect against chemical and mechanical stimuli. On the other hand, cannabinoids exerted an inhibitory effect on the HPT axis (e.g., reduction of TSH level and thyroid weight) (Malhotra et al., 2017). Thus, our results may be due to the inhibitory effect of cannabinoids on the HPT axis (e.g., reduction of TSH level and thyroid weight) (Malhotra et al., 2017) and the anti-nociceptive activity of TRH (Boschi et al., 1983), which may counteract their effects, resulting in no differences in the tail-flick response of normal, hypothyroid, and hyperthyroid rats.

The evidence revealed that exogenous cannabinoid agonists attenuated pain responses in several pain models, such as inflammatory and neuropathic pain (Palazzo et al., 2001). In agreement with our study, Pertwee (2001) reported that a cannabinoid CB1 receptor agonist induced an anti-nociceptive effect, which was blocked by the CB1 receptor antagonist. Behavioral studies have demonstrated that subjects treated with a CB1 receptor antagonist exhibited enhanced behavioral responses to intradermal injections of formalin and nociceptive responses of dorsal horn neurons, suggesting the main role of the CB1 receptor is the normal transmission of nociceptive information (Iversen and Chapman, 2002).

Additionally, the results of this study revealed that intra-third ventricle injection of morphine induced an anti-nociceptive effect in normal, hypothyroid, and hyperthyroid subjects in the tail-flick test. Morphine is a potent analgesic drug that is widely used for the modulation of both acute and chronic pain. The analgesic property of morphine is produced through coupling to μ receptors in the CNS (Ochiai et al., 2016). Several investigations have confirmed that systemic or central injection of morphine effectively blocks pain pathways in different pain tests (Gregoire et al., 2012). Similar to the results of the current study, there are reports that microinjection of morphine into the VTA (Morgan and Franklin, 1990), anterior cingulate cortex (Hao et al., 2005), spinal cord (Thomson et al., 2006), and pallidum (Anagnostakis et al., 1992) induced an analgesic effect. Several studies also reported that endogenous opioid peptides exert regulatory actions on the HPT axis, and exogenous morphine may have different effects than endogenous opioids (Vuong et al., 2010). For instance, Lomax and George (1966; 1970) reported that systemically ineffective doses of morphine injected into the hypothalamus decreased the release of radioiodine from the thyroid glands of rats, which led to the inhibition of thyroid activity. Research reported by Berglund and coworkers (1990) also demonstrated the role of thyroid hormone in the

suppression of TSH by opiates. These investigations revealed that morphine exerts its inhibitory effect on TSH secretion by increasing negative feedback sensitivity to thyroid hormones. Furthermore, Muraki and colleagues (1980) revealed that morphine reduced the release of TRH. It has been reported that sensitivity to morphine was increased in hyperthyroid rats (Edmondson et al., 1990). On the other hand, decreased sensitivity to morphine was observed in hypothyroid rats (Huidobro-Toro et al., 1978). There is a report showing that thyroid hormones enhanced opiate receptor number and native pain sensitivity but reduced the duration of analgesia from morphine (Edmondson et al., 1990). We suggest crosstalk occurs between morphine and thyroid hormones, which may have influenced the pain responses observed in this study.

Furthermore, these results demonstrated that intra-third ventricle co-injection of different doses of AM251 plus an ineffective dose of morphine caused a hyperalgesic effect, whereas intra-third ventricle co-infusion of various doses of ACPA along with an ineffective dose of morphine induced analgesia in normal, hypothyroid, and hyperthyroid rats in the tail-flick test. As mentioned previously, there are relationships between CB1 receptors, opioid receptors, and thyroid hormones (Scavone et al., 2013; Bonnet, 2017). As the administration of morphine and cannabis reduced TSH levels in rats (Malhotra et al., 2017), the pain response observed in hypothyroid and hyperthyroid rats could also be affected. Drugs that target both CB1 and morphine receptors have shared pharmacological profiles. Agonists of both receptor types have been demonstrated to induce anti-nociception and drug reward/reinforcement (Scavone et al., 2013). One study demonstrated that systemic co-injection of cannabinoid and opioid agonists resulted in synergistic anti-nociception (Cichewicz and McCarthy, 2003). CB1 and μ opioid receptors are distributed in many of the same areas in the brain, including the periaqueductal gray, locus coeruleus, dorsal hippocampus, raphe nuclei, medial basal hypothalamus, and cerebral ventricles (Mansour et al., 1994; Suárez et al., 2010; Wiese and Wilson-Poe, 2018). It was shown that cannabinoid and opioid agonists induced anti-nociception by acting on similar structures in the CNS, and a peripheral mechanism was also suggested for both compounds (Scavone et al., 2013). Ibrahim et al. (2005) demonstrated that CB2 receptor activation by AM1241 induced a release of β -endorphin from the cerebral ventricles, and the anti-nociceptive effect of AM1241 in rats was abolished by μ -opioid receptor antagonism and by β -endorphin antiserum.

Furthermore, Zubrzycki and colleagues (2019) showed that a selective μ -receptors antagonist, β -funtrexamine, and a CB₁ receptor antagonist, AM251, infused within the cerebral ventricles permeated via the cerebroventricular lining and induced their effects on the near structures. Pharmacological studies have revealed that the endogenous opioid system could be involved in cannabinoid anti-nociception (Maldonado and Valverde, 2003). On the other hand, cannabinoids may be able to modulate opioid action at several levels within the cell, ranging from direct receptor interactions to modifications in endogenous peptide release or post-receptor interactions through shared signal transduction pathways (Scavone et al., 2013). Therefore, it appears likely that interaction between cannabinoid agents and morphine modulates the pain response in the third ventricle.

Our results also indicated that there was an interaction between ACPA and morphine on pain behavior in normal, hypothyroid, and hyperthyroid rats. Interestingly, using isobolographic analysis, we found a synergistic effect of ACPA and morphine upon induction of an analgesic effect. A probable mechanism for analgesia induced by the co-administration of ACPA and morphine might be due to the functional interaction between opioid and cannabinoid receptors in the CNS. These interactions may be direct, for example, *via* receptor heteromerization, or indirect, for example, *via* signaling crosstalk, which includes agonist-mediated release and synthesis of endogenous ligands that can stimulate downstream receptors. Evidence suggests that interactions between cannabinoid and opioid receptors might mediate several of the behavioral phenomena related to the use of these drugs, such as the induction of acute anti-nociception and the development of tolerance, as well as cross-tolerance to the anti-nociceptive properties of cannabinoid- and opioid-specific ligands (Bushlin et al., 2010).

Our isobolographic analysis revealed a synergistic effect of ACPA and morphine on the production of the anti-nociception effect. We propose that the infusion of drugs into the third ventricle may activate the descending pain pathways due to the distribution of μ receptors and CB1 receptors in the areas related to pain control (Nadal et al., 2013) and the expression of these receptors in the cerebral ventricles (Mansour et al., 1994; Suárez et al., 2010) where they can cross the walls and act in the parenchyma (Suárez et al., 2010), thereby modulating pain behavior. Further investigations are required to clarify the specific mechanisms underlying interactions between morphine and cannabis, as well as the different brain areas involved in the modulation of pain behavior.

CONCLUSION

Consistent with other studies, our results provide evidence of an interaction between CB1 and opioid receptors in the modulation of pain behavior. An interesting outcome from the current investigation was the observed synergistic effect between ACPA and morphine upon induction of analgesic effect in normal, hypothyroid, and hyperthyroid rats. More experiments are required to determine the exact function of cannabis and opioid in neurons as well as the third ventricle in the modulation of pain response in normal, hypothyroid, and hyperthyroid male rats.

REFERENCES

- Aloisi AM, Vodo S, Buonocore M (2013) Pain and thyroid hormones. *Neurosci* 34: 1501–1508.
- Anagnostakis Y, Zis V, Spyraiki C (1992) Analgesia induced by morphine injected into the pallidum. *Behav Brain Res* 48: 135–143.
- Armario A (2010) Activation of the hypothalamic-pituitary-adrenal axis by addictive drugs: different pathways, common outcome. *Trends Pharmacol Sci* 31: 318–325.
- Bakke JL, Lawrence NL, Robinson S (1974) The effect of morphine on pituitary-thyroid function in the rat. *Eur J Pharmacol* 25: 402–406.
- Berglund LA, Millard WJ, Gabriel SM, Simpkins JW (1990) Opiate-thyroid hormone interactions in the regulation of thyrotropin secretion in the rat. *Neuroendocrinology* 52: 303–308.
- Bonnet U (2017) Chapter 47 – Chronic Cannabis Abuse and Thyroid Function, Editor(s): V.R. Preedy, *Handbook of Cannabis and Related Pathologies*, Academic Press, pp: 461–465.
- Boschi G, Desiles M, Reny V, Rips R, Wrighglessworth S (1983) Anti-nociceptive properties of thyrotropin releasing hormone in mice: comparison with morphine. *Br J Pharmacol* 79: 85–92.
- Brent GA (2012) Mechanisms of thyroid hormone action. *J Clin Invest* 122: 3035–3043.
- Bushlin I, Rozenfeld R, Devi LA (2010) Cannabinoid-opioid interactions during neuropathic pain and analgesia. *Curr Opin Pharmacol* 10: 80–86.
- Cheng SY, Leonard JL, Davis PJ (2010) Molecular aspects of thyroid hormone actions. *Endocr Rev* 31: 139–170.
- Cichewicz DL, McCarthy EA (2003) Anti-nociceptive synergy between delta(9)-tetrahydrocannabinol and opioids after oral administration. *J Pharmacol Exp Ther* 304: 1010–1015.
- Edmondson EA, Bonnet KA, Friedhoff AJ (1990) The effect of hyperthyroidism on opiate receptor binding and pain sensitivity. *Life Sci* 47: 2283–2289.
- Fields H (2004) State-dependent opioid control of pain. *Nat Rev Neurosci* 5: 565–575.
- Frohlich E, Wahl R (2019) The forgotten effects of thyrotropin-releasing hormone: Metabolic functions and medical applications. *Front Neuroendocrinol* 52: 29–43.
- Gregoire S, Etienne M, Gaulmin M, Caussade F, Neuzezer D, Ardid D (2012) New method to discriminate sedative and analgesic effects of drugs in the automated formalin test in rats. *Neurosci Res* 72: 194–198.
- Hao Y, Yang JY, Guo M, Wu CF, Wu MF (2005) Morphine decreases extracellular levels of glutamate in the anterior cingulate cortex: an in vivo microdialysis study in freely moving rats. *Brain Res* 1040: 191–196.
- Hendrie CA, Pickles AR (2010) Depression as an evolutionary adaptation: anatomical organisation around the third ventricle. *Med Hypotheses* 74: 735–740.
- Hennessey J (2000) Subacute Thyroiditis. In KR. Feingold, B. Anawalt, et al., editors, *Endotext*. South Dartmouth (MA).
- Hillard CJ, Farber NE, Hagen TC, Bloom AS (1984) The effects of delta 9-tetrahydrocannabinol on serum thyrotropin levels in the rat. *Pharmacol Biochem Behav* 20: 547–550.
- Huidobro-Toro JP, Harris RA, Loh HH, Way EL (1978) Effects of hypothyroidism on the response of the rat colon to morphine. *Eur J Pharmacol* 52: 191–195.
- Hwang JH, Jung HW, Kang SY, Kang AN, Ma JN, Meng XL, et al. (2018) Therapeutic effects of acupuncture with MOK, a polyherbal medicine, on PTU-induced hypothyroidism in rats. *Exp Ther Med* 16: 310–320.
- Ibrahim MM, Porreca F, Lai J, Albrecht PJ, Rice FL, Khodorova A, et al. (2005) CB2 cannabinoid receptor activation produces anti-nociception by stimulating peripheral release of endogenous opioids. *Proc Nat Acad Sci* 102: 3093–3098.
- Iversen L, Chapman V (2002) Cannabinoids: a real prospect for pain relief? *Curr Opin Pharmacol* 2: 50–55.
- Iwen KA, Oelkrug R, Brabant G (2018) Effects of thyroid hormones on thermogenesis and energy partitioning. *J Mol Endocrinol* 60: R157–R170.
- Khakpai F, Ebrahimi-Ghiri M, Alijanpour S, Zarrindast MR (2019) Ketamine-induced antidepressant like effects in mice: A possible involvement of cannabinoid system. *Biomed Pharmacother* 112: 108717.
- Kieffer BL, Evans CJ (2009) Opioid receptors: from binding sites to visible molecules in vivo. *Neuropharmacology* 56: 205–212.
- Kosarmadar N, Ghasemzadeh Z, Rezayof A (2015) Inhibition of microglia in the basolateral amygdala enhanced morphine-induced anti-nociception: Possible role of GABAA receptors. *Eur J Pharmacol* 765: 157–163.
- Langerman L, Zakowski MI, Piskoun B, Grant GJ (1995) Hot plate versus tail flick: evaluation of acute tolerance to continuous morphine infusion in the rat model. *J Pharmacol Toxicol Methods* 34: 23–27.
- Llewellyn BD (2009) Nuclear staining with alum hematoxylin. *Biotech Histochem* 84: 159–177.
- Loeser JD, Treede RD (2008) The Kyoto protocol of IASP basic pain terminology. *Pain* 137: 473–477.
- Lomax P (1970) The effect of marihuana on pituitary-thyroid activity in the rat. *Agents Actions* 1: 252–257.
- Lomax P, George R (1966) Thyroid activity following administration of morphine in rats with hypothalamic lesions. *Brain Res* 2: 361–367.
- Maldonado R, Valverde O (2003) Participation of the opioid system in cannabinoid-induced anti-nociception and emotional-like responses. *Eur Neuropsychopharmacol* 13: 401–410.
- Malhotra S, Heptulla RA, Homel P, Motaghedi R (2017) Effect of marijuana use on thyroid function and autoimmunity. *Thyroid* 27: 167–173.
- Mansour A, Fox CA, Burke S, Meng F, Thompson RC, Akil H, Watson SJ (1994) Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study. *J Comp Neurol* 350: 412–438.
- Morgan MJ, Franklin KB (1990) 6-hydroxydopamine lesions of the ventral tegmentum abolish D-amphetamine and morphine analgesia in the formalin test but not in the tail flick test. *Brain Res* 519: 144–149.
- Muller R, Liu YY, Brent GA (2014) Thyroid hormone regulation of metabolism. *Physiol Rev* 94: 355–382.
- Muraki T, Nakadate T, Tokunaga Y, Kato R (1980) Effect of morphine on the release of thyroid-stimulating hormone stimulated by exposure to cold, thyroidectomy and the administration of thyrotrophin releasing hormone in male rats. *J Endocrinol* 86: 357–362.
- Nadal X, La Porta C, Andreea Bura S, Maldonado R (2013) Involvement of the opioid and cannabinoid systems in pain control: new insights from knockout studies. *Eur J Pharmacol* 716: 142–157.
- Nasehi M, Morteza-Zadeh P, Khakpai F, Zarrindast MR (2016) Additive effect of harmaline and muscimol for memory consolidation impairment in inhibitory avoidance task. *Neuroscience* 339: 287–295.
- Nasehi M, Ostadi E, Khakpai F, Ebrahimi-Ghiri M, Zarrindast MR (2017) Synergistic effect between D-AP5 and muscimol in the nucleus accumbens shell on memory consolidation deficit in adult male Wistar rats: An isobologram analysis. *Neurobiol Learn Mem* 141: 134–142.

- Ness TJ, Jones SL, Gebhart GF (1987) Contribution of the site of heating to variability in the latency of the rat tail flick reflex. *Brain Res* 426: 169–172.
- Ochiai, W, Kaneta, M, Nagae, M, Yuzuhara, A, Li, X, Suzuki, H, et al. (2016) Mice with neuropathic pain exhibit morphine tolerance due to a decrease in the morphine concentration in the brain. *Eur J Pharm Sci* 92: 298–304.
- Palazzo E, Marabese I, de Novellis V, Oliva P, Rossi F, Berrino L, Maione S (2001) Metabotropic and NMDA glutamate receptors participate in the cannabinoid-induced anti-nociception. *Neuropharmacology* 40: 319–326.
- Park YJ, Lee EK, Lee YK, Park DJ, Jang HC, Moore DD (2012) Opposing regulation of cytochrome P450 expression by CAR and PXR in hypothyroid mice. *Toxicol Appl Pharmacol* 263: 131–137.
- Paxinos G, Watson C (2007) *The rat brain in stereotaxic coordinates* (6rd ed.) Academic Press. London. UK.
- Pertwee RG (2001) Cannabinoid receptors and pain. *Prog Neurobiol* 63: 569–611.
- Puopolo M (2019) The hypothalamic-spinal dopaminergic system: a target for pain modulation. *Neural Regen Res* 14: 925–930.
- Rittner HL, Brack A, Stein C (2008) Pain and the immune system. *Br J Anaesth* 101: 40–44.
- Roef G, Lapauw B, Goemaere S, Zmierzak HG, Toye K, Kaufman JM, Taes Y (2012) Body composition and metabolic parameters are associated with variation in thyroid hormone levels among euthyroid young men. *Eur J Endocrinol* 167: 719–726.
- Salazar P, Cisternas P, Codocedo JF, Inestrosa NC (2017) Induction of hypothyroidism during early postnatal stages triggers a decrease in cognitive performance by decreasing hippocampal synaptic plasticity. *Biochim Biophys Acta Mol Basis Dis* 1863: 870–883.
- Salio C, Fischer J, Franzoni MF, Mackie K, Kaneko T, Conrath M (2001) CB1-cannabinoid and μ -opioid receptor co-localization on postsynaptic target in the rat dorsal horn. *Neuroreport* 12: 3689–3692.
- Scavone JL, Sterling RC, Van Bockstaele EJ (2013) Cannabinoid and opioid interactions: implications for opiate dependence and withdrawal. *Neuroscience* 248: 637–654.
- Silva JE (2006) Thermogenic mechanisms and their hormonal regulation. *Physiol Rev* 86: 435–464.
- Starowicz K, Finn DP (2017) Cannabinoids and pain: sites and mechanisms of action. *Adv Pharmacol* 80: 437–475.
- Suárez J, Romero-Zerbo SY, Rivera P, Bermúdez-Silva FJ, Pérez J, De Fonseca FR, Fernández-Llebrez P (2010) Endocannabinoid system in the adult rat circumventricular areas: an immunohistochemical study. *J Comp Neurol* 518: 3065–3085.
- Thomson LM, Zeng J, Terman GW (2006) Differential effect of glutamate transporter inhibition on EPSCs in the morphine naive and morphine tolerant neonatal spinal cord slice. *Neurosci Lett* 407: 64–69.
- Titford M (2005) The long history of hematoxylin. *Biotech Histochem* 80: 73–78.
- Ueda M, Iwasaki H, Wang S, Murata E, Poon KY, Mao J, Martyn JA (2014) Cannabinoid receptor type 1 antagonist, AM251, attenuates mechanical allodynia and thermal hyperalgesia after burn injury. *Anesthesiology* 121: 1311–1319.
- Vuong C, Van Uum SH, O'Dell LE, Lutfy K, Friedman TC (2010) The effects of opioids and opioid analogs on animal and human endocrine systems. *Endocr Rev* 31: 98–132.
- Wiese B, Wilson-Poe AR (2018) Emerging evidence for cannabis' role in opioid use disorder. *Cannabis Cannabinoid Res* 3: 179–189.
- Woodhams SG, Chapman V, Finn DP, Hohmann AG, Neugebauer V (2017) The cannabinoid system and pain. *Neuropharmacology* 124: 105–120.
- Yu D, Zhou H, Yang Y, Jiang Y, Wang T, Lv L, et al. (2015) The bidirectional effects of hypothyroidism and hyperthyroidism on anxiety- and depression-like behaviors in rats. *Horm Behav* 69: 106–115.
- Zubrzycki M, Stasiolek M, Zubrzycka M (2019) Opioid and endocannabinoid system in orofacial pain. *Physiological Res* 68: 705–715.