JPET Fast Forward. Published on March 28, 2006 as DOI: 10.1124/jpet.105.099937
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JPET #99937

Thienorphine is a Potent Long-acting Partial Opioid Agonist: a Comparative Study with Buprenorphine

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Running title: pharmacological characterization of thienorphine

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Text pages: 14

Abstract word count: 209

Tables: 4

Introduction word count: 548

Figures: 7

Discussion word count: 906

References: 28

ABBREVIATIONS:

thienorphine: N-cyclopropylmethyl-7α-[(*R*)-1-hydroxy-1-methyl-3-(thien-2-yl)-propyl]-6,14-endo-ethanotetrahydronororipavine; CHO cell: Chinese hamster ovary cell; CHO-μ cells: Chinese hamster ovary cells stably expressing rat μ-opioid receptor; CHO-δ cells: Chinese hamster ovary cells stably expressing rat δ-opioid receptor; CHO-κ cells: Chinese hamster ovary cells stably expressing rat κ-opioid receptor; GDP: guanosine diphosphate; [^{35}S]GTPγS: guanosine 5'-O-(3-[^{35}S]thio)triphosphate.

Recommended section assignment:

Neuropharmacology

ABSTRACT

A strategy in the development of new treatment for opioid addiction is to find partial opioid agonists with properties of long duration of action and high oral bioavailability. In a search for such compounds, thienorphine, a novel analog of buprenorphine, was synthesized. Here we reported that, like buprenorphine, thienorphine bound potently and nonselectively to μ-, δ- and κ-opioid receptors stably expressed in CHO cells and behaved as a partial agonist at μ-opioid receptor. However, some differences were observed between the pharmacological profiles of thienorphine and buprenorphine. *In vitro*, thienorphine was more potent than buprenorphine in inhibiting [^{3}H]diprenorphine and stimulating [^{35}S]GTPγS binding to rat μ-opioid receptor stably expressed in CHO cells. *In vivo*, thienorphine exhibited a less potent but more efficacious antinociceptive effect with an ED_{50} value of 0.25 mg/kg (s.c.), and more potent anti-morphine effect with an ED_{50} value of 0.64 mg/kg (i.g.), in comparison with buprenorphine. Additionally, the bioavailability of thienorphine was greatly higher than that of buprenorphine after oral administration. Moreover, compared to buprenorphine, thienorphine showed a similar long-lasting antinociceptive effect but a much longer antagonism of morphine-induced lethality (more than 15 days). These results indicate that thienorphine is a potent, long-acting partial opioid agonist with high oral bioavailability, and may have possible application in treating addiction.

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Introduction

Opioid abuse and dependence remain a serious worldwide health problem. The drugs currently in clinical use for treating opioid dependence are either full opioid agonist, methadone and LAAM, or antagonist, naltrexone (Johnson et al., 2003). Although these drugs showed extremely effective in reducing illicit opioid use, they have some drawbacks; the agonist merely substitutes one addiction for another, and the antagonist is unable to retain patients in treatment due to a lack of desired positive subjective effects (Johnson et al., 2003). Buprenorphine, a derivative of thebaine, has unique pharmacological properties; it is a high affinity, low intrinsic activity agonist at μ-opioid receptor, and has an antagonist activity at κ-opioid receptor (Cowan et al., 1977; Dum and Herz, 1981; Negus et al., 1989). Buprenorphine, as a partial agonist, either alone or in combination with naloxone, has been shown to be effective in treating opioid dependence experimentally and clinically (for reviews, see Davids and Gastpar, 2004; Johnson et al., 2003). With its unique pharmacological properties, buprenorphine gains advantages over the agonist or antagonist medication in the treatment of opioid addiction, showing an acceptable effectiveness, as well as a good safety profile, particularly with respect to lower respiratory depression and physical dependence relative to a full μ-opioid receptor agonist (Walsh et al., 1994, 1995).

Buprenorphine is safe and has low abuse liability, whereas its use in the treatment of opioid dependence has been restricted by its very low oral bioavailability (Heel et al., 1979), which results in a somewhat inconvenient administration of sublingual preparations in clinic (Mendelson et al., 1997; Schuh and Johanson, 1999). In addition, it has been shown that buprenorphine can cause dependence both in physical and psychological studies, probably due to its fairly strong

agonist effect at the μ-opioid receptor (Lopatko et al., 2003). In the treatment of opioid addiction, a long-lasting effect of a medication may produce better treatment effectiveness (Kreek, 1992, 1996). Although buprenorphine has a relatively longer duration of action in comparison with other opioids, it still requires daily or alternate-day dosing (Fudala et al., 1990; Amass et al., 1998), which gives rise to great medical burdens and may reduce patient compliance under some circumstance. Therefore, new partial opioid agonists with higher oral bioavailability and longer duration of action than buprenorphine may improve the treatment for opioid addiction.

To obtain an ideal long-lasting treatment effect, development of new formulations that could maintain stable plasma drug levels would be a common consideration. An extended-release formulation of naltrexone has been developed to improve the adherence of antagonist treatment by encapsulating naltrexone into injectable biodegradable polymer microspheres (Bartus et al., 2003). However, little effort has been made to elongate the intrinsic duration of action of opioid compounds by modifying their molecular structures. Recently, several research groups reported that compounds obtained by structural modification of buprenorphine strengthened their bindings to opioid receptors and thus slowed receptor kinetics, resulting in a long-lasting effect (Neilan et al., 2004; Husbands et al., 2005). Inspired by these results, the chemists in our institute have designed and synthesized several dozens of buprenorphine analogs. Among these analogs, thienorphine (Fig.1), N-cyclopropylmethyl-7α-[(*R*)-1-hydroxy-1-methyl-3-(thien-2-yl)-propyl]-6,14-endo-ethanotetrahydronororipavine (Liu et al., 2005), appears to be a potent and long-acting partial agonist. In this study, we characterized the pharmacological activities of thienorphine both *in vitro* and *in vivo*, in comparison with its analog, buprenorphine.

Materials and Methods

Animals.

Male and female Kunming mice (18-22 g) were supplied by Beijing Animal Center (Beijing, China) and maintained on a 12 h light-dark cycle. Animals had free access to food and water. Animal care and procedures were strictly in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Materials.

Thienorphine HCl and buprenorphine HCl were synthesized in our institute (Liu et al., 2005). [^{3}H]diprenorphine (50.0 Ci/mmol) and [^{35}S]GTPγS (1250.0 Ci/mmol) were products of NEN (Boston, MA, USA). Naloxone, GTPγS, GDP and Tris were purchased from Sigma (St. Louis, MO). The following materials were obtained as indicated: RPMI medium and geneticin (Gibco, Grand Island, NY, USA), Fetal bovine serum (HyClone, South Logan, UT, USA), and morphine (Qinghai Pharmaceutic Factory, Xining, China).

Cell Culture and Membrane Preparation.

CHO cells stably expressing the rat μ-, δ- and κ-opioid receptors (CHO-μ, -δ, and -κ, respectively) were generous gifts from Dr. Gang Pei (Shanghai Institute of Cell Biology, Chinese Academy of Sciences, Shanghai, China) and were cultured in RPMI 1640 supplemented with 100 U/ml penicillin, 100 U/ml streptomycin, 200 μg/ml geneticin and 10% fetal bovine serum at 37ºC with humidified atmosphere consisting of 95% air and 5% CO_2. Cell membrane was prepared according to Li et al (2001).

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Receptor Binding Assay.

Competitive binding assays were carried out to determine the binding affinities of opioid compounds for μ-, δ-, and κ-opioid receptors. Membrane protein (100 μg/tube) prepared from CHO-μ, -δ, or -κ cells was incubated in 50 mM Tris-HCl buffer (pH 7.4) containing 2 nM [^{3}H]diprenorphine and different concentrations of compounds in a total volume of 0.5 ml for 30 min at 37ºC. Nonspecific binding was determined with 50 μM naloxone. The binding was terminated by inserting the assay tubes into ice-cold water and membrane bound [^{3}H]diprenorphine was rapidly separated by filtration through GF/C filters. The filters were washed with 5 ml cold Tris-HCl buffer 3 times and filter-bound radioactivity was counted by a liquid scintillation counter.

[^{35}S]GTPγS Binding Assay.

[^{35}S]GTPγS binding assay was used as a functional measure of the agonist efficacy and potency of these compounds for the μ-opioid receptor. Membrane protein (100 μg/tube) prepared from CHO-μ cells was incubated in assay buffer (Tris-HCl 50 mM, NaCl 100 mM, $MgCl_2$ 5 mM, EDTA 1 mM, pH 7.4) containing 100 μM GDP, 0.2 nM [^{35}S]GTPγS, and different concentrations of compounds in a total volume of 0.5ml for 60 min at 28ºC. Nonspecific binding was determined with 40 μM unlabeled GTPγS. Binding was terminated by inserting the assay tubes into ice-cold water and membrane bound [^{35}S]GTPγS was rapidly separated by filtration through GF/C filters. The filters were washed with 5 ml cold assay buffer 3 times and filter-bound radioactivity was counted by a liquid scintillation counter.

Antinociceptive Test.

Hot plate test was used to assess the compounds' antinociceptive effects as previously described (Eddy and Leimbach, 1953) with minor modifications. Briefly, female mice were individually placed on the surface of a hot plate (HUGO SACHS, Germany) maintained at 55 ± 0.1ºC, and the latency was recorded from starting to the end point of jumping, licking or shaking hind paws. A cutoff time of 60 sec was imposed to prevent the possibility of tissue damage. Mice were tested before drug administration and 30 min after subcutaneous administration or 60 min after intragastric administration. Antinociceptive effects of thienorphine and buprenorphine were compared in dose response and time course experiments. To establish the dose-response curves, at least four doses of each compound were used. To determine the duration of action, mice were treated with subcutaneous administration of thienorphine (1.0 mg/kg, ~ ED_{95}) or buprenorphine (0.75 mg/kg, ~ ED_{95}) and tested at different times after injection.

Anti-morphine Assay.

A high dose of morphine produces lethality in mice as a consequence of μ-opioid receptor mediated respiratory depression and this effect can be reversed by an opioid antagonist. This assay was carried out based on the knowledge that partial agonist functions primarily as antagonist in the presence of a full agonist. Mice were treated with intragastric administration of different doses of thienorphine, buprenorphine or vehicle respectively followed 1 h later with 600 mg/kg morphine given subcutaneously. After 24 h, the number of mice survived the acute toxicity of morphine was recorded. To establish the dose-response curves, at least four doses of each

compound were used. To determine the duration of action, mice were treated with intragastric administration of thienorphine (2.6mg/kg, ~ 4 x ED_{50}) or buprenorphine (14mg/kg, ~ 4 x ED_{50}) followed with 600 mg/kg morphine (s.c.) at different times afterward and the number of mice survived was recorded 24 h later.

Data Analysis.

Receptor binding and [^{35}S]GTPγS binding data were analyzed using nonlinear regression analysis with GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). Antinociceptive data are presented as a percentage of the maximum possible effect (% MPE) following the equation of %MPE = (postdrug latency – predrug latency)/(cutoff time – predrug latency) × 100%. The ED_{50} values with 95% confidence limits in antinociceptive and anti-morphine assays were calculated by the method of Bliss (1967).

Results

Binding Profile of Thienorphine to Opioid Receptors.

Thienorphine displayed high affinities for μ-, δ- and κ-opioid receptors, inhibiting the binding of [^{3}H]diprenorphine to these opioid receptors with K_i values of 0.10, 0.27 and 0.23 nM, respectively (Table 1). Similar to buprenorphine, thienorphine showed no selectivity for all three opioid receptors. However, the K_i value of thienorphine to inhibit the binding of [^{3}H]diprenorphine to μ-opioid receptor was about 5-fold lower than that of buprenorphine (0.51nM).

Efficacy and Potency of Thienorphine in Stimulating [^{35}S]GTPγS Binding to μ-Opioid Receptor.

To further investigate the agonist properties of these opioid compounds, stimulation of [^{35}S]GTPγS binding to μ-opioid receptor by thienorphine, buprenorphine or morphine was assayed in cell membrane prepared from CHO-μ cells. As shown in Fig. 2, morphine potently stimulated [^{35}S]GTPγS binding to μ-opioid receptor in a dose-dependent manner (EC_{50} =18.24 nM). The maximal stimulatory response of morphine was designated as the 100 % effect in this experiment. The stimulatory effects of thienorphine and buprenorphine on [^{35}S]GTPγS binding to μ-opioid receptor were different from that of morphine, presenting ceiling effects with EC_{50} values of 0.009 and 0.10 nM, and the maximal stimulatory values of 62.42% and 33.05%, respectively (Fig. 2; Table 2). These results indicate that thienorphine, similar to buprenorphine, is a partial μ-opioid agonist. Notably, thienorphine was more potent than buprenorphine in stimulation of [^{35}S]GTPγS binding to μ-opioid receptor.

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Antinociceptive Effect of Thienorphine.

The antinociceptive effects of thienorphine and buprenorphine were studied in the mice hot plate test. Both thienorphine and buprenorphine presented the typical partial opioid agonist character with a ceiling dose-response curve and in addition a bell shape of the curve (Fig. 3; Table 3). Although thienorphine was less potent than buprenorphine after subcutaneous administration, the efficacy (maximal antinociceptive effect) of thienorphine was higher than that of buprenorphine and it produced an approximately 80% increase in hot plate response latency. Moreover, a marked rightward shift of the dose-response curve for buprenorphine was observed in mice treated with intragastric administration, with ED_{50} value increasing from 0.08 (s.c.) to 2.19 (i.g.) mg/kg. However, the dose-response curve for i.g. thienorphine only slightly shifted to right with ED_{50} increasing from 0.25 (s.c.) mg/kg to 0.83 (i.g.) mg/kg. The i.g./s.c. ED_{50} ratios of thienorphine and buprenorphine were 3.32 and 27.38 respectively.

To study the duration of the antinociceptive effect, mice were treated with s.c. injection of 1.0 mg/kg thienorphine or 0.75 mg/kg buprenorphine, the approximately doses of ED_{95} for both compounds, respectively and tested at different times after injection. As shown in Fig. 4, the antinociceptive effects of these two compounds declined very slowly and thienorphine was more efficacious than buprenorphine throughout the first 8 h after administration.

Antagonist Effect of Thienorphine on Morphine-induced Lethality.

Opioid antagonist effects of thienorphine and buprenorphine were studied in the anti-morphine assay. Morphine at the dose of 600 mg/kg (s.c.) produced 100% lethality in

vehicle-treated mice. Either thienorphine or buprenorphine given orally prior to morphine significantly antagonized the lethal effect of morphine with ED_{50} values of 0.64 mg/kg and 3.50 mg/kg, respectively (Fig. 5). However, the duration of action was different between these two compounds. Pretreatment of mice with 2.6 mg/kg of thienorphine, an approximate dose of 4 x ED_{50}, yielded a fully antagonist effect for two days, and 40% of the effect still remained 15 days after administration (Fig. 6), whereas the effect of buprenorphine at an approximate dose of 4 x ED_{50} (14.0 mg/kg) lasted for less than two days.

Discussion

In this study, we demonstrated that thienorphine, a new derivative of buprenorphine, has several advantages over buprenorphine as shown by its higher binding affinity at μ-opioid receptor *in vitro*, higher antinociceptive efficacy, longer duration of action, and better oral bioavailability *in vivo*.

Similar to buprenorphine, thienorphine behaved like a partial μ-opioid receptor agonist; it had a weaker stimulatory effect than morphine on [^{35}S]GTPγS binding to μ-opioid receptor *in vitro*, and showed a bell-shaped dose-response curve in antinociceptive test *in vivo*. In addition, thienorphine exhibited an antagonist effect at μ-opioid receptor against morphine, a typical full agonist.

Buprenorphine is widely used as an analgesic in clinic (Lewis, 1985). Thienorphine showed higher efficacy and longer duration of action than buprenorphine in the hot plate test, suggesting that thienorphine might be a better analgesic than buprenorphine. However, we also found that the analgesic effect of thienorphine declined very fast (Fig.7). After 4 consecutive administrations of 3 mg/kg, its antinociceptive effect decreased from 72.7% to 28.7%. This result is similar to buprenorphine (48.5% to 15.1%) after 4 consecutive administrations of 2.5 mg/kg. Therefore, the possible use of thienorphine as an analgesic is not predictable now. We also investigated the tolerance rate of its anti-morphine effect (Table 4), and the result is very interesting and unusual. A single dose of 0.045 mg/kg thienorphine antagonized the lethal effect of morphine (600 mg/kg) in 10% of the mice, while repeated administration of 0.045 mg/kg once daily for 8 days (total dose 0.36 mg/kg) protected 80% of the mice. The latter treatment was even more effective than a single high dose of 0.45 mg/kg (50% effective). This result suggested the possible accumulation of the

antagonist effect after repeated administration of thienorphine. The mechanisms underlying the different fashions of tolerance of the agonist and antagonist effects of thienorphine are undefined. Nevertheless, the relative fast tolerance of the agonist effect and the persistent maintenance of the antagonist effect of thienorphine on the μ-opioid receptor would be beneficial for treating opioid addiction.

In the treatment of opioid addiction, a medication with a long duration of action, e.g. several months, is desired. This goal could be achieved by several means. Developing new formulations is a most popular one at present and progresses have been made with the microspere and other control release formulations of naltrexone (Bartus et al., 2003; Comer et al., 2002; Carreno et al., 2003) and buprenorphine (Sobel et al., 2004). The other way is to alter the metabolism procedure of an existing compound by chemical modification of its structure, e. g. methadone and LAAM. These two considerations based mainly on the maintenance of an effective drug concentration in the body. In recent years, several reports have demonstrated that structural modifications of buprenorphine resulted in improvements in opioid receptor binding profile (Neilan et al., 2004; Husbands et al., 2005). These modified compounds are thought to have more powerful binding interactions with opioid receptor and thus slow receptor kinetics, securing a long-lasting effect. Thienorphine is a modified compound from buprenorphine. As expected, it elicits a long-lasting effect. The persistent occupation and activation of the μ-opioid receptor may account for the long duration of agonist effect of thienorphine *in vivo*. However, there will be more difficulties in the explanation of the strikingly longer duration of antagonist effect of thienorphine, which lasted for more than 15 days after a single administration of 2.6 mg/kg. One explanation is that thienorphine may be redistributed and stored in fat tissue because of its high liposolubility, and then released

slowly to maintain the effective drug concentration. However, the functional adaptation at the postreceptor level and changes in receptor sensitivity to ligand and gene expression of the μ-opioid receptor could also account for the observations in this study (Neilan et al., 2004). Further works are needed to address these issues.

Medications employed in the treatment of opioid addiction should avoid using injectable forms of administration to prevent the spread of infectious diseases, such as HIV and hepatitis (Kreek and Vocci, 2002). Buprenorphine has a very low oral bioavailability, and this results in the application of sublingual preparations in clinic. Although this kind of administration achieved somewhat success, new compounds with high oral bioavailability would be more safe and convenient for the patients. The high liposolubility of thienorphine may make it easier to across the biomembranes, and then increase the bioavailability after oral administration. In the hot plate test, the i.g. over s.c. ED_{50} ratio was much lower for thienorphine (3.32) than for buprenorphine (27.38). Similar results were observed in the anti-morphine assay (data not shown). These results suggest that the oral bioavailability of thienorphine is much higher than that of buprenorphine, which would make thienorphine more superior to buprenorphine in the treatment of opioid dependence.

The present study indicates that thienorphine, a structurally new partial μ-agonist, has a higher potency, longer duration of action and better oral bioavailability than buprenorphine. In contrast to common opioid agonists, we observed that thienorphine induced hypoactivity in mice and this effect declined after repeated administration (Zhao et al., 2004). Moreover, co-administration of thienorphine dose-dependently suppressed the development, transfer, and expression of behavioral sensitization to morphine in mice (Zhao et al., 2004). In conclusion, these

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data indicate that thienorphine may be a good candidate to be developed as a new treatment for opioid dependence, although further efforts are needed to discover the possible mechanisms of its unique effects and to investigate the clinical pharmacodynamics and toxicodynamics of this compound.

Acknowledgments

We thank Dr. Chun-He Liu for the synthesis of thienorphine and buprenorphine. We also recognize the outstanding technical assistance provided by Yong-Shao Liu in the animal experiments.

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Footnotes

This work was supported in part by National Basic Research Program and the 863-High Technology Research and Development Program Plan (Grant) of China.

Figure Legends

Fig. 1. Chemical structure of thienorphine and buprenorphine.

Fig. 2. Stimulation of [^{35}S]GTPγS binding to membrane of CHO cells stably expressing rat μ-opioid receptor by thienorphine, buprenorphine, and morphine. [^{35}S]GTPγS binding was performed in the presence of different concentrations of each compound as described in Materials and Methods. The data are expressed as stimulation in percentage relative to the maximum effect produced by morphine. Each data point represents the mean ± S.E.M. of at least three independent experiments performed in duplicate. EC_{50} values and maximal responses are summarized in Table 2.

Fig. 3. Dose-response curves of the antinociceptive effects produced by s.c. and i.g. administrations of thienorphine and buprenorphine in the mice hot plate test. (A) Animals were treated with s.c. (0.156, 0.313, 0.625 or 1.250 mg/kg) or i.g. (0.625, 1.25, 2.50 or 5.00 mg/kg) administration of thienorphine. (B) Animals were treated with s.c. (0.078, 0.156, 0.313, or 0.625 mg/kg) or i.g. (0.78, 3.13, 12.50 or 50.00 mg/kg) administration of buprenorphine. Each data point represents the mean ± S.E.M. n = 10.

Fig. 4. Time course of the antinociceptive effects produced by thienorphine (1.0 mg/kg, s.c.) and buprenorphine (0.75 mg/kg, s.c.) in the mice hot plate test. Each data point represents the mean ± S.E.M. n = 10.

Fig. 5. Dose-response curves of thienorphine and buprenorphine to antagonize the lethal effect of morphine. Animals were pretreated with thienorphine (0.3, 0.5, 1, or 1.5 mg/kg, i.g.) or buprenorphine (1.25, 2.5, 5.0 or 10.0mg/kg, i.g.) followed with morphine administration 1 h later. Morphine was administered subcutaneously at the dose of 600 mg/kg, with which it produced lethality in 100% of vehicle-treated mice (n = 20). Lethality was recorded up to 24 h after morphine administration. Each data point represents the percentage of mice protected against morphine-induced lethality (n = 8-10 mice/data point). *P<0.05, compared to vehicle-treated mice (Fisher's exact probability test).

Fig. 6. Time course of the anti-morphine effects of thienorphine and buprenorphine. Mice were pretreated with 2.6 mg/kg thienorphine or 14.0 mg/kg buprenorphine orally, followed with 600 mg/kg (s.c.) morphine at different times. Each data point represents the percentage of mice protected against morphine-induced lethality (n = 10 mice/data point). Morphine produced 100 % of lethality in vehicle-treated mice (n = 20). *P<0.05, compared to vehicle-treated mice (Fisher's exact probability test).

Fig. 7. Antinociceptive effects of repeated administration of thienorphine and buprenorphine. Female mice were treated with s.c. injections of thienorphine (3.0 mg/kg) or buprenorphine (2.5 mg/kg) twice daily (08:00, 18:00) for two consecutive days and were tested before treatment and 30 min after each administration. Antinociceptive effects are presented as %MPE following the equation described above. Each data point represents the mean ± S.E.M. n = 10.

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TABLE 1

Binding affinities of thienorphine for rat μ-, δ- and κ-opioid receptors stably expressed in CHO cells. Competitive binding assays were performed as described in Materials and Methods. K_i values with 95% confidence limits were calculated by non-linear regression using GraphPad Prism 4.0.

	K_i (95% Confidence Limits)		
	μ	δ	κ
		nM	
Thienorphine	0.10 (0.06-0.17)	0.27 (0.16-0.46)	0.23 (0.10-0.49)
Buprenorphine	0.51 (0.25-1.03)	0.91 (0.49-1.70)	0.21 (0.14-0.31)
Morphine	24.80 (15.26-40.31)	213.9 (135.8-337.0)	133.1 (96.88-182.7)

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TABLE 2

Efficacy and potency of thienorphine in stimulating [^{35}S]GTPγS binding in membrane prepared from CHO cells stably expressing rat μ-opioid receptor. [^{35}S]GTPγS Binding was performed as described in Materials and Methods. EC_{50} values with 95% confidence limits were calculated by non-linear regression using GraphPad Prism 4.0.

Compound	-log EC_{50} ± SE	EC_{50} (95% Confidence Limits)	Maximal Effect
		nM	*%*
Thienorphine	11.05 ± 0.05	0.009 (0.006-0.013)	62.42 ± 0.87
Buprenorphine	10.00 ± 0.21	0.10 (0.02-0.47)	33.05 ± 2.37
Morphine	7.74 ± 0.08	18.24 (11.14-29.88)	100

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TABLE 3

Antinociceptive i.g./s.c. potency ratios of thienorphine and buprenorphine in the hot plate test in the mice. ED_{50} values with 95% confidence limits were calculated by the method of Bliss.

i.g./s.c. refers to the ratio of ED_{50} (i.g.) / ED_{50} (s.c.)

Compound	ED_{50} (95% Confidence Limits)		i.g./s.c.
	s.c.	i.g.	
	mg/kg		
Thienorphine	0.25 (0.09-0.41)	0.83 (0.31-1.25)	3.32
Buprenorphine	0.08 (0.007-0.16)	2.19 (0.49-4.35)	27.38

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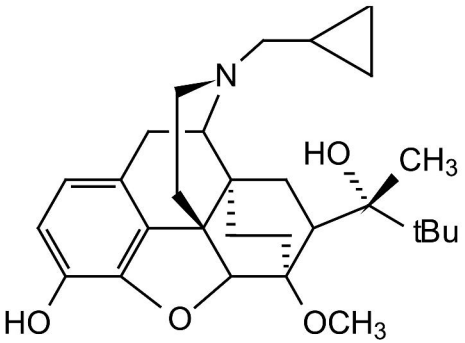
TABLE 4

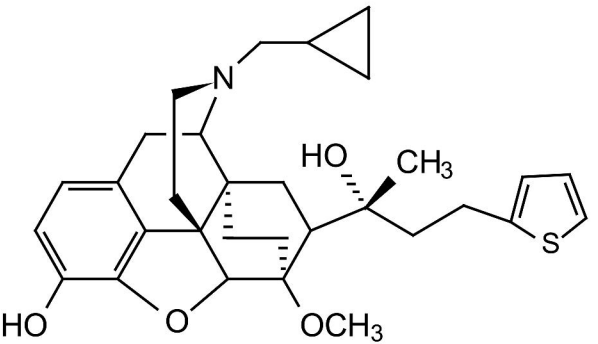
Anti-morphine effects of repeated administration of thienorphine. Mice were pretreated with thienorphine at a dose of 0.045 mg/kg (i.g.) once daily for 1, 4 and 8 days or at a single dose of 0.45 mg/kg (i.g.), followed with 600 mg/kg morphine (s.c.) 1 h after the last thienorphine administration. Data are represented as the percentage of mice protected against morphine-induced lethality (n = 10 in each group).

Dose (mg/kg)	Days	Total dose	Anti-morphine effect
		mg/kg	*%*
0.45	1	0.45	50
0.045	1	0.045	10
0.045	4	0.18	60
0.045	8	0.36	80

Fig. 1



buprenorphine



thienorphine

Fig. 2

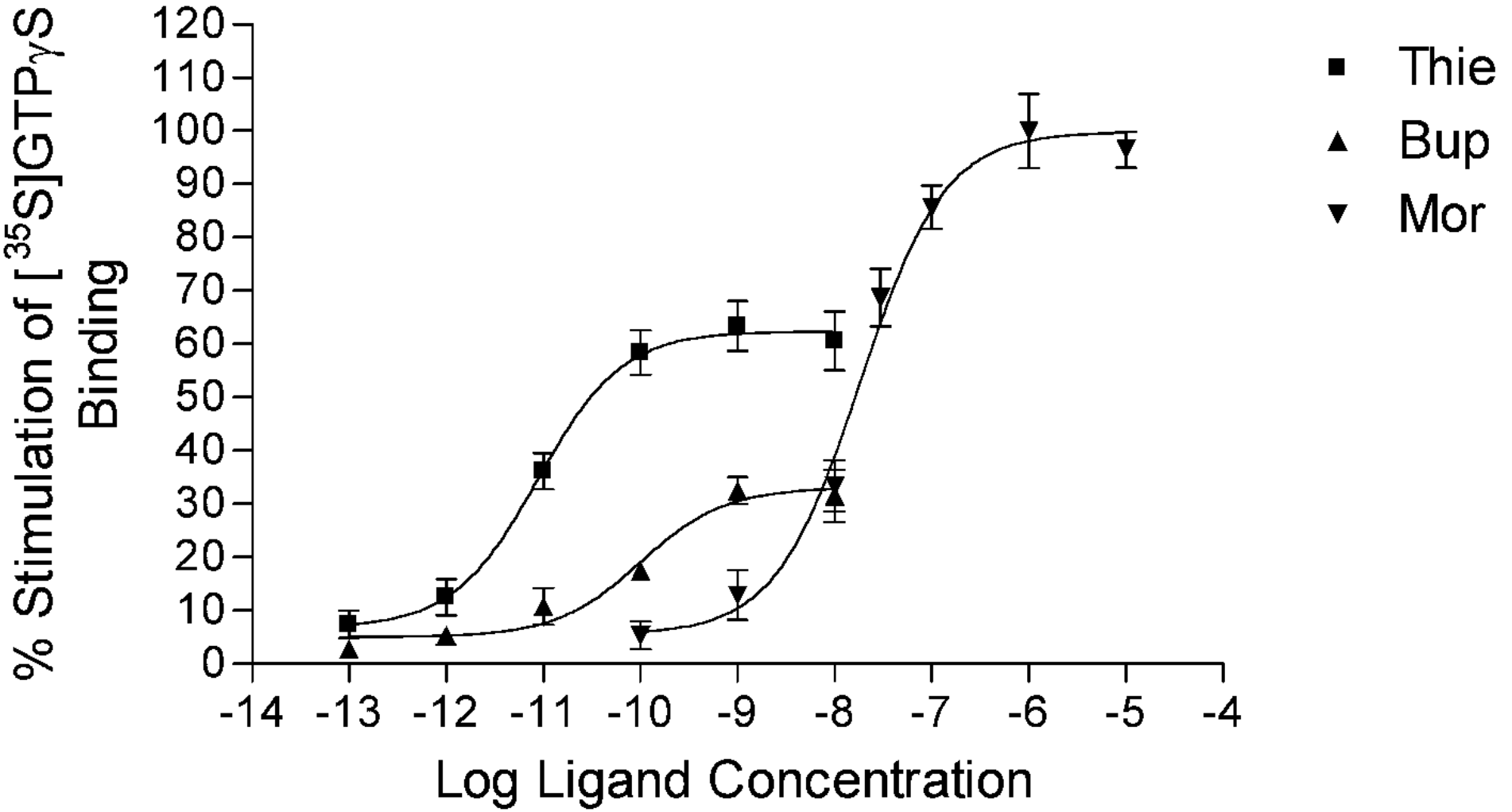

% Stimulation of [35S]GTPγS Binding
120
110
100
90
80
70
60
50
40
30
20
10
0
-14
-13
-12
-11
-10
-9
-8
-7
-6
-5
-4
Log Ligand Concentration
Thie
Bup
Mor

Fig. 3

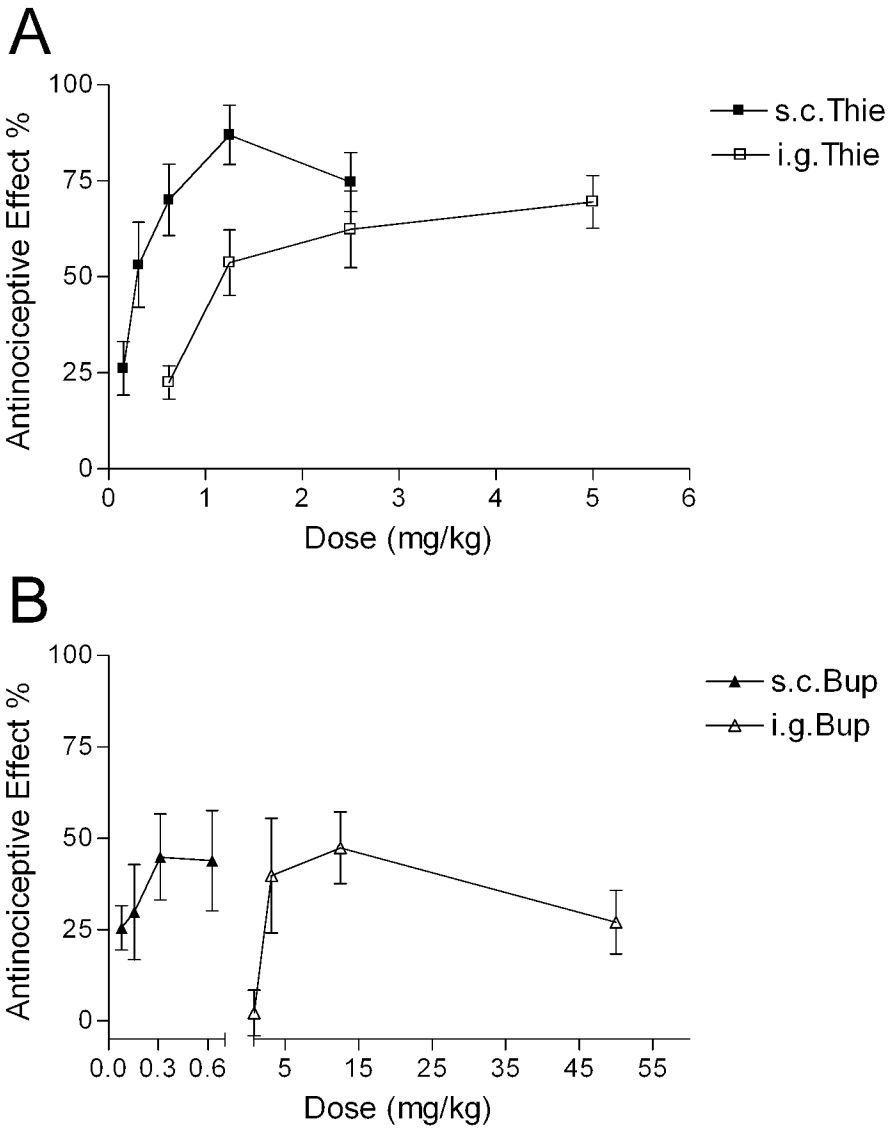

A
Antinociceptive Effect %
100
75
50
25
0
0
1
2
3
4
5
6
Dose (mg/kg)
s.c.Thie
i.g.Thie
B
Antinociceptive Effect %
100
75
50
25
0
0.0 0.3 0.6
5
15
25
35
45
55
Dose (mg/kg)
s.c.Bup
i.g.Bup

Fig. 4

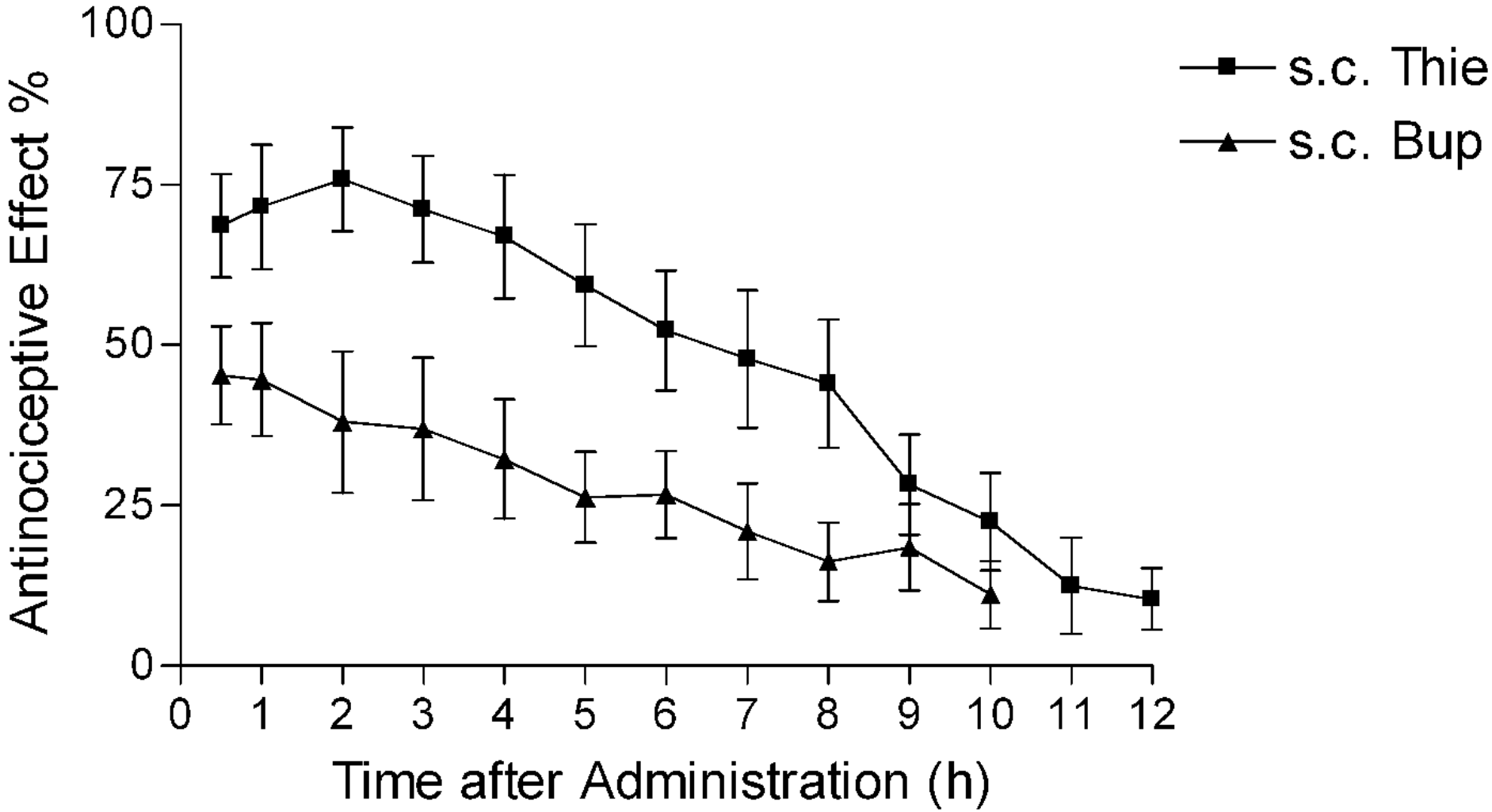

100
75
50
25
0
Antinociceptive Effect %
0
1
2
3
4
5
6
7
8
9
10
11
12
Time after Administration (h)
s.c. Thie
s.c. Bup

Fig. 5

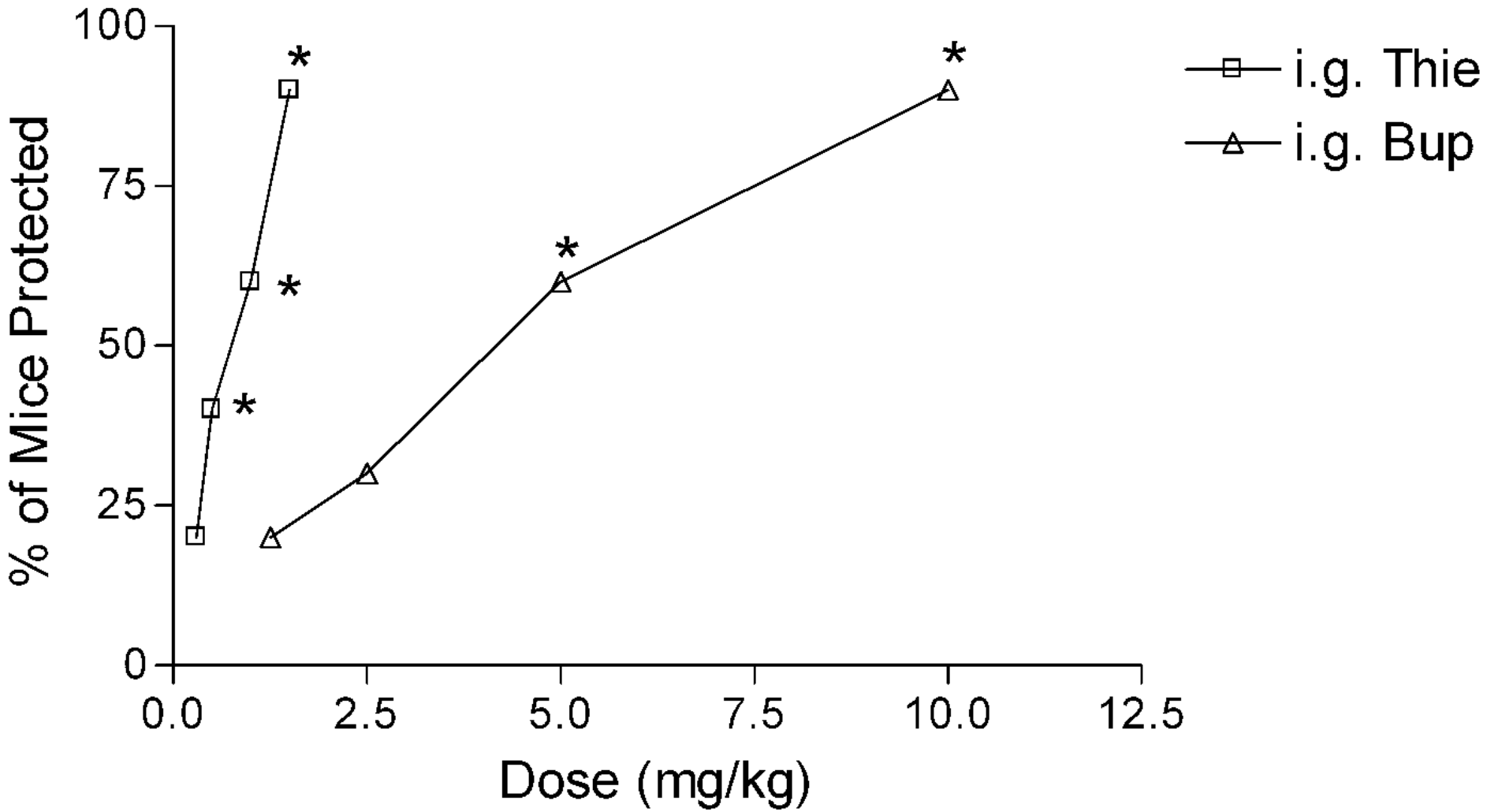

100
75
50
25
0
% of Mice Protected
0.0
2.5
5.0
7.5
10.0
12.5
Dose (mg/kg)
i.g. Thie
i.g. Bup
*
*
*
*
*

Fig. 6

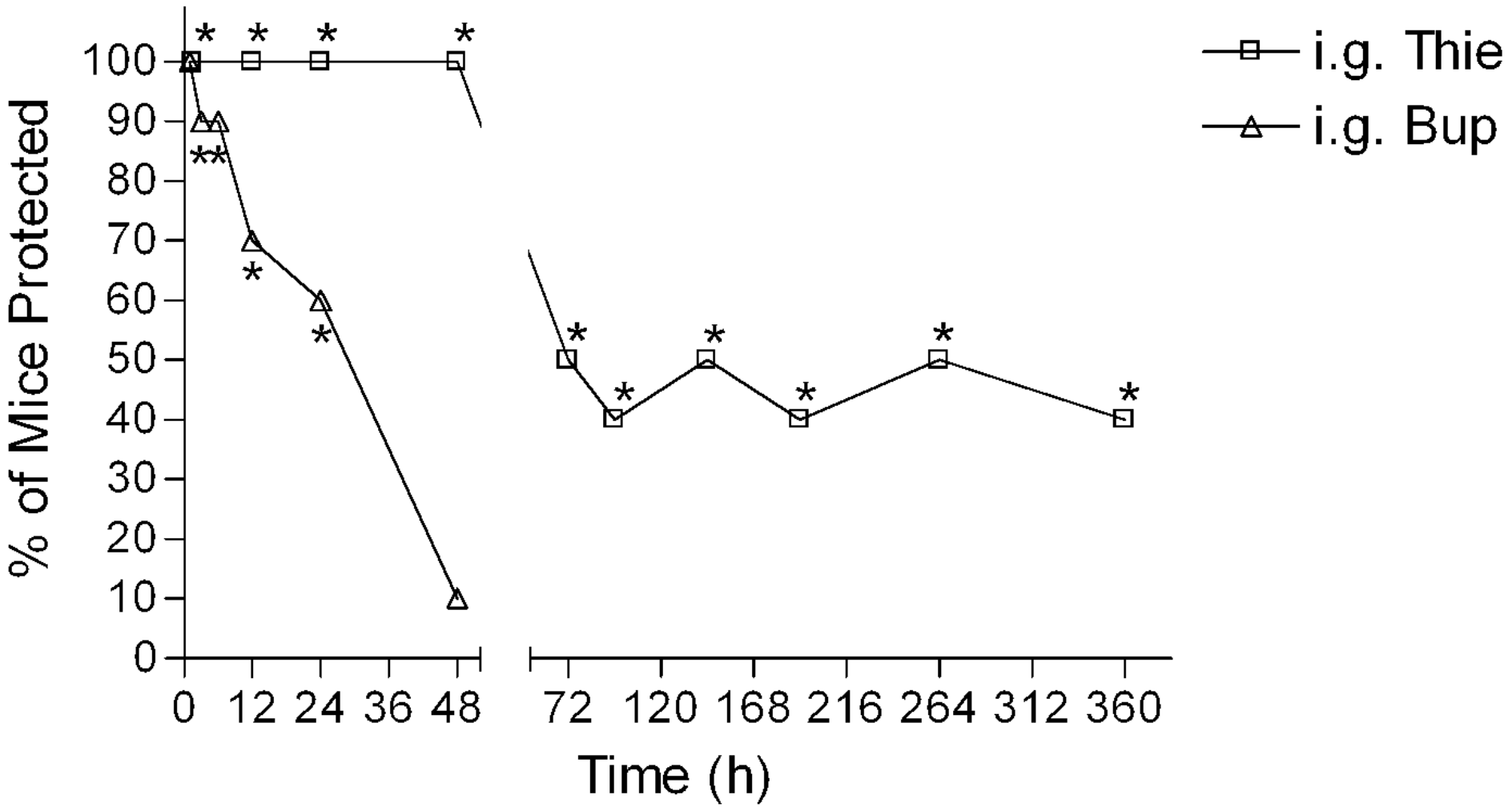
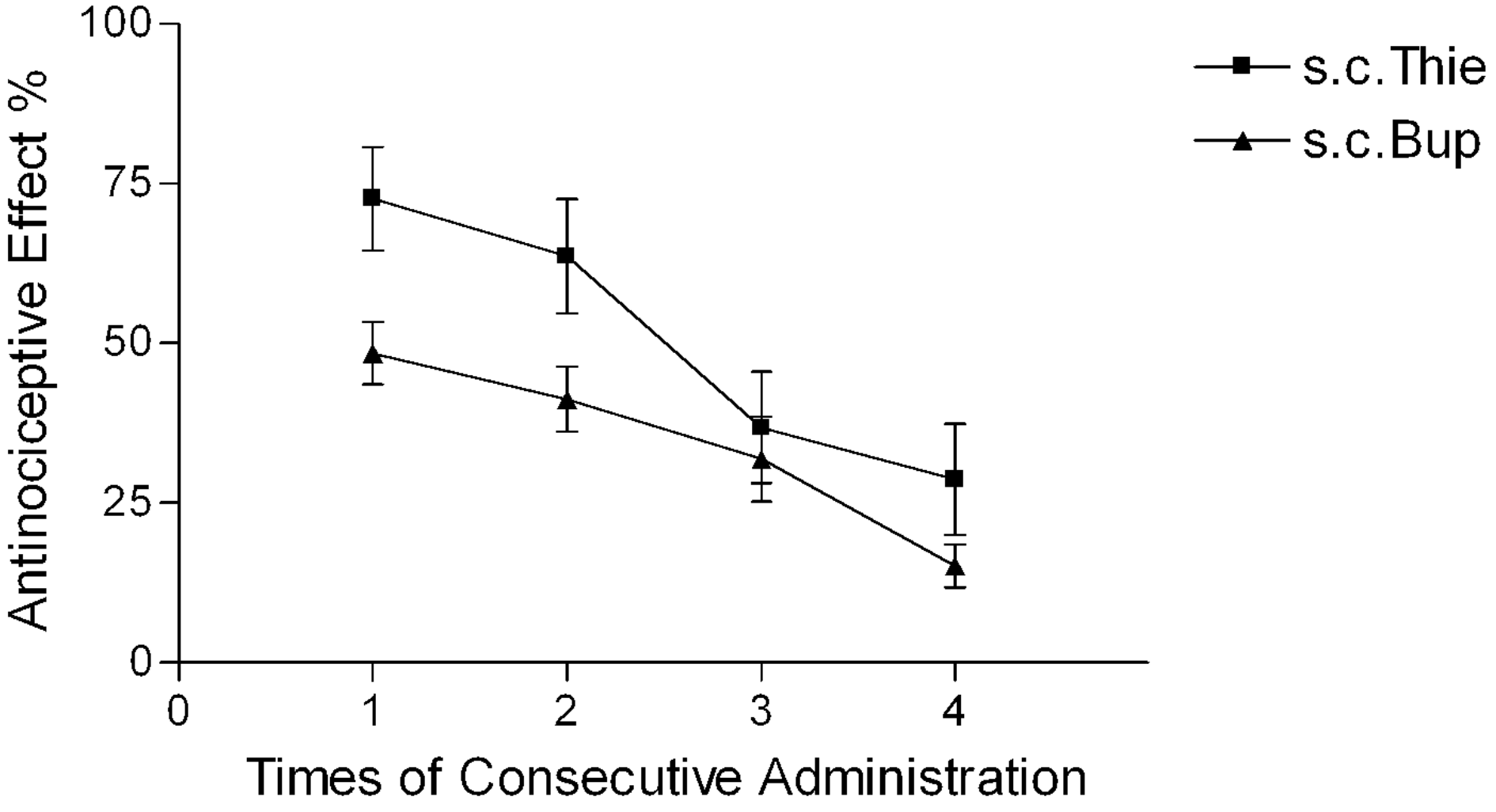

% of Mice Protected
100
90
80
70
60
50
40
30
20
10
0
0 12 24 36 48
72 120 168 216 264 312 360
Time (h)
i.g. Thie
i.g. Bup

Fig. 7


100
75
50
25
0
Antinociceptive Effect %
0
1
2
3
4
Times of Consecutive Administration
s.c.Thie
s.c.Bup