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Central antinociceptive effect of L-ornithine, a metabolite of L-arginine, in rats and mice

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Abstract

L-Arginine produces central antinociception by acting as a precursor of kyotorphin (L-tyrosyl-L-arginine), a [Met^5]enkephalin releaser. This study investigated the antinociceptive activity of L-ornithine, a metabolite of L-arginine. L-Ornithine given s.c. at 300–1000 mg/kg suppressed carrageenin-induced hyperalgesia in rats in a naloxone-reversible manner. L-Ornithine and L-arginine, when given i.c.v. at 10–100 μg/mouse, elicited antinociception even in intact mice, the effects being abolished by naloxone or naltrindole, and potentiated by bestatin, an inhibitor of aminopeptidase and kyotorphinase. The antinociception induced by i.c.v. L-ornithine was also inhibited by i.c.v. L-leucyl-L-arginine, a kyotorphin receptor antagonist, but was resistant to intracisternal anti-kyotorphin serum. L-Tyrosyl-L-ornithine, a synthetic dipeptide, (1–10 μg/mouse, i.c.v.), exerted kyotorphin-like antinociception in mice. These findings suggest that L-ornithine produces L-arginine-like antinociception via kyotorphin receptors. However, this effect does not appear to be mediated by kyotorphin itself, but most likely by L-tyrosyl-L-ornithine, a putative dipeptide.

Keywords: L-Ornithine; L-Arginine; Kyotorphin; Enkephalin; Pain; Antinociception

1. Introduction

The mammalian brain contains all the urea cycle intermediates, whereas enzymes participating in the conversion of L-ornithine into L-citrulline are absent, resulting in an incomplete urea cycle (Garthwaite, 1991). The discovery of nitric oxide (NO) synthase that catalyses the formation of NO and L-citrulline as a co-product from L-arginine (L-Arg) in the brain has indicated an additional pathway for L-Arg metabolism (Bredt and Snyder, 1990, 1992). L-Arg is also converted into kyotorphin, a [Met^5]enkephalin-releasing antinociceptive dipeptide (L-tyrosyl-L-arginine), by kyotorphin synthetase in the brain (Takagi et al., 1979a, b; Ueda et al., 1987b). Thus, there exist multiple pathways for L-Arg metabolism in the brain.

Kyotorphin binds to kyotorphin receptors on [Met^5]enkephalin neurones in the brain, and subsequently elevates the cytosolic Ca^{2+} concentration by activating phospholipase C via G protein (Ueda et al., 1989), followed by enhancement of [Met^5]enkephalin release (Takagi et al., 1979b), thereby activating opioid receptors, especially of the δ-subtype, consequently producing analgesia or antinociception (Kawabata et al., 1993; for review, see Takagi and Ueda, 1988) via the brainstem-spinal monoaminergic systems (Ueda et al., 1987a; Kawabata et al., 1994). Kyotorphin synthetase catalyses the formation of kyotorphin from L-tyrosine and L-Arg in the presence of ATP and Mg^{2+}. The K_m value of kyotorphin synthetase for L-Arg is much higher than its physiological concentration in the brain, predicting that exogenously applied L-Arg can act as an effective precursor of kyotorphin (Ueda et al., 1987b). In fact, systemic administration of L-Arg produces antinociception in rats or mice with carrageenin-induced hyperalgesia, but not in intact ani-

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mals, (Kawabata et al., 1992a, b), and produces analgesia in humans with chronic pain (Harima et al., 1991; Takagi et al., 1990), in a naloxone-reversible manner. L-Arg, when administered centrally, exhibits naloxone-reversible antinociceptive activity even in intact animals (Kawabata et al., 1992a, b, 1993). The antinociceptive effect of L-Arg is also blocked by s.c. preadministration of naltrindole, a δ-opioid receptor-selective antagonist, and by i.c.v. L-leucyl-L-arginine (Leu-Arg), a kyotorphin receptor antagonist, or intracisternal (i. cist.) anti-kyotorphin serum (Kamei et al., 1994; Kawabata et al., 1992b, 1993, 1994), thereby suggesting the involvement of the kyotorphin-[Met^5]enkephalin pathway in the brain (Kawabata and Takagi, 1994). L-Arg is also a substrate for NO synthase. NO appears to play a promoting role in supraspinal nociceptive transmission, since a number of NO synthase inhibitors exhibit potent antinociceptive activity on systemic or i.c.v. administration, the effect being resistant to naloxone (Babbedge et al., 1993; Moore et al., 1991, 1993; Kawabata et al., 1993, 1995; Kawabata and Takagi, 1994). In contrast, an antinociceptive property of brain NO has also been reported (Brignola et al., 1994; Iwamoto and Marion, 1994; Xu and Tseng, 1994; Kawabata et al., 1995). Thus, the role of brain NO in pain processing is complex. Both pro-nociceptive and antinociceptive NO systems may exist in the brain, and only their mixed effects can be detected by i.c.v. administration (Kawabata et al., 1995). Based upon kinetic evidence, however, exogenously applied L-Arg appears incapable of facilitating NO formation in the brain, since brain NO synthase is saturated by L-Arg under physiological conditions (Garthwaite, 1991). Therefore, it seems unlikely that L-Arg-induced antinociception is mediated by increased formation of NO in the brain. Thus, exogenously applied L-Arg acts as an effective precursor of kyotorphin but not of NO, resulting in antinociception.

The aim of this study was to test and characterize the antinociceptive activity of L-ornithine, a metabolite of L-Arg in the brain, compared to that of L-Arg. The present findings indicate that L-ornithine administered systemically or i.c.v. produces L-Arg-like antinociception in rats and mice, via the activation of kyotorphin receptors in the brain.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–300 g) and male ddy mice (20–30 g), purchased from Japan SLC. (Shizuoka), were used. The animals were housed with a 12-h light/12-h dark cycle and fed a standard laboratory diet and tap water ad libitum before the experiments.

2.2. Nociceptive testing

2.2.1. Paw-pressure test (Randall-Selitto test)

The changes in the mechanical nociceptive thresholds of the rats were measured using an analgesia meter (MK-300, Muromachi Kikai Co., Japan); pressure was applied to the right hindpaw of the rat at a rate increasing linearly by 30 g/s. The weight (g) required to elicit nociceptive responses such as squeak and struggle was taken as the nociceptive threshold. A cut-off value of 500 g was used to prevent damage to the paw.

2.2.2. Tail-pressure test (modified Randall-Selitto test)

Changes in the mechanical nociceptive threshold of the mice were assessed using the same analgesia meter as described above, with a plastic plate as a presser (the surface for application of pressure, 3×0.1 cm); the tail of the mouse was put on the center line of a round platform (1.3 cm in diameter) at right angles to the presser, and pressure was gradually applied to the middle part of the tail at a rate increasing linearly by 15 g/s. The nociceptive threshold, taken as the weight (g) required to elicit nociceptive responses such as squeak and struggle, was determined, and a cut-off value of 250 g was used. The threshold for each mouse was measured 6–8 times, and the basal threshold was defined as the mean of the values of the last four stable thresholds. The results are expressed as percentages of the basal threshold.

2.2.3. Tail-flick test

To determine thermal nociception, the mice were tested for responsiveness to radiant heat by use of a tail-flick analgesia meter (MK-330, Muromachi Kikai Co., Japan), in which the intensity of the thermal stimulus was adjusted to obtain baseline latencies of 2.0–2.5 s, and a cut-off latency of 8 s was used. The results are expressed as changes in latencies, which were obtained by subtracting the basal latency (the mean of the values of the last three baseline latencies) from the test latency.

2.3. Determination of antinociceptive effect of systemically administered L-ornithine in rats with carrageenin-induced hyperalgesia

After measurement of the baseline nociceptive threshold for each rat in the paw-pressure test, mechanical hyperalgesia was produced by an intraplantar (i.pl.) injection of 0.1 ml of 1% carrageenin into the right hindpaw of the rat (Kawabata et al., 1992a), and the nociceptive threshold was assessed at 30-min intervals. L-Ornithine at 300–1000 mg/kg was administered s.c. 2 h after carrageenin treatment. Naloxone, 2 mg/kg, was administered s.c. 30 min after s.c. L-ornithine, 1000 mg/kg. Control animals received an s.c. injection of saline in the same volume.

2.4. Determination of antinociceptive effect of centrally administered L-ornithine and its related amino acids in intact mice

2.4.1. Antinociceptive potency of i.c.v. administration of L-Arg, L-ornithine and L-citrulline in mice as assessed by the tail-pressure test or tail-flick test

L-Arg, L-ornithine or L-citrulline was administered i.c.v. (Haley and McCormick, 1957) in a dose range of 3–100 μg/mouse. The mechanical nociceptive threshold in the tail-pressure test was repeatedly assessed at 5, 10, 20, 40 and 60 min after i.c.v. injection. The antinociceptive effect of i.c.v. L-ornithine was also examined with the tail-flick assay.

2.4.2. Characterization of antinociception elicited by i.c.v. L-ornithine and L-Arg in mice as assessed by the tail-pressure test

Naloxone at 1 mg/kg and naltrindole, a δ-opioid receptor-selective antagonist, at 0.1 mg/kg were administered s.c. 5 min before i.c.v. L-ornithine or L-Arg at 100 μg/mouse. Leu-Arg, a kyotorphin receptor antagonist, at 3 μg/mouse was coadministered i.c.v. with L-ornithine or L-Arg (100 μg/mouse). Bestatin, an inhibitor of aminopeptidase and kyotorphinase (Akasaki et al., 1991; Orawski and Simmons, 1992), at a dose of 0.1 μg/mouse was coadministered i.c.v. with L-ornithine or L-Arg at 1 μg/mouse. Anti-kyotorphin serum in a volume of 10 μl without dilution was administered i.cist. (Ueda et al., 1979) 1 h before i.c.v. L-ornithine or L-Arg at 30 μg/mouse. Control animals were injected s.c., i.c.v. or i.cist. with the same volume of saline or control serum.

2.5. Evaluation of antinociceptive potency of centrally administered L-tyrosyl-L-ornithine (Tyr-Orn), a synthetic dipeptide, in mice

The synthetic dipeptide, Tyr-Orn, was administered i.c.v. to mice in a dose range of 1–10 μg/mouse, and the mechanical nociceptive threshold was measured 5, 10, 20, 40 and 60 min later with the tail-pressure test. The antinociceptive effect of i.c.v. Tyr-Orn was also assessed with the tail-flick test. Naltrindole at 0.1 mg/kg was given s.c. 5 min before i.c.v. Tyr-Orn (3 μg/mouse), and Leu-Arg at 3 μg/mouse was coadministered i.c.v. with the same dose of Tyr-Orn. Control animals received an s.c. or i.c.v. injection of saline.

2.6. Chemicals and anti-kyotorphin serum employed

L-Arginine hydrochloride (L-Arg) (Nacalai Tesque, Japan), L-ornithine hydrochloride, L-citrulline (Wako Pure Chem., Japan), λ-carrageenin, D-ornithine hydrochloride, naloxone hydrochloride, naltrindole hydrochloride, kyotorphin acetate, L-leucyl-L-arginine acetate (Leu-Arg), bestatin hydrochloride (Sigma, U.S.A.), L-tyrosyl-L-ornithine hydrochloride (Tyr-Orn) (Peptide Institute, Japan). For injections, λ-carrageenin was dissolved in distilled water, and all other drugs in saline. Anti-kyotorphin serum, which was produced from rabbits immunized with kyotorphin conjugated with bovine serum albumin (Ueda et al., 1987b), was a gift from Dr. Shiomi et al. (Fukuyama University, Japan). The binding activity and specificity of the antiserum have been tested and reported (Kawabata et al., 1994). Control serum was prepared from a non-immunized rabbit.

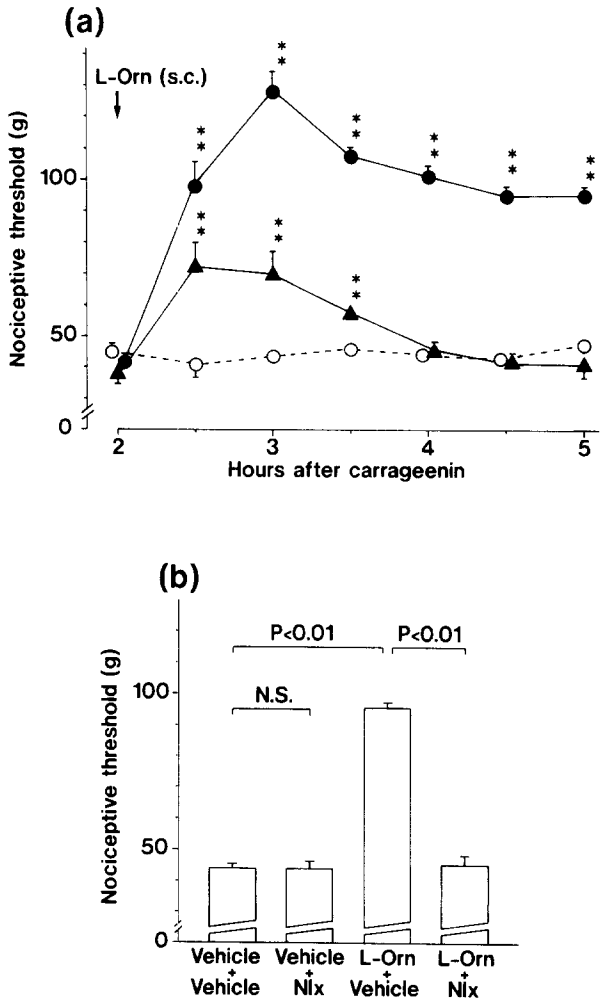

Fig. 1. (a) Antinociceptive effect of s.c. administration of L-ornithine (L-Orn) in rats with carrageenin-induced hyperalgesia, as assessed by the paw-pressure test. L-Orn at 300 (closed triangle) and 1000 (closed circle) mg/kg was administered s.c. 2 h after carrageenin. $^{**}P < 0.01$ vs. vehicle (open circle). $n = 4$. The basal nociceptive threshold before carrageenin was 156.7 ± 2.5 g ($n = 12$). (b) Naloxone (Nlx) antagonism of L-Orn (s.c.)-induced antinociception in rats with carrageenin-induced hyperalgesia in the paw-pressure test. Nlx at 2 mg/kg was administered s.c. 30 min after s.c. L-Orn (1000 mg/kg). Data indicate the threshold 3 h after carrageenin. $n = 4$. N.S., not significant. The basal nociceptive threshold before carrageenin was 167.6 ± 2.2 g ($n = 16$). Values are means with S.E.M.

2.7. Statistical analysis

The results are expressed as means with S.E.M. The statistical significance of differences between groups was analyzed with Student's unpaired *t*-test or by Newman-Keuls' multiple comparison test, and was set at $P < 0.05$.

3. Results

3.1. Opioid-dependent antinociceptive effect of systemically administered L-ornithine in rats with carrageenin-induced hyperalgesia

L-Ornithine, administered s.c. at 300 and 1000 mg/kg, significantly elevated the mechanical nociceptive threshold of the carrageenin-treated hindpaw in rats, as assessed by the paw-pressure test (Fig. 1a), although it was without any effect in intact rats (data not shown). The antinociception produced by L-ornithine (1000 mg/kg, s.c.) was completely antagonized by naloxone (2 mg/kg, s.c.) (Fig. 1b).

3.2. Antinociceptive potency of i.c.v. administered L-Arg, L-ornithine and L-citrulline in mice

L-Arg and L-ornithine, but not L-citrulline, when administered i.c.v. at 100 μg/mouse, significantly elevated the mechanical nociceptive threshold in the tail-pressure test in the mouse, the effect peaking at 10 min (Fig. 2a). The antinociceptive activity of L-ornithine and L-Arg at the peak time was significant and dose-dependent over a dose range of 10–100 μg/mouse,

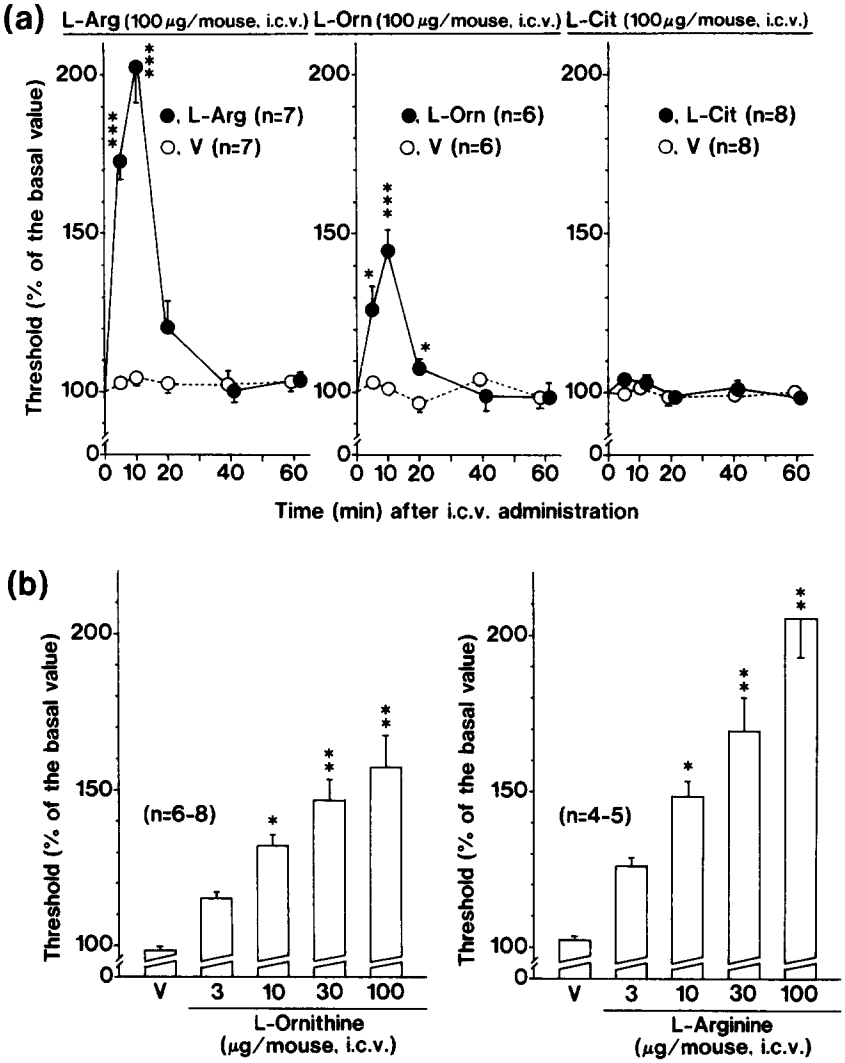

Fig. 2. (a) Comparison of the antinociceptive potency of centrally administered L-arginine (L-Arg), L-ornithine (L-Orn) and L-citrulline (L-Cit) in mice as assessed by the tail-pressure test. L-Arg, L-Orn and L-Cit, at a dose of 100 μg/mouse, were administered i.c.v. to mice. $^{*}P < 0.05$, $^{***}P < 0.001$ vs. vehicle (V). (b) Dose-dependent antinociceptive effects of i.c.v. L-Orn and L-Arg in mice in the tail-pressure test. The data indicate thresholds 10 min after i.c.v. injection. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle (V). Values are means with S.E.M.

although the antinociceptive potency of L-ornithine was somewhat lower than that of L-Arg (Fig. 2b). L-Ornithine, given i.c.v., also suppressed the thermal nociception in mice, as assessed by the tail-flick test; Δ latencies (s) 10 min after administration were 0.44 ± 0.09, 2.56 ± 0.40 ($P < 0.05$ vs. vehicle) and 3.52 ± 0.57 ($P < 0.01$ vs. vehicle) in groups treated with vehicle and with L-ornithine at 10 and 100 μg/mouse, respectively ($n = 4$). In contrast, D-ornithine, given i.c.v., did not modify the tail-flick latency (data not shown).

3.3. Antagonism by opioid receptor antagonists of the antinociception elicited by i.c.v. L-ornithine and L-Arg in mice

Naloxone, preadministered s.c. at 1 mg/kg, which did not produce any effect by itself, completely antagonized the antinociceptive effects of i.c.v. L-ornithine as well as L-Arg at 100 μg/mouse in the tail-pressure test (Fig. 3a). Similarly, naltrindole, a δ-opioid receptor-selective antagonist, preadministered s.c. at 0.1 mg/kg, completely abolished the antinociception induced by i.c.v. L-ornithine or L-Arg (100 μg/mouse) (Fig. 3b).

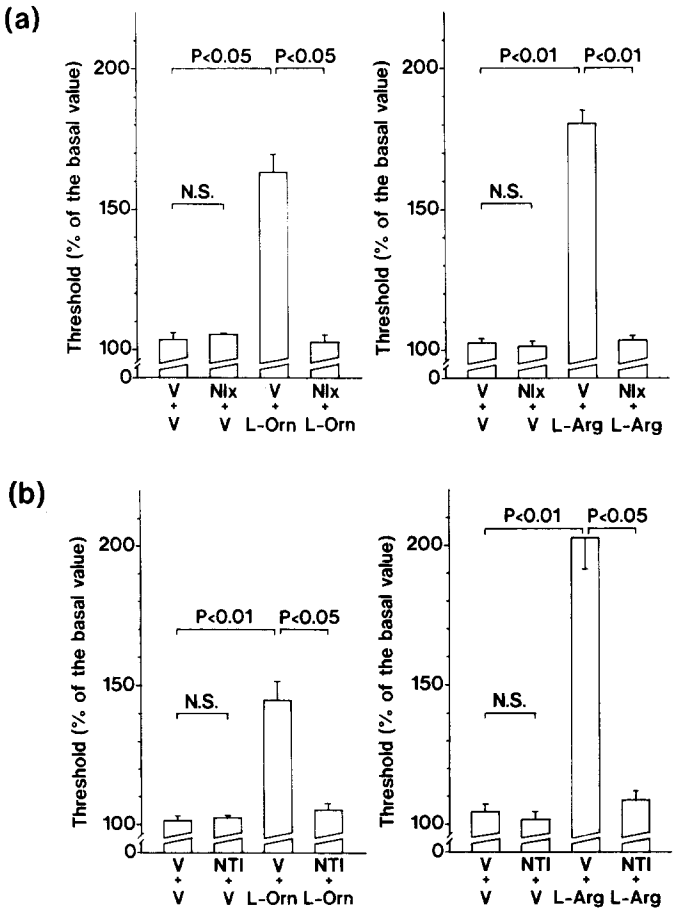

Fig. 3. Antagonism by naloxone (Nlx) (a) and by naltrindole (NTI) (b) of the antinociception produced by i.c.v. L-ornithine (L-Orn) or L-arginine (L-Arg) in mice in the tail-pressure test. Nlx at 1 mg/kg (a) or NTI at 0.1 mg/kg (b) was administered s.c. 5 min before i.c.v. L-Orn or L-Arg at 100 μg/mouse. The data indicate thresholds 10 min after i.c.v. injection; the values are means with S.E.M. $n = 4$–5 (a) or 6–7 (b). V, vehicle; N.S., not significant.

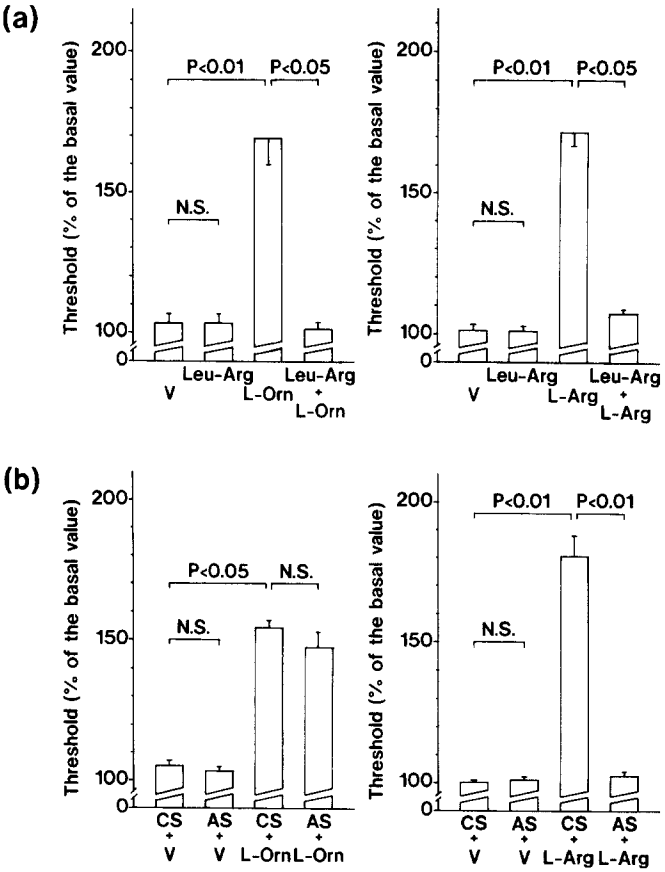

Fig. 4. Effects of L-leucyl-L-arginine (Leu-Arg) (a) and anti-kyotorphin serum (AS) (b) on the antinociception produced by i.c.v. L-ornithine (L-Orn) or L-arginine (L-Arg) in mice in the tail-pressure test. (a) Leu-Arg at 3 μg/mouse was coadministered i.c.v. with L-Orn or L-Arg at 100 μg/mouse. (b) AS or control serum (CS) in a volume of 10 μl was preadministered i.cist. 60 min before i.c.v. L-Orn or L-Arg at 30 μg/mouse. The data indicate thresholds 10 min after i.c.v. injection; the values are means with S.E.M. $n = 4$–6. V, vehicle; N.S., not significant.

3.4. Potentiation by bestatin of i.c.v. L-ornithine- and i.c.v. L-Arg-induced antinociception in mice

Bestatin, an inhibitor of aminopeptidase that degrades both [Met^5]enkephalin and kyotorphin, and of kyotorphinase that specifically degrades kyotorphin, when administered i.c.v. alone at 0.1 μg/mouse, failed to exhibit any significant antinociceptive effect by itself, as assessed by the tail-pressure test. However, the same dose of bestatin, when coadministered i.c.v. with L-ornithine or L-Arg at 1 μg/mouse, markedly potentiated the antinociceptive activity of L-ornithine and of L-Arg, although neither L-ornithine nor L-Arg, given alone at the same dose, were effective. The mechanical nociceptive thresholds (%) at 10 min after i.c.v. injection were: 102.7 ± 2.8, 105.9 ± 1.5, 102.8 ± 1.9 and

163.6 ± 5.4 ($P < 0.01$ vs. vehicle only) in groups treated with vehicle only, L-ornithine (1 μg/mouse), bestatin (0.1 μg/mouse), and L-ornithine plus bestatin, respectively ($n = 5$); 101.5 ± 1.0, 106.0 ± 2.0, 103.1 ± 3.3 and 174.2 ± 4.7 ($P < 0.01$ vs. vehicle only) in groups treated with vehicle only, L-Arg (1 μg/mouse), bestatin (0.1 μg/mouse), and L-Arg plus bestatin, respectively ($n = 5$).

3.5. Effect of a kyotorphin receptor antagonist and anti-kyotorphin serum on the antinociceptive activity of i.c.v. L-ornithine and L-Arg in mice

Leu-Arg, a kyotorphin receptor antagonist, given i.c.v. at 3 μg/mouse, did not affect the mechanical nociceptive threshold in the tail-pressure test by itself. However, the antinociception induced by i.c.v. L-ornithine or L-Arg (100 μg/mouse) was completely abolished by coadministered Leu-Arg at 3 μg/mouse (Fig. 4a). The antinociceptive effect of i.c.v. L-Arg at 30 μg/mouse was also inhibited by i.cist. preadministration of anti-kyotorphin serum, while control serum administered in the same manner was without any inhibitory effect. In contrast, the antinociceptive activity of i.c.v. L-ornithine at 30 μg/mouse was resistant to preadministered anti-kyotorphin serum (Fig. 4b). Thus, L-ornithine-induced antinociception appears to be mediated by kyotorphin receptors but not by increased formation of kyotorphin itself.

3.6. Antinociceptive activity of Tyr-Orn, a synthetic dipeptide, administered i.c.v. in mice

To test the hypothesis that L-ornithine produces antinociception possibly by acting as a substrate alter-

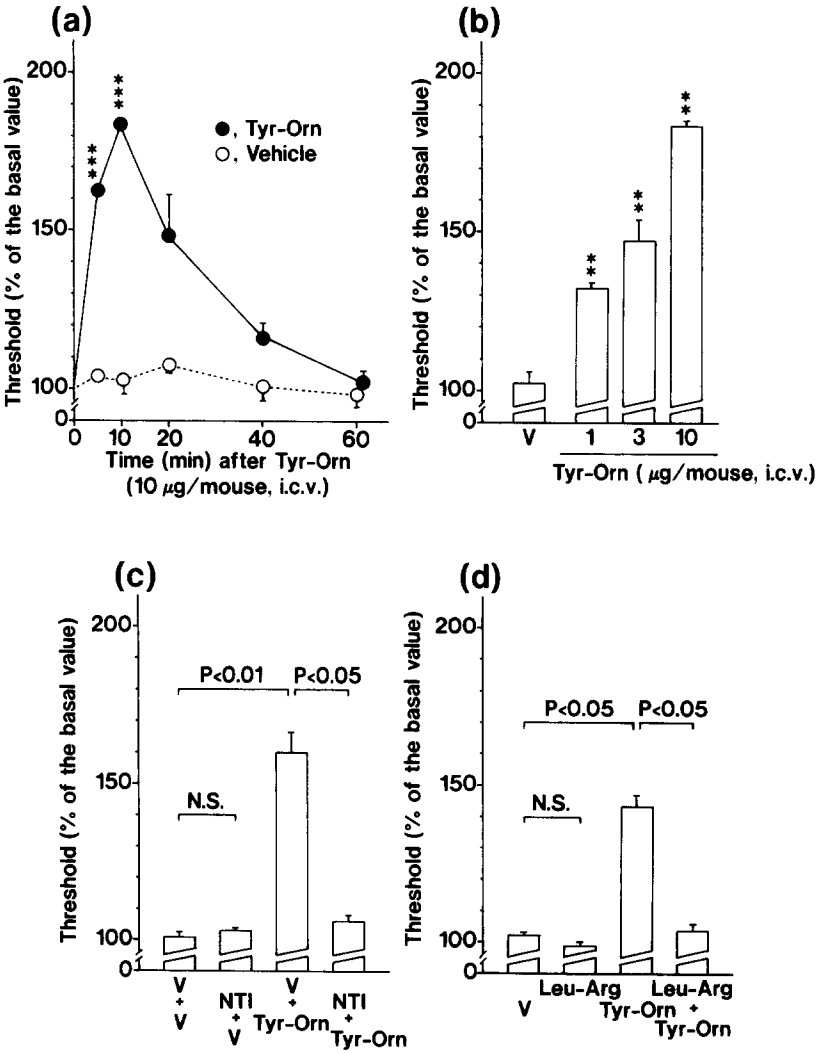

Fig. 5. (a) Time-related antinociceptive effects of i.c.v. L-tyrosyl-L-ornithine (Tyr-Orn) in mice as assessed by the tail-pressure test. Tyr-Orn at 10 μg/mouse was administered i.c.v. to mice. $^{***}P < 0.001$ vs. vehicle. $n = 4$. (b) Dose-related effects of i.c.v. Tyr-Orn in mice in the tail-pressure test. Tyr-Orn at 1–10 μg/mouse was administered i.c.v. to mice. The data indicate thresholds 10 min after i.c.v. injection. $^{**}P < 0.01$ vs. vehicle (V). $n = 4$. (c,d) Antagonism by naltrindole (NTI) and L-leucyl-L-arginine (Leu-Arg) of the antinociceptive effect of i.c.v. Tyr-Orn. NTI was administered s.c. 5 min before i.c.v. Tyr-Orn at 3 μg/mouse, and Leu-Arg at 3 μg/mouse was coadministered i.c.v. with the same dose of Tyr-Orn. The data indicate thresholds 10 min after i.c.v. injection. $n = 6–7$. V, vehicle; N.S., not significant. The values are means with S.E.M.

native to L-Arg for kyotorphin synthetase, we assessed the antinociceptive activity of Tyr-Orn, a synthetic dipeptide that may be expected to be formed from L-ornithine by kyotorphin synthetase, in mice. Tyr-Orn, administered i.c.v. at 10 μg/mouse, significantly elevated the mechanical nociceptive threshold in the tail-pressure test, the effect peaking at 10 min (Fig. 5a). The antinociceptive effect of Tyr-Orn at the peak time was significant and dose-dependent in a dose range of 1–10 μg/mouse (Fig. 5b). Tyr-Orn at 10 μg/mouse, i.c.v., significantly suppressed the thermal nociception, also as assessed by the tail-flick test; Δ latencies (s) at 10 min after i.c.v. injection were 0.13 ± 0.12 and 1.87 ± 0.12 ($P < 0.001$ vs. vehicle) in groups treated with vehicle and with Tyr-Orn at 10 μg/mouse ($n = 6$). The antinociception produced by i.c.v. Tyr-Orn at 3 μg/mouse in the tail-pressure test was completely abolished by s.c. preadministration of the δ-receptor antagonist, naltrindole at 0.1 mg/kg (Fig. 5c), and by coadministration of the kyotorphin receptor antagonist, Leu-Arg at 3 μg/mouse (Fig. 5d).

4. Discussion

In this study, we found that L-ornithine, a metabolite of L-Arg, administered systemically and centrally, mimics the antinociceptive effect of L-Arg in rats and in mice; s.c. administration of L-ornithine produced opioid-dependent antinociception in hyperalgesic, but not intact, rats; i.c.v. administration of L-ornithine as well as L-Arg dose dependently suppressed the mechanical and thermal nociception in intact mice, the effect being reversed by naloxone, naltrindole or Leu-Arg, and potentiated by bestatin, thereby suggesting the involvement of the kyotorphin-[Met^5]enkephalin pathway in the induction of the antinociceptive effect of L-ornithine as well as L-Arg. However, the antinociception produced by L-ornithine does not appear to be mediated by the formation of kyotorphin itself in the brain, since i.cist. anti-kyotorphin serum abolished the antinociceptive effect of i.c.v. L-Arg but not that of i.c.v. L-ornithine. Therefore, we assumed that L-ornithine acts as a substrate alternative to L-Arg for kyotorphin synthetase in the brain, followed by its conversion into a putative antinociceptive dipeptide (possibly Tyr-Orn) analogous to kyotorphin, resulting in antinociception. This hypothesis is strongly supported by the finding that the synthetic dipeptide, Tyr-Orn, administered i.c.v. to mice, exerted a significant antinociceptive effect that was reversed by naltrindol or Leu-Arg. We propose that L-ornithine may act as a substrate alternative to L-Arg for kyotorphin synthetase in the brain, followed by the formation of a kyotorphin-like antinociceptive dipeptide, possibly Tyr-Orn, resulting in antinociception via activation of kyotorphin receptors and subsequently via activation of δ-opioid receptors by endogenous [Met^5]enkephalin.

Systemic (s.c.) administration of L-Arg produces opioid-dependent antinociception in the animals with carrageenin-induced mechanical hyperalgesia but not in intact animals, and the antinociceptive effect of i.c.v. administration of L-Arg is much greater in the former than in the latter (Kawabata et al., 1992a, b). Similarly, significant antinociceptive activity of systemic L-Arg can be detected in animals with prostaglandin E_2 (i.pl.)-induced mechanical hyperalgesia or with heat injury-induced thermal hyperalgesia (unpublished data), and also in diabetic mice (Kamei et al., 1994). The enhancement of the antinociceptive potency of L-Arg in hyperalgesic animals may be due to the induction of kyotorphin synthetase, but not of kyotorphin receptors, since the effective dose range of kyotorphin administered i.c.v. in hyperalgesic mice is not different from that in intact mice (Kawabata et al., 1992b). In the present study, L-ornithine administered s.c. mimicked the above antinociceptive properties of L-Arg, implying involvement of antinociceptive mechanisms resembling those of L-Arg. I.pl. administration of L-Arg also evokes antinociception in the presence of carrageenin-induced inflammation, the effect being mediated by NO but not by kyotorphin, although this peripheral effect of L-Arg does not contribute to the antinociception induced by its systemic administration (Duarte et al., 1990; Kawabata et al., 1992a). We confirmed the lack of such a peripheral effect of L-ornithine at 1000 μg/paw (data not shown). Clinically, L-Arg, when administered i.v., evokes potent analgesia in chronic pain patients in a naloxone-reversible manner (Harima et al., 1991; Takagi et al., 1990). Therefore, we also expect therapeutic significance for L-ornithine as an analgesic, which is considered to be more specific than L-Arg in terms of lack of NO production.

L-Ornithine and L-Arg, but not L-citrulline, given i.c.v., elicited significant antinociception in the mechanical and thermal nociception tests in intact mice. The formation of L-Arg from L-ornithine via L-citrulline in the urea cycle is not responsible for the production of the antinociceptive effect of L-Orn, considering the lack of antinociceptive activity of i.c.v. L-citrulline and the absence of enzymes forming L-citrulline from L-ornithine in the brain (Garthwaite, 1991). The finding that the antinociception induced by i.c.v. L-ornithine and by i.c.v. L-Arg was abolished by naloxone, naltrindole or Leu-Arg, suggests the involvement of opioid receptors, especially δ-receptors, and of kyotorphin receptors, and, in turn, may predict the involvement of endogenous [Met^5]enkephalin, which is released by newly formed kyotorphin. The antinociceptive effects of centrally administered enkephalins and kyotorphin are potentiated by coadministration of bestatin, an

inhibitor of aminopeptidase, an enzyme degrading both enkephalins and kyotorphin, and of kyotorphinase, a specific enzyme for kyotorphin metabolism (Akasaki et al., 1991; Chaillet et al., 1983; Orawski and Simmons, 1992; Ueda et al., 1985). That i.c.v. bestatin markedly potentiated the antinociceptive activity of i.c.v. L-ornithine and of i.c.v. L-Arg further supports the contribution of endogenous [Met^5]enkephalin and kyotorphin to the induction of the effects of the two amino acids. However, the formation of kyotorphin itself in the brain does not appear to participate in the production of the effect of L-ornithine, differing from the antinociceptive mechanism of L-Arg as a precursor of kyotorphin (Kawabata et al., 1992b, 1993, 1994; Kawabata and Takagi, 1994), since i.cist. preadministration of anti-kyotorphin serum abolished the antinociception by i.c.v. L-Arg but not by i.c.v. L-ornithine. Thus, L-ornithine-induced antinociception is not mediated by kyotorphin itself but by kyotorphin receptors.

We assume that L-ornithine may be utilized as a substrate alternative to L-Arg by kyotorphin synthetase in the brain, and may be converted into a kyotorphin-like dipeptide, possibly Tyr-Orn, leading to activation of kyotorphin receptors. Actually, in the present study, i.c.v. administration of the synthetic dipeptide Tyr-Orn produced significant antinociception mediated by kyotorphin receptors and δ-opioid receptors. Furthermore, in our preliminary experiments, L-ornithine, but not L-citrulline, competitively inhibited immunoreactive kyotorphin formation from L-Arg by rat brain kyotorphin synthetase in vitro (unpublished data), supporting the above hypothesis, although the conversion of L-ornithine into Tyr-Orn by kyotorphin synthetase still remains to be demonstrated. In conclusion, L-ornithine appears to mimic the antinociceptive properties of L-Arg, most likely via the formation of Tyr-Orn, a putative antinociceptive dipeptide.

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