Br. J. Pharmacol. (1992), **107**, 1096–1101

L-Leucyl-L-arginine, naltrindole and D-arginine block antinociception elicited by L-arginine in mice with carrageenin-induced hyperalgesia

Atsufumi Kawabata, Yumiko Nishimura & [1]Hiroshi Takagi

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kinki University 3-4-1 Kowakae, Higashi-Osaka 577, Japan

1 Intraplantar injection of carrageenin into the mouse hind paw produced hyperalgesia when measured by the paw pressure test (Randall & Selitto method).

2 Subcutaneous administration of L-arginine (100–1,000 mg kg^{-1}), a possible precursor of kyotorphin which is an endogenous analgesic neuropeptide, inhibited carrageenin-induced hyperalgesia in a dose-dependent manner. This effect was blocked by subcutaneous administration of naloxone, naltrindole, a selective δ-opioid receptor antagonist (enkephalin antagonist), and D-arginine.

3 Intracerebroventricular administration of L-leucyl-L-arginine inhibited the antinociceptive effect of systemically administered L-arginine in hyperalgesic mice.

4 Intracerebroventricular administration of L-arginine (3 and 30 μg per mouse) and kyotorphin (300 ng–3 μg per mouse) produced antinociception in hyperalgesic mice. The antinociceptive effects of L-arginine but not kyotorphin were blocked by intracerebroventricular administration of D-arginine.

5 These results suggest that L-arginine-induced antinociception is mediated by activation of 'kyotorphinergic' nerves followed by activation of the 'opioidergic' (possible 'enkephalinergic') nerves in the central nervous system.

Keywords: L-arginine; kyotorphin; enkephalin; antinociceptive effect; L-leucyl-L-arginine; D-arginine; carrageenin

Introduction

Kyotorphin (L-tyrosyl-L-arginine), an endogenous neuropeptide isolated from bovine brain, produces naloxone-reversible antinociception by enhancing Met-enkephalin release in the brain and spinal cord (Takagi *et al.*, 1979a,b), and is considered to be a neurotransmitter or neuromodulator (Takagi & Ueda, 1988), since it is localized in synaptosomes (Ueda *et al.*, 1982), and released by depolarizing stimuli in a Ca^{2+}-dependent manner (Ueda *et al.*, 1986). It is synthesized from L-tyrosine and L-arginine by a specific enzyme, kyotorphin synthetase, in the presence of ATP and $MgCl_2$ (Ueda *et al.*, 1987), although, like endogenous opioid peptides, it can also be formed from its precursor protein by processing (Yoshihara *et al.*, 1988). In the former pathway, L-arginine may be a rate limiting factor for enzymatic biosynthesis of kyotorphin, since the K_m value for L-arginine is higher than its physiological concentration in the brain. This theory predicts that L-arginine effectively acts as a precursor of kyotorphin *in vivo*, resulting in antinociception.

We have recently demonstrated that subcutaneous administration of L-arginine produced a naloxone-reversible antinociceptive action in rats with carrageenin-induced hyperalgesia (Kawabata *et al.*, 1992). The therapeutic significance of L-arginine is also supported by our clinical finding that intravenous infusion of L-arginine produces potent analgesia in a naloxone-reversible manner in patients with various types of chronic pain (Takagi *et al.*, 1990; Harima *et al.*, 1991).

Here we show that L-arginine-induced antinociception is antagonized by L-leucyl-L-arginine, a kyotorphin receptor antagonist (Ueda *et al.*, 1989), and by naltrindole, a selective δ-opioid antagonist (Portoghese *et al.*, 1988), suggesting that the antinociception is mediated by kyotorphin and enkephalin in the brain, and that D-arginine antagonizes the effect of L-arginine through distinct mechanisms from L-leucyl-L-arginine.

[1] Author for correspondence.

Methods

Animals

Male ddy mice weighing 20–30 g (Japan SLC. Inc.) were given food and water *ad libitum*.

Nociceptive assay

The paw pressure test described by Randall & Selitto (1957) was applied to mice. An analgesia meter was used (MK-300, Muromachi Kikai Co. Ltd, Japan) with a pencil-shaped wooden paw-presser with a dull tip; pressure was gradually applied to the hind paw at an increasing linear rate of 15 g s^{-1}. The weight (g) required to elicit nociceptive responses such as squeak and struggle was determined as a mechanical nociceptive threshold. A cut-off value of 250 g was used to prevent damage to the paw. The nociceptive threshold of each mouse was measured 6–8 times, and only mice with stable thresholds were used in experiments. The control threshold for each mouse was defined as the mean of the values of the last 4 stable thresholds, since the initial 2–4 values were in general, high and unstable. Results are expressed as a percentage of the control threshold. In one experiment, thermal nociception was assayed with a tail flick analgesia meter (MK-330, Muromachi Kikai Co. Ltd., Japan), in which the intensity of the thermal stimulus was adjusted to obtain basal latencies of 2.0–2.5 s.

Induction of hyperalgesia in mice

Hyperalgesia was induced by intraplantar (i.pl.) injection of 25 μl of 1% carrageenin into the right hindpaw. This dose of carrageenin has been reported to elicit paw oedema in the mouse, following a time pattern similar to that seen in the rat (Levy, 1969). The nociceptive threshold was measured at 30 min intervals, unless otherwise stated.

Experimental protocol

Antinociceptive action of systemically administered L-arginine L-Arg (100–1,000 mg kg^{-1}) was given s.c. to mice 30 min after carrageenin treatment. The interaction between L-Arg and three other agents was evaluated according to the following schedules. Naloxone (Nlx, 1 mg kg^{-1}), an opioid antagonist, and naltrindole (NTI, 1 mg kg^{-1}), a δ-selective opioid antagonist, were administered s.c. 30 and 20 min respectively after 1,000 mg kg^{-1} of L-Arg. D-Arg and L-Arg at 1,000 mg kg^{-1} were co-administered s.c. 30 min after carrageenin. Leu-Arg (300 ng per mouse), a KTP antagonist, was given i.c.v. 20 min after 1,000 mg kg^{-1} of L-Arg (50 min after carrageenin).

Antinociception induced by intracerebroventricular administration of L-arginine and kyotorphin L-Arg (30 ng–300 μg per mouse) and KTP (30 ng–3 μg per mouse) were administered i.c.v. to carrageenin-treated (30 min after carrageenin) and non-treated mice. In this case, the nociceptive threshold was assessed 5, 10, 20, 40 and 60 min after i.c.v. injection. D-Arg at doses of 3–30 μg per mouse was co-administered i.c.v. with L-Arg or KTP to carrageenin-treated and non-treated mice.

Control animals received a saline injection s.c., or i.c.v.

Statistical analysis

The results are expressed as means with s.e.mean. Statistical significance between groups was analyzed by Newman-Keuls' multiple comparison test and was set at $P<0.05$.

Chemicals

Naloxone hydrochloride (Nlx), λ-carrageenin as well as kyotorphin (KTP) and L-leucyl-L-arginine (Leu-Arg) as the acetate salt were purchased from Sigma Chem. Co. (U.S.A.). Both L-arginine (L-Arg) and D-arginine (D-Arg) as the hydrochloride salt were from Nacalai Tesque (Japan). Naltrindole hydrochloride (NTI, Sigma) was a gift from Dr K. Takahashi. λ-Carrageenin was dissolved in distilled water and all other chemicals in saline.

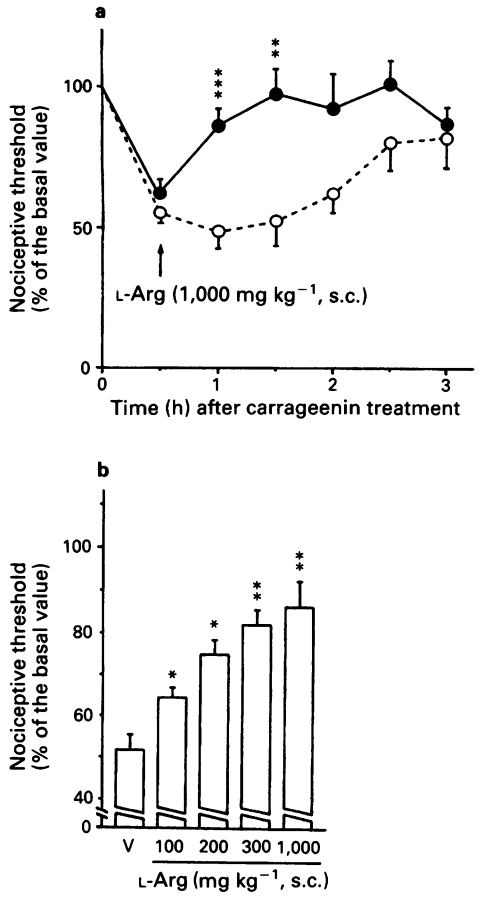

Figure 1 (a) Time-related effects of L-arginine (L-Arg, 1,000 mg kg^{-1}, s.c.) on the nociceptive threshold in carrageenin-induced hyperalgesia in mice. L-Arg was administered s.c. 30 min after carrageenin treatment. (○), Vehicle ($n=7$); (●), L-Arg 1,000 mg kg^{-1} ($n=7$). $^{**}P<0.01$, $^{***}P<0.001$ vs. vehicle. (b) Dose-related antinociceptive effects of L-arginine (100–1,000 mg kg^{-1}) in mice with carrageenin-induced hyperalgesia. Data indicate the nociceptive threshold 30 min after s.c. administration of L-Arg ($n=6$–7) or vehicle ($n=13$) (60 min after carrageenin). $^{*}P<0.05$, $^{**}P<0.01$, vs. vehicle (V).

Results

Carrageenin-induced hyperalgesia and L-arginine (s.c.)-induced antinociception

After an i.pl. injection of carrageenin, the nociceptive threshold of the carrageenin-treated hindpaw decreased almost to 50% of the basal value at 30 min, which was maintained for at least 1–1.5 h, followed by a slow recoevery (Figure 1). That of the contralateral (non-treated) hindpaw showed only a transient and slight tendency toward a decrease (threshold: 79.4 ± 8.7 and 92.4 ± 14.5% at 30 and 60 min after carrageenin, respectively, being not significantly different from the basal value). L-Arg at doses of 100–1,000 mg kg^{-1}, when administered s.c. 30 min after carrageenin, significantly elevated the decreased threshold of the hyperalgesic hindpaw in a dose-dependent manner (Figure 1), without affecting the threshold of the contralateral hindpaw and also without causing any behavioural change. The nociceptive thresholds in intact mice were resistant to the highest dose of L-Arg (1,000 mg kg^{-1}, s.c.). The L-Arg (1,000 mg kg^{-1})-induced antinociception in the hyperalgesic mouse was completely blocked by Nlx (1 mg kg^{-1}, s.c.), when it was administered 30 min after L-Arg (Figure 2).

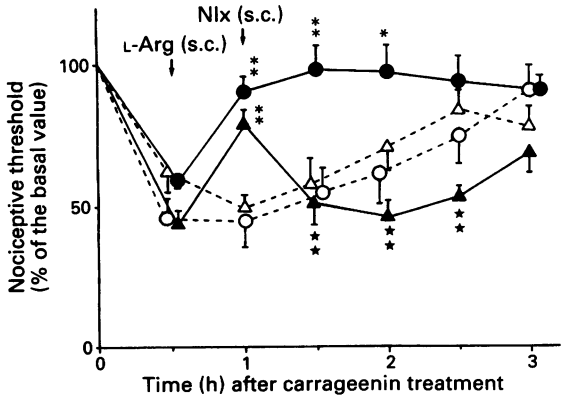

Figure 2 Antagonism by naloxone (Nlx) of L-arginine (L-Arg)-induced antinociception in mice with carrageenin-induced hyperalgesia. L-Arg (1,000 mg kg^{-1}) and Nlx (1 mg kg^{-1}) were administered s.c. 30 and 60 min respectively, after carrageenin treatment. (○) Vehicle + vehicle; (△) vehicle + Nlx; (●) L-Arg + vehicle; (▲) L-Arg + Nlx. $^{*}P<0.05$, $^{**}P<0.01$ vs. vehicle + vehicle; ★★ $P<0.01$ vs. L-Arg + vehicle. $n=6$.

Antagonism by L-leucyl-L-arginine, naltrindole and D-arginine of L-arginine-induced antinociception

In a preliminary experiment, when kyotorphin (KTP, 3 μg per mouse, i.c.v.) and Leu-Arg (300 ng per mouse, i.c.v.), a KTP antagonist, were co-administered, KTP-induced antinociception was completely blocked by Leu-Arg; the threshold at 5 min after administration was 97.5 ± 5.6, 92.9 ± 3.4, 167.9 ± 6.1 and 78.7 ± 9.2%, in groups treated with vehicle, Leu-Arg, KTP and Leu-Arg plus KTP, respectively ($n = 4$), and both the effect of KTP and its antagonism by Leu-Arg were significant ($P<0.01$). The same dose of Leu-Arg completely blocked antinociception induced by L-Arg (1,000 mg kg^{-1}, s.c.) in carrageenin-treated mice, although, when administered alone, it produced no effect on the hyperalgesia (Figure 3). Similarly, naltrindole at 1 mg kg^{-1}, s.c., which completely antagonized KTP (i.c.v.)-induced antinociception in a preliminary experiment, inhibited L-Arg (1,000 mg kg^{-1}, s.c.)-induced antinociception, although alone, it did not show any effect on the hyperalgesia (Figure 3). D-Arg (1,000 mg kg^{-1}, s.c.) blocked the L-Arg (1,000 mg kg^{-1}, s.c.)-induced antinociception, when both agents were co-administered, although D-Arg alone had no antinociceptive activity (Figure 3).

Antinociceptive effects induced by centrally administered L-arginine and kyotorphin

L-Arg, when administered i.c.v. at doses of 3 and 30 μg per mouse, produced rapid and potent antinociception in hyperalgesic mice, which peaked at 5 min (Figure 4a, left). Injections of L-Arg at doses of 30 and 300 μg per mosue i.c.v. induced significant antinociceptive effects even in intact mice, although the effective dose-range was about 10 fold higher than that in hyperalgesic mice. The maximal effect of L-Arg was obtained 20 min after administration in intact mice (Figure 4a, right). In addition, when the effect was evaluated by the tail-flick test, i.c.v. injections of L-Arg at doses of 30 and 300 μg per mouse also exhibited dose-dependent antinociception in intact mice in a similar manner; the threshold 20 min after injection was 2.10 ± 0.06 s, 3.35 ± 0.27 s ($P<0.05$), 4.10 ± 0.65 s ($P<0.01$), in groups treated with vehicle and L-Arg at 30 and 300 μg per mouse, respectively ($n = 4$). L-Arg given s.c. at 1,000 mg kg^{-1} failed to produce such effects (data not shown).

KTP (i.c.v.) at a dose-range of 300 ng–3 μg per mouse produced potent antinociception both in intact and hyperalgesic mice, which peaked at 5 min in both groups of mice. Such effects disappeared 10 min after administration in the former, but persisted even at 70–90 min in the latter (Figure 4b).

Interaction between D-arginine and L-arginine or kyotorphin in the brain

In hyperalgesic mice, L-Arg (3 μg per mouse, i.c.v.)-induced antinociception was completely blocked by co-administration of the same dose of D-Arg which, given alone, did not affect the hyperalgesia (Figure 5a). A similar result was obtained by the co-administration of 30 μg per mouse (i.c.v.) of both drugs to intact mice (Figure 5b).

By contrast, in intact mice, D-Arg even at a dose of 30 μg per mouse (i.c.v.) failed to inhibit the antinociceptive action of KTP (3 μg per mouse, i.c.v.) (data not shown).

Discussion

These results show that carrageenin-induced hyperalgesia in mice is a useful model of persistent pain, which is similar to that in rats: L-Arg (s.c.) elicits naloxone-reversible antinociception in mice with carrageenin-induced hyperalgesia. An i.c.v. injection of a small dose (3 μg per mouse) of L-Arg is also effective in hyperalgesic mice, but not in intact mice. However, in the intact mice, i.c.v. administration of a large dose (30 μg per mouse) of L-Arg elicited antinociception. These results suggest that hyperalgesia elevates the antinociceptive effect of L-Arg, possibly due to the induction of kyotorphin synthetase.

In general, experimental hyperalgesia has been observed during carrageenin-induced inflammation or heat injury in the rat hindpaw (Coderre & Melzack, 1985; Kayer & Guilbaud, 1987), and it involves central and peripheral mechanisms (Coderre & Melzack, 1987; Treede *et al.*, 1992). The peripheral mechanism of carrageenin-induced hyperalgesia is in part due to the increased local formation of bradykinin, since levels of immunoreactive bradykinin increased two fold during carrageenin treatment (Hargreaves *et al.*, 1988). Prostanoids also participate in the peripheral mechanism of pain in that potent suppression by cyclo-

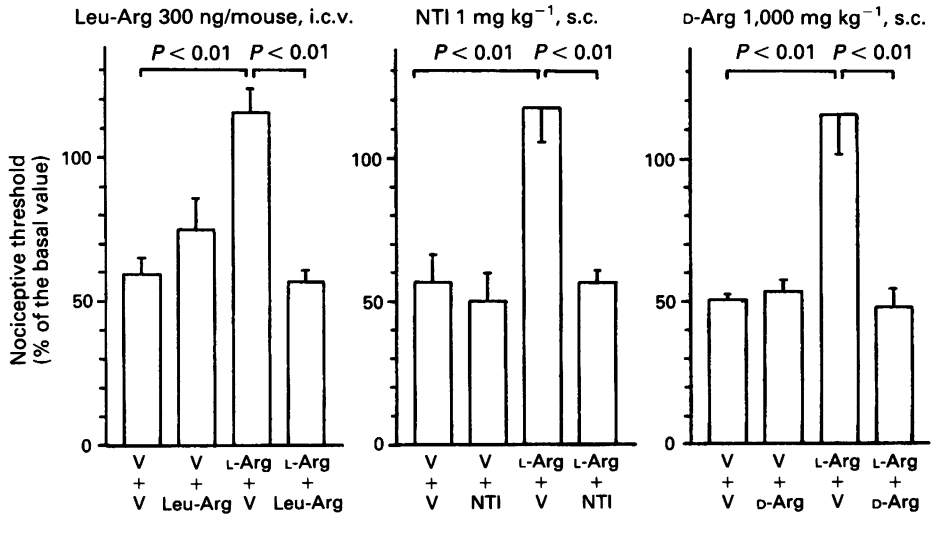

Figure 3 Antagonism of L-arginine (L-Arg)-induced antinociception by L-leucyl-L-arginine (Leu-Arg), naltrindole (NTI) and D-arginine in mice with carrageenin-induced hyperalgesia. L-Arg (1,000 mg kg^{-1}) was administered s.c. 30 min after carrageenin treatment. Leu-Arg (i.c.v.) and NTI (s.c.) were given 20 and 10 min after L-Arg injection, respectively, and D-Arg was co-administered with L-Arg. Data indicate the threshold 60 min after the carrageenin-treatment. V: vehicle. $n = 4$.

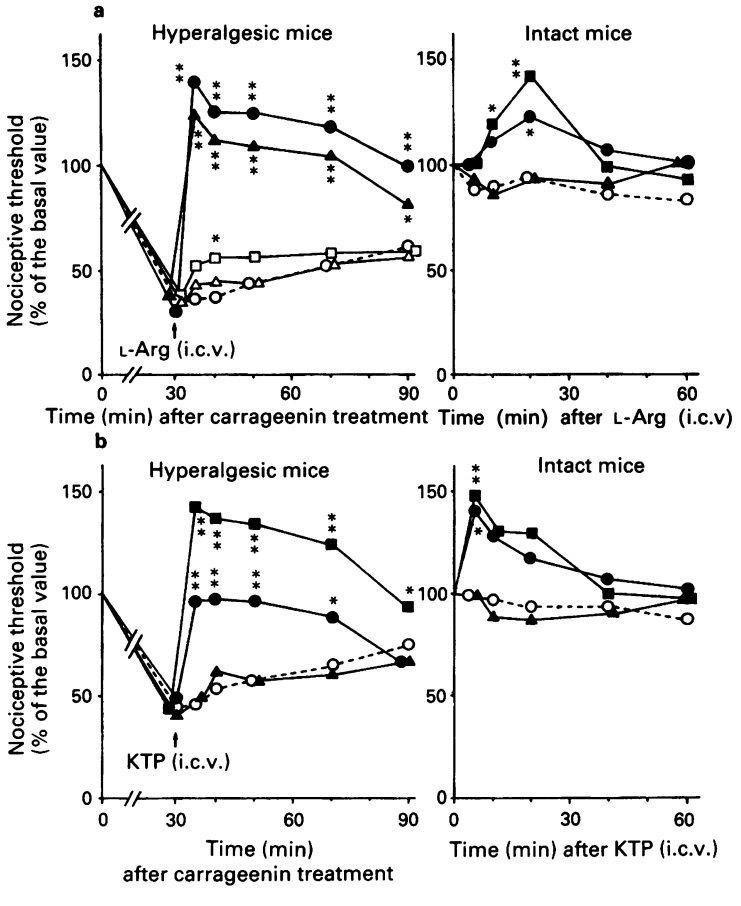

Figure 4 Antinociceptive effects induced by i.c.v. administration of L-arginine (L-Arg) and kyotorphin (KTP) in mice with and without carrageenin-induced hyperalgesia. L-Arg and KTP were administered i.c.v. to hyperalgesic mice (30 min after carrageenin) or to intact mice. (a) (○) Vehicle; (△) L-Arg 30 ng per mouse; (□) L-Arg 300 ng per mouse; (▲), L-Arg 3 μg per mouse; (●), L-Arg 30 μg per mouse; (■), L-Arg 300 μg per mouse. (b) (○) Vehicle; (▲), KTP 30 ng per mouse; (●), KTP 300 ng per mouse; (■), KTP 3 μg per mouse. Data are expressed as means without s.e.mean. $*P<0.05$, $**P<0.01$ vs. vehicle. $n=4$.

oxygenase inhibitors of the hyperalgesia has been reported (Treede *et al.*, 1992). The central mechanism is complex, but a recent study has suggested that C-fibre neuropeptides (e.g. substance P, vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), somatostatin, calcitonin gene-related peptide (CGRP) and galanin) and excitatory amino acids (L-glutamate) are involved in inducing hyperalgesia in the spinal dorsal horn (Coderre & Melzach, 1991). L-Arg appears to inhibit the central neuronal mechanism involving hyperalgesia.

That L-Arg-induced antinociception was blocked by systemic administration of naloxone (Nlx) suggests the involvement of opioid peptides. That L-Arg-induced antinociception was blocked by i.c.v. administration of Leu-Arg, a KTP receptor antagonist, indicates that the antinociception is mediated by KTP formed from L-tyrosine and L-Arg by a KTP synthetase (Ueda *et al.*, 1987). The potency of Leu-Arg as a KTP antagonist is shown by the fact that antinociception induced by an i.c.v. injection of KTP was blocked by an i.c.v. injection of Leu-Arg. As described in the Introduction, L-Arg is a precursor of KTP and administration of L-Arg should increase the KTP level in the brain, which would result in an enhancement of Met-enkephalin-release and antinociception. This possibility is further supported by the present finding that NTI, a selective δ-opioid receptor antagonist, antagonizes the antinociceptive effects of L-Arg as well as KTP. These results suggest the presence of a functional link between the 'kyotorphinergic' system and the 'enkephalinergic' system. The relatively long duration of the antinociceptive action of L-Arg and KTP in hyperalgesia may be due to enhanced recycling of L-Arg; KTP, formed from L-tyrosine and L-Arg in the nerve endings, is released to the synaptic cleft and metabolized by bestatin-sensitive aminopeptidases into L-Arg, which is then incorporated into the nerve endings and re-utilized as a precursor (Ueda *et al.*, 1985; 1986; 1987). Based upon the above evidence, we show a hypothetical scheme of the mechanisms of L-Arg-induced antinociception in Figure 6.

In addition, the role of nitric oxide (NO) formed from L-Arg by a NO synthase in the brain should be considered. According to Bredt & Snyder (1990), purified rat brain NO synthase has high affinity for L-Arg with a K_m of 1.5 μM and it is saturated with L-Arg under normal conditions, since the physiological concentration of L-Arg in the brain is approximately 100 μM (Levy *et al.*, 1967; Norberg & Siesjo, 1975). By contrast, the K_m value of KTP synthetase is 926 μM for L-Arg and 100 μM for L-tyrosine (Ueda *et al.*, 1987), suggesting that KTP synthetase is less active under normal conditions and is activated when sufficient L-Arg accumulates in the brain after it is systemically or i.c.v. administered. Moreover, systemic administration of L-N^G-nitroarginine, a selective NO synthase inhibitor, elicits antinociception in mice by a supraspinal effect which is antagonized by systemic administration of L-Arg (Moore *et al.*, 1991). Spinal inhibition of NO synthase also results in antinociception (Haley *et al.*, 1992). This evidence suggests that the L-Arg-NO pathway in the CNS is not involved in the production of L-Arg-induced antinociception but rather promotes pain transmission at spinal and supraspinal levels.

The peripheral role of the L-Arg-NO pathway in nocicep-

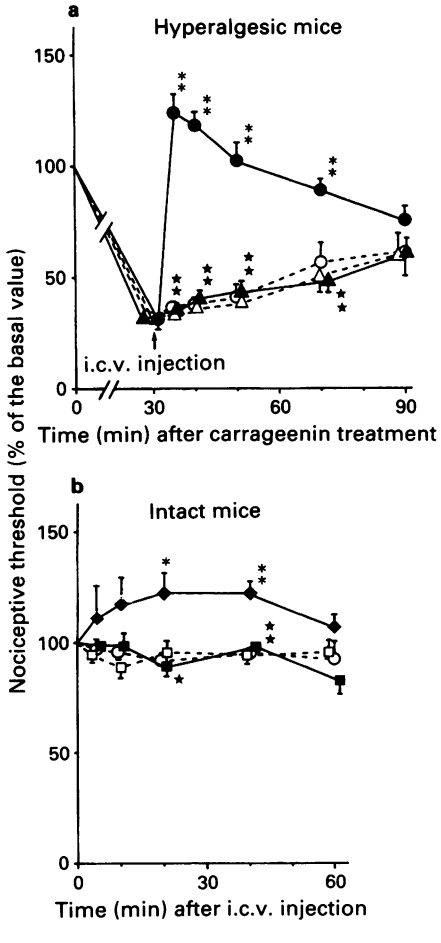

Figure 5 Antagonism of L-arginine (L-Arg, i.c.v.)-induced antinociception in mice with and without carrageenin-induced hyperalgesia by co-administered D-arginine (D-Arg). L-Arg and D-Arg were co-administered i.c.v. to hyperalgesic mice (30 min after carrageenin) or to intact mice. (○), Vehicle; (△), D-Arg 3 μg per mouse; (□), D-Arg 30 μg per mouse; (●), L-Arg 3 μg per mouse; (◆), L-Arg 30 μg per mouse; (▲), L-Arg 3 μg per mouse + D-Arg 3 μg per mouse; (■), L-Arg 30 μg per mouse + D-Arg 30 μg per mouse. $*P<0.05$, $**P<0.01$ vs. vehicle; $\bigstar P<0.05$, $\bigstar\bigstar P<0.01$ vs. L-Arg alone at 3 or 30 μg per mouse. $n=4$.

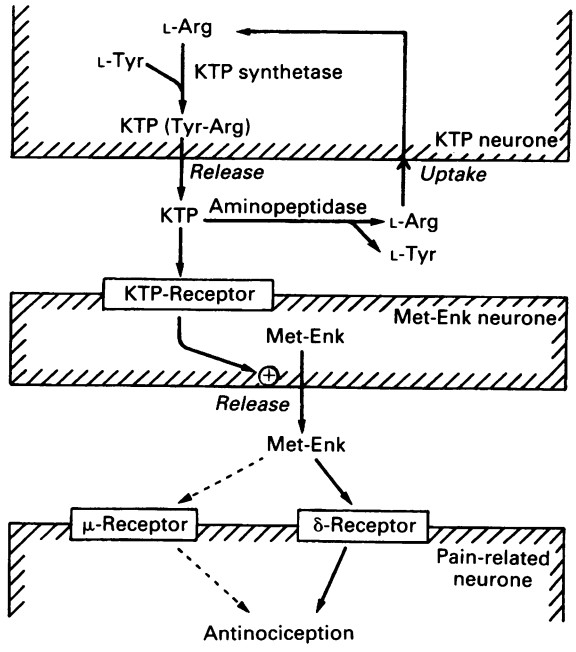

Figure 6 Proposed mechanism of L-arginine-induced antinociception in the central nervous system. Met-Enk, methionine-enkephalin; L-Tyr, L-tyrosine.

tive events is more complex. L-Arg, when injected directly into the carrageenin-treated paw of rats, produces antinociception via the peripheral NO-guanosine 3':5'-cyclic monophosphate (cyclic GMP) pathway (Duarte *et al.*, 1990). However, L-Arg, when injected systemically into rats with a carrageenin-treated hindpaw, produces antinociception which is not mediated by the peripheral NO-cyclic GMP pathway, considering that s.c. L-Arg-induced antinociception was not inhibited by i.pl. methylene blue, a guanylate cyclase inhibitor, and that antinociception elicited by i.pl. L-Arg was reversed by i.pl. methylene blue but was resistant to s.c. Nlx (Kawabata *et al.*, 1992). In contrast, Haley *et al.* (1992) have reported that the number of action potentials of a single dorsal horn neurone, in response to formalin injected into the peripheral receptive field, is reduced by preadministration of L-N^G-nitroarginine into the same site. This suggests that the peripheral L-Arg-NO pathway promotes nociception. Therefore, the peripheral L-Arg-NO system does not appear to be directly involved in the antinociceptive effect of systemic L-Arg in the present study.

A vasodilator effect of the L-Arg-NO system in peripheral blood vessels has been established (Gardiner *et al.*, 1990), which may indirectly contribute to L-Arg (s.c.)-induced antinociception because hypotension in the cat inhibits neural activity in the dorsal horn of the spinal cord (Kitahata, 1975). However, our previous results (Kawabata *et al.*, 1992) do not support this possibility, since L-Arg at 1,000 mg kg^{-1} (s.c.) elicited only a slight and transient hypotension in anaesthetized rats, in contrast to the marked and persistent increase in nociceptive threshold seen in rats with hyperalgesia. With regard to the role of the brain L-Arg-NO system in regulating blood pressure, Mollace *et al.* (1992) have demonstrated that L-Arg at 300 μg per rat (i.c.v.) does not modify blood pressure, and L-N^G-nitroarginine (i.c.v.) causes hypotension in endotoxin-treated but not in control rats. Similarly, Moore *et al.* (1991) have reported that L-N^G-nitroarginine given i.c.v. even at 100 μg per mouse does not significantly alter blood pressure in anaesthetized mice. Therefore, it seems unlikely that centrally mediated circulatory changes exert a role in L-Arg-induced antinociception in mice, although the present study did not actually elucidate whether i.c.v. L-Arg modifies blood pressure or not.

Furthermore, the L-Arg-NO system may also have a role in the modulation of oedema formation by regulating microvascular permeability (Hughes *et al.*, 1990; Ialenti *et al.*, 1992). L-Arg, when injected into the rat paw in combination with carrageenin, enhances increased vascular permeability and oedema volume (Ialenti *et al.*, 1992). However, we have demonstrated that L-Arg administered s.c. 2 h after carrageenin failed to affect the degree of oedema formation but induced definite antinociception (Kawabata *et al.*, 1992). Therefore, systemic L-Arg, after oedema is formed, may be incapable of modifying the inflammation. Also, increases by L-Arg in vascular permeability and oedema volume, if any, appear to promote nociception. In addition, it should be emphasized that the antinociceptive effect of systemic L-Arg is resistant to i.pl. methylene blue as mentioned above (Kawabata *et al.*, 1992). Thus, the L-Arg-NO system in the CNS and peripheral organs appears to play only a minor role, if any, in antinociception induced by systemic and i.c.v. L-Arg.

The mechanism by which D-Arg antagonizes L-Arg-induced antinociception is distinct from those of Leu-Arg and NTI, because D-Arg failed to antagonize KTP-induced antinociception in intact mice. The fact that D-Arg, when

administered i.c.v. as well as s.c. in combination with L-Arg, completely blocked the antinociceptive effect of L-Arg, suggests the existence of an antagonistic site within the CNS, in addition to competitive inhibition by D-Arg of carrier-mediated blood-brain barrier transport of L-Arg. Concerning the mechanism of action of D-Arg in the brain, at least, three mechanisms should be considered: (1) block of L-Arg uptake into central neurones, (2) inhibition of KTP synthetase, and (3) suppression of KTP release from KTP-containing neurones. The first mechanism is probable, since our preliminary experiments do not support the second and third. The first mechanism is being examined in our laboratory.

We thank Morishita Seiyaku Co. Ltd. for financial support. This paper is the second report on 'Pain modulation by neuroactive amino acids'.

References

BREDT, D.S. & SNYDER, S.H. (1990). Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 682–685.

CODERRE, T.J. & MELZACK, R. (1985). Increased pain sensitivity following heat injury involves a central mechanism. *Behav. Brain Res.*, **15**, 259–262.

CODERRE, T.J. & MELZACK, R. (1987). Cutaneous hyperalgesia: Contribution of the peripheral and central nervous systems to the increase in pain sensitivity after injury. *Brain. Res.*, **404**, 95–106.

CODERRE, T.J. & MELZACK, R. (1991). Central neural mediators of secondary hyperalgesia following heat injury in rats: neuropeptides and excitatory amino acids. *Neurosci. Lett.*, **131**, 71–74.

DUARTE, I.D.G., LORENZETTI, B.B. & FERREIRA, S.H. (1990). Peripheral analgesia and activation of the nitric oxide-cyclic GMP pathway. *Eur. J. Pharmacol.*, **186**, 289–293.

GARDINER, M.S., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1990). Regional haemodynamic changes during oral ingestion of N^G-monomethyl-L-arginine or N^G-nitro-L-arginine methyl ester in conscious Brattleboro rats. *Br. J. Pharmacol.*, **101**, 10–12.

HALEY, J.E., DICKENSON, A.H. & SCHACHTER, M. (1992). Electrophysiological evidence for a role in nitric oxide in prolonged chemical nociception in the rat. *Neuropharmacol.*, **31**, 251–258.

HARGREAVES, K.M., TROULLOS, E.S., DIONNE, R.A., SCHMIDT, E.A., SCHAFER, S.C. & JORIS, J.L. (1988). Bradykinin is increased during acute and chronic inflammation: therapeutic implications. *Clin. Pharmacol. Ther.*, **44**, 613–621.

HARIMA, A., SHIMIZU, H. & TAKAGI, H. (1991). Analgesic effect of L-arginine in patients with persistent pain. *Eur. Neuropsychopharmacol.*, **1**, 529–533.

HUGHES, S.R., WILLIAMS, T.J. & BRAIN, S.D. (1990). Evidence that endogenous nitric oxide modulates oedema formation induced by substance P. *Eur. J. Pharmacol.*, **191**, 481–484.

IALENTI, A., IANARO, A., MONCADA, S. & ROSA, M.D. (1992). Modulation of acute inflammation by endogenous nitric oxide. *Eur. J. Pharmacol.*, **211**, 177–182.

KAWABATA, A., FUKUZUMI, Y., FUKUSHIMA, Y. & TAKAGI, H. (1992). Antinociceptive effect of L-arginine on carrageenin-induced hyperalgesia in rats: possible involvement of central opioidergic systems. *Eur. J. Pharmacol.*, **218**, 153–158.

KAYER, V. & GUILBAUD, G. (1987). Local and remote modification of nociceptive sensitivity during carrageenin-induced inflammation in the rat. *Pain*, **23**, 99–107.

KITAHATA, L.M. (1975). Modes and sites of 'analgesic' action of anesthetics on the spinal cord. *Proc. Int. Un. Physiol.*, **13**, 149–170.

LEVY, L. (1969). Carrageenan paw edema in the mouse. *Life Sci.*, **8**, 601–606.

LEVY, G., KANDERA, J. & LAJITHA, A. (1967). Control of cerebral metabolite levels. I. Amino acid uptake and levels in various species. *Arch. Biochem. Biophys.*, **119**, 303–311.

MOLLACE, V., DE FRANCESCO, E.A. & NISTICO, G. (1992). Evidence that pharmacological manipulation of central L-arginine-NO pathway influences blood pressure and heart rate in rats. *Neurosci. Lett.*, **137**, 87–90.

MOORE, P.K., OLUYOMI, A.O., BABBEDGE, R.C., WALLACE, P. & HART, S.L. (1991). L-N^G-nitroarginine methyl ester exhibits antinociceptive activity in the mouse. *Br. J. Pharmacol.*, **102**, 198–202.

NORBERG, K. & SIESJO, B.K. (1975). Cerebral metabolism in hypoxic hypoxia, II. Citric acid cycle intermediates and associated amino acids. *Brain Res.*, **86**, 45–54.

PORTOGHESE, P.S., SULTANA, M. & TAKEMORI, A.E. (1988). Naltrindole, a highly selective and potent non-peptide δ opioid receptor antagonist. *Eur. J. Pharmacol.*, **146**, 185–186.

RANDALL, L.O. & SELITTO, J.J. (1957). A method for measurement of analgesic activity on inflammed tissue. *Arch. Int. Pharmacodyn.*, **111**, 409–419.

TAKAGI, H., HARIMA, A. & SHIMIZU, H. (1990). A novel clinical treatment of persistent pain with L-arginine. *Eur. J. Pharmacol.*, **183**, 1443.

TAKAGI, H., SHIOMI, H., UEDA, H. & AMANO, H. (1979a). Morphine-like analgesia by a new dipeptide, L-tyrosyl-L-arginine (kyotorphin) and its analogue. *Eur. J. Pharmacol.*, **55**, 109–111.

TAKAGI, H., SHIOMI, H., UEDA, H. & AMANO, H. (1979b). A novel analgesic dipeptide from bovine brain is a possible Met-enkephalin releaser. *Nature*, **282**, 410–412.

TAKAGI, H. & UEDA, H. (1988). Kyotorphin as an endogenous pain modulator. In *The Biowarning System in the Brain*. ed. Takagi, H., Oomura, Y., Ito, M. & Otsuka, M. pp. 139–153, Tokyo: University of Tokyo Press.

TREEDE, R.-D., MEYER, R.A., RAJA, S. & CAMBELL, J.N. (1992). Peripheral and central mechanisms of cutaneous hyperalgesia. *Progr. Neurobiol.*, **38**, 397–421.

UEDA, H., GE MING, HAZATO, T., KATAYAMA, T. & TAKAGI, H. (1985). Degradation of kyotorphin by a purified membrane-bound aminopeptidease from monkey brain: potentiation of kyotorphin-induced analgesia by a highly effective inhibitor bestatin. *Life Sci.*, **31**, 1865–1871.

UEDA, H., MATSUMOTO, S., YOSHIHARA, Y., FUKUSHIMA, N. & TAKAGI, H. (1986). Uptake and release of kyotorphin in rat brain synaptosomes. *Life Sci.*, **38**, 2405–2411.

UEDA, H., TATSUMI, K., SHIOMI, H. & TAKAGI, H. (1982). Analgesic dipeptide, kyotorphin (Tyr-Arg) is highly concentrated in the synaptosomal fraction of the rat brain. *Brain Res.*, **231**, 222–224.

UEDA, H., YOSHIHARA, Y., FUKUSHIMA, N., SHIOMI, H., NAKAMURA, A. & TAKAGI, H. (1987). Kyotorphin (tyrosyl-arginine) synthetase in rat brain synaptosomes. *J. Biol. Chem.*, **262**, 8165–8173.

UEDA, H., YOSHIHARA, Y., MISAWA, H., FUKUSHIMA, N., KATADA, T., UI, M., TAKAGI, H. & SATOH, H. (1989). The kyotorphin (tyrosyl-arginine) receptor and a selective reconstitution with purified Gi, measured with GTPase and phospholipase C assays. *J. Biol. Chem.*, **264**, 3732–3741.

YOSHIHARA, Y., UEDA, H., IMAJOH, S., TAKAGI, H. & SATOH, M. (1988). Calcium-activated neural proteases (CANP), a putative processing enzyme of the neuropeptide, kyotorphin, in the brain. *Biochem. Biophys. Res. Commun.*, **155**, 546–553.

(Received June 26, 1992
Revised August 5, 1992
Accepted August 10, 1992)