ELSEVIER

European Journal of Pharmacology 334 (1997) 133–140

ejp

Intracerebroventricular injection of isoproterenol produces its analgesic effect through interleukin-1β production

Kazuki Yabuuchi [a], Emiko Maruta [b], Junki Yamamoto [b], Atsushi Nishiyori [b], Shinya Takami [b], Masabumi Minami [b], Masamichi Satoh [b,*]

[a] *Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan*
[b] *Department of Molecular Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan*

Received 17 July 1997; accepted 22 July 1997

Abstract

The effects of isoproterenol, a β-adrenoceptor agonist, on the production of interleukin-1β in the brain and on mechanical nociception were examined in rats. Intracerebroventricular (i.c.v.) injection of isoproterenol at the dose of 3 μg/rat markedly induced interleukin-1β mRNA in the molecular layer of the hippocampus, medial preoptic area, paraventricular thalamic nucleus, paraventricular hypothalamic nucleus, ventromedial hypothalamic nucleus, dorsomedial hypothalamic nucleus and central gray 1 h after injection. In these regions, interleukin-1β mRNA was expressed mainly in the glial cells. The thresholds to the mechanical stimulation to the hind paw were elevated by i.c.v. administration of isoproterenol (1 to 10 μg/rat). When isoproterenol was given at the dose of 3 μg/rat, the analgesic effect showed two peaks. The first peak was observed at 60 min after injection and the second was observed at 180 min. The second phase of analgesia was antagonized by coadministration of interleukin-1 receptor antagonist. These results suggest that isoproterenol produces an analgesic effect, at least in part, through the induction of interleukin-1β expression in the brain. © 1997 Elsevier Science B.V.

Keywords: Isoproterenol; Interleukin-1β; β-Adrenoceptor; Nociception, mechanical; (Rat)

1. Introduction

In the last decade, the regulation of nociceptive transmission in the spinal cord has been progressively elucidated. The catecholaminergic system, especially the α_2-adrenergic system, was shown to be involved in the descending regulation of nociceptive information (Kuraishi et al., 1985). Furthermore, the intraspinal opioidergic system has been vigorously studied by many groups, including our laboratory (Kuraishi et al., 1983; Ueda et al., 1995). On the other hand, supraspinal monoaminergic systems were reported to play some roles in the regulation of nociceptive transmission (Radouco-Thomas et al., 1967). The involvement of the β-adrenergic system was suggested by Gardella et al. (1970), who showed that intracerebroventricular (i.c.v.) injection of isoproterenol produced analgesia in the rabbit tooth pulp assay. However, the molecular mechanisms for supraspinal regulation of nociceptive transmission remain unclear.

Interleukin-1 is a cytokine which has various effects on the central nervous system such as increasing body temperature (Kluger, 1991), inducing slow wave sleep (Krueger et al., 1984) and activating the hypothalamus–pituitary–adrenal axis (Berkenbosch et al., 1987). We have demonstrated that i.c.v. administration of isoproterenol induces the expression of interleukin-1β mRNA in the rat hypothalamus (Yamaguchi et al., 1991). Furthermore, interleukin-1β, when administered i.c.v., produces analgesic effects in rats (Yabuuchi et al., 1996). These observations suggest that the β-adrenergic system in the brain regulates nociceptive transmission through interleukin-1β production.

In this study, we examined the effects of i.c.v. injection of isoproterenol on the expression of interleukin-1β mRNA in the rat brain using northern blot analysis and in situ hybridization histochemistry. Secondly, we examined the effects of i.c.v. administration of isoproterenol on mechanical nociception using the rat paw-withdrawal test and the

* Corresponding author. Tel.: +81-75-7534526; fax: +81-75-7534586.

0014-2999/97/$17.00 © 1997 Elsevier Science B.V. All rights reserved.

involvement of interleukin-1β in the effect of isoproterenol using interleukin-1 receptor antagonist, an endogenous antagonist protein for interleukin-1 receptors.

2. Materials and methods

2.1. Animals

All experiments using male Sprague–Dawley rats weighing 220–280 g (6–8 weeks old) followed the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983). Animals were kept at a constant ambient temperature (24 ± 1°C) under a 12 h light and dark cycle with free access to food and water.

2.2. Implantation of guide cannula

Under pentobarbital (50 mg/kg i.p.) anesthesia, a stainless steel guide cannula (o.d. 0.7 mm) was stereotaxically (P 0.8, L 1.5, H 2.0) implanted on the right side according to the atlas of Paxinos and Watson (1986). After surgery, the animals were returned to the cages and housed individually. They were allowed to recover for 5 to 7 days until the following experiments.

2.3. Drug administration

Isoproterenol (1, 3 and 10 μg/5 μl) was usually dissolved in saline. In the case of the coadministration with interleukin-1 receptor antagonist (100 ng/5 μl), it was dissolved in phosphate buffered saline (pH 7.6) containing 0.02% bovine serum albumin. Morphine hydrochloride (10 μg/5 μl) was dissolved in saline. Drugs were administered via the injection cannula which reached the right lateral ventricle (P 0.8, L 1.5, H 4.0) when attached to the guide cannula. The drugs were administered i.c.v. in a volume of 5 μl at a constant rate of 5 μl/30 s.

2.4. Preparation of RNA probe

Rat interleukin-1β cDNA including the full length of open reading frame (about 1.4 kbp) was a gift from Dr. S. Nakai (Otsuka Pharmaceutical, Tokushima). The cDNA was subcloned into pBluescript II vector (Stratagene, La Jolla). ^{32}P-Labeled antisense RNA probe was synthesized in the presence of [α^{32}P]UTP (15 TBq/mmol, Amersham) using Riboprobe Systems (Promega, Madison). Northern blot analysis using this RNA probe gave a single band with appropriate length for interleukin-1β mRNA, indicating that the probe used in the present study was specific for interleukin-1β mRNA. ^{35}S-Labeled antisense and sense RNA probes were synthesized in the presence of [α^{35}S]UTP (30 TBq/mmol, Amersham) with the same in vitro transcription system as used for the ^{32}P-labeled probe. Specific activities of the ^{35}S-labeled probes were $1.0–1.2 \times 10^9$ cpm/μg, and the probes were alkaline hydrolyzed to about 250 bases. In situ hybridization histochemistry with sense RNA probe did not give any significant signals on all sections examined (data not shown).

2.5. Northern blot analysis

The dose-dependency of the induction of interleukin-1β mRNA by isoproterenol was examined by northern blotting. Sixty minutes after injection of isoproterenol at doses of 0, 1, 3 or 10 μg/rat, animals were killed by decapitation and the brain was rapidly removed. In addition, the time course of interleukin-1β mRNA induction by 3 μg/rat of isoproterenol was studied at 0, 30, 60, 90 and 120 min after injection. Both right and left sides of the cerebral cortex, hippocampus, thalamus and hypothalamus were excised and total RNA was extracted using Isogen (Nippon Gene, Tokyo) from each brain region pooled from 3 rats. Northern blotting was performed as previously described (Minami et al., 1990). Total RNA samples (20 μg each) were fractionated by electrophoresis on 1% agarose gels containing formaldehyde and transferred onto nylon membranes (Biodyne A, Pall, New York). Blots were prehybridized and then hybridized to ^{32}P-labeled antisense RNA probe for rat interleukin-1β mRNA at 65°C. The expression level of β-actin mRNA was also analyzed by rehybridization to ^{32}P-labeled DNA probe for β-actin mRNA at 42°C. Autoradiograms were quantified using bioimaging analyzer BAS2000 (Fuji, Tokyo). The expression levels of interleukin-1β mRNA were normalized by those of β-actin mRNA.

2.6. In situ hybridization histochemistry

Isoproterenol (3 μg/rat) or saline was injected i.c.v. and the brain was removed 60 min later. Fresh-frozen sections (16 μm thick) were cut on a cryostat and in situ hybridization was conducted as described in our previous report (Yabuuchi et al., 1993). Briefly, sections were fixed with 4% formaldehyde, treated with proteinase K (1 μg/ml) and acetylated in 0.25% acetic anhydride. After dehydration, sections were hybridized with ^{35}S-labeled antisense RNA probe for rat interleukin-1β mRNA. They were washed, dehydrated and dipped in autoradiographic emulsion NTB-3 (Kodak, New York). After 3 weeks of exposure, they were developed and counterstained with cresyl violet.

2.7. Measurement of nociceptive threshold

The nociceptive threshold to mechanical stimulation was measured by the Randall–Selitto method (Randall and Selitto, 1957) with some modifications as previously described (Yabuuchi et al., 1996). Namely, the nociceptive threshold of the hind paw to mechanical stimulation was measured using an analgesimeter (Ugo Basile, Milan) with

a cone-shape piston. The piston was put on the ventral surface of the hind paw and the pressure was loaded at a rate of 32 g/s. The pressure which elicited paw withdrawal behavior was determined as a nociceptive threshold. The rats were habituated to the procedure for measuring the threshold three times per day. After two days of habituation, the threshold of each animal was measured following two additional habituation procedures, and the threshold value was taken as a control. Within 10 min after measuring the control value of the threshold, the drugs were administered as described above and the threshold was measured at 15, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min after administration. In the case of the examination of analgesic effect of morphine for comparative purpose, the threshold was measured at 30, 60, 90 and 120 min after administration. Measurement of nociceptive thresholds was performed between 11.30 and 17.00.

2.8. Materials

Human interleukin-1 receptor antagonist was a gift from Otsuka Pharmaceutical (Tokushima). Proteinase K was purchased from Merck (Darmstadt), salmon sperm DNA and ribonuclease A were from Sigma Chemical (St. Louis) and yeast tRNA was from Boehringer Mannheim (Mannheim). β-Actin cDNA was from Wako Pure Chemical (Osaka). Morphine hydrochloride was from Takeda Chemical (Osaka). DL-Isoproterenol and other chemicals were purchased from Nacalai Tesque (Kyoto).

2.9. Statistical analysis

The threshold to mechanical stimulation at each time point is presented as the mean of % of the control value ± S.E.M. Statistical analyses were performed using the Mann-Whitney's *U*-test. $P < 0.05$ was considered significant.

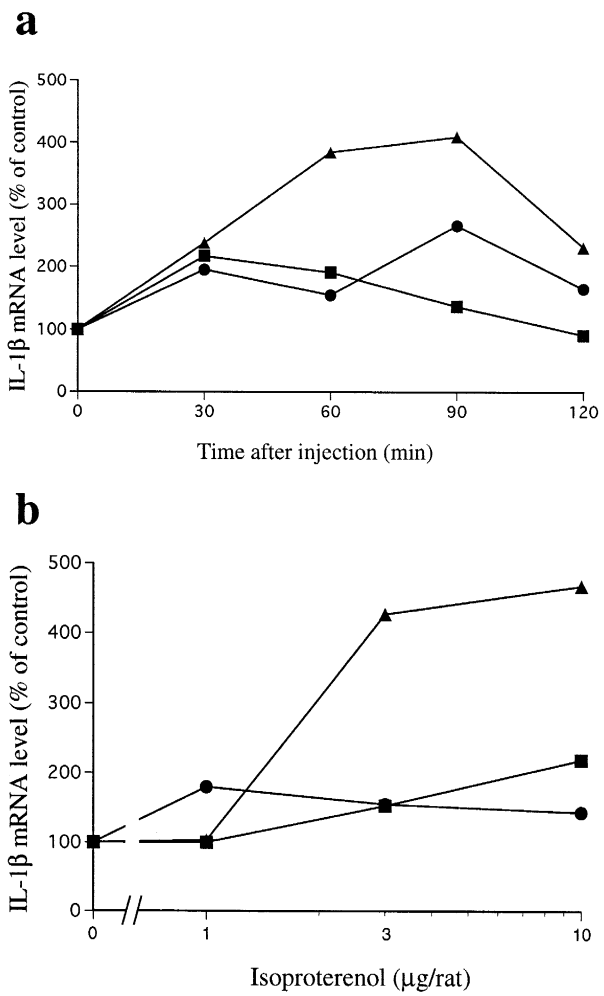

Fig. 1. (a) Time courses of the induction of interleukin-1β mRNA by i.c.v. injection of isoproterenol (3 μg/rat). (b) Dose-dependency of the induction of interleukin-1β mRNA by isoproterenol at 60 min after i.c.v. injection. The level of interleukin-1β mRNA was normalized by that of β-actin mRNA, and presented as % of control which was the level of interleukin-1β mRNA in each brain region of saline-injected rats. ■: hippocampus, ▲: thalamus, ●: hypothalamus.

3. Results

3.1. Northern blot analysis of interleukin-1β mRNA induced by i.c.v. administration of isoproterenol

I.c.v. administration of isoproterenol induced interleukin-1β mRNA in the hippocampus, hypothalamus and thalamus. As insertion of the guide and injection cannula, which caused traumatic damage to the cerebral cortex, intensely induced interleukin-1β mRNA, we did not perform northern blot analysis of interleukin-1β mRNA in the cerebral cortex. In the hippocampus, the induction of interleukin-1β mRNA reached a peak (217% of control) at 30 min after injection of 3 μg of isoproterenol (Fig. 1a). The levels of interleukin-1β mRNA were increased to 384 and 409% of control in the thalamus at 60 and 90 min, respectively (Fig. 1a). In these regions, i.c.v. administration of isoproterenol induced interleukin-1β mRNA in a dose-dependent manner (Fig. 1b). Ten micrograms of isoproterenol increased the levels of interleukin-1β mRNA to 219 and 467% of control in the hippocampus and thalamus, respectively. The induction of interleukin-1β mRNA in the hypothalamus reached a peak at 90 min (266% of control, Fig. 1a) and the maximum induction at 60 min (180% of control) was observed at the dose of 1 μg/rat (Fig. 1b).

3.2. Detailed distribution of interleukin-1β mRNA induced by i.c.v. administration of isoproterenol

The detailed distribution of interleukin-1β mRNA was examined using an in situ hybridization technique. In saline-administered rats, interleukin-1β mRNA was intensely expressed around the injection site in the cerebral

cortex (Fig. 2a). No expression of interleukin-1β mRNA was observed in brain regions other than the injection site, except for the Purkinje cell layer of the cerebellum as previously reported (Yabuuchi et al., 1993). Although intense expression of interleukin-1β mRNA was observed around the injection site also in the cerebral cortex injected

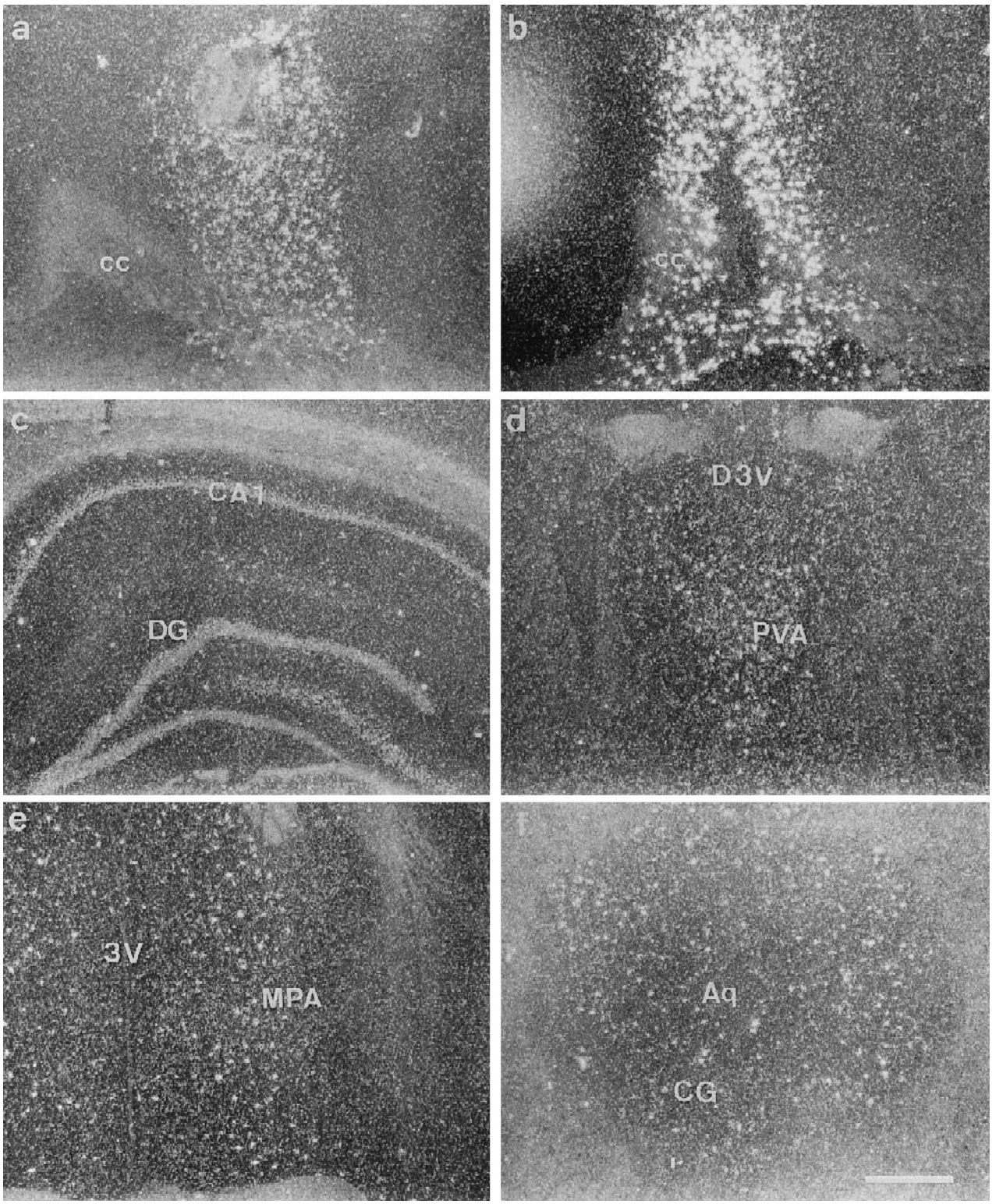

Fig. 2. Induction of interleukin-1β mRNA in the rat brain. Interleukin-1β mRNA was expressed around the sites of injection in both rats injected with saline (a) and isoproterenol (b). Isoproterenol induced interleukin-1β mRNA in the hippocampus (c), paraventricular thalamic nucleus (d), medial preoptic area (e) and central gray (f). 3V; third ventricle, Aq; Aqueduct, CA1; CA1 region of Ammon's horn, cc; corpus callosum, CG; central gray, D3V; dorsal third ventricle, DG; dentate gyrus, MPA; medial preoptic area, PVA; paraventricular thalamic nucleus, anterior. Bar = 500 μm.

with isoproterenol (Fig. 2b), the levels of expression in other fields of the cerebral cortex were very weak. In the hippocampus of the isoproterenol-injected rats, cells expressing interleukin-1β mRNA were sparsely observed in the polymorphic layer (Fig. 2c). Interleukin-1β mRNA was also induced in the amygdala, medial preoptic area (Fig. 2e) and paraventricular thalamic nucleus (Fig. 2d). In the hypothalamus, signals of interleukin-1β mRNA were observed in the paraventricular hypothalamic nucleus, supramammillary nucleus, ventromedial hypothalamic nucleus and dorsomedial hypothalamic nucleus (data not shown). The expression was observed in the central gray (Fig. 2f). In these regions, the signals of interleukin-1β mRNA were accumulated on cells which had relatively small and densely counterstained nuclei (Fig. 3).

3.3. Effects of i.c.v. administration of isoproterenol on mechanical nociception

I.c.v. administration of isoproterenol at the doses of 1, 3 and 10 μg/rat elevated the nociceptive threshold to the paw-pressure stimulation (Fig. 4a). At doses of 1 and 3 μg/rat, the time course of the elevation of nociceptive threshold showed two peaks. The first peak was observed at 60 min after injection, at which time 1 and 3 μg/rat of isoproterenol elevated the nociceptive thresholds to 114.6 $\pm$ 6.0% and 119.9 $\pm$ 6.5% of the pre-injected control level, respectively. These elevations were significant when compared with the saline-injected group. The second peak was observed at 180–210 min after injection of isoproterenol at doses of 1 and 3 μg/rat. At the second peak, 1 and 3 μg/rat of isoproterenol elevated the nociceptive threshold to 106.2 $\pm$ 7.3% and 131.9 $\pm$ 6.5% of control, respectively. On the other hand, 10 μg/rat of isoproterenol elevated the nociceptive threshold with one peak at 180–210 min after injection. The nociceptive threshold was significantly elevated to 119.2 $\pm$ 7.8% and 120.4 $\pm$ 6.4% of control at 180 and 210 min, respectively. For comparison with the analgesic effect of isoproterenol, we examined the analgesic effect of morphine (Fig. 4b). I.c.v. administration of morphine at a dose of 10 μg/rat significantly elevated the nociceptive threshold at 30, 60 and 90 min after injection. The peak of analgesic effect was observed at 30 min, then the nociceptive threshold was elevated to 149.6 $\pm$ 13.8% of control. The threshold in the morphine-injected group became similar to that in the saline-injected group at 120 min after injection.

3.4. Effects of coadministration of interleukin-1 receptor antagonist with isoproterenol

I.c.v. injection of interleukin-1 receptor antagonist (100 ng/rat) alone did not significantly alter the nociceptive threshold compared with the vehicle-injected group (Fig. 5). Isoproterenol (3 μg/rat) dissolved in phosphate buffered saline containing 0.02% bovine serum albumin significantly elevated the nociceptive threshold at 120, 150, 180, 240 and 270 min after injection, and the thresholds were elevated to 109.9 $\pm$ 4.6%, 112.4 $\pm$ 5.4%, 119.8

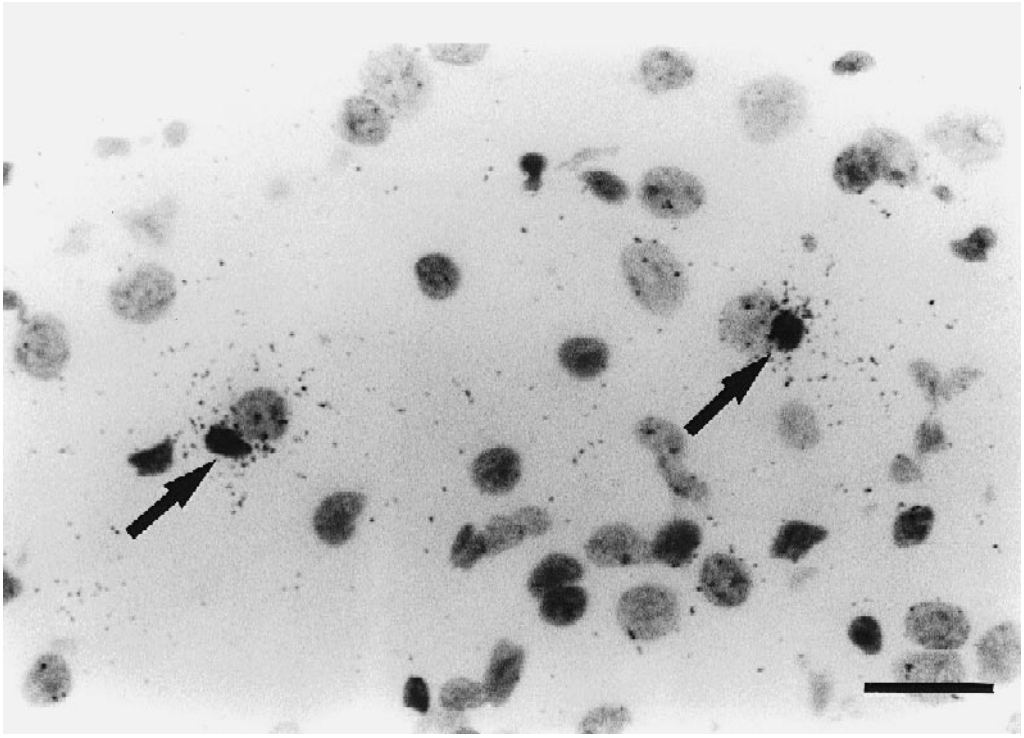

Fig. 3. Cells expressing interleukin-1β mRNA in the medial preoptic area of the isoproterenol (3 μg/rat, 60 min)-injected rat (arrow). Interleukin-1β mRNA was expressed in the cells which had relatively small and densely counterstained nuclei, probably glial cells. Bar = 50 μm.

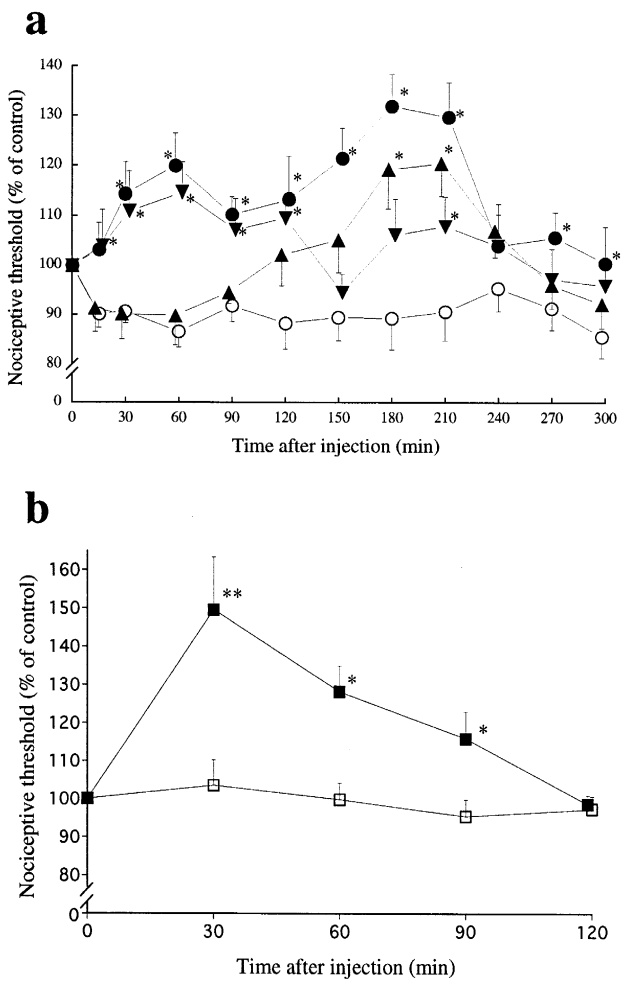

Fig. 4. Time courses of the effects of i.c.v. administration of isoproterenol (a) and morphine (b) on nociceptive threshold to the mechanical stimulation. The changes in threshold are presented as means of % of control ± S.E.M.. ○: Saline ($n=10$), ▼: isoproterenol 1 μg/rat ($n=10$), ●: isoproterenol 3 μg/rat ($n=8$), ▲: isoproterenol 10 μg/rat ($n=9$), □: saline ($n=6$) and ■: morphine 10 μg/rat ($n=5$). $^{*}P<0.05$, $^{**}P<0.01$ compared with the saline-injected group (Mann-Whitney's *U*-test).

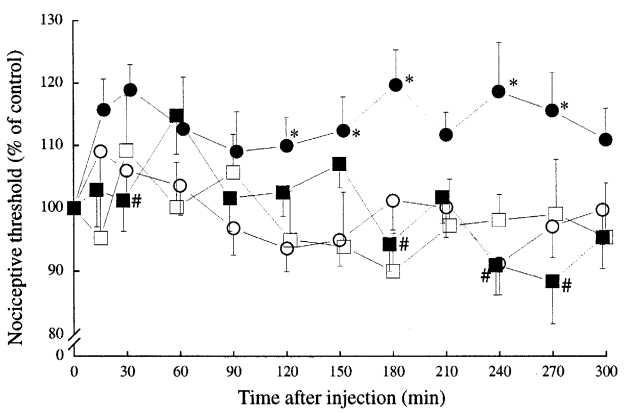

Fig. 5. Time courses of the effects of coadministration of interleukin-1 receptor antagonist (100 ng/rat) with isoproterenol (3 μg/rat) on the nociceptive threshold to mechanical stimulation. ○: Vehicle ($n=10$), □: interleukin-1 receptor antagonist 100 ng/rat ($n=8$), ●: isoproterenol 3 μg/rat ($n=10$) and ■: interleukin-1 receptor antagonist 100 ng/rat + isoproterenol 3 μg/rat ($n=8$). $^{*}P<0.05$ compared with the vehicle-injected group, $^{\#}P<0.05$ compared with the group injected with isoproterenol 3 μg/rat alone (Mann-Whitney's *U*-test).

± 5.6%, 118.6 ± 7.9% and 115.5 ± 6.2% of control, respectively. This analgesic effect was abolished by coadministration with the interleukin-1 receptor antagonist.

4. Discussion

We demonstrated that interleukin-1β mRNA was induced in several brain regions by i.c.v. administration of isoproterenol. In previous studies, we showed that interleukin-1β mRNA was induced by intraperitoneal (i.p.) administration of kainic acid (Minami et al., 1990) and methamphetamine (Yamaguchi et al., 1991). We have reported that the induction of interleukin-1β mRNA by i.p. treatment with methamphetamine, which exerts its pharmacological effects by releasing biogenic amines from the nerve terminals, was significantly inhibited by i.c.v. injection of propranolol, a β-adrenoceptor antagonist (Yamaguchi et al., 1991). Furthermore, we observed that the induction of interleukin-1β mRNA by kainic acid was, at least in part, suppressed by propranolol (unpublished data). These findings combined with the present results strongly suggest that the expression of interleukin-1β mRNA in the brain is regulated by the β-adrenergic system.

Using an in situ hybridization technique, we examined the distribution of interleukin-1β mRNA induced by i.c.v. injection of isoproterenol. In the brain of control rats administered with saline, interleukin-1β mRNA was intensely expressed around the site of injection. Intense expression around the site of injection was also observed in the group treated with isoproterenol. As interleukin-1-like activity was reported to be increased by brain trauma (Yan et al., 1992), the intense expression of interleukin-1β mRNA around the site of injection was considered to be due to the traumatic damage. In other brain regions in control rats, interleukin-1β mRNA was not detected except in the Purkinje cell layer of the cerebellum. I.c.v. administration of isoproterenol markedly induced interleukin-1β mRNA in several brain regions. Interleukin-1β mRNA was expressed in cells which had relatively small and densely counterstained nuclei. Such characteristics suggest that the cells expressing interleukin-1β mRNA were glial cells. Our previous study demonstrated that interleukin-1β mRNA was induced by kainic acid in microglial cells rather than astrocytes (Yabuuchi et al., 1993). This may also be the case in the brain treated with isoproterenol. This possibility was supported by the results of in vitro study which showed the induction of interleukin-1β mRNA and production of interleukin-1β protein in microglia but not in astrocytes following the treatment with isoproterenol (Tomozawa et al., 1995).

I.c.v. injection of isoproterenol resulted in significant elevation of the nociceptive threshold to mechanical stimulation. The analgesic effect of isoproterenol (3 μg/rat) showed two peaks at 60 min and 180–210 min after administration. At the second peak, 3 μg/rat of isoprote-

renol elevated the nociceptive threshold to 131.9 ± 6.5% of control. The elevation of nociceptive threshold was of similar degree observed in the group injected with 10 μg/rat of morphine (149.6 ± 13.8% of control). The second peak of the analgesic effect of isoproterenol was antagonized by coadministration with interleukin-1 receptor antagonist. These results, together with the finding that interleukin-1β mRNA was induced by i.c.v. administration of isoproterenol, suggest that the analgesic effect of isoproterenol was, at least in part, mediated by interleukin-1β produced in the brain. In this context, we previously reported that i.c.v. injection of interleukin-1β (1 and 10 ng/rat) caused analgesia in mechanical nociception (Yabuuchi et al., 1996). Interleukin-1β at a dose of 10 ng/rat elevated the nociceptive threshold to about 130% of the control level. In the present study, a similar degree of elevation in the nociceptive threshold was observed in the rat treated with 3 μg/rat of isoproterenol. These findings suggest that interleukin-1β mRNA was translated and a considerable amount of interleukin-1β protein was produced in the brain. Furthermore, the elevation of the nociceptive threshold by i.c.v. injection of interleukin-1β was observed about 120 min after injection. This is consistent with the present results that the peak of the analgesic effect mediated by interleukin-1β was observed at 180–210 min after i.c.v. injection of isoproterenol, taking into account that the induction of interleukin-1β mRNA by isoproterenol reached the peak 30–60 min after injection. On the contrary, morphine, which can produce its analgesic effect probably without newly synthesized proteins, showed the analgesic effect at 30 min after injection.

Although we reported that i.c.v. injection of interleukin-1β at the lower doses (10–100 pg/rat) caused hyperalgesia, we did not observe any algesic effect of isoproterenol in the present study. The algesic effects of interleukin-1β were observed only at optimal i.c.v. doses (Oka et al., 1993; Yabuuchi et al., 1996). The doses of isoproterenol used in this study possibly produced interleukin-1β beyond the adequate amount to cause algesic effects. Alternatively, algesic effects of interleukin-1β might be masked by the first phase of the analgesic effect of isoproterenol, which seems not to be mediated by interleukin-1β. As well as the first phase of the analgesic effect of isoproterenol, algesic effects of interleukin-1β were observed within 90 min after i.c.v. injection (Yabuuchi et al., 1996).

Oka et al. (1995) reported that microinjection of interleukin-1β into the ventromedial hypothalamic nucleus prolonged the paw-withdrawal latency in the hot plate test. We previously demonstrated that type 1 interleukin-1 receptors are localized on the neuronal cells in the ventromedial hypothalamic nucleus (Yabuuchi et al., 1994). In addition, the present study showed that interleukin-1β mRNA was induced by isoproterenol in this nucleus. These findings suggest the possible involvement of this nucleus in the analgesic effect of isoproterenol which were mediated by interleukin-1β production, while the involvement of the other brain regions in which interleukin-1β mRNA was induced by isoproterenol can not be excluded. Oka et al. (1995) also showed that microinjection of interleukin-1β into the medial and lateral part of the preoptic area and median preoptic nucleus produced the algesic effect in the hot plate test. In the present study, we observed a bell-shaped dose–response curve for the analgesic effect of isoproterenol. This might be due to the opposing effects of interleukin-1β on nociceptive behavior in the ventromedial hypothalamic nucleus and preoptic area. Further studies including the microinjection of isoproterenol into these brain regions are necessary to elucidate which brain regions are involved in the analgesic and/or algesic effects of isoproterenol.

Our previous study (Yabuuchi et al., 1996) revealed that the analgesic effect of interleukin-1β was not altered by the i.c.v. pretreatment with sodium salicylate. This suggested that the analgesic effect of interleukin-1β was not mediated via a cyclooxygenase-dependent pathway. On the other hand, we showed that the analgesic effect of interleukin-1β was inhibited by the prior administration of α-helical corticotropin releasing factor (CRF) [9-41], a CRF receptor antagonist, indicating that the analgesic effect of interleukin-1β are mediated by endogenous CRF. Reportedly, i.c.v. injection of interleukin-1β stimulates CRF neurons in the hypothalamus (Ju et al., 1991) and thereby activates the hypothalamus–pituitary–adrenal axis (Berkenbosch et al., 1987). These findings suggest the possibility that activation of the hypothalamus–pituitary–adrenal axis is involved in the analgesia induced by i.c.v. injection of isoproterenol, which is thought to be mediated, at least in part, by interleukin-1β produced in the brain. Such a possibility should be elucidated by the experiments using glucocorticoid receptor antagonists or adrenolectomized rats. Further experiments are necessary for clarifying the mechanism of analgesic effect of i.c.v. isoproterenol.

Acknowledgements

We would like to thank to Dr. Nakai and Dr. Masui (Otsuka Pharmaceutical. Co. LTD) for gifts of the rat interleukin-1β cDNA and human recombinant interleukin-1 receptor antagonist. This study was supported by a Grant-in-Aid for General Scientific Research and for JSPS fellows from the Ministry of Education, Science, Sports and Culture of Japan, and by a grant from The Naito Foundation.

References

Berkenbosch, F., Oers, J.V., Rey, A.D., Tilders, F., Besedovsky, H., 1987. Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. Science 238, 524–526.

Gardella, J.L., Izquierdo, A., Izquierdo, I., 1970. The analgesic action of catecholamine and of pyrogallol. Eur. J. Pharmacol. 10, 87–90.

Ju, G., Bo-Quan, J., Chuan-Shu, H., 1991. Activation of corticotropin-releasing factor-containing neurons in the paraventricular nucleus of the hypothalamus by interleukin-1 in the rat. Neurosci. Lett. 132, 151–154.

Kluger, M.J., 1991. Fever: Role of pyrogens and cryogens. Physiol. Rev. 71, 93–127.

Krueger, J.M., Walter, J., Dinarello, C.A., Wolff, S.M., Chedid, L., 1984. Sleeping-promoting effects of endogenous pyrogen (interleukin-1). Am. J. Physiol. 246, R994–R999.

Kuraishi, Y., Hirota, N., Sugimoto, M., Satoh, M., Takagi, H., 1983. Effects of morphine on noxious stimuli-induced release of substance P from rabbit dorsal horn in vivo. Life Sci. 33 (Suppl. I), 693–696.

Kuraishi, Y., Hirota, N., Sato, Y., Kaneko, S., Satoh, M., Takagi, H., 1985. Noradrenergic inhibition of the release of substance P from the primary afferents in the rabbit spinal dorsal horn. Brain Res. 359, 177–182.

Minami, M., Kuraishi, Y., Yamaguchi, T., Nakai, S., Hirai, Y., Satoh, M., 1990. Convulsants induce interleukin-1β messenger RNA in rat brain. Biochem. Biophys. Res. Commun. 171, 832–837.

Oka, T., Aou, S., Hori, T., 1993. Intracerebroventricular injection of interleukin-1β induces hyperalgesia in rats. Brain Res. 624, 61–68.

Oka, T., Oka, K., Hosoi, M., Aou, S., Hori, T., 1995. The opposing effects of interleukin-1β microinjected into the preoptic hypothalamus and the ventromedial hypothalamus on nociceptive behavior in rats. Brain Res. 700, 271–278.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, San Diego, CA.

Radouco-Thomas, S., Singh, P., Garcin, F., Radouco-Thomas, C., 1967. Relationship between experimental analgesia and brain monoamines: Catecholamines and 5-hydroxytryptamine. Arch. Biol. Med. Exp. 4, 42–52.

Randall, L.O., Selitto, J.J., 1957. A method for measurement of analgesic activity on inflamed tissue. Arch. Int. Pharmacodyn. 106, 409–419.

Tomozawa, Y., Yabuuchi, K., Inoue, T., Satoh, M., 1995. Participation of cAMP and cAMP-dependent protein kinase in β-adrenoceptor-mediated interleukin-1β mRNA induction in cultured microglia. Neurosci. Res. 22, 399–409.

Ueda, M., Sugimoto, K., Oyama, T., Kuraishi, Y., Satoh, M., 1995. Opioidergic inhibition of capsaicin-evoked release of glutamate from rat spinal dorsal horn slices. Neuropharmacology 34, 303–308.

Yabuuchi, K., Minami, M., Katsumata, S., Satoh, M., 1993. In situ hybridization study of interleukin-1β mRNA induced by kainic acid in the rat brain. Mol. Brain Res. 20, 153–161.

Yabuuchi, K., Minami, M., Katsumata, S., Satoh, M., 1994. Localization of type I interleukin-1 receptor mRNA in the rat brain. Mol. Brain Res. 27, 27–36.

Yabuuchi, K., Nishiyori, A., Minami, M., Satoh, M., 1996. Biphasic effects of intracerebroventricular interleukin-1β on mechanical nociception in the rat. Eur. J. Pharmacol. 300, 59–65.

Yamaguchi, T., Kuraishi, Y., Minami, M., Yabuuchi, K., Satoh, M., 1991. Involvement of central β-adrenoceptors in the induction of hypothalamic interleukin-1β mRNA by methamphetamine. Neurosci. Res. 12, 432–439.

Yan, H.Q., Banos, M.A., Herregodts, P., Hooghe, R., Hooghe-Peters, E.L., 1992. Expression of interleukin (IL)-1β, IL-6 and their respective receptors in the normal rat brain and after injury. Eur. J. Immunol. 22, 2963–2971.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.