ELSEVIER

European Journal of Pharmacology 419 (2001) 183–189

ejp

www.elsevier.nl/locate/ejphar

Role of brain arachidonic acid cascade on central CRF_1 receptor-mediated activation of sympatho-adrenomedullary outflow in rats

Kunihiko Yokotani *, Yoshinori Murakami, Shoshiro Okada, Masakazu Hirata

Department of Pharmacology, Kochi Medical School, Nankoku, Kochi 783-8505, Japan

Received 7 September 2000; received in revised form 4 April 2001; accepted 10 April 2001

Abstract

The present experiments were designed to characterize the mechanisms involved in the corticotropin releasing factor (CRF)-induced activation of central sympatho-adrenomedullary outflow in rats. Intracerebroventricularly (i.c.v.) administered CRF and urocortin (0.5, 1.5 and 3.0 nmol/animal) effectively and dose-dependently elevated plasma levels of adrenaline and noradrenaline, and the effect of urocortin was almost the same as that of CRF. The elevation of catecholamines induced by CRF and urocortin (1.5 nmol/animal) was reduced by CP-154,526(butyl-ethyl-{2,5-dimethyl-7-(2,4,6trimethylphenyl)-7*H*-pyrrolo [2,3-*d*] pyrimidin-4-yl]amine), a selective CRF_1 receptor antagonist, in a dose dependent manner (1.2 and/or 2.4 μmol/animal, i.c.v.), and abolished by indomethacin (1.2 μmol/animal, i.c.v.), an inhibitor of cyclooxygenase. Furegrelate (1.8 μmol/animal, i.c.v.), an inhibitor of thromboxane A_2 synthase, abolished the CRF-induced elevation of adrenaline, but had no effect on the evoked release of noradrenaline. These results suggest that activation of brain CRF_1 receptor facilitates the central sympathetic and adrenomedullary outflow in distinct central pathways in rats: brain thromboxane A_2 is involved in the central adrenomedullary outflow; an active metabolite of arachidonic acid other than thromboxane A_2 (probably prostaglandin E_2) may be involved in the central sympathetic outflow.

Keywords: Catecholamine; Plasma; Corticotropin releasing factor (CRF); CRF_1 receptor; Cyclooxygenase; Thromboxane A_2; Brain; Sympatho-adrenomedullary outflow

1. Introduction

Corticotropin releasing factor (CRF) is a 41 amino acid peptide initially identified as a hypothalamic factor responsible for stimulating corticotropin secretion from the anterior pituitary (Vale et al., 1981). CRF-like immunoreactivity and high affinity CRF receptors are heterogeneously distributed in the brain (Swanson et al., 1983). Recently, Vaughan et al. (1995) have identified and characterized a novel CRF receptor-like ligand, urocortin, which is a 40 amino acid peptide with 75% homology with CRF and localized in brain regions in which CRF_2 receptors are found. Molecular cloning studies indicate the existence of at least two major classes of mammalian CRF receptor, CRF_1 (Chang et al., 1993; Chen et al., 1993; Perrin et al., 1993; Vita et al., 1993) and CRF_2 (Kishimoto et al., 1995; Lovenberg et al., 1995; Perrin et al., 1995; Stenzel et al., 1995). These CRF receptor subtypes are heterogeneously distributed in the brain (Chalmers et al., 1995).

CRF has been shown to take many roles in the brain functions in addition to its action on the hypothalamic pituitary adrenal axis (Menzaghi et al., 1993; Chalmers et al., 1996; Dieterich et al., 1997). Intracerebroventricular administration of CRF to laboratory animals produces a wide spectrum of behavioral and autonomic effects. In this regard, centrally administered CRF elicits the activation of sympatho-adrenomedullary system and oxygen consumption (Brown et al., 1982a,b, 1985), the elevation of blood pressure and heart rate (Fisher et al., 1982), and the activation of adrenal sympathetic efferent nerve activity (Kurosawa et al., 1986). CRF-deficient (knockout) mice also leads to the decreased level of the basal and restraint-induced plasma adrenaline (Jeong et al., 2000). However, the central mechanisms that mediate such responses are largely undefined.

Previously, we reported that the brain arachidonic acid cascade is involved in the central activation of the sympa-

* Corresponding author. Tel.: +81-88-880-2328; fax: +81-88-880-2328.

E-mail address: yokotani@dtn.am400gw.kochi-ms.ac.jp (K. Yokotani).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)00987-6

tho-adrenomedullary outflow (Yokotani et al., 1988, 1995a,b, 1996, 2000). The present study, therefore, was designed to characterize the CRF-induced activation of central sympatho-adrenomedullary outflow in regard to CRF receptor subtype and brain arachidonic acid cascade using anesthetized rats.

2. Materials and methods

2.1. Experimental procedures

Male Wistar rats weighing about 350 g were maintained in an air-conditioned room at 22–24°C under a constant day–night rhythm for more than 2 weeks and given food (laboratory chow, CE-2; Clea Japan, Hamamatsu, Japan) and water ad libitum. Under urethane anesthesia (1.2 g/kg, i.p.), the femoral vein was cannulated for infusion of saline (1.2 ml/h), and the femoral artery was cannulated for collecting blood samples. After these procedures, the animal was placed in a stereotaxic apparatus, as shown in our previous paper (Yokotani et al., 1995a, 2000).

Three hours after the animal was placed in a stereotaxic apparatus, a stainless-steel cannula (0.35 mm outer diameter) was inserted into the right lateral ventricle according to the rat brain atlas of Paxinos and Watson (1986). The stereotaxic coordinates of the tip of cannula was as follows (in mm): AP −0.8, L 1.5, H 4.0 (AP, anterior from the bregma; L, lateral from the midline; H, below the surface of the brain). CRF and urocortin was dissolved in sterile saline and slowly injected into the right lateral ventricle in a volume of 10 μl using a 50 μl Hamilton syringe. Water-soluble indomethacin-Na and furegrelate were dissolved in saline and CP-154,526 was dissolved in 100% dimethyl sulfoxide (DMSO). These reagents were administered into the right lateral ventricle 60 min before the application of CRF in a volume of 10 μl for indomethacin and furegrelate and 2.5 μl for CP-154,526.

All experiments were conducted in compliance with the guiding principles for the care and use of laboratory animals approved by the Kochi Medical School. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Measurement of plasma catecholamines

Blood samples (250 μl) were collected through an arterial catheter. Catecholamines in the plasma were extracted by the method of Anton and Sayre (1962) with a slight modification and were assayed electrochemically by

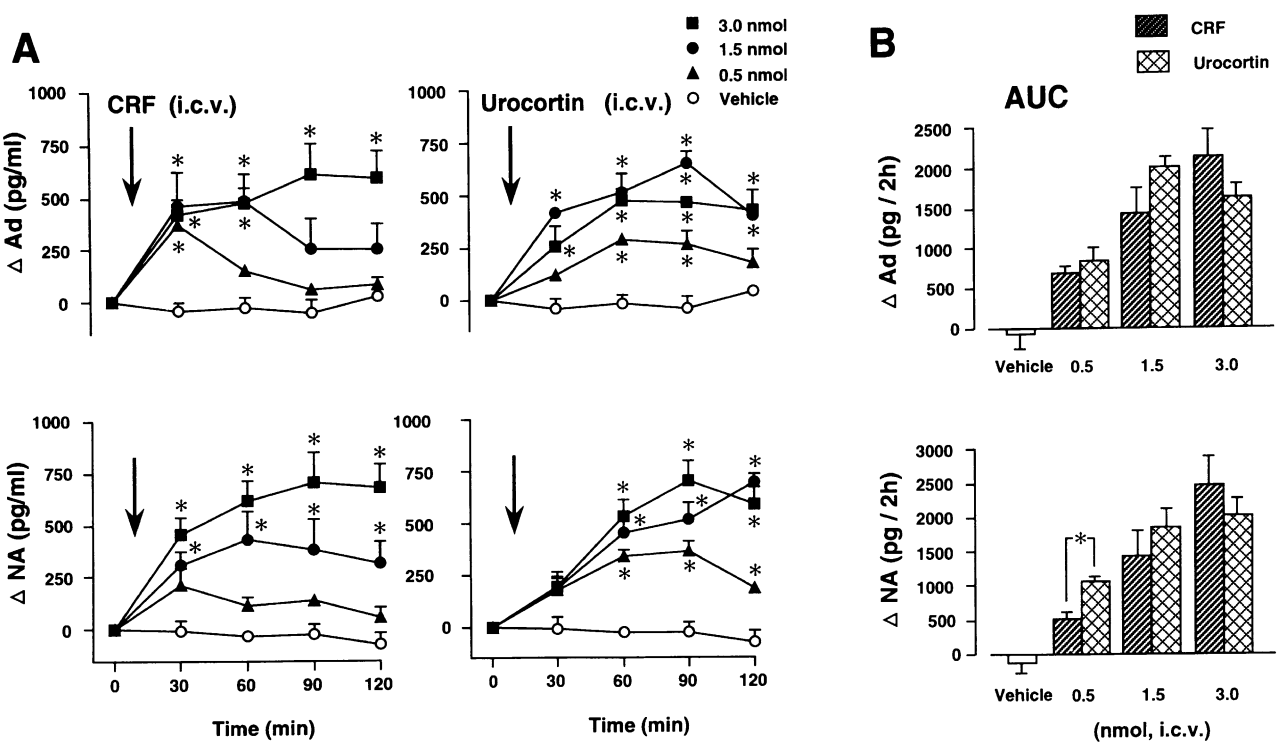

Fig. 1. Effects of CRF and urocortin on plasma levels of adrenaline (Ad) and noradrenaline (NA). ΔAd and ΔNA, increase of adrenaline (Ad) and noradrenaline (NA) above the basal. (A) Arrow indicates intracerebroventricular (i.c.v.) administration of CRF and urocortin (0.5, 1.5 and 3.0 nmol/animal). Left panel (CRF); vehicle (saline 10 μl/animal) ($n = 5$), 0.5 nmol CRF ($n = 4$), 1.5 nmol CRF ($n = 6$)(cited from Fig. 3A), 3.0 nmol CRF ($n = 5$): Right panel (urocortin); vehicle (cited from (A), left panel), 0.5 nmol urocortin ($n = 5$), 1.5 nmol urocortin ($n = 5$), 3.0 nmol urocortin ($n = 5$). The actual values for Ad and NA at 0 min were 144.8 ± 18.1 and 353.6 ± 20.3 pg/ml ($n = 29$), respectively. (B) The area under the curve (AUC) of CRF- and urocortin-induced elevation of plasma Ad and NA is expressed as pg/2 h. Each point represents the mean ± S.E.M. * Significantly different ($P < 0.05$) from vehicle-treated control in (A) and significant difference between CRF and urocortin in (B).

high performance liquid chromatography (Yokotani et al., 1995a). Briefly, after centrifugation, the plasma (100 μl) was transferred to a centrifuge tube containing 30 mg of activated alumina, 2 ml of double deionized water, 1 ng of 3,4-dihydrooxybenzylamine as an internal standard and 1 ml of 1.5 M Tris Buffer (pH 8.6) containing 0.1 M disodium EDTA. The tube was shaken for 5 min and the alumina was washed three times with 4 ml of ice-cold double deionized water. Then catecholamines adsorbed onto the alumina were eluted with 300 μl of 4% acetic acid containing 0.1 mM disodium EDTA. A pump (EP-300: Eicom, Kyoto, Japan), a sample injector (Model-231XL; Gilson, Villiers-le-Bel, France) and an electrochemical detector (ECD-300: Eicom) equipped with a graphite electrode were used with high performance liquid chromatography. Analytical conditions were as follows: detector, +450 mV potential against a Ag/AgCl reference electrode; column, Eicompack CA-50DS, 2.1 × 150 mm (Eicom); mobile phase, 0.1 M NaH_2PO_4–Na_2HPO_4 buffer (pH 6.0) containing 50 mg/l EDTA dihydrate, 750 mg/l 1-octane sulfate sodium (Nacalai Tesque, Kyoto, Japan) and 15% methanol at a flow of 0.22 ml/min. The amount of catecholamines in each sample was calculated using the peak height ratio relative to that of 3,4-dihydrooxybenzylamine, an internal standard. This assay could determine 0.5 pg of adrenaline and noradrenaline accurately.

2.3. Treatment of data and statistics

The area under the curve is used to compare the effects of CRF and urocortin on plasma catecholamine levels (Fig. 1B). All values are expressed as the means ± S.E.M. The data were analyzed by repeated-measure analysis of variance (ANOVA), followed by post hoc analysis with the Bonferroni method for comparing a control to all other means (Figs. 1A and 2A). When only two means were compared, an unpaired Student's *t*-test was used (Figs. 1B, 2B and 3). *P* values less than 0.05 were taken to indicate significance.

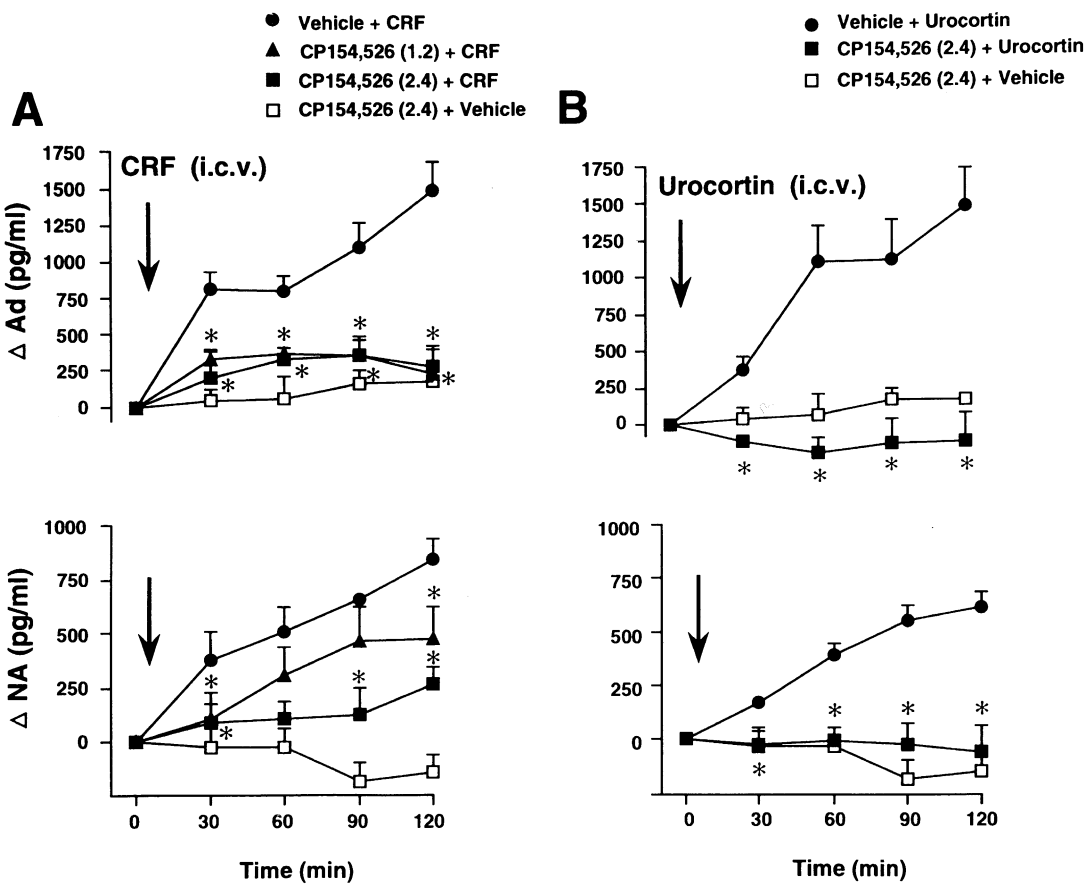

Fig. 2. Effect of CP-154,526 on the CRF- and urocortin-induced elevation of plasma adrenaline (Ad) and noradrenaline (NA). CP-154,526 [1.2 μmol (500 μg) and/or 2.4 μmol (1000 μg)/animal, i.c.v.] was administered 60 min before the administration of CRF or urocortin (1.5 nmol/animal, i.c.v.). (A) vehicle (2.5 μl of 100% DMSO) plus CRF ($n = 5$), CP-154,526 (1.2 μmol/animal) plus CRF ($n = 5$), CP-154,526 (2.4 μmol/animal) plus CRF ($n = 4$), CP-154,526 (2.4 μmol/animal) plus vehicle (10 μl saline) ($n = 4$). (B) vehicle (2.5 μl of 100% DMSO) plus urocortin ($n = 5$), CP-154,526 (2.4 μmol/animal) plus urocortin ($n = 4$), CP-154,526 (2.4 μmol/animal) plus vehicle (10 μl saline) (cited from (A)). * Significantly different ($P < 0.05$) from the group treated with vehicle plus CRF or vehicle plus urocortin. Other conditions were the same as those in Fig. 1. The actual values for Ad and NA at 0 min were 197.7 ± 20.5 and 516.0 ± 45.7 pg/ml in vehicle (2.5 μl of 100% DMSO)-treated group ($n = 10$); 227.0 ± 78.7 and 698.4 ± 143.4 pg/ml in CP-154,526 (1.2 μmol/animal)-treated group ($n = 5$); 260.9 ± 56.2 and 741.9 ± 39.1 pg/ml in CP-154,526 (2.4 μmol/animal)-treated group ($n = 12$), respectively.

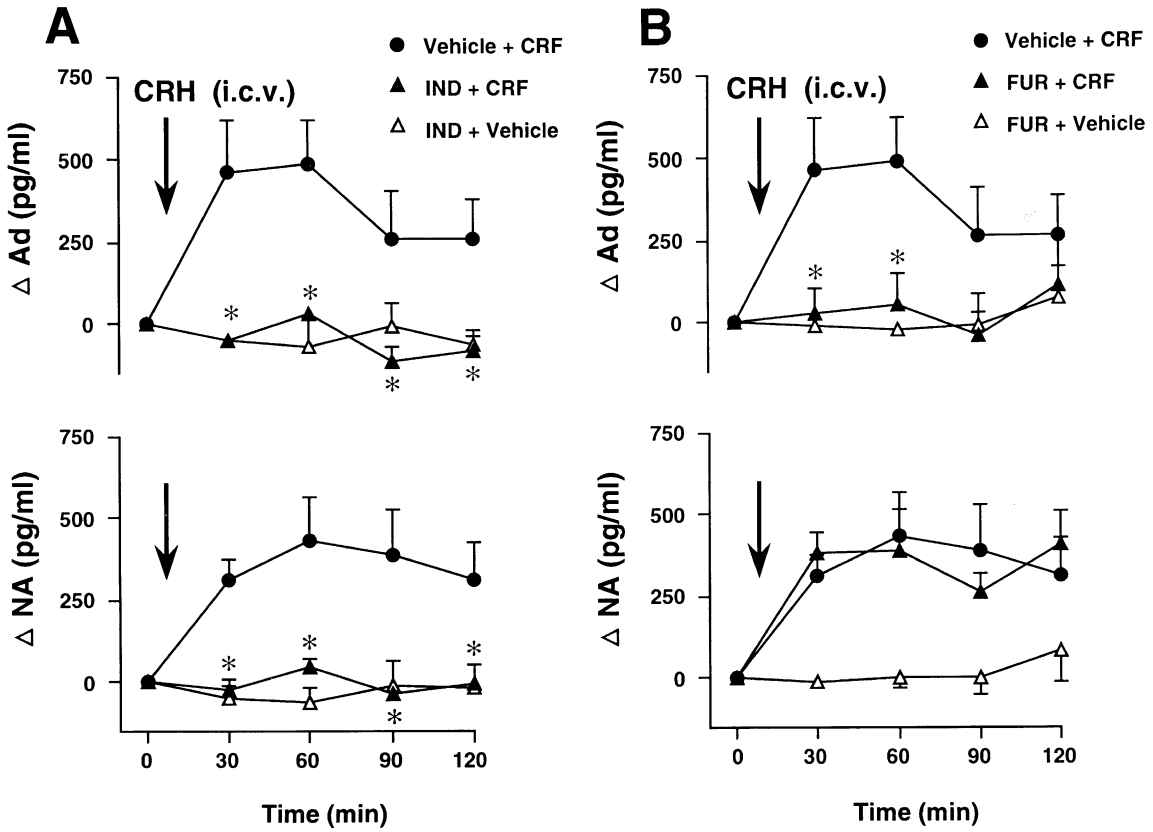

Fig. 3. Effect of indomethacin and furegrelate on the CRF-induced elevation of plasma adrenaline (Ad) and noradrenaline (NA). Indomethacin (IND) [1.2 μmol (500 μg)/animal, i.c.v.] or furegrelate (FUR) [1.8 μmol (500 μg)/animal, i.c.v.] was administered 60 min before the administration of CRF (1.5 nmol/animal, i.c.v.). (A) vehicle (saline 10 μl/animal) plus CRF ($n = 6$), indomethacin plus CRF ($n = 5$), indomethacin plus vehicle (saline 10 μl/animal) ($n = 4$). (B) vehicle (saline 10 μl/animal) plus CRF (cited from (A)), furegrelate plus CRF ($n = 5$), furegrelate plus vehicle (saline 10 μl/animal) ($n = 4$). * Significantly different ($P < 0.05$) from the group treated with vehicle plus CRF in (A) and (B). Other conditions were the same as those in Figs. 1 and 2. The actual values for Ad and NA at 0 min were 142.6 ± 34.1 and 379.8 ± 43.9 pg/ml in vehicle- plus CRF-treated group ($n = 6$), 144.8 ± 48.3 and 406.6 ± 47.8 pg/ml in indomethacin-treated group ($n = 9$), 184.3 ± 40.9 and 408.4 ± 37.6 pg/ml in furegrelate-treated group ($n = 9$), respectively.

2.4. Compounds

The following drugs were used: furegrelate sodium (Biomol Research Lab., Plymouth Meeting, PA, USA); water-soluble indomethacin sodium trihydrate (Merck, Rahway, NJ, USA); CP-153,526 (butyl-ethyl-{2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]amine) (Pfizer, Groton, CT, USA); synthetic corticotropin releasing factor (rat/human) and urocortin (rat) (Peptide Institude, Osaka, Japan). All other reagents were the highest grade available (Nacalai Tesque, Kyoto, Japan).

3. Results

3.1. Effects of CRF and urocortin on plasma catecholamines

Intracerebroventricularly (i.c.v.) administered vehicle (10 μl saline/animal) and blood sampling for five times over a 120-min period did not affect the basal plasma levels of either adrenaline or noradrenaline (Fig. 1A).

Administration of corticotropin releasing factor (CRF) (0.5, 1.5 and 3.0 nmol/animal, i.c.v.) dose dependently elevated plasma levels of adrenaline and noradrenaline (Fig. 1A, left panel). The response of adrenaline reached a maximum 30–60 min after the administration of this peptide (0.5 and 1.5 nmol/animal, i.c.v.) and then gradually declined toward their basal levels, while a higher dose of this peptide (3.0 nmol/animal, i.c.v.) continuously elevated plasma adrenaline levels throughout the experiments. On the other hand, i.c.v. administered CRF gradually elevated plasma levels of noradrenaline in a dose dependent manner; the response reached a maximum 60–90 min after the administration of this peptide and then declined toward their basal levels.

Administration of urocortin (0.5, 1.5 and 3.0 nmol/animal, i.c.v.) also elevated plasma levels of adrenaline and noradrenaline and these responses reached a maximum 60–90 min after the administration of this peptide and then gradually declined toward their basal levels (Fig. 1A, right panel). The maximal response of adrenaline was obtained by 1.5 nmol urocortin. A higher dose of urocortin (3.0 nmol/animal, i.c.v.) did not evoke more responses than

that induced by this peptide (1.5 nmol/animal, i.c.v.). On the other hand, urocortin elevated plasma noradrenaline levels in a dose-dependent manner.

When comparing the relationship between the area under the curve of plasma catecholamines elevation over time and the dose of CRF and urocortin (Fig. 1B), it was observed that the magnitude of the response elicited by 0.5 nmol/animal (i.c.v.) of urocortin was significantly greater than that elicited by the same dose of CRF. At the higher doses employed (1.5 and 3.0 nmol/animal, i.c.v.), however, both peptides were equieffective.

Intravenous administration of CRF and urocortin (3.0 nmol/animal) had no effect on plasma levels of catecholamines.

3.2. Effect of CP-154,526 on the CRF- and urocortin-induced elevation of plasma catecholamines

The pretreatment with vehicle alone (2.5 μl of 100% DMSO, i.c.v.) or CP-154,526 dissolved in 2.5 μl of 100% DMSO slightly but significantly elevated the basal levels of noradrenaline but had no effect on the basal adrenaline levels. Pretreatment with vehicle significantly potentiated the release of adrenaline evoked by CRF or urocortin (1.5 nmol/animal, i.c.v.), but had only a slight effect on the evoked release of noradrenaline (Fig. 2A and B).

CP-154,526 [1.2 μmol (500 μg) and 2.4 μmol (1000 μg)/animal, i.c.v.] reduced the release of adrenaline and noradrenaline evoked by CRF (1.5 nmol/animal, i.c.v.) in a dose-dependent manner, however, the evoked release of adrenaline was more effectively attenuated than that of noradrenaline (Fig. 2A).

CP-154,526 [2.4 μmol (1000 μg)/animal, i.c.v.] also abolished the release of adrenaline and noradrenaline evoked by urocortin (1.5 nmol/animal, i.c.v.) (Fig. 2B).

3.3. Effect of indomethacin and furegrelate on the CRF-induced elevation of plasma catecholamines

Administration of indomethacin [1.2 μmol (500 μg)/animal, i.c.v.] had no effect on the basal plasma levels of both adrenaline and noradrenaline (Fig. 3A). Indomethacin completely abolished the release of both catecholamines evoked by CRF (1.5 nmol/animal, i.c.v.).

Administration of furegrelate [1.8 μmol (500 μg)/animal, i.c.v.] had no effect on the basal plasma levels of catecholamines (Fig. 3B). Furegrelate completely abolished the release of adrenaline evoked by CRF (1.5 nmol/animal, i.c.v.) (Fig. 3B, upper panel). On the other hand, furegrelate had no effect on the release of noradrenaline evoked by this peptide (Fig. 3B, lower panel).

4. Discussion

In the first experiments, we compared the effects of CRF and urocortin on plasma catecholamine levels. The magnitude of the response elicited by a small dose of urocortin was significantly greater than that elicited by the same dose of CRF. At the higher doses, both peptides were equieffective. Gottowik et al. (1997) showed that urocortin has a high affinity to CRF_2 receptor and also has the same affinity as CRF to CRF_1 receptors expressed on rat cerebellum and cells stably transfected with the human CRF_1 and rat CRF_2 receptors. Furthermore, urocortin is about 10 times more effective than CRF in accumulation of cAMP in COS-M6 cells expressing CRF_2 receptor (Vaughan et al., 1995). From these evidence, it is suggested that central CRF_1 receptor is primarily involved in CRF- and urocortin-induced activation of central sympatho-adrenomedullary outflow. However, it remains possible that central CRF_2 receptor is involved in urocortin-induced activation of central sympathetic outflow.

Recently, a highly selective, nonpeptide CRF_1 antagonist, CP-154,526, has been developed (Lundkvist et al., 1996). This antagonist reverses CRF-elicited increase in corticotropin, prevents CRF-induced elevation in locus caeruleus cell firing, and blocks CRF-enhanced startle response in rat (Schulz et al., 1996). In the present study, intracerebroventricularly administered CP-154,526 led to a dose dependent attenuation of the CRF- and urocortin-induced elevation of plasma catecholamines. These results further suggest that CRF_1 receptor is actually involved in CRF- and urocortin-induced activation of central sympatho-adrenomedullary outflow.

The hypothalamus, especially the paraventricular nucleus, has been considered to be the control center of the central sympatho-adrenomedullary outflow (Swanson and Sawchenko, 1980). A retrograde tracer study suggests a possible connection between the sympatho-adrenomedullary system and the paraventricular nucleus, including CRF expressing neuron, through the splanchnic nerve and spinal cord (Jansen et al., 1995). CRF_1 receptor mRNA is in scattered cells in the paraventricular nucleus (Chalmers et al., 1995), however, CRF_1 receptor mRNA and Fos induction are elicited in autonomic part of this nucleus by centrally administration of CRF (Bittencourt and Sawchenko, 2000). This evidence suggests the involvement of hypothalamic CRF_1 receptor in CRF- and urocortin-induced activation of central sympatho-adrenomedullary outflow. In addition, the nucleus tractus solitarius complex is also the major center for autonomic function in the brain stem (Van Zwieten et al., 1995). In this nucleus complex, urocortin-responsive structures include the area postrema, where CRF_2 receptor but not CRF_1 receptor mRNA expression is apparent (Bittencourt and Sawchenko, 2000). From these evidence, it remains possible that urocortin acts on this nucleus complex, thereby activating sympathetic outflow through CRF_2 receptor-mediated mechanisms.

The CRF-induced elevation of plasma catecholamines was abolished by centrally administered indomethacin. Indomethacin is a potent inhibitor of the prostaglandin-forming cyclooxygenase (Insel, 1996). Since indomethacin does not easily penetrate the blood–brain barrier by pe-

ripheral administration, we directly administered this reagent (500 μg/animal) into the lateral ventricule of the rat brain. Previously, we reported that intracerebro-ventricularly administered indomethacin (50 and 500 μg/animal) and diclofenac (an another cyclooxgenase inhibitor) (100 and 500 μg/animal) effectively attenuated centrally administered bombesin-induced elevation of plasma catecholamines, however, the effect of indomethacin was more greater than that of diclofenac (Okuma et al., 1996). The failure of sodium salicylate to block the increase of centrally administerted CRF-induced splenic sympathetic nerve activity (Katafuchi et al., 1997) seems to be due to a small amount and low efficacy of this reagent (100 μg/animal) to block the brain cyclooxgenase or differences in experimental procedures. The present study suggests a role of brain arachidonic acid cascade in the CRF-induced activation of central sympatho-adrenomedullary outflow.

The CRF-induced elevation of plasma adrenaline was abolished by centrally administered furegrelate. Furegrelate is a selective thromboxane synthase inhibitor (Gorman et al., 1983). Recently, we reported that the elevation of plasma adrenaline induced by centrally administered nitric oxide donor (3-morpholino-sydnonimine) or *N*-methyl-D-aspartate was abolished by centrally administered furegrelate, suggesting the involvement of brain thromboxane A_2 in the activation of central adrenomedullary outflow (Murakami et al., 1998; Okada et al., 2000). The present results also suggest the role of brain thromboxane A_2 in the CRF-induced activation of central adrenomedullary outflow. Although CRF-induced elevation of plasma adrenaline, but not noradrenaline, is abolished by centrally administered somatostatin analog, desAA^{1,2,4,5,12,13}-[D-Trp^{8}]somatostatin (Brown et al., 1982b), the relationship between thromboxane A_2-mediated selective activation of adrenomedullary outflow and somatostatin-mediated selective inhibition of adrenomedullary outflow is not apparent from the present results.

Previously, we reported that intracerebroventricularly administered prostaglandin (PG) E_2, but not PGI_2, PGD_2 and $PGF_{2\alpha}$, elevated plasma noradrenaline by activation of brain prostanoid EP_3 receptors in rats (Yokotani et al., 1988, 1995a, 1996). The elevation of plasma noradrenaline induced by centrally administered interleukin-1β or arachidonic acid was also abolished by centrally administered indomethacin (Murakami et al., 1996; Yokotani et al., 1995b, 2000). From this evidence, it seems likely that CRF-induced activation of central sympathetic outflow is mediated by brain PGE_2-mediated mechanisms.

In summary, we demonstrated here that activation of brain CRF_1 receptor primarily facilitates the central sympathetic and adrenomedullary outflow in distinct central pathways in rats: brain thromboxane A_2 is involved in the central adrenomedullary outflow; an active metabolite of arachidonic acid other than thromboxane A_2 (probably prostaglandin E_2) may be involved in the central sympathetic outflow.

Acknowledgements

This work was supported in part by a grant from The President Research Fund of Kochi Medical School.

References

Anton, A.H., Sayre, D.F., 1962. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J. Pharmacol. Exp. Ther. 138, 360–375.

Bittencourt, J.C., Sawchenko, P.E., 2000. Do centrally administered neuropeptides access cognate receptors?: an analysis in the central corticotropin-releasing factor system. J. Neurosci. 20, 1142–1156.

Brown, M.R., Fisher, L.A., Rivier, J., Spiess, J., Rivier, C., Vale, W., 1982a. Corticotropin-releasing factor: effects on the sympathetic nervous system and oxygen consumption. Life Sci. 30, 207–210.

Brown, M.R., Fisher, L.A., Spiess, J., Rivier, C., Rivier, J., Vale, W., 1982b. Corticotropin-releasing factor: actions on the sympathetic nervous system and metabolism. Endocrinology 111, 928–931.

Brown, M.R., Fisher, L.A., Webb, V., Vale, W.W., Rivier, J.E., 1985. Corticotropin-releasing factor: a physiologic regulator of adrenal epinephrine secretion. Brain Res. 328, 355–357.

Chalmers, D.T., Lovenberg, T.W., De Souza, E.B., 1995. Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. J. Neurosci. 15, 6340–6350.

Chalmers, D.T., Lovenberg, T.W., Grigoriadis, D.E., Behan, D.P., De Souza, E.B., 1996. Corticotrophin-releasing factor receptors: from molecular biology to drug design. Trends Pharmacol. Sci. 17, 166–172.

Chang, C.P., Pearse, R.V., O'Connell, S., Rosenfeld, M.G., 1993. Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. Neuron 11, 1187–1195.

Chen, R., Lewis, K.A., Perrin, M.H., Vale, W.W., 1993. Expression cloning of a human corticotropin-releasing-factor receptor. Proc. Natl. Acad. Sci. U. S. A. 90, 8967–8971.

Dieterich, K.D., Lehnert, H., De Souza, E.B., 1997. Corticotropin-releasing factor receptors: an overview. Exp. Clin. Endocrinol. Diabetes 105, 65–82.

Fisher, L.A., Rivier, J., Rivier, C., Spiess, J., Vale, W., Brown, M.R., 1982. Corticotropin-releasing factor (CRF): central effects on mean arterial pressure and heart rate in rats. Endocrinology 110, 2222–2224.

Gorman, R.R., Johnson, R.A., Spilman, C.H., Aiken, J.W., 1983. Inhibition of platelet thromboxane A_2 synthase activity by sodium 5-(3′-pyridinylmethyl)benzofuran-2-carboxylate. Prostaglandins 26, 325–342.

Gottowik, J., Goetschy, V., Henriot, S., Kitas, E., Fluhman, B., Clerc, R.G., Moreau, J.L., Monsma, F.J., Kilpatrick, G.J., 1997. Labelling of CRF_1 and CRF_2 receptors using the novel radioligand, [^{3}H]-urocortin. Neuropharmacology 36, 1439–1446.

Insel, P.A., 1996. Analgesic-antipyretic and antiinflammatory agents and drugs employed in the treatment of gout. In: Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W., Gilman, A.G. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics. McGraw-Hill, New York, NY, pp. 617–658.

Jansen, A.S., Nguyen, X.V., Karpitskiy, V., Mettenleiter, T.C., Loewy, A.D., 1995. Central command neurons of the sympathetic nervous system: basis of the fight-or-flight response. Science 270, 644–646.

Jeong, K.-H., Jacobson, L., Pacák, K., Widmaier, E.P., Goldstein, D.S., Majzoub, J.A., 2000. Impaired basal and restraint-induced epinephrine secretion in corticotropin-releasing hormone-deficient mice. Endocrinology 141, 1142–1150.

Katafuchi, T., Ichijo, T., Hori, T., 1997. Sequential relationship between actions of CRF and PGE2 in the brain on splenic sympathetic nerve activity in rats. J. Auton. Nerv. Syst. 67, 200–206.

Kishimoto, T., Pearse, R.V., Lin, C.R., Rosenfeld, M.G., 1995. A sauvagine/corticotropin-releasing factor receptor expressed in heart and skeletal muscle. Proc. Natl. Acad. Sci. U. S. A. 92, 1108–1112.

Kurosawa, M., Sato, A., Swenson, R.S., Takahashi, Y., 1986. Sympatho-adrenal medullary functions in response to intracerebroventricularly injected corticotropin-releasing factor in anesthetized rats. Brain Res. 367, 250–257.

Lovenberg, T.W., Liaw, C.W., Grigoriadis, D.E., Clevenger, W., Chalmers, D.T., De Souza, E.B., Oltersdorf, T., 1995. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc. Natl. Acad. Sci. U. S. A. 92, 836–840.

Lundkvist, J., Chai, Z., Teheranian, R., Hasanvan, H., Bartfai, T., Jenck, F., Widmer, U., Moreau, J.L., 1996. A non peptidic corticotropin releasing factor receptor antagonist attenuates fever and exhibits anxiolytic-like activity. Eur. J. Pharmacol. 309, 195–200.

Menzaghi, F., Heinrichs, S.C., Pich, E.M., Weiss, F., Koob, G.F., 1993. The role of limbic and hypothalamic corticotropin-releasing factor in behavioral responses to stress. Ann. N. Y. Acad. Sci. 697, 142–154.

Murakami, Y., Yokotani, K., Okuma, Y., Osumi, Y., 1996. Nitric oxide mediates central activation of sympathetic outflow induced by interleukin-1 beta in rats. Eur. J. Pharmacol. 317, 61–66.

Murakami, Y., Yokotani, K., Okuma, Y., Osumi, Y., 1998. Thromboxane A_2 is involved in the nitric oxide-induced central activation of adrenomedullary outflow in rats. Neuroscience 87, 197–205.

Okada, S., Murakami, Y., Nishihara, M., Yokotani, K., Osumi, Y., 2000. Perfusion of the hypothalamic paraventricular nucleus with *N*-methyl-D-aspartate produces thromboxane A_2 and centrally activates adrenomedullary outflow in rats. Neuroscience 96, 585–590.

Okuma, Y., Yokotani, K., Osumi, Y., 1996. Brain prostaglandins mediate the bombesin-induced increase in plasma levels of catecholamines. Life Sci. 59, 1217–1225.

Paxinos, G., Watson, C., 1986. In: Paxinos, G., Watson, C. (Eds.), The Rat Brain in Stereotaxic Coordinates. Academic Press, Boston.

Perrin, M.H., Donaldson, C.J., Chen, R., Lewis, K.A., Vale, W.W., 1993. Cloning and functional expression of a rat brain corticotropin releasing factor (CRF) receptor. Endocrinology 133, 3058–3061.

Perrin, M., Donaldson, C., Chen, R., Blount, A., Berggren, T., Bilezikjian, L., Sawchenko, P., Vale, W., 1995. Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart. Proc. Natl. Acad. Sci. U. S. A. 92, 2969–2973.

Schulz, D.W., Mansbach, R.S., Sprouse, J., Braselton, J.P., Collins, J., Corman, M., Dunaiskis, A., Faraci, S., Schmidt, A.W., Seeger, T., Seymour, P., Tingley, F.D., Winston, E.N., Chen, Y.L., Heym, J., 1996. CP-154,526: a potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. Proc. Natl. Acad. Sci. U. S. A. 93, 10477–10482.

Stenzel, P., Kesterson, R., Yeung, W., Cone, R.D., Rittenberg, M.B., Stenzel-Poore, M.P., 1995. Identification of a novel murine receptor for corticotropin-releasing hormone expressed in the heart. Mol. Endocrinol. 9, 637–645.

Swanson, L.W., Sawchenko, P.E., 1980. Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. Neuroendocrinology 31, 410–417.

Swanson, L.W., Sawchenko, P.E., Rivier, J., Vale, W.W., 1983. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology 36, 165–186.

Vale, W., Spiess, J., Rivier, C., Rivier, J., 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213, 1394–1397.

Van Zwieten, P.A., Hendriks, M.G., Pfaffendorf, M., Bruning, T.A., Chang, P.C., 1995. The parasympathetic system and its muscarinic receptors in hypertensive disease. J. Hypertens. 13, 1079–1090.

Vaughan, J., Donaldson, C., Bittencourt, J., Perrin, M.H., Lewis, K., Sutton, S., Chan, R., Turnbull, A.V., Lovejoy, D., Rivier, C., Rivier, J., Sawchenko, P.E., Vale, W., 1995. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature 378, 287–292.

Vita, N., Laurent, P., Lefort, S., Chalon, P., Lelias, J.M., Kaghad, M., Le Fur, G., Caput, D., Ferrara, P., 1993. Primary structure and functional expression of mouse pituitary and human brain corticotrophin releasing factor receptors. FEBS Lett. 335, 1–5.

Yokotani, K., Yokotani, K., Okuma, Y., Osumi, Y., 1988. Sympathoadrenomedullary system mediation of the prostaglandin E2-induced central inhibition of gastric acid output in rats. J. Pharmacol. Exp. Ther. 244, 335–340.

Yokotani, K., Nishihara, M., Murakami, Y., Hasegawa, T., Okuma, Y., Osumi, Y., 1995a. Elevation of plasma noradrenaline levels in urethane-anaesthetized rats by activation of central prostanoid EP3 receptors. Br. J. Pharmacol. 115, 672–676.

Yokotani, K., Okuma, Y., Osumi, Y., 1995b. Recombinant interleukin-1 beta inhibits gastric acid secretion by activation of central sympatho-adrenomedullary outflow in rats. Eur. J. Pharmacol. 279, 233–239.

Yokotani, K., Okuma, Y., Osumi, Y., 1996. Inhibition of vagally mediated gastric acid secretion by activation of central prostanoid EP3 receptors in urethane-anaesthetized rats. Br. J. Pharmacol. 117, 653–656.

Yokotani, K., Wang, M., Murakami, Y., Okada, S., Hirata, M., 2000. Brain phospholipase A_2-arachidonic acid cascade is involved in the activation of central sympatho-adrenomedullary outflow in rats. Eur. J. Pharmacol. 379, 341–347.