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Role of brain thromboxane A_2 in the release of noradrenaline and adrenaline from adrenal medulla in rats

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Abstract

Plasma noradrenaline reflects the release from adrenal medulla and sympathetic nerves; however, the exact mechanisms of adrenal noradrenaline release remain to be elucidated. The present study was designed to characterize the source of plasma noradrenaline induced by centrally administered vasopressin and corticotropin-releasing hormone (CRH) in urethane-anesthetized rats. Intracerebroventricularly administered vasopressin (0.2 nmol/animal) and CRH (1.5 nmol/animal) elevated plasma levels of noradrenaline and adrenaline. Intracerebroventricularly administered indomethacin [1.2 μmol (500 μg)/animal] (an inhibitor of cyclooxygenase) abolished the elevations of both noradrenaline and adrenaline induced by vasopressin and CRH. Intracerebroventricularly administered furegrelate [1.8 μmol (500 μg)/animal] (an inhibitor of thromboxane A_2 synthase) abolished the elevations of both noradrenaline and adrenaline induced by vasopressin, while the reagent only attenuated the elevation of plasma adrenaline evoked by CRH. Acute bilateral adrenalectomy abolished the elevation of both noradrenaline and adrenaline induced by vasopressin, while the procedure reduced only the elevation of adrenaline induced by CRH. These results suggest that the release of noradrenaline from adrenal medulla and sympathetic nerves is mediated by different central mechanisms. The vasopressin-induced noradrenaline release from adrenal medulla is mediated by brain thromboxane A_2-mediated mechanisms, while the CRH-induced noradrenaline release from sympathetic nerves is mediated by brain prostanoid (other than thromboxane A_2)-mediated mechanisms. The vasopressin- and CRH-induced adrenaline release from adrenal medulla is also mediated by brain thromboxane A_2-mediated mechanisms in rats.
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1. Introduction

The relative importance of sympathetic nerve activity and adrenomedullary secretion in various physiological situations has generally been inferred from measurement of plasma noradrenaline and adrenaline. Noradrenaline and adrenaline have overlapping, but essentially distinct, roles: noradrenaline is the more potent vasoconstrictor, while adrenaline is responsible for metabolic actions such as raising the blood glucose level. Many studies indicate that the ratio between plasma noradrenaline and adrenaline is not fixed. For instance, hypoglycemia causes the elevation of plasma adrenaline (Young et al., 1984; Fujino and Fujii, 1995; Vollmer et al., 1997), while hypotension elevates both catecholamines (noradrenaline>adrenaline) (Brown and Fisher, 1984; Vollmer et al., 2000). Likewise, several neuropeptides, for example, bombesin, corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone and calcitonin gene-related peptide, act within the central nervous system to produce differential changes in the relative concentrations of noradrenaline and adrenaline in plasma (Brown et al., 1979; Fisher et al., 1983; Brown and Fisher, 1984; Brown et al., 1985; Hasegawa et al., 1993; Okuma et al., 1996; Yokotani et al., 2001).

Adrenal medullary chromaffin cells have been shown to secrete noradrenaline as well as adrenaline (Folkow and von Euler, 1954; Vollmer et al., 1997; Yokotani et al., 2002). In both humans and rats, noradrenaline and adrenaline are localized in separate populations of cells with the number of adrenaline-containing cells about 4-fold greater than the number of noradrenaline-containing cells (Verhofstad et al., 1985). Despite the constancy of the stored amounts of

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noradrenaline and adrenaline, the proportion of each catecholamine released seems to vary depending on the strength and type of stimulus, suggesting a separate control of noradrenaline- and adrenaline-containing cells (Vollmer, 1996). Some studies emphasized the mechanisms that would lead to differential release of catecholamines to be dependent on differences in receptors presented in each cell type (Vollmer et al., 1988; Choi et al., 1993; Montiel, 1997) and in neurotransmitters released from the adrenal branch of the splanchnic nerves (Malhotra and Wakade, 1987; Wakade et al., 1991). Recently, it has been reported that the noradrenaline-containing cells and adrenaline-containing cells are innervated by separate groups of preganglionic neurons in the spinal cord (Edwards et al., 1996); however, relatively little is known about the differentially controlling mechanisms of these neurons.

The brain arachidonic acid released by brain phospholipase A_2 is metabolized rapidly to oxygenated products by several distinct enzymes, including cyclooxygenase, prostaglandin E synthase and thromboxane A synthase (Flower and Blackwell, 1976; Irvine, 1982; Axelrod, 1990). Recently, we reported that the brain arachidonic acid cascade is involved in the central regulation of the sympatho-adrenomedullary outflow in rats: intracerebroventricular (i.c.v.) administration of prostaglandin E_2 elevates plasma noradrenaline by activation of the brain prostanoid EP_3 receptors in rats (Yokotani et al., 1995; Murakami et al., 2002a,b), and that injection of thromboxane A_2 mimetic into the paraventricular nucleus of the hypothalamus elevates plasma adrenaline (Murakami et al., 2002a,b). These results suggest that the brain prostaglandin E_2 and thromboxane A_2 are involved in the central regulation of the sympatho-adrenomedullary outflow.

In the present study, we aimed to characterize the mechanisms involved in the adrenal release of noradrenaline and/or adrenaline evoked by vasopressin and CRH in relation to the 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. In some experiments, acute bilateral adrenalectomy or sham operation was done by an abdominal midline incision before each experiment. After these procedures, the animal was placed in a stereotaxic apparatus, as shown in our previous paper (Yokotani et al., 2001).

Three hours after the animal was placed in a stereotaxic apparatus, a stainless-steel cannula (0.35 mm outer diameter) or a double lumens cannula (0.50 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 were 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). Vasopressin and corticotropin-releasing hormone (CRH) were dissolved in sterile saline and slowly injected into the right lateral ventricle in a volume of 5 μl using a 50-μl Hamilton syringe. Water-soluble indomethacin–Na and furegrelate dissolved in sterile saline were also administered into the right lateral ventricle in a volume of 10 μl 60 min before the application of vasopressin or CRH. Correct placement of the cannula was confirmed at the end of each experiment by verifying that a blue dye, injected through the cannula, had spread throughout the entire ventricular system.

All experiments were conducted in compliance with the guiding principles for the care and use of laboratory animals approved by the Kochi Medical School.

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 high-performance liquid chromatography (Okada et al., 2000). 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-dihydroxybenzylamine 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 10 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-dihydroxybenzylamine, an internal standard. This assay could determine 0.5 pg of adrenaline and noradrenaline accurately.

2.3. Treatment of data and statistics

Results are expressed as the mean ± S.E.M. of the net changes above the respective basal values, because of individual variations. The data were analyzed with an unpaired Student's *t*-test. *P* values less than 0.05 were taken to indicate statistical significance.

2.4. Compounds

The following drugs were used: water-soluble indomethacin sodium trihydrate (a kind gift from Merck, Rahway, NJ, USA); furegrelate sodium (Biomol Research Lab., Plymouth Meeting, PA, USA); arginine–vasopressin (vasopressin), corticotropin-releasing hormone (rat/human) (Peptide Institute, Osaka, Japan). All other reagents were the highest grade available (Nacalai Tesque).

3. Results

3.1. Effect of indomethacin on the vasopressin- and CRH-induced elevation of plasma catecholamines

Intracerebroventricularly administered vehicle (10 μl plus 5 μl of saline/animal) and blood sampling five times over a 60- to 120-min period did not affect the basal plasma levels of either noradrenaline or adrenaline (Fig. 1). On the other hand, pretreatment with indomethacin (an inhibitor of cyclooxygenase) [1.2 μmol (500 μg)/animal, i.c.v.] slightly elevated the basal plasma level of noradrenaline and also slightly, but significantly, elevated the plasma levels of adrenaline (Fig. 1).

Previously we reported that vasopressin (0.1, 0.2 and 0.5 nmol/animal, i.c.v.) and CRH (0.5, 1.5 and 3.0 nmol/animal, i.c.v.) dose dependently elevated plasma levels of noradrenaline and adrenaline (Yokotani et al., 2001; Okada et al., 2002). In the present experiments, therefore, we used 0.2 nmol/animal of vasopressin and 1.5 nmol/animal of CRH.

Administration of vasopressin (0.2 nmol/animal, i.c.v.) quickly elevated plasma levels of noradrenaline and adrenaline (adrenaline>noradrenaline). These responses reached a maximum 5–10 min after the administration of the peptide and then declined toward their basal level (Fig. 1A). The vasopressin-induced elevation of both catecholamines was abolished by pretreatment with indomethacin (1.2 μmol/animal, i.c.v.).

Administration of CRH (1.5 nmol/animal, i.c.v.) gradually elevated plasma levels of noradrenaline and adrenaline (adrenaline = noradrenaline). These responses reached a maximum 60 min after the administration of the peptide (Fig. 1B). The CRH-induced elevation of both catecholamines was also abolished by pretreatment with indomethacin (1.2 μmol/animal, i.c.v.).

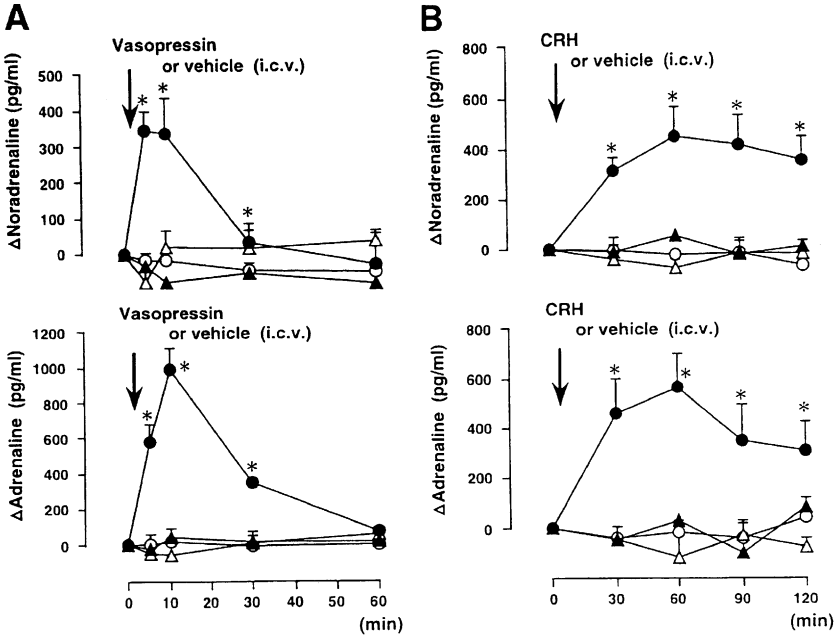

Fig. 1. Effect of indomethacin on the vasopressin- and CRH-induced elevation of plasma catecholamines. Δnoradrenaline and Δadrenaline; increase of noradrenaline and adrenaline above the basal. Indomethacin [1.2 μmol (500 μg)/animal, i.c.v.] or vehicle (10 μl saline/animal) was intracerebroventricularly administered 60 min before the administration of vasopressin (0.2 nmol/animal, i.c.v.) or CRH (1.5 nmol/animal). Arrow indicates i.c.v. administration of vehicle (5 μl saline/animal) or vasopressin in A, and vehicle or CRH in B. A: ○, vehicle plus vehicle ($n=6$); ●, vehicle plus vasopressin ($n=6$); △, indomethacin plus vehicle ($n=5$); ▲, indomethacin plus vasopressin ($n=7$). B: ○, vehicle plus vehicle ($n=5$); ●, vehicle plus CRH ($n=7$); △, indomethacin plus vehicle ($n=6$); ▲, indomethacin plus CRH ($n=6$). Each point represents the mean ± S.E.M. *Significantly different ($P<0.05$) from those treated with indomethacin plus vasopressin in A and indomethacin plus CRH in B. The actual values for noradrenaline and adrenaline at 0 min were 225 ± 22 and 195 ± 21 pg/ml ($n=24$) in vehicle-pretreated group and 301 ± 36 and 264 ± 29 pg/ml ($n=24$) in indomethacin-pretreated group, respectively.

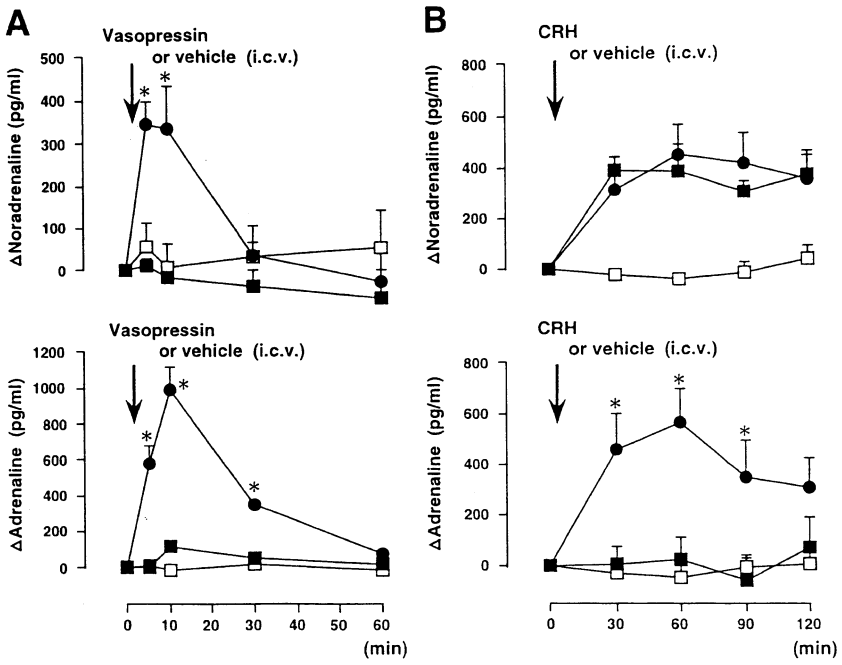

Fig. 2. Effect of furegrelate on the vasopressin- and CRH-induced elevation of plasma catecholamines. Furegrelate [1.8 μmol (500 μg)/animal, i.c.v.] or vehicle (10 μl saline/animal) was intracerebroventricularly administered 60 min before administration of vasopressin or CRH. Arrow indicates the administration of vehicle (5 μl saline/animal, i.c.v.) or vasopressin (0.2 nmol/animal, i.c.v.) in A, and vehicle or CRH (1.5 nmol/animal) in B. A: ●, vehicle plus vasopressin (cited from Fig. 1A); □, furegrelate plus vehicle ($n=5$); ■, furegrelate plus vasopressin ($n=6$). B: ●, vehicle plus CRH (cited from Fig. 1B); □, furegrelate plus vehicle ($n=6$); ■, furegrelate plus CRH ($n=6$). *Significantly different ($P<0.05$) from the group treated with furegrelate plus vasopressin in A and furegrelate plus CRH in B. Other conditions were the same as those of Fig. 1. The actual values for noradrenaline and adrenaline at 0 min were 275 ± 34 and 213 ± 38 pg/ml in the furegrelate-pretreated group ($n=23$), respectively.

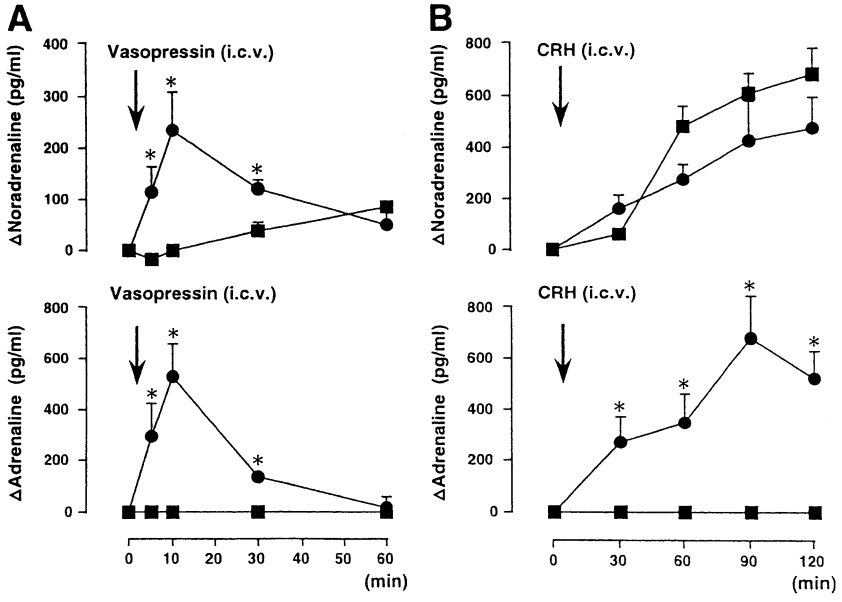

Fig. 3. Effect of acute bilateral adrenalectomy on the vasopressin- and CRH-induced elevation of plasma catecholamines. Arrow indicates the administration of vasopressin (0.2 nmol/animal, i.c.v.) in A and CRH (1.5 nmol/animal, i.c.v.) in B. A: ●, sham-operated group ($n=6$); ■, adrenalectomized group ($n=5$). B: ●, sham-operated group ($n=6$); ■, adrenalectomized group ($n=6$). *Significantly different ($P<0.05$) from those in adrenalectomized group. Other conditions were the same as those of Figs. 1 and 2. The actual values for noradrenaline and adrenaline at 0 min were 265 ± 20 and 223 ± 33 pg/ml in sham-operated group ($n=12$) and 264 ± 20 and 0 pg/ml in bilateral adrenalectomized group ($n=11$), respectively.

3.2. Effect of furegrelate on the vasopressin- and CRH-induced elevation of plasma catecholamines

Administration of furegrelate [1.8 μmol (500 μg)/animal, i.c.v.] had no effect on the basal plasma levels of both catecholamines (Fig. 2). Furegrelate completely abolished the vasopressin (0.2 nmol/animal, i.c.v.)-induced elevation of plasma noradrenaline and adrenaline (Fig. 2A). On the other hand, furegrelate abolished the CRH (1.5 nmol/animal, i.c.v.)-induced elevation of plasma adrenaline, but had no effect on the CRH-induced elevation of plasma noradrenaline (Fig. 2B).

3.3. Effect of bilateral adrenalectomy on the vasopressin- and CRH-induced elevation of plasma catecholamines

The basal plasma levels of noradrenaline and adrenaline were not influenced by sham operation. The basal plasma levels of noradrenaline were not influenced by bilateral adrenalectomy, while the basal plasma adrenaline was not detectable in bilaterally adrenalectomized group (Fig. 3A and B).

Vasopressin (0.2 nmol/animal, i.c.v.)-induced elevations of plasma noradrenaline and adrenaline were abolished by bilateral adrenalectomy (Fig. 3A). CRH (1.5 nmol/animal, i.c.v.)-induced elevation of plasma adrenaline was abolished by bilateral adrenalectomy; however, the peptide-induced elevation of plasma noradrenaline was not influenced by the procedure (Fig. 3B).

4. Discussion

Centrally administered vasopressin rapidly increased the plasma levels of noradrenaline and adrenaline, while centrally administered CRH gradually increased the plasma levels of both catecholamines. These time-related differences in the catecholamine responses to vasopressin and CRH seem to be due to the differences in their signal transductions in the brain. The CRH-induced elevation of catecholamines was attenuated by centrally administered L-N^6-(1-imino-3-butenyl)-ornithine [a selective inhibitor of inducible nitric oxide synthase (Babu and Griffith, 1998)] (Okada and Yokotani, 2002). Brain inducible nitric oxide synthase has been shown to be involved in the interleukin-1β-induced, gradually developing elevation of plasma noradrenaline in rats (Murakami et al., 1996, 2002a,b). In addition, centrally applied nitric oxide donor (3-morpholino-sydnonimine) elevates plasma catecholamines (Murakami et al., 1998).

The elevation of plasma noradrenaline and adrenaline induced by centrally administered vasopressin and CRH was abolished by central pretreatment with indomethacin, an inhibitor of the prostaglandin-forming cyclooxygenase (Insel, 1996). These results suggest the involvement of the brain arachidonic acid cascade in the vasopressin- and CRH-induced activation of the central sympatho-adrenomedullary outflow in rats. The elevation of plasma adrenaline and/or noradrenaline induced by centrally administered interleukin-1β or arachidonic acid has also been shown to be abolished by central pretreatment with indomethacin (Murakami et al., 1996; Yokotani et al., 2000).

Centrally administered prostaglandin E_2 has been shown to elevate plasma noradrenaline by activation of the brain prostanoid EP_3 receptors in rats (Yokotani et al., 1995; Murakami et al., 2002a,b). In addition, injection of thromboxane A_2 mimetic into the paraventricular nucleus of the hypothalamus selectively elevates plasma adrenaline (Murakami et al., 2002a,b). The hypothalamus, especially the paraventricular nucleus, has been considered to be the control center of the sympatho-adrenomedullary outflow (Swanson and Sawchenko, 1980; Jansen et al., 1995). These results suggest the involvement of brain prostaglandin E_2 and thromboxane A_2 in activation of the central sympatho-adrenomedullary outflow in rats.

Then, we examined the effect of furegrelate, a selective inhibitor of thromboxane A_2 synthase (Gorman et al., 1983) on the vasopressin- and CRH-induced elevation of plasma adrenaline. The vasopressin- and CRH-induced elevation of plasma adrenaline was abolished by centrally administered furegrelate. The elevation of plasma adrenaline evoked by centrally administered 3-morpholino-sydnonimine (a nitric oxide donor), *N*-methyl-D-aspartate and arachidonic acid has also been reported to be abolished by centrally administered furegrelate (Murakami et al., 1998; Okada et al., 2000; Yokotani et al., 2000). These results further support the possibility that brain thromboxane A_2 is involved in the vasopressin- and CRH-induced activation of the central adrenomedullary outflow.

On the other hand, centrally administered furegrelate also abolished the vasopressin-induced elevation of plasma noradrenaline, but did not affect the CRH-induced elevation of plasma noradrenaline. These results suggest a different source of plasma noradrenaline (adrenal medulla or sympathetic nerve terminals) evoked by these peptides. To refine this hypothesis, we examined the effect of acute bilateral adrenalectomy on the centrally administered vasopressin- and CRH-induced elevation of plasma catecholamines.

Bilateral adrenalectomy abolished the centrally administered vasopressin- and CRH-induced elevations of plasma adrenaline, indicating the release of adrenaline from the adrenal medulla. On the other hand, bilateral adrenalectomy abolished the vasopressin-induced elevation of plasma noradrenaline, while the CRH-induced elevation of plasma noradrenaline was not influenced by this procedure. These results suggest that the release of noradrenaline from the adrenal medulla and sympathetic nerve terminals is differentially controlled by these peptides: vasopressin evokes the release of noradrenaline from the adrenal medulla, while CRH evokes the release of noradrenaline from the sympathetic nerve terminals in rats.

In summary, we demonstrated here that the vasopressin-induced release of noradrenaline from the adrenal medulla is mediated by brain thromboxane A_2-mediated mechanisms, while the CRH-induced release of noradrenaline from sympathetic nerve terminals is mediated by a brain prostanoid other than thromboxane A_2 (probably prostaglandin E_2). The release of adrenaline from the adrenal medulla by vasopressin and CRH is also mediated by the brain thromboxane A_2-mediated mechanisms in rats.

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