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Brain cyclooxygenase and prostanoid TP receptors are involved in centrally administered epibatidine-induced secretion of noradrenaline and adrenaline from the adrenal medulla in rats

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ABSTRACT

Plasma adrenaline mainly originates from adrenaline-containing cells in the adrenal medulla, whereas plasma noradrenaline reflects not only the release from sympathetic nerves but also the secretion from noradrenaline-containing cells in the adrenal medulla. The present study was undertaken to examine the mechanisms involved in centrally administered epibatidine (a potent agonist of nicotinic acethylcholine receptors)-induced elevation of plasma catecholamines with regard to the brain prostanoid. Intracerebroventricularly (i.c.v.) administered epibatidine (1, 5 and 10 nmol/animal) effectively elevated plasma noradrenaline and adrenaline. The epibatidine (5 nmol/animal, i.c.v.)-induced elevation of both catecholamines was attenuated by hexamethonium (an antagonist of nicotinic acethylcholine receptors) (0.9 and 1.8 μmol/animal, i.c.v.), indomethacin (an inhibitor of cyclooxygenase) (0.6 and 1.2 μmol/animal, i.c.v.) and (+)-S-145 (an antagonist of prostanoid TP receptors) (0.6 and 1.3 μmol/animal, i.c.v.), and abolished by acute bilateral adrenalectomy. On the other hand, intravenously administered epibatidine (5 nmol/animal) was largely ineffective on the plasma levels of catecholamines, and intravenous pretreatment with hexamethonium (1.8 μmol/animal) had no effect on the epibatidine (5 nmol/animal, i.c.v.)-induced elevation of both catecholamines. These results suggest that centrally administered epibatidine activates the brain nicotinic acethylcholine receptors, thereby evoking the secretion of noradrenaline and adrenaline from the adrenal medulla by brain cyclooxygenase- and prostanoid TP receptor-mediated mechanisms in rats.

1. Introduction

Smoking is a leading cause of cardiovascular diseases including high blood pressure (Lakier, 1992) and nicotine is one of the components of cigarette smoke. The effects of peripherally administered nicotine on the sympatho-adrenomedullary system have been clearly shown to evoke the release of noradrenaline and adrenaline from sympatho-adrenomedullary system by activation of peripheral nicotinic acethylcholine receptors (Watts, 1960; Wang et al., 2000; Yokotani et al., 2001, 2002). Centrally administered nicotine also evokes pressor response and elevation of plasma catecholamine by activation of the brain nicotinic acethylcholine receptors in rats (Kiritsy-Roy et al., 1990; Buccafusco and Yang, 1993; Tseng et al., 1993, 1994). However, the effect of microinjected nicotine into the brain seems to vary according to the injected nuclei. Nicotine administered into the rostral ventrolateral medulla increases blood pressure and renal sympathetic nerve activity (Tseng et al., 1993, 1994), whereas nicotine administration into the nucleus tractus solitarius induces hypotension, probably by an enhancement of inhibitory baroreflex (Tseng et al., 1993, 1994; Ashworth-Preece et al., 1998). Chronic treatment of nicotine is able to intensify and accelerate the development of hypertension in spontaneously hypertensive rats (Bui et al., 1994; Ferrari and Fior-Chadi, 2007), in which the central dysregulation of sympatho-adrenomedullary outflow has been suggested to be involved (Barron and Van Loon, 1989; Wyss and Carlson, 2001; Guyenet, 2006). However, the precise mechanisms of this alkaloid-induced central modulation of the sympatho-adrenomedullary outflow are largely undefined.

We recently reported that centrally administered stress-related neurotransmitters such as vasopressin, bombesin and histamine elicit adrenal secretion of both noradrenaline and adrenaline from noradrenaline- and adrenaline-containing cells in the adrenal medulla via the brain thromboxane A_2-mediated mechanisms, whereas centrally administered corticotropin-releasing factor (CRF) and glucagon-like peptide 1 (GLP-1) elicits adrenaline secretion from adrenal adrenaline-containing cells and noradrenaline release from sympathetic nerves via the brain thromboxane A_2- and prostaglandin E_2-mediated mechanisms, respectively, in rats (Okada et al., 2003; Yokotani et al., 2005; Shimizu et al., 2006; Arai et al., 2008). In the present study, therefore, we examined the mechanisms involved in

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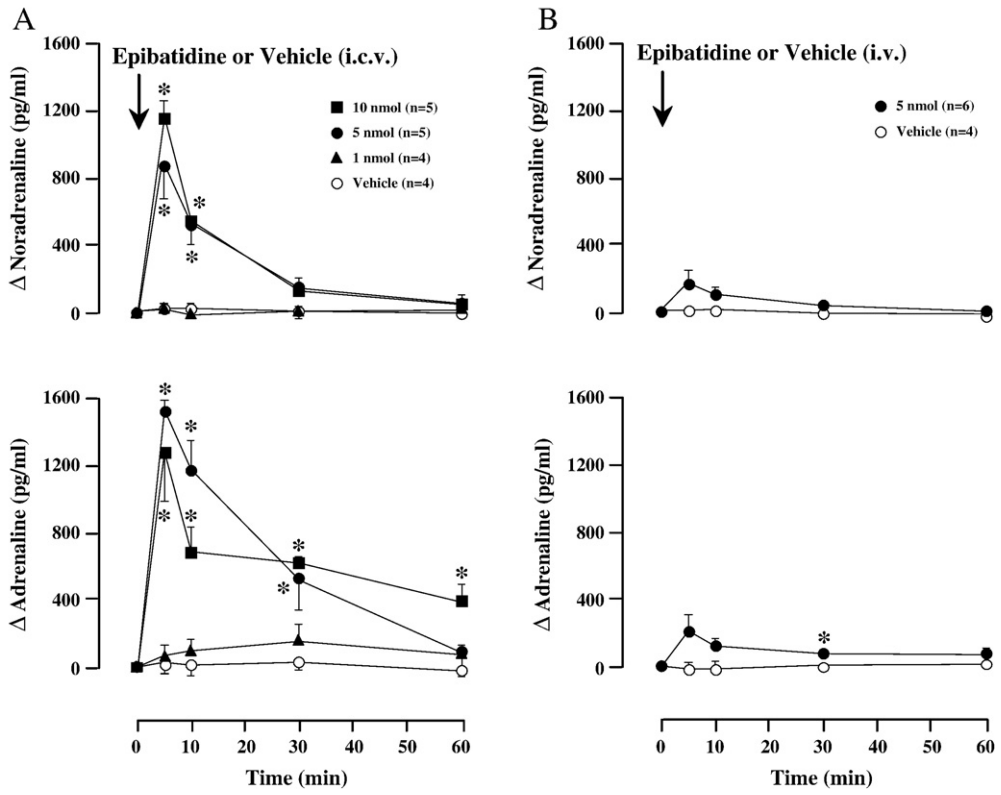

Fig. 1. Effect of centrally and peripherally administered epibatidine on the plasma levels of catecholamines. ΔNoradrenaline and ΔAdrenaline: increments of noradrenaline and adrenaline above the basal level. Each point represents the mean ± S.E.M. (A) Arrow indicates the administration of vehicle (DMF 2.5 μl/animal, i.c.v.) or epibatidine (1, 5 and 10 nmol/animal, i.c.v.). *Significantly different from the vehicle-treated group with the Bonferroni method [noradrenaline; at 5 min, $F(3,14)=20.78$, $P<0.017$; at 10 min, $F(3,14)=13.07$, $P<0.017$: adrenaline; at 5 min, $F(3,14)=6.30$, $P<0.017$; at 10 min, $F(3,14)=7.75$, $P<0.017$; at 30 min, $F(3,14)=4.53$, $P<0.017$; at 60 min, $F(3,13)=5.85$, $P<0.017$]. The actual values for noradrenaline and adrenaline at 0 min were 186 ± 22 and 211 ± 35 pg/ml ($n=18$), respectively. (B) Arrow indicates the administration of vehicle (0.5% DMF/saline 500 μl/animal, i.v.) or epibatidine (5 nmol/animal, i.v.). *Significantly different from the vehicle-treated group with Welch's t-test [adrenaline; at 30 min, $F(3,5)=32.3$, $P<0.05$]. The actual values for noradrenaline and adrenaline at 0 min were 146 ± 9 and 176 ± 48 pg/ml ($n=10$), respectively.

the centrally administered epibatidine (a potent agonist of nicotinic acethylcholine receptors)-induced elevation of plasma catecholamines with regard to the brain prostanoids using urethane-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), epibatidine or hexamethonium, and the femoral artery was cannulated for collecting blood samples. In some experiments, acute bilateral adrenalectomy [plus hydrocortisone (5 mg/kg, i.m.)] or sham-operation (plus 200 μl saline/animal, i.m.) was done just before these cannulations into the femoral artery and vein by an abdominal midline incision (Yokotani et al., 2005; Shimizu et al., 2006; Sasaki et al., 2008). After these procedures, the animal was placed in a stereotaxic apparatus, as shown in our previous papers (Yokotani et al., 1995; Shimizu et al., 2004). The skull was drilled for intracerebroventricular administration of test substances using a stainless-steel cannula (0.3 mm outer diameter). The stereotaxic coordinates of the tip of the cannula were as follows (in mm): AP −0.8, L 1.5, V 4.0 (AP, anterior from the bregma; L, lateral from the midline; V, below the surface of the brain), according to the rat brain atlas (Paxinos and Watson, 1986). Three hours were allowed to elapse before the application of epibatidine or blocking reagents.

Epibatidine dissolved in 100% *N*,*N*-dimethylformamide (DMF) was slowly injected into the right lateral ventricle in a volume of 2.5 μl/animal using a 10-μl Hamilton syringe. Each animal received only one dose of epibatidine or vehicle. Hexamethonium, water-soluble indomethacin-Na and (+)-S-145 dissolved in sterile saline were intracerebroventricularly (i.c.v.) administered in a volume of 5 μl/animal using a 10-μl Hamilton syringe. When blocking reagents were used, epibatidine was i.c.v. administered 30 min after the application of hexamethonium or indomethacin-Na and 60 min after the application of (+)-S-145, due to their slightly elevating effects on the basal plasma levels of catecholamines. When epibatidine was injected intravenously (i.v.), the epibatidine solution (500 μl) dissolved in 0.5% DMF in saline was slowly injected via a cannula inserted into the femoral vein. Intravenous administration of hexamethonium was also carried out via the cannula in a volume of 500 μl saline/animal. Each animal also received only one dose of blocking reagents or vehicle.

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

2.2. Measurement of plasma catecholamines

Blood samples (250 μl) were collected through an arterial catheter and were preserved on ice during experiments. Plasma was prepared immediately after the final sampling. Catecholamines in the plasma were extracted by the method of Anton and Sayre (1962) with a slight modification and were assayed electrochemically with high performance liquid chromatography (HPLC) (Shimizu et al., 2004). Briefly, after centrifugation (1500 *g* for 10 min, at 4 °C), the plasma (100 μl) was transferred to a centrifuge tube containing 30 mg of activated alumina,