Endothelin 1 (ET-1) is a vasoactive peptide that acts via ET-A receptors coupling inducing vascular smooth muscle cell proliferation and contraction. ET-1 is involved in the development and maintenance of hypertension.

Aim of this study was to determine the contribution of Ras farnesyl transferase, mitogen activated protein kinase (MAP kinase) and cytochrome P-450 (CYP450) metabolites to ET-1 induced hypertension.

ET-1 (5 pmol/kg per minute) was chronically infused into the jugular vein by use of mini-osmotic pump for 9 days in male Sprague-Dawley rats. Mean arterial blood pressure (MABP) in ET-1-treated rats was 154±2 mm Hg (hypertensive rats) compared with 132±5 mm Hg in control (normotensive) rats. Infusion of Ras farnesyl transferase inhibitor FPTIII (138 ng/min), MAP kinase inhibitor PD-98059 (694 ng/min) and CYP450 inhibitor 17-ODYA (189 ng/min) significantly attenuated MABP to 148±4.5 mm Hg, 133±5 mm Hg and 141±4.5 mm Hg, respectively. These results suggest that CYP-450 metabolites and Ras/MAP kinase pathway contribute to the development of ET-1 induced hypertension. Further investigation has to be done to confirm whether activation of RAS/MAP kinase pathway by arachidonic acid metabolites plays an important role in the development of ET-1 induced hypertension.
inhibitor PD-98059 (694 ng/min) treated group, 5) ET-1 plus CYP450 inhibitor 17-ODYA (189 ng/min) treated group. Endothelin-1 was obtained from Amersham. Ras farnesyl transferase inhibitor FPTIII, MAP kinase inhibitor PD-98059 and CYP450 inhibitor 17-ODYA were obtained from New England Biolabs.

Methods
Endothelin-1 induced hypertension and blood pressure measurement
ET-1 (5 pmol/kg per minute) was chronically infused into the jugular vein by use of mini-osmotic pump (Alzet model 2002) for 9 days in male Sprague-Dawley rats. Catheters (PE-60) connected to a mini-osmotic pump were inserted into the jugular veins under isoflurane anesthesia. Inhibitors of Ras farnesyl transferase FPTIII (138 ng/min), MAP kinase PD-98059 (694 ng/min), COX indomethacin (145 ng/min), LO baicalein, (124 ng/min) and CYP450 17-ODYA (189 ng/min) were administered by a mini-osmotic pump. Animals were anesthetized with sodium pentobarbital (60 mg/kg), and left femoral artery was exposed surgically. A small incision was made in the femoral artery. A catheter was inserted and connected to a pressure transducer (Grass model 7m, Grass Instruments) for blood pressure measurement. Blood pressure was recorded on a polygraph (model 7D; Grass polygraph). Mean arterial blood pressure (MABP) was expressed as mm Hg.

Statistical analysis
Values are expressed as mean ± SEM. The data were analyzed by 1-way ANOVA, and difference between the means for multiple comparisons was determined by the Newman-Keuls test and a value difference of p<0.05 was considered statistically significant.

RESULTS
Chronic infusion of ET-1 at the rate of 5 pmol/kg per minute in conscious rats for 9 days significantly increased MABP (154±2 mm Hg versus 98±3 mm Hg in the control group, (p<0.001). Infusion of Ras farnesyl transferase inhibitor FPTIII (138 ng/min), MAP kinase inhibitor PD-98059 (694 ng/min) during the ET-1 treatment significantly attenuated MABP to 115±2.5 mm Hg, 109±3 mm Hg (p<0.01) (Figure 1). To determine whether activities of enzymes cyclooxygenase (COX), lipoxigenase (LO) and cytochrome P-450 (CYP-450) and their metabolites are involved in ET-1 induced hypertension, rats were infused with inhibitors of COX (indomethacin, 145 ng/min), LO (baicalein, 124 ng/min) and CYP-450 17-ODYA (189 ng/min) during the treatment by ET-1. CYP450 inhibitor, but not COX and LO inhibitors, attenuated significantly MABP to 118±1.5 mm Hg (p<0.01) (Figure 2).

DISCUSSION
It has been previously reported that Ras/MAP kinase pathway activation by CYP-450 metabolites contributes to the development of DOCA-Salt-induced hypertension [9]. The present study demonstrates that Ras/MAP kinase pathway activated probably by CYP-450 metabolites contributes to the development of ET-1 induced hypertension, as well. Other vasoactive agents such as Norepinephrine (NE) and angiotensin II (ANG II) increase CaM kinase II and cPLA2 activities and thus release arachidonic acid [11]. The metabolites of arachidonic acid, such as 12- and 15-HETE made via cytochrome P-450 and lipoxigenase, through Ras/MEK/MAPK pathway activation contribute to vascular smooth muscle cell proliferation induced by NE or ANG II [12, 13, 14]. ET-1 promotes vascular smooth muscle
cell proliferation through extracellular signal-regulated kinase activation mediated by cPLA2 [6]. ET-1 is involved in the development and maintenance of hypertension [4, 5]. In our study, administration of ET-1 produced significant rise in MABP compared with that in control (normotensive) rats. Infusion of Ras farnesyl transferase inhibitor FPTIII, MAP kinase inhibitor PD-19802, and CYP450 inhibitor 17-ODYA, but not COX inhibitor indometacin or LO inhibitor baicalein, significantly attenuated MABP. Our findings suggest that ET-1 induced hypertension is mediated by CYP450 metabolites and Ras/MAP kinase pathway activation. Similar results have been reported in DOCA-Salt-, norepinephrine- and Angiotensin II-induced hypertension, as well [9,10,12].

CONCLUSION

The present study demonstrates that Ras/MAP kinase pathway activated probably by CYP450 metabolites contributes to the development of ET-1 induced hypertension. It seems to be common mechanism by which ET-1, ANG II and NE induce hypertension. Further investigation has to be done to confirm the activation of RAS/MAP kinase signal transduction pathway induced by different vasoactive agents by arachidonic acid metabolites is a universal mechanism in the development of hypertension.

DECLARATION OF INTEREST

Authors report no conflict of interest related to this study.

REFERENCES