Br. J. Pharmacol. (1994), **111**, 435–438

An endothelium-dependent contraction in canine mesenteric artery caused by caffeine

Hiroshi Jino, Hachiro Usui, Shinji Temma, Hiroaki Shirahase & [1]Kazuyoshi Kurahashi

Pharmacology Division, Radioisotope Research Center, Kyoto University, Kyoto, 606, Japan

1 We examined whether or not caffeine caused an endothelium-dependent contraction (EDC) in canine mesenteric artery and whether the endothelium-dependent contracting factors (EDCF) were arachidonic acid metabolites.

2 Caffeine (1, 3 and 10 mM) caused a transient contraction in endothelium-intact arterial strips. Removal of the endothelium significantly attenuated the caffeine (1 and 3 mM)-induced contraction.

3 Caffeine (1 mM)-induced EDC was not affected by quinacrine and manoalide (phospholipase A_2 inhibitors), indomethacin and aspirin (cyclo-oxygenase inhibitors), ONO-3078 and S-1452 (thromboxane A_2 antagonists) or AA-861 and TMK-777 (lipoxygenase inhibitors).

4 Caffeine (1 mM)-induced EDC was also unaffected by 50-235 (an endothelin A receptor antagonist). In addition, catalase combined treatment with superoxide dismutase, or allopurinol (antioxidant) did not affect the EDC.

5 Gro-PIP and NCDC (phospholipase C inhibitors) did not affect the caffeine-induced EDC. However, wortmannin (a phospholipase D inhibitor) and staurosporine (a protein kinase C inhibitor) attenuated the caffeine-induced EDC.

6 The present experiments demonstrate that caffeine causes an EDC in canine mesenteric artery and suggest that the EDCF mediating this response is probably not arachidonic acid metabolites, endothelin or superoxide. Instead, caffeine-induced EDC may be due to activation of the phospholipase D pathway.

Keywords: Canine mesenteric artery; caffeine-induced endothelium-dependent contraction; endothelium-derived contracting factor

Introduction

Furchgott & Zawadzki (1980) have reported that acetylcholine (ACh) produces an endothelium-dependent relaxation in rabbit thoracic aorta. Nitric oxide is considered to be one of the endothelium-derived relaxing factors (Palmer *et al.*, 1987). In contrast, we have reported that various vasoactive substances (ACh, Usui *et al.*, 1983; 1986; noradrenaline, Usui *et al.*, 1987; arachidonic acid, Shirahase *et al.*, 1987; ATP, Shirahase *et al.*, 1988b; nicotine, Shirahase *et al.*, 1988a; A-23187, Shirahase *et al.*, 1988c) produce an endothelium-dependent contraction (EDC) in canine basilar artery. Since such EDC is attenuated by phospholipase A_2 (PLA_2) inhibitors, cyclo-oxygenase inhibitors, thromboxane A_2 (TXA_2) synthetase inhibitors and TXA_2 antagonists, we have proposed that the endothelium-derived contracting factor (EDCF) is probably TXA_2 (Usui *et al.*, 1986; 1987; Shirahase *et al.*, 1987; 1988a,b,c).

Caffeine causes a transient contraction by releasing Ca^{2+} from the endoplasmic reticulum in skeletal and smooth muscle cells (Endo, 1977; Karaki & Weiss, 1988), but few authors have investigated whether caffeine causes an endothelium-dependent contraction.

We recently observed an EDC produced by caffeine in canine mesenteric, coronary and renal arteries, but not in the basilar artery (Jino *et al.*, 1992). The present experiments were undertaken to elucidate the pharmacological nature of caffeine-induced EDC and to determine whether the EDCF for caffeine-induced EDC in canine mesenteric artery is arachidonic acid metabolites, endothelin or superoxide.

Methods

Mongrel dogs of either sex (8–15 kg) were supplied by the Institute of Laboratory Animals at the Faculty of Medicine of Kyoto University (Kyoto, Japan). The animals were housed in steel cages at ambient temperature with a 12-h light/dark cycle and a humidity of about 60%. They were allowed free access to dog food and water. Mesenteric and basilar arteries were isolated from dogs after anaesthesia with ketamine (50 mg kg^{-1}, i.m.) and exsanguination from the common carotid artery. The isolated arteries were removed and cleaned of excess fat and connective tissue. Then the vessels were prepared as helically cut arterial strips (3 mm in width and 15 mm in length for mesenteric artery and 2 mm in width and 15 mm in length for basilar artery) with ligatures placed at both ends. One end of each strip was attached to a holder and the other to a strain gauge force-displacement transducer (Nihonkoden FD) which was connected to a polygraph (San-ei Instrument, 8S) on which isometric tension was recorded.

Each strip was then placed in a 10 ml tissue organ bath containing a physiological salt solution of the following composition (mM): NaCl 120, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 10. The bathing solution was maintained at 37 ± 0.5°C and was bubbled with 95% O_2 and 5% CO_2 (pH 7.4) throughout the experiments. An initial 1.5 g tension was applied to each strip and an equilibration period of 120 min was allowed before starting the experiments. The resting tension was readjusted periodically until equilibration was achieved. The endothelium was removed by rubbing the intimal surface. The presence of endothelium was initially confirmed by the ability of ACh (10^{-6} M) to cause relaxation during contraction induced by noradrenaline (NA) (10^{-6} M). Any strips that did not relax in response to ACh were discarded. After it was observed that the KCl (60 mM)-induced contraction was no different between endothelium-intact and endothelium-removed strips (so as to detect smooth muscle damage), caffeine was added to the organ bath. The experiments were carried out in parallel using both endothelium-intact and endothelium-denuded strips at the same time. Caffeine was added to the organ bath 3–4 times

[1] Author for correspondence.

with a washout interval of 30 min between additions. After the contractile response reached a steady level, various agents were added to the organ bath 20 min before the addition of caffeine.

Measurement of inositol-1,4,5-triphosphate

After arterial strips had been equilibrated for 120 min in normal Krebs-Henseleit solution, they were stimulated with caffeine (1, 3 and 10 mM) for 15 s and immediately frozen in liquid nitrogen. Frozen tissue samples were then weighed and homogenized in 6% trichloroacetic acid. After centrifugation of homogenate, the supernatant was extracted with diethyl ether to remove the trichloroacetic acid. The content of inositol-1,4,5-triphosphate (IP_3) was determined by a protein binding assay (TRK 1000; Amersham, Tokyo, Japan) according to the method of Langland & Diamond (1990) and was expressed as pmol mg^{-1} wet weight.

Drugs

Caffeine and indomethacin were purchased from Nacalai Tesque, Kyoto, Japan, manoalide from Wako Pure Chemical Industries Ltd., Kyoto, Japan, aspirin, 2-nitro-4-carboxyphenyl-*N*,*N*-diphenylcarbamate (NCDC), quinacrine, allopurinol, superoxide dismutase (SOD), catalase, L-α-glycerophospho-D-*myo*-inositol 4-monophosphate (Gro-PIP) and staurosporine from Sigma Chemical Co., St. Louis, MO, U.S.A. [9,11-Dimethyl-methane-11,12-methano-13,14-dihydro-13-aza-14-oxo-15 (β) cyclophenyl-ω-pentenor-TXA_2 L-arginine salt] (ONO-3708) was kindly provided by Ono Pharmaceutical Co. Ltd., Osaka, Japan, calcium (5Z)-1**R**,2S,-3S,4S-7 [3-phenylsulphonyl-aminobicyclo[2.2.1]hept-2yl]-5-heptenoate hydrate (S-1452) and myriceron caffeoyl ester (50-235) by Shionogi & Co., Osaka, Japan, 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA-861) by Takeda Chemical Industries Ltd., Osaka, Japan, 1-[{5'-(3''-methoxy-4''-ethoxycarbonyloxyphenyl)-2',4'-pentadienoyl} aminoethyl]-4-diphenylmethoxypiperidine (TMK-777) by Terumo Corp., Tokyo, Japan, and wortmannin by Sandoz, Basle, Switzerland.

Statistics

All results are expressed as means ± s.e.mean. Statistical analysis was performed using Student's paired *t* test, and the criterion of significance was a *P* value less than 0.05.

Results

Contractile responses to caffeine in endothelium-intact or endothelium-denuded strips in canine mesenteric artery

The endothelium-dependence of the contractile response to caffeine was examined in canine mesenteric artery. Caffeine (1, 3 and 10 mM) caused a transient contraction of endothelium-intact strips in a concentration-dependent manner (Figure 1). Repeated application of caffeine with a 30 min washout period caused reproducible transient contractions. Caffeine-induced contractions reached a stable level after 3–4 applications of caffeine over 2 h. The contraction produced by caffeine at 1, 3 and 10 mM resulted in a tension of 0.22 ± 0.02 g ($n = 12$), 0.50 ± 0.02 g ($n = 12$) and 0.76 ± 0.07 g ($n = 8$), respectively. As shown in Figure 2, removal of the endothelium significantly ($P < 0.05$) decreased the response to caffeine (1 and 3 mM), but did not affect contraction induced by caffeine (10 mM). Thus, part of contraction induced by caffeine (1 and 3 mM) was EDC and the caffeine (10 mM)-induced contraction was endothelium-independent (Figure 2). The EDC induced by caffeine (1 mM) was subsequently analysed in more detail.

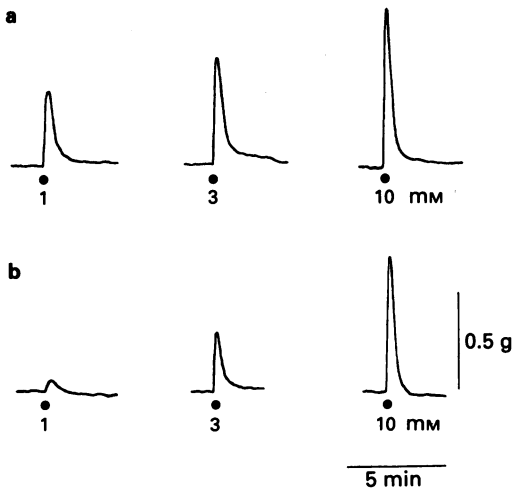

Figure 1 Representative tracings shown caffeine-induced contraction in canine mesenteric artery with (a) and without (b) endothelium.

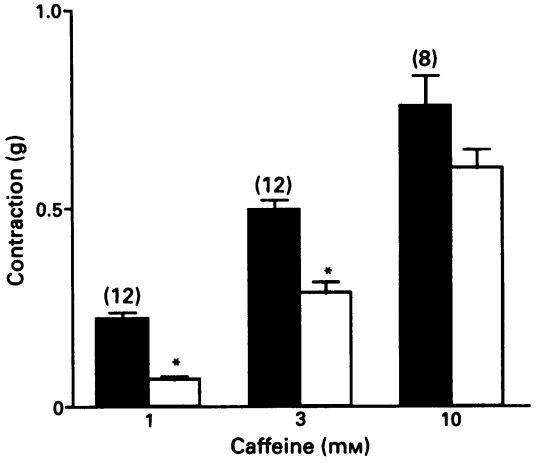

Figure 2 Contractile responses to caffeine (1, 3 and 10 mM) in endothelium-intact (solid columns) or endothelium-denuded (open columns) preparations in canine mesenteric artery. *Indicates a significant difference from the value in endothelium-intact preparations ($P < 0.05$). The number of experiments is shown in parentheses.

Effects of blocking agents on caffeine-induced endothelium-dependent contraction in canine mesenteric artery

We have previously reported that an EDC in canine basilar artery induced by vasoactive substances was attenuated by PLA_2 inhibitors, cyclo-oxygenase inhibitors, TXA_2 synthetase inhibitors, or TXA_2 antagonists (Usui *et al.*, 1986; Shirahase *et al.*, 1987). The effects of these inhibitors or antagonists on the caffeine-induced EDC were examined in the present study. Quinacrine (10^{-6} M) or manoalide (10^{-6} M) (PLA_2 inhibitors), aspirin (2×10^{-4} M) or indomethacin (10^{-6} M) (cyclo-oxygenase inhibitors) and ONO-3708 (10^{-8} M) or S-1452 (10^{-8} M) (TXA_2 antagonists) did not significantly attenuate the caffeine-induced EDC (Table 1). The same concentrations of aspirin and indomethacin augmented the caffeine-induced EDC and prolonged its duration. AA-861 (10^{-5} M) and TMK-777 (10^{-6} M) (5-lipoxygenase inhibitors) had no effect on the EDC. In contrast, ACh-induced EDC in the canine basilar artery was attenuated by quinacrine, manoalide, aspirin, indomethacin, ONO-3708 and S-1452 at the concentration indicated above (Table 1).

It has been reported that endothelin (Yanagisawa *et al.*, 1988) and superoxide (Katusic & Vanhoutte, 1989) are

Table 1 Effects of blocking agents on caffeine (1 mM)-induced EDC in canine mesenteric artery and acetylcholine (ACh, 10^{-7} M)-induced EDC in canine basilar artery

Agents	Concentration (M)	Contraction (%) Caffeine-EDC	ACh-EDC
Phospholipase A_2 inhibitors			
Manoalide	10^{-6}	99 ± 9 (4)	33 ± 8 (4)
	3×10^{-6}	76 ± 9 (5)	23 ± 5 (3)
Quinacrine	10^{-6}	86 ± 5 (3)	35 ± 5 (3)
Cyclo-oxygenase inhibitors			
Aspirin	2×10^{-4}	101 ± 8 (8)	22 ± 3 (3)
Indomethacin	10^{-6}	114 ± 11 (6)	20 ± 5 (3)
Thromboxane A_2 antagonists			
ONO-3708	10^{-8}	82 ± 5 (3)	10 ± 2 (3)
S-1452	10^{-8}	87 ± 8 (3)	15 ± 3 (3)
Lipoxygenase inhibitors			
AA 861	10^{-5}	82 ± 8 (3)	
TMK-777	10^{-6}	72 ± 6 (3)	
Endothelin antagonist			
50-235	10^{-5}	87 ± 8 (4)	
Antioxidants			
SOD (150 u ml^{-1}) + catalase (1000 u ml^{-1})		118 ± 9 (3)	
Allopurinol	10^{-5}	127 ± 7 (3)	

EDC in the absence of any agents was taken as 100%. The numbers in parentheses indicate the number of experiments. Abbreviations in text.

EDCFs. Therefore, we examined whether the caffeine-induced EDC was caused by the release of endothelin or superoxide. Caffeine-induced EDC was not affected by an endothelin A receptor antagonist, 50-235 (10^{-5} M), although the same concentration of this agent antagonized 80% of the endothelin-1 (10^{-8} M)-induced contraction. SOD (150 u ml^{-1}) treatment combined with catalase (1000 u ml^{-1}) or allopurinol (10^{-5} M) had no effect on the caffeine-induced EDC (Table 1).

Effects of phospholipase C inhibitors and a phospholipase D pathway inhibitor on caffeine-induced endothelium-dependent contraction in canine mesenteric artery

We examined whether caffeine-induced EDC was due to the activation of PLC and/or PLD. Gro-PIP (3×10^{-6} and 10^{-5} M) and NCDC (10^{-6} and 3×10^{-6} M) (PLC inhibitors) did not affect caffeine-induced EDC (Figure 3). In addition, caffeine (1, 3 and 10 mM) did not affect the content of IP_3 in endothelium-intact canine mesenteric arterial strips. The IP_3 content in the absence of caffeine was 4.8 ± 0.5 pmol mg^{-1} wet weight ($n = 5$). The content of IP_3 was not significantly altered by the presence of caffeine (1, 3 and 10 mM) and the respective IP_3 levels were 4.4 ± 0.5 pmol mg^{-1} wet weight ($n = 3$), 5.0 ± 1.0 pmol mg^{-1} wet weight ($n = 4$) and 3.0 ± 1.3 pmol mg^{-1} wet weight ($n = 4$). In contrast, wortmannin (3×10^{-7}, 10^{-6} and 3×10^{-6} M) (PLD pathway inhibitor) attenuated the caffeine-induced EDC with decreases of 12 ± 8% ($n = 5$), 41 ± 7% ($n = 5$) and 78 ± 7% ($n = 4$), respectively. However, wortmannin (3×10^{-7} M) did not affect the contraction induced by KCl (70 mM) or by NA (10^{-5} M). The inhibitory effect of wortmannin (10^{-6} M) on the caffeine-induced EDC was significantly ($P<0.05$) more potent than the effect on the contraction induced by KCl (70 mM) with a decrease of 19 ± 3% ($n = 3$) or NA (10^{-5} M) with a decrease of 11.2 ± 5% ($n = 3$). Staurosporine (10^{-8}, 3×10^{-8} and 10^{-7} M) (protein kinase C inhibitor) attenuated the caffeine-induced EDC, and the decreases were 16 ± 4% ($n = 5$), 33 ± 3% ($n = 7$) and 62 ± 4% ($n = 9$), respectively. However, staurosporine (10^{-8} M) did not affect the contraction induced by KCl (70 mM) or by NA (10^{-5} M). Staurosporine (3×10^{-8} M) attenuated the caffeine-induced EDC significantly ($P<0.05$) more potently than the contraction induced by KCl (70 mM) with a decrease of 15 ± 5% ($n = 3$) or NA (10^{-5} M) with a decrease of 2 ± 0.5% ($n = 3$).

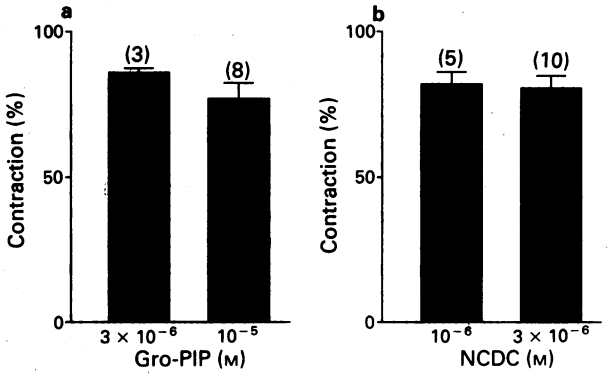

Figure 3 Effects of L-α-glycerophospho-D-*myo*-inositol 4-monophosphate (Gro-PIP, a) and 2-nitro-4-carboxyphenyl-*N*,*N*-diphenylcarbamate (NCDC, b) on caffeine (1 mM)-induced EDC in canine mesenteric artery. EDC in the absence of any agent was taken as 100%. The number of experiments is shown in parentheses.

Discussion

We have previously reported that vasoactive substances (ACh, NA, arachidonic acid, ATP, nicotine and A-23187) cause an EDC in canine basilar artery (Usui *et al.*, 1983; 1986; 1987; Shirahase *et al.*, 1987; 1988a,b,c). There have been few reports on EDC in peripheral arteries. The present study demonstrates that caffeine caused a transient contraction in endothelium-intact strips in canine mesenteric artery, while removal of the endothelium definitely decreased this caffeine-induced contraction. These results clearly indicate that caffeine caused an EDC, as has previously been reported in canine basilar artery. We examined whether the pharmacological nature of the caffeine-induced EDC in canine mesenteric artery was similar to ACh-induced EDC in canine basilar artery.

In canine basilar artery, EDCs induced by various vasoactive substances are attenuated by PLA_2 inhibitors, cyclo-oxygenase inhibitors, TXA_2 synthetase inhibitors and TXA_2 antagonists (Usui *et al.*, 1983; 1986; 1987; Shirahase *et al.*, 1987; 1988a,b,c). In addition, since the TXA_2 content of endothelium-intact preparation is higher than that of endothelium-denuded strips of the canine basilar artery, we have proposed that the EDCF for ACh-induced EDC is probably TXA_2 (Usui *et al.*, 1986; Shirahase *et al.*, 1987). In this study, caffeine-induced EDC was not affected by quinacrine and manoalide (PLA_2 inhibitors), aspirin and indomethacin (cyclo-oxygenase inhibitors), ONO-3708 and S-1452 (TXA_2 antagonists) and AA 861 and TMK-777 (lipoxygenase inhibitors). These results indicate that the EDCF for caffeine-induced EDC is probably not TXA_2 or any of the leukotrienes.

Subsequent to endothelin being identified by Yanagisawa *et al.* (1988), many investigators have reported that it is released from the endothelium of various arteries (Miller *et al.*, 1993) and have proposed that it is one of EDCFs. In addition, it has been reported that superoxide is an EDCF, since A-23187 causes an EDC which is blocked by combined treatment with SOD and catalase in the canine basilar artery (Katusic & Vanhoutte, 1989). However, the caffeine-induced EDC was not affected by 50-235 (an endothelin A receptor antagonist) (Fujimoto *et al.*, 1992) or by allopurinol (antioxidant) and SOD combined treatment with catalase. These results suggest that the caffeine-induced EDC is not due to the release of endothelin and/or the production of superoxide.

It has been reported that bradykinin increases the IP_3 and

cytosolic Ca^{2+} concentration in porcine endothelial cells through a mechanism involving bradykinin-induced hydrolysis of phosphatidylinositol 4,5-diphosphate via PLC activation (Lambert *et al.*, 1986). Therefore, we examined whether the caffeine-induced EDC involved PLC activation in the canine mesenteric artery. Gro-PIP (Cruz-Rivera *et al.*, 1990) and NCDC (Walenga *et al.*, 1980) are PLC inhibitors, and neither agent affected the caffeine-induced EDC. These results indicate that the caffeine-induced EDC is not due to the activation of PLC, and this view is further supported by the finding that caffeine did not increase IP_3 production. Recently, there has been increasing evidence suggesting that PLD plays an important role in intracellular signal transduction in bovine pulmonary artery and aortic endothelial cells (Martin, 1988; Pirotton *et al.*, 1990), primary cultured human abdominal aortic smooth muscle cells and rat aortic smooth muscle cells (Welsh *et al.*, 1990). Wortmannin inhibits the activation of PLD by a chemotactic peptide (formyl-Met-Leu-Phe) in human neutrophils (Reinhold *et al.*, 1990; Bonser *et al.*, 1991). Caffeine-induced EDC was attenuated by wortmannin in a concentration-dependent manner, suggesting that this EDC may involve the activation of the PLD pathway. This view is supported by the findings that staurosporine, a potent protein kinase C inhibitor (Tamaoki *et al.*, 1986), also attenuated the caffeine-induced EDC in a concentration-dependent manner. These results suggest that caffeine activates the PLD pathway to cause the EDC.

Thus, the present experiments suggest that caffeine causes an EDC by the activation of the PLD pathway in endothelial cells and/or smooth muscle cells. The EDCF for this caffeine-induced EDC is probably not arachidonic acid metabolites, endothelin or superoxide, although it may be diacylglycerol. In man, caffeine increases blood pressure by elevating vascular resistance rather than by enhancing cardiac output (Pincomb *et al.*, 1988), and the caffeine-induced EDC may play an important role in this enhancement of vascular resistance.

Supported by a grant from the Smoking Research Foundation, Japan.

References

BONSER, R.W., THOMPSON, N.T., RANDALL, R.W., TATESON, J.E., SPACEY, G.D., HADOSON, H.F. & GARLAND, L.G. (1991). Demethoxyviridin and wortmannin block phospholipase C and D activation in human neutrophil. *Br. J. Pharmacol.*, **103**, 1237–1241.

CRUZ-RIVERA, M., BENNETT, C.F. & CROOKE, S.T. (1990). Glycerol-3-phospho-D-*myo*-inositol 4-phosphate (Gro-PIP) is an inhibitor of phosphoinositide-specific phospholipase C. *Biochim. Biophys. Acta*, **1042**, 113–118.

ENDO, M. (1977). Calcium release from the sarcoplasmic reticulum. *Physiol. Rev.*, **57**, 71–108.

FUJIMOTO, M., MIHARA, S., NAKAJIMA, S., UEDA, M., NAKAMURA, M. & SAKURAI, K. (1992). A novel non-peptide endothelin antagonist isolated from bayberry, Myrica cerifera. *FEBS Letts.*, **305**, 41–44.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.

JINO, H., USUI, H., SHIRAHASE, H. & KURAHASHI, K. (1992). Caffeine causes an endothelium-dependent contraction in canine artery. *Med. Sci. Res.*, **20**, 169–170.

KARAKI, H. & WEISS, G.B. (1988). Calcium release in smooth muscle. *Life Sci.*, **42**, 111–121.

KATUSIC, Z.S. & VANHOUTTE, P.M. (1989). Superoxide anion is an endothelium-derived contracting factor. *Am. J. Physiol.*, **257**, H33–37.

LAMBERT, T.L., KENT, R.S. & WHORTON, A.R. (1986). Bradykinin stimulation of inositol polyphosphate production in porcine aortic endothelial cells. *J. Biol. Chem.*, **261**, 15288–15293.

LANGLAND, J.M. & DIAMOND, J. (1990). The effect of phenylephrine on inositol 1, 4, 5-trisphosphate levels in vascular smooth muscle measured using a protein binding assay system. *Biochem. Biophys. Res. Commun.*, **173**, 1258–1265.

MARTIN, T. (1988). Formation of diacylglycerol by a phospholipase D-phosphatidate phosphatase pathway specific for phosphatidylcholine in endothelial cells. *Biochim. Biophys. Acta*, **962**, 282–296.

MILLER, R.C., PELTON, J.T. & HUGGINS, J.P. (1993). Endothelins – from receptors to medicine. *Trends Pharmacol. Sci.*, **14**, 54–60.

PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.

PINCOMB, G.A., LOVALLO, W.R., PASSEY, R.B. & WILSON, M.F. (1988). Effect of behavior state on caffeine's ability to alter blood pressure. *Am. J. Cardiol.*, **61**, 798–802.

PIROTTON, S., ROBAYE, B., LAGNEAU, C. & BOEYNAEMS, J.M. (1990). Adenine nucleotides modulate phosphatidylcholine metabolism in aortic endothelial cells. *J. Cell. Physiol.*, **142**, 449–457.

REINHOLD, S.L., PRESCOTT, S.M., ZIMMERMAN, G.A. & MCINTYRE, T.M. (1990). Activation of human neutrophil phospholipase D by three separable mechanisms. *FASEB J.*, **4**, 208–214.

SHIRAHASE, H., USUI, H., KURAHASHI, K., FUJIWARA, M. & FUKUI, K. (1987). Possible role of endothelial thromboxane A_2 in the resting tone and contractile response to acetylcholine and arachidonic acid in canine cerebral arteries. *J. Cardiovasc. Pharmacol.*, **10**, 517–522.

SHIRAHASE, H., USUI, H., KURAHASHI, K., FUJIWARA, M. & FUKUI, K. (1988a). Endothelium-dependent contraction induced by nicotine in isolated canine basilar artery – possible involvement of a thromboxane A_2 (TXA_2) like substance. *Life Sci.*, **42**, 437–445.

SHIRAHASE, H., USUI, H., MANABE, K., KURAHASHI, K. & FUJIWARA, M. (1988b). Endothelium-dependent contraction and -independent relaxation induced by adenine nucleotides and nucleoside in the canine basilar artery. *J. Pharmacol. Exp. Ther.*, **247**, 1152–1157.

SHIRAHASE, H., USUI, H., MANABE, K., KURAHASHI, K. & FUJIWARA, M. (1988c). An endothelium-dependent contraction induced by A-23187, a Ca^{++} ionophore in canine basilar artery. *J. Pharmacol. Exp. Ther.*, **247**, 701–705.

TAMAOKI, T., NOMOTO, H., TAKAHASHI, I., KATO, Y., MORIMOTO, M. & TOMITA, F. (1986). Staurosporine, a potent inhibitor of phospholipid/Ca^{++} dependent protein kinase. *Biochem. Biophys. Res. Commun.*, **135**, 397–402.

USUI, H., FUJIWARA, M., TSUBOMURA, T., KURAHASHI, K., NOMURA, S. & MIZUNO, N. (1986). Possible involvement of prostanoids in cholinergic contractile response of canine cerebral artery. In *Neural Regulation of Brain Circulation*. ed. Owman, C. & Hardebo, J.E. pp. 261–272. Amsterdam: Elsevier.

USUI, H., KURAHASHI, K., ASHIDA, K. & FUJIWARA, M. (1983). Acetylcholine-induced contractile response in canine basilar artery with activation of thromboxane A_2 synthesis sequence. *Med. Sci. Res.*, **11**, 418–419.

USUI, H., KURAHASHI, K., SHIRAHASE, H., FUKUI, K. & FUJIWARA, M. (1987). Endothelium-dependent vasocontraction in response to noradrenaline in the canine cerebral artery. *Jpn. J. Pharmacol.*, **44**, 228–231.

WALENGA, R., VANDERHOEK, J.Y. & FEINSTEIN, M.B. (1980). Serine esterase inhibitors block stimulus-induced mobilization of arachidonic acid and phosphatidyl-inositide-specific phospholipase C activity in platelets. *J. Biol. Chem.*, **255**, 6024–6027.

WELSH, C.J., SCHMEICHEL, K., CAO, H. & CHABBOTT, H. (1990). Vasopressin stimulates phospholipase D activity against phosphatidylcholine in vascular smooth muscle cells. *Lipids*, **25**, 675–684.

YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **332**, 411–415.

(Received May 24, 1993
Revised September 24, 1993
Accepted October 6, 1993)