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Effect of captopril on the bradykinin-induced release of prostacyclin from guinea-pig lungs and bovine aortic endothelial cells

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1 In guinea-pig isolated lungs perfused with Krebs solution, captopril (10 μM) inhibited the metabolism of bradykinin (Bk) and the conversion of angiotensin I to angiotensin II, as measured by bioassay. Captopril significantly enhanced Bk-stimulated output of prostacyclin.

2 In bovine aortic endothelial cells grown on microcarrier beads, captopril (10 μM) did not affect the release of prostacyclin or of endothelium-derived relaxing factor (EDRF) induced by Bk.

3 Angiotensin I or angiotensin II did not release prostacyclin from guinea-pig isolated lungs or bovine aortic endothelial cells. They were also ineffective as releasers of EDRF from bovine aortic endothelial cells.

4 Thus, activation of angiotensin converting enzyme is not involved in the release of prostacyclin from guinea-pig isolated lungs or bovine aortic endothelial cells, or in release of EDRF from bovine aortic endothelial cells.

Introduction

Sawada *et al.* (1986) showed that captopril inhibited prostacyclin (PGI_2) release induced by bradykinin (Bk) or angiotensin I (AI) from cultures of human vascular endothelial cells. These authors suggested that PGI_2 generation could be caused by the breakdown of Bk or AI by increased angiotensin converting enzyme (ACE) activity induced by these peptides, as an autoregulatory mechanism.

de Nucci *et al.* (1986) showed that Bk-induced release of eicosanoids from isolated lungs of guinea-pigs exposed to pure oxygen for up to 96 h is increased, probably because of a decrease in Bk metabolism. Since this explanation conflicts with the concept proposed by Sawada, we investigated the effect of captopril on the release of PGI_2 by Bk, AI and angiotensin II (AII) in guinea-pig isolated lungs and endothelial cells (EC) from bovine aorta. In the latter, we also investigated the effect of captopril on release of endothelium-derived relaxing factor (EDRF) induced by Bk and adenosine diphosphate (ADP).

Some of the results were presented to the British Pharmacological Society (de Nucci *et al.*, 1988b).

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Methods

Isolated lungs

Guinea-pigs were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.). After mid-thoracotomy the pulmonary artery was cannulated and perfused for 5 min with 25 ml of heparinised Krebs solution. The lungs were then removed, placed in a jacketed chamber and perfused via the pulmonary artery with warmed (37°C) and gassed (95% O_2 + 5% CO_2) Krebs solution at a constant rate of 5 ml min^{-1}, and left to stabilize for 30 min (Piper & Vane, 1969).

Bradykinin (0.2 μM) was infused for 4 min and the lung effluent was collected, as described previously, for radioimmunoassay of eicosanoids (Bakhle *et al.*, 1985). Each set of lungs received a single infusion. Captopril (10 μM) was dissolved in water and infused for 30 min before challenge with bradykinin.

Endothelial cells

Endothelial cells were isolated by treatment of bovine aortae with 0.02% w/v collagenase. Cells were grown to confluence in plastic vessels and then

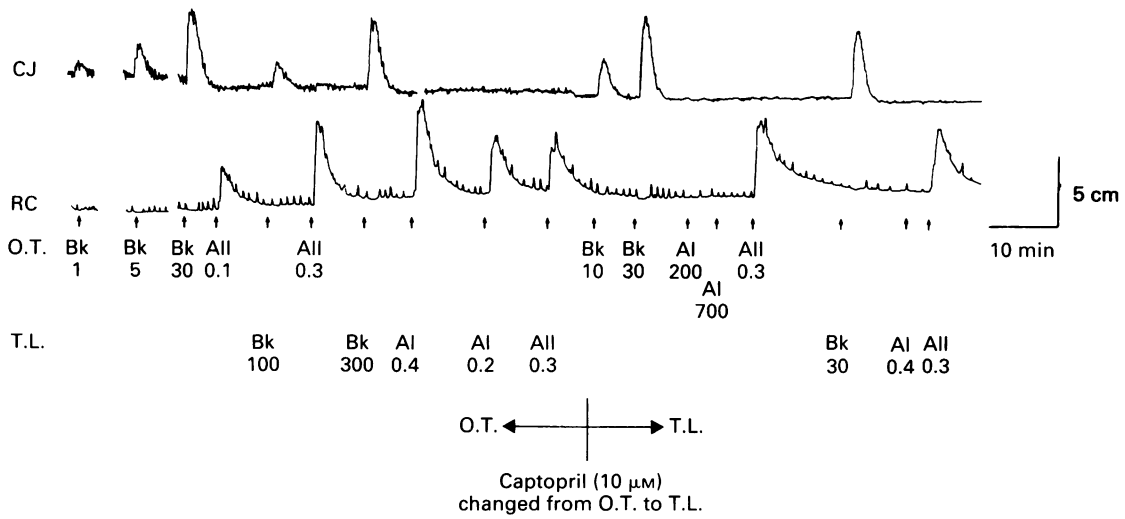

Figure 1 Effect of captopril infusion (10 μM) on angiotensin converting enzyme (ACE) activity as measured by bradykinin (Bk) and angiotensin I (AI) metabolism in guinea-pig isolated lungs. The lung effluent superfused longitudinal strips of cat jejunum (CJ) and rat colon (RC). Doses are expressed in pmol. Drugs were infused over the assay tissues (O.T.) or through the lungs (T.L.). At the point indicated, the captopril infusion was changed from O.T. to T.L. The same pattern of response was observed in 3 other experiments.

removed by treatment with 0.05% w/v trypsin and seeded onto Cytodex 3 microcarrier beads. The beads were stirred for 3–7 days until the cells became confluent (de Nucci *et al.*, 1988a). Then, 2–3 ml of beads covered with endothelial cells (10–20 × 10^6 cells) were packed into a jacketed column and perfused (5 ml min^{-1} at 37°C) with gassed (95% O_2 + 5% CO_2) Krebs buffer which contained superoxide dismutase (SOD, 10 units ml^{-1}).

Assay of prostacyclin

Prostacyclin, measured as 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$), in the lung effluent or in the column effluent was determined by specific radioimmunoassay (RIA) after suitable dilution (1:2–1:10) in RIA buffer but without prior extraction or purification. The specificity of the antisera used in these RIA has been established previously (Salmon, 1978).

Metabolism of bradykinin and angiotensin I

Bk and AII were measured by bioassay. The effluent from the lungs or columns of endothelial cells superfused longitudinal strips of cat jejunum for Bk assay (Ferreira & Vane, 1967) or rat colon for AII assay (Regoli & Vane, 1964). The assay tissues were treated with indomethacin (5.6 μM; Eckenfels & Vane, 1972). Contractions of the assay tissues were recorded with auxotonic levers (Paton, 1957), attached to Harvard isotonic transducers and displayed on a six channel Watanabe Recorder (type WR3101).

The metabolism of Bk and AI was calculated by comparing the contractions of the assay tissues in response to bolus injections or infusions of Bk, AI or AII directly over the tissues (O.T.) with those in response to injections or infusions given either through the lungs (T.L.) (Ferreira & Vane, 1967) or through the column of endothelial cells.

Assay of EDRF

EDRF was bioassayed as described by Gryglewski *et al.* (1986). The effluent from the column of endothelial cells superfused in cascade (Vane, 1964) four spirally cut rabbit aortic strips (RbAs) which were denuded of endothelium. Effluent from the column reached the consecutive RbAs after 1, 4, 7 and 10 s. Drugs were injected as a single bolus over the assay tissues (O.T.) as a control or through the column of endothelial cells (T.C.). Antagonists and superoxide dismutase (SOD) were given as infusions. The assay tissues were first superfused with Krebs solution containing U46619 (30–60 nM) until a stable contraction was evident. They were then calibrated by the relaxant effects of glyceryl trinitrate (GTN) O.T. and the sensitivities of the recordings of the 4 RbAs adjusted electronically to be roughly equal.

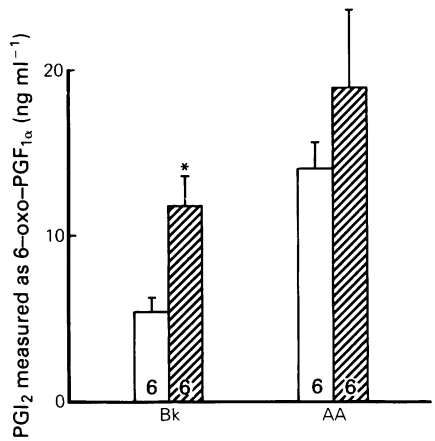

Figure 2 Effect of captopril infusion (10 μM) on prostacyclin release as measured by 6-oxo-prostaglandin $F_{1\alpha}$ (ng ml^{-1}) release induced by bradykinin (Bk, 0.2 μM) or arachidonic acid (AA, 13 μM) from guinea-pig isolated lungs ($n = 6$). Effects of captopril infused through the lungs (hatched columns) were compared with controls (open columns) without captopril. Statistical significance: * $P < 0.01$. Numbers inside the columns show the numbers of animals used.

Materials

The Krebs buffer had the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.17, $CaCl_2.6H_2O$ 2.5, $NaHCO_3$ 25 and glucose 8.4. Bradykinin triacetate salt, angiotensin I and angiotensin II acetate salts (Sigma Chemical Company, Poole, U.K.) were dissolved in saline. Indomethacin (Merck, Sharp & Dohme Ltd., Hertfordshire, U.K.) was dissolved in 5% (w/v) $NaHCO_3$ solution and diluted in Krebs solution before use. Arachidonic acid (Sigma) was stored in hexane solution (10 mg ml^{-1}) at -20°C. The sodium salt was obtained by evaporating the hexane under nitrogen, treating with NaOH (0.5 N, 0.5 ml) and diluting to the required concentration in 50 mM Tris buffer, pH 7.5 for immediate use. Captopril (Squibb) was dissolved in water. 6-oxo-5,6,8,9,11,14,15[^{3}H]-$PGF_{1\alpha}$ (specific activity 150 Ci mmol^{-1}) was purchased from Amersham International (Amersham, Bucks., U.K.). 6-oxo-$PGF_{1\alpha}$ was obtained from the Upjohn Co. (Kalamazoo, U.S.A.). The 6-oxo-$PGF_{1\alpha}$ antiserum was kindly provided by Dr J. Salmon, Wellcome Research Laboratories, U.K. U46619 9,11-dideoxy-9α,11α-methanoepoxyprostaglandin $F_{2\alpha}$; Upjohn Co., Kalamazoo, U.S.A.

Statistics

Results are shown as mean values $\pm$ s.e.mean for n experiments. Student's unpaired t test was used to determine the significance of differences between means and a P value of <0.05 was taken as significant.

Results

Angiotensin converting enzyme activity in guinea-pig isolated lungs

Captopril (10 μM) infused through the guinea-pig isolated lungs substantially inhibited the inactivation of bradykinin. At the same time the conversion of AI to AII was prevented (Figure 1).

Release of prostacyclin

Captopril (10 μM) did not affect the basal release of prostacyclin from guinea-pig lungs (control 0.2 ng ml^{-1}; captopril-treated 0.1 ng ml^{-1} 6-oxo-$PGF_{1\alpha}$, $n = 12$). However, it induced a significant increase of prostacyclin release from guinea-pig lungs stimulated with Bk. Captopril (10 μM) did not increase the release of prostacyclin when lungs were infused with arachidonic acid (AA) (Figure 2). Neither AI nor AII (5 μM) released detectable amounts of prostacyclin from guinea-pig isolated lungs.

Release of EDRF

Bk or adenosine 5'-diphosphate (ADP) released EDRF from the column of endothelial cells as detected by bioassay. Captopril (10 μM) had no effect on the release of EDRF induced by these agonists (Figure 3). The release of PGI_2 induced by Bk from endothelial cells was also unaffected by captopril (control: 9.6 ± 1.2 ng ml^{-1}; captopril treated: 11.3 ± 4.1 ng ml^{-1} 6-oxo-$PGF_{1\alpha}$, $n = 3$).

Angiotensin converting enzyme activity in bovine aortic endothelial cells

There was no removal of AI when it was infused through a column of naked cytodex beads (Figure 4). However, when endothelial cells were present in the column, a small conversion (less than 1%) of AI to AII was observed (Figure 4). Bk infused through a column of either naked beads or endothelial cells was substantially removed (50–60%).

Discussion

We have shown that inhibition of angiotensin converting enzyme (ACE) is associated with an increase

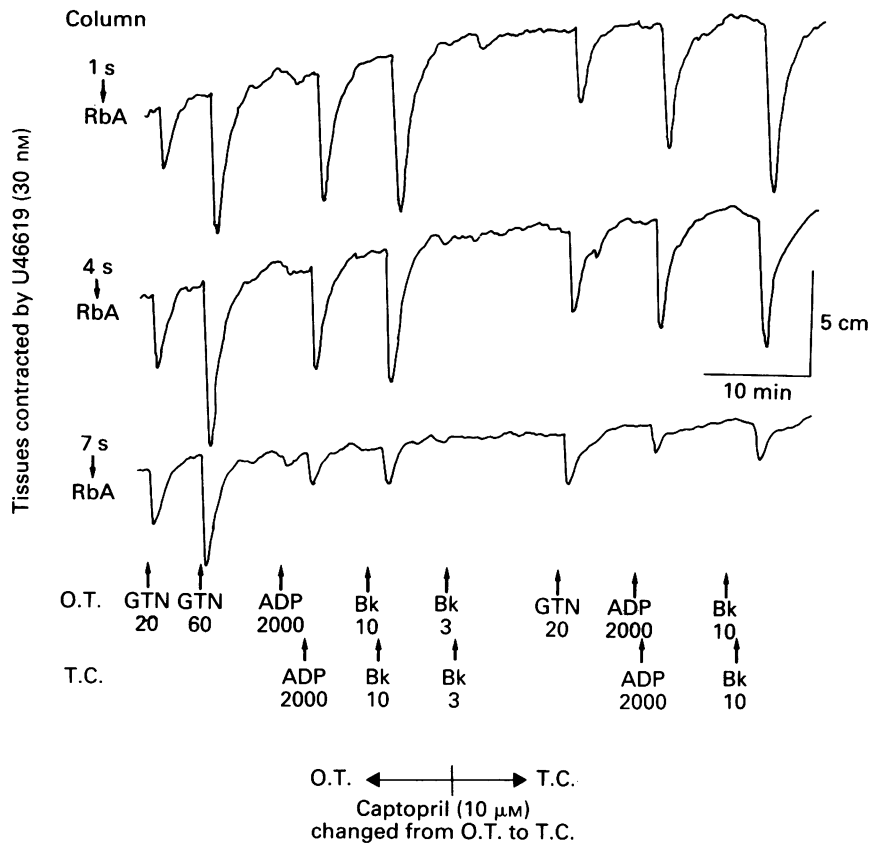

Figure 3 Lack of effect of captopril on the release of EDRF from bovine aortic endothelial cells. The effluent from a column of endothelial cells superfused strips of rabbit aortae (RbA). Bradykinin (Bk) and ADP when injected through the column (T.C.) induced release of EDRF as observed by the relaxation of the RbA. Captopril infused T.C. did not affect the release of EDRF by either Bk or ADP. The same pattern of responses was observed in 3 other experiments. Glyceryl trinitrate (GTN) was used as control. O.T., over the assay tissues. Doses are expressed in pmol. The captopril infusion was changed from O.T. to T.C. at the point indicated.

in eicosanoid output from guinea-pig lungs stimulated with Bk. This result supports the previous hypothesis that enhancement of eicosanoid release from lungs of guinea-pigs exposed to hyperoxia and stimulated with Bk was due to inhibition of ACE (de Nucci *et al.*, 1986). They are also in agreement with the results of Mullane & Moncada (1980) who showed that captopril potentiated the release of PGI_2 by Bk in dogs. The possibility that captopril enhances eicosanoid output by inhibition of prostaglandin dehydrogenase (Bakhle & Pankhania, 1987) is unlikely since it did not alter eicosanoid release after AA.

As previously shown (Gryglewski *et al.*, 1986; de Nucci *et al.*, 1988b) Bk causes release of both prostacyclin and EDRF from endothelial cells. Interestingly, captopril did not potentiate the effects of Bk in causing release of EDRF and prostacyclin in these cells. Alhenc-Galas (1982) also showed that captopril was without effect on prostacyclin production induced by Bk in human cultured endothelial cells. Endothelial cells produce ACE *in vitro* (Mendelsohn & Kachel, 1981), so that a potentiation of the effect of Bk by captopril might be expected, as seen in the guinea-pig isolated lungs. However, there are many more endothelial cells in a guinea-pig isolated lung (10^9) than in our column of endothelial cells (10^7). Thus, our bioassay system may not be sensitive enough to detect inhibition of ACE at this level, because of the very low doses of agonists used. Indeed, we observed conversion of AI by our endothelial cells, but less than 1% was converted by the endothelial cells as compared to over 90% by the isolated lungs.

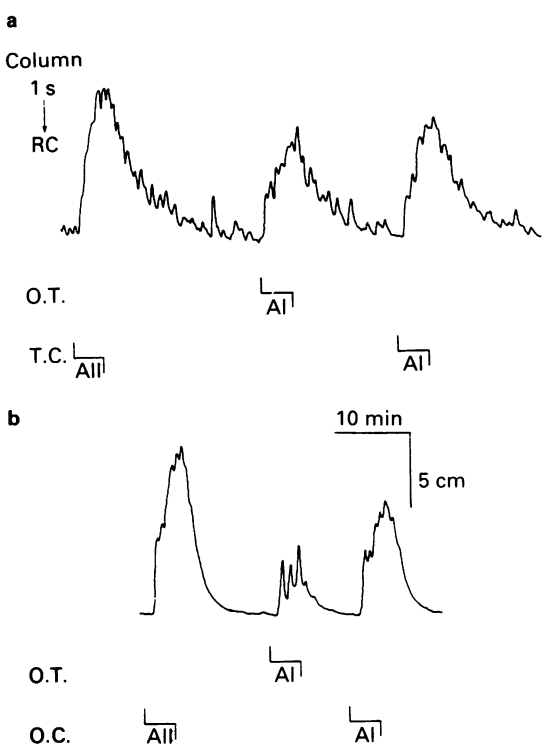

Figure 4 Conversion of angiotensin I (AI) to angiotensin II (AII) by bovine aortic endothelial cells. The effluent of a column of endothelial cells superfused a rat colon (RC). (a) (naked beads) Shows that AI (7.7×10^{-8} M) infused either over the tissues (O.T.) or through the column (T.C.), had the same contractile effect on the RC. (b) (beads with endothelial cells) Shows that AI infused T.C. provoked a larger contraction of the RC than infused O.T., indicating a small conversion to AII. AII 9.6×10^{-10} M was used to induce responses.

Another explanation for the lack of conversion could be that although ACE activity of lung endothelial cells is similar to that of endothelial cells from other vascular beds (Ody & Junod 1977; Johnson *et al.*, 1979), the ACE of our cells grown in culture may have been lost.

We were unable to detect stimulation of prostacyclin production by AII in our endothelial cells. Alhenc-Galas (1982) and Ody *et al.* (1983) also showed that AII had no effect on PGI_2 production by human or piglet cultured endothelial cells. These authors also failed to detect specific angiotensin receptors on the surface of intact cultured endothelial cells. Thus, it is likely that bovine endothelial cells in culture also lack receptors for angiotensin II.

In conclusion, our results show that ACE activity is not required for PGI_2 or EDRF release induced by Bk from bovine aortic endothelial cells. The lack of effect of captopril on the release of these mediators may be associated with the small number of endothelial cells used in these experiments or a loss of ACE activity.

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References

ALHENC-GALAS, F., TSAI, S.J., CALLAHAN, K.S., CAMPBELL, W.B. & JOHNSON, A.R. (1982). Stimulation of prostaglandin formation by vasoactive mediators in cultured human endothelial cells. *Prostaglandins*, **24**, 723–742.

BAKHLE, Y.S., MONCADA, S., DE NUCCI, G. & SALMON, J.A. (1985). Differential release of eicosanoids by bradykinin, arachidonic acid and calcium ionophore A23187 in guinea-pig isolated perfused lung. *Br. J. Pharmacol.*, **86**, 55–62.

BAKHLE, Y.S. & PANKHANIA, J.J. (1987). Inhibitors of prostaglandin dehydrogenase (PhCL28A and PhCK61A) increase output of prostaglandins from rat isolated lung. *Br. J. Pharmacol.*, **92**, 189–196.

DE NUCCI, G., ASTBURY, P., READ, N., SALMON, J.A. & MONCADA, S. (1986). Release of eicosanoids from isolated lungs of guinea-pigs exposed to pure oxygen: effect of dexamethasone. *Eur. J. Pharmacol.*, **126**, 11–20.

DE NUCCI, G., GRYGLEWSKI, R.J., WARNER, T.D. & VANE, J.R. (1988a). Receptor-mediated release of endothelium-derived relaxing factor (EDRF) and prostacyclin from bovine aortic endothelial cells is coupled. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 2334–2338.

DE NUCCI, G., WARNER, T.D. & VANE, J.R. (1988b). Effect of captopril on the release of prostacyclin induced by bradykinin (BK) from guinea-pig lungs or endothelial cells. *Br. J. Pharmacol.*, **93**, 105P.

ECKENFELS, A. & VANE, J.R. (1972). Prostaglandins, oxygen tension and smooth muscle tone. *Br. J. Pharmacol.*, **45**, 451–462.

FERREIRA, S.H. & VANE, J.R. (1967). The disappearance of bradykinin and eledoisin in the circulation and vascular beds of the cat. *Br. J. Pharmacol. Chemother.*, **30**, 417–424.

GRYGLEWSKI, R.J., MONCADA, S. & PALMER, R.M.J. (1986). Bioassay of prostacyclin and endothetium-derived relaxing factor (EDRF) from porcine aortic endothelial cells. *Br. J. Pharmacol.*, **87**, 685–694.

JOHNSON, A.R., NOGUEIRA, L.A. & ERVIN, E.G. (1979). Biochemical activities associated with vascular endothelial cells cultured from human vessel. *Abstracts of the Second World Congress for the Microcirculation*, July 22–29, La Jolla, California, p. 42.

MENDELSOHN, F.A.O. & KACHEL, C. (1981). Production of angiotensin converting enzyme by cultured bovine endothelial cells. *Clin. Exp. Pharmacol. Physiol.*, **61**, S277–280.

MULLANE, K.M. & MONCADA, S. (1980). Prostacyclin mediates the potentiated hypotensive effect of bradykinin following captopril treatment. *Eur. J. Pharmacol.*, **66**, 355–365.

ODY, C. & JUNOD, A.F. (1977). Converting enzyme activity in endothelial cells isolated from pig pulmonary artery and aorta. *Am. J. Physiol.*, **232**, C95–98.

ODY, C., SEILLAN, C., RUSSO-MARIE, F. & DUVAL, D. (1983). Angiotensin II does not elicit any specific prostaglandin secretion in piglet cultured endothelial cells. *Thromb. Res.*, **31**, 219–231.

PATON, W.D.M. (1957). A pendulum auxotonic lever. *J. Physiol.*, **137**, 35P.

PIPER, P.J. & VANE, J.R. (1969). Release of additional factors of anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature*, **223**, 29–35.

REGOLI, D. & VANE, J.R. (1964). A sensitive method for the assay of angiotensin. *Br. J. Pharmacol. Chemother.*, **23**, 351–359.

SALMON, J.A. (1978). A radioimmunoassay for 6-keto-prostaglandin $F_{1\alpha}$. *Prostaglandins*, **15**, 383–397.

SAWADA, S., TOYODA, T., TAKAMATSU, H., NIWA, I., MAEBO, N., TSUJI, H., NAKAGAWA, M. & INCHI, M. (1986). Prostacyclin generation by cultured human vascular endothelial cells with reference to angiotensin I-converting enzyme. *Jap. Circ. J.*, **50**, 242–247.

VANE, J.R. (1964). The use of isolated organs for detecting active substances in the circulating blood. *Br. J. Pharmacol. Chemother.*, **23**, 360–373.

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