Stimulation of cell-surface urokinase-type plasminogen activator activity and cell migration in vascular endothelial cells by a novel hexapeptide analogue of neurotensin

Shin Ushiro[a], Kazushige Mizoguchi[b], Shigeo Yoshida[a], Sei-ichiro Jimi[a], Tadami Fujiwara[b], Masaya Yoshida[b], Edward T. Wei[c], Patrick Kitabgi[d], Sakae Amagaya[b], Mayumi Ono[a], Michihiko Kuwano[a,*]

[a]*Department of Biochemistry, Kyushu University School of Medicine, Maidashi, Fukuoka 812-82, Japan*
[b]*Preclinical Research Department, Central Research Laboratories, Tsumura Co., Ibaraki 300-11, Japan*
[c]*Department of Toxicology, University of California School of Public Health, Berkeley, CA 94720, USA*
[d]*Institut de Pharmacologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique, 660, Route des lucioles, Sophia Antipolis, 06560 Valbonne, France*

Received 8 September 1997; revised version received 24 October 1997

Abstract To investigate if neurotensin (NT) could induce activation of urokinase-type plasminogen activator (uPA) in vascular endothelial cells, we utilized the acetyl-NT (8–13) analogue, TJN-950, in which the C-terminal leucine is reduced to leucinol. TJN-950 inhibited the binding of ^{125}I-NT to membranes of newborn rat brains and of COS-7 cells transfected with rat NT receptor cDNA, but at 10^4 higher doses than NT (8–13). However, TJN-950 was as effective as NT in inducing the fibrinolytic activity in bovine vascular aortic and human umbilical vein endothelial cells, and enhanced the migration of vascular endothelial cells. Moreover, administration of TJN-950 induced neovascularization in the rat cornea in vivo. TJN-950 had no effect on expression of uPA, plasminogen activator inhibitor-1 or uPA receptor mRNA. The binding of ^{125}I-TJN-950 to cell membranes was blocked by unlabeled uPA and TJN-950, but not the amino-terminal or 12–32 fragment of uPA. TJN-950 may enhance uPA activity in vascular endothelial cells by interacting with the uPA receptor, resulting in induction of angiogenesis.

Key words: Neurotensin analogue; Plasminogen activator; Endothelial cell migration

1. Introduction

Neurotensin (NT) is a tridecapeptide that was originally isolated from extracts of the bovine hypothalamus [1,2]. In mammals, NT is widely distributed throughout the central nervous system and digestive tract [3,4]. The biological activities have been shown to reside in the carboxy-terminal hexapeptide of NT [5]. NT has been found to interact with at least two specific membrane receptors. The high affinity receptor, NTR1, is expressed in the brain, intestine and blood mononuclear cells, whereas the low affinity, levocavastine sensitive receptor, NTR2, is expressed in brain and heart [6]. Binding of NT to its high affinity receptors has been reported to be coupled to intracellular calcium release [7].

NT and its acetylated fragment, acetyl-NT (8–13), showed dual activities of decreasing blood pressure and inhibiting vascular leakage/edema formation in peripheral tissues [8–10], but these activities were mediated through independent pathways [11]. We produced a novel analogue of *N*-acetyl-NT hexapeptide, TJN-950 whose C-terminal leucine is reduced, inhibiting the ability of this peptide to bind to the NT receptor, and we found that TJN-950 showed a specific inhibition of vascular permeability without any effect on blood pressure (unpublished data). To investigate whether TJN-950 could modulate uPA activities in vascular endothelial cells, we have now examined the effects of TJN-950 on the activation of uPA, on the migration of human and bovine vascular endothelial cells, as well as on the angiogenesis in the rat cornea.

2. Materials and methods

2.1. Materials

TJN-950 is a hexapeptide analogue of *N*-acetyl-NT (8–13) whose C-terminal leucine is reduced to leucinol. This analogue was supplied by Tsumura Co., Ltd. (Tokyo, Japan). NT was purchased from Peptide Institute, Inc. (Osaka, Japan). Mono[125]iodo[Tyr^3]NT (^{125}I-NT; 200 mCi/mmol) was prepared by McFarlane's procedure. Human high molecular weight uPA and ATF were purchased from American Diagnostica, Inc. (Greenwich, USA). Human uPA [12–32(Ala^{19})] [12] was synthesized by the Peptide Institute, Inc. (Osaka, Japan).

All other chemicals were obtained from commercial sources.

2.2. Iodination of TJN-950

To iodinate TJN-950, 1 μg of this substance was dissolved in 10 μl of 400 mM sodium acetate buffer (pH 5.6) and 2 μl of iodine-125 (100 mCi/ml; Amersham, Tokyo, Japan) and 5 μl of lactoperoxidase (83.3 μg/ml) were added. Three 5 μl aliquots of 0.003% H_2O_2 were added at 5 min intervals. Iodination was terminated by the addition of 35% acetonitrile containing 0.1% trifluoroacetic acid. The mixture was injected onto a TSK gel ODS-80 column (4.6 mm×15 cm, Tosoh Corp., Tokyo, Japan) and purified by HPLC using a mobile phase of 20% acetonitrile, 0.1% trifluoroacetic acid and monitoring with a UV detector coupled to a gamma counter. Fractions counting radioactivity were collected, frozen and dried, and the iodinated peptide was reconstituted in 50 mM Tris-HCl (pH 7.5), 1 mM EGTA, 2 mM

*Corresponding author. Fax: (81) (92) 632-4198.

Abbreviations: acetyl-NT (8–13), *N*-acetylated neurotensin fragment (8–13); ATF, amino-terminal fragment of uPA; BAE, bovine aortic endothelial; bFGF, basic fibroblast growth factor; DMEM, Dulbecco's modified Eagle medium; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HUVE, human umbilical vein endothelial; NT, neurotensin; NTR1 and NTR2, neurotensin receptor 1 and 2; NT (8–13), neurotensin fragment (8–13); PA, plasminogen activator; PAI-1, plasminogen activator inhibitor-1; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; uPA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor

PII S0014-5793(97)01403-8

$MgSO_4$, 10 μM 1,10-phenanthroline. Specific radioactivity was calculated from spectrophotometric and radioactivity measurements.

2.3. Cell culture

BAE cells isolated from bovine aortas [13,14] were cultured in DMEM (Nissui, Tokyo) supplemented with 10% FBS, 100 U/ml penicillin, and 60 μg/ml kanamycin. HUVE cells (Kurabou Co., Osaka, Japan) were cultured in the appropriate medium.

BAE cells were homogenized by suspending 5×10^9 cells in 10 volumes 50 mM Tris-HCl (pH 7.5), 1 mM EGTA, 2 mM $MgSO_4$, 0.002% soybean trypsin inhibitor, 10 μM leupeptin, 10 μM aprotinin in a teflon-glass homogenizer. The membrane fraction was prepared by centrifugation, its protein concentration was measured [15], and it was stored at −80°C. Whole brain homogenate from 7-day-old rats was prepared as described [16].

2.4. Transfection of COS-7 cells

The *Hin*dIII-*Not*I fragment encoding rat NTR1 [17] was ligated into the *Hin*dIII-*Not*I cloning site of the CDM8 vector and transfected into COS-7 cells by the DEAE-dextran precipitation method. Membrane fractions were prepared 48–72 h after transfection as described above.

2.5. Binding assay

The binding of ^{125}I-NT to membranes of newborn rat brains and COS-7 cells was performed at 26°C in 50 mM Tris-HCl buffer (pH 7.5) containing 0.2% bovine serum albumin and 1 mM 1,10-phenanthroline in the presence of 0.05–0.10 nM ^{125}I-NT. Binding of ^{125}I-TJN-950 to BAE cell membranes was performed at 20°C in 50 mM Tris-HCl (pH 7.5), 1 mM EGTA, 2 mM $MgSO_4$, 10 μM 1,10-phenanthroline, and 0.3 nM ^{125}I-TJN-950. Total, non-specific, and specific binding were measured at equilibrium by the filtration technique [18].

2.6. Cell migration assay and fibrin zymography

The cell migration assay and fibrin zymography were performed as described previously [14,19].

2.7. Northern blot analysis

Northern blot analysis was performed as described [20]. Total RNA was isolated, separated by electrophoresis in 1% agarose, 2.2 M formaldehyde gels and blotted onto Hybond-N^+ (Amersham) membranes. The membranes were hybridized with ^{32}P-labeled mouse uPA cDNA [14], bovine PAI-1 cDNA [21] and human uPA receptor (uPAR) cDNA (the kind gift of K. Matsuo, Taiho Research Center) probes, and then with a human GAPDH probe (Japanese Cancer Research Resource Bank). Hybridization was visualized by autoradiography, and densitometric analysis was performed using a BAS 2000 Fujix imaging analyzer (Fujix, Tokyo).

2.8. Corneal pocket assay

The corneal assay was performed essentially as described [22]. Briefly, 5 μl hydron pellets (Interferon Sciences, New Brunswick, NJ) containing 250 nmol TJN-950 were implanted into the cornea of anesthetized rats. After 7 days, the animals were killed, and corneal vessels were photographed. Six rats were used to asses the effect of TJN-950.

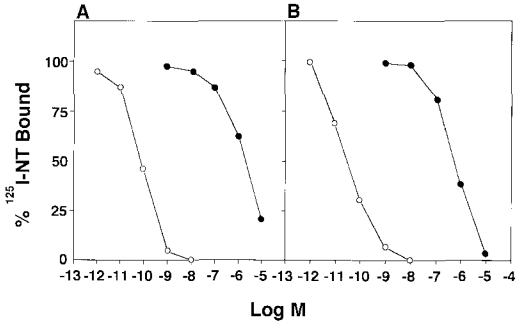

Fig. 1. Binding characteristics of TJN-950 and NT (8–13) for high affinity rat NT receptors. A: Membranes from newborn rat brains. B: Membranes from COS-7 cells transfected with high affinity rat NTR (NTR1) cDNA. Binding of ^{125}I-NT was performed in the absence or presence of TJN-950 (●) or NT (8-13) (○). Each value is the mean of three independent experiments.

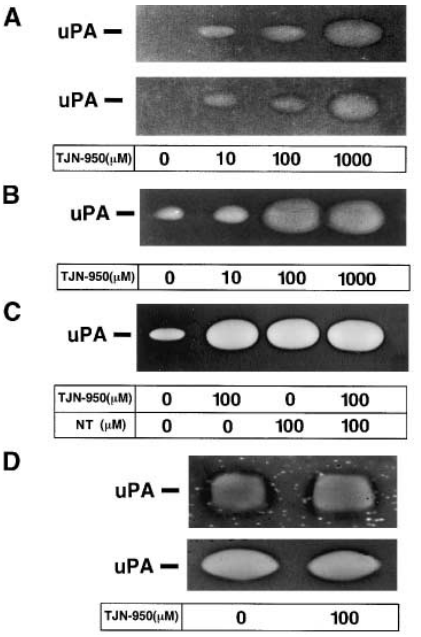

Fig. 2. Effects of TJN-950 on uPA activity. Confluent monolayers were incubated in DMEM containing 10% FBS with increasing doses of TJN-950 for 24 h. For fibrin zymography, cell homogenates or culture media were separated by non-reducing SDS-PAGE and transferred to fibrin-agar indicator gels. A: uPA activity in cell lysate (upper panel) and medium (lower panel) of BAE cells. Transparent bands had an apparent molecular weight of 52 kDa, consistent with that of bovine uPA. B: uPA activity in cell lysates of HUVE cells; the band had an apparent molecular weight of 54 kDa, consistent with that of human uPA. C: BAE cells treated with 100 μM TJN-950, NT or both for 24 h. D: Effect of TJN-950 on uPA secreted into conditioned medium of BAE cells (upper panel) and on purified uPA (lower panel).

3. Results and discussion

We compared the ability of TJN-950 and NT (8–13) to compete with the binding of ^{125}I-NT to NT receptors in two assay systems: the membrane fractions prepared from newborn rat brains (Fig. 1A) and the membrane fraction of rat NTR1 cDNA-transfected COS-7 cells (Fig. 1B). At a concentration of 10^{-9} M, NT (8–13) showed almost complete inhibition of ^{125}I-NT binding in both assay systems (Fig. 1), and the inhibitory kinetics for NT (8–13) were almost identical to those for non-radioactive NT (data not shown). In contrast, TJN-950 was less effective at inhibiting the binding of ^{125}I-NT, showing 10^4-fold lower affinity for the NTR1 than NT (8–13) (Fig. 1). From the dose-response kinetics the IC_{50} in newborn rat brains was 2.1×10^{-6} M for TJN-950 and 6.8×10^{-11} M for NT (8–13) (Fig. 1A); in COS-7 cells transfected with NTR1 cDNA, the IC_{50} was 7.7×10^{-7} M for TJN-950 and 7.1×10^{-11} M for NT (8–13) (Fig. 1B).

Utilizing fibrin zymographic analysis, we next examined whether TJN-950 could modulate PA in vascular endothelial cells. Treatment of BAE cells for 24 h with 10–1000 μM TJN-950 induced a dramatic increase in uPA activity in both cell

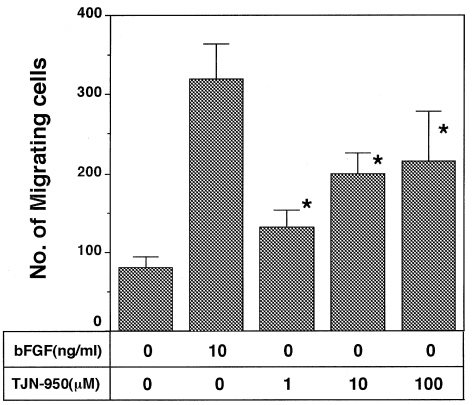

Fig. 3. Effect of TJN-950 on endothelial cell migration in vitro. Confluent monolayers of BAE cells were scraped with a razor blade, and incubated for 24 h in DMEM supplemented with 1% FBS and 10 ng/ml bFGF or 1–100 mM TJN-950. The number of migrating cells was counted in successive 100-mm sections from the denuded edge in four random fields (1000×1000 mm^2 per field). *Significant difference ($P < 0.01$) compared to control.

lysates and medium (Fig. 2A). In contrast, reverse zymographic analysis showed no change in 45 kDa PAI-1 activity (data not shown). TJN-950 also enhanced uPA activity in HUVE cells in a dose-dependent manner (Fig. 2B). In BAE cells, similar levels of PA activity were induced by 100 μM TJN-950 and 100 μM NT, and co-administration of both reagents did not further enhance uPA activity (Fig. 2C), suggesting that the induction of uPA activity by TJN-950 and NT occur via a similar pathway. To determine if TJN-950 can directly activate uPA, we added TJN-950 to the conditioned medium of BAE cells or to purified uPA (Fig. 2D). TJN-950 had no effect on uPA activity in either case, indicating that the TJN-950-induced uPA activation is not due to a direct interaction.

Migration of vascular endothelial cells is closely associated with their PA activity [23,24]. We therefore assayed the ability of TJN-950 to enhance the migration of BAE cells. Treatment with 1, 10 and 100 μM TJN-950 significantly stimulated the migration of BAE cells (Fig. 3). As a comparison, the positive control, 10 ng/ml bFGF, stimulated a 3-fold enhancement of cell migration (Fig. 3). TJN-950-induced enhancement of cell migration was almost completely blocked by co-administration of anti-uPA antibody (data not shown), suggesting the involvement of uPA in TJN-950-induced cell migration.

To examine if TJN-950 could induce angiogenesis in vivo, we implanted hydron pellets impregnated with TJN-950 into rat corneas. TJN-950 (250 nmol) elicited an angiogenic response, but administration of PBS alone did not (Fig. 4). TJN-950 could thus induce angiogenesis in the in vivo model system.

We next determined whether TJN-950-induced activation of uPA is due to enhanced expression of uPA-encoded mRNA. Treatment of BAE cells for 24 h with 100 μM TJN-950 induced no change in the levels of uPA and PAI-1 mRNA (Fig. 5A). TJN-950 had no effect on uPA, PAI and uPAR mRNA expression in HUVE cells (Fig. 5B). Thus, it appeared that TJN-950-induced activation of uPA was not due to an up-regulation of uPA and uPAR genes expression or to a down-regulation of PAI-1 gene expression.

Concomitant binding of pro-uPA or latent uPA to uPAR

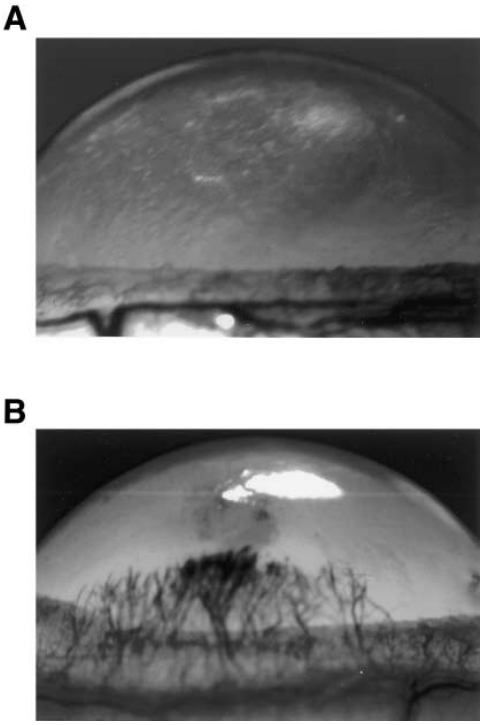

Fig. 4. The effect of TJN-950 on in vivo angiogenesis in rat cornea. Hydron pellets were formulated as described in Section 2 and implanted into rat corneas. Seven days later vessels in the region of the pellet implant were photographed. Pellets contained (A) PBS and (B) TJN-950.

has been shown to induce preferential and effective activation of uPA [25]. We therefore assayed the binding of ^{125}I-TJN-950 to BAE cell membranes in the presence of various doses of TJN-950 or purified uPA (Fig. 6A). Almost identical competition kinetics were observed for TJN-950 and the uPA preparation, suggesting that they have similar binding sites on vascular endothelial cell membranes. To further examine whether TJN-950 directly interacted with uPAR, we assayed the binding of ^{125}I-TJN-950 to BAE cell membranes in the presence of uPA peptides essential for its binding to uPAR.

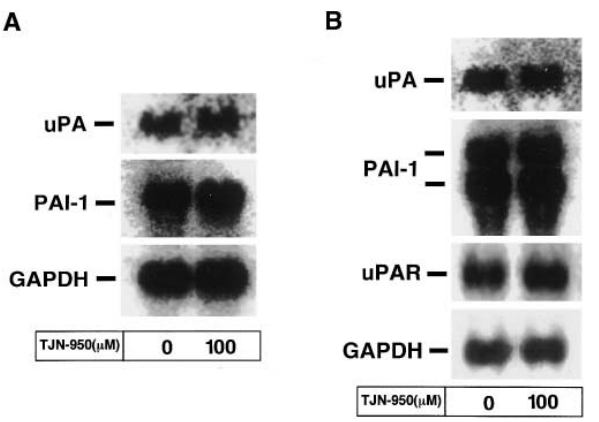

Fig. 5. Effect of TJN-950 on expression of uPA, PAI-1 and uPAR mRNA in vascular endothelial cells. BAE (A) or HUVE (B) cells were incubated with 100 mM TJN-950 for 24 h, and their total RNAs were extracted and assayed for expression of uPA, PAI-1 and uPAR message. As a control, levels of GAPDH mRNA were also determined.

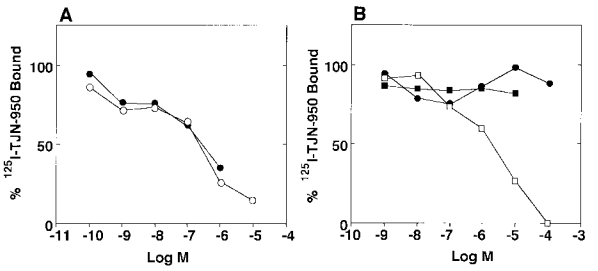

Fig. 6. Competitive assay for ^{125}I-TJN-950 binding to BAE cell membranes. A: Membranes incubated with ^{125}I-TJN-950 and indicated concentrations of non-radioactive TJN-950 (○) or uPA (●). B: Membranes incubated with ^{125}I-TJN-950 and indicated concentrations of non-radioactive TJN-950 (□), ATF (●) or uPA [12–32(Ala^{19})] (■). Each value is the mean of three duplicate experiments.

Exogenous administration of ATF (up to 10^{-4} M) or uPA [12–32(Ala^{19})] (up to 10^{-5} M) did not appreciably inhibit ^{125}I-TJN-950 binding, however. In contrast, 10^{-5} M TJN-950 inhibited binding by almost 80% (Fig. 6B).

In this study, we utilized a novel analogue of acetyl-NT (8–13), TJN-950, to investigate the effects of NT on vascular endothelial cells. Although TJN-950 induced the activation of uPA in these cells, it did not do so by increasing expression of uPA- and uPAR-encoded mRNA, or by decreasing the expression of PAI-1-encoded message. These results suggest that the mechanism of NT-induced uPA activation may not occur at the transcriptional level. TJN-950 lost its affinity to NTR1 which NT or acetyl-NT (8–13) binds. TJN-950, however, showed similar affinity to uPAR as NT. Activation of uPA by TJN-950 thus appeared to be mediated through its interaction with uPAR. However, the precise mechanism of how TJN-950 or NT interacts with uPAR remains unclear at present.

The PA-plasmin system is required for the migration of vascular endothelial cell and tubular morphogenesis (angiogenesis) [23,24]. Inhibition of PA by irsogladine or nortriterpenoid has been shown to result in the inhibition both of cell migration and of angiogenesis in vitro as well as in vivo [20,26,27]. These results suggest that activation of the PA-plasmin system in vascular endothelial cells is closely coupled to cell migration and angiogenesis. We have shown here that the NT analogue, TJN-950, also enhanced vascular endothelial cell migration, providing further evidence that these two activities may be coupled. Moreover, administration of TJN-950 resulted in a potent angiogenic response in rat cornea in vivo. TJN-950 was supposed to induce angiogenesis in both in vitro and in vivo models.

uPA is a serine protease that regulates the blood coagulation cascade, extracellular matrix degradation and remodeling, angiogenesis, tumor invasion and repair of skin wounds [28–30]. uPAR is a highly glycosylated 55–65 kDa protein linked to the plasma membrane by glycosyl-phosphatidylinositol [31], which is expressed in various types of cells including vascular endothelial cells [32]. Activation of pro-uPA to uPA occurs when pro-uPA is bound to its receptor, and the uPAR-bound uPA is fully active at the cell surface [25,33–35]. The uPA-uPAR site, at which plasmin is efficiently generated, may provide a target for the treatment of inflammatory disorders, tumor invasion/metastasis, and angiogenic diseases [36]. The NT analogue, TJN-950, which may activate uPA activity though its interaction with uPAR on vascular endothelial cells, may thus be useful in the treatment of such disorders that involve uPA.

References

[1] Carraway, R. and Leeman, S.E. (1973) J. Biol. Chem. 248, 6854–6861.
[2] Carraway, R. and Leeman, S.E. (1976) J. Biol. Chem. 251, 7045–7052.
[3] Emson, P.C., Goedert, M. and Mantyh, P.W. (1981) in: Handbook of Chemical Neuroanatomy, Vol. 4, GABA and Neuropeptides in CNS (Björklund, A. and Høkfelt, T., Eds.), pp. 355–405, Elsevier, Amsterdam.
[4] Rosell, S. and Rokaeus, A. (1981) Clin. Physiol. 1, 3–20.
[5] Carraway, R.E. and Leeman, S.E. (1985) in: Peptides: Chemistry, Structure and Biology (Walter, R. and Meinhafer, J., Eds.), pp. 679–685, Ann Arbor Science, Ann Arbor, MI.
[6] Chalon, P., Vita, N., Kaghad, M., Guillemot, M., Bonnin, J., Delpech, B., Le Fur, G., Ferrara, P. and Caput, D. (1996) FEBS Lett. 386, 91–94.
[7] Schaeffer, P., Laplace, M.C., Savi, P., Pflieger, A.-M., Gully, D. and Herbert, J.-M. (1995) J. Biol. Chem. 270, 3409–3413.
[8] Gao, G.C. and Wei, E.T. (1993) J. Pharmacol. Exp. Ther. 265, 619–625.
[9] Gao, G.C. and Wei, E.T. (1995) Regul. Peptides 56, 41–48.
[10] Gao, G.C. and Wei, E.T. (1995) Regul. Peptides 58, 117–121.
[11] Granier, C., van Rietschoten, J., Kitabgi, P., Poustis, C. and Freychet, P. (1982) Eur. J. Biochem. 124, 117–124.
[12] Appella, E., Robinson, E.A., Ullrich, S.J., Stoppelli, M.P., Corti, A., Cassani, G. and Blasi, F. (1987) J. Biol. Chem. 262, 4437–4440.
[13] Abe, T., Okamura, K., Ono, M., Kohno, K., Mori, T., Hori, S. and Kuwano, M. (1993) J. Clin. Invest. 92, 54–61.
[14] Jimi, S., Ito, K., Kohno, K., Ono, M., Kuwano, M., Itagaki, Y. and Ishikawa, H. (1995) Biochem. Biophys. Res. Commun. 211, 476–483.
[15] Mills, A., Demoliou-Mason, C.D. and Barnard, E.A. (1988) J. Neurochem. 50, 904–911.
[16] Kitabgi, P., Poustis, C., Granier, C., Van Rietschoten, J., Rivier, J., Morgat, J.-L. and Freychet, P. (1980) Mol. Pharmacol. 18, 11–19.
[17] Tanaka, K., Masu, M. and Nakanishi, S. (1990) Neuron 4, 847–854.
[18] Bozou, J.-C., Rochet, N., Magnaldo, I., Vincent, J.-P. and Kitabgi, P. (1989) Biochem. J. 264, 871–878.
[19] Ushiro, S., Ono, M., Izumi, H., Kohno, K., Taniguchi, N., Higashiyama, S. and Kuwano, M. (1996) Jpn. J. Cancer Res. 87, 68–77.
[20] Ushiro, S., Ono, M., Nakayama, J., Fujiwara, T., Komatsu, Y., Sugimachi, K. and Kuwano, M. (1997) Int. J. Cancer 72, 657–663.
[21] Ito, K., Ryuto, M., Ushiro, S., Ono, M., Sugenoya, A., Kuraoka, A., Shibata, Y. and Kuwano, M. (1995) J. Cell. Physiol. 162, 213–224.
[22] Yoshida, S., Ono, M., Shono, T., Izumi, H., Ishibashi, T., Suzuki, H. and Kuwano, M. (1997) Mol. Cell. Biol. 17, 4015–4023.
[23] Sato, Y. and Rifkin, D.B. (1988) J. Cell Biol. 107, 1199–1205.
[24] Sato, Y., Okamura, K., Morimoto, A., Hamanaka, R., Hamaguchi, K., Shimada, T., Ono, M., Kohno, K., Sakata, T. and Kuwano, M. (1993) Exp. Cell Res. 204, 223–229.
[25] Vassalli, J.-D., Baccino, D. and Belin, D. (1985) J. Cell Biol. 100, 86–92.
[26] Sato, Y., Morimoto, A., Kiue, A., Okamura, K., Hamanaka, R., Kohno, K., Kuwano, M. and Sakata, T. (1993) FEBS Lett. 322, 155–158.
[27] Ono, M., Kawahara, N., Goto, D., Wakabayashi, Y., Ushiro, S., Yoshida, S., Izumi, H., Kuwano, M. and Sato, Y. (1996) Cancer Res. 56, 1512–1516.
[28] Danø, K., Andreasen, P.A., Grondahl-Hansen, J., Kristensen, P., Nielsen, L.S. and Skriver, L. (1985) Adv. Cancer Res. 44, 139–266.

[29] Mignatti, P. and Rifkin, D.B. (1993) Physiol. Rev. 73, 161–195.
[30] Rømer, J., Bugge, T.H., Pyke, C., Lund, L.R., Flick, M.J., Degen, J.L. and Danø, K. (1996) Nat. Med. 2, 287–292.
[31] Ploug, M., Rønne, E., Behrendt, N., Jensen, A.L., Blasi, F. and Danø, K. (1991) J. Biol. Chem. 266, 1926–1933.
[32] Wojta, J., Zoellner, H., Gallicchio, M., Hamilton, J.A. and McGrath, K. (1992) Biochem. Biophys. Res. Commun. 188, 463–469.
[33] Ellis, V., Wun, T.-C., Behrendt, N., Rønne, E. and Danø, K. (1990) J. Biol. Chem. 265, 9904–9908.
[34] Stephens, R.W., Pollanen, J., Tapiovaara, H., Leung, K.-C., Sim, P.-S., Salonen, E.-M., Rønne, E., Behrendt, N., Danø, K. and Vaheri, A. (1989) J. Cell Biol. 108, 1987–1995.
[35] Manchanda, N. and Schwartz, B.S. (1991) J. Biol. Chem. 266, 14580–14584.
[36] Fazioli, F. and Blasi, F. (1994) Trends Pharmacol. Sci. 15, 25–29.