Original Article

Antiproliferative Action of an Angiotensin I-Converting Enzyme Inhibitory Peptide, Val-Tyr, *via* an L-Type Ca^{2+} Channel Inhibition in Cultured Vascular Smooth Muscle Cells

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Recent antihypertensive studies have demonstrated that small peptides with angiotensin I-converting enzyme (ACE) inhibitory activity had an ability to lower or to modulate a pressor blood pressure response in mild hypertensive subjects. However, the underlying mechanisms still remain unclear. Based on our previous finding that a small peptide, Val-Tyr (VY), was accumulated in the rat aorta and kidney as well as in the circulating blood system, we here investigated whether antihypertensive small peptides exert an antiproliferative effect on serum- or mitogen-induced human vascular smooth muscle cells (VSMCs). Treatment with some ACE inhibitory small peptides (VY, Ile-Trp [IW], and Ile-Val-Tyr [IVY]) had diverse effects on serum-stimulated VSMC proliferation that were independent of their ACE inhibitory activity, though only VY exerted a potent antiproliferative action. VY also showed a greater inhibition of WST-8 incorporation in response to angiotensin (Ang) II-stimulation than the other two small peptides. The attenuation of Ang II-stimulated WST-8 incorporation by VY was not affected by Ang II receptor antagonists (losartan and saralasin ([Sar^1, Ile^8]-Ang II)), indicating that the antiproliferative action of VY may not be due to the peptide's antagonistic effect against Ang II receptors. Treatment with VY had a significant inhibitory effect on the WST-8 incorporation induced by the stimulation of a voltage-gated L-type Ca^{2+} channel agonist, Bay K 8644. Even in the presence of a K^+ channel blocker (paxillin) the inhibition was apparent, suggesting that VY inhibited the proliferation of VSMCs by serving as a natural L-type Ca^{2+} channel blocker, but not as a K^+ channel agonist. (*Hypertens Res* 2005; 28: 545–552)

***Key Words*: Ca^{2+} channel, angiotensin I-converting enzyme, antihypertensive peptide, vascular smooth muscle cells**

Introduction

Angiotensin (Ang) II is a potent vasopressor hormone formed from Ang I by the action of Ang I-converting enzyme (ACE). The hemodynamic and proliferative effects of Ang II are caused by diverse intracellular signal responses in both endothelial cells (EC) and vascular smooth muscle cells (VSMCs) (*1–3*). To retard the development of vascular lesions, the proliferation of VSMCs can be inhibited in any of three ways: 1) by suppressing the pressor Ang II production from Ang I, *i.e.*, ACE inhibition; 2) by inhibiting Ang II binding to Ang II type

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1 (AT_1)-receptors, *i.e.*, AT_1-receptor-mediated signaling block; and 3) by suppressing Ca^{2+} influx, *i.e.*, voltage-gated L-type Ca^{2+} channel-blocking action. Numerous therapeutic drugs, including ACE inhibitors (*4–6*) and AT_1-receptor antagonists (*7*, *8*), have been developed to accomplish the first two effects, while Ca^{2+} channel blockers have been used to retard vascular lesions by the third mechanism (*9*). Current studies on antihypertensive treatment have focused on the development of AT_1-receptor antagonists (*7*, *8*, *10*), since they modulate the renin-angiotensin system (RAS) more specifically than ACE inhibitors, and do not affect bradykinin and/or substance P levels, which are responsible for the well recognized side-effects of ACE inhibitors, such as dry cough and angioneurotic edema (*11*). Ca^{2+} channel blockers are also among the novel antihypertensive agents in that they have diverse therapeutic actions, including cardiodepressive and antiarrhythmic effects.

In the course of the development of antihypertensive drugs, several studies have examined natural ACE inhibitors with a view toward prophylaxis of hypertension disease through medicinal foods (*12–14*). Clinical evidence of some antihypertensive foods has provided the efficacy of the food intake on blood pressure (BP) regulation (*15–17*). In our previous, randomized double-blind placebo-controlled study in which a sardine peptide drink was given to volunteers with mild essential hypertension (*15*), Val-Tyr (VY) was shown to have ACE inhibitory activity and the ability to improve hypertension through the suppression of human RAS. More recently, other peptides with ACE inhibitory activity, such as Ile- (or Val)-Pro-Pro (*16*) or Leu-Lys-Pro-Asn-Met (*17*), have also been revealed to have a mild antihypertensive effect in humans. On the other hand, although there have been many studies on the ACE inhibition of natural peptides, there has been no report on whether these peptides might also have effects on regional BP regulatory systems, such as the heart and vascular tissues, that were significant target organs of ACE inhibitory drugs (*18*). Our recent study—in which the VY peptide did not inhibit the enhanced circulating human RAS in transgenic hypertensive mice bearing both the human renin gene and human angiotensinogen gene, although VY significantly lowered the BP of the hypertensive mice—has led us to an alternative strategy for elucidating peptide-induced antihypertensive actions (*19*). The observation that the VY peptide is accumulated in organs such as the aorta and kidney at a concentration higher (0.26 μg/rat) than that in the blood (0.046 μg/rat) (*20*, *21*) also supports the view that the peptide may play a role in regulating regional BP control systems. In addition, the report by Masuda *et al*. (*22*) revealed that Val-Pro-Pro was detected at a level of 4.3 μg/rat-aorta at 6 h after administration. However, the physiological role of these three peptides in vascular functions still remains unclear. In this report, therefore, we examined the ability of ACE inhibitory peptides, including VY, to inhibit the proliferation of VSMCs, and also revealed a novel antihypertensive mechanism of bioactive small peptides in cultured VSMCs.

Methods

Materials

Ang II, Bay K 8644, verapamil, captopril and paxillin were purchased from Sigma Chemical Co. (St. Louis, USA), losartan potassium from Banyu Co. (Tokyo, Japan), saralasin ([Sar^1, Ile^8]-Ang II) from Nacalai Tesque Co., Ltd. (Kyoto, Japan), and the small peptides, VY and Ile-Trp (IW), from Kokusan Chemical Works, Ltd. (Tokyo, Japan). Ile-Val-Tyr (IVY) was synthesized by an Fmoc-solid phase peptide synthesis method, and its sequence was confirmed on a PPSQ 21 amino acid sequencer (Shimadzu Co., Ltd., Kyoto, Japan). WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt) for viable cell counting was purchased from Dojindo Co., Ltd. (Kumamoto, Japan). All other chemicals were of analytical-reagent grade and used without further purification.

Cell Cultures

Human VSMCs were purchased from Sanko Junyaku Kogyo Co. (Tokyo, Japan) and cultured in smooth muscle cell growth medium-2 (SmBM) containing 5% fetal bovine serum (FBS), human epidermal growth factor (0.5 ng/ml), bovine insulin (5 μg/ml), human basic fibroblast growth factor-B (1 ng/ml), and GA-1000 (30 μg/ml of gentamicin sulfate and 15 ng/ml of amphotericin-B). The SmBM corresponds to 10 mmol/l HEPES buffer (pH 7.4) containing 145 mmol/l NaCl, 5 mmol/l KCl, 1 mmol/l Na_2HPO_4, 1 mmol/l $CaCl_2$, 0.5 mmol/l $MgSO_4$ and 5 mmol/l glucose. Cell preparations in 24-well tissue culture plates were kept at 37°C in a 5% CO_2/95% O_2 humidified atmosphere. For all experiments, VSMCs were grown to 70–90% confluence and made quiescent by serum-free SmBM for 24 h. Cells between passages 7 and 8 were used in this study.

Cell Counts and Cell Proliferation Assay

Cell numbers were determined by the Trypan blue dye exclusion method using a hemacytometer. VSMCs seeded in 24-well tissue culture plates (1.5×10^5 cells/well) were made quiescent by serum-free SmBM for 24 h to remove any serum cytokines or growth factors. After the 24 h-treatment, the medium was replaced by 5% FBS-SmBM in the presence or absence of peptides. The medium was exchanged for fresh medium every 48 h, and on day 5 of incubation the cells were removed by treatment with 0.1% trypsin/EDTA solution and the number of viable VSMCs was counted using a hemacytometer. Values are expressed as the means from two wells from triplicate experiments.

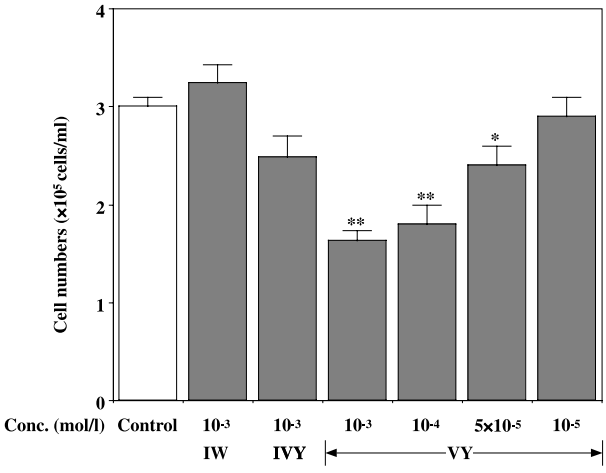

Fig. 1. *Effect of ACE inhibitory peptides on the proliferation of cultured VSMCs on day 5 of incubation. VSMCs seeded in 24-well tissue culture plates (1.5×10^5 cells/well) were made quiescent by serum-free SmBM for 24 h. After the 24 h-treatment, the medium was replaced by 5% FBS-SmBM in the presence or absence (control) of peptides. The medium was exchanged for fresh medium every 48 h during the 5-day incubation. The peptides used in this series of experiments were IW (1 mmol/l), IVY (1 mmol/l), and VY (0.01–1 mmol/l). Each value is the mean±SEM (*n=6). *$p<0.05$ and **$p<0.01$ compared with the control cells by the Tukey-Kramer's* t*-test.*

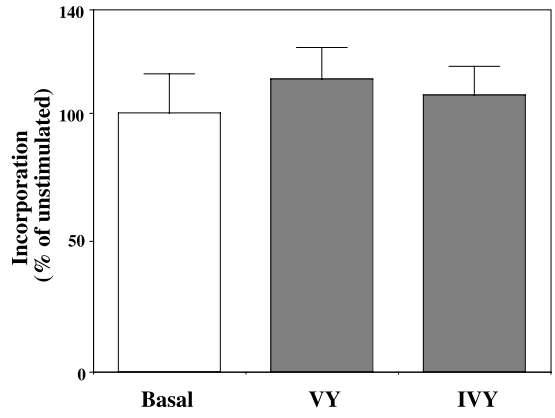

Fig. 2. *Cytotoxicity test of VY and IVY in VSMCs. VSMCs grown to subconfluence in 24-well tissue culture plates were made quiescent and inoculated onto 96-well tissue culture plates. Cells (1×10^4 cells/well) incubated in serum-free SmBM for 24 h were treated for 48 h in the presence or absence (basal) of 1 mmol/l VY or 1 mmol/l IVY. After the incubation, WST-8 incorporation was determined at 450 nm to evaluate cytotoxicity. Each value is the mean±SEM (*n=6). *There were no significant differences between the groups at the* $p<0.05$ *level.*

WST-8 Incorporation Assay

For cytotoxicity and mitogen-stimulated VSMC proliferation studies, a WST-8 incorporation experiment was performed, in which the rate of cell proliferation or DNA synthesis was determined by a nonradioactive colorimetric method on the basis of the cleavage of a tetrazolium salt rather than by a [^{3}H]thymidine incorporation assay (*23*). VSMCs grown to subconfluence in 24-well tissue culture plates were made quiescent and inoculated onto 96-well tissue culture plates at a density of 1×10^4 cells/well. Cells incubated in serum-free SmBM for 24 h were treated for 48 h with mitogens in the presence or absence of antagonists and/or peptides. The mitogens were added a few minutes after the addition of antagonists and/or peptides. The medium was replaced with fresh medium 24 h after plating, and then an additional 24 h-incubation was performed. After the incubation, 0.5 µmol of WST-8 per well was added to the growth medium and incubated for 3 h. The WST-8 incorporation was determined by a Wallac 1420 Multilabel counter (Perkin Elmer Co., Tokyo, Japan) at 450 nm.

Analyses of Data

Each result is expressed as the mean±SEM. Statistical differences between control and sample groups were evaluated by using a two-way analysis of variance (ANOVA) followed by the Tukey-Kramer's *t*-test for a *post hoc* analysis. Statistical differences between groups were analyzed by Student's *t*-test. Values of $p<0.05$ were considered statistically significant. Analyses were performed with Stat View J 5.0 software (SAS Institute Inc., Cary, USA).

Results

Inhibition of VSMC Proliferation by ACE Inhibitory Peptides

We first investigated the effect of small peptides that are known to have *in vitro* ACE inhibitory activity at a concentration of 1 mmol/l on VSMC proliferation in 5% FBS-SmBM for 5 days. The peptides used in this series of experiments were IW, VY and IVY. IW (IC_{50} of 2.0 µmol/l) (*24*) and IVY (IC_{50} of 0.48 µmol/l) (*14*) were used because, among the dipeptides and tripeptides so far reported, they show two of the highest levels of ACE inhibitory activities. VY (IC_{50} of 26 µmol/l) was used (*25*) because it has been shown to have an antihypertensive effect in mild hypertensive subjects (*15*), and to accumulate in aortic tissue after oral administration in rats (*20*). As shown in Fig. 1, among the three peptides, only VY caused a marked decrease in the serum-stimulated VSMC growth, to 54% of the number of serum-stimulated cells (control). This is the first finding that even a small natural peptide could inhibit VSMC proliferation. Although IVY also tended

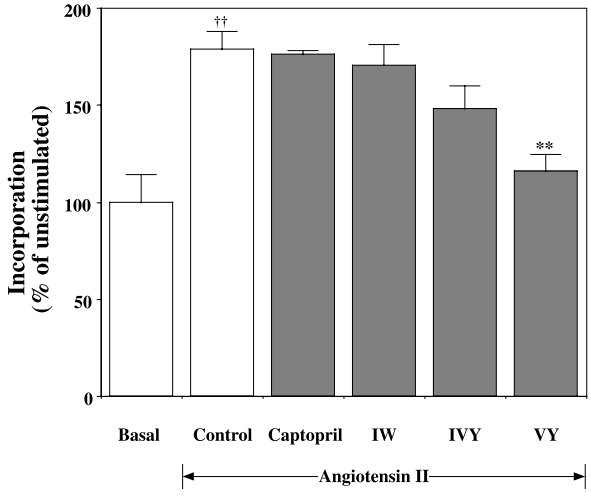

Fig. 3. *Effects of small peptides on angiotensin (Ang) II-induced VSMC proliferation. VSMCs at a density of 1×10^4 cells/well were treated for 48 h with 1 μmol/l Ang II in the presence of 1 mmol/l peptides. Ang II was added a few minutes after the addition of peptides. After the incubation, the WST-8 incorporation was determined at 450 nm to evaluate the inhibition of mitogenic increase in the incorporation by peptides. The peptides used in this series of experiments were IW, VY, and IVY. Captopril (1 μmol/l) was also used as a typical ACE inhibitor. Each value is the mean±SEM (*n*=6). Significant differences between the basal and control groups or between the control and peptide groups were analyzed by the Tukey-Kramer's* t*-test.* ††$p<0.01$ *compared with the basal value, and* **$p<0.01$ *compared with the control value.*

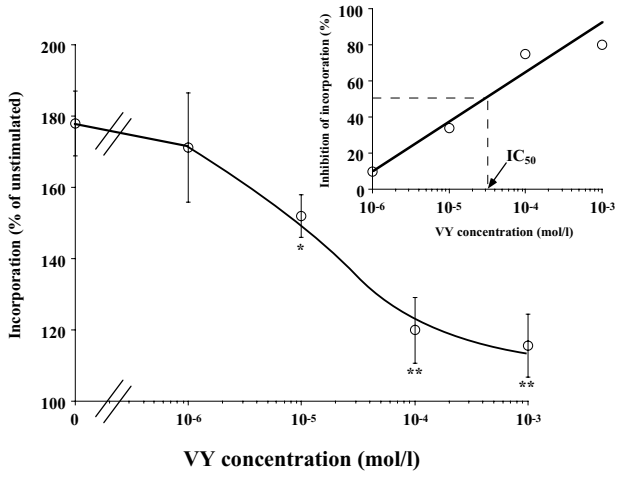

Fig. 4. *Concentration-dependence of the inhibitory action of VY on the WST-8 incorporation induced by 1 μmol/l Ang II in VSMCs. The concentration of VY was set at 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} mol/l. Each value is the mean±SEM (*n*=6).* *$p<0.05$ *and* **$p<0.01$ *compared with the control (0 mol/l VY) by the Tukey-Kramer's* t*-test.*

to cause a slight decrease in the growth, to 82% of the level in the control cells, IW, an ACE inhibitory peptide, did not elicit an antiproliferative effect, indicating that ACE inhibition is not responsible for the inhibition of the serum-stimulated proliferation. Cytotoxicity experiments of VY and IVY (Fig. 2), in which a WST-8 incorporation assay was done for VSMCs treated in serum-free SmBM for 48 h in the presence or absence of 1 mmol/l of the peptides, revealed that the peptide-induced antiproliferative action was not due to any cytotoxic action. Figure 1 also shows that the VY-induced antiproliferation effect was elicited in a concentration-dependent manner. A significant VY-induced decrease of 21% ($p<0.05$ *vs.* control), 41% ($p<0.01$) and 46% ($p<0.01$) in the cell number was observed at final VY concentrations of 0.05, 0.1 and 1 mmol/l, respectively; at least 50 μmol/l of VY was required to inhibit the serum-stimulated VSMC proliferation.

Antiproliferative Action Induced by Peptides in Ang II-Stimulated VSMCs

In order to clarify the antiproliferative mechanism of VY on the serum-stimulated VSMC growth, effects of VY on the signal-transduction pathway *via* AT_1-receptor were examined. As shown in Fig. 3, Ang II induced a significant mitogenic effect on the VSMCs treated for 48 h in serum-free SmBM; 1 μmol/l Ang II increased WST-8 incorporation by 179±9% (control) above the basal level (without Ang II). In the presence of the peptides at a concentration of 1 mmol/l, we observed a potent suppression of the WST-8 incorporation into the Ang II-stimulated VSMCs for VY with a reduction to 65% of the control. IVY also tended to suppress an increase in the incorporation by the Ang II stimulation (though a significant difference was not observed), whereas IW had no effect on the Ang II-induced WST-8 incorporation. Captopril as a typical ACE inhibitor (IC_{50} of 20 nmol/l) (*14*) also showed no influence on the incorporation at a concentration of 1 μmol/l, strongly suggesting that the antiproliferative effect induced by VY was not a result of ACE inhibition. Inhibition of the Ang II-stimulated WST-8 incorporation by VY was elicited in a concentration-dependent manner over the range 1 μmol/l to 1 mmol/l, as shown in Fig. 4. The concentration at which VY induced 50% inhibition of the WST-8 incorporation stimulated by 1 μmol/l Ang II (the IC_{50} value) was estimated to be 33±6 μmol/l.

Ang II-stimulated VSMCs in serum-free SmBM were treated with 1 mmol/l VY for 48 h in the presence or absence of 1 μmol/l of an AT_1-receptor selective antagonist (losartan) and 1 μmol/l of a nonselective Ang II receptor antagonist (saralasin). As shown in Fig. 5, VY inhibited the WST-8 incorporation into the Ang II-stimulated VSMCs, irrespective of the presence of losartan. The VY-induced inhibition of WST-8 incorporation was also independent of saralasin, indi-

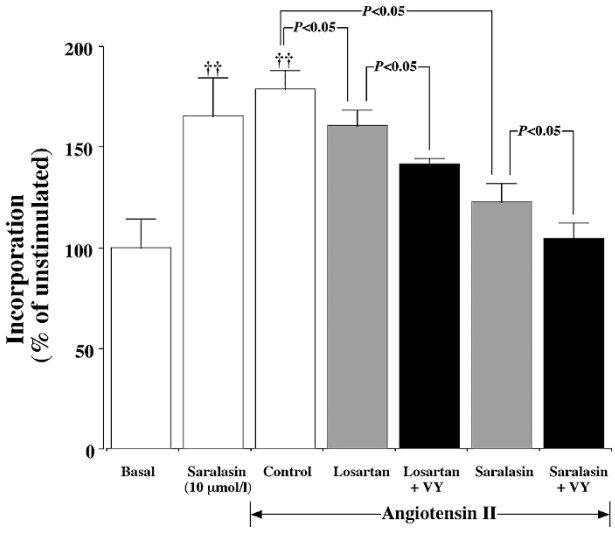

Fig. 5. *Influence of receptor antagonists on the WST-8 incorporation into Ang II-stimulated VSMCs in response to VY. Ang II-stimulated VSMCs in serum-free SmBM were treated with 1 mmol/l VY for 48 h in the presence or absence of an AT_1-receptor selective antagonist (losartan) and a non-selective Ang II receptor antagonist (saralasin; [Sar^1, Ile^8]-Ang II). Each value is the mean±SEM (*n*=6). Significant differences between groups were analyzed by the Tukey-Kramer's* t-*test at* $p<0.05$. $^{††}p<0.01$ *compared with the basal value.*

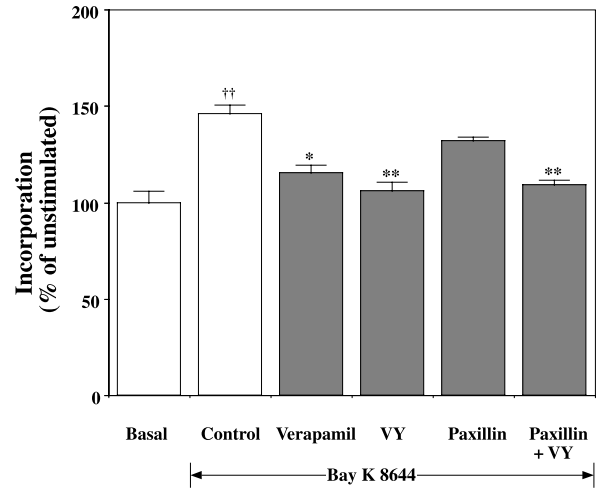

Fig. 6. *Influence of channel blockers on the WST-8 incorporation into 1 μmol/l Bay K 8644-stimulated VSMCs in response to VY. Bay K 8644-stimulated VSMCs in serum-free SmBM were treated with 1 mmol/l VY for 48 h in the presence or absence of 1 μmol/l of a Ca^{2+} channel blocker (verapamil) and 1 μmol/l of a K^+ channel blocker (paxillin). Each value is the mean±SEM (*n*=6). Significant differences between the basal and control groups (in the presence of 1 μmol/l Bay K 8644) or between the control and peptide groups were analyzed by the Tukey-Kramer's* t-*test.* $^{††}p<0.01$ *compared with the basal value, and* $*p<0.05$ *and* $**p<0.01$ *compared with the control value.*

cating that some action of the peptide other than an antagonistic effect against Ang II receptors may be responsible for its antiproliferative effect on VSMCs.

Antiproliferative Action Induced by Peptides in Bay K 8644-Stimulated VSMCs

We next examined whether the small peptide VY achieved its inhibition of the proliferation of VSMCs by suppressing increases of the intracellular Ca^{2+} concentration. Bay K 8644 was used as a mitogen or voltage-gated L-type Ca^{2+} channel agonist to assess the inhibition of extracellular Ca^{2+} influx by VY. Figure 6 shows the effect of VY on WST-8 incorporation for VSMCs in serum-free SmBM that contained 1 mmol/l $CaCl_2$ in the presence or absence of 1 μmol/l Bay K 8644 for 48 h. The results showed that Bay K 8644 elicited a significant ($p<0.01$) increase in the WST-8 incorporation; 1 μmol/l Bay K 8644 increased the WST-8 incorporation by 146±24% (control) above a basal level (without Bay K 8644). It was also revealed that 1 mmol/l VY significantly ($p<0.01$) inhibited the increase in the WST-8 incorporation stimulated by 1 μmol/l Bay K 8644, as 1 μmol/l verapamil (a therapeutic L-type Ca^{2+} channel blocker) inhibited. In contrast, the presence of 1 μmol/l paxillin, which is a K^+ channel blocker, did not affect the inhibitory action of VY. This finding provided the first evidence that VY achieved its inhibition of VSMC proliferation by acting as a voltage-gated L-type Ca^{2+} channel blocker, but not as a K^+ channel agonist. As shown in Fig. 7, VY inhibited the incorporation in a concentration-dependent manner over the range of 10–1,000 μmol/l. The tentatively estimated IC_{50} value for inhibiting the incorporation by VY was 55±4 μmol/l (IC_{50} of verapamil: 30±2 nmol/l).

Discussion

In the present study, we provided the first evidence that a small peptide with ACE inhibitory activity had the additional antihypertensive effects of inhibiting the proliferation of VSMCs, inhibiting mitogen-stimulated DNA synthesis in a concentration-dependent manner, and reducing serum-stimulated cell growth without cytotoxicity. Vascular functional and structural alternations are critical for hypertension disease including vascular or arteriosclerotic lesions (*26*). VSMC proliferation, hypertrophy or migration is induced by Ang II and/or norepinephrine stimulation, and Ang II has also been shown to act as an autocrine/paracrine mediator in the RAS and to play a prominent role in the pathogenesis of vascular lesions *via* upregulation of the proliferation of VSMCs (*27*). Thus, an appropriate inhibition of Ang II production or Ang II-stimulated signaling pathways would be beneficial for diseases involving vascular lesions. Studies have shown that

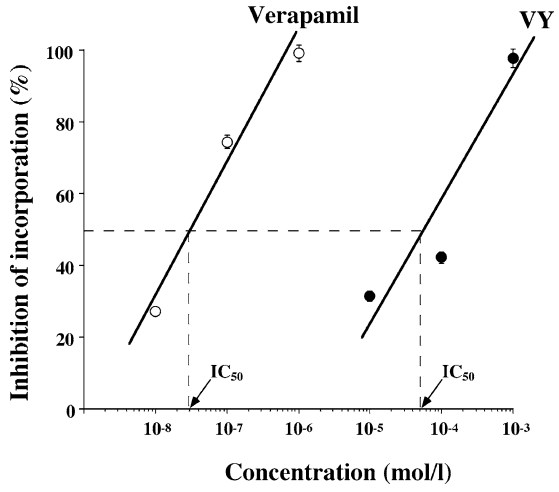

Fig. 7. *Concentration-dependence of the inhibitory action of VY and verapamil on the WST-8 incorporation induced by 1 μmol/l Bay K 8644 in VSMCs. The concentration was, respectively, set at 10^{-5}, 10^{-4}, and 10^{-3} mol/l for VY, and 10^{-8}, 10^{-7}, and 10^{-6} mol/l for verapamil. Each value is the mean±SEM (*n*=6).*

Ang II-induced VSMC proliferation could be successfully prevented by an AT_1-receptor antagonist (*7*), a Ca^{2+} channel blocker (*28*), troglitazone (*29*), Ang(1–7) (*30*), quercetin (*31*), docosahexaenoic acid (DHA)/eicosapentaenoic acid (EPA) (*32*), genistein (*33*) and β-sitosterol (*34*). The antiproliferative mechanism of the latter natural compounds was considered to be due, at least in part, to the inhibition of such intracellular signal-transduction pathways as matrix metalloprotease-9 (MMP-9) secretion (*31*), phosphorylation of cyclin/cyclin dependent kinases (CDKs) (*32*), and expressions of NAD(P)H oxidase and AT_1 receptor (*33*), but the precise mechanism remains poorly understood. Additionally, there has been no report that small peptides had the potential to inhibit VSMC proliferation in the manner of the Ang(1–7) peptide (*30*).

Our present findings show that, among the ACE inhibitory di- and tri-peptides tested, only VY induced a potent VSMC growth inhibition in 5% FBS-SmBM for 5 days (Fig. 1). The lack of an association between the antiproliferative effect and ACE inhibitory activity, and the fact that VY had no cytotoxic effect on VSMCs (Fig. 2), indicated that some action of the peptide other than ACE inhibition may have been responsible for its antiproliferative effect on VSMCs. Freeman *et al*. (*30*) have reported the antiproliferative effect of a hepta-peptide, Ang(1–7), in VSMCs, which is mediated by an Ang(1–7)-sensitive receptor distinct from AT_1 or AT_2 receptors (*30*, *35*). However, it seems unlikely that VSMCs possess a small peptide (VY)-specific receptor that can influence the growth, because the smaller peptide would not act as a ligand for the G protein-coupled receptor.

Ang II stimulation was shown to promote DNA synthesis in VSMCs in serum-free medium, and the maximal increase in [^{3}H]thymidine incorporation was observed at an Ang II concentration of 1.75 μmol/l (*36*). The effect in response to Ang II was coupled to AT_1 receptor-mediated signaling transduction pathways. In the present study, the increase in WST-8 incorporation by 1 μmol/l Ang II was prevented by 1 mmol/l VY, whereas captopril as an ACE inhibitor did not affect the incorporation (Fig. 3). The finding that an ACE inhibitor had no effect on the Ang II-stimulated VSMC incorporation was in agreement with the result by Watanabe *et al*. (*36*), suggesting that the role of ACE inhibitors was restricted to the suppression of Ang II production, and did not include inhibition of Ang II-signaling pathways in VSMCs. Although the VY-induced antiproliferative effect (IC_{50} of 33±6 μmol/l) was much weaker than those of des-[Asp^1]-Ang I (*37*) and Ang(1–7) (*30*), the present study reveals that the "ACE inhibitory" peptide played a physiological role in regulating the VSMC growth. The antiproliferative action of VY on the Ang II-stimulated VSMCs was observed even in the presence of an Ang II receptor antagonist (saralasin or losartan) (Fig. 5). This indicates that the VY-induced antiproliferative action may not be responsible for the antagonistic effect against Ang II receptors.

Hence, we focused on another VSMC proliferative pathway. Apparently, VY inhibited the WST-8 incorporation in the L-type Ca^{2+} channel agonist (Bay K 8644)-stimulated VSMCs in a concentration-dependent manner over the range of 0.01–1 mmol/l (Fig. 7), just as verapamil (a voltage-gated L-type Ca^{2+} channel blocker) did. The effect induced by VY was not abolished by paxillin as a K^+ channel blocker (Fig. 6). Thus, it seems likely that VY may inhibit extracellular Ca^{2+} influx *via* L-type Ca^{2+} channels in the manner of verapamil (*38*). On the other hand, Umemoto *et al*. (*39*) reported that another Ca^{2+} antagonist, amlodipine, inhibited the vascular oxidative stress induced by activated NAD(P)H oxidase, which implies that other mechanisms such as oxidative stress, inositol phosphate-3 binding (*3*), or myosin light chain kinase activation by facilitation of Ca^{2+}/calmodulin signaling, may likewise contribute to the VY-induced antiproliferative action. Further experiments are currently in progress to elucidate the mechanism of antiproliferation induced by small peptides in VSMCs.

In conclusion, we demonstrated that a small peptide, VY, exerted an antiproliferative effect on VSMCs. The effect of VY did not involve ACE inhibition, but may have been due to an inhibition of L-type Ca^{2+} influx. These findings suggest the possibility of developing novel medicinal foods to prevent diseases involving vascular lesions, such as arteriosclerosis, insulin-resistance and vascular restenosis.

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