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5-Hydroxytryptamine induces transient Ca^{2+} influx through Ni^{2+}-insensitive Ca^{2+} channels in rat vascular smooth muscle cells

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Abstract

The effects of Ni^{2+}, a non-selective cation channel inhibitor, on 5-hydroxytryptamine (5-HT)- and angiotensin II (Ang II)-induced intracellular Ca^{2+} dynamics in rat aortic smooth muscle cells were investigated. Ni^{2+} (1 mM) significantly inhibited the transient increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) induced by Ang II (100 nM) in aortic smooth muscle cells, as measured using fura-2. However, Ni^{2+} did not suppress the transient increase in Ca^{2+} influx induced by 5-HT (10 μM), while significantly suppressed the sustained increase. Ca^{2+} influx evoked by high KCl (80 mM), thapsigargin (TG) (1 μM) or depletion of intracellular Ca^{2+} store was almost completely suppressed by Ni^{2+}. Ni^{2+} had no effect on 5-HT-induced inositol triphosphate production and Ca^{2+} release from the intracellular store(s). These results suggest that 5-HT, but not Ang II, induces transient Ca^{2+} influx through Ni^{2+}-insensitive Ca^{2+} channels, which are distinguishable from the voltage-dependent or store-operated Ca^{2+} channels.

Keywords: 5-HT (5-hydroxytryptamine serotonin); Ca^{2+} influx; Ca^{2+} channel; Ni^{2+}; Smooth muscle cell, vascular; Angiotensin II

1. Introduction

Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in vascular smooth muscle cells plays a regulatory role as a second messenger in the mechanism of vasocontraction (see Karaki et al., 1997 for a review). A number of mechanisms regulate intracellular Ca^{2+} dynamics induced by various vasoactive agonists in vascular smooth muscle cells (see Himpens et al., 1995; Hughes, 1995 for reviews). Receptor stimulation by the agonists of vascular smooth muscle cells induces both the release of Ca^{2+} from intracellular stores and the influx of extracellular Ca^{2+} through Ca^{2+} channels on the plasma membrane. It has been well established that inositol 1,4,5-triphosphate (IP_3) induces the release of Ca^{2+} from sarcoplasmic reticulum, an intracellular Ca^{2+} store, in vascular smooth muscle cells (Himpens et al., 1995). However, the precise mechanisms involved in Ca^{2+} influx are largely unknown, though Ca^{2+} can enter the cell through both voltage-dependent Ca^{2+} channels and voltage-independent Ca^{2+}. Although the classification and terminology are not yet established, the voltage-independent channels can be further subdivided into four channels based on the mode of activation (Fasolato et al., 1994; Felder et al., 1994; Hughes, 1995; Clementi and Meldolesi, 1996). Receptor stimulation by its agonist can induce Ca^{2+} influx through receptor-operated Ca^{2+} channels which are integral or in close physical association with the receptor (Von der Weid et al., 1993). The activation of a receptor can also cause channel opening through heterogeneous family of second messenger-operated channels which are mediated by some diffusible second messengers including Ca^{2+} itself (Wang et al., 1993), or through G protein-operated channels (Xiong et al., 1991). The depletion of Ca^{2+} from internal stores by pharmacological tools such as thapsigargin (TG) can induce Ca^{2+} influx through store-operated channel or capacitative Ca^{2+} entry pathway (Putney, 1990) in some cells including vascular smooth muscle cells (Casteels and Droogmans, 1981; Xuan et al., 1992).

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In cardiovascular system, 5-hydroxytryptamine (5-HT) causes very strong smooth muscle contraction, which is considered to be associated with cardiovascular diseases such as hypertension or vasospasm (Frishman et al., 1995). 5-HT causes an increase in $[Ca^{2+}]i$ of smooth muscle cells via 5-HT_2 receptor by causing both the release of Ca^{2+} from intracellular stores and the influx of extracellular Ca^{2+} (Doyle et al., 1986; Kanaide et al., 1987; Hirafuji et al., 1998). One of the intracellular stores is the IP_3-sensitive store, since 5-HT increased IP_3 production in rat vascular smooth muscle cells (Doyle et al., 1986; Kanaide et al., 1987; Hirafuji et al., 1998). 5-HT-induced Ca^{2+} influx may occur through both voltage-independent and -dependent channels sensitive to L-type Ca^{2+} antagonist. However, the precise mechanism of 5-HT-induced Ca^{2+} influx is not yet fully defined. In the course of the study to clarify it, we were interested to examine the effect of Ni^{2+}, a nonselective cation channel blocker (Fox et al., 1987; Hagiwara et al., 1988), on the intracellular Ca^{2+} dynamics stimulated by 5-HT, because the voltage-independent channels have different sensitivity and permeability to various divalent cations (Fasolato et al., 1994; Hughes and Schachter, 1994). In this paper, we demonstrate that Ni^{2+} is a useful pharmacological tool to distinguish a form of 5-HT receptor-mediated Ca^{2+} influx channels from the voltage-dependent or store-operated Ca^{2+} channels.

2. Materials and methods

2.1. Cell culture

Vascular smooth muscle cells were isolated from aortic media of male Wistar rats (6–7 weeks old) as described previously (Hirafuji et al., 1998). Briefly, the thoracic aortic media was incubated with collagenase (1.0 mg/ml) and elastase (0.5 mg/ml) for 90 min at 37°C. Cells were suspended in Dulbecco's modified Eagle medium containing 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were cultured in the medium for 6–8 days with medium change every 2–3 days, and then 2% fetal calf serum for 2 days to render them quiescent. Primary cultured cells were used throughout this study.

2.2. Measurement of intracellular $[Ca^{2+}]_i$

Intracellular $[Ca^{2+}]_i$ was measured as described previously (Hirafuji and Shinoda, 1992; Hirafuji et al., 1998). Cells seeded and grown on coverglasses (8 × 16 mm) were loaded with 5 μM fura-2 acetoxymethyl ester for 45 min at room temperature in Hanks' balanced salt solution containing 0.1% bovine serum albumin and 10 mM HEPES (HBSS; pH 7.4). The fluorescence at 505 nm emission wavelength alternately excited at 340 and 380 nm was measured in a fluorescence spectrophotometer (Hitachi F-4000, Japan). Cells were continuously perfused at 1 ml/min with HBSS, to which test drugs were added. R_{max}, the maximal fluorescence ratio, was measured by exposing cells to 10 μM ionomycin in the presence of 5 mM Ca^{2+}, followed by perfusion with Ca^{2+}-free HBSS containing 1 mM EGTA to obtain R_{min}, the minimum ratio. The cells were finally exposed to 0.05% Triton X-100 to obtain the autofluorescence. After the subtraction of autofluorescence, $[Ca^{2+}]_i$ was calibrated according to the equation of Grynkiewicz et al. (1985), assuming the K_d of the Ca^{2+}-fura-2 interaction to be 225 nM in the cytosolic environment.

Ni^{2+} may quench the fura-2 fluorescence if permeated into the cells, giving artificial changes in $[Ca^{2+}]_i$. However, asymmetrical changes in the emission fluorescence at two excitation wavelengths were not observed when cells were exposed to Ni^{2+} in the absence or the presence of stimuli.

2.3. Determination of inositol phosphates

Vascular smooth muscle cells grown in 35-mm culture dishes were incubated for 24 h in the culture medium containing 10 μCi/ml *myo*-[^{3}H]inositol. Cells were washed three times with HBSS and preincubated for 3 min at 37°C. The medium was then removed and replaced with 1 ml HBSS containing 10 μM 5-HT. The reaction was stopped by adding 4% perchloric acid (final concentration) and chilled on ice for 30 min. The medium was then transferred to vials and centrifuged at $1600 \times g$. The supernatant was neutralized with KOH–HEPES. Inositol triphosphates (IP_3) were separated by chromatography using AG1-X8 resin as described previously (Tanimura et al., 1991).

2.4. Materials

Fetal calf serum, penicillin, streptomycin and Dulbecco's modified Eagle medium were obtained from Gibco; fura-2 acetoxymethyl ester, nickel chloride and HEPES from Dojin; ionomycin from Calbiochem; 5-HT creatinine sulfate, collagenase and elastase from Sigma; angiotensin II (Ang II) from Peptide Institute; TG from Research Biochemicals International; bovine serum albumin (fraction V) from Boehringer Mannheim; AG1-X8 resin from Bio-Rad; *myo*-[^{3}H]inositol (s.a. 117 Ci/mmol) from Amersham.

2.5. Statistical analysis

Results are expressed as means ± S.E. of replicate experiments. Statistical analysis of the results was performed using unpaired Student's *t*-test. *P* values less than 0.05 were considered as significant.

3. Results

3.1. Effects of Ni^{2+} on Ang II- and 5-HT-induced intracellular Ca^{2+} dynamics

Fig. 1A demonstrated representative tracings showing Ang II-induced intracellular Ca^{2+} dynamics in cultured rat vascular smooth muscle cells. Ang II at a maximal concentration of 0.1 μM induced a rapid and transient increase in $[Ca^{2+}]_i$, which was returned to the basal level within 5 min after the stimulation. Removal of extracellular Ca^{2+} (1.3 mM) decreased the basal level. Under this condition, Ang II caused a small and transient increase in $[Ca^{2+}]_i$. Likewise, the exposure of cells to 1 mM Ni^{2+} lowered the basal level, and Ang II caused a small transient increase in $[Ca^{2+}]_i$ in the presence of Ni^{2+}.

Fig. 1B demonstrated representative results showing 5-HT-induced intracellular Ca^{2+} dynamics. 5-HT at a maximal concentration of 10 μM induced a rapid and transient elevation in $[Ca^{2+}]_i$, which was maximal by 1 min after the stimulation followed by the sustained increase. When extracellular Ca^{2+} was removed, the basal level of $[Ca^{2+}]_i$ lowered, and the small transient increase was observed in response to 5-HT. Exposure to 1 mM Ni^{2+} caused the decrease of the basal level as was the case for Ang II. However, in the presence of Ni^{2+}, 5-HT still caused a transient increase in $[Ca^{2+}]_i$ comparable to the effect in the presence of extracellular Ca^{2+}, while the sustained increase after the transient increase was remarkably suppressed.

Fig. 2A and B summarized the effect of Ni^{2+} on $[Ca^{2+}]_i$ in rat aortic smooth muscle cells stimulated by 0.1 μM Ang II and 10 μM 5-HT. As shown in Fig. 2A, Ni^{2+} at 1 and 2 mM significantly inhibited the basal $[Ca^{2+}]_i$ before stimulation, the peak $[Ca^{2+}]_i$ induced by Ang II, and $[Ca^{2+}]_i$ at 5 min after the stimulation. In contrast, as shown in Fig. 3B, Ni^{2+} at 1 and 2 mM did not significantly inhibit the peak $[Ca^{2+}]_i$ induced by 5-HT, whereas significantly inhibited the basal level and the level at 5 min after the stimulation.

Fig. 3 demonstrated the effect of Ni^{2+} on the peak $[Ca^{2+}]_i$ as a function of 5-HT concentration. Ni^{2+} (1 mM) significantly inhibited $[Ca^{2+}]_i$ induced by 5-HT at concentrations lower than 0.1 μM, whereas had no effect on $[Ca^{2+}]_i$ induced by 5-HT at concentrations higher than 1 μM.

3.2. Effects of Ni^{2+} on KCl- and TG-induced intracellular Ca^{2+} dynamics

Fig. 4A demonstrated the effects of depolarizing concentration of KCl (80 mM) on intracellular Ca^{2+} dynamics

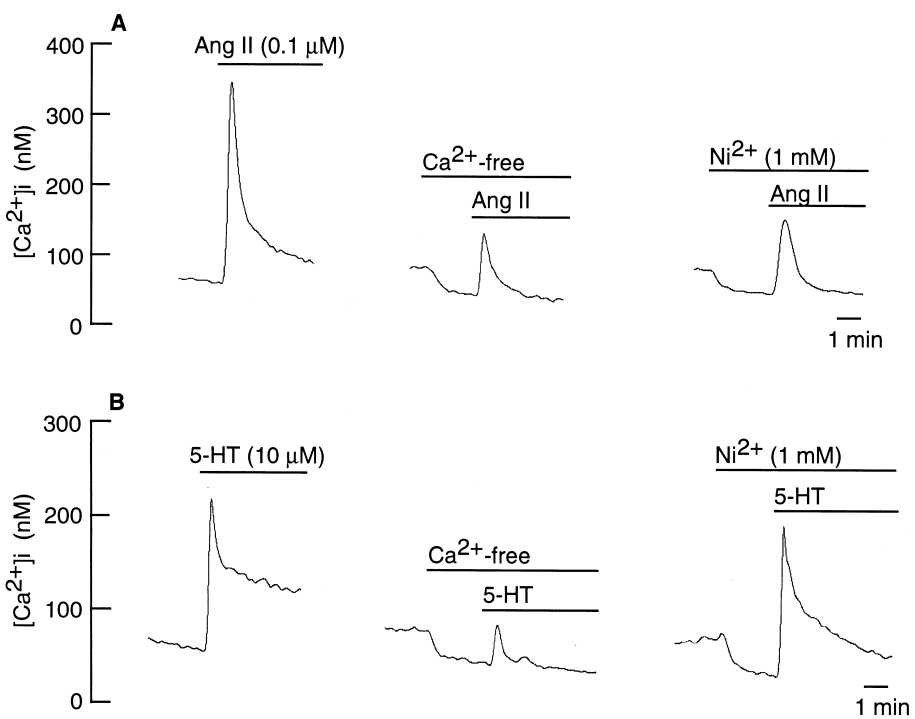

Fig. 1. Effect of Ni^{2+} on Ang II (Ang II)- and 5-HT-induced intracellular Ca^{2+} dynamics in rat aortic smooth muscle cells. Cells were stimulated with 0.1 μM Ang II (A) or 10 μM 5-HT (B) in HBSS containing 1.3 mM Ca^{2+}, or after exposure to Ca^{2+}-free HBSS (+1 mM EGTA) or HBSS containing 1 mM Ni^{2+} for 3 min. Representative tracings in an experiment of each agonist. HBSS: Hanks' balanced salt solution (pH 7.4) containing 10 mM HEPES and 0.1% bovine serum albumin.

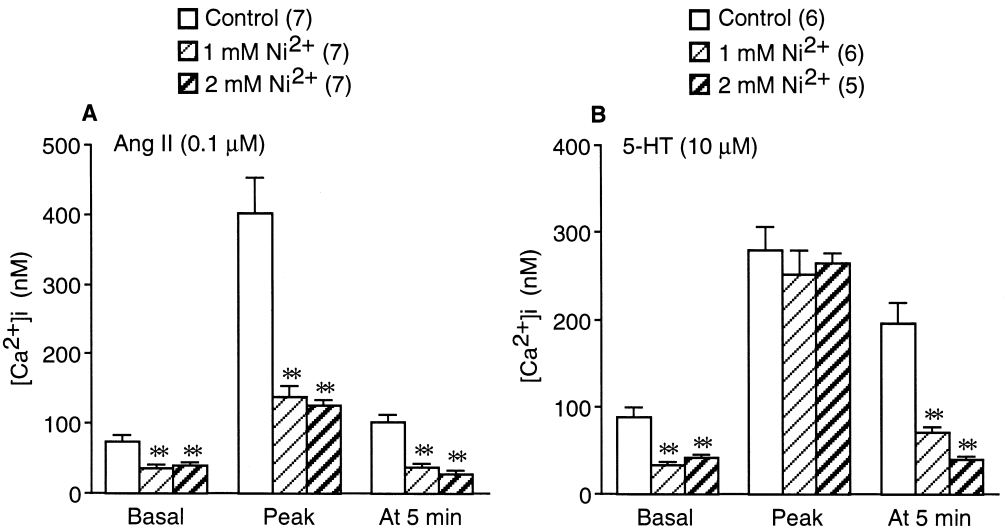

Fig. 2. Effect of Ni^{2+} on Ang II (Ang II)- and 5-HT-induced $[Ca^{2+}]_i$ in rat aortic smooth muscle cells. Cells were stimulated with 0.1 μM Ang II (A) or 10 μM 5-HT in the absence or the presence of 1 and 2 mM Ni^{2+}. Cells were exposed to Ni^{2+} 3 min before to and during the stimulation. Basal: the basal $[Ca^{2+}]_i$ before stimulation; Peak: the peak $[Ca^{2+}]_i$ after stimulation; After 5 min: $[Ca^{2+}]_i$ 5 min after stimulation. Each column represents mean ± S.E. of (n) experiments. $^{**}P < 0.01$ vs. each control.

in cultured rat aortic smooth muscle cells. KCl at 80 mM induced a transient increase in $[Ca^{2+}]_i$, which gradually returned to the basal level. This increase was completely suppressed when extracellular Ca^{2+} was removed. Exposure to 1 mM Ni^{2+} decreased the basal $[Ca^{2+}]_i$ level, and also completely suppressed the effect of KCl.

Fig. 4B demonstrated the effect of TG on intracellular Ca^{2+} dynamics. TG induced an immediate increase followed by a gradual and continuous increase in $[Ca^{2+}]_i$. When extracellular Ca^{2+} was removed, a small transient increase was observed in response to TG. When cells were stimulated with TG in the presence of 1 mM Ni^{2+}, the same change in $[Ca^{2+}]_i$ to that obtained in the absence of extracellular Ca^{2+} was observed.

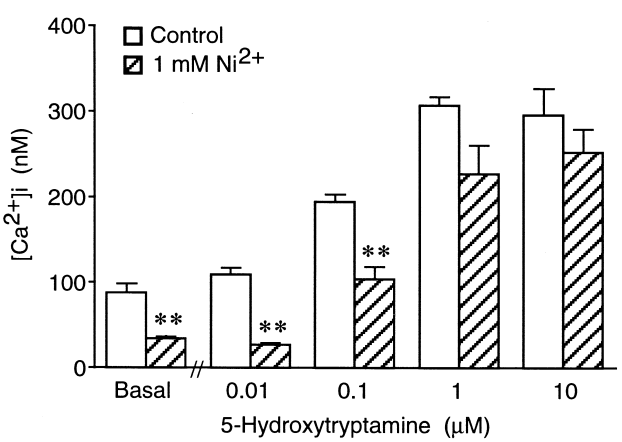

Fig. 3. Effect of Ni^{2+} on 5-HT-induced peak $[Ca^{2+}]_i$ in rat aortic smooth muscle cells. Cells were stimulated with various concentrations of 5-HT in the absence or the presence of 1 mM Ni^{2+}. Cells were exposed to Ni^{2+} 3 min before to and during the stimulation. Each column represents mean ± S.E. of five to six experiments. $^{**}P < 0.01$ vs. each control.

3.3. Effect of Ni^{2+} on Ca^{2+} influx evoked by internal store depletion

When cells were stimulated with Ang II or 5-HT in the absence of extracellular Ca^{2+} (1.3 mM), the transient Ca^{2+} mobilization from the internal stores was observed as demonstrated in Fig. 1. As demonstrated in Fig. 5, when cells were thereafter exposed again to the buffer containing 1.3 mM Ca^{2+}, a rapid Ca^{2+} influx into the cells was observed. Ni^{2+} significantly suppressed the increase in $[Ca^{2+}]_i$ induced by Ca^{2+} re-exposure in cells stimulated with both Ang II (from 127.0 ± 19.8 to 37.4 ± 2.9 nM, $n = 5$, $p < 0.01$) and 5-HT (from 133.7 ± 21.9 to 41.8 ± 4.9 nM, $n = 5$, $p < 0.01$).

3.4. Effects of Ni^{2+} on 5-HT-induced Ca^{2+} mobilization from internal stores

5-HT stimulation in the absence of extracellular Ca^{2+} (1.3 mM) induced Ca^{2+} mobilization from the internal store(s), as demonstrated in Fig. 1B. Ni^{2+} at 1 mM had effect on neither the basal $[Ca^{2+}]_i$ before stimulation, the peak $[Ca^{2+}]_i$ induced by 10 μM 5-HT, nor $[Ca^{2+}]_i$ at 5 min after the stimulation (data not shown).

The effect of Ni^{2+} on 5-HT-induced phosphatidylinositol metabolism in aortic smooth muscle cells was also investigated. The stimulation with 5-HT at a concentration of 10 μM for 1 min significantly increased the production of IP_3 from 71.2 ± 13.5 to 192.0 ± 28.2 dpm/dish ($n = 4$,

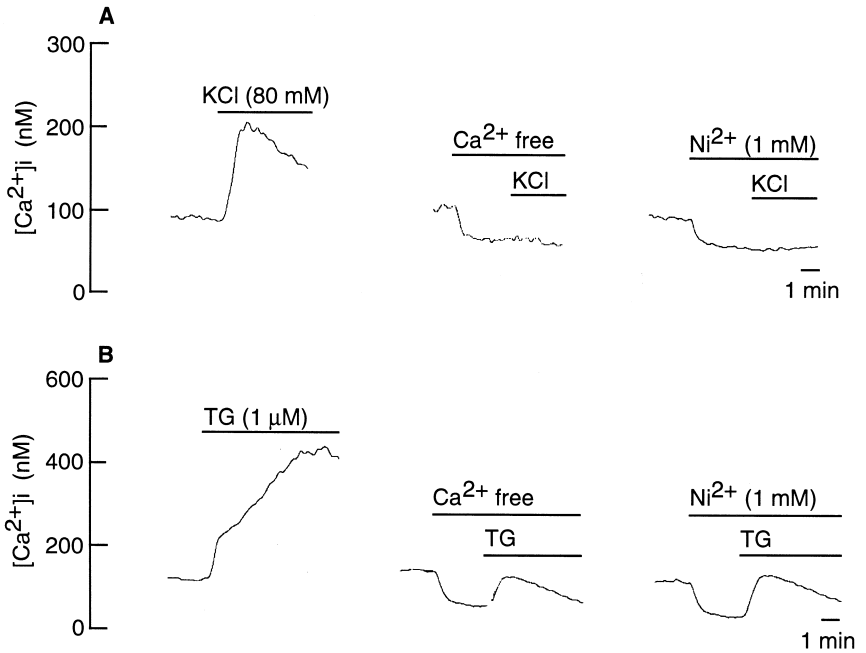

Fig. 4. Effect of Ni^{2+} on KCl- and TG-induced intracellular Ca^{2+} dynamics in rat aortic smooth muscle cells. Cells were stimulated with 80 mM KCl (A) or 1 μM TG (B) in HBSS containing 1.3 mM Ca^{2+}, or after exposure to Ca^{2+}-free HBSS (+1 mM EGTA) or HBSS containing 1 mM Ni^{2+} for 3 min. Representative tracings in an experiment of each agent.

$p < 0.01$). The increasing effect of 5-HT was not significantly altered when cells were stimulated in the presence of 1 mM Ni^{2+} (from 74.2 ± 9.4 to 160.4 ± 24.9 dpm/dish, $n = 4$, $p < 0.05$) and in the absence of extracellular Ca^{2+} (from 77.9 ± 6.6 to 167.2 ± 26.6 dpm/dish, $n = 4$, $p < 0.05$), although slightly suppressed.

4. Discussion

5-HT induces a biphasic changes in intracellular Ca^{2+} dynamics, i.e., a transient peak increase followed by a smaller sustained increase in $[Ca^{2+}]_i$ in rat aortic smooth muscle cells in primary culture (Hirafuji et al., 1998). The

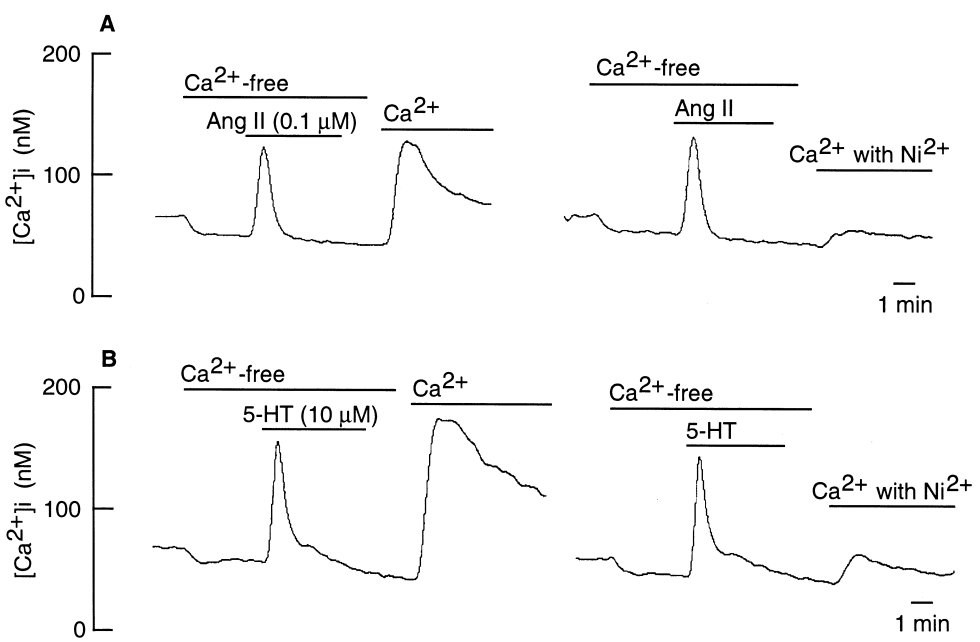

Fig. 5. Effect of Ni^{2+} on intracellular Ca^{2+} dynamics evoked after Ang II- and 5-HT-induced internal Ca^{2+} store depletion in rat aortic smooth muscle cells. Cells were stimulated with 0.1 μM Ang II (A) or 10 μM 5-HT (B) in the absence of extracellular Ca^{2+} (1.3 mM), and then re-exposed to HBSS containing Ca^{2+} without (left) or with (right) 1 mM Ni^{2+}. Representative tracings of an experiment of each agonist.

effect of 5-HT is mediated via 5-HT_2 receptor subtypes, and accompanied by the production of intracellular IP_3 (Doyle et al., 1986; Kanaide et al., 1987; Hirafuji et al., 1998). 5-HT induced a smaller transient increase in the absence of extracellular Ca^{2+}, indicating that 5-HT-induced Ca^{2+} transient is due to the influx of extracellular Ca^{2+} as well as the mobilization of Ca^{2+} from the intracellular stores such as IP_3-sensitive store. However, the 5-HT-induced Ca^{2+} influx pathways are still unclear. Diltiazem, a blocker of L-type Ca^{2+} channel, at 1 μM did not suppress the 5-HT-induced Ca^{2+} transient, and only partially suppressed even at a high concentration of 10 μM (Hirafuji et al., 1998). Similar result has been obtained with nifedipine, another L-type Ca^{2+} channel blocker (Capponi et al., 1987). Therefore, it is likely that 5-HT induces a transient Ca^{2+} influx mainly through voltage-independent Ca^{2+} channels.

Ni^{2+} is known as a non-selective cation channel blocker, and inhibits voltage-dependent T-type Ca^{2+} channels at lower concentrations, and additionally L-type channels and Na^+/Ca^{2+} exchange at higher concentrations (Fox et al., 1987; Hagiwara et al., 1988). Both type of Ca^{2+} channels has been shown to be present in rat aortic smooth muscle cells, by using the whole voltage-clamp method (Hirakawa et al., 1995). In the course of the study to clarify the pathways for 5-HT-induced transient Ca^{2+} influx in vascular smooth muscle cells, we were interested to examine the effect of Ni^{2+} on the Ca^{2+} influx. Exposure of cells to depolarizing concentration of KCl at 80 mM induced a gradual increase in $[Ca^{2+}]_i$. TG induced an immediate increase followed by a gradual continuous increase in $[Ca^{2+}]_i$. When cells were exposed to the depolarizing concentration of KCl and TG in the presence of 1 mM Ni^{2+}, the intracellular Ca^{2+} dynamics observed were similar to those observed in the absence of extracellular Ca^{2+}. TG, an inhibitor of sarcoplasmic reticulum Ca^{2+}–ATPase, is known as a pharmacological tool to induce Ca^{2+} influx through store-operated Ca^{2+} channels, though partially through L-type Ca^{2+} channel blocker-sensitive channels (Xuan et al., 1992). Ca^{2+} influx through store-operated Ca^{2+} channels was also evoked when cells were exposed to the buffer containing Ca^{2+} following the stimulation with Ang II or 5-HT in the absence of extracellular Ca^{2+}. Ni^{2+} also significantly inhibited these Ca^{2+} influxes. Therefore, it is obvious that Ni^{2+} inhibit Ca^{2+} influx through store-operated Ca^{2+} channels as well as the voltage-dependent channels as a cation channel blocker in vascular smooth muscle cells.

The present study also indicated that intracellular Ca^{2+} dynamics induced by a maximal concentration (0.1 μM) of Ang II in the presence of Ni^{2+} was similar to that induced in the absence of extracellular Ca^{2+}. Ang II induced extracellular Ca^{2+} influx as well as Ca^{2+} mobilization from intracellular stores. Previous studies have indicated that Ang II stimulates voltage-dependent Ca^{2+} channels in vascular smooth muscle cells, (Ohya and Sperelakis, 1991; Morel et al., 1996). Therefore, these results suggest that Ang II-induced Ca^{2+} transient is largely through Ni^{2+}-sensitive channels such as voltage-dependent or store-operated Ca^{2+} channels. Since the sustained increase induced by 5-HT was significantly suppressed by Ni^{2+}, it is mainly due to Ca^{2+} influx through these Ca^{2+} channels as well. Vasopressin and endothelin receptor stimulations have been shown to promote Ca^{2+} influx mainly through the non-L-type and receptor-mediated Ca^{2+} entry pathway, which is inhibited by Ni^{2+} in cultured A10 smooth muscle cells (Simpson et al., 1990).

However, in contrast to the case of Ang II, vasopressin or endothelin, the Ca^{2+} transient induced by 5-HT was not inhibited by Ni^{2+}. Therefore, it could be due to Ca^{2+} influx through Ni^{2+}-insensitive Ca^{2+} channels other than L-type or store-operated Ca^{2+} channels, in addition to Ca^{2+} mobilization from intracellular Ca^{2+} stores. The higher concentration of 5-HT is used, the more contribution of the Ni^{2+}-insensitive channel to the Ca^{2+} influx was observed. Consistent to our results, the transient rise in $[Ca^{2+}]_i$ evoked by 5-HT has been shown to be independent of the entry of Ca^{2+} through voltage-sensitive Ca^{2+} channels in rat aortic smooth muscle cells (Capponi et al., 1987) and rat cerebrovascular smooth muscle cells (Wang et al., 1991). Na^+/Ca^{2+} exchange as well as plasma membrane Ca^{2+}–ATPase is important for intracellular Ca^{2+} extrusion process in vascular smooth muscle cells (Karaki et al., 1997). The Ca^{2+} mobilization from intracellular stores is at least in part through IP_3-sensitive store (Doyle et al., 1986; Kanaide et al., 1987; Hirafuji et al., 1998). The levels of IP_3 produced by 5-HT stimulation under the condition with and without extracellular Ca^{2+}, and in the presence of Ni^{2+} were almost the same. Further, Ni^{2+} had no effect on the Ca^{2+} transient induced by 5-HT in the absence of extracellular Ca^{2+}. Therefore, it is ruled out the possibility that 5-HT-induced Ca^{2+} transient in the presence of Ni^{2+} is due to altered Ca^{2+} release from the intracellular store(s) or the inhibition of Na^+/Ca^{2+} exchange.

Several mechanisms that activate the voltage-independent Ca^{2+} channels have been reported. Although their classification and terminology are not yet established, the voltage-independent channels are usually subdivided into four types: receptor-operated channel, second messenger-operated channel, G protein-operated channel, and store-operated channel (Fasolato et al., 1994; Felder et al., 1994; Hughes, 1995; Clementi and Meldolesi, 1996). All these types of Ca^{2+} channels seems to be present in vascular smooth muscle cells (Hughes, 1995). In endothelial cells, pharmacological evidences indicate that the Ca^{2+} influx pathway activated by receptor agonist is indistinguishable from store-operated Ca^{2+} channels (Schilling et al., 1992), while others suggested that these two pathways are distinguishable because of differences in the permeability to Mn^{2+} and the sensitivity to SKF 96365 (1-[3-(4-methoxyphenyl) propoxyl]-1-(4-methoxyphenyl)ethyl-1*H*-imida-

sole HCl), a putative inhibitor of receptor-operated Ca^{2+} channels (Li and Van Breemen, 1996). Our results indicated that Ni^{2+}-insensitive channel activated by 5-HT receptor stimulation is distinguishable from store-operated Ca^{2+} channels in vascular smooth muscle cells.

The functional roles of voltage-independent Ca^{2+} channels in the regulation of vascular smooth muscle contraction are still unclear, although the sustained Ca^{2+} influx through the voltage-dependent channels is necessary for the maintenance of contraction in many cases (Karaki et al., 1997). Ca^{2+} influx through the Ni^{2+}-insensitive Ca^{2+} channels seems to be not directly linked to the contraction, because our preliminary experiments showed that Ni^{2+} at 2 mM completely suppressed the 5-HT-induced tension development of rat aortic strips (Kawahara et al., 1998), while had no effect on Ca^{2+} transient as demonstrated here. It is possible that 5-HT stimulation induces the opening of the Ni^{2+}-insensitive channels that facilitate depolarization and subsequent gating of the L-type channels. It can also induce Ca^{2+} influx through store-operated Ca^{2+} channels as a consequence of phosphatidylinositol hydrolysis. Protein kinase C can positively regulate the activation of L-type Ca^{2+} channels by 5-HT stimulation (Hirakawa et al., 1995; Hirafuji et al., 1998), and induce Ca^{2+} sensitization of contractile elements (Karaki et al., 1997). Elevation of intracellular Ca^{2+} may further induce Ca^{2+}-induced Ca^{2+} release from the ryanodine-sensitive stores (Saida and Van Breemen, 1983; Morel et al., 1996).

In conclusion, the present study suggests that 5-HT induces transient Ca^{2+} influx through Ni^{2+}-insensitive Ca^{2+} channels, which are distinguishable from the voltage-dependent or store-operated Ca^{2+} channels. Since this effect is mediated via $5\text{-}HT_2$ receptor subtype coupled to a G protein, the Ni^{2+}-insensitive channel could be either or both of second messenger-operated or G protein-operated Ca^{2+} channels. The second messenger-operated channels appear to comprise a heterogeneous family of channels, regulated by a variety of second messengers such as IP_3, inositol tetraphosphates (IP_4), cyclic GMP, protein kinase C or Ca^{2+} itself (Clementi and Meldolesi, 1996). Our results indicate that Ni^{2+} is a useful pharmacological tool to further investigate the nature and the regulatory mechanism of receptor-mediated Ca^{2+} influx pathway in 5-HT-stimulated vascular smooth muscle cells.

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