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GS354 and GS389: New Type of Calcium Channel Blockers

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ABSTRACT

The inhibitory effects of GS354 and GS389 on cytosolic Ca^{2+} level ($[Ca^{2+}]_i$; measured with fura-2 fluorescence) and muscle tension in vascular smooth muscle of rat thoracic aorta were investigated. Both GS354 and GS389 inhibited the contractions induced by high K^+ or by norepinephrine. The vasodilator effect of GS354 was accompanied by a decrease in $[Ca^{2+}]_i$. The inhibitory effect on high K^+-stimulated $[Ca^2]_i$ was antagonized by a Ca^{2+} channel activator, Bay K8644. However, the inhibitory effect on muscle tension was not antagonized by Bay K8644. These results suggest that GS354 inhibits Ca^{2+} channels to decrease $[Ca^{2+}]_i$ and also decreases Ca^{2+} sensitivity of contractile elements. The inhibitory effects of GS389 was accompanied by the increase in tissue fluorescence. This increment was not due to fura-2 fluorescence but to endogeneous pyridine nucleotides, suggesting that GS389 has an effect to inhibit mitochondrial function. Because of this interference, effects of GS389 on $[Ca^{2+}]_i$ was obscured. However, since sequential addition of Bay K8644 in the presence of GS389 further increased the fluorescence but not muscle tension, this compound seems to have the effects to inhibit Ca^{2+} channels and to decrease Ca^{2+} sensitivity of contractile elements.

Key Words: Vascular smooth muscle, Relaxation, Calcium channel

INTRODUCTION

Tetrahydroisoquinolines(THI) have various pharmacological actions such as antiplatelet aggregation action, calcium antagonistic action, cardiac stimulating action, bronchial as well as vascular smooth muscle relaxation action (Asoke *et al.* 1985, Ruppert *et al.* 1982, Ko *et al.* 1990, Park *et al.*, 1984, Chang *et al.* 1986). The prototype of THI is papaverine, of which chemical stucture provides us a very important and useful information for drug design to develop selectively acting drugs, since it shares all of these actions mentioned above. Trimetoquinol, for example, is a bronchoselective beta adrenergic agonist developed by modifying papaverine structure which is useful for the treatment of moderated bronchial asthma(Iwasawa *et al.* 1967). Chemical modification, therefore, of THIs to develop more potent and selective acting drugs has been made by many investigators (Davis *et al.* 1984, Kantelip *et al.* 1988, Chang *et al.* 1990, Pierre *et al.* 1991). Recently we have synthesized a papaverine analog, GS389, 1-(4'-methoxyben-zyl)-6.7-dimethoxy-1,2,3,4-tetrahydroisoquin-oline and GS354, N-(3'4'-dimethoxy pheny-lethyl)-4-methoxy phenylacetamide(Fig. 1). We were interested in the mechanism of GS354 and GS389 since other isoquinoline compound such as H-7, HA-1077 and ML-9 have

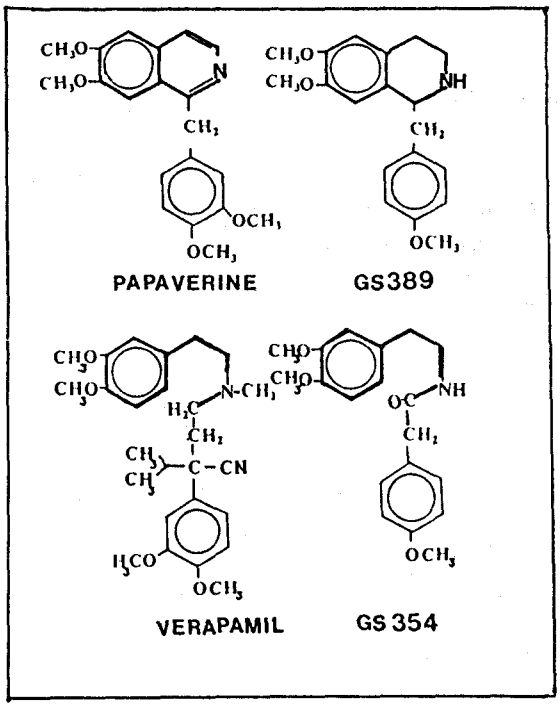

Fig. 1. Structural similarities of GS354, GS389, verapamil and papaverine. Bold line of upper layer indicates tetrahydroisoquinoline skeleton.

unique inhibitory effects on smooth muscle. This, therefore, has been the purpose of the present experiments.

MATERIALS AND METHODS

Materials

Norepinephrine hydrochloride, phenylephrine hydrochloride, Bay K8644, indomethacin N^{ω}-nitro-l-arginine metyl ester hydrochloride were purchased from Sigma, ^{125}I cAMP and cGMP kit from Amersham. GS354 and GS389 synthesized by our laboratory.

Muscle preparations

Male Wistar rats(200~300g) were killed by a blow on the neck and exsangination. The thoracic aorta was removed and placed in physiological salt solution. Adhered fat and connective tissues were removed and transverse rings(2.5mm wide) or helical strips(2.5 × 15mm) were prepared according to Chung *et al.* (1987). Each ring or strip was mounted using tungsten wire and connected to transducer for recording.

Solutions

The normal physiological salt solution contained(mM), NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.18; KH_2PO_4 1.18; $NaHCO_3$, 24.9; glucose, 10; EDTA, 0.03. Isosmotic 64.7mM K^+ solution was made by substituting 60 mM NaCl in the normal solution with equimolar KCl. These solutions were saturated with a mixture of 95% O_2 and 5% CO_2 at 37°C.

Tension recording

Muscle tension was recorded isometrically with a force displacement transducer(FT-03) connected to a physiograph(Grass 7E). Passive tension of 1g was initially applied and tissues were allowed to equilibrate in a 10ml bath for more than 90min before commencing the experiment. GS compounds were cumulatively added when the contractile tension induced by a stimulant reached a steady level.

Cytosolic Ca^{2+} levels

Cytosolic Ca^{2+} levels $[(Ca^{2+})_i]$ was measured simultaneously with muscle contraction as described by Ozaki *et al.*(1987) and Sato *et al.* (1988) using a fluorescent Ca^{2+} indicator, fura-2 (Grynkiewicz *et al.* 1985). The muscle ring was loaded with 5μM acetoxymethyl ester of fura-2 for 3hr in the presence of 0.02% Cremophore EL at room temperature(23~25°C) and then placed in a tissue bath at 37°C. The muscle was illuminated alternatively(48HZ) with 340nm and 380nm light and 500nm emission was detected with a fluorimeter(CAF-100, JASCO, Tokyo, Japan). The amounts of the 500nm fluorescence induced by the 340nm excitation(F380) were measured and the ratio of these two fluorescence(R340/380) was calculated. We used the relative R340/380 value as an indicator of $[Ca^{2+}]_i$, taking R340/380 in resting muscle as 0% and that in hign K^+-simtulated muscle as 100%. In order to know the effects of test compounds on endogenous fluorescence and also on the fura-2 fluorescence, was always monitored F340 and F380. In some experiments, fluorescence in

muscle without fura-2 loading was also examined to monitor the effects of test compounds on endogenous fluoresence. In a preliminary experiments, we have confirmed that these GS compounds do not have fluorescence or absorbance of fura-2 fluorescence.

Measurement of cAMP and cGMP

cAMP and cGMP were assayed using ^{125}I RIA kit. Rings of 2~3mm width were cut from rat aorta. After recording their wet weight, rings were equilibrated for 60min in Krebs solution and bubbled with 95% O_2 and 5% CO_2 at 37°C. Phenylephrine was added to each group of vascular rings, 100μM NNA was pretreated before addition of phenylephrine. After quick freezing with clamps precooled on liquid nitrogen. Samples were processed according to the protocol described in the brocheur.

RESULTS AND DISCUSSION

Effects of GS354

Fig. 2. shows the concentration response curves for GS354 and GS389 on the contractions induced by high K^+ or norepinephrine in rat aorta. GS354 and GS389 inhibited the contractions induced by 64.7mM KCl and 1μM norepinephrine. The inhibitory effect on high K^+ induced contraction was stronger than that on norepinephrine induced contraction in case of GS354.

By contrast, other isoquinoline derivatives, H-7, HA-1077 and ML-9, inhibited the contractions induced by K^+ and norepinephrine with similar potencies(Karaki *et al.* 1990). From these results, we decided to use 30μM GS compounds in the following experiments. Fig. 3A shows the effect of 30μM GS354 on high K^+ induced contraction and$[Ca^{2+}]_i$ in rat aorta. GS 354 inhibited high K^+ induced contraction and $[Ca^{2+}]_i$ almost in parallel. The inhibitory effect on $(Ca^{2+})_i$ was antagonized by the addition of 0.3μM Bay K8644 although muscle tension was not recovered. Fig. 3B shows the effect of GS354 on 1μM norepinephrine induced contraction and $[Ca^{2+}]_i$ in rat aorta. 30μM GS354 inhibited the norepinephrine induced contraction with almost parallel decreases in$[Ca^{2+}]_i$.

Previously, it has been shown that a Ca^{2+}

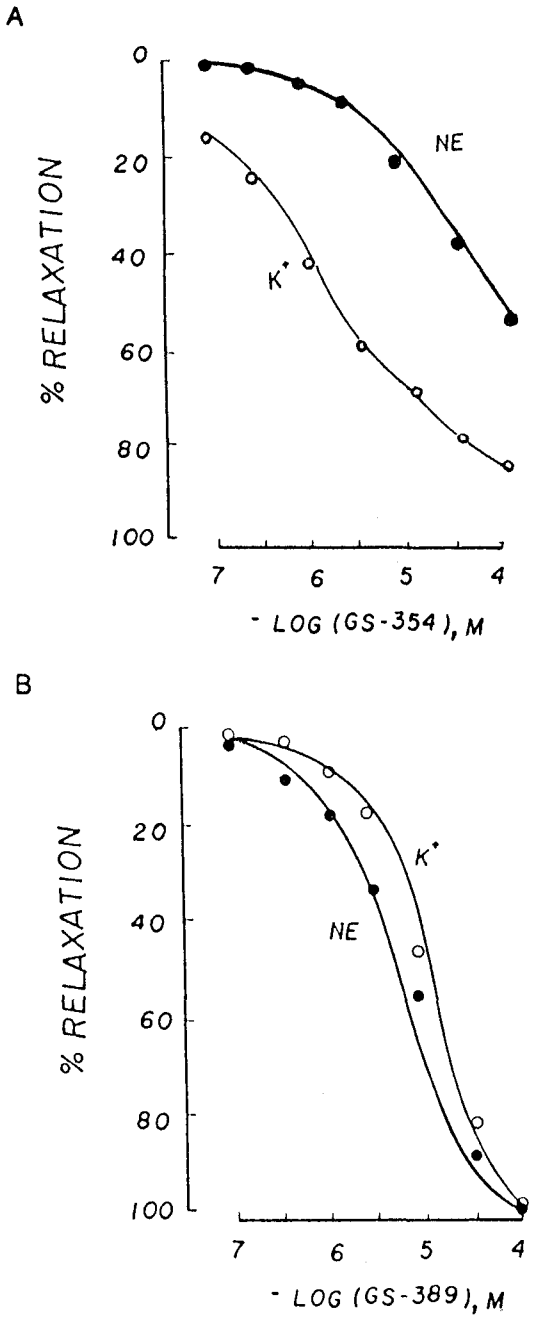

Fig. 2. Dose-response curves of GS354(A) and GS389(B) on high K^+ and norepinephrine induced contraction in rat aorta.

channel blocker, verapamil, inhibits high K^+ induced contraction more strongly than norepi-

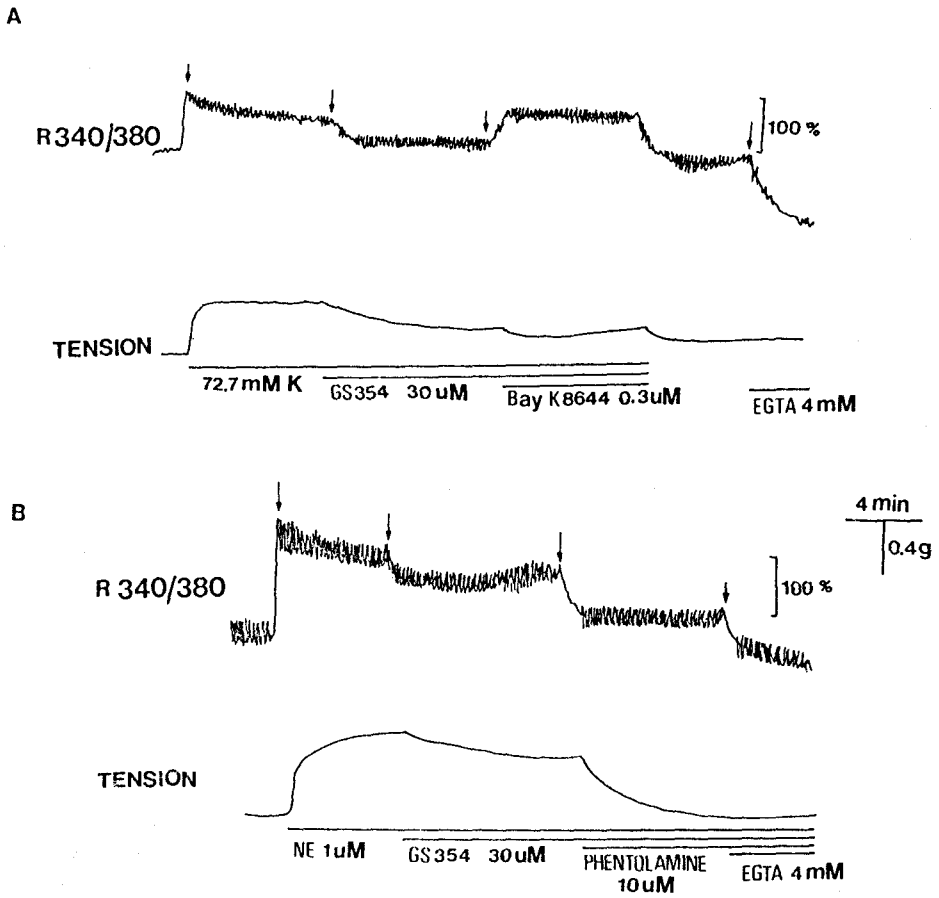

Fig. 3. Effects of GS354 on $[Ca^{2+}]_i$ measured with fura-2 and muscle tension in isolated rat aorta stimulated by high K^+(A) and norepinephrine(B).

nephrine induced contraction, inhibits the $[Ca^{2+}]_i$ stimulated by high K^+ or phenylephrine, and the inhibition is antagonized by By K8644 (Karaki *et al.* 1990). These results are simlar to those obtained with GS354 suggesting that this compound has an effect to inhibit Ca^{2+} channels. Although Bay K8644 antagonized the inhibitory effect of GS354 on $[Ca^{2+}]_i$, it did not antagonize the inhibitory effect on muscle tension. This results suggest that GS354 inhibits the Ca^{2+} sensitivity of contractile elements. Furthermore, GS354 inhibited the norepinephrine stimulated$[Ca^{2+}]_i$ and muscle tension almost in parallel. It has been shown that verapamil inhibits norepinephrine stimulated $[Ca^{2+}]_i$ more strongly that contraction because norepinephrine induced contraction is due to increase in $[Ca^{2+}]_i$ and also Ca^{2+} sensitivity of contractile elements, and that verapamil selectively inhibit the increase in $[Ca^{2+}]_i$ with little effect on Ca^{2+} sensitivity. The difference between the effects of GS354 and verapamil support the suggestion the GS354 inhibits Ca^{2+} sensitivity.

Effects of GS389

Fig. 4A and B show the effect of 30μM GS389 on the contraction and $[Ca^{2+}]_i$ stimulated by high K^+ or norepinephrine. Surprisingly, GS389 seemed to increase $[Ca^{2+}]_i$ (or increased fluorescence ratio) although it inhibited the contractions induced by high K^+ and norepinephrine. Examination of F340 and F380 in Fig. 4A

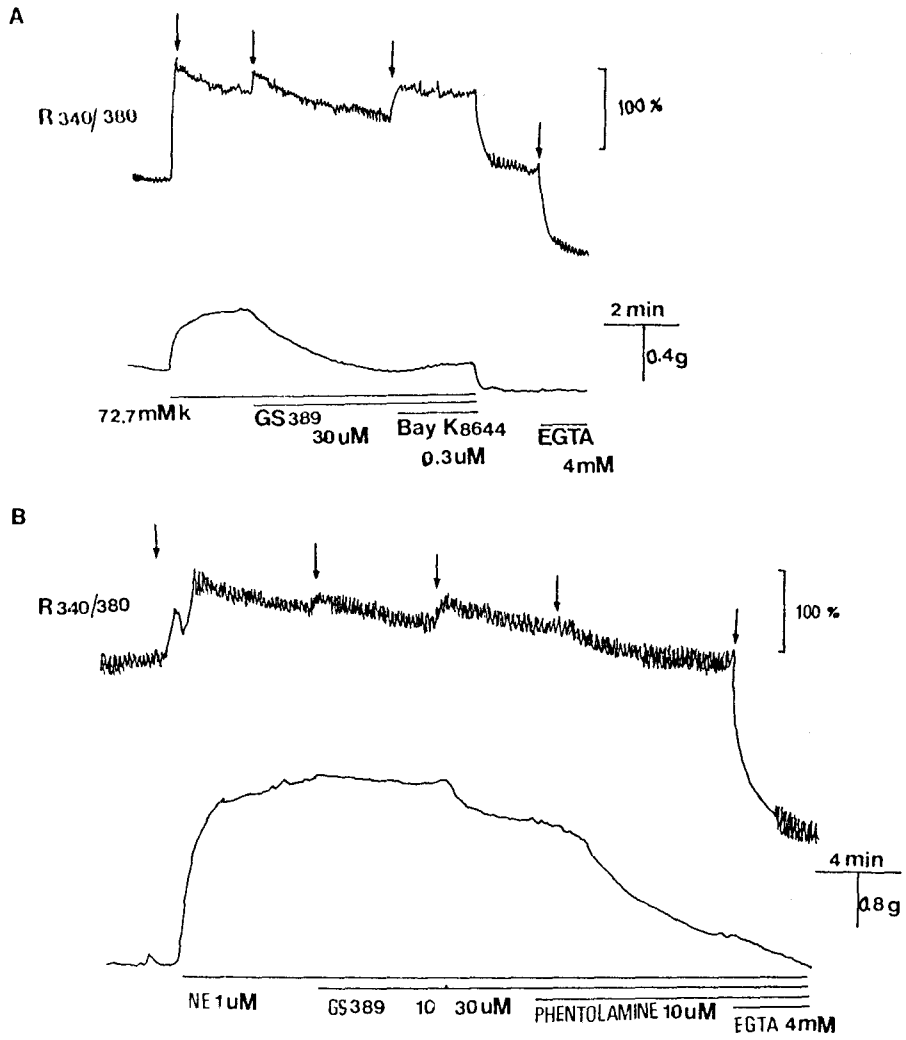

Fig. 4. Effects of GS389 on $[Ca^{2+}]_i$ measured with fura-2 and muscle tension in isolated rat aorta stimulated by high K^+(A) and norepinephrine(B).

suggested that this increase in fluorescence ratio is not due to the increase in $[Ca^{2+}]_i$ because both F340 and F380 increased on the addition of GS389 although increased in $[Ca^{2+}]_i$ should decrease F380 and increase F340. In order to know the reason for the GS389 induced increase in fluorescence, we measured the effects of this compound on the endogenous fluorescence of rat aorta without fura-2 loading. Results in Fig. 5 indicate that high K^+ stimulation increased both F340 and F380 without changing R340/380.

When GS389 and high K^+ were added simultaneously, however, F340, F380 and R340/380 were increased. This result suggest that the increase of internal fluoresence substances, possibly pyridine nucleotides and flavoproteins, are increased by GS389. This results further suggest that GS389 inhibits mitochondrial function resulting in a change in metabolic state of the muscle.

However, this effect does not seem to play a

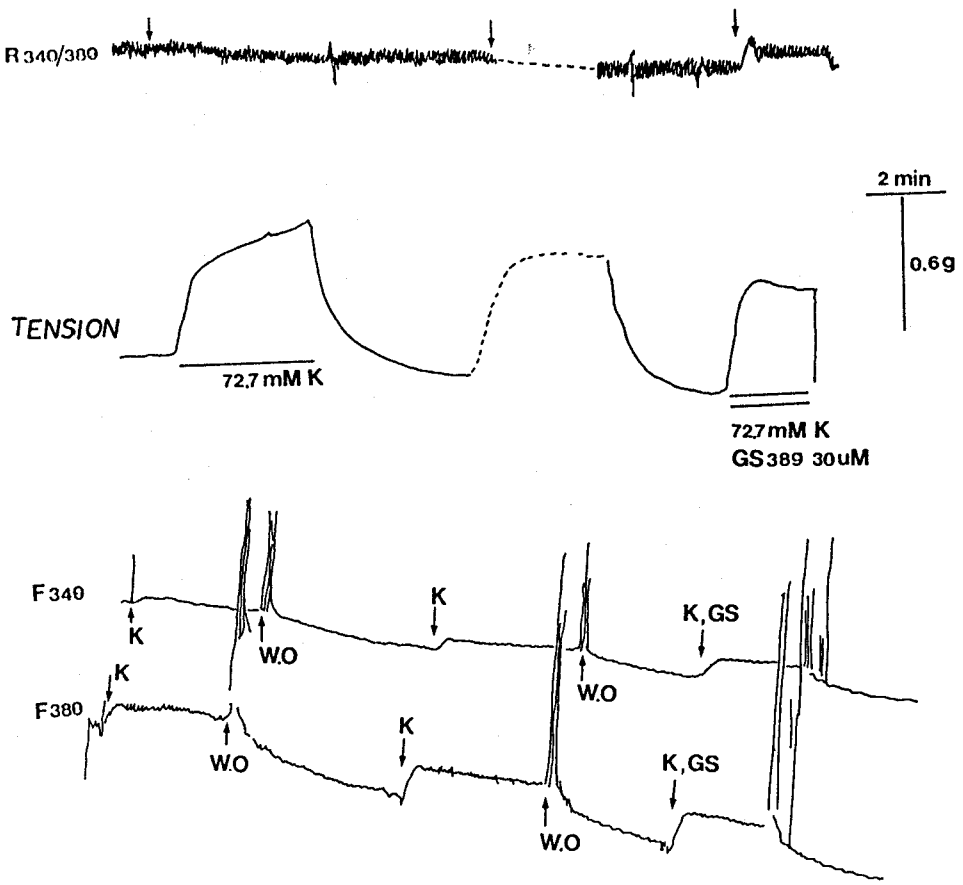

Fig. 5. Effects of GS389 on endogenous fluoresecence. High K^+ stimulation increased both F340 and F380 without changing R340/380. GS389 increased F340, F380 and R340/380 when added high K^+ simultaneously, indicating GS389 increases internal fluorescent substances.

Table 1. Effects of GS389 on cyclic nucleotide levels in rat aorta(unit: fmol/mg wet weight)

Treatment	Endothelium	cAMP	cGMP
Control	+	62.35 ± 1.44	8.1 ± 0.03
GS389	+	79.90 ± 4.12	13.7 ± 0.07*
PE + GS389	+	113.45 ± 12.36	20.0 ± 0.08*
PE + NNA + GS389	+	88.00 ± 3.19	4.3 ± 0.01§
PE + GS389	−	90.55 ± 1.27	4.5 ± 0.06§
PE + NNA + GS389	−	74.80 ± 1.12	0.9 ± 0.02§

NNA: N^w-nitro-l-arginine methly ester, PE: Phenylephrine

Cyclic nucleotides in tissues were checked at 30 sec after incubating GS389.

*: significantly greater than control($P<0.5$)

§: significantly smaller than control($P<0.05$)

major role on the inhibitory effect a weak inhibitory effect in rat aorta. Addition of Bay K8644 further increased the flourescence in GS389 treated muscle with little effect of muscle tension(Fig. 4A). Examination of F340 and F380 suggest that this increase is due to increase in $[Ca^{2+}]_i$. Consequently, GS389 may have an effect quite similar to that of GS354, that is, inhibition of Ca^{2+} channels and Ca^{2+} sensitivity of contractile elements. GS389 increased cGMP more in endothelium intact rings than endothelium rubbed rings and also NNA, EDRF inhibitor, antagonized the effect of GS389. However, cAMP level was not affected (Table 1). This increment of cGMP may be responsible for lowering Ca^{2+} sensitivity of contractile element (Godfraind 1986, Karaki, 1989). In summary, GS354 and GS389 inhibited both muscle tension evoked by norepinephrine and high K^+ and cytosolic Ca^{2+} levels. GS389 also increased cGMP not cAMP this result imply that GS389 may inhibit cGMP specific phosphodiesterase, which awaits further investigation.

REFERENCES

Asoke M, Stephen SN, Hebatalla MA, Sayed AA, Jane C, Douglas JS, Duana DM and Dennis RF: *Effects of trimetoquinol analogs for antagonism of endoperoxide/thromboxane A_2-mediated responses in human platelet and rat aorta. J Pharmacol Exp Ther 232: 1-9, 1985*

Chang KC, Lim JK and Park CW: *Synthesis of higenamine and its cardiovascular effect in rabbit: Evidence for beta adrenergic agonist. Kor J Pharmacol 22: 96-104, 1986*

Chang KC, Sohn DR, Chung WS, Chung SY and Lee YS: *Approaches to the mechanism of relaxing effect of GS389 on rat aorta. Abstract. The 9th Korea-Japan Joint seminar on Pharmacology 1990*

Chung SY, Chang KC and Lim JK: *Responsiveness of muscarinic and alpha adrenergic activation on endothelial cell in isolated canine renal arteries. Kor J Pharmacol 25(1): 43-51, 1989*

Davis CW and Walker KA: *1-(4-aminophenyl) isoquinoline derivatives potent inhibitors of calcium-dependent and calcium independent phosphodiesterase from rat cerebral cortex. Biochem Pharmacol 33: 1205-1212, 1984*

Godfraind T: *EDRF and cyclic GMP control gating of receptor-operated calcium channels in vascular smooth muscle. Eur J Pharmacol 126: 341-343, 1986*

Grynkirwicx G, Poenie M and Tsien RY: *A new generation of Ca^{2+} indicator with greatly improved fluorescence properties. J Biol Chem 260: 3440-3450, 1985*

Iwasawa Y, Kiyomoto A: *Studies on tetrahydro-isoquinoline(THI) 1. bronchodilator activity and structure activity relationship. Jpn J Pharmacol 17: 243-252, 1967*

Kantelip JP, Talmant JM and Duchene-Marullaz D: *Effects of dipeototeverine, a new calcium antagonist on sinoatrial node and atrioventricular conduction in conscious unsedated dogs. J Cardiovas Pharmacol 12: 432-436, 1988*

Karaki H: *Ca^{2+} localization and sensitivity in vascular smoth muscle. TIPS 10: 320-325, 1989*

Karaki H: *Effects of HA 1077 on cytosolic Ca^{2+} level and contraction in vascular smooth muscle. Abstract Jpn Pharmacol meeting, 1991*

Ko FN, Chen IS, Wu SJ, Lee LG, Haung TF and Tao CM: *Antiplatelet effects of Cherythrine chloride isolated from Zanthxylum simulanes. Biophysica Acta 1052: 360-365, 1990*

Ozaki H, Sato K, Satoh T and Karaki H: *Simultaneous signals and mechanical activity using fluorescent dye fura 2 in isolated strips of vascular smooth muscle. Jpn J Pharmacol 45: 429-433, 1987*

Sato M, Nagao T, Yamaguchi I, Nakajima H and Kiyomoto A: *Pharmacological studies on a new 1, 5-benzothiazepine derivative(CRD 401), Arzneim. Forsch 21: 1338-1402, 1988*

Park CW, Chang KC and Lim JK: *Effects of higenamine on isolated heart adrenoceptor of rabbit. Arch Pharmacodyn Ther 267: 279-288, 1984*

Pierre L, Philippe L and Marie CF: *Diproteverine (BRL40015): A new type of calcium antagonist with potential antianginal pectoris. Eur J Pharmacol 192: 317-327, 1991*

Ruppert D, Weithnab KU: *HL725, an extremely potent inhibitor of platelet phosphodiesterase and induced platelet aggregation in vitro. Life Sci 31: 2037-2043, 1982*

Sato K, Ozaki H and Karaki H: *Multiple effects of caffeine on contraction and cytosolic free Ca^{2+} levels in vascular smooth muscle of rat aorta. Naunyn-Schmidebergs Arch Pharmacol 338: 443-448, 1988*

= 국문요약 =

GS354, GS389: 새로운 칼슘 길항제

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GS354와 GS389의 세포내 칼슘{$[Ca^{2+}]_i$: Fura-2의 형광으로 측정}과 근장력 변화에 대하여 백서의 흉부동맥을 사용하여 검토하였다. GS354와 GS389 모두 고농도의 포타슘과 노어에피네프린에 의한 수축을 억제시켰다. GS354의 혈관이완은 $[Ca^{2+}]_i$의 감소가 동반되었고 고농도의 포타슘에 의한 $[Ca^{2+}]_i$증가억제 현상도 칼슘통로 활성제인 Bay K8644에 의하여 길항되었다. 그러나 혈관이완 작용은 Bay K8644에 의해 억제 되지 않았다. 이러한 사실은 GS354는 칼슘통로를 차단하여 $[Ca^{2+}]_i$을 감소시키며 또한 수축기구에 대한 칼슘의 감수성을 낮추는 것을 암시하는 결과이다. 한편 GS389는 세포내 형광성을 증가시켰으나 이것은 Fura-2에 의한 형광이 아니라 내인성 피리딘 뉴클레오타이드에 의한것으로서 나타났다. 이것은 미토콘드리아 기능을 억제하는 것을 의미하며 이러한 현상때문에 GS389에 대한 $[Ca^{2+}]_i$의 측정이 곤란하였으나 계속하여 Bay K8644를 첨가하여 본 결과 형광은 더욱 증가 되었으나 혈관이완은 역전되지 않았다. 이러한 사실은 GS389가 칼슘통로를 억제하며 아울러 수축기구에 대한 칼슘의 감수성을 낮추는 것을 암시하는 결과로 사료된다.