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PARADOXICAL AUGMENTATION OF (-)BAY K 8644-INDUCED CALCIUM INFLUX BY NITRENDIPINE

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(-)Bay K 8644 produced a concentration-dependent contraction of porcine coronary artery rings with the maximal contraction at 10^{-6} M. Pretreatment of the rings with 10^{-6} M nitrendipine inhibited (-)Bay K 8644-induced contraction, while pretreatment with 10^{-8} M nitrendipine potentiated the contraction elicited by (-)Bay K 8644. (-)Bay K 8644 (10^{-6}M) significantly stimulated Ca^{2+} influx. Although 10^{-8} M nitrendipine never stimulated Ca^{2+} influx, Ca^{2+} influx induced by (-)Bay K 8644 was significantly potentiated by pretreatment with 10^{-8} M nitrendipine. Pretreatment with 10^{-6} M nitrendipine significantly decreased Ca^{2+} influx in tissues treated with (-)Bay K 8644. Our results suggest that the increased Ca^{2+} influx might be involved in the mechanisms by which (-)Bay K 8644-induced contraction was potentiated by pretreatment with nitrendipine.

Calcium entry from the extracellular space to the cytoplasm through voltage-dependent Ca^{2+} channels leads to a change in the intracellular Ca^{2+} concentration and is thought to play a critical role in excitation-contraction coupling in vascular smooth muscle. Calcium entry blockers, a group of compounds containing at least three structural groups, phenylalkylamines, benzothiazepines and the DHPs, act on the Ca^{2+} channels to produce an inhibition of Ca^{2+} influx (1). Although Ca^{2+} channel activators, or agonists, have the opposite effect of Ca^{2+} entry blockers (2), and competitively interact at mutual binding sites (3), the predominant interaction between both Ca^{2+} channel modulators has yet to be determined. Isometric contraction by the calcium agonist DHP (-)Bay K 8644 has been reported to be potentiated by

ABBREVIATIONS: DHP, 1,4-dihydropyridine; NTD, nitrendipine; PSS, physiologic saline solution; ED_{50}, median effective concentration.

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pretreatment with the antagonist DHPs NTD and nimodipine (4,5). We have suggested that attenuation of the release of endothelium-derived relaxing factors by the antagonists might explain at least in part these observations (6,7). Direct Ca^{2+} influx studies on this paradoxical phenomenon are required. Thus, we examined the interaction between the agonist specific stereoisomer (-)Bay K 8644 and NTD in isolated pig coronary arteries using both isometric tension and calcium influx studies.

METHODS

Isometric tension study. Fresh porcine hearts were obtained from a local abattoir and the right coronary artery was dissected. Loose connective and adipose tissues were trimmed away and the artery was cut into circumferential rings (length = 6 mm). Each ring was mounted isometrically in a 25 ml muscle bath containing PSS having the following composition (millimoles/L): 126.9 NaCl, 4.7 KCl, 1.6 $CaCl_2$, 1.17 $MgSO_4$, 1.8 KH_2PO_4, 18.0 $NaHCO_3$, and 5.5 glucose. The solution was continuously saturated with 95% O_2 and 5% CO_2 at 37 ± 0.5°C (pH = 7.35 ± 0.5.). The upper end of each ring was connected to the lever of a force-displacement transducer (model FT03 Grass Instrument Co.). Isometric contractions were displayed on an ink-writing polygraph. The coronary rings, under a resting tension of 5 g, were equilibrated for 2 hours in normal PSS before beginning the experiments. A set of four arterial rings was used from the same animal for the experiments. The contraction induced by 35 mM KCl was recorded first to test the response of the tissues. After washing with PSS several times, the tissues were further equilibrated for an hour. Then coronary rings were pretreated for 60 min with 10^{-8}, 10^{-7}, or 10^{-6} M NTD or the equivalent volume of solvent. Cumulative concentration-response curves with (-)Bay K 8644 were constructed, and each concentration was allowed to produce an equilibrium response before the next was added. In these experiments, control tissues in solvent alone was without effect except for a slight reduction in tension at the highest concentration employed. The solvent effects were subtracted from the responses of the test tissues.

^{45}Ca influx study. Coronary rings were equilibrated in normal PSS for 2 hours. These rings were then pretreated for 30 min in normal PSS containing either 10^{-8} or 10^{-6} M NTD while control rings were pretreated in normal PSS containing the solvent vehicle. The solutions were replaced and either 10^{-6} M (-)Bay K 8644 or the solvent was added. Therefore, there were six treatments, consisting of control, 10^{-8} or 10^{-6} M NTD in the absence and presence of 10^{-6} M (-)Bay K 8644 (Table 1). Then rings were exposed to ^{45}Ca (2 μCi/ml) control or experimental solution for 2 min. The tissues were then bathed in ice-cold Ca free PSS containing 2 mM EGTA for 45 min in order to remove extracellular ^{45}Ca. The tissues were subsequently blotted, weighed and incubated overnight in 3 ml of 5 mM EDTA at room temperature. Seven ml of scintillation cocktail containing Triton X-100 were added to the vials after which they were analyzed for ^{45}Ca in a liquid scintillation counter (Beckman, LS 8100). Calcium influx was calculated based on the specific activity of the appropriate solution.

Concentration-response curves were analyzed for statistical differences using Analysis of Variance (ANOVA, Human Systems Dynamics, Northridge, CA). Student's t-test by paired comparisons was used to evaluate the possible significant difference between ED_{50}'s, maximal contraction and Ca^{2+} influxes.

TABLE 1

The Experimental Protocol of ^{45}Ca Influx Study

	Time Course 0-30'	30' → 60'	60' → 62'
Control	PSS	PSS	PSS + ^{45}Ca
NTD 10^{-8} M	NTD 10^{-8} M	NTD 10^{-8} M	NTD 10^{-8} M + ^{45}Ca
NTD 10^{-6} M	NTD 10^{-6} M	NTD 10^{-6} M	NTD 10^{-6} M + ^{45}Ca
(-)Bay K 8644 10^{-6} M	PSS	(-)Bay K 8644 10^{-6} M	(-)Bay K 8644 10^{-6} M + ^{45}Ca
(-)Bay K 8644 10^{-6} M with NTD 10^{-8} M	NTD 10^{-8} M	(-)Bay K 8644 10^{-6} M NTD 10^{-8} M	(-)Bay K 8644 10^{-6} M NTD 10^{-8} M + ^{45}Ca
(-)Bay K 8644 10^{-6} M with NTD 10^{-6} M	NTD 10^{-6} M	(-)Bay K 8644 10^{-6} M NTD 10^{-6} M	(-)Bay K 8644 10^{-6} M NTD 10^{-6} M + ^{45}Ca

See Text for Details.

RESULTS

Isometric tension study. (-)Bay K 8644 produced a concentration dependent increase in tension (Fig. 1). Equilibrium tension at each concentration of (-)Bay K 8644 was reached after approximately an hour and the maximal

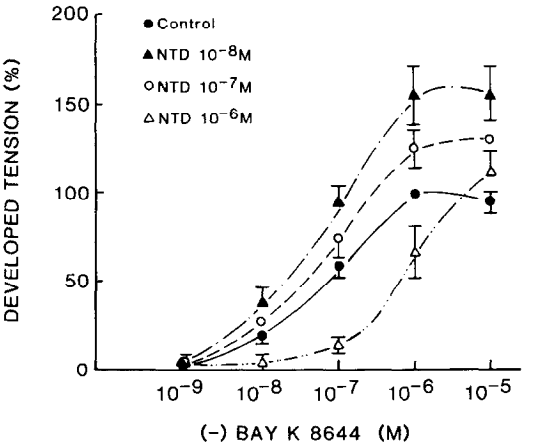

FIGURE 1. Effect of different concentrations of NTD on the (-)Bay K 8644 concentration-response curve. Following pretreatment with NTD for 60 min, (-)Bay K 8644 was added in a cumulative manner. Contractions relative to 10^{-6} M (-)Bay K 8644-induced maximum contractions in control tissues are presented. Each point represents the mean response ± S.E.M. of 7 experiments. ●: control ▲, ○, and Δ: pretreated with 10^{-8}, 10^{-7} and 10^{-6} M of NTD, respectively.

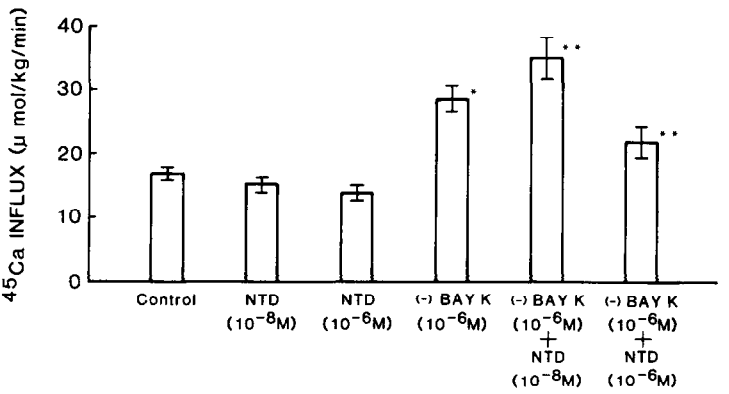

FIGURE 2. Ca^{2+} influx in pig coronary arteries. See text and table for details. Each point represents the mean ± S.E.M. of 11-24 experiments. *: significantly different from control; **: significantly different from (-)Bay K (10^{-6} M); (-)Bay K:(-)Bay K 8644.

contraction was obtained at a concentration of 10^{-6} M of the agonist. The ED_{50} for (-)Bay K 8644 in control tissues was 5.0 x 10^{-8} M. There was slight, or no, change in tension after treatment of tissues with different concentrations of NTD. Compared with control tissues pretreated with solvent, (-)Bay K 8644 produced a significantly greater maximum tension in tissues pretreated with 10^{-8} M of NTD (Fig. 1). Concentration-response curves for (-)Bay K 8644 were significantly different between control and 10^{-8} M NTD pretreated tissues (ED_{50} = 4.5 x 10^{-8} M). However, there was no difference between the ED_{50}'s for (-)Bay K 8644 in test and control tissues. When tissues were pretreated with 10^{-6} M NTD, the contractile effect of (-)Bay K 8644 was significantly depressed compared with control tissues (Fig. 1). The concentration-response curve for (-)Bay K 8644 was shifted to the right and the maximal contraction was not significantly different. The ED_{50} of the coronary rings pretreated with 10^{-6} M NTD (ED_{50} = 1.0 x 10^{-6} M) was significantly different from control rings ($P < 0.01$). The apparent ED_{50} of test tissues pretreated with 10^{-7} M NTD was 8.8 x 10^{-8} M.

^{45}Ca influx study. The data for ^{45}Ca influx experiments are summarized in Fig. 2. ^{45}Ca influx in porcine coronary artery in normal PSS was 16.9 ± 1.0 μ mol/kg/min. Neither 10^{-8} M (15.1 ± 1.2 μ mol/kg/min) nor 10^{-6} M NTD (13.8 ± 1.0 μ mol/kg/min) caused significant changes of ^{45}Ca influx in the PSS. 10^{-6} M

Bay K 8644 caused a significant stimulation of ^{45}Ca influx (28.6 ± 2.4 μ mol/kg/min) in these tissues. This 10^{-6} M Bay K 8644-induced stimulation of ^{45}Ca influx was significantly augmented by pretreatment with 10^{-8} M NTD (34.8 ± 3.7 μ mol/kg/min). In contrast, pretreatment with NTD 10^{-6} M significantly depressed ^{45}Ca influx in tissues treated with 10^{-6} M (-)Bay K 8644 (21.8 ± 2.5 μ mol/kg/min). We also examined ^{45}Ca in tissues with and without endothelium. No significant difference in ^{45}Ca influx in the control state was found between rings with intact endothelium (17.7 ± 1.2 μ mol/kg/min, n=10). (-)Bay K 8644-stimulation of Ca^{2+} influx in the control rings (26.2 ± 2.0 μ mol/kg/min, n=12) was not significantly different from that in endothelium-denuded rings (23.2 ± 2.1 μ mol/kg/min, n=12).

DISCUSSION

Ca^{2+} channel modulators act effectively on vascular smooth muscle in which the free Ca^{2+} ions needed for excitation-contraction coupling are mainly supplied from the extracellular space rather than from intracellular storage sites. Ca^{2+} channel activators produce contraction by acting primarily on potential operated Ca^{2+} channels, resulting in enhanced Ca^{2+} entry through the Ca^{2+} channels (8-10). Our study, as well as previous studies (4,5,11), confirmed the concentration-dependent agonist action of (-)Bay K 8644 on coronary smooth muscle.

A low concentration of NTD (10^{-8} M) did not change isometric tension nor Ca^{2+} influx into coronary rings in PSS containing 4.7 mM KCl. However, a paradoxical interaction between (-)Bay K 8644 and NTD in the pig coronary artery was observed. This study demonstrates that the Ca^{2+} channel blocker, NTD, stimulates both the contraction and Ca^{2+} influx induced by (-)Bay K 8644 in isolated porcine coronary arteries at 10^{-8} M, while NTD inhibited both effects at 10^{-6} M. We have recently provided several reports concerning the paradoxical potentiation of Ca^{2+} agonistic action by Ca^{2+} antagonists. Dube et al. (4,5) showed a surprising potentiation of (-)Bay K 8644-induced contractions by diltiazem as well as by nimodipine and NTD in inverted iso-

lated porcine coronary arteries. Williams et al. (6) also recently reported that the contraction induced by (+)202-791, a Ca^{2+} agonist, was significantly greater in non-inverted coronary rings with intact endothelium pretreated with 10^{-8} or 10^{-7} M (-)202-791, a Ca^{2+} antagonist, than in control tissues pretreated with only solvent. This could not be observed in tissues without endothelium, suggesting an important role for the endothelium in the potentiation phenomenon. Multiple effects induced by different components involving the endothelium as well as vascular smooth muscle should however be considered in the interpretation of the results of isometric tension studies. Obviously Ca^{2+} influx data should provide critical information concerning the mechanism of this paradoxical potentiation. Although it is possible that NTD inhibited the release of endothelium-derived relaxing factor(s) in the contractility studies, resulting in potentiation of the contraction, it would not be a reason for the increased Ca^{2+} influx observed here unless the endothelium is involved in the regulation of Ca^{2+} influx of vascular smooth muscle. There may be little or no correlation between this ^{45}Ca uptake data and the previous contractility data (6). It is not possible to simply assume that the calcium uptake in the contracting smooth muscle will be the same as that in tissues in the present study. Similarly, the endothelium may not play an important role in Ca^{2+} uptake, while it is known to be important in vascular smooth muscle tone and contractility. Our results suggest that the mechanism of the paradoxical potentiation of agonist-induced contraction by antagonist will involve increased Ca^{2+} influx in addition to the inhibition of the release of endothelium-derived relaxing factor(s).

Several possible mechanisms related to the potentiation of Ca^{2+} influx induced by (-)Bay K 8644 in the presence of a low dose of NTD can be considered. It has been suggested that DHP receptors have multiple binding sites with different affinities (12,13). Glossmann et al. (14) suggested that all individual binding sites can exist in one high and two low affinity states and the inactivated state is stabilized by DHP antagonists, the resting state by agonists. If this is the case, an explanation could involve the possibility

that the preferential binding of an antagonist, NTD, to the receptor in an inactivated state might lead to the increased binding of agonist, (-)Bay K 8644 to the calcium channel in the resting state which is available for opening. Recently, Kokubun et al. (15) demonstrated positive or homotropic cooperative interactions between (+)202-791 (channel activator) and [^{3}H]-(+)PN200-110 (channel blocker) in cardiac myocytes, indicating that at least two high affinity binding sites for DHPs are associated with voltage-dependent Ca^{2+} channels. Although they could only study the effects of a channel activator on the binding of a radiolabeled blocker, the activator-induced increase in the binding affinity of the blocker seems to be paralleled by a blocker-induced increase in the affinity of the activator resulting in their synergistic stimulation of Ca^{2+} currents.

While several possible theories exist to explain the present results, further examination is required to clarify the mechanism by which the (-)Bay K 8644-induced agonistic effect on the Ca^{2+} channel was potentiated by pretreatment with a low dose of the antagonist, NTD.

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