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The mode of vasorelaxant action of 2-aminoisoquinoline, 1.3 (2H.4H)-dione, a novel 'intracellular calcium inhibitor'

R.K. Hester, N. Satake, S. Shibata,[1], S. Ueda & J. Yamahara

Department of Pharmacology, School of Medicine, University of Hawaii, 1960 East West Rd, Honolulu, HI and Department of Medical Pharmacology & Toxicology, College of Medicine, Texas A & M University, College Station, TX, U.S.A.

1 In rabbit aorta, pretreatment with 2-aminoisoquinoline, 1.3 (2H.4H)-dione (AQ, 10^{-5} M and 10^{-4} M) shifted the concentration-response relationship to noradrenaline (NA, 10^{-9} M to 10^{-4} M) in a parallel manner whereas the agent (10^{-4} M) failed to affect the response to potassium and only slightly depressed Ca^{2+}-induced contractions in a Ca^{2+}-free medium in the presence of K^+ (40 mM).

2 Ca^{2+}-entry blockers such as nifedipine and diltiazem (10^{-6} M and 10^{-5} M) had very weak or no apparent effects on the response to NA but markedly attenuated or abolished the K^+- and Ca^{2+}-induced contractions.

3 Following incubation of tissues for 15 min in a Ca^{2+}-free medium with low EGTA (0.01 mM) and methoxyverapamil (D600, 10^{-5} M), NA (3×10^{-7} M) caused a phasic (transient) contraction and the subsequent application of Ca^{2+} (2 mM) resulted in a tonic contraction. This NA-induced, Ca^{2+}-dependent, D600-insensitive contraction was inhibited by AQ (10^{-5} M and 10^{-4} M) in a concentration-dependent manner. This suggests that the inhibitory action of AQ may be related to Ca^{2+} entry through specific receptor activated pathways.

4 Following incubation of tissues for 30 min in a Ca^{2+}-free medium with high EGTA (2.0 mM), NA (10^{-5} M) caused a contraction of rabbit aorta which is dependent upon release of intracellular Ca^{2+}, but the response was 50% to 60% less than that in a normal medium. This contraction was inhibited by AQ (10^{-5} M and 10^{-4} M) and nitroglycerin (10^{-5} M) but not by nifedipine or diltiazem. The inhibitory action of combined treatment with AQ and nitroglycerin (10^{-5} M) on the response to NA was not different from that of either agent alone.

5 These results suggest that AQ may have inhibitory actions on the release of intracellular Ca^{2+} and also on Ca^{2+}-entry through D600-insensitive, receptor-activated Ca^{2+} pathways in rabbit aorta.

Introduction

In the screening process of a new vasorelaxant agent, we have found that in rabbit aorta, 2-aminoisoquinoline, 1.3 (2H.4H)-dione (AQ) inhibited the noradrenaline (NA)-induced contraction but had no apparent effect on the K^+-induced contraction.

It is believed that in vascular smooth muscle a K^+-induced contraction is primarily dependent upon Ca^{2+} influx across the sarcolemma, whereas a NA-induced contraction has a significant component that is directly dependent upon the mobilization of intracellular Ca^{2+} (van Breemen *et al.*, 1972; Weiss, 1977).

Therefore, the present experiments were undertaken to define whether or not the mechanism of the relaxant action of AQ on vascular smooth muscle is primarily related to inhibition of mobilization of intracellular Ca^{2+}.

[1]Author for correspondence (University of Hawaii).

Methods

Male New Zealand white rabbits weighing 1.5–2.0 kg were killed by a blow to the head. The chest was opened to remove the thoracic aorta. After excess fat and connective tissues were removed, the aortae were cut into helical strips, about 5 mm in width and 15 mm in length. The preparation was mounted vertically in

organ baths containing 20 ml of Krebs solution of the following composition (mM): NaCl 120.3, KCl 4.8, $CaCl_2$ 1.2, $MgSO_4.7H_2O$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 24.2, and glucose 5.8 at pH 7.4. The tissue bath solution was maintained at 37°C and bubbled with a 95% O_2 and 5% CO_2 gas mixture. Ligatures were placed around both ends of the muscle strips, one attaching the muscle to a glass holder and the other to a transducer adjusted to give initial stretched tensions of 1.5 g. Isometric tension changes were recorded through force-displacement transducers (FT-03) connected to a six channel Grass polygraph.

Agonists were added to the bath 10 min after treatment with antagonists. Ca^{2+}-free medium was prepared by omitting $CaCl_2$ from the solution. In the Ca^{2+}-free medium experiment, tissues were incubated in this medium for 15 or 60 min (washed every 5 min with Ca^{2+}-free medium) before the addition of drugs. In other experiments, ethyleneglycol *bis* (β-aminoethyl ether) N,N'-tetraacetic acid (EGTA; 0.01 or 2.0 mM) was included in the Ca^{2+}-free medium.

The following drugs were used: 2-aminoisoquinoline, 1.3-(2H.4H)-dione (AQ, Kyoto College of Pharmacy); nifedipine (Pfizer); diltiazem (Tanabe); methoxyverapamil (D600, AG Knoll); nitroglycerin (Parke-Davis); noradrenaline bitartrate (Sigma); 5-hydroxytryptamine creatine phosphate (5-HT, Sandoz); and histamine diphosphate (Mann). The drugs were dissolved in distilled water except for AQ and nifedipine which were dissolved in acetone and ethanol respectively and were diluted with deionized water to make final solutions. The final concentrations of acetone and ethanol in the bath did not exceed 0.1% and had no effect on muscle contraction.

The concentrations of drugs were expressed as final bath concentrations. Results are expressed or plotted as the mean ± s.e.mean. Student's *t* test was used for statistical analysis, $P < 0.05$ being considered as significant.

Results

Contractile responses to noradrenaline and potassium

Pretreatment with AQ at 10^{-6} M and 10^{-5} M inhibited the contractile response to NA (10^{-9} M–10^{-4} M) in a concentration-dependent fashion and shifted the con-

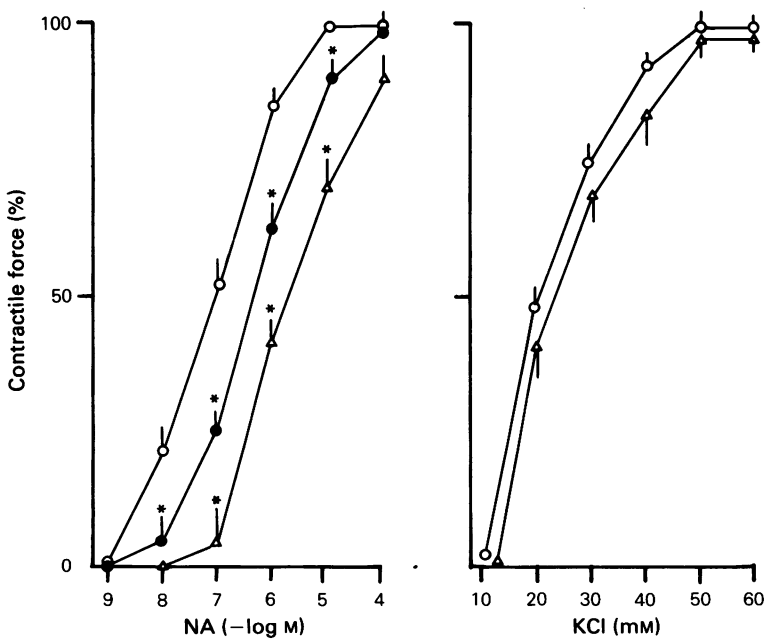

Figure 1 The effect of 2-aminoisoquinoline, 1.3 (2H.4H)-dione (AQ) on the contractile responses to noradrenaline (NA) and KCl in rabbit aortic strips. Tissues were pretreated with AQ for 10 min and then agonists were added to the bath. The maximum contractions induced by NA (10^{-5}M) and KCl (60 mM) in the untreated control preparation are 3.7 ± 0.6 g and 3.8 ± 0.5 g, respectively and expressed as 100% of contractile force. NA (○); NA + AQ at 10^{-5}M (●), and 10^{-4}M (△). Each value is the mean of 7 experiments with s.e.mean shown by vertical lines. *Significantly different from control ($P < 0.05$).

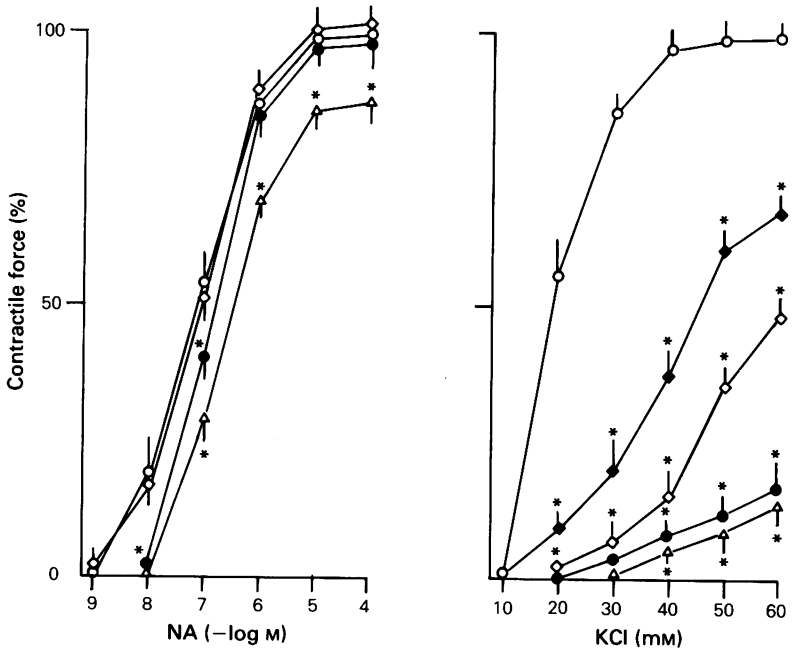

Figure 2 The effects of nifedipine (NF) and diltiazem (DZ) on the contractile responses to noradrenaline (NA) and KCl in rabbit aortic strips. Tissues were pretreated with 2-aminoisoquinoline, 1.3 (2H.4H)-dione (AQ) for 10 min and then the agonists were added to the bath. The maximum contractions induced by NA (10^{-5}M) and KCl (60 mM) in the untreated control preparation were 3.8 ± 0.6 g and 3.5 ± 0.7 g respectively and expressed as 100% of contractile force. Agonists (NA or KCl) alone (○), agonist + NF at 10^{-6}M (●) and 10^{-5}M (△), agonist + DZ at 10^{-6}M (◆) and 10^{-5}M (◇). Each value is the mean of 7 experiments with s.e.mean shown by vertical lines. *Significantly different from control ($P < 0.05$).

trol concentration-response curve for NA to the right in a parallel manner (Figure 1). Nifedipine at 10^{-5}M produced moderate inhibition of the response to NA at all concentrations, but at 10^{-6}M, it reduced only the responses to NA at low concentrations (10^{-8}M and 10^{-7}M) (Figure 2). On the other hand, diltizaem at 10^{-5}M had no significant inhibitory effect on the response to NA.

Even at 10^{-4}M, AQ failed to inhibit the contractile response to potassium (10 mM–60 mM) (Figure 1). However, both nifedipine (10^{-6}M and 10^{-5}M) and diltiazem (10^{-6}M and 10^{-5}M) markedly inhibited or nearly abolished the response to potassium, respectively (Figure 2).

Ca^{2+}-induced contraction of K^+-depolarized tissue in a Ca^{2+}-free medium

Following incubation for 60 min in a Ca^{2+}-free medium containing potassium (40 mM), the addition of Ca^{2+} (0.05 mM–3.0 mM) caused a concentration-dependent contraction (Figure 3). AQ at 10^{-4}M but not 10^{-5}M slightly inhibited the contractile response to low concentrations of Ca^{2+} (0.05 mM–1 mM) (Figure 3). On the other hand, nifedipine (10^{-6}M–10^{-5}M) and diltiazem (10^{-6}M–10^{-5}M) markedly inhibited or nearly abolished the Ca^{2+}-induced contractions, respectively (Figure 3).

Noradrenaline-induced contraction in a Ca^{2+}-free medium plus high EGTA

Incubation of tissues for 30 min in a Ca^{2+}-free medium with EGTA (2 mM) has usually been used to characterize agonist-induced Ca^{2+} release in vascular smooth muscle (van Breemen *et al.*, 1972; Weiss, 1977; Karaki *et al.*, 1979; Ishida *et al.*, 1980; Shibata *et al.*, 1984). The response to a high concentration of NA (10^{-5}M) in this medium was decreased to approximately 57% of the control response in a normal Ringer medium (Figure 4). Under similar experimental conditions, AQ at 10^{-5}M and 10^{-4}M further decreased the residual contractile response to NA in a concentration-dependent manner, whereas nifedipine (10^{-5}M) and diltiazem (10^{-4}M) did not (Figure 4). The effect of combining AQ (10^{-4}M) with nifedipine (10^{-5}M) or

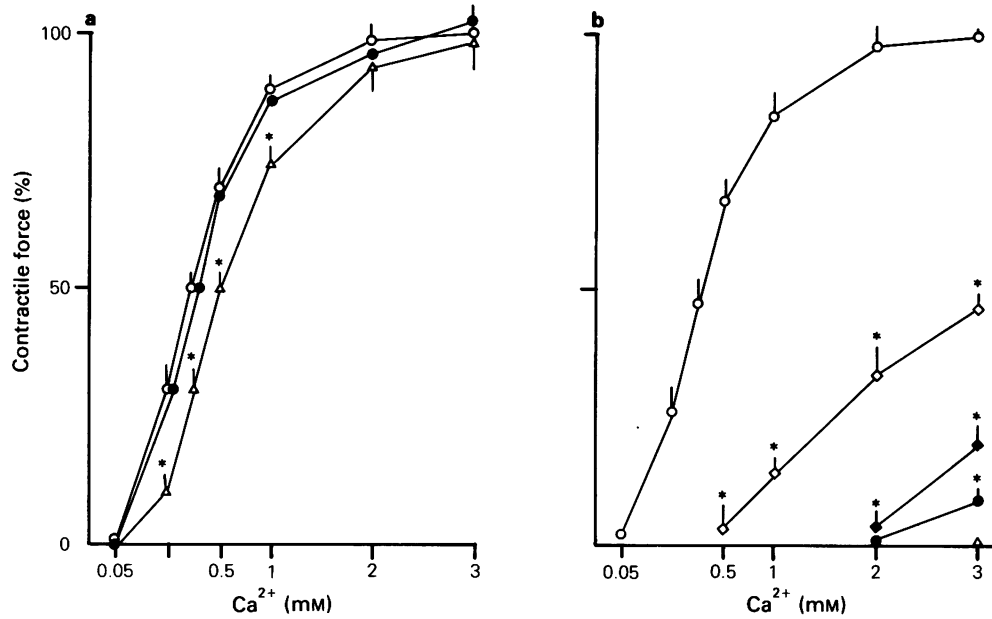

Figure 3 The effects of 2-aminoisoquinoline, 1.3 (2H.4H)-dione (AQ), nifedipine (NF) and diltiazem (DZ) on the response of rabbit aorta to Ca^{2+} in a Ca^{2+}-free medium with potassium (40 mM). Tissues were incubated in a Ca^{2+}-free medium for 60 min and then Ca^{2+} (0.05 mM–3.0 mM) was cumulatively added to the bath. Each value is the mean of 5 experiments with s.e.mean shown by vertical lines. The maximum contraction induced by Ca^{2+} (3 mM, 3.1 ± 0.5 g) was expressed as 100% of the contractile force. Ca^{2+} + DZ at 10^{-6}M (◇) and 10^{-5}M (◆). (a) Ca^{2+} alone (○), Ca^{2+} + AQ at 10^{-5}M (●) and 10^{-4}M (△); (b) Ca^{2+} + NF at 10^{-6}M (●) and 10^{-5}M (△). *Significantly different from control ($P < 0.05$).

diltiazem (10^{-4} M) on the residual response to NA was not different from that of AQ alone (Figure 4). Nitroglycerin (10^{-5} M) also decreased the residual NA-induced contraction to a level similar to that in the presence of AQ. The inhibitory effect of combined treatment with AQ and nitroglycerin was not different from that of treatment with either agent alone (Figure 4).

Ca^{2+}-induced contraction in the Ca^{2+}-free medium containing low EGTA, noradrenaline and methoxyverapamil

Following incubation of tissues for 15 min in a Ca^{2+}-free medium with low EGTA (0.01 mM) and D600 (10^{-5} M) added to inhibit any potential-dependent Ca^{2+} entry (Hester, 1975), the addition of NA (3×10^{-7} M) results in a phasic (transient) contraction (Figure 5). Although the maximum phasic contractile response in this Ca^{2+}-free, low EGTA medium to NA was slightly different from the NA-induced contraction in a normal medium, the difference was not significant. Also, this NA-induced phasic contraction could not be elicited more than once without re-exposure to Ca^{2+}. Following the phasic contraction induced by NA in a Ca^{2+}-free medium plus EGTA and D600, subsequent addition of Ca^{2+} (2 mM) resulted in a sustained contraction which was not significantly different in magnitude from the NA-induced contraction in normal or Ca^{2+}-free plus low EGTA medium. This method has been used previously in rabbit arota to separate vasodilator effects on Ca^{2+} release from any effects on NA-mediated, Ca^{2+} dependent but D600-insensitive Ca^{2+} entry (Hester, 1985). In the presence of AQ (10^{-5} M and 10^{-4} M) this Ca^{2+} dependent, D600-insensitive contraction was inhibited in a concentration-dependent manner (Figure 5).

Discussion

The present experiments have demonstrated in rabbit aorta, that AQ, a new 2-aminoisoquinoline derivative, readily inhibited contractile responses to NA without affecting responses to added potassium or Ca^{2+} (K^+-depolarized preparation in a Ca^{2+}-free medium). On the other hand, Ca^{2+}-entry blockers such as nifedipine and diltiazem nearly abolished the K^+- and Ca^{2+}-induced contraction with little or no apparent effect on

the response to NA. These results illustrate that the vascular effects of AQ are obviously different from the action of the conventional organic Ca^{2+}-entry blockers.

It has been suggested that in vascular smooth muscle both K^+- and Ca^{2+}-induced contractions are primarily related to an increase in Ca^{2+} influx from the extracellular space as a result of membrane depolarization (for review see Weiss, 1977). Conversely, the NA-induced contraction in vascular smooth muscle is partially attributed to the facilitation of release of sequestured cellular Ca^{2+} (van Breemen *et al.*, 1972). Furthermore, exogenous NA can cause contraction of certain large conduit vessels without any apparent membrane depolarization (Droogmans *et al.*, 1977; Holman & Surprenant, 1979) and it therefore appears that the response to NA can occur independent of major effects on membrane potential. Actually, the current study reaffirms that in rabbit aorta, the NA-induced contraction is much more resistant to the inhibitory action of Ca^{2+}-entry blockers than to K^+- and Ca^{2+}-induced contractions as shown by our previous experiments (Ishida *et al.*, 1980; Ozaki *et al.*, 1981; Furuta *et al.*, 1983; Takagi *et al.*, 1983; Shibata *et al.*, 1984). Karaki *et al.* (1984) suggested that in rabbit aorta there seem to be two Ca^{2+} channels, one of which is activated by high K^+ and inhibited by Ca^{2+}-entry blocker, while the other is activated by NA and not affected by the Ca^{2+}-entry blockers.

In the present study, NA elicited a phasic contraction in Ca^{2+}-free medium containing D600 and low EGTA (0.01 mM: Figure 5) which is similar to, but less depressed in magnitude, than the phasic response to NA noted in a Ca^{2+}-free medium containing high EGTA (2 mM). These NA-induced contractions are transient and cannot be elicited more than once without re-exposure of tissues to Ca^{2+}. The greater depression of the phasic contraction in the Ca^{2+}-free medium with high EGTA is probably primarily related to the 200 fold increase in the concentration of EGTA and secondarily, to the 2 fold increase in the duration of exposure. The rate and extent of Ca^{2+}

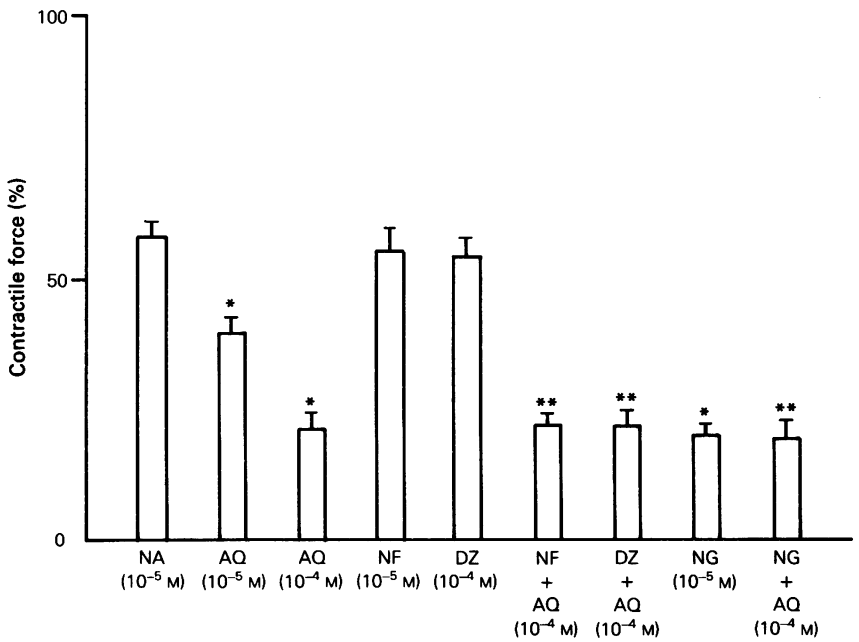

Figure 4 The effects of single treatments with 2-aminoisoquinoline, 1.3 (2H.4H)-dione (AQ, 10^{-5}M and 10^{-4}M), nifedipine (NF, 10^{-5}M), diltiazem (DZ, 10^{-4}M) and nitroglycerin (NG, 10^{-5}M) and the combined treatments with two agents (AQ and NF, AQ and DZ, AQ and NF) on the response to NA in a Ca^{2+}-free medium with EGTA (2 mM). Tissues were incubated in a Ca^{2+}-free medium with EGTA for 30 min and then noradrenaline (NA, 10^{-5}M) was added to the bath. The test agents were added to the bath 10 min before the application of NA (10^{-5} M). Each value is the mean of 5 experiments with s.e.mean shown by vertical lines. The contraction induced by NA (10^{-5} M, 3.7 ± 0.4 g) in a normal Ringer medium was expressed as 100% of the contractile force. *Significantly different from the response to NA; **significantly different from the response to NA in the tissue treated with NF or DZ.

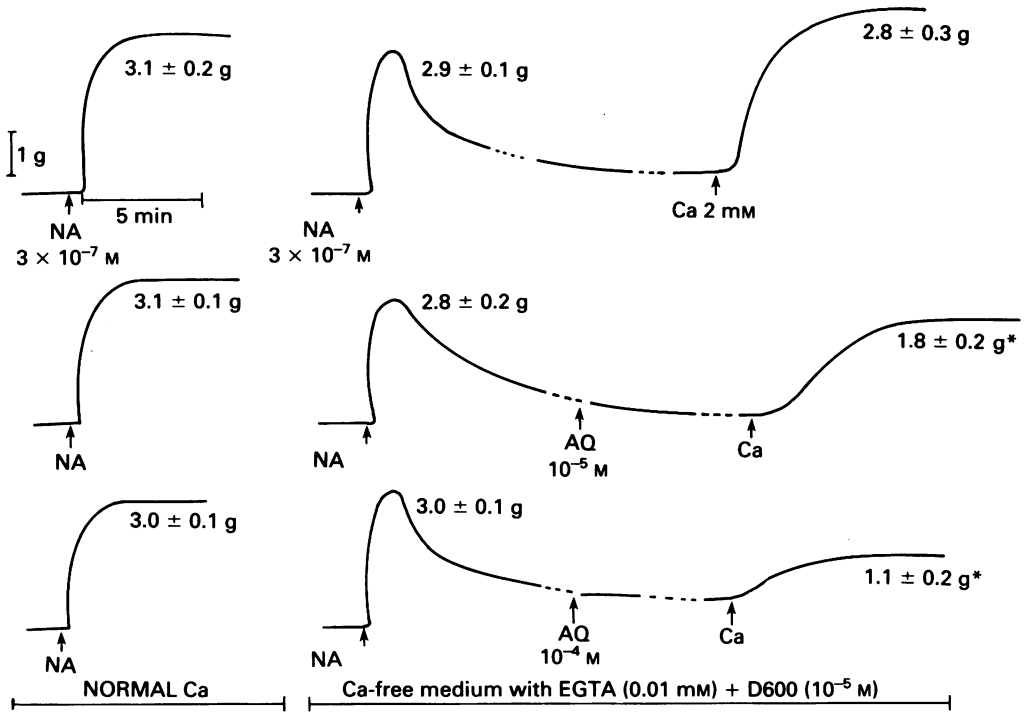

Figure 5 The effect of 2-aminoisoquinoline, 1.3 (2H.4H)-dione (AQ) on the Ca^{2+}-induced contraction in a Ca^{2+}-free media containing low EGTA, noradrenaline (NA) and methoxyverapamil (D600). Tissues were incubated for 15 min in a Ca^{2+}-free medium with low EGTA (0.01 mM) and D600 (10^{-5} M) before application of NA (3×10^{-7} M). The amplitudes of maximum contractions induced by NA and Ca^{2+} under different experimental conditions are indicated as g tension (mean ± s.e.mean, $n = 5$) in the figure. Ca^{2+} (2 mM) was added to the bath 10 min after the addition of NA. *Significantly different from control experiment without AQ pretreatment, and are representative inhibitory effects of AQ on the response to Ca^{2+} in a Ca^{2+}-free medium containing low EGTA, NA and D600.

depletion in a Ca^{2+}-free medium containing Ca^{2+} chelators is directly related to the concentration and time of exposure to Ca^{2+}-free solutions plus chelator (Wheeler & Weiss, 1979), as well as to the number of rinses with these solutions. However, both results support the concept that the NA-induced contraction of aortic strips is at least initially mediated through the mobilization of intracellular Ca^{2+}.

In addition, the subsequent addition of Ca^{2+} to a Ca^{2+}-free plus low EGTA medium after the NA-induced phasic contraction results in a sustained contraction even in the presence of a Ca^{2+} entry blocker (Hester, 1985). D600 was included to eliminate the complicating influence of any potential-dependent Ca^{2+} entry (Karaki *et al.*, 1979; 1974; Hester, 1985) and the concentration of EGTA was lowered to allow this Ca^{2+} dependent response to be maximal (Hester, unpublished observations). Thus, since release has already occurred, the resulting response to added Ca^{2+} presumably reflects NA-induced, D600-insensitive tension resulting from a specific receptor-activated Ca^{2+} entry pathway. This Ca^{2+} entry pathway is probably not involved in refilling the emptied stores as a consequence of the initial NA-induced release. Karaki *et al.* (1979) have demonstrated in rabbit aorta that refilling of releaseable Ca^{2+} stores does not occur in the continued presence of NA as is the case in this experimental protocol. This NA-induced, Ca^{2+}-dependent, D600-insensitive contraction was inhibited in a concentration-dependent manner by AQ. Therefore, since this Ca^{2+}-dependent tonic contraction seems to result from an inward translocation of Ca^{2+} and AQ was added subsequent to NA-induced Ca^{2+} release and prior to Ca^{2+} addition, but still in the presence of NA, the inhibitory action of AQ may partially be related to blockade of this D600-insensitive Ca^{2+} entry pathway activated by specific receptor stimulation. The exact step or mechanism whereby AQ interrupts the coupling of receptor activation to this Ca^{2+} entry pathway cannot, however, be delineated with the current study.

Moreover, it has recently been reported that agents

which possibly interfere with the mobilization of intracellular Ca^{2+}, such as nitroglycerin, nitroprusside, nicorandil and MDI (2-substituted methylenedioxindenes) inhibited the NA-induced contraction observed in a Ca^{2+}-free medium (Rahwan *et al.*, 1977; Hester *et al.*, 1979; Ozaki *et al.*, 1981; Heaslip & Rahwan, 1984; Weishaar *et al.*, 1983; Shibata *et al.*, 1984; Hester, 1985). The present results also demonstrate that AQ, like nitroglycerin, resulted in further inhibition of NA-induced contraction in a Ca^{2+}-free medium containing a high concentration of EGTA (2 mM), whereas nifedipine and diltiazem failed to affect similar NA responses. Thus, the inhibitory action of AQ is in part associated with the interference of release of Ca^{2+} from a cellular store. Also, combined treatment with AQ and nitroglycerin did not cause any further suppression of the NA-induced contraction in a Ca^{2+}-free medium as that with either agent alone, suggesting a similar site of action for both agents. The present experiments suggest that in rabbit aorta, AQ may have inhibitory actions on release of cellular Ca^{2+}, as well as Ca^{2+} entry through a specific receptor-activated, D600-insensitive pathway.

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References

DROOGMANS, B., RAEYMAEKERS, L. & CASTEELS, R. (1977). Electro- & pharmacomechanical coupling in the rabbit ear artery. *J. gen. Physiol.*, **70**, 129–148.

FURATA, T., SHIBATA, S., KODAMA, T. & YAMADA, K. (1983). Cardiovascular effects of FR34235, a new dihydropyridine slow channel blocker in isolated rabbit myocardium & aorta. *J. cardiovasc. Pharmac.*, **5**, 836–84.

HEASLIP, R.J. & RAHWAN, R.G. (1983). Norepinephrine induced contractions of the rat aorta in the absence of extracellular calcium-II. Effects of calcium antagonists. *Gen. Pharmac.*, **14**, 505–512.

HESTER, R.K. (1985). The effects of 2-nicotinamidoethyl nitrate on agonist-sensitive Ca^{2+} release and Ca^{2+} entry in rabbit aorta. *J. Pharmac. exp. Ther.*, **233**, 100–111.

HESTER, R.K., WEISS, G.B. & FRY, W.J. (1979). Differing effect of nitroprusside & D600 on tension & ^{45}Ca fluxes in canine renal arteries. *J. Pharmac. exp. Ther.*, **208**, 155–160.

HOLMAN, M.E. & SURPRENANT, S.M. (1979). Some properties of the excitatory junction potentials recorded from saphenous arteries of rabbits. *J. Physiol.*, **287**, 337–351.

ISHIDA, Y., OZAKI, H., SHIBATA, S. (1980). Vasorelaxant action of caroverine fumurate (a quinoxaline derivative), a calcium blocking agent. *Br. J. Pharmac.*, **71**, 344–348.

ITO, Y., KITAMURA, K. & KURIYAMA. (1980). Nitroglycerin and catecholamine actions on smooth muscle cells of the canine coronary artery. *J. Physiol.*, **309**, 171–184.

KARAKI, H., KUBOTA, H. & URAKAWA, N. (1979). Mobilization of stored calcium for phasic contraction induced by norepinephrine in rabbit aorta. *Eur. J. Pharmac.*, **56**, 237–245.

KARAKI, H., NAKAGAWA, H. & URAKAWA, N. (1984). Comparative effects of verapamil & sodium nitroprusside on contraction and ^{45}Ca uptake in the smooth muscle of rabbit aorta, rat aorta & guinea-pig taenia coli. *Br. J. Pharmac.*, **81**, 393–400.

OZAKI, H., SHIBATA, S., KITANO, H., MATSUMOTO, P. & ISHIDA, Y. (1981). A comparative study of the relaxing effect of nitroprusside & verapamil on human umbilical vessels. *Blood Vessels*, **18**, 321–329.

RAHWAN, R.G., FAUST, M.M. & WITIAK, D.T. (1977). Pharmacological evaluation of new calcium antagonists: 2-substituted 3-dimethylamino-5,6-methylendioxyindenes. *J. Pharmac. exp. Ther.*, **201**, 126–137.

SHIBATA, S., SATAKE, N., TAKAGI, T., KERFOOT, F. & SUH, T.K. (1984). Relaxing effect of nicorandil (N-2-(hydroxyethyl)-nicotinamide nitrate), a new anti-angina agent, on the isolated vascular smooth muscle. *Eur. J. Pharmac.*, **99**, 219–226.

TAKAGI, T., SATAKE, N. & SHIBATA, S. (1983). The inhibitory action of FR34235 (a new Ca^{2+} entry blocker) as compared to nimodipine & nifedipine on the contractile response to norepinephrine, potassium & 5-hydroxytryptamine in rabbit basilar artery. *Eur. J. Pharmac.*, **90**, 297–299.

VAN BREEMEN, C., FARINAS, B.R., GERBA, P. & MCNAUGHTON, E.G. (1972). Excitation-contraction coupling in rabbit aorta studied by the lanthanum method for measuring cellular calcium influx. *Circulation Res.*, **30**, 44–54.

WEISHAAR, R.E., QUADE, M., SCHEUDEN, J.A. & KAPLAN, H.R. (1983). The methylenedioxyindenes, a novel class of "intracellular calcium antagonists": Effects on contractility & on processes involved in regulating intracellular calcium homeostasis. *J. Pharmac. exp. Ther.*, **227**, 767–778.

WEISS, G.B. (1977). Calcium & contractility in vascular smooth muscle. In *Advances in General and Cellular Pharmacology*. Vol. II, ed. Narahashi, T. & Bianchi, C.P. pp. 71–154. New York: Plenum.

WHEELER, E.S. & WEISS, G.B. (1979). Correlation between response to norepinephrine and removal of ^{45}Ca from high-affinity binding sites by extracellular EDTA in rabbit aortic smooth muscle. *J. Pharmac. exp. Ther.*, **211**, 353–359.

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