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Effects of Calcium and Its Antagonists on the Canine Mesenteric Circulation

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SUMMARY We studied metabolic and circulatory responses of the intestinal circulation to intraarterial infusions of solutions containing calcium chloride or calcium antagonists in anesthetized dogs. Measurements included blood flow to the terminal ileum, arteriovenous oxygen content difference, distribution of radiolabeled microspheres to the mucosal-submucosal compartment and intraluminal pressure. Calculated parameters included oxygen consumption and fractional mucosal-submucosal blood flow. Both calcium antagonists, nifedipine and diltiazem, increased intestinal blood flow, mainly to the mucosa-submucosa, depressed intestinal motility, and did not change oxygen consumption. Thus, both agents appear to act mainly on resistance vessels without increasing the nutrient circulation. Calcium chloride (1.0-500.0 g/kg per min) had a mild constrictor effect; at a dose of 1000.0 g/kg per min, calcium chloride became a dilator agent in the mesenteric circulation. The dilator effect of the highest dose of calcium was reversed by digoxin, suggesting the involvement of Na\(^+\),K\(^-\)-ATPase. Nifedipine completely blocked calcium-induced constriction of the intestinal circulation and partly inhibited norepinephrine-induced constriction. Studies on isolated mesenteric arterial smooth muscle revealed that nifedipine relaxed KCl-contracted strips in the presence of external calcium and relaxed norepinephrine-contracted strips in both the presence and absence of external calcium. These in vitro findings suggest that calcium antagonists interfere with the release of calcium from intracellular sites as well as with the slow inward current of calcium. Circ Res 48: 692-700, 1981
shown to influence the two sources of Ca\textsuperscript{2+} differently. For instance, contraction induced by depolarizing concentrations of potassium is the result of an augmented flux of Ca\textsuperscript{2+} from the extracellular space, whereas the vasoconstriction induced by noradrenaline involves primarily the release of intracellular Ca\textsuperscript{2+} (Hudgins and Weiss, 1968; Seidel and Bohr, 1971; Godfraind and Kaba, 1972; Bohr, 1978).

Effects of Ca\textsuperscript{2+} on the intestinal circulation have not been studied extensively and vascular responses appear minimal (Chou et al., 1963; Dabney et al., 1967; Pawlik et al., 1975). Furthermore, calcium ionophores are minimally active (Hanley et al., 1975; Lanciault et al., 1976). Recently, a group of chemically different compounds was introduced which are believed to exert a negative inotropic effect and relax vascular smooth muscle by a selective inhibition of membrane Ca\textsuperscript{2+} flux, i.e., by reducing the slow inward current (Fleckenstein, 1977; Fleckenstein and Fleckenstein-Grin, 1977).

Effects of calcium antagonists in the intestinal circulation have received little attention (Schwaiger et al., 1977; Fleckenstein and Fleckenstein-Grin, 1977). Since such pathological changes also occur in the mesenteric vasculature (Marston, 1977; Bynum and Jacobson, 1979) a search among the calcium antagonists for a potentially useful agent for the management of intestinal ischemia would appear justified.

The aim of our present study was to investigate the effects of calcium and two calcium antagonists, nifedipine and diltiazem, on the intestinal circulation, its oxygen consumption, and the distribution of intestinal blood flow in vivo. Attempts also were made to elucidate the mechanism of action of nifedipine on mesenteric arterial smooth muscle in vitro.

### Methods

A total of 29 mongrel dogs of both sexes, weighing between 16 and 28 kg (average 20.6 kg) were used in this study. Of these animals, 25 were studied under in vivo conditions, and four dogs were used to obtain mesenteric arteries for in vitro experiments. All animals were deprived of food for 24 hours and anesthetized with intravenous (iv) injection of sodium pentobarbital (30 mg/kg).

#### In Vivo Experiments

After endotracheal intubation, ventilation was maintained with a positive pressure respirator (Harvard Apparatus) at a rate adjusted to body weight. A femoral artery was cannulated and connected to a pressure transducer (Hewlett-Packard) for recording the arterial pressure and for siphoning arterial blood into one cuvette of a spectrophotometric oxygen content difference analyzer (A-VOX Systems) (Shepherd and Burgar, 1977). Another femoral artery was used to introduce a catheter into the left ventricle for injection of radiolabeled microspheres. Both femoral veins were also catheterized to return the blood from the oxygen analyzer and for injections of drugs and supplemental anesthetic.

After midline laparotomy, a distal trunk of the superior mesenteric artery supplying the terminal ileum was exposed and its side branch cannulated for intraarterial (ia) infusions of the substances under study. An electromagnetic flow probe of 2.0 to 3.0 mm i.d. was positioned around the mesenteric artery and connected to a blood flow amplifier (Zepeda Instruments). The flowmeter was calibrated in each experiment by transient occlusion of the mesenteric artery distal to the flow probe. A side branch of the superior mesenteric vein was also cannulated to siphon venous blood continuously from the segment under study to the oxygen analyzer. Both ends of the ileal segment were ligated to exclude collateral circulation.

A wide-bore, saline-filled, multihole catheter was inserted through the proximal end into the lumen of the ileal segment and was connected to a pressure transducer (Hewlett-Packard) for measurements of intraluminal pressure. The measured parameters included the systemic arterial pressure, arteriovenous oxygen content difference (A-V\textsubscript{O\textsubscript{2}}), blood flow (BF), and intraluminal pressure (IP). These measurements were recorded continuously on a direct writing recorder (Hewlett-Packard, model 7759A).

Intestinal oxygen consumption (V\textsubscript{O\textsubscript{2}}) was calculated as the product of A-V\textsubscript{O\textsubscript{2}} and BF in ml O\textsubscript{2}/min per 100 g of intestine. After heparin (250 USP/kg, Panheprin, Abbott) was injected, the cuvettes of the oxygen analyzer were perfused with arterial and venous blood by a constant flow pump (Holter) at 5.5 ml/min.

Changes in blood flow distribution within the intestinal wall were assessed with four radiolabeled microspheres (\textsuperscript{51}Ce, \textsuperscript{52}Cr, \textsuperscript{85}Sr, and \textsuperscript{46}Sc) of 15 ± 3 \textmu m diameter (3M Co.) injected into the left ventricle during a control period and during administration of drugs. After each experiment, control and experimental intestinal segment were removed, rinsed, and weighed. Segments were opened along the mesenteric border and cut into portions about 10 cm long. The mucosal and submucosal layers were stripped from the muscularis and counted in a γ spectrometer (Packard Instruments). Corrections were made for overlap in the energy spectra. Fractional blood flow to the mucosal-submucosal compartment (FF\textsubscript{m-s}) was calculated as the product of BF and the percentage of microspheres distributed to the mucosa-submucosa (m-s) and was expressed in ml/min per 100 g of intestine.

Following surgery and a 30-minute stabilization period, dose-response curves for ia infusions of nifedipine (Pfizer Pharmaceuticals), diltiazem (Mar-
Laplace). Contractile activity was determined as isometric tension on a Grass model 7 polygraph. For development of tension by imposing a resting passive tension was reapplied and the strips were suspended in 20 ml of physiological salt solution (PSS) aerated with 95% O2:5% CO2 gas mixture and maintained at 37°C. The composition of the PSS (in mm): NaCl, 130.0; NaHCO3, 14.9; KCl, 4.7; KH2PO4, 1.19; MgSO4 • 7H2O, 1.17, CaCl2 • 2H2O, 1.6; CaNa2 versenate, 0.026; and dextrose 5.5. Two modifications of PSS also were used, namely, CaCl2-free PSS, and CaCl2-free PSS to which 100 μM EGTA (ethylene glycol-bis-(beta-aminoethyl-ether)-N,N'-tetracetic acid) had been added to reduce further the activity of any contaminating Ca2+.

Reproducibility of contractile responses to both agonists in each type of PSS was also evaluated in separate experiments in which strips were repetitively contracted at 20-minute intervals. Cumulative dose-response curves (10^-9 to 10^-5 M) for nifedipine were determined in strips contracted with 30 mM KCl or 0.1 μM norepinephrine in normal PSS, in strips contracted with 0.1 μM norepinephrine in Ca2+-free PSS and Ca2+-free PSS with EGTA, and in strips 20 minutes after obtaining dose-response relationships for nifedipine and washing the chambers several times with appropriate PSS. The response of strips to KCl and norepinephrine were assessed again a few times during the subsequent 2 to 3 hours following drug washout.

In an additional series of experiments, an attempt was made to explore the unexpected vasodilator effect of the highest dose of CaCl2. First, the effects of iv infusions of comparably hyperosmotic (1100 mOsm) solutions of NaCl (3.6%) or mannitol (20%) were assessed. Second, the dilator dose of CaCl2 was infused after iv administration of digoxin (Lanoxin, Burroughs-Wellcome Co., 50 μg/kg). Extreme care was taken to minimize the exposure of nifedipine to light. This was accomplished by weighing the drug in a dark room on a balance having an illuminated dial and dissolving the agent in a solution (ethanol 15%, polyethylene glycol 15% in saline) contained in a flask covered with aluminum foil. The infusion pump syringe containing dissolved nifedipine and the polyethylene tubine conveying the drug to the mesenteric artery were wrapped in aluminum foil. The pump, syringe, tubing, and dog were covered with surgical towels throughout the experiment. In another series of experiments, the effects of nifedipine (0.1 μg/kg per min) on responses to CaCl2 (500.0 μg/kg per min) or norepinephrine (0.1 μg/kg per min) were investigated by infusing each of the constrictors with nifedipine. The duration of infusion with each agent was 10 minutes.

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In Vivo Studies

Control values for 25 dogs were: BF 65.6 ± 4.7 ml/min per 100 g of intestinal tissue, A-V02 3.1 ± 0.3 ml O2/ml of blood, V02 2.0 ± 0.2 ml O2/min per 100 g of intestine, the fraction of total blood flow which perfused the muscularis 30.6 ± 3.5%, m-s 69.4 ± 3.5%, FBFm,s 45.0 ± 3.4 ml/min per 100 g of intestine, and IP 4.8 ± 0.7 mm Hg. Systemic arterial pressure was not changed significantly during any series of tests. The weight of intestinal segments averaged 208.5 g.

Infusion of the three doses of nifedipine produced a dose-dependent increase in BF and decreases in A-V02 and IP. The two higher doses of nifedipine also caused increases in m-s and FBFm,s. The calculated dose of the drug producing a 50% increase in BF was 0.36 μg/kg per min or 1 x 10^-9 mol/kg per min (Fig. 1). Diltiazem evoked similar responses, but was less potent. The calculated dose of diltiazem causing a 50% increase in BF was 3.3 μg/kg per min or 7.3 x 10^-9 mol/kg per min (Fig. 2). After cessation of-challenged with 50 mM potassium chloride (KCl) until the response obtained was reproducible. Then strips were contracted by adding either KCl to give a concentration of 30 mM or 0.1 μM norepinephrine (Levophed bitartrate, Winthrop Laboratories) in order to compare the potency of agonists, and to assess possible changes in tension related to time. Reproducibility of contractile responses to both agonists in each type of PSS was also evaluated in separate experiments in which strips were repetitively contracted at 20-minute intervals. Cumulative dose-response curves (10^-9 to 10^-5 M) for nifedipine were determined in strips contracted with 30 mM KCl or 0.1 μM norepinephrine in normal PSS, in strips contracted with 0.1 μM norepinephrine in Ca2+-free PSS and Ca2+-free PSS with EGTA, and in strips 20 minutes after obtaining dose-response relationships for nifedipine and washing the chambers several times with appropriate PSS. The response of strips to KCl and norepinephrine were assessed again a few times during the subsequent 2 to 3 hours following drug washout.

Measurements used for statistical analyses under in vivo conditions were obtained at the time of injections of microspheres to correlate changes in distribution of blood flow with those of total blood flow or oxygen consumption. Results have been expressed as the mean percentage of control values ± standard errors of the means (SEM). Changes from control values under both in vivo and in vitro conditions were analyzed with a paired t-test and the differences between series of experiments were analyzed with a pooled t-test. Differences were considered significant at a probability of less than 0.05. Only those changes which were significant are cited in the Results section.

Results

In Vivo Studies

Control values for 25 dogs were: BF 65.6 ± 4.7 ml/min per 100 g of intestinal tissue, A-V02 3.1 ± 0.3 ml O2/ml of blood, V02 2.0 ± 0.2 ml O2/min per 100 g of intestine, the fraction of total blood flow which perfused the muscularis 30.6 ± 3.5%, m-s 69.4 ± 3.5%, FBFm,s 45.0 ± 3.4 ml/min per 100 g of intestine, and IP 4.8 ± 0.7 mm Hg. Systemic arterial pressure was not changed significantly during any series of tests. The weight of intestinal segments averaged 208.5 g.

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infusion of either agent, all measured parameters returned to control within a few minutes.

CaCl₂ produced a dose-dependent decrease in BF up to a dose of 500.0 µg/kg per min. At a dose of 1,000.0 µg/kg per min, CaCl₂ caused a significant increase in BF. A-Vo₂ was increased at all doses of CaCl₂, although at lowest and highest doses the changes were not significant. Vo₂ changed significantly only at the 1,000.0 µg/kg per min dose, when it increased (Fig. 3). IP increased in a dose-dependent manner with the 100.0, 500.0, and 1,000.0 µg/kg per min doses of CaCl₂. The intestinal contractions were of both rhythmic and tonic type and were observed mainly at the beginning of infusion of each dose. Since the distribution of microspheres was not changed significantly at any dose, changes in FBFm-s paralleled those of BF. These data were omitted for the sake of clarity.

The lowest dose of nifedipine, infused together with the highest constrictor dose of CaCl₂, completely blocked the effects of CaCl₂. Thus, the effects of nifedipine + CaCl₂ were not significantly different from those of nifedipine alone (Fig. 4). Nifedipine also partially inhibited the vasoconstrictor response to norepinephrine. Control infusion of the catecholamine decreased BF, Vo₂, m-s and FBFm-s and increased A-Vo₂. Norepinephrine infused with nifedipine produced significantly smaller changes in all parameters except Vo₂ than did norepinephrine alone (Fig. 5).

Digoxin reduced BF and Vo₂ and increased A-Vo₂ (Fig. 6). CaCl₂ (1,000.0 µg/kg per min) infused 10-15 minutes after injection of digoxin exerted a small vasoconstrictor effect; thus, BF decreased and A-Vo₂ increased, whereas Vo₂ decreased when compared with control values before digoxin. BF and A-Vo₂ were significantly different from values observed with digoxin alone.

In Vitro Experiments

KCl (30 mM) and norepinephrine (0.1 µM) were equipotent in contracting strips of mesenteric artery in normal PSS, i.e., contractile tensions obtained with both agonists were not significantly different. As shown in Figure 7 in normal PSS, mean control
tension obtained with KCl was 8.2 ± 0.8 g and that obtained with norepinephrine was 9.6 ± 1.1 g in the same strips. In calcium-free PSS, control tension obtained with KCl was reduced to 1.5 ± 0.2 g and in the same strips norepinephrine induced a tension of 7.4 ± 1.2 g. These values were significantly different (P < 0.001). In calcium-free PSS with EGTA, KCl-contracted strips developed a tension of 0.7 ± 0.1 g, whereas norepinephrine-induced contractions of the same series of strips averaged 5.0 ± 1.1 g. These values again were significantly different (P is less than 0.005).

Additional control tests involved repetitive challenges with either agonist in normal PSS and Ca²⁺-free PSS with EGTA. Contractile responses to consecutive challenges with KCl were not different from one another and were very well sustained so long as the excess of KCl was not washed out. Responses to consecutive challenges with norepinephrine in normal PSS were also not different from one another and were well sustained; however, in Ca²⁺-free PSS and Ca²⁺-free PSS with EGTA, contractile responses to norepinephrine reached their maxima within 1-2 minutes and then decreased at a steady rate. Therefore, in experiments in which norepinephrine was used as the contractile agonist, before assessing the effects of nifedipine, the rate of spontaneous decrease in tension was established for each strip over a period of 30-35 minutes, and decreases in tension obtained with increasing concentrations of nifedipine then were
Figure 6 Comparison of the effects of CaCl₂ (dilator dose) before and after digoxin. + indicates significant difference between digoxin alone and digoxin + CaCl₂. Other symbols as in Figure 1.

corrected for the spontaneous decrease in tension during the control test for each corresponding period of time.

Nifedipine administered in concentrations increasing from $10^{-9}$ to $10^{-5}$ M every 5 minutes produced a concentration-dependent relaxation of KCl-contracted strips in normal PSS and of strips contracted with norepinephrine in normal PSS (Fig. 8). The ED₅₀ (dose producing 50% relaxation) under these conditions was $7.1 \times 10^{-7}$ M in KCl contracted strips and $3.3 \times 10^{-8}$ M in norepinephrine-contracted strips. The same concentrations of nifedipine relaxed strips contracted by norepinephrine in Ca²⁺-free PSS and in Ca²⁺-free PSS with EGTA. After assessing the dose-response to nifedipine, the strips were washed several times and challenged again after 20 minutes. All responses were significantly depressed (Fig. 7, hatched bars) up to 3 hours after the experiments were terminated.

Discussion

The results of our in vivo studies indicate that both nifedipine and diltiazem are potent dilators of the intestinal circulation. Comparison of doses producing a 50% increase in blood flow to the ileum shows that nifedipine is about 7 times more potent than diltiazem on a molar basis. It appears that both agents dilate arteriolar resistance vessels in the intestinal circulation, while decreasing the tone...
of large arteries as an additional effect. The latter
effect has been suggested in the coronary circula-
tion (Fleckenstein and Fleckenstein-Grün, 1977)
but cannot alone account for such large increases
in blood flow to the gut as were observed with these
drugs. Vasodilation occurred mainly in the mucosa-
submucosa with the calcium antagonists indicating
that these compounds produce a redistribution of
intestinal blood flow to the mucosal-submucosal
compartment. This redistribution may have been
related to the decrease in intestinal motor activity
observed with these drugs, reflecting the important
role of calcium in mediating contractile activity of
both vascular and visceral smooth muscle (Anderson et al., 1972).

Dose-related increases in blood flow observed
with the calcium antagonists were accompanied by
proportional decreases in oxygen extraction across
the intestinal circulation and resulted in no change
in oxygen consumption by the gut. The lack of
change in oxygen uptake suggests that the main
micro-circulatory effect of these compounds, relax-
ation of arterioles, was not accompanied by any
apparent relaxation of precapillary sphincters
which regulate the nutrient circulation. The in-
creased blood flow which bypasses the nutrient
circulation thereby represents a functional arterio-
venous shunting. It is also possible that Ca\(^{2+}\) antag-
onists depress intestinal function(s) and, therefore,
reduce oxygen use by the gut, as has been shown in
the myocardium (Fleckenstein, 1977; Fleckenstein
and Fleckenstein-Grün, 1977). Were this the case,
the decrease in oxygen utilization would nullify any
effect of opening the precapillary sphincters. The
more likely of these two explanations cannot be
certified from our data.

Previous studies of the interactions of calcium
agonists with cardiac glycosides in the coronary
and intestinal circulations showed that the antago-
nists appear to have different effects in the normal
and digitalis-poisoned circulations.

In contrast to the high potency of calcium antag-
onists in the intestinal circulation, Ca\(^{2+}\) itself
evoked very small constrictor effects in this vascular
bed. Chou et al. (1963) reported a 10% increase in
total intestinal resistance and a 20% increase in
small-vessel resistance in response to an isotonic
CaCl\(_2\) infusion at a rate of about 8 ml/min. The
same group reported later that Ca\(^{2+}\) relaxed visceral
smooth muscles but had no significant effect on
intestinal resistance (Dabney et al., 1967). Infusion
of CaCl\(_2\) at 300 µg/kg per min into the mesenteric
artery was reported to decrease both blood flow and
oxygen consumption by 12% (Pawlik et al., 1975).
Maximal constriction obtained with CaCl\(_2\) in the
present study corresponded to about a 20% decrease
in blood flow. The dose-dependent decrease in
blood flow was accompanied by an increase in ox-
ygen extraction and no change in oxygen uptake,
typical of a weak vasoconstrictor effect in this cir-
culation. The highest dose of CaCl\(_2\) (1,000 µg/kg per
min) produced, unexpectedly, a reproducible intes-
tinal vasodilation accompanied by an increase in
oxygen consumption. In additional experiments not
reported here still higher doses of CaCl\(_2\) (2,000.0
and 5,000.0 µg/kg per min) prompted progressively
greater dilator responses. We thought this vasodi-
lation might be a function of the hyperosmolarity of
the CaCl\(_2\) solution infused into the artery, since
administration of hyperosmolar glucose or NaCl
was shown to decrease vascular resistance in the
feline intestinal circulation (Levine et al., 1978). In
our study, however, solutions of NaCl or mannitol
of the same osmolarity as the 1,000.0 µg/kg per min
solution of CaCl\(_2\) infused i.a. did not change any
circulatory or metabolic parameter. The vasodilator
effect of the 1,000.0 µg/kg per min dose of calcium
may be related to its effect on the membrane-bound
Na\(^{+}\),K\(^{-}\)-ATPase of the muscle cell. This enzyme is
blocked by cardiac glycosides (Akerha, 1977), and
this effect probably accounts for the vasoconstrictor
effect of digitalis in the intestinal circulation (Paw-
lik and Jacobson, 1974; Schwaiger et al., 1979). After
blockade of Na\(^{+}\),K\(^{-}\)-ATPase by digoxin, the dilator
effect of the highest dose of CaCl\(_2\) was converted to
a constrictor effect.

None of the doses of CaCl\(_2\) changed the distri-
bution of intestinal blood flow, suggesting that vas-
oconicastriction or vasodilation was proportional in all
intestinal layers. The three higher doses of CaCl\(_2\)
also stimulated intestinal motility, a finding which
is not in agreement with reports of others wherein
a decrease in tone of intestinal musculature was
observed with Ca\(^{2+}\) (Dabney et al., 1967). The stim-
ulation of intestinal motility by Ca\(^{2+}\) is, however,
consistent with the inhibitory effects on motility we
observed with calcium antagonists and suggests
that qualitative similarities exist between the role
of Ca\(^{2+}\) in contractility of vascular and nonsvascular
smooth muscle. The suggestion that effects of ions
on visceral smooth muscle may interfere with their
action on vascular resistance (Chou et al., 1963;
Dabney et al., 1967) appears unlikely, since a dose-
related increase in intraluminal pressure was found
with the three highest doses of CaCl\(_2\), whereas
intestinal blood flow was first diminished and then
augmented (at highest dose when the amplitude of
intestinal contractions was highest). Our observa-
ations suggest different sensitivities of vascular and
visceral smooth muscle to the increments of plasma
Ca\(^{2+}\) concentrations.

Ca\(^{2+}\) may act through several mechanisms in
vascular smooth muscles. First, a rise in extracel-
ular Ca\(^{2+}\) increases the transmembrane concentra-
tion gradient, the membrane Ca\(^{2+}\) influx, and the
intracellular concentration of the ion. This effect is
not very pronounced, probably because much of the
Ca\(^{2+}\) entering the cell is easily sequestered in intra-
cellular stores before it can activate contractile
proteins. Second, Ca\(^{2+}\) may stabilize the cell mem-
brane or may stimulate Na\(^{+}\), K\(^{-}\)-ATPase. Inhibition
of vascular tone in vitro by supranormal extracel-
lular concentrations of Ca²⁺ has been shown previously (Winquist and Bevan, 1980). Whether this effect occurs over the entire range of concentrations and thus minimizes the direct constrictor effect or occurs only at very high concentrations remains to be established in studies of the direct effects of calcium on isolated enzymes.

Nifedipine completely blocked the circulatory effects of the highest constrictor dose of calcium but only partially reduced the constrictor response of the intestinal circulation to norepinephrine. It is possible that reduction of norepinephrine-induced constriction reflects the ability of nifedipine to block increased Ca²⁺ influx which accounts in part for the constrictor action of the drug (Seidel and Bohr, 1971; Godfraind and Kaba, 1972; Bohr, 1978; Johansson, 1978; Meisher et al., 1980). Nifedipine is commonly believed to interfere with the slow inward current, i.e., transmembrane Ca²⁺ influx, or to reduce the number of slow channels (Fleckenstein, 1977; Fleckenstein and Fleckenstein-Grün, 1977; Bayer and Ehara, 1978; but an effect on intracellular calcium release has also been proposed (Church and Zsoter, 1980). Under in vitro conditions, nifedipine appeared to be a very effective relaxant of mesenteric arterial strips contracted with either norepinephrine or KCl and produced almost complete relaxation whether or not extracellular calcium was present in the medium. It is well established that KCl-induced constriction uses extracellular calcium whereas norepinephrine-mediated constriction depends on both extra- and intracellular Ca²⁺ (Hudgins and Weiss, 1968; Seidel and Bohr, 1971; Godfraind and Kaba, 1972; Bohr, 1978; Meisher et al., 1980) van Breeman et al., 1979). The ED₅₀ of nifedipine fed norepinephrine-contracted strips was more than one order of magnitude lower than for KCl-contracted strips. This difference may mean that nifedipine relaxes vascular smooth muscle both by blocking Ca²⁺ influx and by preventing Ca²⁺ release from intracellular stores.

To exclude Ca²⁺ influx, we performed studies on the norepinephrine-nifedipine interaction after removing Ca²⁺ from the external medium. In both Ca²⁺-free PSS and Ca²⁺-free PSS with EGTA, norepinephrine still produced constriction, in contrast to KCl. This differential effect of Ca²⁺ removal on vascular smooth muscle from rat aorta was demonstrated previously by Hudgins and Weiss (1968), and our results confirm their findings. Contractions obtained with norepinephrine after removal of Ca²⁺ were not sustained. This finding is consistent with the suggestion that the tonic response to norepinephrine is due to increased uptake of external Ca²⁺ in contrast to the phasic (initial) response which depends upon release of Ca²⁺ from internal stores (Godfraind and Kaba, 1972). The difference probably is not discrete because phasic responses to norepinephrine in normal, Ca²⁺-free and Ca²⁺-free PSS with EGTA differ significantly. On the other hand, a part of the tonic response may also depend on intracellular Ca²⁺ release since the decrease in tension after norepinephrine was relatively slow in the absence of Ca²⁺. Addition of nifedipine in increasing concentrations accelerated the spontaneous decay of tension.

Nifedipine irreversibly blocked any subsequent responses to KCl or norepinephrine in any type of PSS. Thus, the drug interferes not only with KCl-induced contraction and the tonic response to norepinephrine, both of which depend mainly on extracellular Ca²⁺, but also with the phasic response to the catecholamine. In the absence of external Ca²⁺ (as is the case in Ca²⁺-free PSS with EGTA), the phasic response depends entirely on intracellular Ca²⁺. It appears that Ca²⁺ antagonism by nifedipine involves blockade of both Ca²⁺ influx and Ca²⁺ release from intracellular sites. Increased Ca²⁺ efflux is also possible as a mechanism of nifedipine-induced relaxation (Fleckenstein and Fleckenstein-Grün, 1977). Our results generally confirm the results of studies of Ca²⁺ fluxes under the influence of calcium antagonists (Church and Zsoter, 1980). The quantitative contribution of the preceding mechanisms to the vasodilator effects of these compounds cannot be established at this point.

In Ca²⁺-free media, maximum relaxation of norepinephrine-contracted strips is much less than that of norepinephrine or KCl-contracted strips in the presence of Ca²⁺. Furthermore, at low concentrations of nifedipine, relaxation of strips contracted with norepinephrine in all types of PSS is similar to relaxation of strips contracted with KCl. These findings support the speculation that Ca²⁺ antagonists in low doses interact in a major way with the intracellular free Ca²⁺ pool. When the concentration of the drug is high, these drugs also affect membrane calcium flux, and produce additional relaxation. It is possible that studies of calcium fluxes in vascular smooth muscle might bear upon these speculations, although recent experiments (Church and Zsoter, 1980) using such techniques did not appear to yield definitive results.

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