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A comparison of the effects of the calcium entry blockers, verapamil, diltiazem and flunarizine against contractions of the rat isolated aorta and portal vein

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1 The actions of the chemically distinct calcium entry blockers, verapamil (Ver), diltiazem (Dlz) and flunarizine (Flu) have been compared in rat isolated aorta and portal vein.

2 KCl-induced contractions of the rat aorta depend exclusively upon extracellular Ca^{2+}, whereas, those induced by noradrenaline (NA) rely upon Ca^{2+} from intra- and extracellular sources. The NA-induced contraction was pharmacologically dissected under Ca^{2+}-free conditions revealing a contraction dependent upon intracellular Ca^{2+} (EGTA-resistant response) or a low concentration of prazosin which left a contraction which was mediated by extracellular Ca^{2+} (prazosin-resistant response).

3 The portal vein produced spontaneous rhythmic contractions and a sustained contraction to NA and KCl; however, all responses appeared to depend exclusively upon extracellular Ca^{2+}.

4 In the aorta, contractions which might be expected to depend upon Ca^{2+}-entry through voltage-operated channels (KCl-induced contraction) showed similar sensitivities to Ver, Dlz and Flu whereas, marked differences in the sensitivity to these agents was noted against contractions which appear to depend upon Ca^{2+}-entry through receptor-operated channels (prazosin-resistant response). Only Dlz reduced contractions mediated by intracellular Ca^{2+} (EGTA-resistant response).

5 In the portal vein, Ver and Dlz caused similar pronounced reductions of spontaneous and NA or KCl-induced contractions. In contrast, these contractions of the portal vein were unaffected by Flu except at a concentration of 10 μM. However, contractions induced by addition of Ca^{2+} (0–14 mM) to previously depolarized portal veins could be reduced by Flu (100 nM–10 μM).

6 The present study indicates that in the rat aorta, contractions mediated by intracellular Ca^{2+} and depolarization or receptor-activated Ca^{2+} entry can be pharmacologically dissected and that these processes show different sensitivities to calcium entry blockade. Of the agents tested, Ver displays the properties most commonly associated with an ideal calcium entry blocker. Ca^{2+}-activation mechanisms in the portal vein differ from those in the aorta resulting in a different spectrum of selectivity of the calcium entry blockers studied.

Introduction

Contractions of vascular smooth muscle may be initiated by elevating the concentration of free intracellular Ca^{2+}, but this can arise from mobilization of intracellular stores of Ca^{2+} or by influx of Ca^{2+} from the extracellular environment through voltage-operated channels activated by membrane depolarization, or through receptor-operated channels activated by agonist-receptor combination (Weiss, 1985). However, the relative importance of these different sources of Ca^{2+} for contraction varies considerably in blood vessels from different anatomical positions, even under similar experimental conditions.

In the rat isolated aorta, contractions induced by high concentrations of noradrenaline (NA) are biphasic; the early fast component of contraction

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depends upon release of intracellular Ca^{2+} while the slowly developing sustained phase of contraction depends upon influx of extracellular Ca^{2+} (Yamashita *et al.*, 1977). Accordingly, under Ca^{2+}-free conditions, a transient contraction may be induced in the rat aorta by NA and this is principally mediated by intracellular Ca^{2+} (Heaslip & Rahwan, 1982). KCl has also been shown to produce a biphasic contraction in the rat aorta (Nghiem *et al.*, 1982), albeit with a less definite separation between fast and slow components than of corresponding NA-induced responses. In addition the confluent fast and slow components of the KCl-induced contraction both depend exclusively upon extracellular Ca^{2+} (Yamashita *et al.*, 1977; Heaslip & Rahwan, 1982).

Although the rat aorta is normally quiescent *in vitro* in the absence of stimulation the rat isolated portal vein shows spontaneous rhythmic contractions and will also respond with a sustained contraction to NA or KCl. Previous studies have indicated that in the rat portal vein Ca^{2+} arising from an intracellular source does not contribute significantly to contraction (Axelsson *et al.*, 1967).

It is generally accepted that organic blockers of calcium entry prevent stimulated influx of Ca^{2+} in vascular muscle but have no effect upon passive entry of Ca^{2+} from the extracellular environment through leak channels or upon release of Ca^{2+} from intracellular stores (Flaim, 1982). However, there appear to be differences in the selectivity of individual calcium entry blockers. Consequently, the present study was designed to compare the actions of verapamil (Ver), diltiazem (Dlz) and flunarizine (Flu), calcium entry blockers with different chemical structures, upon contractions of the rat aorta and portal vein. In order to elucidate the mechanisms responsible for differences in selectivity, attempts have been made to isolate pharmacologically contractions mediated by Ca^{2+} release or influx through voltage- and receptor-operated channels.

Methods

The thoracic aorta and portal vein were removed from male Wistar rats (200–280 g) that had been killed by cervical dislocation. Cylindrical segments of the aorta, 8 mm in length, were suspended between two parallel stainless steel wires while portal veins were secured in a longitudinal axis by cotton threads 15 mm apart. The aortae and portal veins were mounted under resting tensions of 3 and 0.5 g respectively, in Krebs solution of the following composition (mM): NaCl 118.40, KCl 4.75, $CaCl_2$ 2.50, $MgSO_4 \cdot 7H_2O$ 1.18, KH_2PO_4 1.19, $NaHCO_3$ 25.00 and glucose 11.66, maintained at 37°C and gassed with 5% CO_2 in O_2; EDTA (10 μM) and ascorbic acid (50 μM) were included in the Krebs solution in order to prevent oxidation of NA (Maxwell *et al.*, 1983). All tissues were equilibrated for one hour before experimentation and during this period the bathing fluid was changed every 20 min. Following equilibration, contractions of the aorta and portal vein were elicited by NA (1 μM) or KCl (60 mM) every 30 min until responses varied by less than 10%. In the aorta contractions were quantified by measuring the peak of the contractile response. In the portal vein, contractile activity was measured by electronically integrating the contractile responses using digital planimeters manufactured to a design similar to that described by Illingworth & Naylor (1980). Since significant release of intracellular Ca^{2+} in vascular muscle only occurs following stimulation with higher concentrations of agonists (Van Breemen *et al.*, 1982) all concentrations of stimulants used in this study produced contractions which were approximately 90% of maximum. Moreover, all responses investigated in the present study could be elicited at 30 min intervals over a period of 8 h with less than 10% variation.

Modified Krebs solutions

Contractile responses to NA were also elicited in the aorta under Ca^{2+}-free conditions by incubation for 4 min with Krebs solution from which Ca^{2+} had been omitted and 0.5 mM EGTA added; these Ca^{2+}-free conditions were maintained during the response (EGTA-resistant response). Tissues were returned to Krebs containing Ca^{2+} for 30 min before eliciting further responses. The Ca^{2+}-free conditions used in the present study abolished KCl-induced contractions of the aorta and all responses of the portal vein. In some preparations of the aorta, NA-induced contractions were elicited following 30 min incubation in normal Krebs containing 2 nM prazosin (prazosin-resistant response, see Figure 1c). All physiological saline used subsequently in this series of experiments contained 2 nM prazosin. In this manner, EGTA-resistant and prazosin-resistant responses could be reproduced every 30 min over a period of 8 h with less than 10% variation.

In some preparations of the portal vein, contractions were also induced by adding Ca^{2+} (0.5–14 mM) to the bathing solution after depolarizing the tissue: for the latter purpose a K-rich Ca^{2+}-free physiological saline of the following composition was used (mM): NaCl 17.11, KCl 99.83, $MgCl_2$ 1.21, $NaHCO_3$ 14.88 and glucose 11.66 (Van Nueten *et al.*, 1978). A transient contraction of the portal vein was observed upon contact with the Ca^{2+}-free depolarizing medium. Consequently, the tissue was allowed to relax to the resting tension before any attempt was

made to construct a Ca^{2+} concentration-response curve.

The effects of calcium entry blockers

The actions of cumulative addition of Ver, Dlz or Flu were investigated upon contractions induced in the aorta by KCl or NA in the presence or absence of Ca^{2+} or prazosin. Similarly, spontaneous contractions and responses induced in portal vein by NA and KCl were investigated in the presence of these agents. Each concentration of Ver and Dlz was incubated for 30 min and a 90 min incubation period was used for each concentration of Flu.

Statistical analysis

Values are presented as arithmetic means with the associated s.e.mean for the number (n) of experiments noted. Statistical evaluation of results was carried out by use of Student's unpaired t test. Values $P < 0.05$ were considered significant.

Drugs and chemicals

Ver, Dlz, Flu and prazosin were gifts from Abbott, Synthelabo, Jansson and Pfizer respectively. All other agents were obtained from BDH or Sigma.

Results

Contractions of the aorta

Contractions of the rat aorta elicited by NA and KCl in normal Krebs solution were both of a similar magnitude (see Figure 1a and b respectively). In the present study NA-induced contractions of the rat aorta were clearly biphasic consisting of an early fast component followed by a slowly developing maintained response (see Figure 1a). Contractions of the rat aorta to KCl also appeared biphasic (see Figure 1b) though compared to the NA-induced contraction, the transition between fast and slowly developing components was not well defined.

The use of oxygenated isolated ring segments of rat aorta could be expected to preserve a functional endothelium as demonstrated by enface silver staining and relaxation of NA-induced contractions by acetylcholine.

Following 30 min incubation with Krebs solution containing 2 nM prazosin there was no change in the magnitude of the sustained phase of the contraction induced by NA, but the early fast component of the response was abolished (prazosin-resistant response, Figure 1c). In contrast, incubation with Ca^{2+}-free Krebs solution containing 0.5 mM EGTA abolished

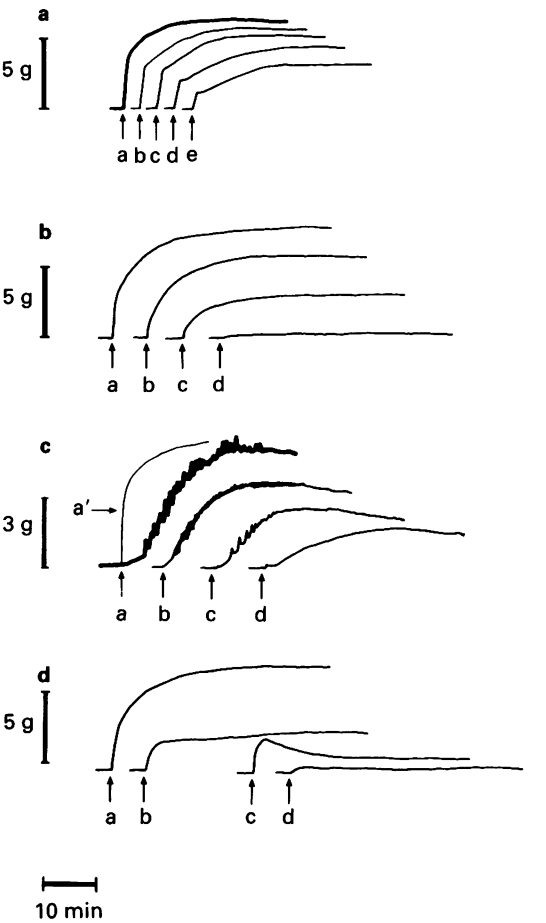

Figure 1 Representative contractions of the rat aorta: time scales apply to all figures. (**a**) Contractions to 1 μM noradrenaline (NA), (a) control; (b), (c), (d) and (e) are responses following incubation with 10 nM, 100 nM, 1 μM and 10 μM verapamil respectively. (**b**) Contractions to 60 mM KCl, (a) control; (b), (c) and (d) are responses following incubation with 0.1 μM, 0.5 μM and 1 μM verapamil respectively. (**c**) Responses to 1 μM NA (a') in unmodified Krebs solution; (a) in the presence of 2 nM prazosin (prazosin-resistant responses); (b), (c) and (d) are prazosin-resistant responses following incubation with 10 nM, 100 nM and 1 μM verapamil respectively. (**d**) Responses to 60 mM KCl, (a) control; (b), (c) and (d) are responses following incubation with 0.1 μM, 1 μM and 10 μM flunarizine.

the sustained component of the response to NA but left a rapid transient contraction which reached at peak 41.6 $\pm$ 1.7% ($n = 47$) of the control response (EGTA-resistant response).

Effects of verapamil Sustained contractions to KCl and NA were reduced in a concentration-dependent

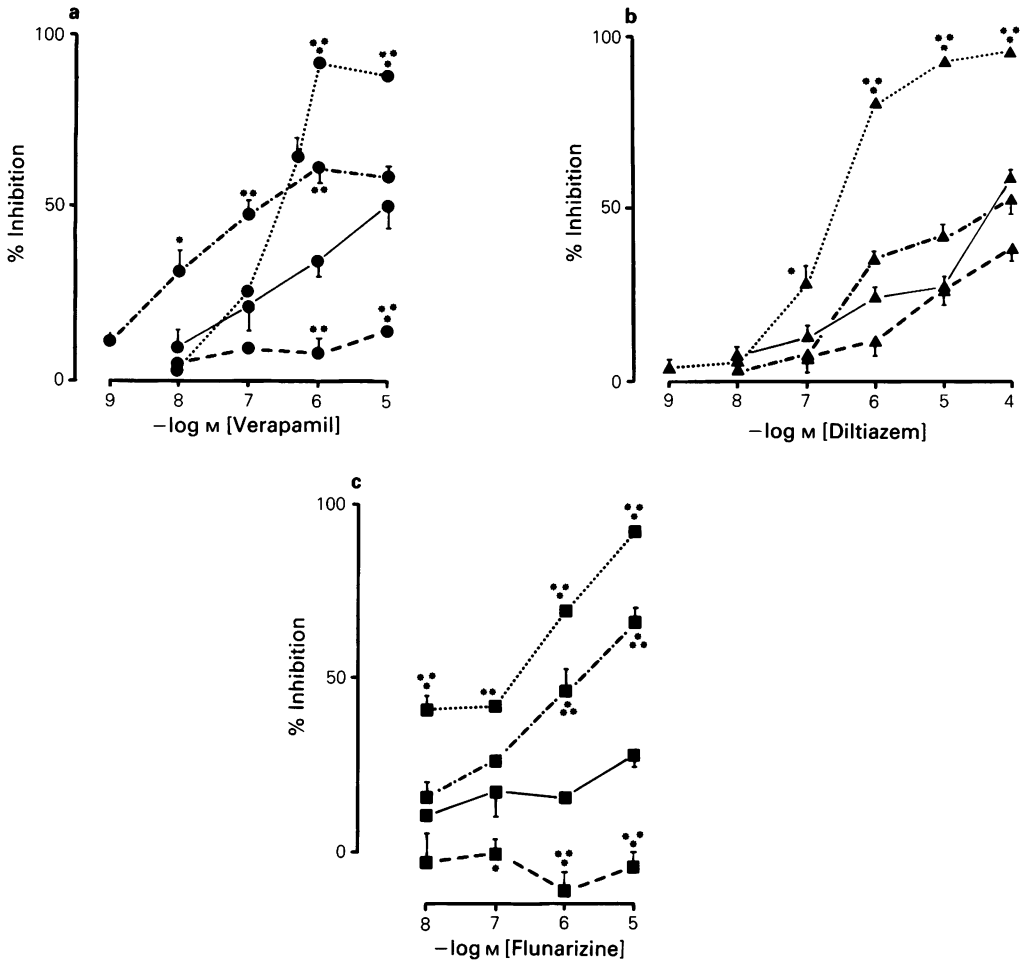

Figure 2 Effects of (a) verapamil (1 nM–10 μM), (b) diltiazem (1 nM–10 μM) and (c) flunarizine (10 nM–10 μM) on sustained contractions of the rat aorta induced by noradrenaline (NA) and KCl in normal Krebs solution and by NA in the presence of prazosin (prazosin-resistant response) and in the absence of Ca^{2+} (EGTA-resistant response). In (a) (● —— ●) shows contraction to NA ($n = 6$); (●·····●) contraction to KCl ($n = 6$–18); (● — — ●) EGTA resistant response ($n = 6$) and (● · — · ●) the prazosin-resistant response ($n = 6$–8). In (b) (▲——▲) shows the contraction to NA ($n = 6$); (▲·····▲) contraction to KCl ($n = 6$); (▲--▲) EGTA-resistant response ($n = 12$) and (▲— · ▲) prazosin-resistant response ($n = 6$). In (c) (■ —— ■) shows contraction to NA ($n = 6$), (■ ····· ■) contraction to KCl ($n = 6$); (■ — — ■) EGTA-resistant response ($n = 6$) and (■— · — · ■) prazosin-resistant response ($n = 6$). In (a–c) the ordinate scale shows the percentage inhibition of the control response and the abscissa scale the concentration of the agent used. Symbols represent the arithemetic mean of n results and vertical bars the s.e.mean. Significant (*$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$) differences from the inhibition of NA-induced responses.

manner by Ver (10 nM–10 μM) (see Figure 2a). However, verapamil at the higher concentrations (> 100 nM) caused a significantly ($P < 0.001$) greater reduction of contractions to KCl than to NA (see Figure 2a): 1 μM Ver caused a 34.1 ± 6.9% ($n = 6$) and a 92.0 ± 1.1% ($n = 18$) reduction of responses to NA and KCl respectively. The prazosin-resistant response was significantly more sensitive to Ver than the corresponding contraction to NA (see Figure 2a) at all but the highest concentration of verapamil but significantly less sensitive than the response to KCl, at least at concentrations of Ver of 1 μM and 10 μM ($P < 0.001$, see Figure 2a): Ver (1 μM) caused a 61.3 ± 3.4% ($n = 6$) reduction of the prazosin-

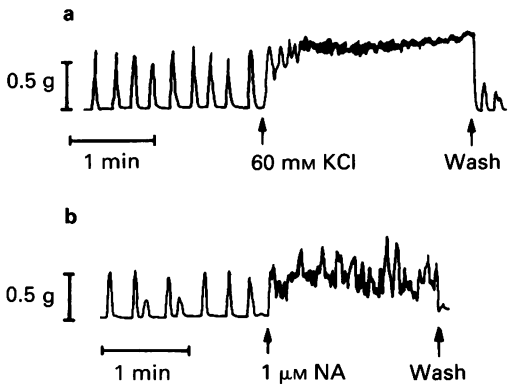

Figure 3 Examples of spontaneous activity of the rat portal vein and sustained contractions induced by (a) KCl and (b) noradrenaline (NA). Time and tension scale apply to all figures.

resistant response. The EGTA-resistant response was virtually insensitive to the actions of Ver: only a $7.9 \pm 4.1\%$ ($n = 6$) reduction of this response resulted from incubation with 1 μM Ver (see Figure 2a).

Effects of diltiazem Dlz (>100 nM) also produced a concentration-dependent reduction of the sustained contraction induced by KCl (see Figure 2b) which was significantly more pronounced than the reduction of the corresponding NA-induced responses; 10 μM Dlz produced a $92.5 \pm 0.8\%$ ($n = 6$) reduction of the KCl-induced response. NA-induced responses were reduced by Dlz but there was no consistent or significant difference between the effects of Dlz (10 nM–10 μM) upon the response to NA or the prazosin-resistant and EGTA-resistant responses (see Figure 2b); 10 μM Dlz reduced these responses by $28.5 \pm 5.2\%$ ($n = 12$), $42.0 \pm 3.3\%$ ($n = 6$) and $27.6 \pm 2.5\%$ ($n = 6$) respectively.

Effects of flunarizine Sustained contractions to KCl could also be reduced in a concentration-dependent manner by Flu (>1 μM) (see Figure 2c). In concentrations up to 1 μM, Flu appeared to reduce preferentially the latter sustained component of the KCl-induced contraction (see Figure 1d(c)): the rapidly developing component of the KCl contraction (≤1 min following stimulation) appeared less affected by Flu (≤1 μM). Exposure to Flu (10 nM–10 μM) caused a significantly greater reduction ($P < 0.001$, >1 μM Flu) of the prazosin-resistant response to NA than of corresponding responses to NA in the absence of prazosin (see Figure 2c), although the reductions were smaller than those of the responses to KCl. For example, 10 μM Flu caused a $92.0 \pm 1.0\%$ ($n = 12$), $65.9 \pm 4.5\%$ ($n = 6$) and a

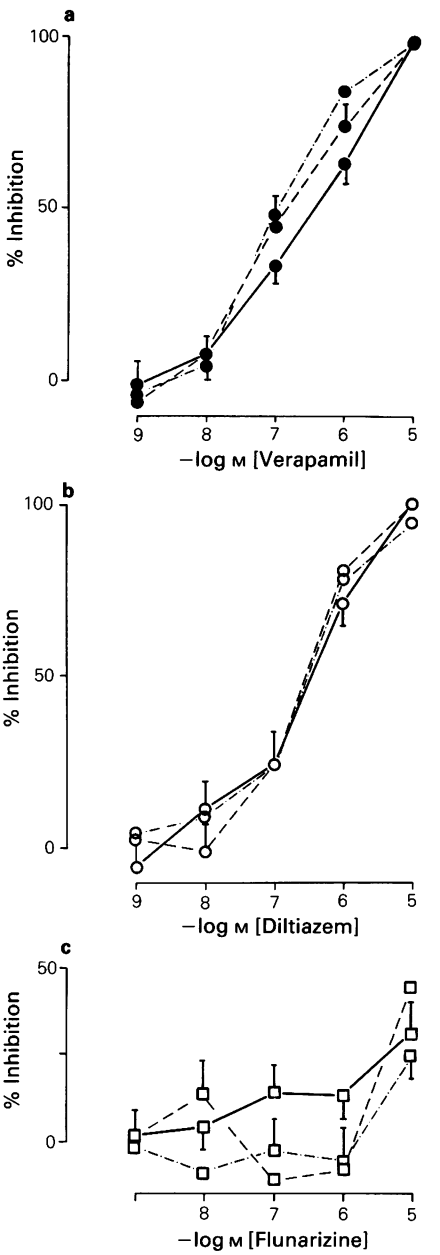

Figure 4 Effect of (a) verapamil (1 nM–10 μM), (b) diltiazem (1 nM–10 μM) and (c) flunarizine (1 nM–10 μM) on spontaneous activity (——, $n = 10$–12) and sustained contractions induced by noradrenaline (– – –, $n = 5$–6) and KCl (–·–·, $n = 5$–6) in the rat isolated longitudinal portal vein. In (a–c) the ordinate scale shows the percentage inhibition of the control response and the abscissa scale the concentration (M) of the agent used. Symbols represent the arithmetic mean of n results and vertical bars the s.e.mean.

27.5 ± 3.3% ($n = 6$) reduction of the KCl-induced contraction, prazosin-resistant response and contraction to NA respectively. EGTA-resistant responses were totally insensitive to all concentrations of Flu examined (see Figure 2c).

The approximate IC_{50} (M) for Ver, Flu and Dlz against prazosin-resistant responses were 6.770, 5.600 and 4.387 respectively compared to 6.790, 6.721 and 6.553 respectively against KCl-induced responses.

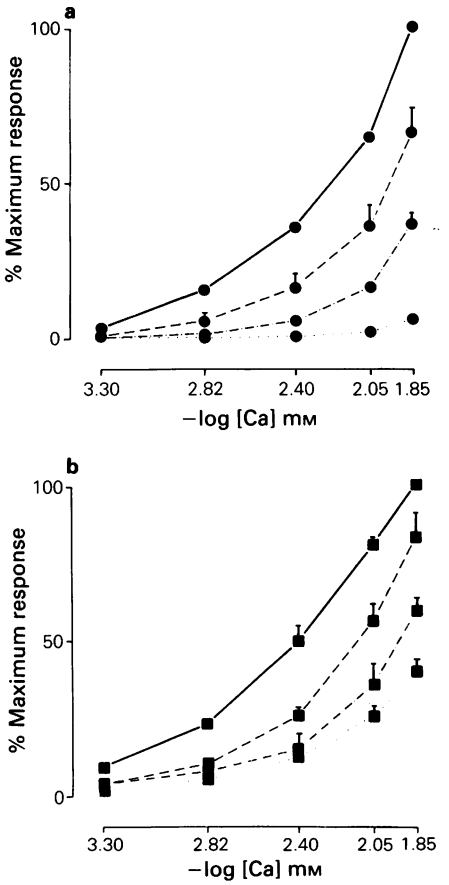

Figure 5 Effects of (a) verapamil ($n = 5$–6) and (b) flunarizine ($n = 6$) on Ca^{2+}-induced contractions (0.5–14 mM; $-\log[Ca^{2+}]$ 3.30–1.85) in depolarized rat portal vein. Symbols represent the arithmetic mean of n results and vertical bars represent s.e.mean. In (a) (●——) control; (●– –) 10 nM, (●–·–) 100 nM and (●·····) 1 μM verapamil. In (b) (■——) control, (■– –) 100 nM, (■–·–) 1 μM and (■·····) 10 μM flunarizine. In (a) and (b) the ordinate scale shows the percentage maximum response and the abscissa scale the concentration of Ca^{2+} used to stimulate tissues.

Contractions of the portal vein

Under the conditions used in the present study the unstimulated portal vein showed rhythmic spontaneous contractions (4–6 min^{-1}, Figure 3). Addition of 1 μM NA or 60 mM KCl caused a rapid increase in tension followed by a sustained tonic contracture with superimposed phasic activity (Figure 3.)

Both Ver and Dlz (1 nM–10 μM) produced concentration-dependent reductions both of spontaneous and KCl- and NA-induced contractions of the portal vein (see Figure 4a and 4b respectively). At each concentration of verapamil or diltiazem tested there was no significant difference between the effects upon induced or spontaneous contractions. All responses were abolished by addition of 10 μM of verapamil or diltiazem (see Figure 4a and 4b respectively). In contrast to Ver and Dlz, Flu was largely without effect upon KCl- and NA-induced and spontaneous contractile responses of the portal vein (Figure 4c). However, 10 μM flunarizine did produce small reductions of spontaneous contractions by 31.4 ± 9.4% ($n = 10$) and of contractions induced by KCl by 24.9 ± 6.6% ($n = 5$) and NA by 44.9 ± 2.7% ($n = 6$).

Cumulative addition of Ca^{2+} (0.5–14 mM; $-\log[Ca^{2+}]$ 3.30–1.85, Figure 5) to depolarized portal veins under Ca^{2+}-free conditions caused graded increases in tension. Exposure to Ver for 30 min (10 nM–1 μM, Figure 5a) or Flu for 90 min (100 nM–10 μM, Figure 5b) caused concentration-dependent reductions of the response to Ca^{2+}. The concentration-response curves for Ca^{2+} were displaced to the right both by Ver (Figure 5a) and Flu (Figure 5b). Furthermore, the log shifts at the EC_{50} produced by 10 nM Ver and 100 nM Flu were similar (0.37 ± 0.06, $n = 5$ and 0.36 ± 0.06, $n = 6$ respectively). Ver (1 μM) and Flu (10 μM) reduced contractions to 14 mM Ca^{2+} by 94.1 ± 1.7% ($n = 6$) and 60.0 ± 4.0% ($n = 6$) respectively.

Discussion

Contractions in aorta

Following stimulation with an agonist, receptor-operated channels allow entry of extracellular Ca^{2+} into vascular muscle while voltage-operated Ca^{2+} channels open in response to changes in membrane potential (Weiss, 1985). Release of intracellular Ca^{2+} apparently mediates the fast component of NA-induced contractions of the rat aorta and this component may be selectively preserved as a transient

contraction, in the absence of extracellular Ca^{2+} (Yamashita *et al.*, 1977; Heaslip & Rahwan, 1982). On the other hand, Downing and associates (1983) have shown that the fast component of the NA-induced contraction of the rat aorta is selectively inhibited by prazosin, while the maintained contraction which remains in the presence of prazosin is abolished by Ca^{2}-free conditions, indicating an exclusive dependence upon extracellular Ca^{2+}. A similar action of prazosin has also been reported by Cauvin & Malik (1984). Such effects are apparently unrelated to the action of prazosin as a selective α_1-adrenoceptor blocking agent (Downing *et al.*, 1983).

Thus, in order to elucidate the actions of Ver, Dlz and Flu, their effects have been examined in the present study upon contractions of the aorta which are mediated by (1) Ca^{2+}-entry through voltage-operated channels (KCl-induced contraction), (2) Ca^{2+}-entry induced by receptor activation (prazosin-resistant response), (3) release of intracellular Ca^{2+} (EGTA-resistant response) and (4) a combination of release of intracellular Ca^{2+} and receptor. activated influx of extracellular Ca^{2+} (NA contraction in the absence of prazosin). It should be noted that since the endothelium would be expected to be functional in the models examined in the present study, vasoactive moieties produced by the endothelium under *in vivo* physiological conditions would also be expected to influence similarly the overall contractility of tissues investigated in this study; however, their contribution has not been assessed.

The calcium entry blockers examined in this study each belong to a distinct chemical subgroup (Spedding, 1985). However, these agents produced similar reductions of the KCl-induced contractions of the aorta, indicating that voltage-operated channels in this tissue have a uniform sensitivity to all of these agents. Cauvin and co-workers (1983) have also indicated that there are only relatively small variations in the sensitivity to calcium entry blockers of voltage-operated Ca^{2+} channels even when comparisons are made between vascular muscle from different species. However, it is worth noting that in the rat aorta, Flu, in contrast to Dlz and Ver caused a preferential reduction of the sustained component of the contraction to KCl. Using rat aorta, Godraind & Dieu (1981) have previously quantified this effect showing that 2 min after stimulation with KCl (fast component of contraction) the IC_{50} for Flu against contraction and ^{45}Ca influx were larger than corresponding parameters measured 33 min later (sustained phase of contraction). Several workers have suggested that a heterogeneous population of voltage-operated Ca^{2+} channels exist in vascular (Jones, 1981; Ratz & Flaim, 1982; Hogestatt & Andersson, 1984) smooth muscle. In intestinal smooth muscle, action potential firing has been associated with the fast component of contractions to KCl, whereas tonic contractions were accompanied by a sustained membrane depolarization (see Hogestatt & Andersson, 1984). Wadsworth & Moss (1982) have demonstrated that Flu preferentially reduces contractions of the rabbit portal vein which result from sustained membrane depolarization. It is therefore possible that similar spike and sustained changes in membrane potential occur in the aorta in response to stimulation with KCl and that this accounts for the observed actions of flunarizine.

As indicated above, KCl-induced contractions and prazosin-resistant responses to NA in the rat aorta are mediated exclusively by extracellular Ca^{2+} (Downing *et al.*, 1983). However, the calcium entry blockers examined in the present study produced widely different reductions of the prazosin-resistant response, and none of the agents tested abolished such contractions. It seems reasonable to assume that the agents used did reduce contraction by blockade of NA-induced Ca^{2+} entry rather than by some alternative mechanism, since NA-induced ^{45}Ca influx has been shown to be reduced by Ver (Karaki *et al.*, 1984) and Flu (Godraind & Dieu, 1981) in the rat aorta and by Dlz (Van Breemen *et al.*, 1981) in the rabbit aorta. If NA had produced significant depolarization of the rat aorta resulting in activation of voltage-operated Ca^{2+} channels then the prazosin-resistant response would be expected to have been as sensitive to calcium entry blockade as the KCl-induced contraction. Thus, the present results suggest that contraction of the rat aorta which is mediated by Ca^{2+} entry through receptor-operated channels differs in its sensitivity to the calcium entry blockers. Cauvin and co-workers (1983) have also reported large variations between the sensitivities to calcium entry blockade of receptor-operated Ca^{2+} channels of vascular muscle from a number of species.

If it is assumed that the EGTA-resistant response of the rat aorta under conditions of zero extracellular Ca^{2+}, was principally dependent upon Ca^{2+} arising from intracellular sites, then the fact that these EGTA-resistant responses were insensitive to Ver and Flu support the role of these agents as selective blockers of stimulated Ca^{2+} influx: the small but significant reduction of the EGTA-resistant response by Dlz would appear to indicate an additional role for this agent as a blocker of contraction mediated by intracellular Ca^{2+}. This observation is consistent with a previous report by Van Breemen and associates (1981) who showed that Dlz acted as a weak intracellular Ca^{2+} antagonist in the rabbit aorta.

Contractions in portal vein

In the present study the isolated portal vein contracted spontaneously and also responded with a sustained contracture to both NA and KCl. A previous report (Fiol de Cuneo *et al.*, 1983) demonstrated that both agonist- and KCl-induced contractions of the rat portal vein consisted of phasic and tonic components: these components of contraction were also evident in the present study but they were not assessed separately. All contractions of the rat portal vein, both spontaneous and KCl or NA-induced, were abolished by a short period of Ca^{2+} withdrawal in the present study, indicating that they were exclusively dependent upon extracellular Ca^{2+}. Other workers (Sutter, 1976; Ebeigbe, 1982) have observed components of contractions to NA and KCl of the rat portal vein which are resistant to Ca^{2+}-free conditions and have implied that intracellular Ca^{2+} contributes to contraction of this vessel. However, in each of these studied, contractions were completely abolished by relatively short (<5 min) periods in the absence of extracellular Ca^{2+}. Furthermore, caffeine, an agent thought to release intracellular stores of Ca^{2+} in vascular muscle did not elicit contraction in the rat portal vein (Andersson *et al.*, 1974), and structures associated with intracellular storage of Ca^{2+} are poorly developed in this vessel (Ebeigbe, 1979). Thus, the evidence available strongly suggests that in the rat portal vein, intracellular Ca^{2+} is not utilized for contraction.

It is striking that in the present study, Ver and Dlz both produced similar reductions of spontaneous and NA- and KCl-induced contractions of the portal vein, in a manner reminiscent of the actions of these agents against KCl-induced contractions in the rat aorta. Previous investigations of the portal vein have also shown that Ver and Dlz produce similar effects upon agonist- and KCl-induced contractions (Fiol de Cuneo *et al.*, 1983; Campbell *et al.*, 1986). Equally striking is the general insensitivity of these responses to Flu. This observation indicates that Flu is either incapable of blocking Ca^{2+} entry in the rat portal vein or that the mechanisms gating entry of Ca^{2+} in this vessel are different from those in the aorta since in the latter, KCl- and NA-induced responses were sensitive to Flu (see above). The former possibility is unlikely since the present study showed that Flu, like Ver, readily reduced contractions induced by addition of Ca^{2+} to portal veins that had been depolarized in the absence of Ca^{2+}: such an action is attributable to calcium entry blockade (Hof & Vuorela, 1983). That Flu was capable of blocking contraction caused by addition of Ca^{2+} to depolarized portal veins, but did not reduce contraction induced by depolarization in the presence of Ca^{2+} may be somewhat surprising. However, Spedding (1982) showed that the calcium entry blocking properties of Flu are potentiated by depolarization of smooth muscle: it may be that a protracted period of incubation in a depolarizing medium allows Flu access to its site of action.

The spontaneous phasic activity of the portal vein and contractions to KCl have both been shown to be associated with changes in membrane potential (Axelssen *et al.*, 1967; Weston, 1978) which would be expected to activate voltage-operated channels (Weiss, 1985). Furthermore, Weston (1978) demonstrated that 1 μM NA causes significant depolarization of the rat isolated portal vein. Thus, it could be argued that all contractions of the rat portal vein examined in the present study were mediated by Ca^{2+} entry through voltage-operated channels which could be blocked by Ver and Dlz, and that these voltage-operated channels must be different from those operating in the aorta, in order to account for the insensitivity of the portal vein to Flu. However, the hypothesis is not consistent with the findings of Mantel and co-workers (1975) who found that contractions of the rat portal vein could be evoked by adrenaline (50 nM–100 μM) even when the tissue was simultaneously contracted with KCl (106 mM), indicating that separate pathways are activated by agonists and KCl in this preparation. Consequently the observation that Ver and Dlz both had comparable effects on NA- and KCl-induced contractions of portal vein, indicate that these agents produced relatively non-specific blockade of receptor- and voltage-operated channels in the portal vein in the present study, as was previously suggested by Fiol de Cuneo and associates (1983).

General conclusions

The present investigation compared the actions of three structurally distinct calcium entry blockers in two isolated preparations of vascular muscle. It is clear that Ca^{2+} activation mechanisms in the aorta depend upon the method of stimulation, but such processes are not identical to those operating in the portal vein. The results indicate that in the aorta the calcium entry blockers studied produce a uniform blockade of responses mediated by Ca^{2+} entry through voltage-operated channels but that verapamil produces a more selective reduction of responses mediated by Ca^{2+} entry through receptor-operated channels than either Dlz or Flu.

They also indicate that calcium activation mechanisms in the rat portal vein are different from those operating in the aorta in as much as receptor- and depolarization-induced contractions in the portal vein were reduced equally by verapamil and diltiazem. Of the three agents investigated, Ver appears to exhibit properties which most nearly conform to

those expected of a selective calcium blocker, in the tissues studied. Dlz also appears to be capable of blocking contractions mediated by release of intracellular Ca^{2+}. while the calcium entry blocking properties of Flu are apparently tissue-specific and are not effective on rat portal vein.

In vivo, it is clear that the mechanisms underlying contraction or spasm of any blood vessel are likely to vary depending upon the prevailing pathological conditions since these may differentially affect Ca^{2+} influx or release of intracellular Ca^{2+} e.g. release of potassium or catecholamines. It is clear from the present study that different calcium entry blockers may be more or less effective against these processes. Moreover, the present findings also highlight the difficulties likely to be encountered when screening compounds for calcium entry blocking properties on isolated vascular preparations, for the choice of a model vessel might dictate the apparent activity of a compound. The present demonstration, that by applying a variety of different pharmacological procedures to the rat aorta it is possible to study the effects of Ca^{2+} entry blockers upon several different aspects of Ca^{2+} utilization for contraction, in one and the same tissue, indicates that the rat aorta can serve as an effective screen for calcium blocking compounds.

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