Effects of oxodipine on ^{45}Ca movements and contractile responses in vascular smooth muscle

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1 The inhibitory effects of oxodipine, a new dihydropyridine, were compared with those of nifedipine on contractile responses in rat isolated aortic strips and on spontaneous mechanical activity in portal vein segments.

2 In rat isolated aorta oxodipine ($\text{IC}_{50} = 7.8 \pm 1.8 \times 10^{-9} \text{ M}$) and nifedipine ($\text{IC}_{50} = 8.5 \pm 2.5 \times 10^{-9} \text{ M}$) dose-dependently inhibited the contractile responses induced by high K (80 mM), whereas responses to noradrenaline (NA, 10^{-6} M) were only slightly affected ($\text{IC}_{50} > 10^{-7} \text{ M}$). These inhibitory actions were observed with both drugs added either before or after the induced contractions.

3 Contractile responses induced by addition of Ca to 0Ca high-K solution were also dose-dependently inhibited by oxodipine ($\text{IC}_{50} = 4.5 \pm 2.5 \times 10^{-9} \text{ M}$). However, oxodipine up to 10^{-6} M did not modify the contractile responses obtained in strips incubated in 0Ca when Ca was added in the presence of NA.

4 Oxodipine and nifedipine also inhibited the development of spontaneous mechanical activity in portal vein segments.

5 Oxodipine inhibited ^{45}Ca uptake stimulated by high K ($\text{I}_{50} = 8.7 \pm 2.5 \times 10^{-9} \text{ M}$) or by NA ($\text{I}_{50} > 10^{-7} \text{ M}$). However, it did not modify ^{45}Ca uptake and ^{45}Ca efflux in resting strips or ^{45}Ca efflux stimulated by NA.

6 These results indicate that the effects of oxodipine on vascular smooth muscle may be due to the blockade of Ca entry through potential- and receptor-operated channels; it was at least 10 times more selective for potential-operated channels. Oxodipine did not modify Ca entry through passive leak channels and NA-induced intracellular Ca release.

Introduction

It is now well recognized that calcium entry blockers (CEBs) are effective in the prophylaxis and treatment of coronary artery disease, arterial vasospasm and hypertension (Godfraind *et al.*, 1984; Opie, 1984; Epstein *et al.*, 1985) which indicates that vascular smooth muscle is one of the tissues in which these compounds show their characteristic effects. These therapeutic effects of CEBs in vascular smooth muscle fibres have been attributed to inhibition of Ca entry through voltage- and receptor-operated channels (Bolton, 1979; Cauvin *et al.*, 1983; Fleckenstein, 1983; Godfraind *et al.*, 1986). In aortic strips dihydropyridine CEBs exhibit a potent antagonism against depolarization-induced contractile responses (Fleckenstein, 1983; Cauvin *et al.*, 1983), whereas their antagonistic effect against noradrenaline-induced contractions occurs only at high concentrations of these compounds (Schumann *et al.*, 1975;

Golenhofen & Hermstein, 1975; Bolton, 1979). Oxodipine [4-(2,3-methylen-dioxyphenyl)-1,4-dihydro-2,6-dimethyl,3-carboxyethyl-5-carboxymethyl-pyridine] is a new dihydropyridine derivative (Tejerina *et al.*, 1987) which in contrast to nifedipine is not photolabile. The present study was undertaken to examine the effects of oxodipine on rat isolated aortic strips and portal vein segments and to compare its effects with those of nifedipine, the best known dihydropyridine.

Methods

Experimental procedure

Sprague-Dawley rats of either sex (200–250 g) were killed by a blow on the head. The thoracic aorta and

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the portal vein were dissected and placed immediately in a modified Krebs-Henseleit solution (KS) of the following composition (mM): NaCl 118, KCl 4.7, NaHCO₃ 25, MgCl₂ 1.2, CaCl₂ 1.8 and glucose 11. The Ca-free KS was prepared by adding 2 mM EGTA instead of 1.8 mM CaCl₂. After excess fat and connective tissue were removed, the aortae were cut into helical strips of about 1.0 mm width and 20 mm in length. Aortic strips and vein segments were mounted vertically under 2 g and 1 g of tension, respectively, in organ baths containing 10 ml KS. The tissue bath was maintained at 34°C and bubbled with 95% O₂-5% CO₂ gas mixture. Contractile responses were recorded isometrically by a force-displacement transducer (Grass FT03) connected to a Grass polygraph. Each preparation was allowed to equilibrate for at least 2 h before initiation of experimental procedures and during this period the incubation media were changed every 15 min.

After equilibration the following experiments were performed: (1) In some experiments aortic strips were exposed to single submaximal concentrations of KCl (80 mM) and noradrenaline (NA, 10⁻⁶ M). An initial 25 min control contraction was obtained in each experiment with the appropriate stimulating agent and strips were then rested for a minimum of 45 min. Control contractile responses for each agonist were obtained at the beginning of the experiment until two successive responses were almost identical in height. This was followed by exposure to oxodipine or nifedipine for a 10 min period in both cases before the addition of KCl or NA and during the 25 min re-exposure to the stimulating agents. Only one agonist was used in each experiment. The results of these experiments are expressed as a percentage of the maximal control agonist-induced contractile responses. (2) To determine whether oxodipine and nifedipine could relax an existing contraction, aortic strips were contracted by single submaximal concentrations of NA or KCl. When the contractile response to either agonist was maximum, oxodipine or nifedipine was added in progressively increasing cumulative concentrations (10⁻⁷ M-5 × 10⁻⁴ M). Strips were allowed to reach a new steady-state tension before each successive concentration of the relaxant drug. The results were expressed as percentage of the maximal control agonist-induced responses. (3) In additional experiments dose-response curves to CaCl₂ (1-5 mM) were obtained in aortic strips incubated in Ca-free KS containing 2 mM EGTA for 2 h and then in Ca-free high-K (80 mM) solution for 10 min before adding the drugs (Barrigón *et al.*, 1984). The results were expressed as a percentage of the maximal contractile response induced by 5 mM CaCl₂. (4) To study the effects of oxodipine on a membrane store of Ca mobilized by NA the experimental protocol

employed was similar to that previously described by Ebeigbe & Aloamaka (1985). Strips were stimulated by 10⁻⁵ M NA. When the contractile response was stable, strips were rinsed with Ca-free KS for 10 min ('Ca-washing') followed by 5 min exposure to 1.6 mM Ca KS ('Ca-loading') and finally, exposure to Ca-washing KS for 1 min, followed by 10⁻⁵ M NA stimulation. The above protocol was repeated in the presence of oxodipine, added 5 min into the 'Ca-washing' period or 2 min at the end of the 'Ca-loading' period. Control tissues incubated with ethanol (i.e. solvent for oxodipine and nifedipine) were always run in parallel in each of these experimental designs, the change in responsiveness of the control strips during the whole time course of these experiments being 6.9 ± 4.6%.

Measurements of ⁴⁵Ca movements

Techniques employed to estimate ⁴⁵Ca movements in aortic strips were as previously described by Godfraind (1983) and Barrigón *et al.* (1984). To determine ⁴⁵Ca uptake, aortic strips were bisected and in each experiment half of the aorta served as control and the other half as the experimental strip. Both halves were equilibrated for 1 h under 1 g of tension in Tris Buffer solution (TS) of the following composition (mM): NaCl 160, KCl 4.6, CaCl₂ 1.8, MgCl₂ 1.2, glucose 11 and tris-hydroxymethyl-amino-methane 6.0. This solution was adjusted to pH 7.4 with 4 N HCl and gassed with 100% O₂. After equilibration strips were further incubated for 5 min in TS containing ⁴⁵Ca (specific activity 0.4 µCi m⁻¹; The Radiochemical Centre, Amersham) either in the presence or absence of oxodipine and then for another 5 min in the same solution with or without the addition of 80 mM KCl or 10⁻⁶ M NA. At this time strips were washed in 50 mM LaCl₃-Ca-free TS for 5 min to remove extracellular bound Ca. Afterwards strips were removed, blotted and weighted. The strips were then placed in scintillation vials and 0.5 ml of Soluene-100 (Packard) added and the strips digested overnight at 50°C. Radioactivity was assayed in a liquid scintillation counter (Intertechnique Model SL-3000) as previously described (Barrigón *et al.*, 1983; 1984).

To study ⁴⁵Ca efflux in non-stimulated aortic strips, preparations were labelled with ⁴⁵Ca TS (sp. act. 2 µCi ml⁻¹) for 2 h and then placed in successive tubes containing 2 ml of Ca-free TS every min for the 30 min duration of the washout. In some vials oxodipine was added to the Ca-free TS. The radioactivity lost into the tubes and present at the end of the experiment was measured as described by Barrigón *et al.* (1984). The data obtained were plotted as desaturation curves which illustrate the decline of

tissue ^{45}Ca with time. ^{45}Ca efflux stimulated by NA (10^{-5} M) was studied following the technique previously described by Godfraind (1983). Aortic strips were preincubated for 2 h in ^{45}Ca containing KS ($2\mu\text{Ci ml}^{-1}$), oxodipine being added or not during the last 20 min. The strips were then transferred to non-radioactive KS also with or without oxodipine and 5 min later to the same solution containing or not containing NA (10^{-6} M). Afterwards strips were treated as for ^{45}Ca uptake experiments.

Drugs

The following drugs were used: oxodipine (IQB, S.A.), nifedipine (Bayer) and noradrenaline bitartrate (Sigma). Oxodipine and nifedipine were initially dissolved in 99% ethanol and further dilutions were made in KS, but control experiments demonstrated that the highest ethanol level used at 10^{-6} M have no effect on vascular smooth muscle contraction. Values were expressed as the mean $\pm$ s.e.mean and statistical analysis was performed by use of Student's *t* test. The differences between control and experimental values were considered significant when $P < 0.05$. Dose-response slopes were analysed to give the concentration of oxodipine or nifedipine producing a 50% inhibition of the maximal contractile response (IC_{50}) or of the maximal NA and high-K dependent ^{45}Ca uptake (I_{50}) using a linear regression analysis (method of least squares).

Results

Effects on resting and spontaneous mechanical activity

The effects of oxodipine and nifedipine were studied on changes in base-line tension and spontaneous mechanical activity in aortic strips and portal veins, respectively. Both drugs had no effect on resting tension but inhibited in a dose-dependent manner the rate and amplitude of spontaneous myogenic activity in portal veins. The IC_{50} values for oxodipine and nifedipine inhibition of the amplitude of spontaneous activity were $2.5 \pm 1.2 \times 10^{-8}\text{ M}$ and $7.0 \pm 0.5 \times 10^{-8}\text{ M}$, respectively, this difference being statistically significant ($P < 0.05$).

Effect on contractions induced by KCl and NA

The inhibitory effect of oxodipine and nifedipine on contractile responses induced by KCl (80 mM) and

NA (10^{-6} M) were quantified and compared in isolated aortic strips. Both agonists produced equipotent submaximal contractile responses, $642.8 \pm 36.0\text{ mg}$ and $842.0 \pm 88.9\text{ mg}$, respectively ($n = 8$; $P > 0.05$), whereas maximal contractile responses were obtained with 100 mM KCl ($700.2 \pm 28.5\text{ mg}$, $n = 8$) and 10^{-5} M NA ($940.8 \pm 32.9\text{ mg}$, $n = 8$). The effects of oxodipine, 10^{-9} M – 10^{-7} M , on the time courses of the KCl- and NA-induced contractions are shown in Figure 1. Oxodipine produced a dose-dependent inhibition of the contractile response induced by both stimulating agents. However, whereas 10^{-7} M oxodipine almost suppressed the contractile response induced by KCl, this concentration left unaltered about 50% of the response induced by NA. The IC_{50} values for oxodipine and

Figure 1 Time course of the contractions induced by (a) high K (80 mM) and (b) noradrenaline (10^{-5} M) in rat aortae pretreated or not with oxodipine. Each point represents the mean of 8 experiments; vertical lines indicate s.e.mean. (●) Controls; (○) after oxodipine, 10^{-9} M , (▲) 10^{-8} M and (△) 10^{-7} M .

Figure 2 Relaxation of high-K- (●, 80 mM) and noradrenaline-induced (○, 10⁻⁵ M) contractions by oxodipine. Ordinate scale: relaxation (% of control values). Each point represents the mean of 8 experiments; vertical lines indicate s.e.mean.

nifedipine-induced depression of high-K-induced responses were $7.8 \pm 1.8 \times 10^{-9}$ M and $8.5 \pm 2.5 \times 10^{-9}$ M ($P > 0.05$), respectively, which indicates that both drugs are almost equipotent on the contractile responses induced by 80 mM KCl.

Figure 2 shows the relaxant effect of cumulatively increasing concentrations of oxodipine on aortic strips previously contracted with either 80 mM KCl or 10⁻⁶ M NA. Cumulative increases in the concentration of oxodipine and nifedipine (10⁻¹⁰ M–10⁻⁶ M) to strips previously contracted with KCl resulted in a dose-dependent relaxation with an IC₅₀ of $3.0 \pm 2.1 \times 10^{-9}$ M and $7.5 \pm 2.1 \times 10^{-9}$ M, respectively ($P > 0.05$). Conversely, at the highest concentration tested (10⁻⁶ M) oxodipine and nifedipine relaxed strips previously contracted with NA by only $15.3 \pm 3.2\%$ and $26.1 \pm 1.8\%$ of its maximum, respectively.

Effects on Ca-induced contractions

The inhibitory effects of oxodipine on the [Ca]_o-dependent contractile responses are shown in Figure 3. Following incubation for 2 h in a Ca-free high-K (80 mM) KS, the addition of Ca to the bathing media (1–5 mM) caused a contraction of the aortic strips in a dose-dependent manner. The maximum contractile response induced by 5 mM CaCl₂ was similar to that induced by 10⁻⁶ M NA (1001.2 ± 83.4 mg as compared to 842.0 ± 88.9 mg,

Figure 3 Effects of oxodipine on the restoration of the isometric contractions of aortic strips by addition of calcium (1–5 mM) to Ca-free high-K (80 mM) medium. Ordinate scale: percentage of the maximum control contractions obtained with the highest concentration of calcium in each experiment. Each point represents the mean of 6 experiments; vertical lines indicate s.e.mean. (●) Controls; (○) after oxodipine 10⁻¹⁰ M, (▲) 10⁻⁹ M and (△) 10⁻⁸ M.

$n = 8$, $P > 0.05$). Oxodipine, 10⁻¹⁰ M–10⁻⁸ M, not only produced a progressive decrease in the amplitude of Ca-induced contractions of K-depolarized aortic strips but also caused a progressive shift of Ca concentration-response curves downward and to the right. Regression analysis of the inhibition yielded an IC₅₀ of $4.5 \pm 2.5 \times 10^{-9}$ M.

In another group of experiments the effects of oxodipine were studied on the contractile responses induced by NA in strips which were incubated for 10 min in Ca-free KS, then in 'Ca-loading' KS for 5 min and finally in Ca-free KS for 1 min. At this time stimulation with NA (10⁻⁵ M) resulted in a phasic contraction of an amplitude of 729.0 ± 72.9 mg ($n = 6$), presumed to reflect Ca mobilization from a membrane-bound store (Karaki *et al.*, 1979). In 6 experiments oxodipine, 10⁻⁶ M, had no effect on the magnitude of the NA-induced phasic contraction when applied before 'Ca-loading' (619.7 ± 71.5 mg, $P > 0.05$) or during 'Ca-loading' (625.0 ± 50.2 mg, $P > 0.05$).

Effects on ⁴⁵Ca uptake and ⁴⁵Ca efflux

The effects of oxodipine, 10⁻⁸ M–10⁻⁶ M, were studied on the La³⁺-resistant ⁴⁵Ca uptake in resting,

Table 1 Effect of oxodipine on lanthanum-resistant ^{45}Ca uptake in resting aortae and in aortae stimulated with 80 mM KCl or 10^{-6} M noradrenaline

Drug Concentration (M)	^{45}Ca uptake ($\mu\text{mol kg}^{-1}$ wet wt.)		
	Unstimulated (controls)	KCl	Noradrenaline
0	86.0 $\pm$ 6.8	317.0 $\pm$ 11.0	215.2 $\pm$ 10.2
10^{-10}		299.1 $\pm$ 12.3	203.1 $\pm$ 14.3
10^{-9}		281.2 $\pm$ 12.8*	190.6 $\pm$ 10.7
10^{-8}	85.2 $\pm$ 5.8	203.5 $\pm$ 10.0***	170.6 $\pm$ 12.5**
10^{-7}	88.6 $\pm$ 6.9	110.4 $\pm$ 10.5***	148.5 $\pm$ 10.5***

Results shown are means $\pm$ s.e.mean, $n = 9$

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

non-stimulated, as well as in aortic strips stimulated by 80 mM KCl and 10^{-5} M NA. Oxodipine, 10^{-7} M, had no significant effects on ^{45}Ca uptake in resting, non-stimulated aortic strips (86.0 ± 6.8 as compared to $88.6 \pm 6.9 \mu\text{mol kg}^{-1}$ aorta; $n = 6$, $P > 0.05$), but produced (Table 1) a significant dose-dependent inhibition of ^{45}Ca uptake stimulated by KCl (80 mM) and NA (10^{-6} M). In order to analyse the dose-dependency of the inhibitory effects of oxodipine on high-K and NA-induced ^{45}Ca uptake the procedure of Meisner *et al.* (1981) was used. ^{45}Ca uptake was measured in unstimulated strips and from these, averaged values for the increase in Ca uptake over control by the stimulating agent, in the absence as well as the presence of oxodipine were calculated. % inhibitions of ^{45}Ca uptake by oxodipine were then obtained to produce Figure 4. Oxodipine, 10^{-10} M– 10^{-7} M dose-dependently inhibited ^{45}Ca uptake stimulated by 80 mM KCl (Figure 4a). Concentration-inhibition curves for contraction and ^{45}Ca uptake were almost superimposed and thus IC_{50} and I_{50} values were similar ($7.8 \pm 1.8 \times 10^{-9}$ M and $8.7 \pm 2.5 \times 10^{-9}$ M, respectively). Oxodipine also decreased ^{45}Ca uptake in strips stimulated by 10^{-5} M NA. As is shown in Figure 4b, concentration-inhibition curves for contraction and ^{45}Ca uptake were also superimposed (IC_{50} and $\text{I}_{50} \geq 10^{-7}$ M, respectively). These results seem to indicate that even when oxodipine-induced inhibition of high K- and NA-induced contractions can be related to the blockade of Ca entry induced by both agonists, ^{45}Ca uptake stimulated by NA was more resistant to oxodipine inhibition than high K-stimulated ^{45}Ca uptake.

The effects of oxodipine were also studied on ^{45}Ca efflux in non-stimulated as well as in aortic strips stimulated by 10^{-5} M NA. In non-stimulated strips and following exposure for 2 h to ^{45}Ca -labelled TS the strips were rinsed with Ca-free-TS for the first 18 min of the 30 min washout and with Ca-free-TS

containing 10^{-6} M oxodipine for the final 12 min. The desaturation curves show (Figure 5) that oxodipine had no effect on the percentage of ^{45}Ca remaining in the aorta.

Furthermore, even at the highest concentration tested, 10^{-6} M, oxodipine had no effect on the tissue content of ^{45}Ca after 2 h incubation in ^{45}Ca -containing solution ($0.975 \pm 0.11 \text{ mmol kg}^{-1}$ wet wt.) as compared to the values obtained in control tissues ($0.980 \pm 0.09 \text{ mmol kg}^{-1}$ wet wt.; $n = 8$, $P > 0.05$). When 10^{-5} M NA was added for 2 min, a significant decrease ($P < 0.05$) of similar magnitude in residual ^{45}Ca content was observed both in control ($0.685 \pm 0.06 \text{ mmol kg}^{-1}$ wet wt.) and oxodipine-pretreated strips ($0.711 \pm 0.08 \text{ mmol kg}^{-1}$ wet wt.; $n = 8$, $P > 0.05$). These results indicate that oxodipine does not modify NA-stimulated ^{45}Ca efflux.

Discussion

The results of this paper demonstrate that oxodipine, a new dihydropyridine derivative, exhibits on rat isolated aortic strips and portal vein segments effects similar to those previously described with nifedipine (Godfraind, 1983) and other dihydropyridines (Godfraind, 1981; Cauvin *et al.*, 1983; Fleckenstein, 1983). Thus, the mechanism through which oxodipine relaxes vascular smooth muscle fibres seems to be related to its ability to inhibit the influx of extracellular Ca through potential- (POCs) and receptor-operated channels (ROCs) of the cell membrane.

Different experimental results demonstrated that oxodipine inhibited Ca entry through POCs: (1) oxodipine inhibited in a dose-dependent manner the contractile responses induced by K depolarization or by increases of extracellular Ca in K-depolarized aortic strips. These responses are entirely dependent

Figure 4 Inhibitory effect of oxodipine on (a) high K (80 mM) and (b) noradrenaline-induced (10^{-6} M) ^{45}Ca uptake (○) and contractile force (●) in rat isolated aortae. Ordinate scales: oxodipine-sensitive component as % of the maximum. Each point represents the mean of at least 6 experiments; vertical lines indicate s.e.mean.

upon the influx of extracellular Ca through POCs (Hudgins & Weiss, 1968; Godfraind & Kaba, 1969) and have been used to provide a simple means of studying drugs with possible Ca entry blocking properties (Haeusler, 1972; Godfraind, 1981). (2) Oxodipine was more potent than nifedipine at inhibiting the spontaneous myogenic activity in rat portal veins. This myogenic activity is also dependent on extracellular Ca (Sigurdson *et al.*, 1975) and has been related to Ca entry through POCs (Golenhofen & Lammel, 1972). (3) At the same range

Figure 5 Effect of oxodipine, 10^{-6} M, on ^{45}Ca efflux from rat isolated aortic strips. Muscles were incubated for 2 h in ^{45}Ca before washout in Ca-free solution. Each point represents the mean of 6 experiments. (●) Controls. (○) Oxodipine-treated strips (17 to 30 min).

of concentrations at which it inhibited K-induced contractions, oxodipine also inhibited dose-dependently the ^{45}Ca uptake stimulated by high K without altering resting ^{45}Ca uptake. Moreover, concentration-inhibition curves for K-induced contractions and ^{45}Ca uptake were almost superimposed. This indicates that an inhibition of Ca entry through POCs was responsible for the inhibitory effect of oxodipine on contractile responses induced by high K and Ca in aortic strips, as well as for the suppression of the myogenic activity in rat portal veins. However, as has been previously described with other CEBs (Cauvin *et al.*, 1983; Godfraind, 1983; Godfraind *et al.*, 1986), even at high concentrations oxodipine had no effect on ^{45}Ca uptake in resting aortic strips. This suggests that Ca entry through passive membrane leak channels is insensitive to oxodipine.

Furthermore, most of the CEBs are relatively more potent at inhibiting K-induced contractions than NA-induced contractions (Godfraind, 1981; Cauvin *et al.*, 1983; Kazda *et al.*, 1983) and this is also true for oxodipine. In fact, oxodipine inhibited NA-induced contractions at concentrations about three orders of magnitude higher than those required to inhibit Ca-induced contractions in K-depolarized aortic strips. NA-induced contractions can be resolved into two components, an initial phasic component which is due to the release of Ca from intracellular stores (Hudgins & Weiss, 1968; Godfraind & Kaba, 1969) and a tonic component which is associated with an increase in Ca influx through ROCs (Hudgins & Weiss, 1968; Deth & Van Breemen, 1974). In the present experiments the inhibitory effect of oxodipine on total contractile

responses to NA paralleled its attenuation of the tonic component. In fact, oxodipine-sensitive ^{45}Ca influx and contractions showed a similar concentration-response relationship, suggesting that oxodipine-induced inhibition of NA contractions can be related to the blockade of Ca entry through ROCs. However, if at the high concentrations used NA depolarized the membrane potential of vascular smooth muscle fibres, the inhibitory effect of oxodipine on NA-induced contractions could be attributed to inhibition of Ca entry through both POCs and ROCs. Furthermore, when these results are compared with those obtained in aortic strips stimulated by high K, it is evident that oxodipine was at least two orders of magnitude more selective at inhibiting Ca entry through POCs than through ROCs. These results confirm recent data in rat cultured aortic smooth muscle cells (Ruegg *et al.*, 1988) demonstrating that ^{45}Ca uptake stimulated by high K was blocked by CEBs (various DHPs, verapamil, diltazem), their potency reflecting the potencies in inhibiting K-induced contractions. However, ^{45}Ca uptake stimulated by angiotensin II and vasopressin was not blocked by these concentrations of CEBs. Therefore, the present results support the concept that POCs are more sensitive to CEBs than the ROCs (Cauvin *et al.*, 1983; Godfraind *et al.*, 1986).

However, the phasic component of the NA-induced contractions was unaffected suggesting that the release of Ca from intracellular stores is insensitive to oxodipine. To obtain further information on the effect of oxodipine on the NA-sensitive Ca store, the effects of oxodipine on NA-induced phasic contractions were studied. The phasic contractile response induced by NA in Ca-free media is similar

to those found in rat (Ebeigbe & Alaomaka, 1985) and rabbit aorta (Karaki *et al.*, 1979) and has been attributed to mobilization of Ca from a membrane-bound store. In the present study oxodipine did not affect the amplitude of the NA-induced phasic contraction in Ca-free medium whether applied before or during 'Ca-loading'. This result suggests that oxodipine had no effect on the release of Ca from a membrane-bound store induced by NA and/or that the Ca store responsible for the phasic contraction is filled with extracellular Ca via an oxodipine-insensitive pathway. A similar explanation was proposed by Karaki *et al.* (1979) to explain why verapamil and D600 in concentrations of 10^{-6} M did not affect this phasic response. Furthermore, at the maximum concentration tested which inhibited by almost 70% the NA-dependent ^{45}Ca uptake, oxodipine had no effect on ^{45}Ca efflux in resting, non-stimulated, as well as in aortic strips stimulated by NA. These results strongly suggest that at the concentrations at which it inhibits Ca entry, oxodipine fails to inhibit NA-induced intracellular Ca release. Similar results have been found in the presence of PY 108-068 (Hof *et al.*, 1982) and nifedipine (Godfraind, 1983; Kanmura *et al.*, 1983).

In conclusion, oxodipine is a new dihydropyridine derivative which like nifedipine inhibits Ca entry through voltage- and receptor-operated channels. However, oxodipine failed to inhibit Ca entry through passive leak channels and NA-induced intracellular Ca release.

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