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Characteristics of Ca^{2+} channel blockade by oxodipine and elgodipine in rat cardiomyocytes

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Abstract

The two novel dihydropyridines, oxodipine and elgodipine greatly depressed the KCl-induced contraction of rabbit aorta and decreased the cardiac force of contraction of rat ventricular strips with lower potency. Both compounds markedly shortened cardiac action potentials. In rat cultured neonatal ventricular myocytes, oxodipine and elgodipine decreased the L-type Ca^{2+} current (I_{CaL}) with IC_{50} of 0.24 and 0.33 μM respectively while oxodipine was slightly more potent on the T-type Ca^{2+} current (I_{CaT}) than elgodipine (IC_{50} = 0.41 vs. 2.18 μM). Both compounds were less potent in inhibiting I_{CaL} of adult cardiomyocytes. Oxodipine exhibited mostly a tonic block of both currents while elgodipine induced mainly a use-dependent block. Oxodipine and elgodipine increased by at least one order of magnitude their inhibitory potency on I_{CaT} and I_{CaL} when the cells were partially depolarized. We conclude that the mechanisms of inhibition of Ca^{2+} channels by these two dihydropyridines are different and suggest that the underlying mechanism of vascular selectivity is the voltage-dependent block of I_{CaL}, with the use-dependent inhibition of Ca^{2+} currents by elgodipine further contributing to this selectivity. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Heart; Patch-clamp; Ca^{2+} channel antagonist; 1,4-Dihydropyridine; Vascular selectivity

1. Introduction

Nifedipine and 1,4-dihydropyridine derivatives are well known calcium channel blockers and are used in clinics since long for the treatment of several cardiovascular disorders (Nayler, 1988). These compounds bind with high affinity to the α_1-subunit of L-type Ca^{2+} channels (Glossmann and Striessnig, 1990; McDonald et al., 1994; Triggle, 1996). It was shown in early patch-clamp studies that this binding to Ca^{2+} channels is state-dependent, such as binding affinity increases when Ca^{2+} channels are in the inactivated state (Bean, 1984).

Despite the fact that 1,4-dihydropyridines form the best-characterized class of calcium channel antagonists (Glossmann and Striessnig, 1990), there is still a need for more vascular selective compounds due to the potentially life-threatening effects of their cardiac depressant action (Packer, 1989; Waters, 1991; Opie, 1992a; Furberg et al., 1995). A second generation of 1,4-dihydropiridine agents has grown up that share the characteristic of being more vascular selective and therefore has a potential therapeutic advantage (Nayler, 1988; Opie, 1992b). Recently, two new 1,4-dihydropiridines that show high vascular selectivity, oxodipine (4-(2,3 methylenedioxyphenyl)-1,4-dihydro-2,6-dimethyl-3 carboxyethyl-5-carboxymethyl-pyridine) and elgodipine ((isopropyl,2-[*N*-methyl-*N*-(4-fluorobenzyl)amino]ethyl,2,6-dimethyl-4-[2′,3′-methylene-dioxyphenyl]-1,4-dihydropyridine3,5-dicarboxylate) have been developed.

Oxodipine has been reported to strongly depress contraction in aorta and mesenteric resistance vessels by inhibiting Ca^{2+} entry through both potential- and receptor-operated pathways, even if it was at least 10 times more potent on potential-operated channels (Tamargo and Tejerina, 1989; Tejerina et al., 1992). These marked vasodilator effects were achieved at concentrations significantly lower than those required for cardiodepressant effects. Oxodipine reduces the cardiac action potential duration without affecting amplitude and maximal rate of the depolarization phase nor the resting membrane potential. Oxodipine, however, decreases amplitude and duration of the

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slow response action potential induced in high-K solution (Tejerina et al., 1987). In rat portal vein myocytes, Baron et al. (1994) showed that both (+) and (−) oxodipine depress the high-threshold Ca current, I_{CaL} (IC_{50} 10 nM) in a voltage-dependent manner while both enantiomers appeared without effect on the low-threshold Ca current, I_{CaT}.

Elgodipine also possesses strong vasodilatory actions, decreasing blood pressure and increasing coronary blood flow (Tamargo et al., 1991). These effects were attributed to depression of Ca^{2+} entry through potential-operated channels in smooth muscle (Tejerina et al., 1987; Chulia et al., 1995). Like oxodipine, elgodipine slows atrio-ventricular conduction (Alloui et al., 1994). A potent coronary dilator properties of elgodipine, without significant depression of cardiac contraction (Sassen et al., 1990; Drieu la Rochelle et al., 1994), could be the basis of its beneficial effects in patients with coronary artery disease (Suryapranata et al., 1992). Elgodipine inhibits both I_{CaT} and I_{CaL} in portal vein myocytes although it is more potent on I_{CaL} (Leprêtre et al., 1994). In this tissue, it shows no use-dependent effects; instead, its effects are strongly voltage-dependent indicating high affinity of elgodipine for the inactivated state of Ca^{2+} channels. Both oxodipine and elgodipine possess significantly higher affinity for the vascular L-type Ca^{2+} channel than for the cardiac L-type Ca^{2+} channel (Rakotoarisoa et al., 1994).

The present study was undertaken to compare the pharmacological properties of oxodipine and elgodipine on vascular and cardiac preparations and to characterize their mechanisms of blockade of I_{CaL} in isolated adult rat ventricular cardiomyocytes. We also studied the effects of both dihydropyridines in rat cultured neonatal ventricular cardiomyocytes since these cells exhibit I_{CaT} (Gomez et al., 1994). Cultured cardiomyocytes provide an excellent model to study I_{CaT} for which a pathological role has been suggested since this channel type is re-expressed during hypertrophy (Nuss and Houser, 1993) and myocardial infarction (Qin et al., 1995).

2. Materials and methods

2.1. Aortic ring preparations and Langendorff-perfused rat hearts

Abdominal aortic rings were dissected from rabbits (New Zealand) under pentobarbital anaesthesia (30 mg/kg). They were fixed to a force transducer and placed in bath chamber continuously perfused (10 ml/min) with a Tyrode solution of the following composition (mM): NaCl, 140; KCl, 2.5; $CaCl_2$, 2; $MgCl_2$, 0.5; hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 10 and glucose, 5 (pH = 7.4, gassed with O_2). Preparations were left to stabilize, under a load of 0.8–1.0 g, for 1 h before the beginning of the experiment. Contraction was induced either by substituting NaCl with KCl (140 mM) or by adding norepinephrine (10 μM) to the bathing solution and was left to stabilize for 8–10 min before application of drugs. Experiments were conducted at 35°C.

Hearts were dissected out from pentobarbital anaesthetized adult Wistar rats and mounted in a Langendorff column. They were perfused at constant flow (10 ml/min) with the Tyrode solution. The apex was attached to a force transducer and the hearts were left to contract spontaneously under a constant load of 1 g. The aortic cannula was used as a reference electrode and the active electrode was fixed by a needle to the left ventricular surface. The surface electrogram was recorded with a biophysical preamplifier. The R–R interval was measured as the time interval between two contiguous R waves of the surface electrogram. It is expressed as the average of five contiguous R–R intervals after spontaneous steady-state heart rate was reached under control conditions or after perfusion with each drug concentration.

2.2. Right ventricular strips

Small ventricular strips were dissected out from neonatal (3–4 days) and adult rat hearts. Besides electrical activity, in adult rat right ventricular strips contraction was recorded after fixing one end of the preparation to the bath chamber and the other to a force transducer. In all cases, strips were continuously perfused (10 ml/min) with normal Tyrode solution and stimulated with 2 ms pulses at a frequency of 1.25 Hz. Standard microelectrode technique was used to record action potentials (Alvarez et al., 1981). Maximal rate of depolarization of the action potential was obtained by differentiating the microelectrode amplifier output with a RC circuit (time constant = 50 μs).

2.3. Isolation of adult ventricular cardiomyocytes

Single rat ventricular cells were dispersed by a collagenase/trypsin enzymatic method similar to that previously described for rabbit heart cells (Alvarez et al., 1992). In brief, rat hearts were cannulated through the aorta and retrogradly perfused with a physiological solution for 5 min (mM): NaCl, 112.0; KCl, 2.5; $CaCl_2$, 1.8; $MgCl_2$, 0.6; HEPES, 10.0; glucose, 5.0; Na_2-pyruvate 5.0; pH 7.4. Thereafter, the hearts were perfused with a low-Ca^{2+} solution ($CaCl_2$ 30 μM) for 5 min. The hearts were then perfused for 30 min with the same solution containing collagenase (Boehringer Mannheim) and trypsine (Merck) at 1.5 mg/ml and 0.4 mg/ml respectively and supplemented with minimum essential medium (1 μl/ml MEM; Sigma), creatine (5 mM) and taurine (20 mM). At the end of this period, the right ventricle was cut off and gently shook in the same solution without enzymes. Isolated myocytes were kept in this physiological solution (Ca^{2+} = 1 mM) at room temperature (21–23°C) and used within 6–8 h.

2.4. Neonatal cell culture

Single ventricular cardiomyocytes were enzymatically dispersed from newborn (2–3 days) Wistar rat hearts as previously described by Pucéat et al. (1995). Hearts were removed from anaesthetized animals and the ventricles minced and then treated in repeated 5-min incubations at room temperature with 0.1% (w/v) trypsin (Difco) in a Ca- and Mg-free PBS added with 0.4% ethylenediaminotetraacetic acid (EDTA) and adjusted to pH 7.4. Myocytes and fibroblasts were separated by differential attachment to plastic. More specifically, after incubation for 30 min at 37°C, 95% of the adherent cells were fibroblasts. Myocytes mainly present in the supernatant were spun down. The cell pellet was resuspended in Eagle's minimum essential medium (MEM) supplemented with antibiotics, 10% newborn calf serum (Gibco), 5.5 mM glucose and 25 mM $NaHCO_3$. Myocytes were plated at a density of 10^5 cells/cm^2 on glass coverslips. To prevent fibroblast proliferation, 1 μM arabino-furanosyl-cytosine (Sigma) was added 48 h after plating and subsequently every 48 h. The cells were serum-starved for 24 h before any experiment. Experiments were performed on cells after 4–5 days in culture.

2.5. Patch-clamp recordings

To record T- and L-type Ca^{2+} currents (I_{CaT} and I_{CaL} respectively), the 'whole cell' variant of the patch-clamp method was used (Hamill et al., 1981; Rubio et al., 1993). Na^+ and K^+ currents were completely blocked by tetrodotoxin (extracellular, 50 μM) and Cs (intracellular and extracellular; see below), respectively. Currents were evoked by 200-ms voltage-clamp pulses to −40 mV (I_{CaT}) or to +10 mV (I_{CaL}) from a holding potential of −90 mV at 0.125 Hz. Current amplitude was estimated as the difference between peak inward current and the current level at the end of the 200-ms pulse. Membrane capacitance (Cm) was estimated by applying 10 ms/2 mV hyperpolarizing pulses from a holding potential of −80 mV. Capacitive spikes were fitted to a simple exponential and Cm was calculated according to:

$$Cm = \tau_m \cdot I_0 / V_m (1 - I_{ss}/I_0)$$

where τ_m is the membrane time constant, I_0 is the maximal amplitude of the capacitive current spike, I_{ss} is the current at the end of the 10 ms pulse (steady-state) and V_m is the amplitude of the voltage step (2 mV).

Pulse generation and data acquisition were done, using computer facilities and ACQUIS1 software (version 2.0, CNRS License, France). Current were filtered at 10 kHz and digitized at 20 kHz.

The composition of the extracellular solution for single cells was (mM): NaCl, 30; TEACl, 110; $CaCl_2$, 5.4; $MgCl_2$, 2; glucose, 10; HEPES, 10; pH was adjusted to 7.4 at 21°C. The pipette ('intracellular') solution contained (mM): CsCl, 100; tetraethylammonium-Cl (TEA), 20; Na_2GTP, 0.4; Na_2ATP, 5; ethyleneglycol-bis-(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid (EGTA), 10; HEPES, 10; pH was adjusted to 7.2 with CsOH. Oxodipine and elgodipine were dissolved in absolute ethanol to obtain a 10^{-2} M stock solution.

2.6. Statistical evaluation

When it was possible, results were analysed by the Student's *t*-test and are expressed as means and standard deviations. The criterion for significance was $P < 0.05$.

3. Results

Under our experimental conditions, we first checked the relative potencies of oxodipine and elgodipine on contraction and electrical activity by performing experiments on vascular smooth muscle and cardiac ventricular multicellular preparations.

3.1. Effects of oxodipine and elgodipine on aortic and ventricular contractions

Both oxodipine and elgodipine exerted a concentration-dependent depression of the KCl-induced contraction of rabbit aortic rings. Elgodipine was, however, about three orders of magnitude more potent than oxodipine. Concentration-response curves, based on the Hill function, were fitted to the experimental data obtained after applying drug concentrations from 10^{-4} to 10^2 μM; the results are presented in Table 1. As can be seen, both compounds depressed the aortic contraction with greater potency than the ventricular muscle one. However, the vascular selectivity index (IC_{50} ventricular/IC_{50} aorta) was greater for elgodipine (≈ 680) than for oxodipine (≈ 72).

Although our main interest was to study the effects of oxodipine and elgodipine on voltage-operated Ca^{2+} channels, we also checked the effects of these compounds on the aortic contraction controlled by receptor-operated channels. Oxodipine (10 μM) decreased the norepinephrine-

Table 1
Estimated IC_{50} values (in μM) of the inhibitory effects of oxodipine and elgodipine on the KCl-induced contraction of rabbit aorta and on the force of contraction of rat ventricular muscle

Tissues	Oxodipine				Elgodipine			
	n	IC_{50}	Range[a]	*N*	*n*	IC_{50}	Range[a]	*N*
Aorta	10	0.12	0.06–0.23	0.59	10	0.00065	0.0005–0.0009	0.48
Ventricular muscle	12	8.6	5.2–14.9	0.46	12	0.44	0.20–0.94	0.45

n: Number of preparations.
N: Hill number.
[a]95% confidence limits.

induced contraction of rabbit aorta by 56.5 ± 2.9% ($n = 5$). Elgodipine was, however, without effects as already reported by Chulia et al. (1995).

3.2. Effects of oxodipine and elgodipine on cardiac electrical activity

In Langendorff-perfused rat hearts, oxodipine ($n = 6$) and elgodipine ($n = 5$) decreased the spontaneous sinus rate in a concentration-dependent manner. Concentrations as low as 1 nM of oxodipine and elgodipine significantly increased the R–R interval. At 10 μM, R–R interval was increased by more than 200 ms by both oxodipine and elgodipine. In this experimental series, the ventricular force of contraction (spontaneous rate) was decreased by both drugs with an IC_{50} of 0.58 μM (range 0.1–3.2; Hill coefficient, $N = 0.4$) for oxodipine and 0.81 μM (range 0.22–2.8; $N = 0.4$) for elgodipine.

When applying 10 μM oxodipine or elgodipine on neonatal and adult rat ventricular strips stimulated at 75/min, the only characteristic affected was the action potential duration. In both neonatal and adult ventricular preparations, as expected from a Ca^{2+} channel antagonist, the decrease in action potential duration was more marked at the 0 mV repolarization level (D_0). Table 2 summarizes the results. Oxodipine was more effective in decreasing D_0 in neonatal ventricle while elgodipine was more effective on D_0 in adult ventricle. Both drugs were less potent on action potential duration at −60 mV (D_{-60}) such as oxodipine even slightly increased D_{-60} in some preparations (mean increase +4.3 ± 5.0% (ns) over control action potential duration). Note that neonatal ventricular cells displayed a less negative resting potential and a longer action potential duration than adult ventricular cells.

3.3. Effects of oxodipine and elgodipine on Ca^{2+} currents in neonatal and adult rat ventricular cardiomyocytes

In rat cultured neonatal cardiomyocytes, I_{CaT} evoked from −90 to −40 mV had a mean density of 1.4 ± 0.3 pA/pF (Cm = 35.2 ± 2.1 pF; $n = 36$). The time-to-peak of I_{CaT} was 4.8 ± 0.3 ms, inactivation time course was best fitted by a single exponential with a time constant of 22.2 ± 7.8 ms. I_{CaL} evoked from −90 to +10 mV had a density of 12.9 ± 1.9 pA/pF (Cm = 35.6 ± 3.4 pF; $n = 44$). Its time-to-peak was 5.7 ± 0.7 ms. Inactivation time course of I_{CaL} in these cells was best fitted by two exponentials whose time constants were 7.3 ± 1.1 ms (τ_1) and 39.5 ± 4.8 ms (τ_2). In adult cardiomyocytes I_{CaL} density was 8.78 ± 1.1 pA/pF (Cm = 103.4 ± 20.3 pF; $n = 25$). Time-to-peak I_{CaL} was 5.4 ± 0.5 ms, and τ_1 and τ_2 were 8.3 ± 2.4 ms and 34.5 ± 6.6 ms respectively. Contributions of the fast and slow components to the total amplitude of I_{CaL} elicited in both cultured neonatal and adult cardiomyocytes were variable from cell to cell and ranged from 60 to 70% for the fast component and from 30 to 40% for the slow component. These values are similar to those previously reported under similar experimental conditions (Furukawa et al., 1992; Scamps et al., 1990) despite I_{CaL} density in adult cardiomyocytes is smaller than in neonatal cardiomyocytes (compare with Gomez et al., 1994). Both I_{CaT} and I_{CaL} were present in 80% of the neonatal cells studied. No neonatal cells were found lacking I_{CaL}.

As discussed below, it is difficult to establish pure current/voltage relationships for I_{CaT}. However in the few cells in which these relations were established, the contribution of I_{CaT} to the total current at +10 mV (test pulse for I_{CaL}) was only 10% or less (compare with Alvarez and Vassort, 1992; Balke et al., 1992).

Fig. 1 illustrates the effects of oxodipine and elgodipine on I_{CaT} and I_{CaL} elicited in neonatal cardiomyocytes and on I_{CaL} in adult cardiomyocytes under steady state conditions, at drug concentrations that inhibit Ca^{2+} currents by more than 50%. Table 3 summarizes IC_{50} values of the inhibitory action of both dihydropyridines on I_{CaT} and I_{CaL} of cultured neonatal cardiomyocytes and on I_{CaL} of adult cardiomyocytes. Elgodipine was five times less potent on I_{CaT} than oxodipine. Both compounds were less effective on adult I_{CaL} than on neonatal I_{CaL}.

Table 2
Alterations of the action potential characteristics of neonatal and adult rat ventricular muscle induced by 10 μM oxodipine or elgodipine

Tissues/Drugs	n	RP	OS	V_{max}	D_0	D_{60}
Neonate control	12	−74.8 ± 5.8 (mV)	21.3 ± 5.2 (mV)	117.3 ± 9.0 (V/s)	26.2 ± 5.7 (ms)	79.3 ± 7.9 (ms)
+Oxodipine%	6	0	0	0	−53.3 ± 8.6[a]	−8.2 ± 3.6
+Elgodipine%	6	0	0	0	−30.0 ± 6.0[a]	+10.3 ± 3.1[a]
Adult control	15	−85.4 ± 3.34 (mV)	−25.4 ± 4.0 (mV)	126.0 ± 8.5 (V/s)	8.6 ± 2.3 (ms)	28.1 ± 3.4 (ms)
+Oxodipine%	8	0	0	0	−20.0 ± 7.4[a]	+4.3 ± 5.0
+Elgodipine%	7	0	−2.7 ± 1.0	0	−37.2 ± 4.8[a]	−22.8 ± 7.8[a]

n: Number of preparations.
RP: resting potential.
OS: overshoot.
V_{max}: maximum rate of depolarization.
D_0 and D_{-60}: action potential duration at 0 and −60 mV, respectively.
In the presence of the drugs, values are expressed as percent decrease (−) or increase (+) from controls. [a]$P < 0.05$.

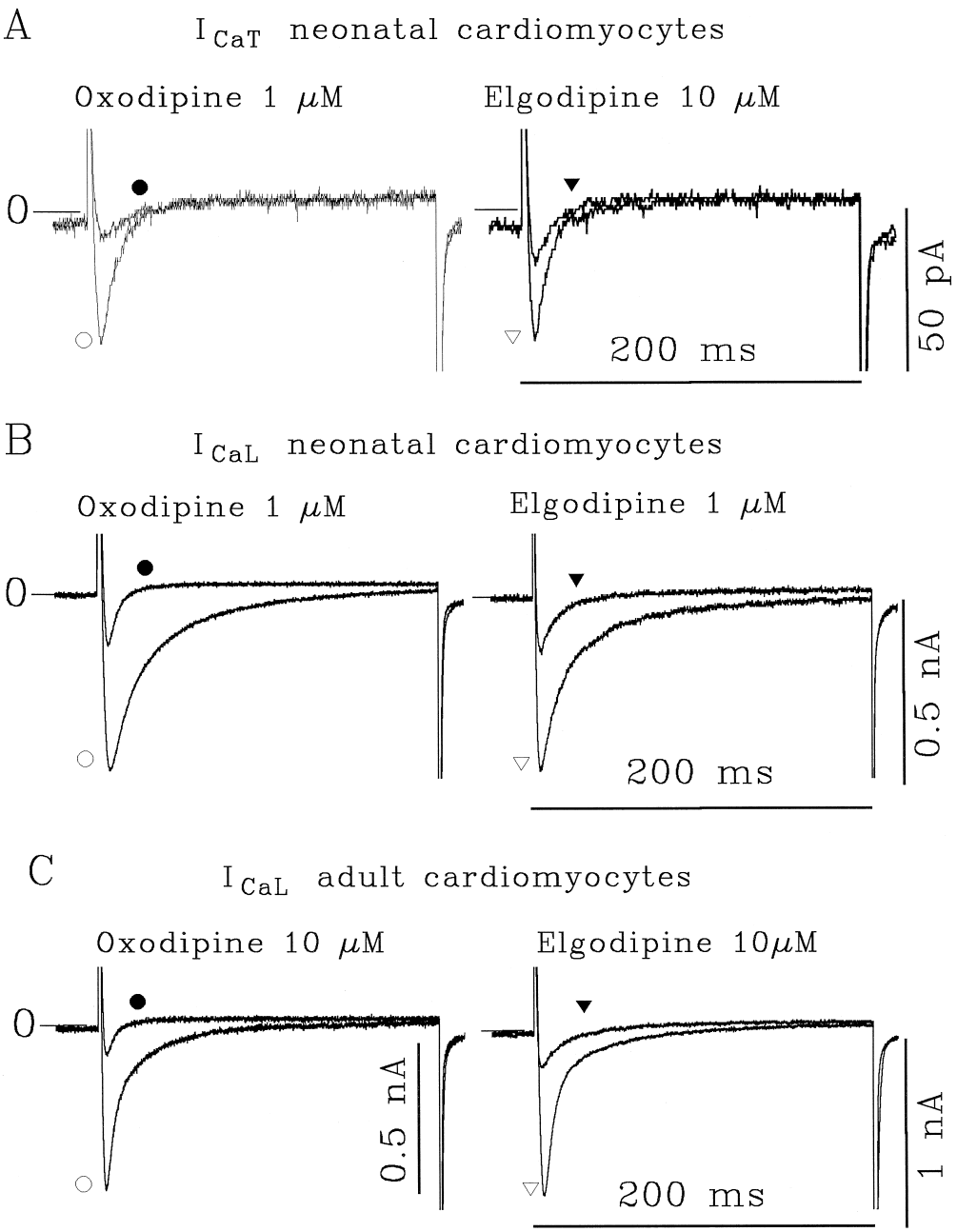

Fig. 1. Effects of oxodipine and elgodipine on Ca^{2+} currents (I_{CaT} and I_{CaL}) in single neonatal and adult rat ventricular cardiomyocytes. A and B: Effects of the two drugs on I_{CaT} and I_{CaL} elicited in cultured neonatal cardiomyocytes. C: Effects of the two drugs on I_{CaL} elicited in adult cardiomyocytes. Currents were evoked from a holding potential of −90 mV to −40 mV (I_{CaT}) or to +10 mV (I_{CaL}). Filled symbols: currents recorded in the presence of drug at the indicated concentration.

Oxodipine and elgodipine at concentrations near their respective IC_{50} values also decreased the inactivation time constants of both I_{CaT} and I_{CaL}. However, inactivation time constant of I_{CaT} in neonatal cardiomyocytes was only slightly decreased from 23.6 ± 7.8 ms to 18.3 ± 5.3 ms by 1 μM oxodipine ($n = 9$; ns) and from 22.4 ± 5.5 ms to 16.4 ± 6.5 ms by 10 μM elgodipine ($n = 10$; ns). Fast and slow I_{CaL} inactivations were significantly accelerated by oxodipine (1 μM): τ_1 was decreased from 7.3 ± 1.1 ms to 5.1 ± 1.7 ms and τ_2 was decreased from 38.0 ± 4.2 ms to 18.4 ± 3.5 ms ($n = 9$; $P < 0.05$). However, elgodipine was without effects on the inactivation time course of I_{CaL} in these cells: τ_1 and τ_2 were 10.2 ± 2.6 ms and 39.0 ± 8.0 ms in control conditions and 9.6 ± 2.5 ms and 36.7 ± 11.0 ms ($n = 9$; ns), respectively in the presence of 1 μM elgodipine.

In adult cardiomyocytes, however, both drugs significantly decreased the inactivation time constants of I_{CaL}: oxodipine (10 μM) decreased τ_1 and τ_2 from 9.16 ± 2.6 ms and 35.0 ± 7.4 ms to 4.9 ± 1.8 ms and 19.6 ± 6.4 ms,

Table 3
Estimated IC_{50} values (in μM) of the inhibitory effects of oxodipine and elgodipine on I_{CaT} and I_{CaL} in neonatal and adult rat cardiomyocytes held at the same membrane potential (−90 mV)

Current type	Oxodipine			Elgodipine		
	IC_{50}	Range[a]	*N*	IC_{50}	Range[a]	*N*
I_{CaT} neonate	0.41	0.36–0.48	1.09	2.18	1.6–2.93	1.01
I_{CaL} neonate	0.24	0.12–0.51	0.77	0.33	0.15–0.70	0.72
I_{CaL} adult	4.15	3.29–5.33	0.59	8.54	5.2–14.8	0.54

N: Hill number.
Values obtained from 5–10 cells at each concentration in the range from 10^{-2} to 10^{2} μM for each drug.
[a]95% confidence limits.

respectively ($n = 12$; $P < 0.05$). Elgodipine (10 μM) decreased τ_1 and τ_2 from 7.4 ± 2.0 ms and 34.2 ± 5.7 ms to 4.8 ± 1.0 ms and 21.4 ± 7.0 ms, respectively ($n = 11$; $P < 0.05$). The amplitude of the fast component was usually more decreased by both drugs (by 70 to 85%) than the amplitude of the slow component (by 35 to 50%).

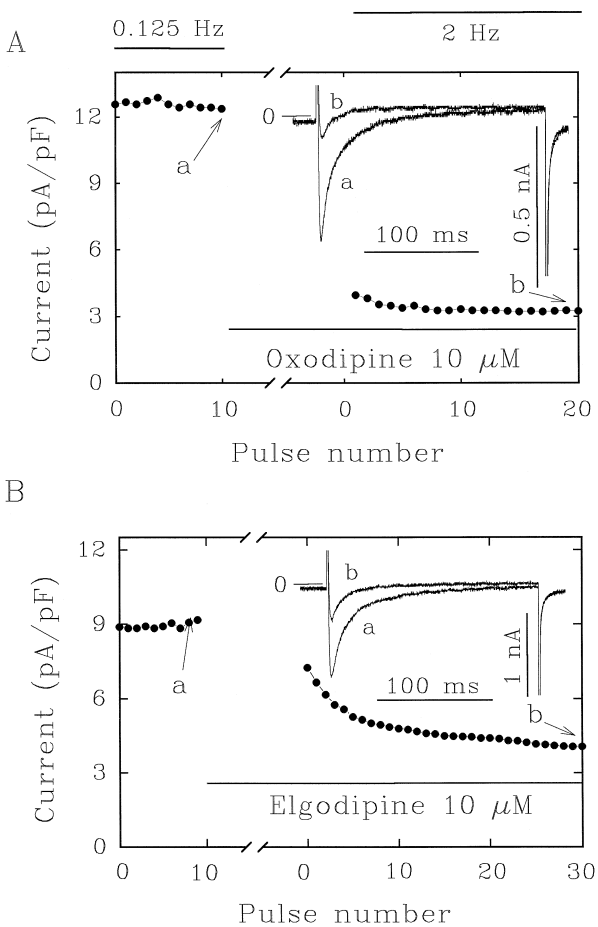

Fig. 2. Tonic and use-dependent effects of oxodipine and elgodipine on I_{CaL} in neonatal (A) and adult (B) cardiomyocytes. The rate-dependent effects were qualitatively similar in neonatal and adult cardiomyocytes. Bars on the top of the figure indicate the stimulation frequencies. Points represent peak current densities as a function of pulse number. Oxodipine induced a tonic block of I_{CaL} while elgodipine induced both a weak tonic and a marked use-dependent block of I_{CaL}. Stimulation was stopped for 3 min before the drug was added.

Table 4
Tonic and use-dependent inhibition of oxodipine and elgodipine on I_{CaT} and I_{CaL} in neonatal and adult rat cardiomyocytes

Drug/current	Use-dependent block	Tonic block	Total block
Oxodipine/I_{CaT} neonate	11.2 ± 2.4	88.8 ± 2.4	80.4 ± 2.4
Elgodipine/I_{CaT} neonate	78.6 ± 5.3	21.4 ± 5.3	67.0 ± 4.0
Oxodipine/I_{CaL} adult	10.6 ± 3.1	89.4 ± 3.1	71.8 ± 2.4
Elgodipine/I_{CaL} adult	59.2 ± 3.7	40.8 ± 3.7	61.4 ± 4.9

Tonic and use-dependent blocks are expressed as percent of total block. The latter is expressed as percent of control Ca^{2+} current elicited at 2 Hz.
Oxodipine and elgodipine were both used at a concentration of 1 μM on I_{CaT} and 10 μM on I_{CaL}. $n \geq 4$ in each condition.

3.4. Tonic and use-dependent effects of oxodipine and elgodipine on I_{CaT} and I_{CaL}

To study the frequency-dependent effects of oxodipine and elgodipine we applied the following voltage-clamp

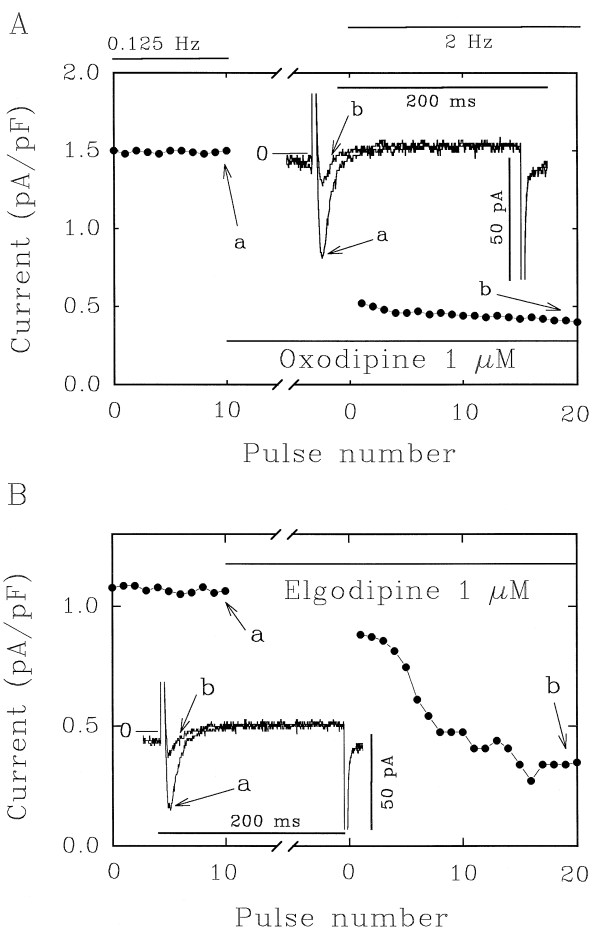

Fig. 3. Tonic and use-dependent effects of oxodipine and elgodipine on I_{CaT} in single cultured neonatal cardiomyocytes. The stimulation protocol was the same as in Fig. 2, except that voltage-clamp pulses were to −40 mV instead of to +10 mV. The behaviour of both drugs on I_{CaT} was the same as for I_{CaL}.

protocol. Under control conditions a train of 10 pulses, to −40 mV for I_{CaT} or to +10 mV for I_{CaL}, was applied at a frequency of 0.125 Hz from a holding potential of −90 mV. Stimulation was then stopped, and the membrane potential was held at −90 mV for 3 min during which the cell was superfused with either the control solution or a drug-containing solution. Stimulation was then reinitiated at the same or a higher frequency (2 Hz). Tonic block was estimated as the difference between peak control Ca^{2+} current (T or L) and peak Ca^{2+} current at the first pulse after drug superfusion. Use-dependent blockade was considered to be the difference between peak Ca^{2+} current for the first and the 20th (or 30th) pulses after drug exposure. Ca^{2+} currents can undergo positive or negative staircases following an abrupt increase in stimulation frequency (see for review, McDonald et al., 1994). For this reason, frequency-dependent changes of Ca^{2+} currents under control conditions were taken into account for estimating the use-dependent action of the drugs. Total block was estimated as the difference between peak Ca^{2+} currents at the 20th (or 30th) pulse under control and drug-containing conditions. At the end of each protocol a washout period of at least 10 min was allowed to occur to reach 'steady-state' Ca^{2+} current value. In the case of I_{CaT}, complete washout was considered if this current recovered its control amplitude (I_{CaT} does not show run-down). For I_{CaL}

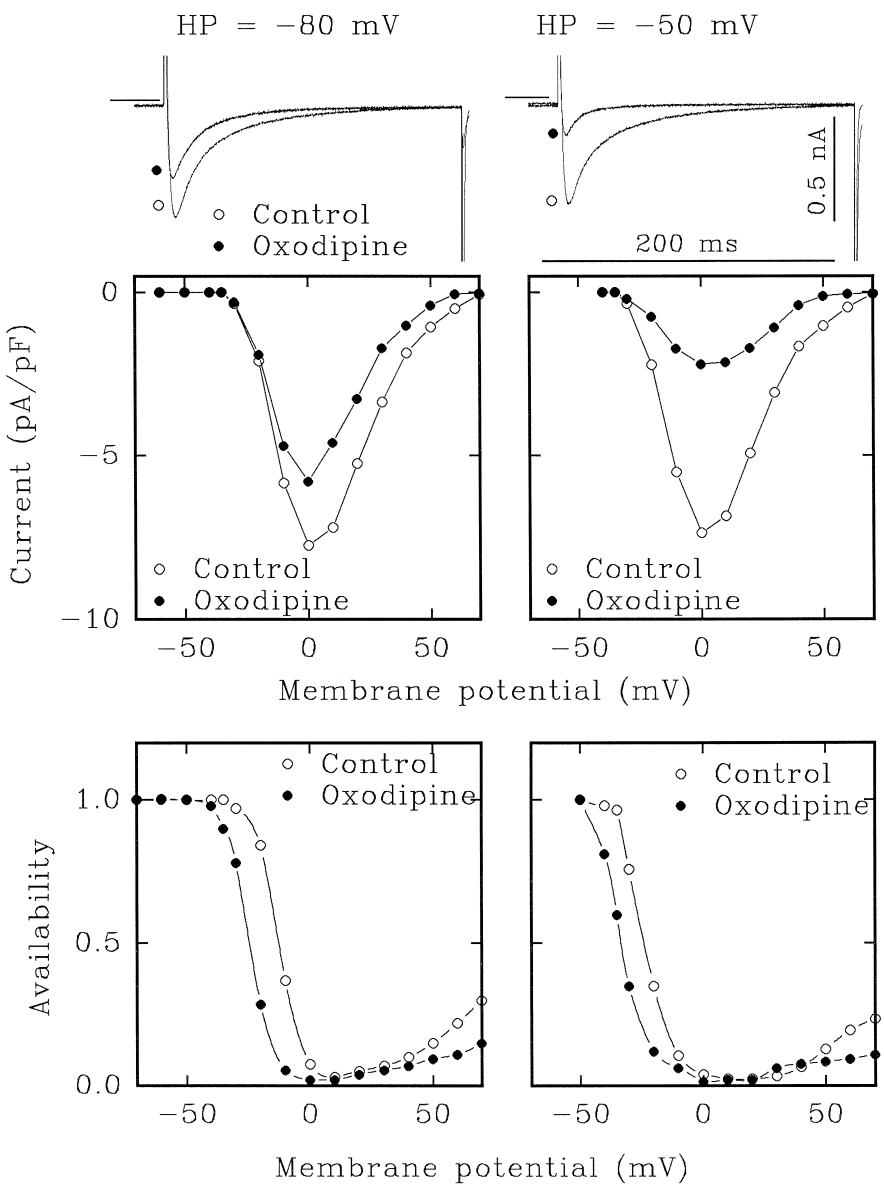

Fig. 4. Voltage-dependent effects of oxodipine on I_{CaL}. Top panel: oxodipine (1 μM) induced inhibition of I_{CaL} elicited by a +10 mV depolarization was enhanced when the cell was clamped from a reduced holding potential (−50 instead of −90 mV). Middle panel: current/voltage relationships obtained from the two holding potentials. Bottom panel: availability curves for I_{CaL} obtained from the two different holding potentials. Note the leftward shift in I_{CaL} availability induced by oxodipine. Adult ventricular myocyte.

complete washout was considered if the current recovered its control amplitude or its control frequency response pattern, if a run-down was evident.

An example of the frequency-dependent effects of 10 μM oxodipine and 10 μM elgodipine on I_{CaL} is illustrated for neonatal (Fig. 2A) and adult (Fig. 2B) cardiomyocytes. The first I_{CaL} elicited after the rest period in the presence of oxodipine was greatly reduced with respect to control indicating a strong tonic block (68%). With further stimulation at high frequency, I_{CaL} was only slightly further reduced. The use-dependent block then represented an additional 20% decrease of I_{CaL} (taking into account the 5% positive staircase in control conditions) but only a 7% of total block. Elgodipine (Fig. 2B) showed a weaker tonic block (20%) and an additional 45% use-dependent inhibition of I_{CaL} that represented 56% of total block. Tonic and use-dependent blocks, relative to total block of I_{CaL} were similar whether recorded on neonatal or adult cardiomyocytes. However as noted above, both drugs were more potent on steady-state I_{CaL} (0.125 Hz) in neonatal cardiomyocytes. Therefore for sake of simplicity, Table 4 summarizes the results obtained on I_{CaL} of adult cardiomyocytes using 10 μM of either drug. It is to note here that in three cells in which I_{CaL} was evoked at a high rate

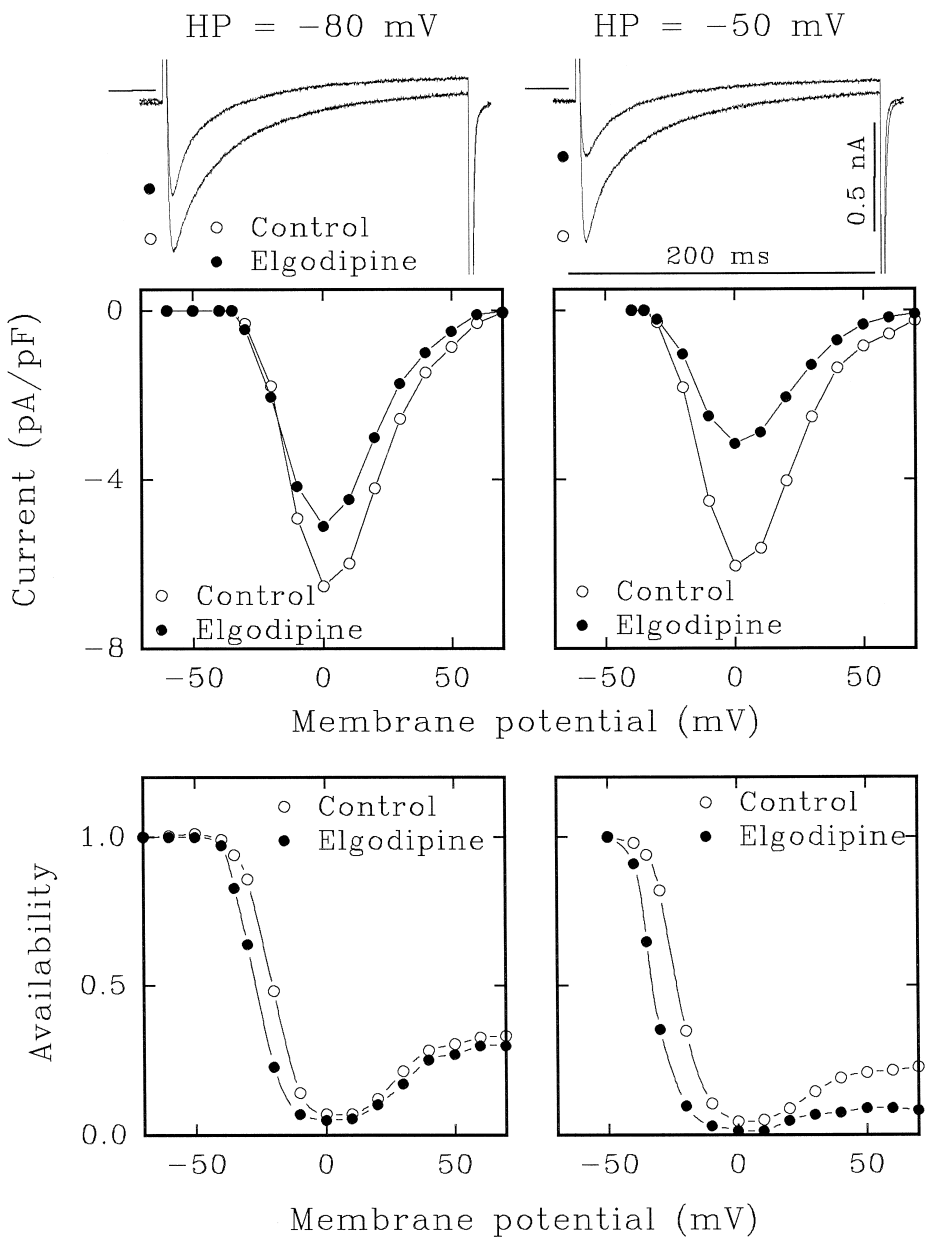

Fig. 5. Voltage-dependent effects of elgodipine on I_{CaL}. Top panel: Elgodipine (1 μM)-induced to +10 mV from two different holding potentials (−90 and −50 mV). An inhibition of I_{CaL} elicited by a +10 mV depolarization was enhanced when the cell was clamped from a reduced holding potential −50 instead of −90 mV. Middle panel: current/voltage relationships obtained from the two holding potentials. Bottom panel: availability curves for I_{CaL} obtained from the two different holding potentials. Note the leftward shift in I_{CaL} availability induced by elgodipine. Adult ventricular myocyte.

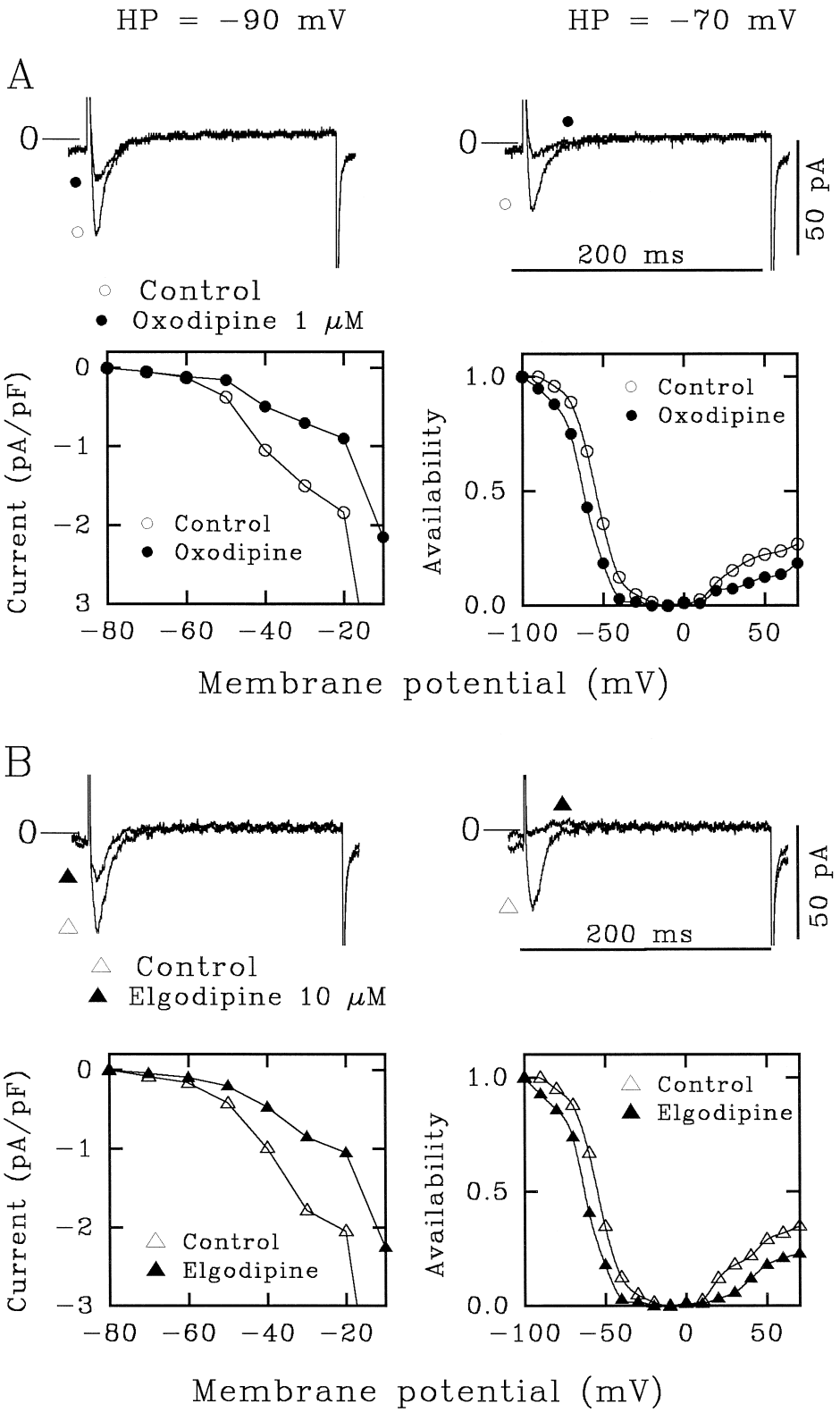

Fig. 6. Voltage-dependent effects of oxodipine (panel A) and elgodipine (panel B) on I_{CaT} in cultured neonatal cardiomyocytes. At the top of each panel are shown selected traces of I_{CaT} evoked by depolarizations to −40 mV from holding potentials of −90 mV or −70 mV. Both drugs induced an enhanced inhibition when cells were clamped from the reduced holding potential. At the bottom of each panel are presented the current/voltage relationships in the range −80 to −20 mV and the respective availability curves. Note there is I_{CaL} activation threshold in the potential is above −30 mV. Both drugs induced a slight leftward shift in the availability curves.

from a holding potential of −80 mV, the elgodipine-induced decrease of I_{CaL} reached 77.6 ± 5.1%.

Oxodipine and elgodipine inhibited I_{CaT} following the same frequency-dependent patterns as showed on I_{CaL}. Oxodipine induced essentially a tonic block and elgodipine a use-dependent block of I_{CaT}. However, because I_{CaT} always showed a negative staircase pattern following an abrupt increase in stimulation frequency under control conditions in neonatal cells, use-dependent effects of both drugs on I_{CaT} appeared diminished (Table 4). In the cells exemplified in Fig. 3, the tonic block of I_{CaT} induced by 1 μM oxodipine and elgodipine was 64% and 16%, respectively. Use-dependent block by oxodipine and elgodipine further decreased I_{CaT} by 25% and 61%, respectively; i.e., 13% and 82% of total blocks, respectively.

3.5. Voltage-dependent effects of oxodipine and elgodipine on Ca^{2+} currents

Current/voltage relationships and availability curves were determined by standard double-pulse protocols. From a holding potential of −90 mV at a frequency of 0.125 Hz, a 200-ms prepulse to various membrane potentials (−90 to +70 mV) was followed by a test pulse, to +10 mV for I_{CaL} or to −40 mV for I_{CaT}, after a 5-ms interval at −90 mV. Availability curves were fitted from −90 to 0 mV for I_{CaL} and from −90 to −50 mV for I_{CaT} by a Boltzman distribution of the type: $f_\infty = 1/1 + \exp[(V_m - V_f)/k]$, where V_f is the potential for half-inactivation and k the slope factor. In both neonatal and adult cardiomyocytes, I_{CaL} inhibition by oxodipine and elgodipine were more marked at positive potentials, reflecting a voltage-dependent action of these drugs on the L-type Ca^{2+} channel. Consequently, current/voltage and availability curves were shifted leftwards by both drugs. In adult ventricular cardiomyocytes, oxodipine and elgodipine (1 μM) shifted V_f of I_{CaL} from −19.8 ± 2.7 mV to −27.6 ± 1.3 mV ($n = 9$; $P < 0.05$) and from −19.4 ± 1.8 mV to −27.3 ± 1.1 mV ($n = 8$; $P < 0.05$), respectively when the cells were clamped at a holding potential of −90 mV (Figs. 4 and 5). These voltage-dependent effects were also evident as an enhanced inhibition of I_{CaL} by both drugs when adult ventricular cardiomyocytes were clamped from a more less negative holding potential. The inhibitory actions of oxodipine and elgodipine (1 μM) were enhanced when cells were held at −50 mV (Figs. 4 and 5). In these conditions, V_f was shifted to −34.0 ± 1.6 mV and to −31.1 ± 2.3 mV by oxodipine ($n = 6$) and elgodipine ($n = 6$), respectively. Note that a concentration of 1 μM is below the IC_{50} for inhibition of I_{CaL} of adult cardiomyocytes by oxodipine and elgodipine (see Table 3). In this experimental series, I_{CaL} evoked at +10 mV from a holding potential of −90 mV was inhibited by 46.3 ± 6.8% and 30.0 ± 18.0% by 1 μM oxodipine and elgodipine, respectively. When the holding potential was set at −50 mV, I_{CaL} was inhibited by 67.2 ± 5.2% and 50.5 ± 14.0% by oxodipine and elgodipine, respectively. At 10 μM oxodipine and elgodipine inhibited I_{CaL} by 63.5 ± 5.7% ($n = 5$) and 53.0 ± 17.0% ($n = 5$), respectively from a holding potential of −90 mV while from a holding potential of −50 mV, the two drugs inhibited I_{CaL} by 90.0 ± 3.4% and 77.0 ± 13.0% respectively. Qualitatively similar results were obtained in four cultured neonatal cardiomyocytes (data not shown).

It is difficult to routinely establish current/voltage curves for I_{CaT} since changes in the holding potential or the use of I_{CaL} blockers give poor estimates of pure whole cell I_{CaT} (Alvarez and Vassort, 1992). Thus, in some neonatal cardiomyocytes, we monitored the current/voltage relation up to −30 mV (usually the threshold for I_{CaL} is positive to −30 mV) together with the availability curve. Under these experimental conditions, both oxodipine and elgodipine exhibited voltage-dependent actions on I_{CaT} (Fig. 6). The effects were, however, less marked than those observed on I_{CaL}: V_f for I_{CaT} was shifted to more hyperpolarized potentials from −55.8 ± 1.7 mV to −60.5 ± 1.3 mV and from −55.7 ± 1.0 mV to −60.0 ± 1.6 mV ($n = 4$) by oxodipine (1 μM) and elgodipine (10 μM), respectively. The effects of both drugs on I_{CaT} evoked from two different holding potentials were studied in three cells. Oxodipine (1 μM) and elgodipine (10 μM) inhibited I_{CaT} evoked from −90 mV by 65.0 ± 5.0% and 81.0 ± 4.2%, respectively and by 77.8 ± 2.5% and 91.6 ± 2.9% when evoked from −70 mV (not shown).

4. Discussion

The present results show that the two dihydropyridines, oxodipine and elgodipine used in the same concentration range block both the T- and L-type Ca^{2+} currents in neonatal and adult rat cardiomyocytes. Oxodipine and elgodipine were both more potent to inhibit I_{CaL} in neonatal than in adult cells, whereas oxodipine was more potent than elgodipine on I_{CaT} recorded in neonates. The mechanisms of action of the two drugs are different since oxodipine behaves essentially as a tonic blocker of both current types, while elgodipine exhibits a smaller tonic block and a prominent use-dependent block. Both compounds also demonstrate an increased inhibition potency of I_{CaT} and I_{CaL} when either current is evoked from reduced holding potentials. This voltage-dependent effect might be the basis of the vascular selectivity of both dihydropyridines.

As already reported (Tejerina et al., 1987, 1992; Tamargo and Tejerina, 1989; Tamargo et al., 1991; Chulia et al., 1995), we found that oxodipine and elgodipine behave as classical Ca^{2+} channel antagonists, blocking the contraction of cardiac and vascular smooth muscles and decreasing the spontaneous sinus rhythm. However both drugs, and particularly elgodipine, are more potent inhibitors of smooth muscle contraction. In addition, oxodipine also blocked the norepinephrine-induced contraction in aortic rings, suggesting that it also interacts with receptor-operated channels. It is to note that while the IC_{50} value

obtained for elgodipine is comparable to those previously reported, our IC_{50} estimate for oxodipine (0.12 μM) is much larger (0.0009 to 0.008 μM; Tejerina et al., 1987, 1992; Tamargo and Tejerina, 1989; Tamargo et al., 1991; Chulia et al., 1995). Different preparations and/or different experimental conditions could explain this discrepancy. The vascular selectivity indexes we estimated were respectively 680 and 72 for elgodipine and oxodipine, indicating a stronger vascular selectivity of elgodipine as usually reported. These values are in the same order of magnitude of those reported for other second generation dihydropyridines (for example, nitrendipine, felodipine, niludipine; see Nayler, 1988). Comparison of our results with other studies on oxodipine and elgodipine is not possible since vascular selective indexes for these dihydropyridines are not currently reported. However, Rakotoarisoa et al. (1994) measuring the displacement by oxodipine and elgodipine of the isradipine binding to cardiac and vascular membranes, found that oxodipine was more vascular selective than elgodipine.

Oxodipine and elgodipine decreased action potential duration in both neonatal and adult ventricular strips, without effects on the resting potential or on the maximal rate of depolarization. This effect was more marked on action potential duration at 0 mV level (D_0), as expected from an antagonist action on Ca^{2+} channels. We should mention that some correlation exists between the decrease of action potential duration and the decrease of contractile force of adult ventricular strips by oxodipine and elgodipine. Elgodipine was slightly more potent than oxodipine in decreasing D_0 of action potential and the contractile force. However, it was not possible to correlate this effect with the decrease in I_{CaL} of adult cardiomyocytes since oxodipine was found to be more potent than elgodipine in inhibiting I_{CaL} (see below). The present experiments do not allow us to suggest other possible actions to explain this lack of correlation. Nevertheless, it should be considered that contrary to what occurs with elgodipine, oxodipine had no significant effects on action potential duration at −60 mV. It thus allows for a lesser decrease of contractile force since there exists a direct relationship between action potential duration and contractile force (Bers, 1991).

It is interesting to note that oxodipine was more potent than elgodipine in decreasing D_0 of neonatal ventricular action potential and that both drugs inhibited equally I_{CaL} in neonatal cardiomyocytes. The specificity of action of oxodipine and elgodipine on D_0 could be explained by the more potent inhibitory effect of I_{CaT} by oxodipine (Table 3). This result suggests a significant role of I_{CaT} in determining action potential duration, at the plateau level, in neonatal cardiomyocytes. Besides the well-known reduced K^+ permeability in neonatal cardiomyocytes (Sperelakis and Haddad, 1995), the existence of a prominent 'window' current for I_{CaT} (Vassort and Alvarez, 1994; Alvarez et al., 1996) at positive potentials could be of importance in determining action potential duration in these cells.

Oxodipine and elgodipine decreased I_{CaL} in neonatal and adult cardiomyocytes but they were at least one order of magnitude more potent on I_{CaL} in neonatal cardiomyocytes (Table 3). It is interesting to note that I_{CaL} density was larger in neonatal than in adult cardiomyocytes (12.9 pA/pF vs. 8.78 pA/pF; see also Cohen and Lederer, 1988). We have no definite explanation for the different potencies in neonatal and adult cells but it should be remembered that Ca^{2+} channel properties (or probably expression of channel isoforms) change during development (e.g., Cohen and Lederer, 1988; Osaka and Joyner, 1991; Huynh et al., 1992). In fact, T-type Ca^{2+} channels are expressed in neonatal but not in adult rat cardiomyocytes (Gomez et al., 1994; present results). Expression of different L-type Ca^{2+} channel subunits and/or association of different channel subunits with cytoskeleton could probably explain this differential sensitivity of the estimated L-type Ca^{2+} current. Another difference is that while in neonatal cardiomyocytes oxodipine but not elgodipine decreased the time constants for I_{CaL} inactivation, in adult cardiomyocytes both drugs accelerated I_{CaL} inactivation time course.

There was a good correlation between the IC_{50} for inhibition of cardiac contraction and of I_{CaL} of adult cardiomyocytes by oxodipine (8.6 μM and 4.6 μM, respectively). However, the IC_{50} for the inhibition of I_{CaL} by elgodipine was $\approx$ 20 times greater than the IC_{50} for the inhibition of cardiac contraction. As noted above, it is not always simple to correlate these findings but since cardiac contraction was evoked at a higher frequency (1.25 Hz vs. 0.125 Hz in single cells), use-dependent effects of elgodipine (see below) could contribute to increase its potency. Thus, when I_{CaL} was evoked at the higher frequency (Table 4) elgodipine blockade of I_{CaL} was increased to 61%; furthermore, this effect reached 77% when the holding potential was −80 mV.

Both compounds inhibit I_{CaT} with IC_{50} in the micromolar range, thus giving support to the idea that cardiac T-type Ca^{2+} channels are not insensitive to dihydropyridines (Vassort and Alvarez, 1994; McDonald et al., 1994). Particularly, elgodipine was shown to inhibit both I_{CaT} and I_{CaL} in portal vein myocytes although it was one order of magnitude less potent on I_{CaT} (Leprêtre et al., 1994; these results). Both oxodipine and elgodipine inhibit I_{CaL} in portal vein myocytes with IC_{50} at least two orders of magnitude lower than those we found here for cardiomyocytes (Baron et al., 1994; Leprêtre et al., 1994).

The major finding of the present study is the voltage-dependent block of I_{CaT} and I_{CaL} by oxodipine and elgodipine. Blockade of both current types was enhanced when currents were evoked from reduced holding potentials. This was also reflected by a shift in the hyperpolarizing direction of the half-inactivation potential (V_f) of I_{CaL} and I_{CaT}. We should note that this shift was relatively small ($\simeq$ 8 mV for I_{CaL}) when compared to results with other dihydropyridines (typically 15–20 mV; e.g., Sanguinetti

and Kass, 1984; Uehara and Hume, 1985) a fact that could, in part, be related to the short prepulse duration (200 ms) we used. Furthermore when I_{CaL} was evoked form a reduced holding potential, V_f was further shifted leftward (15 mV). However, the shifts in V_f for I_{CaT} were similar ($\simeq 5$ mV) whether I_{CaT} was evoked from hyperpolarized (-90 mV) or depolarized (-70 mV) holding potentials although this could be due to the weak change in holding membrane potential. These evidences indicate that, as all dihydropyridines (see Nayler, 1988), oxodipine and elgodipine interact with inactivated Ca^{2+} channels. This interaction could also explain the decrease in the inactivation time constants of I_{CaT} and I_{CaL} induced by oxodipine and elgodipine.

This voltage-dependent inhibition of Ca^{2+} channels could be the basis of the high vascular selectivity of oxodipine and elgodipine. In vascular smooth muscle, resting potentials are lower than in cardiac muscle and the time during which Ca^{2+} channels are inactivated is longer than in cardiac muscle (Bolton, 1979). Thus, interaction of these dihydropyridines with inactivated Ca^{2+} channels is favoured in smooth muscle. It could be possible that cardiovascular selectivity of these dihydropyridines could also arise from differences in the molecular structures of cardiac and vascular smooth muscle Ca^{2+} channels (Rakotoarisoa et al., 1994; Welling et al., 1997). In contrary to the results, these authors found that oxodipine was more vascular selective than elgodipine. Although experimental conditions are different it could be possible that the use-dependent action of elgodipine contribute to enhance its inhibitory action on the vascular smooth muscle contraction.

One major difference between oxodipine and elgodipine effects on Ca^{2+} channels is the use-dependent behaviour: oxodipine is almost an exclusive tonic blocker of I_{CaT} and I_{CaL} while elgodipine behaves as a tonic and use-dependent blocker of both current types. From the present experiments it is not possible to elucidate the underlying mechanism of this difference in terms of structure-activity relationship. However in terms of the 'modulated receptor' hypothesis (Hille, 1977), these results reflect an interaction of oxodipine and elgodipine with rested available Ca^{2+} channels and also with inactivated Ca^{2+} channels. It could be suggested that oxodipine has a higher affinity than elgodipine for rested channels which, after drug binding, enter in an absorbed inactivated state with high drug affinity. The unblocking rate constant should then be large enough to confer this lack of use-dependent behaviour of oxodipine. Following this reasoning, resting Ca^{2+} channels should have less affinity for elgodipine, so tonic block is smaller. Also inactivated Ca^{2+} channels should have a lower affinity for elgodipine thus allowing for some recovery from block between pulses. Progressive accumulation of channels in an inactivated drug-bound state after successive pulses could account for the use-dependent behaviour of elgodipine. Here it is to note that in opposition to our results in cardiac cells, Leprêtre et al. (1994) found that elgodipine was a pure tonic blocker of I_{CaT} and I_{CaL} in portal vein myocytes. It is not simple to explain this difference although it could be speculated that the different molecular structures of portal vein and cardiac myocytes calcium channels (see above) are responsible for the tonic or use-dependent action of elgodipine on these cell types.

In conclusion, our results indicate that oxodipine and elgodipine are potent selective Ca^{2+} channel antagonists. Their vascular selectivity is attributable to the voltage-dependent action of these dihydropyridines as checked on cardiac Ca^{2+} channels; furthermore, the use-dependent inhibtion of Ca^{2+} currents by elgodipine could also contribute to confer this molecule a higher vascular selectivity.

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