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Effect of CA^{2+} Antagonists on Mechanical Function in the Neonatal Heart

MASASHI SEGUCHI, JAY M. JARMAKANI, BARBARA L. GEORGE, AND JENNIFER A. HARDING

Department of Pediatrics, Division of Cardiology, UCLA Medical Center, Los Angeles, California 90024

ABSTRACT. The effect of verapamil and diltiazem on mechanical function was studied in the isolated arterially perfused neonatal and adult rabbit heart. The negative inotropic effect of these drugs in the newborn was significantly greater than in the adult. At concentrations 10^{-7} M of verapamil and 10^{-6} M of diltiazem, resting tension was significantly increased in the newborn, but not in the adult. In both age groups, verapamil and diltiazem inhibited the positive inotropy of staircase, but did not alter the inotropic effect of hyperosmolarity (116 mM mannitol). The positive inotropy of paired electrical stimulation was eliminated by these drugs in the newborn, but not in the adult. These data suggest that the neonatal heart as compared with the adult heart is more dependent on transsarcolemmal calcium influx for contraction and transsarcolemmal calcium efflux for relaxation. (*Pediatr Res* 20: 838–842, 1986)

Abbreviations

SR, sarcoplasmic reticulum
RT, resting tension
DT, developed tension
+dT/dt (max), maximal rate of tension development
TPT, time to peak tension
½ RT, half relaxation time
PES, paired electrical stimulation

The negative inotropic effect of verapamil and diltiazem, "calcium antagonists" is attributed to a reduction of slow inward current (1–3). Several authors have reported the effect of verapamil and diltiazem on mechanical function in adult heart (4–8), however, similar data in the neonatal heart are quite sparse and controversial (9–11). Our previous data (12–14) showed that the amount of contractile calcium and SR in the newborn are significantly less than in the adult. Therefore, the newborn might be more dependent on extracellular calcium for excitation-contraction coupling than the adult. This study was designed to determine the effect of verapamil and diltiazem (Ca^{2+} channel blockers) on myocardial mechanical function in arterially perfused newborn and adult rabbit heart and will assess Ca^{2+} influx via the slow inward current in these age groups.

METHOD

Experimental preparation. Experiments were performed utilizing the isolated arterially perfused newborn (3–5 days old) and adult (6–12 months old) New Zealand White rabbit hearts. The details of these preparations have been described previously (12). In brief, the rabbit was heparinized (150 U/kg body weight) and anesthetized with sodium pentobarbital (50 mg/kg body weight). After thoracotomy, the heart was rapidly excised. We utilized the Langendorff preparation in the newborn and the septal preparation in the adult. We have studied mechanical function parameters in the newborn septal and ventricular preparations. All variables of mechanical function parameters (g/g muscle) were similar in the two preparations. The aorta in the newborn and the septal branch of the left coronary artery in the adult were cannulated with PE-50 polyethylene cannula and secured with a silk suture. The muscle was perfused with well-oxygenated Krebs-Henseleit solution at 2.5 ml/g tissue/min and stimulated at 40 beats/min. The temperature was maintained at 27 ± 0.5° C. This temperature was chosen because both mechanical function and myocardial high energy phosphate were constant for 5 h under these conditions (15, 16). Tension was measured using a Statham force transducer and a bridge amplifier (Accudata 113, Honeywell, Inc., Denver, CO). The first derivative of tension with respect to time was derived using an analog differentiator (Accudata 132, Honeywell, Inc.). The following parameters of mechanical function were monitored with a Brush 220 recorder (Gould, Cleveland, OH): DT, RT, +dT/dt (max), TPT, and ½ RT. TPT was defined as the time from the onset to the peak of developed tension; ½ RT, the time required for tension to fall to 50% of the maximal value.

The control (Krebs-Henseleit) solution contained in mM: NaCl 118, KCl 6, $CaCl_2$ 1.5, glucose 6, $MgCl_2$ 1, $NaHCO_3$ 24, and NaH_2PO_4 0.435. This solution, equilibrated with 95% O_2 and 5% CO_2, had an oxygen content of 1.9–2.2 vol% by Lex-O_2-Con (Lexington Instruments) determinations. The pH and P_{CO_2} ranged from 7.30 to 7.40 and from 35 to 45 mm Hg, respectively. In each age group, the effects of verapamil and diltiazem on mechanical function at pH 7.30 and P_{CO_2} of 40 mm Hg were not significantly different from those at pH 7.40 and P_{CO_2} 45.

Experimental protocol. Initially, the heart muscles were perfused with the control solutions for 60 min to allow for stabilization of mechanical function. During the initial 40 min of each experiment, the muscle length was adjusted so that the tension was equal to maximal tension. After this initial period, the RT, DT, and +dT/dt (max) remained stable. RT, DT, and +dT/dt (max) were expressed as gram per gram tissue and percent of control values.

Dose response. The cumulative dose response of verapamil and diltiazem on mechanical function was investigated in the newborn and adult heart. The concentrations of verapamil were 2.5×10^{-8} M, 5×10^{-8} M, 10^{-7} M, 2.5×10^{-7} M, 5×10^{-7} M, 10^{-6} M and 5×10^{-6} M, and those of diltiazem were, 2.5×10^{-8} M, 10^{-7} M, 5×10^{-6} M. Muscles were cumulatively perfused with either verapamil or diltiazem solutions following stabilization of mechanical function. The drug solutions were prepared and consisted of our control solution with the particular drug

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Address reprint requests to Jay M. Jarmakani, M.D., UCLA Medical Center, Department of Peds/Cardiology, 10833 Le Conte Avenue, Los Angeles, CA 90024.
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concentration under investigation. Each concentration was consistently perfused until the mechanical function reached a steady state.

Inotropic Interventions. The effect of several inotropic interventions was examined in the control muscles and in muscles perfused with verapamil or diltiazem. These interventions included the following.

Stair case. The newborn and adult heart muscle were initially perfused with the control solution and were paced at a rate of 40 beats/min. After stabilization of mechanical function, the stimulation rate was increased from 40 to 60 beats/min and the relative changes in the parameters of mechanical function were recorded for 10 min. At this point, the stimulation rate was returned to 40 beats/min and all parameters of mechanical function returned to control values. The muscle was then perfused with solution that contained verapamil (10^{-7} M) or diltiazem (10^{-6} M). After 40 min mechanical function reached a new steady state and again the stimulation rate was increased from 40 to 60 beats/min. The absolute and relative change of mechanical function in muscles perfused with verapamil and diltiazem were compared with those in the control muscle. The effect in the newborn was compared with that in the adult.

Hyperosmolarity. Hyperosmolar solution was obtained by adding 116 mM mannitol to the control solution. Initially, the muscle was perfused with the control solution. After mechanical function reached steady state, the perfusion was switched from the control solution to the hyperosmolar solution for 30 min, and then returned to the control solution. Mechanical function returned to control values, and perfusion was changed from control perfusate to perfusate that contained verapamil (10^{-7} M) or diltiazem (10^{-6} M). At this new steady state, mannitol was added to the solution. The effect of hyperosmolar solution in muscles perfused with verapamil and diltiazem was compared with that in the muscles perfused with the control solution.

PES. The response to PES was determined by subjecting the muscle to a repetitive pattern of 10 paired stimuli. In a preliminary study, the interval between the paired stimuli was progressively increased from 300 to 800 ms in increments of 50 ms. The maximal inotropic effect of PES occurred when the interval between the paired stimuli was 500 ms. This interval was used in the present study to determine the effect of PES in newborn and adult muscles perfused with the control solution and solutions containing verapamil or diltiazem.

Statistical analysis. Results were expressed as means ± SE. Statistical analysis was performed using the two-tailed *t* test for paired and unpaired data. Confirmation was obtained using nonparametric methods (Wilcoxon's signed rank test or Wilcoxon's rank sum test) (17).

RESULTS

Baseline Contractility. The baseline data of DT, +dT/dt (max), and ½ RT in the adult were significantly ($p < 0.001$) greater than in the newborn (Table 1). There were no significant differences in TPT and RT between the two age groups.

Dose Response Relationship. The effect of verapamil and diltiazem on +dT/dt (max) is shown in Figures 1 and 2. Consecutive doses of verapamil and diltiazem reduced +dT/dt (max) in the newborn and in the adult. The negative inotropic effect of verapamil (10^{-7} M and 2.5×10^{-7} M) and diltiazem (10^{-7} M, 5×10^{-7} M, and 10^{-6} M) in the newborn was significantly greater ($p < 0.05$) than in the adult. The effect of 10^{-7} M verapamil and

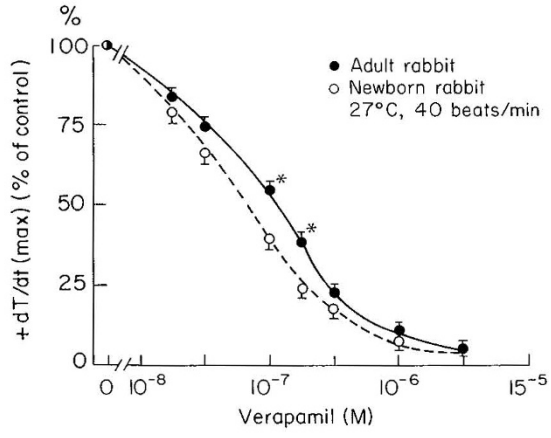

Fig. 1. Dose response relationship of verapamil on dT/dt (max) in the newborn (○, $n = 6$) and adult (●, $n = 5$). +dT/dt (max) is expressed as percentage of control values. Significant difference between newborn and adult; *$P < 0.001$.

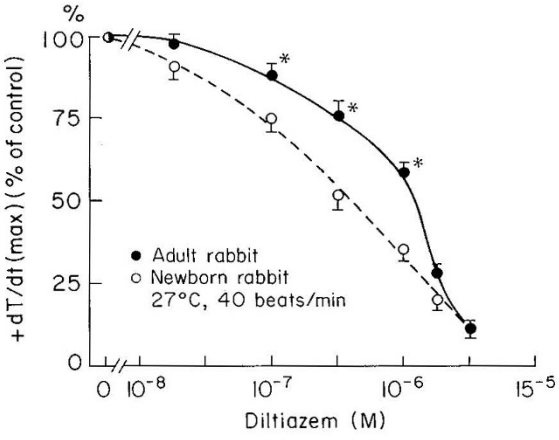

Fig. 2. Dose response relationship of diltiazem on +dT/dt (max) in the newborn (○, $n = 8$) and adult (●, $n = 5$). +dT/dt (max) is expressed as a percentage of control values. Significant difference between newborn and adult; $p < 0.05$.

Table 1. *Effect of Verapamil 10^{-7} M and diltiazem 10^{-6} M on mechanical function (mean ± SE)**

		DT (g/g muscle)	RT (g/g muscle)	+dT/dt (max) (g/s/g muscle^{-1})	1/2 RT (ms)	TPT (ms)
Adult	Control	19.4 ± 2.8	1.8 ± 0.2	100 ± 23	240 ± 14	355 ± 26
	Verapamil 10^{-7} M	11.6 ± 2.2†	2.0 ± 0.4	59 ± 16†	235 ± 17	320 ± 16
Newborn	Control	7.3 ± 0.8‡	2.0 ± 0.2	40 ± 3‡	155 ± 13‡	285 ± 15‡
	Verapamil 10^{-7} M	3.5 ± 0.5†	2.8 ± 0.3†	17 ± 2†	148 ± 13	280 ± 5
Adult	Control	17.6 ± 3.2	2.2 ± 0.3	89 ± 14	218 ± 18	350 ± 14
	Diltiazem 10^{-6} M	9.6 ± 1.8†	2.4 ± 0.3	45 ± 5†	215 ± 22	340 ± 24
Newborn	Control	6.9 ± 0.8	1.8 ± 0.2	35 ± 3	175 ± 17	300 ± 13‡
	Diltiazem 10^{-6} M	2.9 ± 0.6†	2.8 ± 0.3†	15 ± 1†	175 ± 19	280 ± 16

* Values are significantly different from adult values; $n = 5$ per group.
† Significantly ($p < 0.01$) different from control.
‡ Control newborn.

10^{-6} M diltiazem on mechanical function are shown in Table 1. Verapamil and diltiazem caused a significant decrease in DT and +dT/dt (max) in the newborn and the adult ($p < 0.005$). A significant ($p < 0.01$) increase in RT was observed in newborn heart perfused with verapamil and diltiazem, but not in the adult heart.

Stair case. The effect of stair case is shown in Figure 3. In the control group, stair case increased +dT/dt (max) by 17 ± 1 g/s/g muscle (46 ± 4% of control) in the newborn, and 42 ± 7 g/s/g muscle (44 ± 7% of control) in the adult. In muscles perfused with verapamil (10^{-7} M); however, stair case increased +dT/dt (max) by 4 ± 2 g/s/g muscle (24 ± 10% of value at 40 beats/min) in the newborn and 12 ± 3 g/s/g muscle (20 ± 5 of values at 40 beats/min) in the adult. In muscles perfused with diltiazem (10^{-6} M) the positive inotropic effect of stair case was only 1.4 ± 0.4 g/s/g muscle (9 ± 2% of values at 40 beats/min) in the newborn and 9 ± 2 g/s/muscle (20 ± 4% of values at 40 beats/min) in the adult. Prior exposure to verapamil and diltiazem significantly depressed the positive inotropic effect of staircase, and this depression in the newborn was greater than in the adult.

Hyperosmolarity. The effect of mannitol in muscles perfused with the control solution produced a significant ($p < 0.025$) increase in dT/dt (max) of 14 ± 2 g/s/g muscle (36 ± 4% of control) in the newborn, and 22 ± 4 g/s/g muscle (23 ± 4%) in the adult. Mannitol following verapamil (10^{-7} M) perfusion increased dT/dt (max) by 12 ± 2 g/s/g (70 ± 12% of prehyperosmolar values) in the newborn and 36 ± 2 g/s/g (61 ± 3% of prehyperosmolar value) in the adult. In addition, mannitol in muscles perfused with diltiazem (10^{-6} M) increased dT/dt (max) by 21 ± 2 g/s/g muscle (139 ± 4% of prehyperosmolar values) in the newborn and 48 ± 9 g/s/g muscle (107 ± 20% of prehyperosmolar values) in the adult. In both the newborn and adult muscle, prior exposure to verapamil (10^{-7} M) and diltiazem (10^{-6} M) did not attenuate the effect of hyperosmolarity (Fig. 4).

Effect of PES. Figure 5 shows the effect of PES during control and with Ca^{2+} antagonists. In the control muscles, the inotropic effect of PES in the newborn (67 ± 6% of control or 27 ± 2 g/s/g muscle) was significantly less than in the adult (143 ± 11% of control or 142 ± 11 g/s/g muscle). At the verapamil concentration of 10^{-7} M, the inotropic effect of PES in the adult (133 ± 16 g/s/g muscle = 225 ± 27% of control) was not significantly different from that in the control muscles and was significantly ($p < 0.005$) attenuated in the newborn (12 ± 2 g/s/g muscle = 68 ± 12% of control). As for diltiazem (10^{-6} M), similarly, the inotropic effect of PES in the newborn (7 ± 1 g/s/g muscle = 49 ± 13% of control) was significantly decreased ($p < 0.005$), but not in the adult (102 ± 3 g/s/g muscle = 227 ± 7% of control).

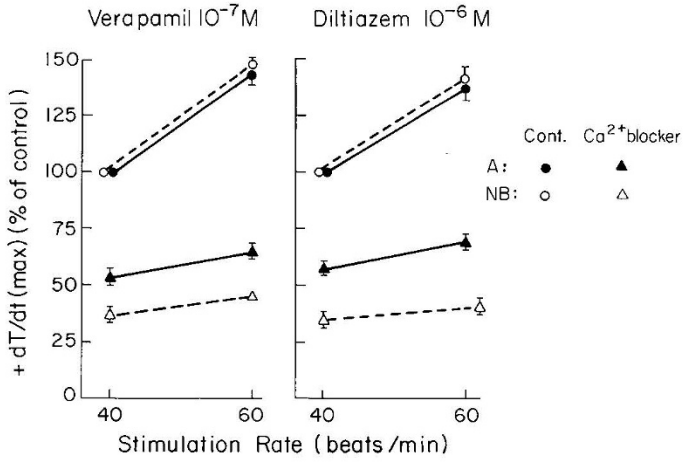

Fig. 3. Effect of verapamil 10^{-7} M and diltiazem 10^{-6} M on the inotropism of stair case. Newborn: (verapamil, $n = 5$; diltiazem, $n = 4$): before (○) and after (△) calcium antagonist. Adult (verapamil, $n = 5$; diltiazem, $n = 4$): before (●) and after (▲) calcium antagonist. The positive inotropic effect became less in the two age groups after verapamil and diltiazem ($p < 0.005$).

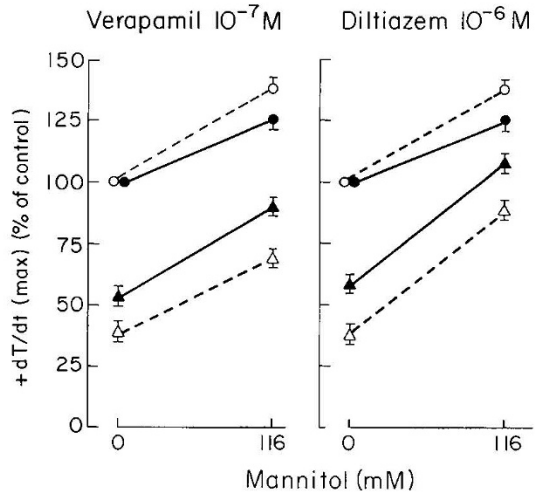

Fig. 4. Effect of verapamil 10^{-7} M and diltiazem 10^{-6} M on the inotropism of hyperosmolarity with mannitol (116 mM). Newborn (verapamil, $n = 5$; diltiazem, $n = 4$): before (○) and after (△) calcium antagonist. Adult (verapamil, $n = 5$; diltiazem, $n = 4$): before (●) and after (▲) calcium antagonist.

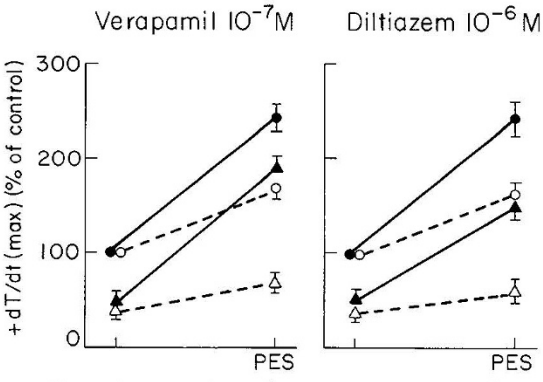

Fig. 5. Effect of verapamil 10^{-7} M and diltiazem 10^{-6} M on the inotropism of PES. Newborn (verapamil, $n = 5$; diltiazem, $n = 4$): before (○) and after (△) calcium antagonist. Adult (verapamil, $n = 5$; diltiazem, $n = 4$): before (●) and after (▲) calcium antagonist. In the newborn the positive inotropic effect of PES became less after verapamil and diltiazem ($p < 0.005$).

DISCUSSION

This study was designed to determine the effect of verapamil and diltiazem on mechanical function in the isolated newborn and adult myocardium. Previous reports (3, 7, 8, 18) showed that verapamil and diltiazem inhibit the slow inward current, and produce a negative inotropic effect in the adult. Gibson *et al.* (11) showed that verapamil effect on cardiac output in the immature dog (2 to 12 days) was greater than in the adult. Boucek *et al.* (9) demonstrated that the effects of verapamil, nifidipine, and diltiazem on dP/dt was greater in the 13- to 26-day-old rabbit than in the adult; and these findings are contrary to previous preliminary report by George *et al.* (10). In addition, the tubular system and dyadic junction are fully developed in the 14- to 21-day-old rabbit (19). For these reasons it is important to determine the effect of Ca^{2+} channel blockers in the newborn rabbit before the appearance of the tubular system and maturation of the dyadic system (19). We therefore designed this study. In the present study, the negative inotropism of verapamil and

diltiazem were observed cumulatively in dose response in the newborn and adult. Our results indicate that the effect in the newborn was significantly greater than in the adult. This finding confirms previous reports by Gibson *et al.* (11) and Boucek *et al.* (9). Although Boucek *et al.* (9) used the blood perfused Langendorff preparation and we used the cristoloid perfusate, the effect of both verapamil and diltiazem in our groups is comparable to that reported by Boucek *et al.* (9). Furthermore, the T-tubular system is absent in our newborn group and present in the immature (13- to 16-day-old) group studied by Boucek *et al.* (9, 19). The fact that the effect of verapamil and diltiazem is similar in the two groups suggest that the t-tubules are not the reason for the age related difference in the effect of Ca^{2+}-channel blockers. Jarmakani *et al.* (12) showed that sarcolemmal permeability to divalent cations was similar in the newborn and adult, but total contractile Ca^{2+} in the newborn was less than in the adult. Nakanishi and Jarmakani (14) showed that the calculated value of SR calcium uptake (μmol/g muscle) in the newborn was 60% of the adult value. These data suggest that the neonatal heart is more dependent on transsarcolemmal calcium influx for excitation-contraction coupling because of the lower amount of contractile calcium and the less quantitative development of SR than in the adult.

It is unknown as to why an increase in RT is observed in the newborn at the concentrations 10^{-7} M verapamil and 10^{-6} M diltiazem. The increased resting tension may be due to a decreased tissue ATP which is unlikely in the well-oxygenated muscle, or to an increase free cytoplasmic Ca^{2+} during relaxation. The latter may be seen when Ca^{2+} sequestration by the SR and Ca^{2+} efflux are less than SR Ca^{2+} release and Ca^{2+} influx. Verapamil and diltiazem block the slow inward Ca^{2+} current and may inhibit Ca^{2+} influx via Na^{+}-Ca^{+} exchange (20). Thus Ca^{2+} influx is reduced in muscles perfused with these drugs. However, verapamil and diltiazem may inhibit the potassium channel and this effect in the newborn may be greater than in the adult. This will result in membrane depolarization and enhanced Ca influx.

It is also conceivable that Ca^{2+} sequestration and/or efflux are depressed in the newborn more than in the adult. Verapamil and diltiazem have no effect on the SR Ca uptake while diltiazem may enhance Ca^{2+} efflux via Na-Ca exchange. An age-dependent difference in Ca^{2+} efflux via Na^{+}-Ca^{2+} exchange in muscles perfused with verapamil and diltiazem may explain an increased cytoplasmic Ca^{2+} concentration and the subsequent increase in the resting tension. The sarcolemmal Ca^{2+} ATPase role in Ca^{2+} efflux is not significant and it may not be responsible for the increased RT. Thus we speculate that the increased RT in the newborn may be due to a decrease in potassium current or Ca^{2+} efflux via Na-Ca exchange.

Myocardial contractility is dependent on several factors which include: 1) contractile Ca^{2+}, 2) the amount of contractile proteins, 3) the sensitivity of the troponin-tropomyosin system to changes in calcium concentration within the cell, and 4) the interaction between actin and myosin. In our study, each experiment serves as its own control where the amount of contractile proteins are the same and we assumed that the sensitivity of troponin-tropomyosin system to calcium as well as the interaction between actin and myosin remained constant. Thus the contractile force is dependent on the sum of sarcolemmal Ca^{2+} influx and Ca^{2+}-stimulated SR Ca^{2+} release. It should be noted that Ca^{2+} influx is the sum of slow inward Ca^{2+} current and Ca^{2+} influx via Na-Ca exchange. In this study we used several interventions which primarily affect Ca^{2+} contribution from slow inward Ca^{2+} current (stair case), Na-Ca exchange (hyperosmolar solutions), or the sarcoplasmic reticulum (paired electrical stimulation).

It is known that stair case, an increase in stimulation rate, will have a positive inotropic effect on the mammalian heart muscle by increasing the transsarcolemmal calcium influx (21). In the present study, stair case produced a positive inotropic effect in the newborn and adult during control (K-H solution). There was, however, no age-related difference in this positive inotropic effect. After exposure to verapamil (10^{-7} M) and diltiazem (10^{-6} M), the positive inotropism was significantly depressed in both age groups. This is probably due to verapamil and diltiazem inhibition of the transsarcolemmal calcium influx.

Because Ca^{2+}-stimulated SR Ca release is dependent on Ca influx, a change in Ca^{2+} influx will cause a proportional change in SR Ca^{2+} release and this will result in a similar stair case effect in the two age groups.

Hyperosmolarity is well known to increase cardiac tension (22–25). Tillisch and Langer (23) suggested that hyperosmolarity elicited cellular dehydration, which increased $(Na)_i$, in turn stimulated Na-Ca exchange, and subsequently led to an increased (Ca_i). Lado *et al.* (25) measured the effect of hyperosmolarity of the bathing solution on intracellular sodium and calcium activities, tension of sheep cardiac Purkinje strands, and ventricular muscle using microelectrodes made from barosilicate glass. In hypertonic solutions, tension development was increased, as was intracellular sodium and calcium activities. In our study, hyperosmolarity caused a significant positive inotropic effect, and the increase in dT/dt (max) was significantly greater in the adult than in the newborn. With prior addition of verapamil (10^{-7} M) and diltiazem (10^{-6} M), the positive inotropic effect of hyperosmolarity could not be prevented in both age groups. Supposing Na-Ca exchange plays an important role in the effect of hyperosmolarity, which produces a positive inotropy along with a rise in intracellular sodium and calcium, verapamil (10^{-7} M) and diltiazem (10^{-6} M) cannot prevent the calcium influx through the Na-Ca exchange pathway. In fact, the inotropic effect of hyperosmolar solution in newborn and adult muscles treated with verapamil or diltiazem was greater than that in muscles perfused with the control solution. The finding is consistent with the findings of Takeo *et al.* (20) who showed that diltiazem enhanced Ca influx via Na-Ca exchange.

Although we do not understand the precise mechanism which causes inotropy due to PES, there seems to be a dependency on calcium release from intracellular stores (26). In the present study, the positive inotropic effect during control in the newborn was significantly less than that in the adult. Maylie (27) demonstrated, that in the neonatal cat myocardium compared to the adult, the immaturity of the SR could be a possible source of decreased contractility during post-extrasystolic potentiation in the newborn. In fact, the calculated value of SR Ca uptake in the newborn was 60% of the adult rabbit (14). Thus, the age-related difference in the PES effect might rely on the maturity of the SR. At verapamil (10^{-7} M) and diltiazem (10^{-6} M), the positive inotropic effect of PES was reduced in the newborn, but not in the adult. In the newborn, the decrease of slow inward current from verapamil and diltiazem attenuated SR Ca^{2+} release in the newborn because the threshold for SR Ca release in the newborn is greater than in the adult. Verapamil and diltiazem effects are similar to a reduction in Ca^{2+} concentration in the perfusate which has been shown to attenuate the positive inotropic effect of PES in the newborn and enhance it in the adult (27). Thus verapamil and diltiazem attenuate Ca influx and subsequent SR Ca^{2+} release in the newborn more than in the adult.

In conclusion, the present data showed age-related differences in mechanical function to calcium antagonists through dose response, stair case, hyperosmolarity, and PES. These results suggest that contractile force in the neonatal heart is more dependent on the slow inward calcium current than in the adult, and is consistent with our previous data (12, 14).

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