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Effects of Calcium Channel Blockers on Arachidonate-Induced Sudden Death in Rabbits[1] (41107)

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Abstract. The effects of the calcium channel blockers, nisoldipine and verapamil, on arachidonate-induced sudden death were investigated in rabbits. Sodium arachidonate (2 mg/kg) was injected into the vena cava producing death within 3 min (2.5 ± 0.3 min) in all untreated rabbits. Seventy-five percent of the rabbits pretreated by nisoldipine (0.2 mg/kg) were protected from arachidonate-induced sudden death, showed inhibition of thromboxane synthesis, and the absence of intravascular thrombosis in pulmonary vessels. Verapamil also protected rabbits from sudden death but to a lesser extent than that of nisoldipine. Inhibition of thromboxane synthesis and prevention of platelet-induced pulmonary thrombosis appear to be related to the effects of the calcium channel blockers in arachidonate-induced sudden death.

Sodium arachidonate is the precursor of a variety of bisenoic prostaglandins (PG) including PGE_2, $PGF_{2\alpha}$, thromboxane A_2, and prostacyclin. Sodium arachidonate induces platelets to aggregate (1) and leads to sudden death (2). The cause of sudden death may be a combination of intravascular platelet aggregation in the pulmonary circulation, arterial vasoconstriction, and bronchoconstriction mediated by the synthesis of prostanoids, particularly by thromboxane A_2, from arachidonic acid.

Since calcium is required for constriction of vascular smooth muscle (3) and platelet aggregation (4), calcium channel blockers might protect against sudden death. Thus, we investigated the effect of the calcium blockers, nisoldipine and verapamil on sodium arachidonate-induced sudden death in rabbits.

Methods. Forty-two male New Zealand rabbits weighing 2.5 to 3.5 kg were anesthetized with sodium pentobarbital (30 mg/kg) injected intravenously. A cannula was inserted into the trachea and connected to a Statham P23AC pressure transducer, in order to monitor changes in airway pressure. Polyethylene catheters were introduced into the left common carotid artery and left external jugular vein to monitor systemic blood pressure and central venous pressure, respectively, using Statham P23AC pressure transducers. Another catheter was inserted into the inferior vena cava via a femoral vein to infuse drugs. Needle electrodes were placed subcutaneously for the recording of the electrocardiogram (ECG) on standard lead III. All pressures and the ECG were recorded on a Grass Model 7 oscillograph.

Ten minutes after the end of all surgical procedures, a control blood sample was drawn from the left carotid artery catheter for the radioimmunoassay of thromboxane B_2, and a dose of one of the calcium blockers was infused for 15 min. This was followed by injection of sodium arachidonate over 5 min after the end of the infusion of the calcium antagonist. Two to three minutes after the end of the injection of 2 mg/kg sodium arachidonate, the second blood sample was drawn from the left carotid artery catheter. This corresponded to a time just before the rabbits died or at an equivalent time in survivors. Systemic blood pressure was observed in survivors until it returned to control values, the recovery usually being complete within 30 to 45 min.

The calcium channel blockers, nisoldipine and verapamil, were dissolved in 100% ethanol. One milliliter of the ethanol

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solution containing a dose of 0.05, 0.1, and 0.2 mg/kg of nisoldipine or 0.05, 0.1, 0.2, and 0.5 mg/kg of verapamil was infused intravenously over a 15-min period using a Harvard infusion pump. Sodium arachidonate was dissolved in 2 ml of a 1 m*M* sodium carbonate solution and injected intravenously over a 1-min period. Vehicle controls (i.e., 1 ml of 100% ethanol) were used when no drug was given.

A 3-ml blood sample was drawn from the arterial catheter for the measurement of plasma thromboxane B_2 (TB_2). Thirty microliters of 15% EDTA and 200 μl of 100 m*M* sodium meclofenamate were added to each blood sample to protect against thromboxane formation during the collection of the blood. These blood samples were centrifuged at 2500*g* for 15 min at 4°. The plasma was separated and stored at −20° in a freezer until the assay was performed. A specific radioimmunoassay method was used to assay thromboxane B_2 concentration (5). The antibody to thromboxane B_2 has very low cross-reactivity to other prostanoids (see legend to Fig. 2).

Lung tissue was obtained at the end of the experiment for microscopic examination of pulmonary blood vessels. Photomicrographs were prepared from these sections at a magnification of 430×.

To investigate the effect of calcium channel blockers on liver lysosomal integrity and pancreatic proteolysis, cat liver and pancreatic homogenates were prepared and studied as previously described (4). Large granule fractions (LGF) were isolated from cat liver homogenates according to the method of Bridenbaugh *et al.* (7). LGF suspensions were incubated at 37° with either calcium blocker (nisoldipine 0.2 and 1 μg/ml, or verapamil 0.2 and 1 μg/ml) or an equivalent volume of ethanol diluted in 0.9% NaCl as a vehicle control. Cathepsin D (8) and β-glucuronidase (9) activities of the liver LGF were determined in the presence and absence of 0.1% Triton X-100, a nonionic detergent that lyses all lysosomal membranes, in order to calculate the ratio of the free to the total enzyme activity (10). Homogenates of cat pancreatic tissue were prepared as previously described (11). Pancreatic homogenates were incubated at 37° for 30 min with calcium channel blockers or an equivalent volume of 0.9% NaCl. The incubates were then deproteinized with 0.5% trichloroacetic acid and the deproteinized incubates were assayed for free amino−nitrogen groups by the ninhydrin method (12).

Significance of the difference was examined by using Student's *t* test. *P* values less than 0.05 were considered statistically significant.

Results. The influence of various doses of nisoldipine and verapamil on survival rates in sodium arachidonate-induced sudden death is shown in Table I. Nisoldipine demonstrated a survival rate of 75% at a dose of 0.2 mg/kg. Verapamil increased the survival rate to 40% at a dose of 0.1 mg/kg, but higher doses were not as effective. The highest survival rate induced by verapamil was lower than the maximum produced by nisoldipine. All untreated rabbits injected with 2 mg/kg of sodium arachidonate were given ethanol (but no infusion of a calcium channel blocker) died within 3 min after the injection of arachidonate.

Figure 1 presents photomicrographs of sections of pulmonary tissue taken from control rabbit lung (Na-Ar) which was injected only with sodium arachidonate and from a surviving rabbit (nisoldipine + Na-Ar) which was infused with 0.2 mg/kg nisoldipine before the injection of sodium arachidonate. The photomicrograph of the untreated rabbit (Na-Ar) shows clear intravascular platelet thrombosis. In contrast, the surviving rabbit (Nisoldipine + Na-Ar) did not show intravascular platelet thrombi in any of the six sections studied from this rabbit. These were typical findings comparable to those obtained in three other rabbits studied in each group.

Figure 2 illustrates the changes in plasma thromboxane B_2 concentrations in three groups of rabbits given sodium arachidonate. Thromboxane B_2 is the single stable metabolite of thromboxane A_2, and thus TB_2 concentrations reflect thromboxane A_2 levels. In rabbits untreated with a calcium antagonist and challenged with sodium arachidonate, TB_2 concentrations increased 10-fold within 3 min. All of these animals died. In the group of rabbits given 0.05

TABLE I. INFLUENCE OF NISOLDIPINE AND VERAPAMIL ON SURVIVAL RATE IN ARACHIDONATE-INDUCED SUDDEN DEATH

Dose of calcium channel blocker (mg/kg)	Number of surviving animals[a]	Number of fatalities	Percentage survival
Control (without calcium antagonist)	0	6	0%
Nisoldipine			
0.05	0	4	0%
0.10	2	5	29%
0.20	6	2	75%
Verapamil			
0.05	0	3	0%
0.10	2	3	40%
0.20	1	4	25%
0.50	0	4	0%

[a] All animals having a MABP above 75 mm Hg at 2 hr were considered survivors.

mg/kg nisoldipine, an ineffective dose of this agent in improving survival, there was also a 10-fold increase in TB_2 concentrations. Those rabbits receiving 0.2 mg/kg nisoldipine, a dose that produced a 75% survival rate, plasma TB_2 concentrations were increased only 2.5-fold, a significantly smaller increase than that observed in the other two groups. The two nonsurvivors in this group exhibited increases in TB_2 concentrations to 8.5 and 10.2 pmole/ml. Although, not shown because of the small numbers involved, those rabbits given 0.1 or 0.2 mg/kg verapamil exhibited increases in TB_2 values of 6- to 7-fold.

The effect of the calcium channel block-

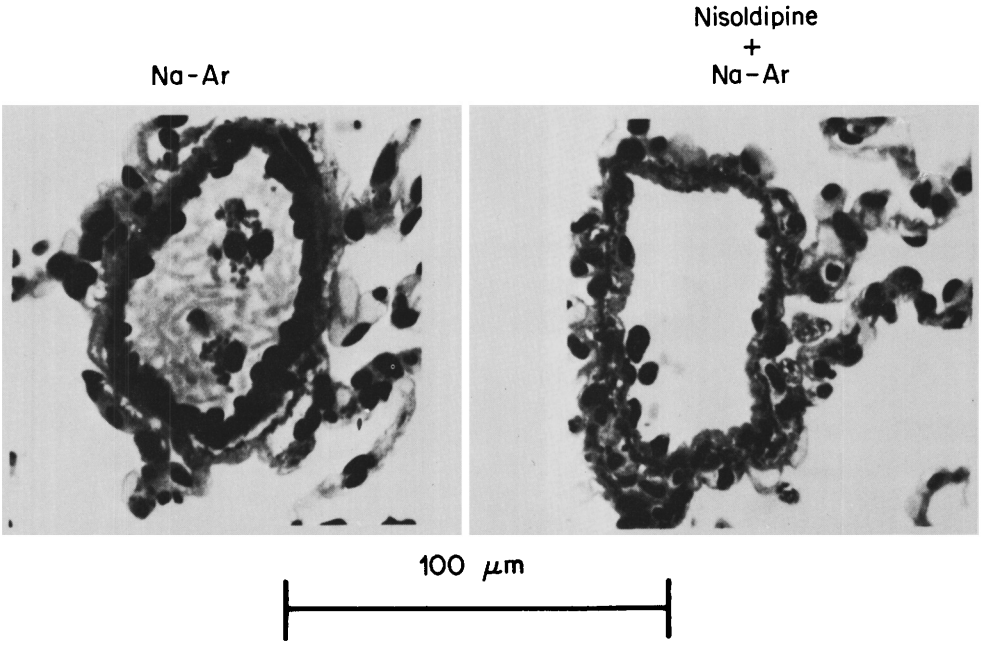

FIG. 1. Photomicrographs of typical sections of rabbit pulmonary tissue each showing a pulmonary vessel. In the left panel, (Na-Ar), sodium arachidonate was given along, and the vessel is thrombosed. In the right panel (nisoldipine + Na-Ar), nisoldipine 0.2 mg/ml was given prior to the arachidonate, and the vessel is patent and appears normal.

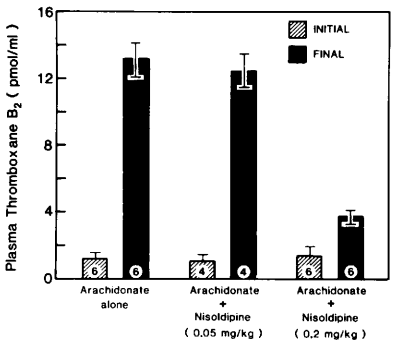

FIG. 2. Plasma thromboxane B_2 (TB_2) concentrations expressed in picomoles per milliliters. All values are means ± SEM. Numbers at the bottom of the bars indicate numbers of samples assayed. Initial = just prior to arachidonate (or saline in the case of the control group). Final = just prior to death or 2–3 min after arachidonate injection. Final values are significantly elevated over initial values in the first two groups ($P < 0.001$). The arachidonate + nisoldipine (0.2 mg/kg) group exhibited a smaller increase ($P < 0.05$) which was significantly lower ($P < 0.01$) than the increase observed in the other two groups. Cross-reactivity of the thromboxane antibody was as follows: thromboxane B_2 = 100%, PGD_2 = 0.06%, 6,15-diketo $PGF_{1\alpha}$ = 0.05%, PGE_2, $PGF_{2\alpha}$, 6 keto-$PGF_{1\alpha}$ = <0.05%, arachidonic acid = <0.01%.

ers on isolated liver lysosomal membrane integrity and on pancreatic homogenate proteolysis are shown in Table II. Neither calcium antagonist showed significant changes in the rate of lysosomal hydrolase release from liver large granule fractions (i.e., lysosome enriched fractions), indicating that neither antagonist stabilizes lysosomal membranes at the concentrations employed. Although verapamil did not alter pancreatic proteolysis, nisoldipine significantly reduced the rate of proteolysis at concentrations of 0.2 and 1 μg/ml. These findings suggest that nisoldipine may have important metabolic effects that are different from verapamil. Some of these differences may explain the superior protection of nisoldipine in arachidonate-induced sudden death.

Discussion. Our experiments show that the rabbits infused with calcium channel blockers survived sudden death induced by sodium arachidonate (2 mg/kg) at a rate of 75% by pretreatment with nisoldipine (0.2 mg/kg) and at a rate of 40% by pretreatment with verapamil (0.1 mg/kg). Rabbits challenged with sodium arachidonate (2 mg/kg) without the previous administration of a calcium channel blocker all died within 3 min. Silver *et al.* (2) showed that injection of sodium arachidonate (1.4 mg/kg) into the marginal ear veins of rabbits produced death within 3 min. We injected 2 mg/kg of sodium arachidonate into the vena cava and found this to result in sudden death in all untreated rabbits. Clearly, calcium channel blockers given prior to arachidonate are effective in preventing rabbits from sudden death. In our studies, nisoldipine was more effective than verapamil, an agent that may have adverse side effects, which may hamper its effectiveness.

Furthermore, we showed that the increase in thromboxane B_2 concentrations in response to arachidonate challenge was significantly lower in the rabbits infused

TABLE II. EFFECT OF CALCIUM CHANNEL BLOCKERS ON LIVER LYSOSOMAL INTEGRITY AND ON PANCREATIC PROTEOLYSIS

Agent	Concentration (μg/ml)	Liver LGF rate of hydrolase release[a]		Pancreatic proteolysis[a]
		β-Glucuronidase	Cathepsin D	
Verapamil	0.2	−0.5 ± 1.1	−0.9 ± 0.8	+0.5 ± 0.3
	1.0	−1.2 ± 1.0	−1.3 ± 1.4	+0.1 ± 0.2
Nisoldipine	0.2	−0.9 ± 0.9	−1.0 ± 0.9	−0.7 ± 0.3*
	1.0	−1.4 ± 1.6	+0.2 ± 2.5	−0.9 ± 0.2**

Note. All values are mean percentage changes ± SEM for from 5 to 10 experiments. LGF = large granule fraction.

[a] Positive number indicates stimulation, negative number indicates inhibition of hydrolase release or proteolysis.

* $P < 0.05$.

** $P < 0.01$.

with 0.2 mg/kg of nisoldipine than in untreated rabbits. Moreover, there was no intravascular platelet aggregation or thrombosis in pulmonary vessels of nisoldipine-pretreated rabbits. These results suggest that the calcium blocker, nisoldipine, inhibits the synthesis of thromboxane B_2 and the subsequent aggregation of platelets. In addition, coronary vasodilation by the calcium channel blockers may be part of the protective mechanism. Although aspirin is reported to protect all rabbits from the lethal effect of sodium arachidonate (13, 14), the protective effect of calcium blockers on arachidonate-induced sudden death is not universal. The difference between the effect of calcium blockers on arachidonate-induced sudden death from that of aspirin and other cyclooxygenase inhibitors (e.g., sodium meclofenamate) (14), may be due to differences in the protective mechanisms of the two groups of agents as well as in the duration of the pretreatment period. Anti-inflammatory agents may be more effective by virtue of their total inhibition of thromboxane A_2 synthesis, whereas calcium channel blockers are only partial thromboxane antagonists (15).

Our results show differences in potency between nisoldipine and verapamil on survival rates in arachidonate-induced sudden death. Survival rates of rabbits treated with nisoldipine were higher than those of verapamil. Furthermore, verapamil did not affect pancreatic proteolysis but nisoldipine was effective in this regard. The difference in pancreatic proteolysis between these two calcium channel blockers may be related to the difference in survival rates. However, there are other pharmacodynamic differences between these two agents. Nisoldipine is 4 to 10 times more potent a vasodilator than verapamil, and has a more moderate negative inotropic effect (16). In addition, nisoldipine exerts a prominent preferential vasodilator effect on the coronary vasculature in contrast to verapamil which has a comparable dilator effect on all vascular beds (16). Thus, the mechanism of protection by nisoldipine probably reflects its inhibition of external calcium influx into smooth muscle cells. The coronary vasodilator effect of nisoldipine occurs even in the absence of changes in blood pressure, suggesting that there would be no prominent coronary steal phenomenon with doses of nisoldipine that do not exert a marked hypotension. Both calcium channel blockers effectively blocked the pressor effect of angiotensin in anesthetized rabbits for 30 min after intravenous doses that protect in sudden death, so that 15 min pretreatment is an effective time of administration of these calcium channel blockers.

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