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Comparison of Verapamil and Nifedipine in Thrombosis Models[1] (42390)

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Abstract. Calcium blockers and calmodulin antagonists have been reported to inhibit the aggregation of blood platelets *in vitro*. In the present study, the effects of two calcium blockers, verapamil and nifedipine, were compared in several rodent thrombosis models. In rat and mouse platelet-rich plasma, preincubation with either verapamil or nifedipine had a dose-dependent inhibitory effect on collagen-induced aggregation ($P < 0.01$). The concentration required for 50% inhibition of rat platelet aggregation was 0.91×10^{-4} *M* for verapamil and 1.77×10^{-4} *M* for nifedipine. In *in vivo* thrombosis models in mice, acute pretreatment with nifedipine had a significant, dose-dependent protective effect ($P < 0.05$). At a dose of 500 μg/kg, nifedipine inhibited thrombotic sudden death provoked by arachidonic acid, a thromboxane agonist (U46619), or a combination of collagen and epinephrine. *In vivo* platelet depletion induced by U46619 was also inhibited by this calcium blocker. Thus, nifedipine is protective against a variety of thrombotic stimuli, and its antiplatelet aggregatory effect apparently extends to the *in vivo* situation. In contrast, no *in vivo* antithrombotic activity was observed for verapamil. Two additional calcium blockers, perhexilene and diltiazem, and three calmodulin antagonists, W-7, chlorpromazine, and trifluoperazine, were also tested in the U46619-induced thrombotic sudden death model. Of these, only diltiazem (5 and 10 mg/kg) had an acute protective effect.

Calcium-blocking drugs are a valuable new class of agents for the therapy of cardiovascular disease in that calcium blockers can increase myocardial perfusion, prevent vasospasm and arrhythmias, and reduce systemic blood pressure. Their actions are thought to be primarily due to effects on coronary arterial and peripheral vascular tone and cardiac conduction. The extent to which these actions are manifested varies with the specific calcium blocker employed. For instance, the antiarrhythmic action of verapamil is more pronounced than that of nifedipine, whereas the latter agent has a more pronounced vasodilator action (1). Differences between the effects of the specific agents are an important consideration in the clinical use of calcium blockers.

A potentially significant action which has received relatively little attention is the antithrombotic effect of these drugs. This effect is not totally unexpected, since Ca^{2+} has a central role in both platelet aggregation and the release reaction. Verapamil inhibits the *in vitro* aggregation of human (2–5) and rat (6) blood platelets by various agonists. Similarly, nifedipine (5, 7), diltiazem, and perhexilene (5) inhibit *in vitro* platelet aggregation. Calcium-blocking drugs have been shown to inhibit aggregation produced by many agonists, including ADP, collagen, thrombin, arachidonic acid, and the calcium ionophore, A23187 (1–7). A related drug, trifluoperazine, which blocks the interaction of Ca^{2+} with the intracellular calcium-binding protein calmodulin, also inhibits aggregation of human platelets (8).

Although several studies show clearly that some calcium blockers have *in vitro* antiaggregatory effects, few studies in *in vivo* thrombosis models have been performed. Okamatsu *et al.* (9) investigated the effects of nisoldipine and verapamil on thrombotic sudden death induced by iv arachidonate in rabbits. Nisoldipine protected against the sudden death, but the effect of verapamil was not clear. The protection by nisoldipine was associated with inhibition of the rise in concentration of thromboxane (the proaggregatory metabolite of arachidonic acid) which otherwise occurs following arachidonate injection. Occlusive pulmonary thrombosis was also prevented. Pumphrey *et al.* (10) studied the effects of verapamil on platelet deposition on synthetic and

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autologous arterial grafts in dogs. The calcium blocker significantly reduced the arterial thrombosis. In a related model, nifedipine and nicardipine have been found to suppress diet-induced atherogenesis in rabbits (11, 12), a process which might involve platelets. In fact, the fall in circulating platelet count occurring during diet-induced atherogenesis is inhibited by these drugs (12).

Platelets have demonstrated or hypothetical roles in many cardiovascular pathologies, including vasospasm, atherosclerosis, coronary thrombosis, acute myocardial infarction and various other ischemic disease states. Because calcium blockers are used therapeutically in such states, it is important to know how they affect platelet function and thus, hemostatic balance *in vivo,* including any possible differences between the agents in antithrombotic efficacy.

The objectives of the present study were to compare the effects of two clinically used calcium blockers, nifedipine and verapamil, in *in vitro* and *in vivo* rodent models of thrombosis. Their effects on the aggregation of rat and mouse platelets induced by collagen were studied *in vitro,* and the effects of pretreatment with these calcium blockers were investigated in three *in vivo* thrombosis models in mice. The models selected were thrombotic sudden death induced by arachidonic acid (13, 14), a thromboxane agonist (15), and a combination of collagen and epinephrine (16). In each case, injection of the thrombotic agent results in sudden death, characterized by intravascular platelet aggregation, respiratory distress, and occlusive pulmonary thrombosis. Additional calcium-blocking drugs and calmodulin antagonists were also tested in the thromboxane agonist-induced sudden death model.

Methods. *In vitro studies.* Mature, male Sprague–Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were anesthetized with sodium amytal (100 mg/kg, ip). Blood was collected from the inferior vena cava into 3.8% sodium citrate (one part anticoagulant to nine parts blood). Platelet-rich plasma (PRP) was prepared by centrifugation of whole blood at 250*g* for 15 min. After separation of PRP, platelet-poor plasma (PPP) was prepared by centrifugation at 1500*g* for 15 min.

Aggregation was measured by the turbidimetric technique of Born (17) in a dual-channel aggregometer (Payton Associates, Buffalo, N.Y.), using a stirring speed of 900 rpm and a temperature of 37°C. The aggregometer was calibrated for each animal to measure aggregation as percentage change in optical density with PPP as the 100% standard and PRP as the 0% standard. Aliquots (0.5 ml) of PRP were incubated at 37°C for 3 min with verapamil hydrochloride (Knoll Pharmaceuticals Co., Whippany, N.J.), nifedipine (Pfizer Pharmaceuticals, New York, N.Y.), or the appropriate vehicle. Verapamil was dissolved in 0.9% saline; the vehicle for nifedipine was 15% polyethylene glycol 400 and 15% ethanol in distilled water. All solutions were prepared at concentrations at which the required dose would be administered in 10 μl.

After the incubation period, 25 μl of 1 mg/ml collagen (Hormon-Chemie, Munich, FRG) was added to yield a final concentration of 50 μg/ml PRP. The aggregation curve was recorded for 7 min. Aggregation was measured as the maximum change in optical density occurring within 7 min. For each rat, three concentrations of a calcium blocker, as well as the proper control, were tested in separate PRP samples. Each concentration of calcium blocker was tested in six animals.

For PRP samples incubated with the calcium blockers, the percentage inhibition of aggregation compared to a vehicle pretreated sample was calculated. Results were subjected to regression analysis of log calcium blocker concentration and percentage inhibition, and for each calcium blocker, the correlation coefficient was determined (18). The concentration of verapamil or nifedipine which inhibited aggregation 50% (IC_{50}) was calculated from the regression equation.

The effects of verapamil and nifedipine on aggregation of mouse platelets were also examined. Blood was collected into sodium citrate (as above) from groups of 12 to 20 male CD-1 mice (Charles River Breeding Laboratories) and pooled. PRP was prepared and aggregation was measured with techniques similar to those used in rats, above, and as previously described (19). The aggregating stimulus was collagen, at a concentration of 50 μg/ml PRP. Verapamil and nifedipine were tested in mouse PRP at the IC_{50} calculated for rat PRP (0.91×10^{-4} *M* and 1.77×10^{-4} *M,* respectively); nifedipine was also tested at 3.54×10^{-4} *M.*

In vivo studies. Mature male CD-1 mice (Charles River Breeding Laboratories) were anesthetized with sodium amytal (100 mg/kg, ip) and the jugular vein was surgically exposed for iv injections. Mice were treated with an iv injection of verapamil, nifedipine, or vehicle. Verapamil was dissolved in 0.9% saline; nifedipine was dissolved in 15% ethanol and 15% polyethylene glycol 400 in distilled water and was then diluted 1:3 in 0.9% saline just prior to use. The calcium blockers were prepared at final concentrations such that the required dose was delivered at an injection volume of 5 μl/g body weight.

After treatment with verapamil, nifedipine, or vehicle for 5 min, mice were injected intravenously with one of three thrombotic challenges (arachidonic acid, thromboxane agonist, or collagen/epinephrine combination) at an approximate LD80 to LD90 dose. Arachidonic acid (Nu-Chek-Prep, Inc., Elysian, Minn.) and U46619 (Upjohn Co., Kalamazoo, Mich.), a thromboxane agonist, were dissolved in 100 m*M* sodium carbonate; the collagen (Hormon-Chemie)/epinephrine (Parke-Davis, Morris Plains, N.J.) combination was prepared in 0.9% saline. The thrombotic agents were prepared at concentrations such that the appropriate doses (75, 0.8, and 1 mg/kg:0.12 mg/kg, respectively) were contained in a 5 μl/g body weight injection volume.

Two additional drugs with calcium-blocking activity, diltiazem and perhexilene maleate (20), and three calmodulin antagonists, W-7, chlorpromazine hydrochloride, and trifluoperazine (21), were also tested against the U46619 challenge in mice. Diltiazem (Marion Laboratories, Kansas City, Mo.), chlorpromazine hydrochloride (Sigma Chemical Co., St. Louis, Mo.), and trifluoperazine (Sigma Chemical Co.) were dissolved in 0.9% saline and administered at iv doses of 2, 5, and 10 mg/kg, 0.2 and 2 mg/kg, and 1 and 10 mg/kg, respectively, 5 min prior to U46619. W-7 (*N*-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide hydrochloride, Sigma Chemical Co.) and perhexilene maleate (Sigma Chemical Co.) were dissolved in 10% DMSO in saline and were administered at sc doses of 2, 12.5, and 20 mg/kg, and 4 and 40 mg/kg, respectively, 1 hr prior to U46619 challenge. In all cases, controls were pretreated using the identical vehicle, route of administration, and time of injection.

Survival rate and time to death of animals which died within 1 hr were recorded. The 1-hr observation period was selected on the basis of preliminary studies, which indicated that survival beyond this point was associated with recovery in nearly 100% of animals. Survival rate was compared between drug and vehicle-treated groups with the χ^2 statistic. Each of the 30 treatment and 30 control groups consisted of 15 mice.

In additional groups of nine experimental and control mice treated with the high doses of nifedipine, verapamil, or the appropriate vehicle, blood samples were obtained from the plantar surface of a hind foot 4 min after the pretreatment. U46619 was injected 5 min after the pretreatment, and a second blood sample was obtained 2 min following this challenge from the inferior vena cava. These samples were processed for platelet count determination by phase contrast microscopy. For each animal, the percentage decline in platelet count following challenge was calculated; this parameter was compared between the control and test groups by the Student *t* test.

Results. Both calcium channel blockers inhibited collagen-induced platelet aggregation in rat PRP in a dose-dependent manner (Table I). The dose-response curves were relatively steep, and in each case, the correlation coefficient was significant at the 0.01 level ($r = 0.72$ and 0.79 for verapamil and nifedipine, respectively). The IC_{50} values for verapamil and nifedipine were 0.91×10^{-4} *M* and 1.77×10^{-4} *M*, respectively, as determined by regression analysis. Thus, verapamil was more

TABLE I. EFFECTS OF VERAPAMIL AND NIFEDIPINE ON COLLAGEN-INDUCED AGGREGATION IN RAT PLATELET-RICH PLASMA

Species	Calcium blocker	Concentration (10^{-4} *M*)	*n*	Inhibition (% ± SEM)
Rat	Verapamil	0.10	6	6.6 ± 3.6
		0.51	6	14.8 ± 3.6
		1.02	6	68.1 ± 9.6
Rat	Nifedipine	0.72	6	0.3 ± 11.3
		1.44	6	30.6 ± 13.3
		2.89	6	83.1 ± 9.3
Mouse	Verapamil	0.91	5	58.3 ± 14.9
Mouse	Nifedipine	1.77	4	6.8 ± 4.9
		3.54	4	56.0 ± 9.5

potent than nifedipine in terms of *in vitro* antiaggregatory activity.

When IC_{50} doses of the calcium blockers were tested in mouse PRP, verapamil (0.91×10^{-4} *M*) produced approximately the expected degree of inhibition, but nifedipine (1.77×10^{-4} *M*) produced only a slight inhibition (Table I). The higher dose of nifedipine resulted in substantial inhibition of mouse platelet aggregation.

In contrast to the *in vitro* studies, nifedipine, but not verapamil, was protective against *in vivo* thrombotic challenges. When male mice were pretreated with the nifedipine vehicle prior to challenge with iv arachidonate, thromboxane agonist, or collagen/epinephrine combination, survival rates in these control groups ranged from 0 to 27% (not illustrated). Pretreatment with nifedipine resulted in dose-dependent protection against each of the three challenges, compared to the control groups (Fig. 1). The 500-μg/kg dose was significantly protective ($P < 0.05$) in all cases, whereas the 200-μg/kg dose had a beneficial effect against sudden death induced by the thromboxane agonist and the collagen/epinephrine combination. The lower dose of nifedipine, 50 μg/kg, had no effect in any of the three sudden death models.

Verapamil, at doses of up to 500 μg/kg, had no significant effect on survival following any of the three thrombotic challenges (Fig. 1). The toxicity of the iv verapamil itself at doses above 1 mg/kg precluded testing at higher doses; this verapamil toxicity does, however, provide evidence of the biological activity of the drug. Survival in the groups of mice pretreated with verapamil ranged from 7 to 20%; survival in control groups pretreated with the appropriate vehicle prior to challenge with the thrombotic agent was 0 to 20%. Of the five additional agents tested in the U46619-induced sudden death model, only diltiazem provided significant protection (Table II).

In vivo platelet counts were substantially lower after mice were challenged with U46619, compared to normal counts (Table III). When mice were pretreated with nifedipine, platelet depletion was significantly inhibited ($P < 0.01$). Verapamil, however, had no significant effect on the fall in platelet count.

Discussion. Antithrombotic effects of calcium blockers would have clinical relevance in the choice of agents for cardiovascular indications involving platelets. The present results confirm the *in vitro* inhibitory action of verapamil and nifedipine on platelet aggregation, and provide evidence that the effect of nifedipine extends to the *in vivo* situation. Both of the calcium blockers inhibited collagen-induced aggregation of rat and mouse platelets at relatively high concentrations, verapamil being 1.95-fold more potent than nifedipine on a molar basis or 1.37-fold more potent on a microgram per milliliter basis in rat PRP.

In vivo, however, verapamil had no acute protective effect against any of the thrombotic challenges used, whereas nifedipine inhibited sudden death provoked by all three challenges and inhibited platelet depletion induced by the thromboxane agonist. It should be noted that these effects were observed at high intravenous doses of the drug, which could reduce blood pressure to levels inconsistent with survival in

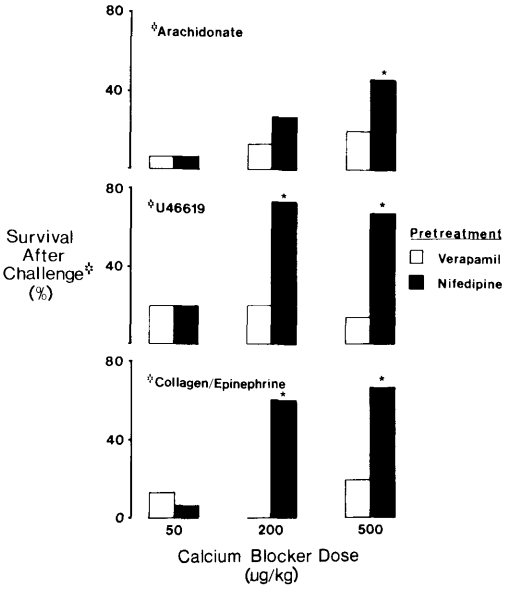

FIG. 1. Effects of nifedipine and verapamil on survival of mice following intravenous thrombotic challenge. An approximate LD80 to LD90 dose of arachidonate, U46619 (a thromboxane agonist), or a combination of collagen and epinephrine was administered following pretreatment with iv verapamil or nifedipine. Nifedipine pretreatment was significantly protective ($P < 0.05$) against sudden death provoked by each of the three thrombotic challenges at the 500 μg/kg dose. Verapamil had no protective effect in any of these thrombosis models. Each of the 18 illustrated test groups and the 18 corresponding control groups (not illustrated) consisted of 15 animals. *$P < 0.05$ compared to the corresponding control group.

larger mammals and patients. Of the two additional calcium blockers and three calmodulin antagonists studied, diltiazem significantly protected against the thromboxane agonist. Thus, in addition to nifedipine, other calcium blockers might have salutory actions in thrombosis models.

The acute protective action of nifedipine against three diverse thrombotic challenges supports the hypothesis that nifedipine is antithrombotic *in vivo,* or alternatively, suggests that nifedipine protects against the sequelae of thrombosis. In either case, it appears that nifedipine has a beneficial effect in induced thrombotic episodes. Sudden death induced by injection of arachidonic acid, thromboxane agonists, and collagen/epinephrine in mice or rabbits has clearly been associated with intravascular platelet aggregation (13–16, 22). The *in vitro* platelet aggregation data and the inhibition of platelet depletion *in vivo,* and earlier work with nifedipine (12) and the related drugs, nisoldipine (9) and nicardipine (12), suggest that nifedipine might indeed have some *in vivo* antithrombotic activity.

The mechanisms whereby nifedipine protects against the three thrombotic challenges are presumably similar. The protection is probably not due to a direct effect on arachidonic acid metabolism, since nifedipine was protective against the direct injection of a thromboxane agonist, and against collagen and epinephrine, which only partially exert their aggregatory actions via arachidonate metabolites. In each of the models, activation of platelets is thought to initiate the sequence of events resulting in sudden death. Changes in intraplatelet calcium levels play an important role in platelet aggregation and release, and thus pretreatment by the calcium blocker nifedipine would have a beneficial effect.

TABLE II. EFFECTS OF CALCIUM BLOCKERS PERHEXILENE AND DILTIAZEM, AND CALMODULIN ANTAGONISTS W-7, CHLORPROMAZINE, AND TRIFLUOPERAZINE ON SUDDEN DEATH INDUCED BY U46619

Pretreatment	Dose (mg/kg)	Mortality (% of control)
Diltiazem (iv)	2.0	57.1[a]
	5.0	14.3*
	10.0	38.5*
Perhexilene (sc)	4.0	100.0
	40.0	80.0
W-7 (sc)	2.0	86.7
	12.5	107.8
	20.0	92.9
Chlorpromazine (iv)	0.2	100.0
	2.0	85.7
Trifluoperazine (iv)	1.0	92.9
	10.0	107.8

[a] Mortality in control groups ranged from 87 to 100%. Control and test groups each consisted of 15 mice.
* $P < 0.01$, compared to vehicle-treated controls.

TABLE III. EFFECTS OF VERAPAMIL AND NIFEDIPINE ON PLATELET DEPLETION INDUCED BY THROMBOXANE AGONIST (U46619) IN MICE

Calcium blocker	Dose (μg/kg)	*n*	U46619-induced platelet depletion (% ± SEM)
Verapamil	0 (vehicle)	9	57.5 ± 6.9
	500	9	50.8 ± 6.0
Nifedipine	0 (vehicle)	9	62.2 ± 6.1
	500	9	31.2 ± 5.2*

* $P < 0.01$, compared to vehicle-treated controls.

The discrepancy between the *in vitro* and *in vivo* effects of verapamil are difficult to explain. The short pretreatment period used in the *in vivo* models (5 min) was selected to allow comparison with *in vitro* aggregation experiments performed in this study as well as earlier studies. In preliminary work, no protective effect of verapamil was seen when the pretreatment time was varied from 30 sec to 5 min. A previous report on the effect of oral verapamil on arachidonate induced sudden death in rabbits (9) also failed to demonstrate any statistically significant beneficial action of this drug in terms of survival. In contrast, *ex vivo* studies, in which verapamil is administered to experimental subjects prior to blood collection and platelet aggregation measurements, have indicated that verapamil is inhibitory under those circumstances. We are currently examining the effects of verapamil and nifedipine in thrombosis models when given chronically and by different routes of administration.

A possible explanation for the disparate findings in the present studies is the potential involvement of vasoconstriction or endothelial damage in the *in vivo* models. Nifedipine, in the intact animal, might inhibit these addi-

tional aspects of a thrombotic episode to a greater extent than verapamil. Other potential explanations include metabolism of nifedipine to a product with greater platelet inhibitory activity, and possible major differences in drug distribution *in vivo*. Further studies are required to evaluate the mechanism of the antiplatelet effects of these agents in the intact animal.

If the antithrombotic effects of calcium channel blockers extend to the human, new applications for these drugs might be possible. Furthermore, differences in the antithrombotic actions of calcium blockers could provide new criteria for the appropriate use of these drugs in cardiovascular diseases involving platelets.

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1. Henry PD. Comparative pharmacology of calcium antagonists: Nifedipine, verapamil and diltiazem. Amer J Cardiol **46**:1047–1058, 1980.
2. Owen NE, Feinberg H, Le Breton GC. Epinephrine induces Ca^{2+} uptake in human platelets. Amer J Physiol **239**:H483–H488, 1980.
3. Chierchia S, Crea F, Bernini W, Gensini G, Parodi O, de Caterina R, Maseri A. Antiplatelet effects of verapamil in man. Amer J Cardiol **47**:399, 1981. [Abstract]
4. Ikeda Y, Kikuchi M, Toyama K, Watanabe K, Ando Y. Inhibition of human platelet functions by verapamil. Thromb Haemostasis **45**:158–161, 1981.
5. Ono H, Kimura M. Effect of Ca^{2+}-antagonistic vasodilators, diltiazem, nifedipine, perhexiline and verapamil, on platelet aggregation *in vitro*. Arzneim Forsch **31**:1131–1134, 1981.
6. Ribiero LGT, Brandon TA, Horak JK, Ware JA, Miller RR, Solis RT. Inhibition of platelet aggregation by verapamil: Quantification by *in vivo* and *in vitro* techniques. J Cardiovasc Pharmacol **4**:170–173, 1982.
7. Johnsson H. Effects by nifedipine (Adalat) on platelet function *in vitro* and *in vivo*. Thromb Res **21**:523–528, 1981.
8. Rao GHR, Reddy KR, White JG. Influence of trifluoperazine on platelet aggregation and disaggregation. Prostaglandins Med **5**:221–234, 1980.
9. Okamatsu S, Peck RC, Lefer AM, Effects of calcium channel blockers on arachidonate-induced sudden death in rabbits. Proc Soc Exp Biol Med **166**:551–555, 1981.
10. Pumphrey CW, Fuster V, Dewanjee MK, Murphy KP, Vlietstra RE, Kaye MP. A new *in vivo* model of arterial thrombosis: The effect of administration of ticlopidine and verapamil in dogs. Thromb Res **28**: 663–675, 1982.
11. Henry PD, Bentley KI. Suppression of atherogenesis in cholesterol-fed rabbit treated with nifedipine. J Clin Invest **68**:1366–1369, 1981.
12. Willis AL, Nagel B, Churchill V, Whyte MA, Smith DL, Mahmud I, Puppione DL. Antiatherosclerotic effects of nicardipine and nifedipine in cholesterol-fed rabbits. Arteriosclerosis **5**:250–255, 1985.
13. Silver MJ, Hoch W, Kocsis JJ, Ingerman CM, Smith JB. Arachidonic acid causes sudden death in rabbits. Science **183**:1085–1087, 1974.
14. Kohler C, Wooding W, Ellenbogen L. Intravenous arachidonic acid in the mouse: A model for the evaluation of antithrombotic drugs. Thromb Res **9**:67–80, 1976.
15. Myers A, Penhos J, Ramey E, Ramwell P. Thromboxane agonism and antagonism in a mouse sudden death model. J Pharmacol Exp Ther **224**:369–372, 1983.
16. DiMinno G, Silver MJ. Mouse antithrombotic assay: A simple method for the evaluation of antithrombotic drugs *in vivo;* potentiation of antithrombotic activity by ethyl alcohol. J Pharmacol Exp Ther **225**:57–60, 1983.
17. Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature (London) **194**:927–929, 1962.
18. Snedecor GW, Cochran WG. Statistical Methods. Ames, Iowa State Univ. Press, 6th ed., pp135–198, 1967.
19. Torres Duarte AP, Ramwell P, Myers AK. Sex differences in mouse platelet aggregation. Thromb Res **43**:33–39, 1986.
20. Flaim SF. Comparative pharmacology of calcium blockers based on studies of vascular smooth muscle. In: Flaim SF, Zelis R, eds. Calcium Blockers: Mechanism of Action and Clinical Applications. Baltimore, Urban & Schwarzenberg, pp155–178, 1982.
21. Triggle DJ. Biochemical pharmacology of calcium blockers. In: Flaim SF, Zelis R, eds. Calcium Blockers: Mechanism of Action and Clinical Applications. Baltimore, Urban & Schwarzenberg, pp121–134, 1982.
22. Lefer AM, Burke SE, Smith JB. Role of thromboxanes and prostaglandin endoperoxides in the pathogenesis of eicosanoid induced sudden death. Thromb Res **32**: 311–320, 1983.

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