Regulation of the tension of human chorionic vasculature by histamine and prostaglandin $F_{2\alpha}$

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The calcium dependence of potassium chloride–, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)–, and histamine-induced contractions of human chorionic vasculature segments was investigated. In physiologic buffer that contained 1.5 mM calcium chloride, 60 mM potassium chloride induced a rapid and sustained contraction of the vasculature. Potassium chloride–induced contractions were completely inhibited by the calcium channel blockers diltiazem and nifedipine or by excluding Ca^{2+} from the medium. Histamine (100 μM) induced a rapid increase in vascular tension in physiologic buffer which rapidly faded or desensitized after maximal tension was obtained. The maximal contractile responses to histamine were reduced approximately 50% by diltiazem and nifedipine in physiologic buffer or by suspension in calcium-free medium (0CaPB). Pretreatment of vessels with 20 mM caffeine in 0CaPB completely abolished histamine-dependent contractile responses. Prostaglandin $F_{2\alpha}$ (100 nM)–induced increases in vascular tension developed slowly but remained maximal for at least 40 minutes. Contractile responses to $PGF_{2\alpha}$ were reduced 50% to 65% by diltiazem and nifedipine in physiologic buffer or by suspension in 0CaPB. Caffeine pretreatment failed to alter the contractile response to $PGF_{2\alpha}$ in 0CaPB. The differences in responsiveness of potassium chloride, histamine, and $PGF_{2\alpha}$ under the various conditions used suggest that these agents act by different mechanisms to elicit contractions in chorionic vessels. The potential roles of $PGF_{2\alpha}$, histamine, and calcium channel blockers in modulating the fetoplacental circulation is discussed.

(Key words: Placenta, vascular tension, autacoids, calcium channel blockers)

Fetal distress has been defined as a state of jeopardy of the fetus that, if untreated, leads to significant neonatal morbidity and mortality. Early investigative efforts correlated fetal distress with insufficient oxygen supply to the fetus secondary to a reduction in either uteroplacental or fetoplacental blood flow. Hypoxemia in the fetus results in a shift from aerobic to anaerobic metabolism with the accumulation of lactic acid which ultimately causes fetal brain cells to swell and rupture. With sufficient brain cell necrosis, fetal damage or death will occur.

The mechanisms regulating fetoplacental

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blood flow in the term human infant are not completely understood, but factors regulating the small-diameter resistance vessels of the placenta are believed to be important in this regard.[1] Because placental vasculature lacks direct innervation, fetoplacental blood flow appears to be regulated by vasoactive substances.[2] The levels of numerous vasoconstrictive substances—such as prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)—increase dramatically in the fetoplacental circulation at the time of labor,[3-6] and contract chorionic and umbilical cord vasculature.[7-11]

The walls of the resistance arteries supplying individual exchange villi have been shown to be a site of synthesis of prostaglandins in the human fetus. In addition, the release of prostaglandins by placental tissue has been demonstrated in the presence of acute hypoxia.[12] Therefore, vasoconstrictive agents, such as $PGF_{2\alpha}$, may be involved in regulating fetoplacental blood flow by constricting the resistance vessels in the chorion and villi. A reduction in fetal blood flow induced by these agents could contribute to fetal distress and hypoxia during labor.

Current efforts to treat such problems amount to controlling the maternal symptoms and are not aimed at the primary disorder. If these vasoactive agents could be shown to have direct effects on the regulatory role of the blood flow, antagonists might promote reversal of this distress. Because vascular smooth muscle and myometrial contractions are inhibited by calcium channel blockers,[13,14] these vasorelaxing agents may improve perfusion to the fetus and decrease fetal distress and, therefore, warrant further study.

In this study, the contractile effects of $PGF_{2\alpha}$, histamine, and potassium chloride on segments of human chorionic plate vasculature were investigated. Although it has not been previously reported, we found that histamine strongly contracted these vessels. The dependence of these agents on extracellular and intracellular calcium sources for their contractile effects was also investigated.

Materials and methods

Human placentas were obtained from normal uncomplicated term pregnancies of women delivered vaginally or by cesarean section. The following criteria were used to define normal pregnancies: a gestational age of between 37 and 42 weeks; no overt maternal medical complications; and no neonatal or gross placental abnormalities, including the absence of meconium staining. Placental vessels (arteries and veins) were dissected from the chorionic plate and placed in physiologic buffer at 5°C within 10 to 30 minutes of delivery. Physiologic buffer consisted of 10 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) (pH, 7.4), 130 mM sodium chloride, 5 mM potassium chloride, 0.3 mM potassium dihydrogen phosphate (KH_2PO_4), 10 mM glucose, 1 mM magnesium chloride, and 1.5 mM calcium chloride. Placental vessels were maintained at 5°C and used over a period of 0 to 3 days.

Vessels were cut into two 6-mm tubular segments. One half was used for the control and the other half for drug treatment studies. Tubular vessel segments (average diameter, 1 to 3 mm) were then fitted with triangles of No. 4 surgical steel wire, horizontally suspended in oxygenated water baths containing 18 mL of physiologic buffer at 37°C, and attached to a Grass transducer (Grass Instruments, Quincy, Mass).[11] The vascular preparations were equilibrated for 30 minutes and the tension was adjusted every 15 minutes by 1.0 g increments until a final resting tension of approximately 1.0 g was obtained.

Each set of vessels was standardized with 60 mM potassium chloride solution at the beginning of each experiment. Subsequently, the vessels were washed with physiologic buffer, or modified physiologic buffer and allowed to relax and equilibrate. After 60 minutes, the vessels were challenged with either histamine or $PGF_{2\alpha}$. Next, various drugs were added directly to the bath medium to determine the presence or absence of calcium dependence on the contractile responses produced by histamine or $PGF_{2\alpha}$. Changes in tension were recorded with a Narco physiograph (Narco BioSystems, Inc, Houston, Tex). Studies showed that storing the vessels in physiologic buffer at 5°C for up to 3 days did not alter their responsiveness to potassium chloride, or $PGF_{2\alpha}$, or histamine. The dose response studies were performed by adding cumulative doses of $PGF_{2\alpha}$, or histamine in physiologic buffer to the chamber bath.

Results

Effects of potassium chloride on tension

In preliminary studies, the effect of membrane depolarizing concentrations of potassium chloride on chorionic vascular tension was examined. Potassium chloride (60 mM) induced tension increases ranging from 1.4 to 3.0 g in physiologic buffer which contained 1.5 mM calcium chloride (*Figure 1*). Half-maximal contractile responses induced by potassium chloride were obtained within 2 to 3 minutes and maximal contractions were sustained for at least 40 minutes. Vessels that were first contracted in 60 mM potassium chloride medium relaxed to baseline tension within 10 minutes when suspended in physiologic buffer or in calcium-free medium (0CaPB) containing 0.1 mM (ethylene glycol bis (β-aminoethyl ether) N, N'-tetraacetic acid (EGTA) (*Figure 1*). Preincubating the vessels with 10 μM diltiazem or 1 μM nifedipine almost completely blocked (>90%) the contractile response caused by 60 mM potassium chloride (*Figure 1* and *Table*). Furthermore, potassium chloride was unable to induce contractions in 0CaPB (*Figure 1* and *Table*). Together, these observations suggest that potassium chloride–induced contractions were totally dependent on extracellular Ca^{2+} influx through voltage-dependent, L-type channels.[15]

Effects of $PGF_{2\alpha}$ and histamine on tension in physiologic buffer

Prostaglandin $F_{2\alpha}$ (10 to 150 nM) induced slow but relatively strong increases in chorionic vasculature tension (*Figure 1*). Half-maximal increases in tension were obtained with approximately 25 nM $PGF_{2\alpha}$, and maximal contractions were obtained with 100 nM $PGF_{2\alpha}$ (*Figures 1* and *2*). Maximal tension changes were sustained for at least 40 minutes without fading in the presence of $PGF_{2\alpha}$.

Histamine (50 to 100 μM) was found to increase chorionic vasculature tension as well (*Figures 1* and *2*). Half-maximal and maximal increases in tension were induced by approximately 32 μM and 80 μM histamine, respectively. The rate of histamine-induced contractions was more rapid than with $PGF_{2\alpha}$, but maximal histamine-induced contractions were not sustained for more than 2 to 5 minutes. In the presence of histamine, the vessels spontaneously relaxed to half maximal tension in 10 to 20 minutes. The muscle tension then remained above baseline for at least 90 minutes in most vessel preparations (*Figure 3*). If vessels were first challenged with 100 μM histamine to produce maximal tension and then immediately washed with physiologic buffer before the vessels relaxed, the maximal tension increase of a second dose of histamine was only 63% of the first. Immediate washing with physiologic buffer and a third histamine challenge produced an even smaller response (39%) (*Figure 3*). These studies indicate that histamine responses in chorionic vessels are subject to desensitization, consistent with studies in other vascular beds.[16] The histamine responses were strongly attenuated by 5 μM pyrilamine, an H_1 antagonist, but not by 5 μM cimetidine, an H_2 antagonist, showing that histamine acts on H_1 receptors to induce contractions in these vessels.

Calcium dependence of $PGF_{2\alpha}$ and histamine

The dependence of $PGF_{2\alpha}$- and histamine-induced contractions on external calcium was examined by suspending tissue in 0CaPB or in physiologic buffer containing diltiazem or nifedipine. In 0CaPB, the vessels relaxed slightly by 0.1 g before establishing a new baseline. The maximal tension induced by 100 nM $PGF_{2\alpha}$ on 0CaPB was only 0.8 g or 33% of the maximal response obtained in physiologic buffer (*Table 1* and *Figure 1*). However, the maximal contraction obtained in 0CaPB was sustained for at least 40 minutes. Pretreatment of vessels with 20 mM caffeine in 0CaPB to deplete sarcoplasmic reticulum Ca^{2+} did not affect the responsiveness to 100 nM $PGF_{2\alpha}$ (*Figure 4*). However, caffeine by itself provoked a small and transient increase in tension, presumably because of the release of intracellular Ca^{2+}.

Maximal histamine-induced contractions were approximately 50% lower in 0CaPB than in physiologic buffer (*Table* and *Figure 1*). In the absence of extracellular Ca^{2+}, the vessels completely relaxed to baseline within 20 to 30 minutes (*Figures 1* and *4*). In vessels preincubated with 20 mM caffeine in 0CaPB, histamine no longer had any significant contracchannel blockers, nifedipine and diltiazem, was tested. In vessels preincubated 10 minutes in physiologic buffer with 1 μM nifedipine or 10 μM diltiazem, the contractile responses to 100 nM $PGF_{2\alpha}$ were 49% and 46% of the maximal responses obtained in physiologic buffer (*Table* and *Figure 1*). Contractile responses to 100 μM histamine were 49% to 57% of maximum in vessels preincubated with nifedipine and diltiazem, respectively.

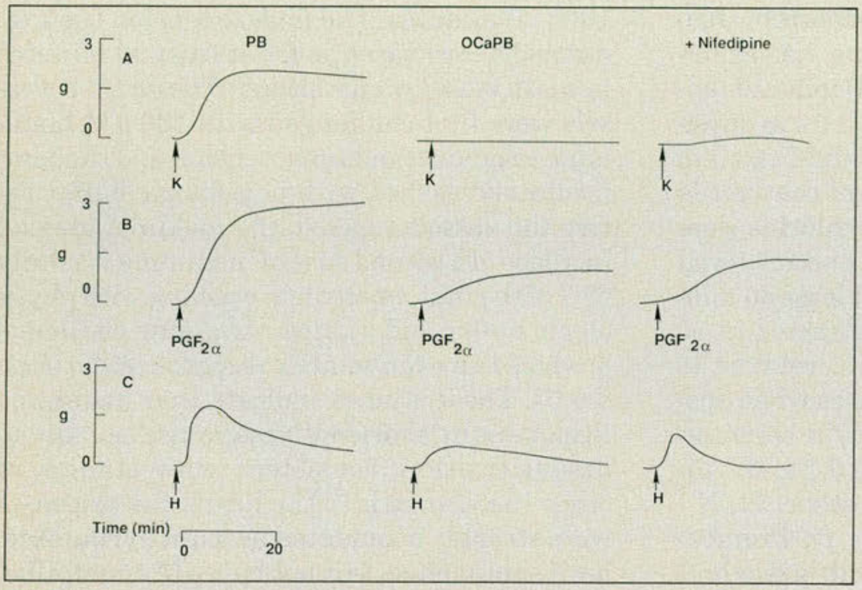

Figure 1. *Contractile responses induced by 60 mM potassium chloride (K), 100 nM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and 100 μM histamine (H) in physiological buffer (PB), calcium-free medium (0CaPB), and physiologic buffer + 1 μM nifedipine. Tracings are representative of one experiment. Similar results have been obtained in at least two other separate experiments.*

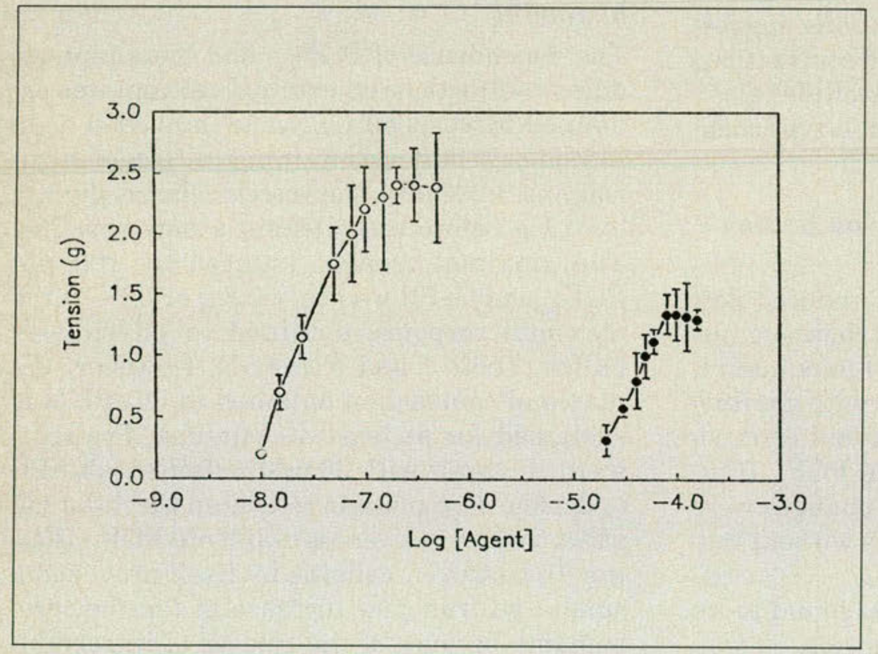

Figure 2. *Dose dependence for $PGF_{2\alpha}$ (*open circle*) and histamine (*solid circle*) in human chorionic vasculature. Results are representative of 3 experiments performed in quadruplicate. Values represent the mean ± SD.*

tile effect, although in control vessels preincubated in 0CaPB without caffeine, histamine increased tension by 0.6 g (*Figure 4*).

The effect of pretreating vessels with maximally effective concentrations of the calcium

Discussion

In this study, potassium chloride, $PGF_{2\alpha}$, and histamine were shown to produce strong contractile responses in human chorionic vasculature. Since calcium is known to play an important role in the activation of smooth muscle contractile proteins,[15,17-21] the calcium requirements for these vasoconstrictor-induced contractions were examined. Although this study originally focused on the contractile responses to $PGF_{2\alpha}$ and histamine, comparative studies with potassium chloride were also included because the mechanism of potassium chloride–induced contractions is better understood.

High concentrations of extracellular potassium chloride depolarize vascular smooth muscle cells to "open" voltage-dependent L-type calcium channels, whereby extracellular Ca^{2+} can enter the cell down its concentration gradient to stimulate contractile proteins. Calcium channel blockers such as diltiazem or nifedipine inactivate L-type channels and, therefore, prevent Ca^{2+} influx into the cells. Consistent with this mechanism, potassium chloride–dependent contractions of human chorionic vessels were shown here to be prevented by diltiazem and nifedipine in physiologic buffer or abolished in Ca^{2+}-free medium.

Pharmacologic effects of $PGF_{2\alpha}$ and histamine

In agreement with previous studies,[6-10] $PGF_{2\alpha}$ strongly contracted human placental chorionic vessels. The rate of induction of contractile responses to $PGF_{2\alpha}$ were relatively slow, but maximal contractions were sustained and, thus, were not characterized by desensitization (*Figure 1*). In comparison, histamine induced more rapid contractile responses which were relatively transient in duration. Exposure of the vasculature to single or repeated doses of histamine resulted in the loss of contractile responsiveness (*Figures 1* and *3*). The mechanism(s) for desensitization are not well understood, but increases in intracellular calcium and/or stimulation of protein kinases have been implicated.[15,22,23]

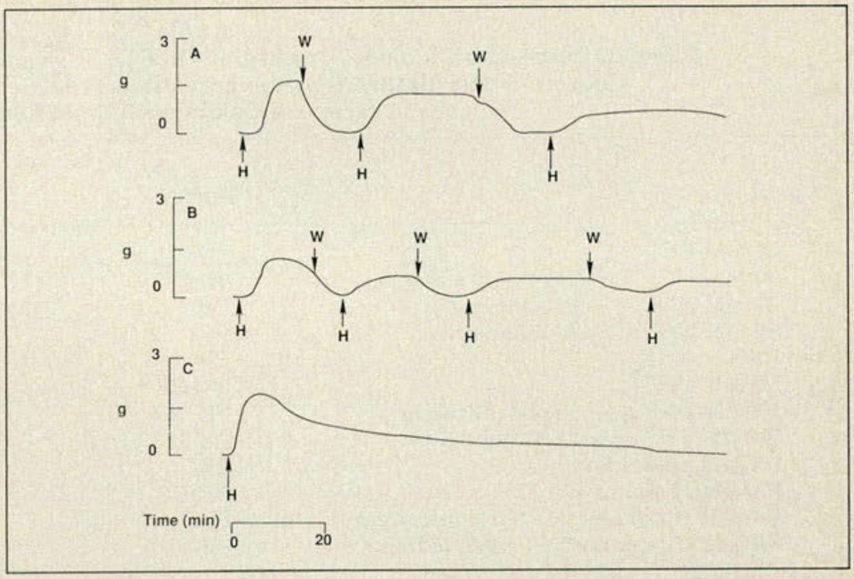

Figure 3. *Effect of repetitive and single doses of histamine on chorionic vessel tension. A and B represent effects of multiple doses of 100 μM histamine (H) on contractions. Vessels are washed (W) between doses with physiologic buffer. C shows the time course of a single dose of 100 μM histamine.*

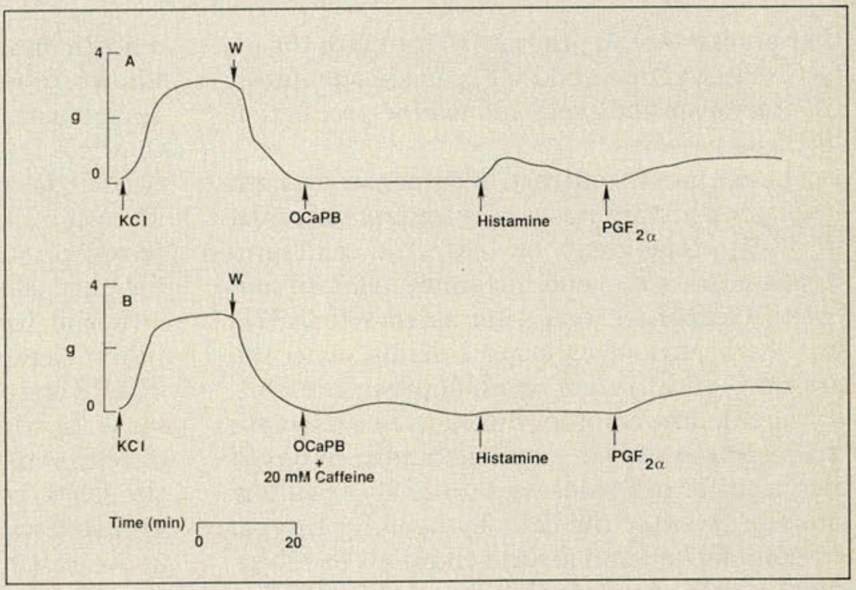

Figure 4. *Histamine and $PGF_{2\alpha}$ contractile responses in vessels suspended in 0CaPB (A) or in 0CaPB + 20 mM caffeine (B). Paired vessels were first contracted with 60 mM potassium chloride in physiologic buffer and then suspended in 0CaPB. After pretreatment with either 0CaPB or 0CaPB + 20 mM caffeine, the medium was replaced with 0CaPB, and 100 nM $PGF_{2\alpha}$. Results are representative of four experiments.*

Increased intracellular calcium could not be solely responsible for desensitization because potassium chloride– and $PGF_{2\alpha}$-induced responses did not desensitize. However, the response to histamine faded faster in 0CaPB than in physiologic buffer, suggesting that dissipation of intracellular calcium stores may be contributory. Clearly, further studies will be required to resolve the mechanism(s) involved in desensitization, and a comparison of the biochemical responses evoked by $PGF_{2\alpha}$ and histamine may be useful to further understand the mechanisms regulating contraction in human chorionic vessels. The results of this investigation suggest that $PGF_{2\alpha}$ and histamine must invoke different biochemical mechanisms (although some pathways may be shared) to contract chorionic vasculature. Fur-

Table
Effect of Potassium Chloride, Prostaglandin $F_{2\alpha}$, ($PGF_{2\alpha}$), and Histamine on Human Chorionic Vasculature Tension in Calcium-Free Medium (0CaPB) and in the Presence of Calcium Channel Blockers*

Agent		Buffer	% Maximal tension	% Inhibition
A	60 mM KCl	PB*	100	0
	60 MM KCl	0CaPB	0	100
	60 mM KCl + 10 μM diltiazem	PB	5 ± 5	95
	60 mM KCl + 1 μM nifedipine	PB	0	100
B	100 nM $PGF_{2\alpha}$	PB	100	0
	100 nM $PGE_{2\alpha}$	0CaPB	33 ± 15	67
	100 nM $PGF_{2\alpha}$ + 10 μM diltiazem	PB	54 ± 11	46
	100 nM $PFG_{2\alpha}$ + 1 μM nifedipine	PB	51 ± 9	49
C	100 μM Histaimine	PB	100	0
	100 μM Histamine	0CaPB	45 ± 11	55
	100 μM Histamine + 10 μM diltiazem	PB	43 ± 15	57
	100 μM Histamine + 1 μM nifedipine	PB	51 ± 5	49

*Results represent the mean ± SD of results from at least three separate experiments. In each experiment, a pair of vessels was used as a control and a matched pair was treated with either Ca^{2+} channel blockers or 0CaPB. In all experiments, vessels were first contracted with 60 mM KCl in physiologic buffer to determine viability. Responses are calculated relative to the maximal response of the agent tested.
PB = physiologic buffer.

ther studies are in progress to compare the effects of histamine and $PGF_{2\alpha}$ on phosphoinositide turnover and cyclic nucleotide production in these vessels.

The maximal contractile responses to both $PGF_{2\alpha}$ and histamine were approximately 50% to 70%, dependent on external calcium. Prostaglandin $F_{2\alpha}$- and histamine-induced contractile responses were attenuated 46% to 67% in the absence of external calcium or in the presence of diltiazem or nifedipine. The ability of calcium channel blockers to attenuate the responses of $PGF_{2\alpha}$ and histamine in physiologic buffer indicates that these agents allow calcium to enter the cells by opening L-type, voltage-dependent calcium channels or receptor-operated channels.[15] To account for $PGF_{2\alpha}$- and histamine-induced contractile responses in the absence of external calcium, these agents may either stimulate the release of calcium from intracellular stores or increase the sensitivity of contractile elements to calcium.

Prostaglandins have been observed to indirectly open receptor-operated Ca^{2+} channels and/or to release intracellular calcium in certain types of smooth muscle cells,[24,25] and in human placental arteries.[13,14] Histamine also increases cytosolic Ca^{2+} in certain vascular smooth muscle beds.[13,22] Caffeine, an agent known to release and deplete Ca^{2+} from the sarcoplasmic reticulum,[15] was shown here not to affect the contractile response to $PGF_{2\alpha}$ in 0CaPB but abolished the histamine responses. Therefore, histamine but not $PGF_{2\alpha}$ appears to rely on sarcoplasmic reticulum Ca^{2+} stores for part of its effect. It is likely that $PGF_{2\alpha}$ either utilizes a caffeine insensitive intracellular calcium pool or the response observed in 0CaPB may not be dependent on calcium release. In other vascular beds, various vasoconstrictors may activate G proteins to increase the Ca^{2+} sensitivity of myosin light chain kinase[26] without increases in cytosolic Ca^{2+} above resting levels.

Clinical aspects of histamine and calcium channel blockers

To our knowledge, this is the first report showing that histamine induces contractions of the human chorionic vasculature. Although the physiologic relevance of histamine-mediated contractions is unknown, certain speculations can be made. For instance, if histamine is released in response to antigens in pregnancy or in response to labor (as are prostaglandins), circulating or locally produced histamine could constrict chorionic vessels and reduce fetoplacental blood flow. Potentially, histamine could also constrict other vessels—such as umbilical arteries and veins—further compromising perfusion. Because histamine affects H_1 receptors in these vessels, predictably H_1 antagonists may oppose the effect of histamine and improve perfusion. At this time, the effects of antihistamines on fetoplacental blood flow are unknown, but theoretically these agents may have some usefulness in treating fetal hypoxia.

As noted by Maigaard and coworkers,[13] nifedipine markedly attenuated the contractile responses induced by $PGF_{2\alpha}$. Diltiazem was also found here to effectively attenuate $PGF_{2\alpha}$ responses, and both nifedipine and diltiazem attenuated responses to histamine. Because $PGF_{2\alpha}$ and possibly histamine may increase in the fetal circulation during labor, calcium channel blockers may improve fetoplacental perfusion. However, decreases in chorionic vascular resistance and a decrease in maternal vascular resistance could simultaneously decrease maternal uterine blood pressure and attenuate exchange of gases and nutrients across the uteroplacental unit. This dual action could thus offset any beneficial effects of vasodilation of chorionic vessels on the fetal side.

Diltiazem, in contrast to nifedipine, slightly depresses myocardial function by a direct mechanism [27] and, therefore, potentially could have some depressing effect on the fetal heart. Another point is that reflex tachycardia is not as great with diltiazem as with nifedipine, and this difference may have some effect on the clinical outcome when these agents are used. The use of calcium channel blockers will require extensive clinical investigation and, therefore, they should be used cautiously until a better understanding of their pharmacologic responses can be ascertained.

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$PGF_{2\alpha}$ was donated by the Upjohn Company, Kalamazoo, Mich. Histamine, caffeine, pyrilamine, and EGTA were obtained from Sigma Chemical Company, St Louis, Mo. Diltiazem was supplied by Marion Laboratories, Inc, Kansas City, Mo, and nifedipine was obtained from the Pfizer Company, Brooklyn, NY.

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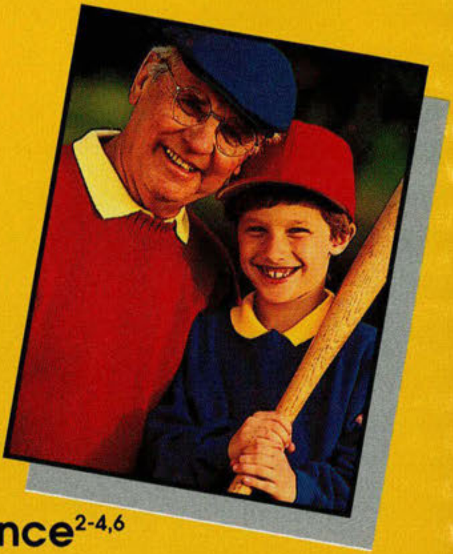

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BRIEF SUMMARY

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WARNINGS

1. **Cardiac Conduction.** CARDIZEM prolongs AV node refractory periods without significantly prolonging sinus node recovery time, except in patients with sick sinus syndrome. This effect may rarely result in abnormally slow heart rates (particularly in patients with sick sinus syndrome) or second- or third-degree AV block (six of 1,243 patients for 0.48%). Concomitant use of diltiazem with beta-blockers or digitalis may result in additive effects on cardiac conduction. A patient with Prinzmetal's angina developed periods of asystole (2 to 5 seconds) after a single dose of 60 mg of diltiazem.
2. **Congestive Heart Failure.** Although diltiazem has a negative inotropic effect in isolated animal tissue preparations, hemodynamic studies in humans with normal ventricular function have not shown a reduction in cardiac index nor consistent negative effects on contractility (dp/dt). Experience with the use of CARDIZEM alone or in combination with beta-blockers in patients with impaired ventricular function is very limited. Caution should be exercised when using the drug in such patients.
3. **Hypotension.** Decreases in blood pressure associated with CARDIZEM therapy may occasionally result in symptomatic hypotension.
4. **Acute Hepatic Injury.** In rare instances, significant elevations in enzymes such as alkaline phosphatase, LDH, SGOT, SGPT, and other phenomena consistent with acute hepatic injury have been noted. These reactions have been reversible upon discontinuation of drug therapy. The relationship to CARDIZEM is uncertain in most cases, but probable in some. (See PRECAUTIONS.)

PRECAUTIONS

General. CARDIZEM (diltiazem hydrochloride) is extensively metabolized by the liver and excreted by the kidneys and in bile. As with any drug given over prolonged periods, laboratory parameters should be monitored at regular intervals. The drug should be used with caution in patients with impaired renal or hepatic function. In subacute and chronic dog and rat studies designed to produce toxicity, high doses of diltiazem were associated with hepatic damage. In special subacute hepatic studies, oral doses of 125 mg/kg and higher in rats were associated with histological changes in the liver which were reversible when the drug was discontinued. In dogs, doses of 20 mg/kg were also associated with hepatic changes; however, these changes were reversible with continued dosing.

Dermatological events (see ADVERSE REACTIONS section) may be transient and may disappear despite continued use of CARDIZEM. However, skin eruptions progressing to erythema multiforme and/or exfoliative dermatitis have also been infrequently reported. Should a dermatologic reaction persist, the drug should be discontinued.

Drug Interaction. Due to the potential for additive effects, caution and careful titration are warranted in patients receiving CARDIZEM concomitantly with any agents known to affect cardiac contractility and/or conduction. (See WARNINGS.)

Pharmacologic studies indicate that there may be additive effects in prolonging AV conduction when using beta-blockers or digitalis concomitantly with CARDIZEM. (See WARNINGS.)

As with all drugs, care should be exercised when treating patients with multiple medications. CARDIZEM undergoes biotransformation by cytochrome P-450 mixed function oxidase. Coadministration of CARDIZEM with other agents which follow the same route of biotransformation may result in the competitive inhibition of metabolism. Dosages of similarly metabolized drugs, particularly those of low therapeutic ratio or in patients with renal and/or hepatic impairment, may require adjustment when starting or stopping concomitantly administered CARDIZEM to maintain optimum therapeutic blood levels.

Beta-blockers: Controlled and uncontrolled domestic studies suggest that concomitant use of CARDIZEM and beta-blockers or digitalis is usually well tolerated. Available data are not sufficient, however, to predict the effects of concomitant treatment, particularly in patients with left ventricular dysfunction or cardiac conduction abnormalities.

Administration of CARDIZEM (diltiazem hydrochloride) concomitantly with propranolol in five normal volunteers resulted in increased propranolol levels in all subjects and bioavailability of propranolol was increased approximately 50%. If combination therapy is initiated or withdrawn in conjunction with propranolol, an adjustment in the propranolol dose may be warranted. (See WARNINGS.)

Cimetidine: A study in six healthy volunteers has shown a significant increase in peak diltiazem plasma levels (58%) and area-under-the-curve (53%) after a 1-week course of cimetidine at 1,200 mg per day and diltiazem 60 mg per day. Ranitidine produced smaller, nonsignificant increases. The effect may be mediated by cimetidine's known inhibition of hepatic cytochrome P-450, the enzyme system probably responsible for the first-pass metabolism of diltiazem. Patients currently receiving diltiazem therapy should be carefully monitored for a change in pharmacological effect when initiating and discontinuing therapy with cimetidine. An adjustment in the diltiazem dose may be warranted.

Digitalis: Administration of CARDIZEM with digoxin in 24 healthy male subjects increased plasma digoxin concentrations approximately 20%. Another investigator found no increase in digoxin levels in 12 patients with coronary artery disease. Since there have been conflicting results regarding the effect of digoxin levels, it is recommended that digoxin levels be monitored when initiating, adjusting, and discontinuing CARDIZEM therapy to avoid possible over- or under-digitalization. (See WARNINGS.)

Anesthetics: The depression of cardiac contractility, conductivity, and automaticity as well as the vascular dilation associated with anesthetics may be potentiated by calcium channel blockers. When used concomitantly, anesthetics and calcium blockers should be titrated carefully.

Carcinogenesis, Mutagenesis, Impairment of Fertility. A 24-month study in rats and a 21-month study in mice showed no evidence of carcinogenicity. There was also no mutagenic response in in vitro bacterial tests. No intrinsic effect on fertility was observed in rats.

Pregnancy. Category C. Reproduction studies have been conducted in mice, rats, and rabbits. Administration of doses ranging from five to ten times greater (on a mg/kg basis) than the daily recommended therapeutic dose has resulted in embryo and fetal lethality. These doses, in some studies, have been reported to cause skeletal abnormalities. In the perinatal/postnatal studies, there was some reduction in early individual pup weights and survival rates. There was an increased incidence of stillbirths at doses of 20 times the human dose or greater.

There are no well-controlled studies in pregnant women; therefore, use CARDIZEM in pregnant women only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers. Diltiazem is excreted in human milk. One report suggests that concentrations in breast milk may approximate serum levels. If use of CARDIZEM is deemed essential, an alternative method of infant feeding should be instituted.

Pediatric Use. Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

Serious adverse reactions have been rare in studies carried out to date, but it should be recognized that patients with impaired ventricular function and cardiac conduction abnormalities have usually been excluded.

In domestic placebo-controlled angina trials, the incidence of adverse reactions reported during CARDIZEM therapy was not greater than that reported during placebo therapy.

The following represent occurrences observed in clinical studies of angina patients. In many cases, the relationship to CARDIZEM has not been established. The most common occurrences from these studies, as well as their frequency of presentation are: edema (2.4%), headache (2.1%), nausea (1.9%), dizziness (1.5%), rash (1.3%), asthenia (1.2%). In addition, the following events were reported infrequently (less than 1%):

Cardiovascular: Angina, arrhythmia, AV block (first degree), AV block (second or third degree — see conduction warning), bradycardia, bundle branch block, congestive heart failure, ECG abnormality, flushing, hypotension, palpitations, syncope, tachycardia, ventricular extrasystoles.

Nervous System: Abnormal dreams, amnesia, depression, gait abnormality, hallucinations, insomnia, nervousness, paresthesia, personality change, somnolence, tremor.

Gastrointestinal: Anorexia, constipation, diarrhea, dysgeusia, dyspepsia, mild elevations of alkaline phosphatase, SGOT, SGPT, and LDH (see hepatic warnings), thirst, vomiting, weight increase.

Dermatological: Petechiae, photosensitivity, pruritus, urticaria.

Other: Amblyopia, CPK elevation, dry mouth, dyspnea, epistaxis, eye irritation, hyperglycemia, hyperuricemia, impotence, muscle cramps, nasal congestion, nocturia, osteoarticular pain, polyuria, sexual difficulties, tinnitus.

The following postmarketing events have been reported infrequently in patients receiving CARDIZEM: alopecia, erythema multiforme, extrapyramidal symptoms, gingival hyperplasia, hemolytic anemia, increased bleeding time, leukopenia, purpura, retinopathy, and thrombocytopenia. There have been observed cases of a generalized rash, characterized as leukocytoclastic vasculitis. In addition, events such as myocardial infarction have been observed which are not readily distinguishable from the natural history of the disease in these patients. A definitive cause and effect relationship between these events and CARDIZEM therapy cannot yet be established. Exfoliative dermatitis (proven by rechallenge) has also been reported.

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