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CORONARY ANAPHYLAXIS *in vitro*

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1 The reactivity of isolated coronary arteries and cardiac veins of the calf to selected chemical mediators of anaphylaxis and to sensitizing antigen (horse plasma) was studied.

2 Both the coronary arteries and cardiac veins contracted to bradykinin, 5-hydroxytryptamine (5-HT), prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, histamine and carbachol.

3 Isoprenaline and PGE_1 relaxed vessels which had been partially contracted by $PGF_{2\alpha}$, PGE_2, histamine, 5-HT, bradykinin, carbachol or antigen.

4 Horse plasma (antigen) contracted coronary vessels obtained from sensitized calves, but not from control calves. Subsequent antigen 'challenge' produced 'desensitization' (tachyphylaxis). After 1 or 2 h of rest, the anaphylactic response partially recovered although there was no change in tissue reactivity to the exogenous agonists.

5 Specific doses of atropine, mepyramine (H_1-blocker) and methysergide (5-HT antagonist) did not modify the anaphylactic reaction in coronary arteries, suggesting a negligible role for these biogenic amines.

6 Compound PRD-92-EA (a new anti-allergic agent) exhibited non-specific receptor blocking activity towards histamine, 5-HT and carbachol and inhibited the coronary anaphylactic reaction.

Introduction

The heart is considered to be a target organ of immediate hypersensitivity in the guinea-pig (Capurro & Levi, 1975; Liebig, Bernauer & Peskar, 1975; Anhut, Bernauer & Peskar, 1977). The cardiac anaphylactic reaction is characterized by a long-lasting decrease in coronary flow (Wilcox & Andrus, 1938; Andrus & Wilcox, 1939; Hahn & Bernauer, 1970; Capurro & Levi, 1975) which suggests that coronary vasoconstriction may be a characteristic feature of cardiac anaphylaxis.

Anaphylactic reactivity has been demonstrated in isolated vascular smooth muscle (Lecomte, 1958; Eyre, 1971; Chand & Eyre, 1977a,b; and reviewed by Chand & Eyre, 1978a). Therefore it was decided to investigate the pharmacological reactivity of sensitized isolated coronary arteries and veins to antigen and to some chemical mediators.

Methods

All animals used in this study were male Jersey or Guernsey calves between 3 and 12 weeks of age (25 to 65 kg) which had been sensitized to horse plasma as previously described (Eyre, Lewis & Wells, 1973). Calves were killed by intravenous overdose of pentobarbitone sodium (40 mg/kg); the heart was removed into cold Krebs solution. The coronary arteries and great cardiac (coronary) veins were dissected out, separated free of fat and cut helically following the methods previously described (Furchgott & Bhadrakom, 1953; Eyre, 1971). Each single vessel strip was bisected longitudinally to produce 'twin' strips of approximately 2 to 3 cm × 2 to 3 mm in size. Each was mounted in a separate bath containing Krebs solution mixed with 5% CO_2 in oxygen at 37°C. Tissues were allowed to equilibrate for at least 1 h under a resting tension of 2.0 g for the artery and 1.0 g for the vein. Control arteries and veins from unsensitized calves were set up in the same way.

Single or cumulative dose-responses to two to four agonists were recorded with an E & M isotonic myograph transducer connected to an E & M Desk Model, 4-channel Physiograph (DMP-4B) pen recorder (Narco Instrument, Houston, Texas). Both strips usually produced approximately equal responses to agonists. Any pair which exhibited marked differences in sensitivity was discarded.

After establishing dose-responses curves to randomly injected agonists, a predetermined dose of an

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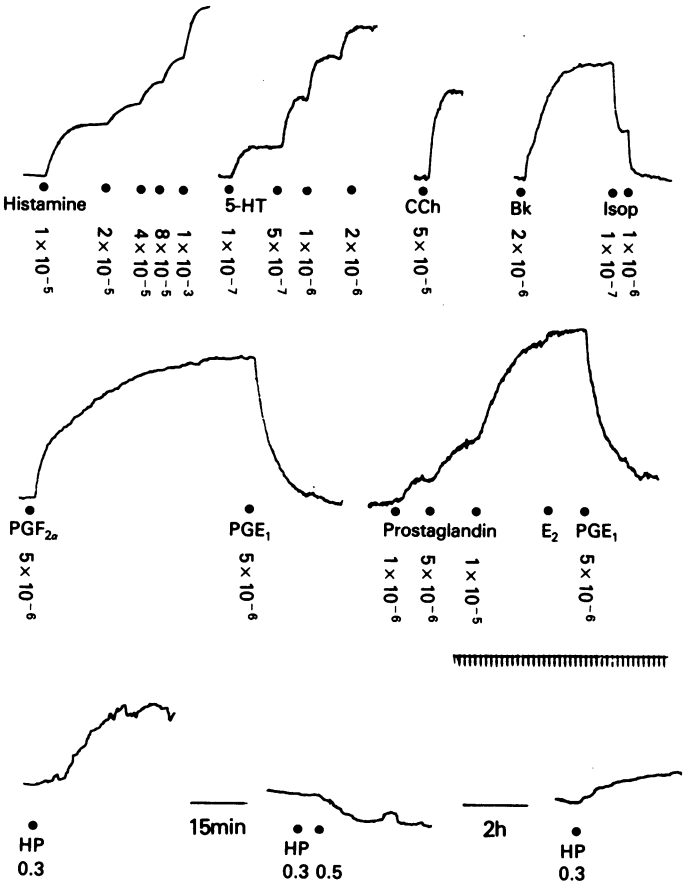

Figure 1 Isolated helical strip of the interventricular branch of the left coronary artery taken from an 8 week old Jersey calf sensitized to horse plasma. It contracted to histamine, 5-hydroxytryptamine (5-HT), carbachol (CCh), bradykinin (Bk), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), PGE_2 and horse plasma (HP), and relaxed to isoprenaline (Isop) and PGE_1 in Krebs-Henseleit solution mixed with 5% CO_2 in O_2 at 37°C (resting tension = 2 g). The second antigenic challenge induced tachyphylaxis 'desensitization'. After a resensitization interval of 2 h, the anaphylactic response was partially recovered. Doses of the agonists are expressed in molar (M) final bath concentration. Horse plasma in ml in 30 ml bath. Time marker indicates minutes.

antagonist was added to one of the strips and left in contact for 30 min. Each agonist was retested in both strips as before. The effectiveness and specificity of antagonist was determined by measuring the dose-ratio: the ratio of equiactive doses of agonists in the presence and absence of antagonist (Gaddum, Hameed, Hathway & Stephens, 1955). Both the strips were challenged with horse plasma (0.1 ml/10 ml) to produce a Schultz-Dale anaphylactic contraction. The antigen-induced contraction of each strip was measured and the degree of inhibition caused by the antagonist was expressed as percentage reduction of the unantagonized (control) response.

Drugs

The drugs used in this study were: histamine diphosphate, 5-hydroxytryptamine creatinine sulphate, bradykinin triacetate, acetylcholine chloride, carbamylcholine chloride and atropine sulphate (Sigma Chemical Co., St. Louis, Mo.).

The following drugs were obtained as gifts: mepyramine maleate from Poulenc Ltd., Montreal, Quebec; methysergide bimaleate from Sandoz, Basle, Switzerland; 5,5-dimethyl-11-oxo-5-H, 11-H-(2)-benzopyrano (4, 3-g) (1) benzopyran-9-carboxylic acid ethanolamine salt (PRD-92-EA) from Pharma Research,

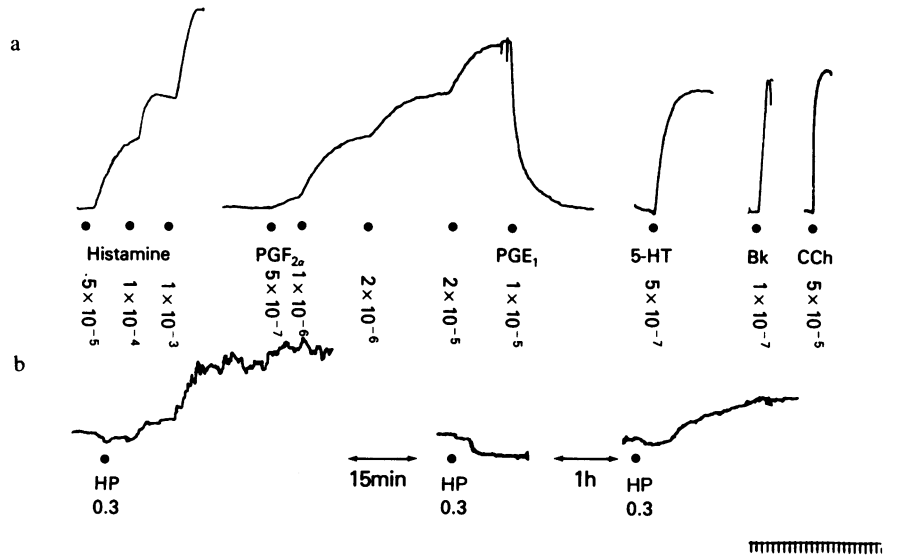

Figure 2 Isolated helical strip of the great cardiac vein obtained from a horse plasma-sensitized calf in 30 ml isolated bath containing Krebs-Henseleit solution maintained at 37°C, mixed with 5% CO_2 in O_2 (resting tension = 1 g). The vein contracted to histamine, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 5-hydroxytryptamine (5-HT), bradykinin (Bk) and carbachol (CCh) and relaxed to PGE_1 (a). It contracted to horse plasma (HP, 0.3 ml in 30 ml); subsequent challenge with horse plasma produced slight relaxation (b). After a 'resensitization interval' of 1 h, the anaphylactic response partially recovered. Doses of the agonists are expressed in molar (M) final bath concentration. Time marker indicates minutes.

Pointe Claire, Quebec; PGE_1, E_2 and $F_{2\alpha}$ from Upjohn Co., Kalamazoo, Michigan.

Results

Reactivity of the coronary blood vessels to selected agonists

Strips of coronary artery [right (n = 18); circumflex (n = 20) and interventricular branches of the left coronary artery (n = 25)] obtained from the sensitized or nonsensitized calves contracted to the following agonists within the given threshold molar (M) dose-ranges: histamine 5×10^{-7} to 10^{-6}; bradykinin: 10^{-8} to 5×10^{-8}; 5-HT: 10^{-8} to 10^{-7}; acetylcholine, carbachol: 2×10^{-6} to 10^{-5}; PGE_2: 10^{-7} to 5×10^{-7}; $PGF_{2\alpha}$: 5×10^{-7} to 10^{-6}. Strips which were partially contracted by histamine, 5-HT, bradykinin, PGE_2, $PGF_{2\alpha}$ or antigen relaxed to isoprenaline (10^{-7} to 10^{-6} M) and to PGE_1 (5×10^{-7} to 10^{-6} M). Typical responses to these agonists are depicted in Figure 1. The great cardiac vein strips (n = 14) showed similar reactivity to the same agonists (Figure 2).

Table 1 Dose-ratios of histamine, 5-hydroxytryptamine (5-HT) and carbachol; and percentage inhibition of Schultz-Dale reaction in calf coronary artery *in vitro*, in the presence of antagonists

		Dose-ratios			*% inhibition of anaphylactic response*
Antagonist	*Bath conc.*	*Histamine*	*5-HT*	*Carbachol*	
Mepyramine maleate	50 ng/ml	27 ± 21* (5)	1.5 ± 1 (5)	2 ± 1.5 (4)	0 (5)
Atropine sulphate	100 ng ml	3 ± 2 (4)	2 ± 1 (4)	240 ± 151* (4)	0 (4)
Methysergide bimaleate	20 ng/ml	1 (5)	137 ± 121* (5)	1 (4)	0 (5)
PRD-92-EA	50 μg/ml	12 ± 9* (5)	15 ± 11* (4)	19 ± 7* (5)	100 (3)* R (2)

* $P < 0.05$.
Values are mean ± s.d. R = reversal (i.e. weak relaxation to antigen challenge).

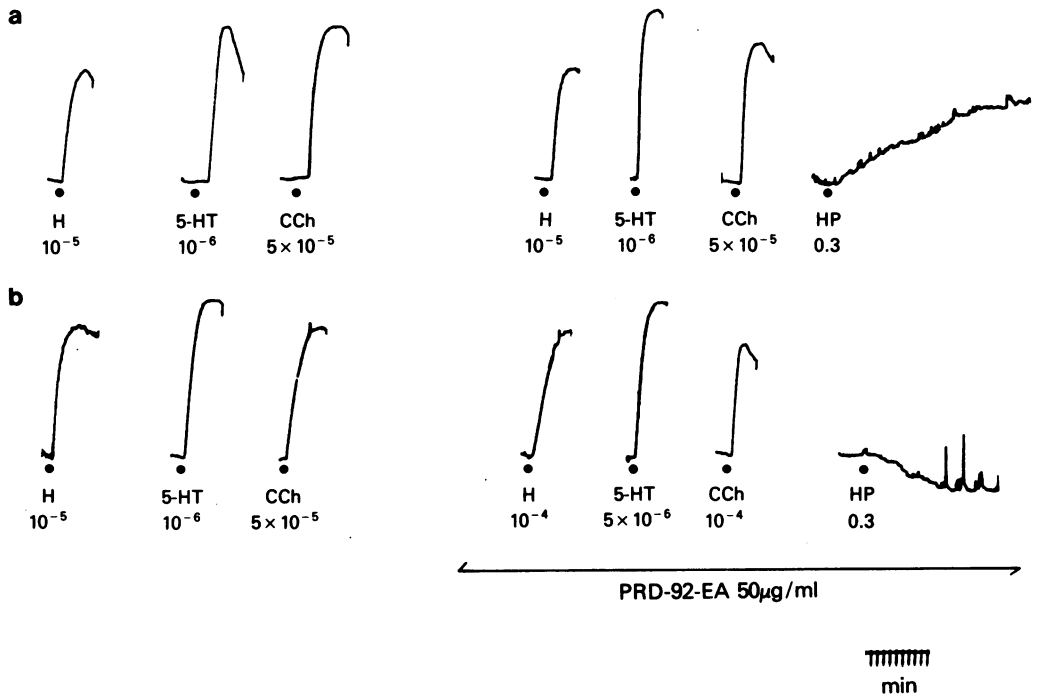

Figure 3 A pair of 'twin' strips of isolated interventricular branch of the left coronary artery taken from a 10 week old Guernsey calf sensitized to horse plasma. Both the strips contracted to histamine (H), 5-hydroxytryptamine (5-HT) and carbachol (CCh) in 30 ml Krebs-Henseleit solution mixed with 5% CO_2 in O_2 at 37°C (resting tension = 2 g). In the second strip (b) of this pair, PRD-92-EA (50 μg/ml) was present in the bath fluid between the arrows and inhibited contractile responses to histamine (dose-ratio = 10), 5-HT (dose-ratio = 5) and carbachol (dose-ratio = 2) and reversed the Schultz-Dale anaphylactic reaction. Agonist doses are expressed in molar (M) final bath concentrations. Horse plasma (HP) doses in ml in 30 ml bath. Time marker indicates minutes.

Anaphylactic (Schultz-Dale) reaction

Antigen-induced contractions were produced in strips taken from three branches of the left coronary artery. Six strips out of 9 from the right; 12 out of 15 from the circumflex and 15 out of 19 from the interventricular branch contracted to horse plasma (1% v:v), producing contractions between 40 and 52% of the magnitude of the histamine maximum contraction (Figure 1). Four out of seven great cardiac veins exhibited Schultz-Dale contraction (25 ± 20% of histamine maximum) (Figure 2). None of the coronary arteries ($n = 12$) or cardiac veins ($n = 7$) obtained from the nonsensitized calves showed any reaction to horse plasma (0.1 ml per 10 ml) although they exhibited identical reactivity to the exogenous agonists.

After 15 min a second antigenic challenge with the same or a higher dose of horse plasma produced either no response or a mild relaxation (Figures 1 and 2). Frequent washings and allowing the tissues to rest ('resensitization interval') for 1 to 2 h usually resulted in 25 to 50% recovery of the initial anaphylactic response.

The effects of antagonists on anaphylaxis of interventricular branch of left coronary arteries

Mepyramine maleate (50 ng/ml) selectively blocked contractions to histamine, 2-methylhistamine and 4-methylhistamine (not included in Table 1) without influencing responses to 5-HT, carbachol or horse plasma (Table 1).

Atropine sulphate (100 ng/ml) selectively antagonized responses to carbachol and also weakly inhibited histamine but did not modify the responses to 5-HT or horse plasma (Table 1).

Methysergide bimaleate (20 ng/ml) specifically antagonized 5-HT-induced responses with no effect on

contractile responses to histamine, carbachol or horse plasma (Table 1).

PRD-92-EA (50 μg/ml) almost equally blocked the responses to histamine, 5-HT and carbachol. It either completely inhibited (3) or reversed (2) the anaphylactic response ($n = 5$) (Figure 3; Table 1).

Discussion

The bovine coronary artery and vein were found to contract to bradykinin, 5-HT, PGE_2, $PGF_{2\alpha}$, histamine, acetylcholine and carbachol. Similar reactivity has been reported in calf pulmonary vasculature (Eyre, 1971).

Isoprenaline and PGE_1 invariably relaxed the artery and vein irrespective of the spasmogen (histamine, 5-HT, PGE_2, $F_{2\alpha}$, Bk or antigen) used. It was reported earlier that the bovine coronary artery contracted to PGE_2 and $PGF_{2\alpha}$ and relaxed to PGE_1 (Kalsner, 1975; Needleman Kulkarni & Raz, 1977).

Anaphylactic contraction, desensitization and recovery of the anaphylactic responses of the coronary blood vessels to antigen is in accord with similar observations on a number of tissues (reviewed by Chand & Eyre, 1978a).

The results of this investigation are compatible with local 'anaphylactic' reactions in coronary blood vessels (coronary vasoconstriction) during systemic hypersensitivity. The coronary spasm may contribute to the electrocardiographic abnormalities associated frequently with anaphylaxis in man and animals (Criep, 1931; Booth & Patterson, 1970; Criep & Woehler, 1971; Capurro & Levi, 1975).

Earlier, Eyre (1971) reported a partial blockade of anaphylactic responses of calf pulmonary vein by mepyramine and methysergide. However, in the present investigation both antihistamine and antitryptamine agents were ineffective on coronary anaphylactic contractions. The discrepancy is difficult to explain but it is possible that histamine and 5-HT are less important in coronary vascular anaphylaxis than in other organ/systems.

Compound PRD-92-EA is an agent that has marked anti-allergic properties (Stewart, Devlin & Freter, 1974) which are associated with inhibition of mast cell degranulation and a broad spectrum of receptor blocking activity towards agonists which include histamine, 5-HT and carbachol (Possanza, Bauen & Stewart, 1974, 1975; Chand & Eyre, 1976; Holroyde & Eyre, 1977). Compound PRD-92 inhibited anaphylactic contraction of the coronary artery virtually completely. This drug was also reported to inhibit passive cutaneous anaphylaxis in rat, allergic bronchoconstriction in monkey (Stewart *et al.*, 1974), Schultz-Dale reaction of chicken ileum (Chand & Eyre, 1976) and pulmonary vein (Eyre, unpublished observations) and systemic anaphylaxis in adult domestic fowl (Chand & Eyre, 1978b). The spectrum of receptor blocking activity coupled with the inhibition of synthesis and release of mediators suggests that compound PRD-92-EA may be an important anti-allergic agent of the future.

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