VASOCONSTRICTOR RESPONSE TO ARACHIDONIC ACID IN THE ISOLATED HIND LIMB OF THE DOG

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1 Arachidonic acid (AA) (25–200 µg/kg) produced a dose-related increase in perfusion pressure in dog isolated hind limbs perfused with either blood or a platelet-free perfusate.
2 Prostaglandin E₂ produced vasodilatation while prostaglandin F₂₅ produced no vascular change at these administered doses.
3 Phentolamine did not alter the arachidonic acid response, eliminating possible α-adrenoceptor mediation.
4 Aspirin and indomethacin blocked the vasoconstrictor response to AA.
5 This study indicates that a vasoactive intermediate in prostaglandin synthesis can be elaborated in the absence of platelets.

Introduction

Arachidonic acid (AA), the precursor of the bisenoic prostaglandins E₂ and F₂₅, consistently produces a systemic hypotensive response when administered intravenously to cats (Jaques, 1959), rats (Cohen, Sztokalo & Hinsch, 1973), rabbits (Ichikawa & Yamada, 1962) and dogs (Rose, Johnson, Ramwell & Kot, 1974). Evidence has been presented that suggests the hypotensive response is produced by one or more potent vasoactive intermediates in the pathway leading to prostaglandin synthesis (Rose et al., 1974).

Hamberg, Hedqvist, Strandberg, Svensson & Samuelsson (1975) have shown that intermediates in prostaglandin synthesis constrict the pulmonary arteries in cats. This observation implies that the initial hypotensive response is due to decreased return to the left ventricle and decreased cardiac output. These authors have suggested that conversion of a vasoconstrictor intermediate to its degradation products, particularly prostaglandin E₂, produces a longer lasting reduction in blood pressure due to peripheral vasodilatation. The well-known enhancement of platelet aggregation by AA (Silver, Hoch, Kocsis, Ingerman & Smith, 1974) suggests that another possible mechanism for acute systemic hypotension could be momentary obstruction of the pulmonary vasculature.

The responses of systemic blood vessels to AA are thus not demonstrable in whole animal preparations which reveal only the result of a complex interplay of central and peripheral effects. The purpose of the present study was to isolate a peripheral regional component of the hypotensive response to AA. The dog hind limb was used to investigate this potentially important phenomenon.

Methods

Mongrel dogs of either sex (13 to 26 kg in body weight) were anaesthetized with sodium pentobarbitone (30 mg/kg) and intubated with auffed endotracheal tube. The right femoral artery was catheterized for systemic arterial pressure recording. The right femoral vein was catheterized to withdraw blood for the extracorporeal circuit. The left hind limb circulation was isolated from the remainder of the systemic circulation. The sciatic nerve was left intact. The superficial limb musculature was severed and an adjustable metal band was tightened around the femur to prevent any cross-circulation via the deep musculature.

Autologous blood anticoagulated with heparin (8 mg/kg) was collected in a reservoir and maintained at 37°C. With a peristaltic pump (Sigma), blood from the reservoir was pumped at a constant flow rate (3 ml kg⁻¹ min⁻¹) through the femoral artery catheter, circulated through the limb, and via the venous catheter to a small oxygenator (Temptrol). A mixture of 98% air and 2% CO₂ was used to aerate the blood and maintain arterial blood gases and pH in the physiological range. The blood was returned to the reservoir to be reperfused. Arterial and venous
pressures in the isolated limb were monitored at their respective catheters. All test substances were administered in random sequence. Each agent was administered by bolus injection into the tubing between the pump and arterial supply to the limb. Control injections of vehicle solution produced no injection artifact. Tracer studies using radioiodinated serum albumin demonstrated no cross-circulation between the closed limb circuit and the systemic circulation.

The sodium salts of arachidonic acid (5,8,11,14-eicosatetraenoic acid), >99% pure from porcine liver (Sigma & Nu-Chek), and linoleic acid (99% Sigma) were prepared by dissolving them with sodium carbonate (100 mM) during constant stirring under N₂, in the absence of light. The resulting solutions were water clear and used only on the day of preparation. Prostaglandins were generously provided by The Upjohn Co. Solutions of prostaglandin (1 mg/ml) in ethanol were evaporated to dryness under N₂. The residue was dissolved in 0.9% w/v NaCl solution (saline).

In two animals, phenotamine (250 µg/kg body weight) was introduced into the isolated hind limb to block α-adrenoceptors. Both aspirin (25 mg/kg) and indomethacin (2.5 mg/kg) were used to block prostaglandin synthetase enzymes. Acetylsalicylic acid powder was dissolved in modified Tyrode solution and pH was adjusted to 7.35 with sodium hydroxide. Indomethacin (2 mg/ml) was made by combining a solution of 50 mg indomethacin in 5 ml distilled water with 15.6 mg Na₂CO₃ in 5 ml distilled water. This solution was mixed for 10 min, diluted to a final volume of 25 ml, and filtered (pH 7.74).

In platelet-free studies, the isolated limb was perfused with Perfudex (Pharmacia), a 5% dextran electrolyte solution with HCO₃⁻ (25 mM) and CaCl₂ (25 mM) added. Platelet counts of the effluent indicate that 85% of the original platelets (2.3 x 10⁹/mm³) were eliminated by this procedure. The effluent was not recirculated but drained off in order to eliminate any trapped platelets released from the vascular bed.

Results

Increasingly larger doses of AA were administered to seven hind limb preparations to establish a dose-response curve (Figure 1). The percentage increase in mean limb arterial perfusion pressure with doses of 25, 50, 100, 150 and 200 µg/kg were 3.1 ± 1.6 (s.e.), 11.5 ± 3.9, 17.9 ± 2.9, 20.9 ± 3.8 and 19.9 ± 6.6%, respectively. Two trials at 400 µg/kg resulted in a mean increase of 18.0%.

An example of the vasoconstrictor effect of AA (150 µg/kg) in the isolated limb, is shown in Figure 2. The isolated limb arterial pressure rose substantially above the control values in an average time-to-onset of seven seconds. At the same time, the systemic arterial pressure and limb venous pressure remained the same. A similar response was produced by noradrenaline (1 µg/kg). Phenotamine completely blocked the pressor response to noradrenaline, but did not alter the vasoconstrictor response to AA.

In three platelet-free preparations dosages of 100 and 150 µg/kg of AA were administered in the same manner. Rapid increases in perfusion pressure were again observed. The percentage changes in mean femoral arterial pressure were 14.6 ± 4.7 and 21.6 ± 7.1%. Blood perfusion experiments in the same animals showed increases of 17.9 ± 2.9 and 20.9 ± 3.8% respectively for the same doses (Figure 1).

Prostaglandin E₂ and F₂α were administered in a dose range that consistently elicited a systemic response in the intact dog (1–6 µg/kg). Initial administrations of prostaglandin E₂ produced marked vasodilatation. However, subsequent doses in the same limb produced no response. Prostaglandin F₂α had no effect on limb perfusion pressure. Periodic monitoring of arterial blood gases indicated values within the physiological range (PaO₂ 138.1 ± 6.7, PaCO₂ 26.4 ± 0.9, pH 7.35 ± 0.02). Linoleic acid, used as a control fatty acid, was administered in a dose of 150 µg/kg. No vascular response was produced. Both aspirin (n=3) and indomethacin (n=5) induced a transient vasodilatation in which the perfusion pressures returned to control values within 1 minute. These synthetase inhibitors completely blocked the vascular response to AA in the isolated limb.

Discussion

Earlier studies have shown that both AA and prostaglandin E₂ produce a systemic hypotensive
response in dogs. However, they differ in cardiac and pulmonary vascular effects (Rose et al., 1974; Wicks, Rose, Johnson, Ramwell & Kot, 1976).

In order to investigate the more direct effects of AA on a representative systemic vascular bed, the isolated hind limb preparation of the dog was selected for study. The systemic hypotensive response observed in intact animals and the results of AA infusion studies by Ryan & Zimmerman (1974) suggested that there would be a marked decrease in perfusion pressure in the limb due to a decrease in arterial resistance. The opposite was the case. AA caused the isolated limb perfusion pressure to rise substantially above the control values, indicating a transient increase in limb vascular resistance.

The possibility that the vascular response to AA is mediated by the release of endogenous noradrenaline or direct stimulation of α-adrenoceptors is unlikely since phenolamine administration did not modify this response. Furthermore, pulmonary vasoconstriction produced by AA, similar to the response in the isolated hind limb, was not affected by β-adrenoceptor, cholinocceptor or 5-hydroxytryptamine antagonists (Wicks et al., 1976). These data suggest that other receptors must be present that mediate the vasoconstriction induced by AA.

The increase in perfusion pressure could be explained by platelet aggregation at arteriolar and capillary sites. AA has been conclusively shown to induce platelet aggregation both in vitro (Silver, Smith, Ingerman & Kocsis, 1973; Vargaftig & Zirins, 1973; Rose et al. 1974) and in vivo preparations (Silver et al., 1974; Furlow & Bass, 1975) in several different species. The results of experiments using the platelet-free perfusate strongly suggest that the increased perfusion pressure is independent of the release of vasoactive platelet products or mechanical obstruction of small vessels by platelet aggregates.

Clearly, some product in the biosynthetic pathway from AA to the biseionic prostaglandins is vasoconstrictor in the canine hind limb. Both prostaglandin E2 and F2α were administered in a dose range that consistently elicits a systemic response in the intact dog. The initial injections of prostaglandin E2 produced vasodilatation of the hind limb which is in agreement with Kadowitz (1972) and others. The failure to elicit a response upon subsequent injections of prostaglandin E2 may be due to the limb vasculature being maximally dilated as suggested by Daugherty (1971). In contrast to the studies of Ducharme, Weeks & Montgomery (1968) and Greenberg & Sparks (1969), prostaglandin F2α did not induce vasoconstriction in the isolated hind limb. However, the techniques employed by these investigators were substantially different. Brody & Kadowitz (1974) suggest that low concentrations of prostaglandin F2α may not exert an effect on the vascular resistance of the limb.

The vascular responses to AA in the isolated hind limb were blocked by aspirin and indomethacin, synthetase inhibitors of the endoperoxide intermediates (Samuelsson & Hamberg, 1974; Malmsten, Hamberg, Svensson & Samuelsson, 1975). Therefore, vasoconstriction is not a direct effect of AA. Linoleic acid produced no vascular response indicating that the increased perfusion pressure is not due to a non-specific fatty acid effect.

These findings suggest that the substance producing
the increase in vascular resistance is not AA, or pro-
staglandin E₂ or F₂α, but a vasoactive intermediate. 
Endoperoxide intermediate compounds have been 
isolated by Nugteren & Hazelhof (1973) and 
Hamberg, Svensson, Wakabayashi & Samuelsson 
(1974). Although these substances are unstable, they 
are more potent vasoconstrictors than their respective 
prostaglandins (Hamberg et al., 1975). Thromboxane 
A₂, a further metabolite of AA, also has vasoactive 
properties (Kolata, 1975; Tuwemo, Strandberg, 
Hamberg & Samuelsson, 1976). These compounds 
are possible alternatives to AA and prostaglandins E₂ and 
F₂α, as vasoactive substances responsible for the 
observed increases in hind limb vasoconstriction.

A discrepancy exists between the systemic 
hypotensive response to AA in the intact dog and the 
vasoconstrictor response to AA in the isolated limb.

Although a reduction in cardiac output was suggested by 
Hamberg et al. (1975) as a possible explanation for 
the systemic response to AA, the hypothesis does not 
appear likely. Ascending aortic blood flow measured 
during systemic administration of AA showed little 
change or an increase in cardiac output. Vasodilata-
tion in the splanchnic circulation (Anggard & 
Larsson, 1974) may in a large part account for the 
systemic hypotensive response.

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