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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 E_2 produced vasodilatation while prostaglandin $F_{2\alpha}$ 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 E_2 and $F_{2\alpha}$, 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 E_2, 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 a cuffed 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% CO_2 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 N_2, 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 N_2. The residue was dissolved in 0.9% w/v NaCl solution (saline).

In two animals, phentolamine (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 Na_2CO_3 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 HCO_3^- (25 mM) and $CaCl_2$ (25 mM) added. Platelet counts of the effluent indicate that 85% of the original platelets ($2.3 \times 10^5/mm^3$) 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). Phentolamine completely blocked the pressor response to noradrenaline, but did not alter the vasoconstrictor response to AA.

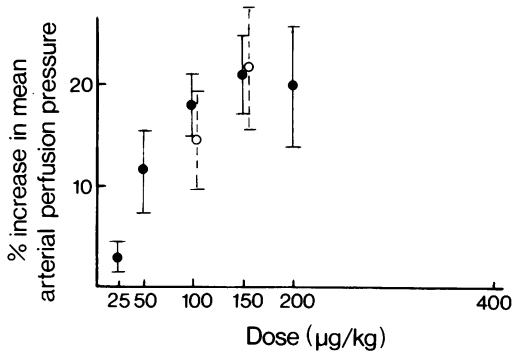

Figure 1 Percentage increase in mean arterial perfusion pressure as a function of the dose of arachidonic acid administered to the isolated hind limb preparation of the dog: (●) represent values obtained with autologous blood as perfusate ($n=7$); (○) are values with a platelet-free perfusate ($n=3$). Vertical lines show s.e. means.

In three platelet-free preparations doses 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 E_2 and $F_{2\alpha}$ were administered in a dose range that consistently elicited a systemic response in the intact dog (1–6 μg/kg). Initial administrations of prostaglandin E_2 produced marked vasodilatation. However, subsequent doses in the same limb produced no response. Prostaglandin $F_{2\alpha}$ had no effect on limb perfusion pressure. Periodic monitoring of arterial blood gases indicated values within the physiological range (PaO_2 138.1 ± 6.7, $PaCO_2$ 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 E_2 produce a systemic hypotensive

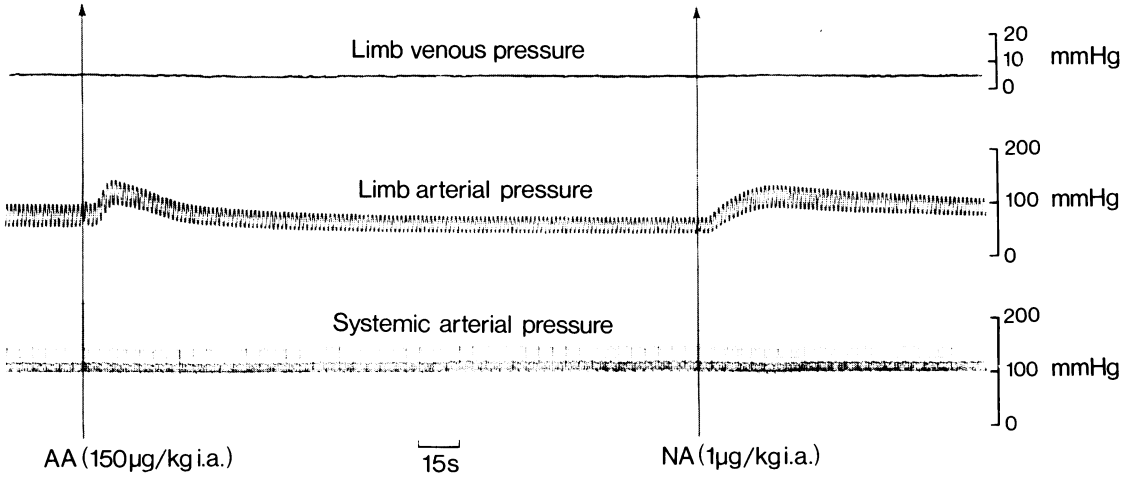

Figure 2 Effects of arachidonic acid (AA, 150 μg/kg) and noradrenaline (NA, 1 μg/kg) on femoral venous pressure, femoral arterial pressure and systemic arterial pressure in the isolated hind limb preparation of the dog. Arrows indicate points of intra-arterial injection.

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 phentolamine 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, cholinoceptor 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 bisenoic prostaglandins is vasoconstrictor in the canine hind limb. Both prostaglandin E_2 and $F_{2\alpha}$ were administered in a dose range that consistently elicits a systemic response in the intact dog. The initial injections of prostaglandin E_2 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 E_2 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 $F_{2\alpha}$ 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 $F_{2\alpha}$ 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 prostaglandin E_2 or $F_{2\alpha}$, 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 A_2, a further metabolite of AA, also has vasoactive properties (Kolata, 1975; Tuvemo, Strandberg, Hamberg & Samuelsson, 1976). These compounds are possible alternatives to AA and prostaglandins E_2 and $F_{2\alpha}$ 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. Vasodilatation in the splanchnic circulation (Anggard & Larsson, 1974) may in a large part account for the systemic hypotensive response.

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