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Effects of Arachidonic Acid on Systemic Arterial Pressure, Myocardial Contractility and Platelets in the Dog (38408)

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Arachidonic acid (AA) is the precursor of the bis-unsaturated, or bisenoic, prostaglandins, PGE_2 and $PGF_{2\alpha}$. As such, it has been the subject of recent cardiovascular investigations (1, 2), although its activity on smooth muscle has been known for over a decade (3, 4). Arachidonic acid is thought to exert an effect through its conversion to prostaglandins (1, 2, 5).

Our objectives were to establish the acute single dose of AA which would provide a reproducible cardiovascular effect in the dog without development of tachyphylaxis, to study the responses of systemic arterial pressure, myocardial contractility, and platelets to this dose, and to compare these effects with those produced by other fatty acids (particularly dihomo-γ-linolenic acid, the precursor of the monoenoic prostaglandins) and PGE_2.

Materials and Methods. Mongrel dogs of either sex were anesthetized with sodium pentobarbital (30 mg/kg) and maintained on the Harvard respirator. The chest was opened and a Walton–Brodie strain-gauge arch was sutured to the right ventricular wall for recording myocardial contractility. The femoral artery was catheterized for simultaneous systemic arterial pressure recording using a strain-gauge transducer and two-channel direct-writing recorder. A femoral vein catheter was advanced into the inferior vena cava for rapid injection of test substances.

The sodium salts of arachidonic acid (5, 8, 11, 14-eicosatetraenoic acid, > 99% pure from porcine liver, Sigma), dihomo-γ-linolenic acid (8, 11, 14-eicosatrienoic acid, > 99% pure, Nu-Chek), oleic acid (99%, Sigma) and linoleic acid (99%, Sigma) were prepared by dissolving in sodium carbonate 100 m*M* with constant stirring under nitrogen, in the absence of light. The resulting solutions were water clear and were stored for no more than 2 days in the dark under nitrogen. Prostaglandins were generously donated by the Upjohn Co. One milligram/milliliter solutions in ethanol were evaporated to dryness under nitrogen, and the residue taken up in normal saline. Acetylsalicylic acid powder (Merck) was dissolved in modified Tyrode's solution and the pH adjusted to 7.35 with sodium hydroxide.

For platelet studies, blood was collected from the inferior vena cava catheter 1 min before and 1 min after injection of the agent under study. Blood was drawn into a syringe containing 3.8% trisodium citrate (one part anticoagulant to nine parts blood). Platelet-rich plasma (PRP) was then prepared at room temperature by centrifugation (400*g* for 15 min). Platelet counts were determined by phase contrast microscopy on a Spencer Neubauer slide. Platelet-rich plasma was adjusted to approximately 100,000 platelets/mm^3 with platelet-poor plasma (prepared by centrifugation at 1500*g* for 15 min) from the same specimen. Platelet aggregation in response to ADP (from equine muscle, Sigma; 1 mg/ml in normal saline) was assessed in a temperature-controlled (37°) aggregometer (Payton Associates) with constant stirring (900 rpm). Additions to the PRP did not exceed 10 μl and were made after 2 min of temperature equilibration.

Results. Arachidonic acid was given in increasingly larger doses in six dogs until an acute intravenous dose was obtained (300 μg/kg) which produced a profound drop in systemic arterial pressure and from which recovery always rapidly ensued. No tachyphylaxis to this dose was observed on repeated injections at 5 min intervals for up to six doses. This dose of AA was used in all subsequent studies.

In 14 dogs, the typical response to AA was a sudden drop in systemic arterial pressure occurring an average of 15.2 sec (SE ± 1.3) after injection into the inferior vena cava. The systolic pressure fell 28.5% (SE ± 4.5) and the diastolic pressure fell 43.8% (SE ± 4.5). A widening of pulse pressure was noted in every experiment. A typical response is shown in the left-hand portion of Fig. 1.

The effect of AA on myocardial contractility was variable and followed by several seconds the arterial pressure change. Myocardial contractility was increased slightly in four dogs, (mean 22.5%, SE ± 4.6) reduced slightly in eight (mean 24.1%, SE ± 5.3), and unchanged in two. No changes in heart rate were noted, nor were there any arrhythmias.

The duration of the AA effect on blood pressure averaged 4.6 min (SE ± 0.6).

In two dogs, AA was injected into the left atrium or a pulmonary vein and the typical arterial pressure response was noted in 2–3 sec.

Linoleic and oleic acids were given in the same dose (300 μg/kg) in various sequences with AA, to rule out nonspecific fatty acid effects. Neither linoleic acid nor oleic acid had any perceptible effect on systemic arterial pressure or myocardial contractility in the six dogs in which each was tested. Dihomo-γ-linolenic acid was tested in two dogs in a dose of 300 μg/kg and in two dogs in a dose of 600 μg/kg with no perceptible effect on systemic arterial pressure or myocardial contractility. One dog received a dose of 1800 μg/kg. This caused a fall in arterial pressure from 125/100 to 100/70. The duration of this effect was 1 min.

In three dogs, after determining the AA response, acetylsalicylic acid, aspirin (55 μg/kg), was given in one dose intravenously, and AA was retested at 30 and 45 min. In one dog, aspirin blockade of AA was total and complete at both tests. In a second, there was a 15 mm Hg vasodepressor response of 10-sec duration at the 30-min test, but blockade was total at 45 min. In the third dog, there were slight vasodepressor responses of 10–15 mm Hg of 10-sec duration at both 30 and 45 min after pretreatment with aspirin.

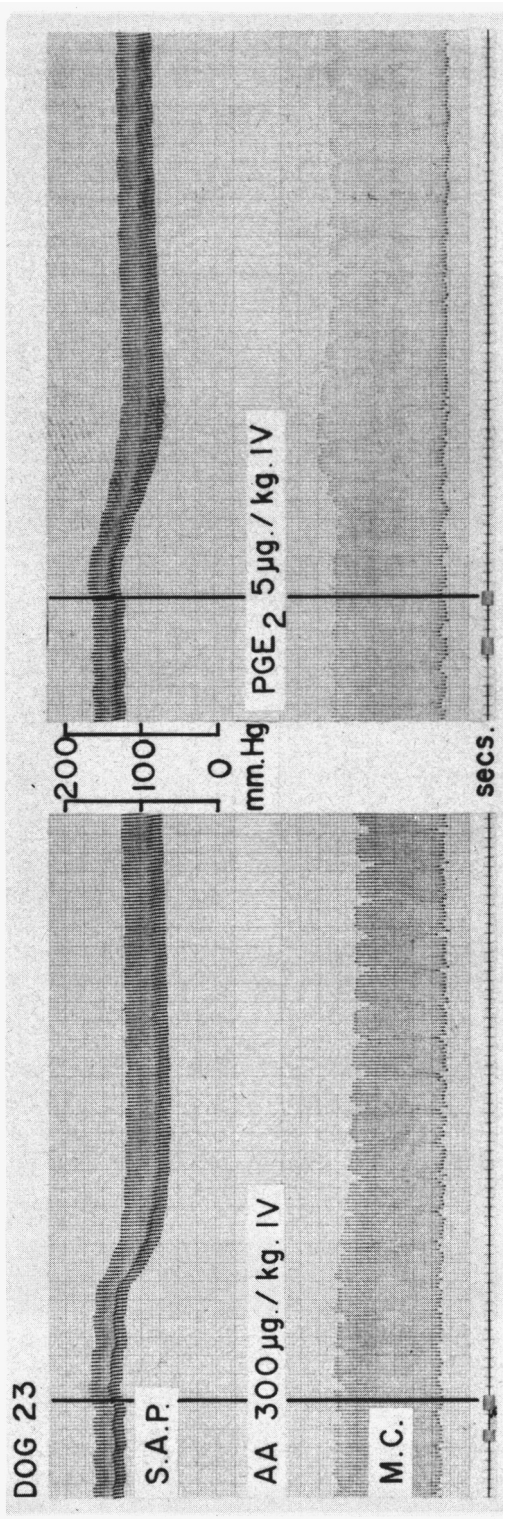
PGE_2 (5 μg/kg) was administered in the same manner to eight dogs. The typical response was an immediate (mean 4.5 sec, SE ± 0.2) drop in systemic arterial pressure (mean systolic 19.4%, SE ± 1.8, mean diastolic 41%, SE ± 8.5) to levels comparable to the AA response, with a consistent and simultaneous increase in myocardial contractility (mean 30%, SE ± 8.5). A typical PGE_2 response is shown in the right-hand portion of Fig. 1. On retesting with PGE_2 after aspirin (three dogs), there was no evidence of blockade or attenuation of the PGE_2 response due to this substance.

$PGF_{2\alpha}$ was tested in two dogs before and after aspirin. The hypertensive response was neither blocked nor attenuated.

In three dogs, platelet counts 1 min after AA were reduced an average of 62.9% (SE ± 9.2). The aggregability response to ADP (5 μg/ml) was enhanced fourfold in post-AA PRP as compared to control PRP. Following aspirin, AA induced only a 9.0% drop in platelets (SE ± 1.1). In two dogs, following PGE_2, there was no fall in platelet count, but aggregability was increased twofold.

Discussion. The vasodepressor and positive inotropic responses to PGE_2 in the dog are well documented (6). While the fall in systemic arterial pressure following AA (300 μg/kg iv) was equivalent to the fall noted with PGE_2 (5 μg/kg iv), there were qualitative differences in the responses demonstrated in these experiments. First, there was a longer interval between the injection of AA and its effect (15 sec) than for PGE_2 (4.5 sec). Second, while PGE_2 produces a consistent increase in myocardial contractility, the effect of AA on this measurement was variable and weak. The timing observation suggests the possibility that a biosynthetic conversion of AA to an active vasodilator is taking place, but the different response in myocardial contractility suggests that the active agent, at least in the myocardium, may not be PGE_2.

Aspirin is known to block the formation of endoperoxide (prostaglandin G_2) from AA, the known step in the biosynthesis of PGE_2 and $PGF_{2\alpha}$. Cohen, Sztokalo, and Hinsch (1) noted a fall in blood pressure and heart rate in spontaneous hypertensive rats given AA, and these effects were inhibited by aspirin and indomethacin. The peak conversion of AA to PGG_2 has been reported as 15–30 sec, whereas PGE_2 and $PGF_{2\alpha}$ do not appear until after 2 min (7). In addition, AA induces platelet aggregation and thrombocytopenia by way of PGG_2 formation (8). PGE_2 and $PGF_{2\alpha}$ do not aggregate unstimulated platelets (9). These observations support the view that some intermediate in prostaglandin



synthesis, prior to PGE_2 and possibly PGG_2, mediates the cardiovascular effects of AA.

The PGE_1 and $PGF_{1\alpha}$ precursor, dihomo-γ-linolenic acid, is much less potent than AA in its cardiovascular effects. From these preliminary observations, it appears that close to 10 times the AA dose will be required for equidepressor responses to dihomo-γ-linolenic acid. This relative lack of effectiveness of dihomo-γ-linolenic acid, which has been previously reported to be inactive in inducing platelet aggregation (10), suggests a possible correlation between the vasodepressor and platelet effects of AA.

Summary. The bisenoic prostaglandin precursor, arachidonic acid (AA), in a single dose intravenously, produced a marked vasodepressor response in dogs, and a weak and variable effect on myocardial contractility. This response differed from the vasodepressor effect of PGE_2 in that the onset of effect was delayed (15 sec for AA, 4.5 sec for PGE_2) and PGE_2 always caused a pronounced increase in myocardial contractility. Arachidonic acid caused thrombocytopenia and increased aggregability of platelets. All AA effects were inhibited by aspirin. The monoenoic prostaglandin precursor, dihomo-γ-linolenic acid, in doses equivalent to that of AA, had no effects. The data suggest that AA exerts its effects through conversion to an intermediate in the biosynthesis of PGE_2 and not PGE_2 itself.

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FIG. 1. Simultaneous recordings of systemic arterial pressure (SAP) and myocardial contractility (MC) in a dog receiving the doses of AA and PGE_2 shown. The vertical line represents the instant of introduction of the substance into the inferior vena cava. The vasodepressor effect of AA begins in 12 sec, that of PGE_2 in 4 sec. Myocardial contractility is depressed with AA, increased with PGE_2.

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