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Cardiovascular and Platelet Responses in the Dog to the Monoenoic Prostaglandin Precursor Dihomo-γ-linolenic Acid[1] (38727)

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Dihomo-γ-linolenic acid (DGLA) is the precursor of the monoenoic prostaglandins, PGE_1 and $PGF_{1\alpha}$ (1, 2). It occurs in animal tissues in much lesser amounts than arachidonic acid (AA) (3), the precursor of the bisenoic prostaglandins, PGE_2 and $PGF_{2\alpha}$ (4, 5). It has only recently become available in pure form for study and there are few published reports of its physiologic or pharmacologic effects (6–8). In the course of studies on the cardiovascular responses to AA in open-chest dogs, DGLA had no discernible effects on blood pressure or myocardial contractility in the dose that for AA (300 μg/kg) had produced consistent, reproducible depressor effects (9). A much larger intravenous dose (1.8 mg/kg) of DGLA was required for a roughly equivalent effect on systemic arterial pressure.

We selected 2.0 mg/kg as the size of an acute intravenous dose of DGLA that produces significant and reproducible cardiovascular responses without tachyphylaxis. The following investigation was pursued in order to characterize the effects of DGLA on systemic arterial pressure, myocardial contractile force, and platelet aggregability in the dog; to compare these responses with those to PGE_1 and $PGF_{1\alpha}$ (5 μg/kg), as well as to other fatty acids (linoleic and palmitoleic) in doses similar to that of DGLA, as control observations; and to analyze the differences in responses to the two PG precursors, AA and DGLA.

Materials and Methods. Twelve mongrel dogs of either sex were anesthetized with sodium pentobarbital (30 mg/kg) and respirations were maintained by the Harvard respirator via an endotracheal tube. The common carotid arteries were exposed bilaterally and identified with loose ligatures, to be used in testing the efficacy of ganglionic blockade. The chest was opened through a left lateral incision in the fourth intercostal space, 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 2-channel direct-writing recorder. A femoral vein catheter was advanced into the inferior vena cava for rapid injection of test substances.

The sodium salt of dihomo-γ-linolenic acid (8, 11, 14-eicosatrienoic acid; >99% pure; NuChek) was prepared by dissolving in sodium carbonate 100 m*M* with constant stirring under nitrogen, in the absence of light. The resulting solution, 10 mg/ml, was water clear and was stored for no more than 2 days in the dark under nitrogen. Sodium salts of linoleic acid (99%; Sigma) and palmitoleic acid (99%; Sigma) were prepared in a similar manner and in the same concentrations. Prostaglandins were generously donated by the Upjohn Company. Hexamethonium chloride (Schwarz/Mann) was prepared in aqueous solution in concentration of 50 mg/ml. Practolol was generously supplied by Ayerst Laboratories, Inc. Acetylsalicylic acid (aspirin) powder (Merck) was dissolved in modified Tyrode's solution and the pH adjusted to 7.35 with sodium hydroxide.

Two dogs were tested with DGLA in bolus intravenous doses of 2.5 mg/kg. In the remaining 10 dogs, the dose of DGLA was 2.0 mg/kg. All PGE_1 and $PGF_{1\alpha}$ doses were 5 μg/kg. All doses of DGLA and prostaglandins were given at 5-min intervals. The 10 dogs were divided into two groups of five: five dogs received DGLA, PGE_1, and $PGF_{1\alpha}$ before and after ganglionic blockade (hexamethonium 2–3 mg/kg) and beta-adrenergic blockade (practolol 2 mg/kg). Ganglion blockade was tested by bilateral

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carotid arterial occlusion for 30 sec to 1 min. Beta-adrenergic blockade was tested with isoproterenol (Isuprel) 0.5 μg/kg iv. In all cases, the efficacy of blockade was assured. The other five dogs were tested with DGLA, PGE_1, and $PGF_{1\alpha}$ before, 30 min and 45 min after aspirin, 100 mg/kg iv.

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. PRP 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. The systemic arterial pressure (SAP) response to DGLA was consistently biphasic in character. A typical example is shown on the left side of Fig. 1. In two dogs given 2.5 mg/kg, the onset of the first brief drop in SAP was 4 sec, with the longer, sustained drop occurring at 12 and 14 sec, respectively. The magnitude of the SAP fall was 18 and 31% systolic, and 38 and 41% diastolic. The consistent increase in myocardial contractility (MC) was often, but not always, noted to occur in two phases coincident with the depressor effects. In the two dogs receiving the 2.5 mg/kg dose, the magnitude of the MC increase was 23 and 46%, respectively. At the maximal heart rate (HR) response, in these two dogs, the HR increased from 150 to 170, and from 180 to 210, respectively. In each dog, the total duration of effect was 4 min (the longest lasting effect was the SAP response).

At the 2.0 mg/kg dose level the responses were as follows in 10 dogs: The mean onset of initial effect was 4.3 sec (SE ± 0.34) and the onset of the sustained depressor effect was 15.9 ± 0.54 sec. The mean maximal fall in systolic SAP was 34 ± 5.1%, and 49 ± 5.5% for diastolic SAP. MC was increased 28 ± 4.2%. HR changes were variable, showing increases in four, drops in two, and no change in four. The mean total duration of effect was 5.2 ± 0.45 min.

In 11 dogs, the SAP response to PGE_1 (5 μg/kg) was depressor, occurring 4.8 ± 0.37 sec after injection (Fig. 1). The mean

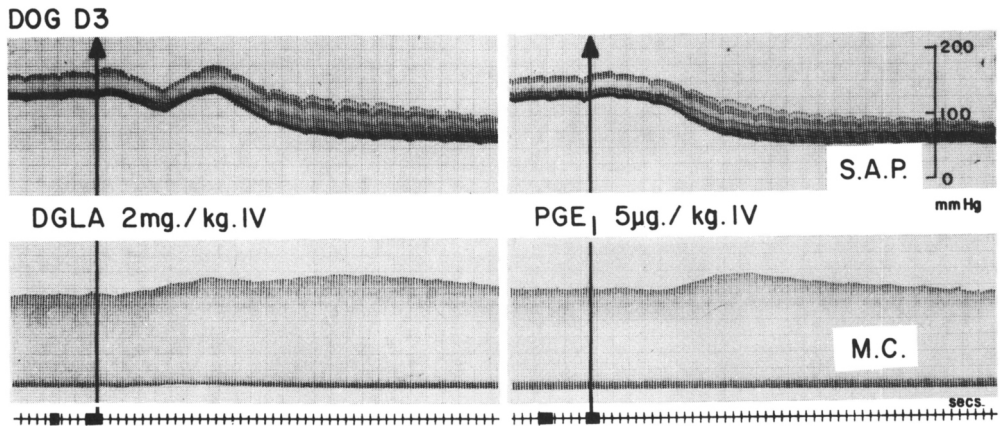

FIG. 1. Simultaneous recordings of systemic arterial pressure (SAP) and myocardial contractile force (MC) in a dog receiving the doses of DGLA and PGE_1 shown. The vertical lines represent the instant of introduction of the substance into the inferior vena cava. Note the biphasic SAP response to DGLA. MC is increased by both agents.

maximal fall in systolic SAP was 37 ± 3.5% and 53 ± 3.8% for diastolic SAP. MC was increased 16 ± 3.0%. HR was significantly increased in two dogs, 170 to 190, and 180 to 210. The mean duration of effect was 5.0 ± 0.27 min.

$PGF_{1\alpha}$ (5 μg/kg) produced a slight fall followed by a slight rise in SAP in 11 dogs. The predominant response was depressor in three and pressor in seven, with no significant change in one. The magnitude of the response was a mean of +4 ± 3.4% systolic and 5.5 ± 3.4% diastolic. MC was consistently increased (mean 15.2 ± 3.5%). The onset of the first effect was 5.0 ± 0.52 sec and the duration of effect was 4.0 ± 0.3 min.

After effective ganglionic blockade with hexamethonium, and after effective β-adrenergic blockade with practolol, there were no qualitative differences in SAP and MC responses to DGLA (five dogs) and PGE_1 (four dogs) from the control responses. Results are summarized in Table I. The $PGF_{1\alpha}$ SAP response became much more predominantly pressor after the blockades with the same increases in MC as before blockade (four dogs, Table I).

After aspirin (five dogs), the DGLA response, in both SAP and MC was markedly attenuated, and sometimes abolished, at 30 min and 45 min. The duration of the positive responses ranged from 5 to 10 sec. There were no significant differences in the same five dogs in responses to PGE_1 and $PGF_{1\alpha}$, before and after aspirin. The results of these experiments are summarized in Table II.

As control fatty acids, linoleic acid (four dogs), and palmitoleic acid (two dogs) were given iv in the same concentration and dose as used for DGLA. Linoleic acid caused one depressor response, one pressor response, and no change in SAP in two dogs. There were no MC responses to linoleic acid. Palmitoleic acid caused a drop in SAP and MC in one dog and no change in either modality in the other. The two control fatty acids produced changes that were in no way similar to the biphasic SAP and positive inotropic responses to DGLA.

Platelet studies showed that DGLA (2 mg/kg) induced a significant decrease (mean 47.8 ± 5.4%) in the aggregability response to ADP (2–5 μg/ml). This was observed in the four dogs tested within 1 min after administration of DGLA. The platelet desensitization persisted after SAP and MC responses to DGLA were complete. Aspirin abolished further effects of DGLA on platelets, but did not restore platelet aggregability to control levels. Platelet counts were unaffected by DGLA in the 2.0 mg/kg dose (four dogs) and reduced

TABLE I. EFFECTS ON SYSTEMIC ARTERIAL PRESSURE (SAP) AND MYOCARDIAL CONTRACTILITY (MC) OF DGLA, PGE_1 AND $PGF_{1\alpha}$ BEFORE AND AFTER GANGLIONIC BLOCKADE (HEXAMETHONIUM) AND β-ADRENERGIC BLOCKADE (PRACTOLOL).

Agent	*n*	% Change systolic SAP	% Change diastolic SAP	% Change MC
Control				
DGLA	5	−29.6 ± 7.6[a]	−44.0 ± 8.9	+22.8 ± 2.7
PGE_1	4	−38.5 ± 5.4	−52.8 ± 8.5	+22.5 ± 4.1
$PGF_{1\alpha}$	4	−3.5 ± 4.9	−5.0 ± 5.5	+10.0 ± 2.5
After ganglionic blockade				
DGLA	5	−20.8 ± 4.0	−31.0 ± 3.2	+32.0 ± 9.1
PGE_1	4	−31.3 ± 5.2	−50.3 ± 6.3	+14.8 ± 5.4
$PGF_{1\alpha}$	4	+28.5 ± 11.1	+42.0 ± 19.7	+17.0 ± 4.4
After ganglionic blockade plus β-adrenergic blockade				
DGLA	5	−21.6 ± 1.9	−34.0 ± 3.4	+16.8 ± 3.4
PGE_1	4	−41.0 ± 4.6	−58.3 ± 4.3	+7.5 ± 3.6
$PGF_{1\alpha}$	4	+17.5 ± 4.2	+25.0 ± 7.4	+14.0 ± 2.1

[a] SE.

TABLE II. EFFECTS ON SAP AND MC OF DGLA, PGE_1 AND $PGF_{1\alpha}$ BEFORE AND AFTER ASPIRIN (100 MG/KG IV).

Agent	*n*	% Change systolic SAP	% Change diastolic SAP	% Change MC	Duration (min)
Control					
DGLA	5	−38.6 ± 7.3[a]	−53.2 ± 7.0	+32.2 ± 7.9	6.1 ± 0.56
PGE_1	5	−37.2 ± 6.0	−52.8 ± 5.6	+8.2 ± 3.4	5.4 ± 0.48
$PGF_{1\alpha}$	5	+9.6 ± 5.5	+14.4 ± 9.6	+20.6 ± 6.5	4.6 ± 0.50
30 Min after aspirin					
DGLA	5	−11.0 ± 3.3	−19.8 ± 6.8	+6.8 ± 2.4	0.79 ± 0.43
45 Min after aspirin					
DGLA	5	−7.6 ± 2.8	−12.8 ± 5.9	+5.8 ± 2.4	0.37 ± 0.28
PGE_1	5	−36.8 ± 3.6	−54.2 ± 5.5	+3.4 ± 1.6	5.4 ± 0.40
$PGF_{1\alpha}$	5	+8.0 ± 5.2	+4.2 ± 9.1	+20.0 ± 4.4	3.4 ± 0.40

[a] SE.

by 11.5% in one dog at the 2.5 mg/kg dose. The control fatty acids induced no changes in platelet count or aggregability at doses of 2.0 mg/kg delivered in the same concentration as that for DGLA.

Discussion. We found that the bisenoic PG precursor, AA, in a single (300 μg/kg) dose intravenously in dogs, produced a marked depressor response and a variable effect on MC (9). This response differed from the depressor 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 MC. AA caused thrombocytopenia and increased aggregability of platelets, and both the cardiovascular and platelet effects were inhibited by aspirin. The data indicated that the effects of AA could not be explained totally by biosynthetic conversion to PGE_2 and that the endoperoxide intermediate (10) was a possible mediator of these responses.

In doses equivalent to that used for AA, DGLA had no effect. However, when given in the relatively large 2.0 mg/kg dose, the response was approximately equidepressor to 300 μg/kg AA, and 5 μg/kg of PGE_1 or PGE_2. The characteristic biphasic SAP effect of DGLA suggested, at first, that this material was contaminated and that we were dealing with two substances. However, the purity of the DGLA used in these experiments was confirmed by three thin-layer chromatographic systems (10–12). No prostaglandins or peroxides were detected. Thus, the SAP response to DGLA is specific for this fatty acid but the later, more sustained depressor effect may be, in part, due to partial conversion to an endoperoxide intermediate or to PGE_1.

The positive inotropic response to AA (300 μg/kg) is a reflex response to hypotension and is always abolished completely by ganglionic blockade with hexamethonium (13). In contrast, the positive inotropic effect of DGLA is unaffected by either ganglionic or β-adrenergic blockade. Thus, it has a direct myocardial effect and does not occupy the β-receptors of the myocardium. The MC response to DGLA was more marked than it was to PGE_1 and $PGF_{1\alpha}$ when the data for all dogs are compared.

Aspirin, even in the large doses employed in this study, does not block the SAP and MC responses to DGLA as effectively as it blocks responses to AA. These observations support the suggestion that the initial DGLA response is specific for DGLA but that the later, sustained response is due to prostaglandin biosynthesis which is blocked by aspirin.

DGLA has previously been reported to be inactive in inducing platelet aggregation, in contrast to AA (8, 12). Our studies confirm that the SAP and MC responses to DGLA are independent of platelet effects since DGLA induces no thrombocytopenia and decreases sensitivity to aggregation. These platelet data are consistent with the biosynthesis of PGE_1 or the monoenoic PG-endoperoxide which are both potent

inhibitors of induced platelet aggregation (14).

An additional observation noted in these experiments is the potentiation of the pressor effect of $PGF_{1\alpha}$ after ganglionic blockade. Before the block, $PGF_{1\alpha}$ shows a variable SAP effect, sometimes the initial depressor effect dominating. After the block, the pressor response is dominant and marked. This phenomenon has also been observed with $PGF_{2\alpha}$ in the same dose (13). In the doses employed, $PGF_{1\alpha}$ had the same effect on MC as PGE_1 when the data for all dogs are compared.

Summary. The monoenoic prostaglandin precursor, dihomo-γ-linolenic acid (DGLA), in a single dose intravenously (2.0 mg/kg) in dogs, produced a biphasic alteration in systemic arterial pressure (SAP) with a predominant and marked depressor effect. This SAP response is approximately equidepressor to the effect of PGE_1 5 μg/kg. DGLA had a positive inotropic effect, causing a greater increase in myocardial contractility than PGE_1 in an equidepressor dose. The effect of DGLA on MC was not altered by ganglion blockade or β-adrenergic blockade. Aspirin blocked the sustained depressor response to DGLA but not an initial drop in SAP and increase of MC of very short duration. Aspirin had no effect on PGE_1 or $PGF_{1\alpha}$ responses. DGLA caused no thrombocytopenia, but caused a decrease in sensitivity to platelet aggregation. Control fatty acid injections produced variable effects with no resemblances to DGLA responses. It is concluded that DGLA produces direct depressor and positive inotropic responses as well as responses which may be due to conversion to an endoperoxide formed in the biosynthesis of prostaglandins. In contrast, in equidepressor doses, arachidonic acid (AA), the bisenoic prostaglandin precursor, produces a delayed, single-phase depressor effect which may be due to endoperoxide formation alone. Further, the effect of AA on MC is reflex and is blocked by hexamethonium.

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