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Modification of the Depressor Response to Arachidonic Acid in Dogs by Gonadal Steroids (40221)

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Arachidonic acid (AA) is well known to give rise to a cascade of prostanoate and non-prostanoate compounds which are active on many tissues including both platelets and vascular tissue (1). Intravenous administration of AA in mice induces a dose dependent hypoxia which is sex dependent and potentiated by testosterone but not by estradiol (2). Preliminary studies in rats indicate that a similar sex difference may be observed in the vasodepressor response to arachidonate and that this effect too is testosterone and not estradiol dependent (3).

In addition, progesterone, another gonadal steroid, has been reported to promote the inactivation of prostaglandins by increasing the activity of prostaglandin-15-dehydrogenase in the rabbit lung, leading to reduction of the vasodepressor response to intravenous PGE_2. Bedwani and Marley (4) demonstrated that lung inactivation of PGE_2 was increased in rabbits which were pretreated with progesterone, but not by pretreatment with estradiol or cortisone, and that ovariectomy had little effect. In rats, Blackwell and Flower (5) showed that progesterone had little effect on PGE_2 metabolism by the kidney, but that prostaglandin oxidation by kidneys of ovariectomized rats was 116% higher than the control group, and, that estradiol reduced metabolism to 17%. In consequence, we have attempted to clarify the effect of these gonadal steroid hormones, in dogs, by comparing the effects of progesterone, estrogen, and testosterone treatment on the response of the systemic arterial pressure to arachidonic acid, PGE_2, and $PGF_{2}\alpha$.

A preliminary report was presented to the American Physiological Society (6).

Materials and methods. Mongrel dogs ($n = 42$) of either sex (14–22 kg) were anesthetized with sodium pentobarbital (30 mg/kg iv) and allowed to breathe room air spontaneously. The right common carotid artery was cannulated with a polyethylene catheter and pressure was measured directly with a pressure transducer (Statham P23Db), and a direct writing recorder. To study pulmonary metabolism, intraarterial injection of drugs was made into the left ventricle via a catheter advanced from the femoral artery. The position of the left ventricular catheter was verified by direct pressure recordings. Intravenous injections were made into a catheter advanced into the inferior vena cava from the femoral vein.

Single intramuscular injections of either progesterone (2 mg/kg) ($n = 10$), estrogen (1 mg/kg) ($n = 10$), testosterone (10 mg/kg) ($n = 11$), or inert vehicle (sesame oil) were given 68–90 hr before the experiment. Arachidonic acid was administered in doses of 100 and 200 μg/kg (ia) and (iv). PGE_2 was administered in doses of 0.025, 0.05, 0.1, 0.25 and 0.5 μg/kg (ia) and 1, 2 and 3 μg/kg (iv), and $PGF_{2}\alpha$ was given in doses of 1 μg/kg (ia) and 5 μg/kg (iv). These doses produced a submaximal response whether the compounds were pressor or depressor. The sodium salt of arachidonic acid (99% pure; Nuchek) was prepared by dissolving in 100 ml sodium carbonate (0.1 *M*) with constant stirring under nitrogen, in the absence of light. PGE_2 and $PGF_{2}\alpha$ (tromethamine salt) were supplied by Upjohn and 1 mg/ml stock solutions were prepared in ethanol. Saline solutions of PGE_2 and $PGF_{2}\alpha$ were prepared daily.

The effect of sex of the animal on the response to the vasoactive agents was studied first. No significant differences were observed with a single ia or iv dose of arachidonic acid (100 & 200 μg/kg); PGE_2 (0.025, 0.05, 0.1, 0.25, 0.5 μg/kg ia; 1, 2, 3 μg/kg iv); and $PGF_{2}\alpha$ (1 μg/kg ia; 5 μg/kg iv) between females and males ($P < 0.05$). Therefore, the data for the effects of hormone treatment presented here are a combination of both male and female responses. This approach

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was considered valid since the doses of steroid hormone administered were designed to override the basal hormonal status of the animal for the period of the experiment. Subsequent calculations showed no significant differences between females and males following hormone treatment. Data were analyzed using Student's *t* test for grouped data and expressed as arithmetic means ± SE of the mean (SE).

Results. PGE_2. The depressor response following administration of PGE_2 (ia and iv) to the control group of dogs was dose related (Fig. 1) (Table I). The dose-response curves of the three treatment groups were similar, except that testosterone and progesterone tended to decrease the response. Thus, the depressor response to 0.1 μg/kg of PGE_2 (ia) in animals treated with testosterone was significantly less than that of both the control and estrogen groups ($P < 0.05$) (Fig. 1) (Table I). Following 0.5 μg/kg of PGE_2 (ia) the response was significantly less in progesterone treated dogs when compared with that of the estrogen groups (Fig. 1) (Table I). Significant differences were obtained following intravenous administration of PGE_2 at 3.0 μg/kg where the response of testosterone treated dogs was less than that of the control group ($P < 0.05$) (Table I). Both routes of administration of PGE_2 yielded vasodepressor responses of the same rank order, namely, estrogen, control, progesterone and testosterone.

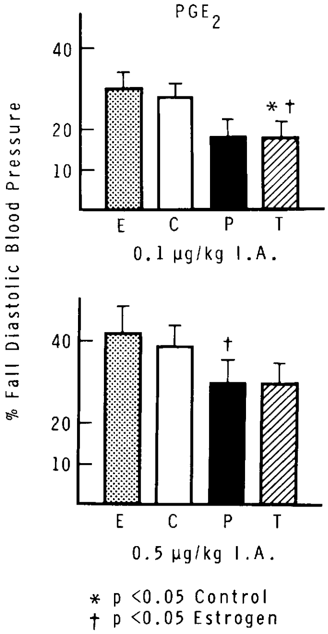

FIG. 1. The percent fall in diastolic blood pressure in response to two different doses of PGE_2 administered ia. All doses are μg/kg and submaximal. Depressor response to 0.1 μg/kg of PGE_2 (ia) in testosterone treated dogs was significantly less than both estrogen and control groups ($P < 0.05$). Response to intra-arterial administration of 0.5 μg/kg PGE_2 was significantly reduced in progesterone treated dogs compared with estrogen treated animals.

$PGF_{2}\alpha$. The pressor responses following administration of $PGF_{2}\alpha$ are shown in Table I. The response to intra-arterial administration of a submaximal dose of 1 μg/kg and the intravenous injection of a submaximal dose of 5 μg/kg were similar. No significant differences were observed in the responses to $PGF_{2}\alpha$ in the different steroid treatment groups.

Arachidonic acid. The depressor response following intravenous administration of 200 μg/kg of AA was significantly reduced in progesterone treated animals compared to both the control groups and to estrogen treated dogs ($P < 0.05$) (Fig. 2). Similarly, intra-arterial injections of AA (200 μg/kg) were significantly attenuated by progesterone when compared with both control or estrogen treated animals. Testosterone also significantly diminished the depressor response to intra-arterial AA when compared to the estrogen group ($P < 0.05$) but not when compared to control ($P > 0.05$) (Fig. 2). No significant differences were observed with 100 μg/kg of AA (Table I).

Discussion. Earlier work (7) showed that the systemic vascular response to iv administration of AA was not attenuated by pulmonary transit, in contrast to PGE_2 and $PGF_{2}\alpha$. In the present study, this observation was confirmed and, in addition, it was shown that steroid hormone treatment did not modify the iv/ia dose response ratio for AA (Fig. 2). Thus, gonadal hormone treatment did not appear to modify pulmonary metabolism of AA. However, significant differences were observed in systemic pressure *between* hormone groups following treatment with a single dose of steroid. There was a significant difference in response to 200 μg/kg of AA, both ia and iv, in dogs treated with estrogen

TABLE I. THE EFFECTS OF GONADAL STEROIDS ON BLOOD PRESSURE RESPONSES FOLLOWING PGE_2, $PGF_2\alpha$ AND ADMINISTRATION ia AND iv.[a]

Agent	Dose (μg/kg)	Route	Estrogen	Control	Proges.	Test.
PGE_2	.025	ia	7.6 ± 0.8	12.4 ± 2.2	8.8 ± 2.8	12.7 ± 4.1
	.05	ia	16.8 ± 2.2	22.2 ± 2.2	17.3 ± 3.4	16.8 ± 3.6
	0.1	ia	28.4 ± 3.7	27.6 ± 3.2	17.2 ± 3.7	17.3 ± 3.4*†
	.25	ia	39.0 ± 3.4	35.0 ± 2.6	31.8 ± 4.6	29.6 ± 6.0
	0.5	ia	40.5 ± 2.3	39.1 ± 4.4	29.9 ± 4.9†	29.4 ± 4.8
	1.0	iv	9.1 ± 2.2	13.4 ± 2.9	12.2 ± 3.5	12.8 ± 3.5
	2.0	iv	21.4 ± 4.2	26.9 ± 3.7	23.2 ± 3.8	18.8 ± 3.1
	3.0	iv	35.4 ± 5.1	38.8 ± 3.7	31.5 ± 5.8	22.1 ± 5.2*
AA	100	ia	5.5 ± 1.2	9.1 ± 1.6	4.1 ± 1.3	3.0 ± 1.1
	100	iv	2.5 ± 0.9	6.6 ± 2.3	2.1 ± 1.0	2.4 ± 0.8
$PGF_2\alpha$	1.0	ia	10.2 ± 2.4	15.9 ± 2.2	13.0 ± 2.0	12.4 ± 1.7
	5.0	iv	11.8 ± 3.3	14.2 ± 3.0	13.6 ± 2.4	12.3 ± 2.9

[a] Numbers represent % fall in diastolic blood pressure ± SE for AA and PGE_2 and % rise in diastolic blood pressure ± SE for $PGF_2\alpha$. All P values were >0.05 unless otherwise noted. * $P < 0.05$ control. † $P < 0.05$ estrogen.

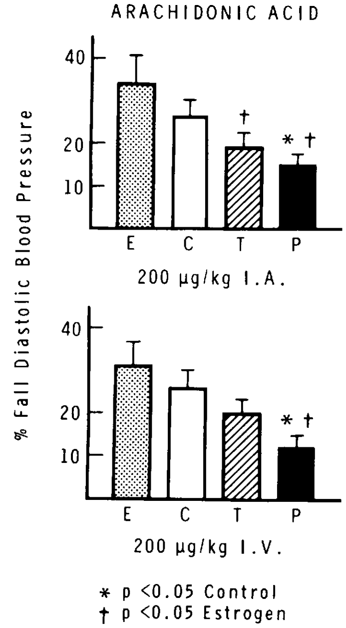

FIG. 2. The percent fall in diastolic blood pressure in response to arachidonic acid administered ia and iv. Doses are μg/kg and submaximal. Response to 200 μg/kg AA (iv and ia) was significantly reduced in progesterone treated animals when compared with both estrogen and control groups ($P < 0.05$). Testosterone treatment significantly reduced response to 200 μg/kg AA (ia) when compared to the estrogen treated groups.

when compared to those treated with progesterone ($P < 0.05$) (Fig. 2). The response to 200 μg/kg of AA (ia and iv) was also significantly less in the progesterone treated dogs when compared to control animals. Testosterone pretreatment significantly reduced the depressor response to 200 μg/kg of AA (ia) when compared to estrogen treated animals, but not when compared to control. However, most testosterone treated animals responded (8 of 11) to both ia and iv administration with attenuation of the expected drop in blood pressure as compared to controls. In this regard, testosterone treated dogs resembled the progesterone treated animals, but differed from those treated with estrogen. Whether estrogen potentiates the vasodepressor response to AA cannot be determined at this stage, as the data were not significantly different from the controls ($P > 0.05$) (Fig. 2). Possibly, chronic treatment with estrogen may be necessary to obtain a convincing difference. The rank order of hormone effect following administration of 200 μg/kg of AA was estrogen, control, testosterone, progesterone.

The results of the effect of gonadal steroid treatment on the vascular response to prostaglandins was more variable (Fig. 1 and Table I). Significant differences were observed in the vasodepressor response to PGE_2 in three instances following ia, and one instance following iv administration (Fig. 1) (Table I). The rank order of steroid effect was the same for both routes of administration of PGE_2, namely, estrogen, control, progesterone and testosterone (Fig. 1) (Table I). Thus, estrogen appears to have an opposite effect to progesterone or testosterone. Our data reflect some of the conclusions of Bedwani and Marley (4) and Sun and Armour (8), who reported an increase in prostaglandin-15-dehydrogenase activity in lungs of progesterone treated and pregnant rabbits, which is associated with a decreased systemic pressure response to intravenous PGE_2. Blackwell and Flower (5) were not able to observe a change in kidney prostaglandin-15-dehydrogenase activity in ovariectomized rats treated with progesterone, but they observed decreased PGE_2 metabolism in kidneys of ovariectomized rats treated with estradiol. Thus, these studies (4, 5, 8) can be taken together as indicating that estrogen and progesterone may have opposite effects on prostaglandin metabolism.

The effect of hormone treatment may also be due to (i) an effect on blood vessels or myocardial function (9), (ii) an alteration of receptor sites, (iii) an alteration of the profile of vasoactive products of AA as well as (iv), a change in the catabolism of the vasoactive components of the arachidonic acid cascade. However, the fact that testosterone has been reported previously to increase the pressor response to norepinephrine of spinal cats (10), rats (3), and dogs (11) appears to argue for a mechanism which may be independent of the nature of the agonist. Thus the attenuation of the vasodepressor response to PGE_2 in progesterone treated rats which Bedwani and Marley (4) ascribed to increased prostaglandin-15-dehydrogenase activity may be a nonspecific effect of progesterone and may not necessarily be related to PGE_2 as such.

Summary. Single intramuscular injections of either progesterone (2 mg/kg), estrogen (1 mg/kg), testosterone (10 mg/kg), or vehicle (control) were given to mongrel dogs of both sexes. Arachidonic acid (100 and 200 μg/kg), PGE_2 (0.025, 0.05, 0.1, 0.25 ia and 1-3 μg/kg iv), and $PGF_{2}\alpha$ (1 μg/kg ia and 5 μg/kg iv) were injected into the inferior vena cava (iv) or left ventricle (ia) 68–90 hr after hormone treatment. Changes in the systemic pressure responses were observed. The depressor response to AA (200 μg/kg) was significantly reduced in progesterone treated animals when compared to estrogen treated and control groups ($P < 0.05$). Similarly, testosterone treatment reduced the response to ia administration of AA (200 μg/kg) when compared to estrogen treatment. Similar effects were observed using PGE_2. No significant differences in pressor responses to $PGF_{2}\alpha$ were observed following gonadal hormone treatment. In all treatment groups the ia/iv dose response ratio to AA was unaltered, which implies that a single dose of gonadal hormone does not modify pulmonary AA metabolism.

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