PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 953–957 (1975)

Effects of Ganglionic and β-Adrenergic Blockade on Cardiovascular Responses to the Bisenoic Prostaglandins and their Precursor Arachidonic Acid[1] 38934)

PETER A. KOT, MALCOLM JOHNSON, PETER W. RAMWELL, AND JOHN C. ROSE

Department of Physiology and Biophysics, Georgetown University Medical Center, Washington, D.C. 20007

The bisenoic prostaglandins, PGE_2 and $PGF_{2\alpha}$, increase myocardial contractile force, but have opposite effects on systemic arterial pressure in the dog (1). PGE_2 produces a vasodepressor response and $PGF_{2\alpha}$ produces a pressor response. Arachidonic acid (AA), the precursor of the bisenoic prostaglandins, has variable effects on myocardial contractile force, but consistently produces a decline in systemic arterial pressure (2).

The specific objectives of the present study were to determine whether the cardiac responses to these agents observed in the dog are due to direct action on the heart or are reflex in nature, and to examine blood pressure and cardiac responses to these agents after blockade of the autonomic ganglia and the β-receptors of the heart.

Materials and Methods. Mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg) and maintained on intermittent positive respiration with a Harvard respirator. A left thoracotomy was performed and a Walton-Brodie strain gauge arch was sutured to the right ventricular wall for measurement of myocardial contractile force. A femoral artery and vein were catheterized; the former for direct measurement of systemic arterial pressure and the latter for administration of test substances into the inferior vena cava. Through a midline neck incision both common carotid arteries were isolated and tagged with loose ligatures.

The sodium salt of arachidonic acid (5, 8, 11, 14-eicosatetraenoic acid; >99% pure from porcine liver; Sigma) was prepared by dissolving the free acid in sodium carbonate (100 m*M*) with constant stirring under nitrogen in the absence of light. The resulting solution was water clear and was stored for no more than 2 days in the dark under nitrogen. The prostaglandins used in this study were generously supplied by the Upjohn Company. Solutions in ethanol (1 mg/ml) were evaporated to dryness under nitrogen, and the residue was dissolved in normal saline. Hexamethonium chloride (Schwarz/Mann) was prepared in aqueous solution in a concentration of 50 mg/ml. Practolol (Practol) was generously supplied by Ayerst Laboratories, Inc.

Test substances were administered as bolus injections intravenously in the following dose levels; AA 300 μg/kg and 900 μg/kg, PGE_2 5 μg/kg, and $PGF_{2\alpha}$ 5 μg/kg. These doses were selected because our previous studies indicated that such levels administered intravenously produced a profound change in systemic arterial pressure from which recovery always rapidly ensued. AA 300 μg/kg is equidepressor with PGE_2 5 μg/kg (2). Sufficient time was allowed to elapse (approx 5 min) between each injection to permit systemic arterial pressure and myocardial contractile force to return to control values.

Following observation of the initial response to each of these agents, ganglionic blockade was produced with hexamethonium chloride 2 mg/kg given intravenously. Both common carotid arteries were temporarily occluded just prior to, and shortly after, the administration of the hexamethonium to verify that the baroreceptor reflexes were inoperative. The same doses of AA, PGE_2, and $PGF_{2\alpha}$ were repeated after ganglionic blockade.

In three dogs, the hemodynamic responses produced by these agents were measured after ganglionic blockade and beta-adrenergic blockade with practolol (2 mg/kg intravenously). Isoproterenol (Isuprel) (0.5 μg/kg) was administered intravenously immediately before and after

[1] Supported in part by the Educational Foundation of America.

practolol administration to verify that the myocardial responses to this agent were completely blocked. The same doses of AA, PGE_2 and $PGF_{2\alpha}$ were administered after ganglion and cardiac beta-receptor blockade were established.

Results. A. Responses before ganglionic blockade. Both AA and PGE_2 consistently produced a decrease in systemic arterial pressure. The magnitude of the changes in systolic and diastolic pressure were comparable for these two substances in the doses used (Table I). The mean time of onset of the depressor response was delayed with AA (14.6 sec) as compared to that observed with PGE_2 (8.6 sec).

$PGF_{2\alpha}$ caused a rise in both the systolic (26 ± 4 mmHg) and diastolic (32 ± 5 mmHg) levels of systemic arterial pressure.

Myocardial contractile force responses were variable following AA administration. In one animal, myocardial contractile force decreased and in another animal there was no change. In the remaining 11 animals, myocardial contractile force increased, but the magnitude of the increase was less than that observed following PGE_2 despite comparable decreases in systemic arterial pressure (Table I).

Following $PGF_{2\alpha}$, myocardial contractile force increased in 11 of 13 animals; in two animals contractile force remained unchanged. In the two animals exhibiting no change in myocardial contractile force, alterations in systemic arterial pressure were not significantly different than in the other animals of the group.

In three animals the intravenous dose of AA was increased to 900 μg/kg. The magnitude of the decline in systolic and diastolic pressure levels was greater than the 300 μg/kg dose. The mean decreases in systolic and diastolic pressure levels were 40 and 61 mmHg, respectively. The myocardial contractile force increased a mean of 12.5%.

B. Responses after ganglion blockade with hexamethonium. The percentage of change in systemic arterial pressure following AA administration was not significantly altered by ganglionic blockade. PGE_2 did not produce as great a fall in systemic arterial pressure after ganglionic blockade. The time of onset of the depressor response was delayed from a mean of 14.6 ± 0.8 sec to 19.6 ± 1.9 sec for AA and from 8.6 ± 0.7 sec to 10.9 ± 1.9 sec for PGE_2. However, $PGF_{2\alpha}$ caused a pressor response which was nearly three times greater than that observed before blockade. The mean increase in systolic and diastolic pressure levels were 28 ± 4 and 34 ± 5 mmHg before blockade and 73 ± 11 and 107 ± 15 mmHg after blockade.

The effect of AA on myocardial contractile force was diminished or completely abolished by ganglionic blockade (Table I). Figure 1 shows responses to AA (300 μg/kg) in the same animal before and after the administration of hexamethonium. The depressor response was not altered by ganglionic blockade, but the increase in cardiac contractile force observed before blockade was eliminated.

The responses to PGE_2 were not appreciably affected by ganglionic blockade as shown in Fig. 1. A prominent positive inotropic effect on the heart was still present after hexamethonium.

Ganglionic blockade also did not diminish the myocardial contractile force response produced by $PGF_{2\alpha}$. As shown in Table I, the percentage of increase in cardiac contrctile force was somewhat enhanced following $PGF_{2\alpha}$ administration in the presence of ganglionic blockade.

C. Responses after ganglionic and β-adrenergic blockade. In three animals, the cardiac effects of AA, PGE_2, and $PGF_{2\alpha}$ were studied after ganglionic and β-adrenergic blockade. At the intravenous dose levels indicated previously, all three compounds produced an increase in myocardial contractile force. The mean increases in myocardial contractile force were 12% following AA, 18% following PGE_2, and 48% following $PGF_{2\alpha}$.

Discussion. Considerable species variation exists in the cardiovascular actions of PGE_2 and $PGF_{2\alpha}$. In near-term pregnant sheep, PGE_2 and $PGF_{2\alpha}$ had no significant effects on the maternal or fetal circulations, suggesting that sheep are insensitive to prostaglandins (3). In unanesthetized calves, however, $PGF_{2\alpha}$ administration produced a significant increase in pulmonary artery

TABLE I. EFFECTS OF GANGLIONIC BLOCKADE ON THE HEMODYNAMIC RESPONSES TO ARACHIDONIC ACID (AA), PGE_2, AND $PGF_{2\alpha}$.

Agent and dose	% Change in systemic arterial pressure				% Change in myocardial contractile force	
	Pre-GN blockade		Post-GN blockade			
	Systolic	Diastolic	Systolic	Diastolic	Pre-GN	Post-GN
AA ($n = 13$)[a] 300 μg/kg	−27 ± 4	−39 ± 4	−28 ± 4	−38 ± 5	+9.0 ± 3.5	−3.7 ± 3.4
PGE_2 ($n = 12$) 5 μg/kg	−28 ± 2	−46 ± 2	−17 ± 3	−29 ± 4	+26.3 ± 5.7	+29.8 ± 6.8
$PGF_{2\alpha}$ ($n = 12$) 5 μg/kg	+28 ± 4	+34 ± 5	+73 ± 11	+107 ± 15	+24.1 ± 5.0	+38.1 ± 4.8

[a] n = number of animals.

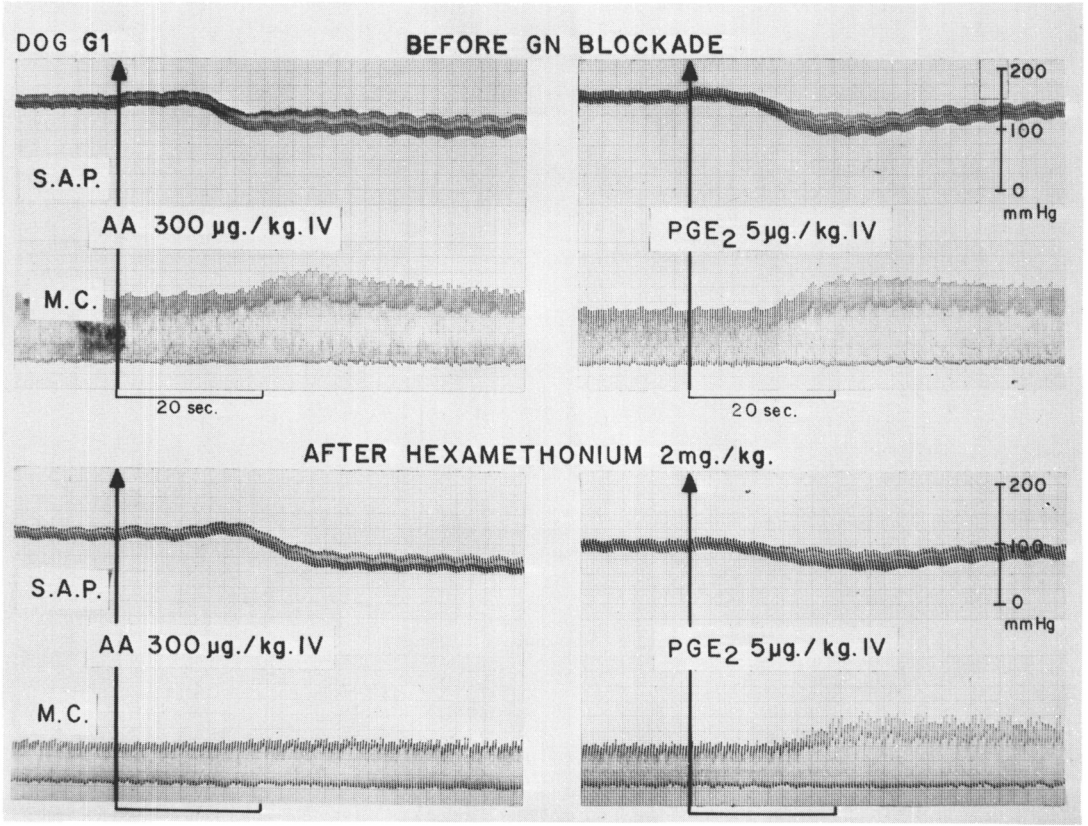

FIG. 1. Simultaneous recordings of systemic arterial pressure (SAP) and myocardial contractile force (MC) in a dog receiving the doses of AA and PGE_2 shown. The vertical lines represent the instant of introduction of the substance into the inferior vena cava. Note the difference in myocardial contractile force response to AA after ganglionic blockade with hexamethonium.

pressure, but no discernible effects on systemic arterial pressure (4). PGE_2 in the same species produced a fall in systemic arterial pressure, which was attributed to arteriolar dilatation since cardiac output was not affected.

In the cat and rabbit (5, 6), $PGF_{2\alpha}$ is a depressor agent. The fall in systemic arterial pressure observed in the cat was attributed to a combination of factors that included reduced left ventricular output secondary to pulmonary vasoconstriction, bradycardia, and a mild vasodilating action on muscle vessels. Koss and Nakano (7) also concluded that only part of the depressor activity of $PGF_{2\alpha}$ in the cat could be attributed

to direct vasodilatation of peripheral vessels. In the dog, $PGF_{2\alpha}$ is a moderate pressor agent (1). Both PGE_2 and $PGF_{2\alpha}$ have previously been shown to increase myocardial contractility in the dog (8).

Results of the present investigation are consonant with previous observations on the hemodynamic effects of PGE_2 and $PGF_{2\alpha}$ in the dog not subjected to ganglionic blockade. PGE_2 produced a consistent depressor response and an increase in myocardial contractile force. $PGF_{2\alpha}$ increased systemic arterial pressure as well as myocardial contractile force.

AA administration as previously observed in this laboratory was associated with a consistent decrease in systemic arterial pressure and a variable effect on myocardial contractile force (2).

With the baroreceptor reflexes inoperative, AA (300 μg/kg iv) did not produce any significant increase in myocardial contractile force, suggesting that the increased myocardial contractile force observed before blockade represented a reflex response which was secondary to the fall in systemic arterial pressure. However, the larger dose of AA (900 μg/kg), which is close to the LD_{50} dose in rabbits (9), elicited an increase in myocardial contractile force after blockade. Whether this represents a direct cardiac effect of AA when given in large doses or is indicative of increased conversion of AA into an intermediate endoperoxide or into PGE_2 and $PGF_{2\alpha}$ could not be determined in these experiments.

Ganglionic blockade did not alter the hemodynamic effects of PGE_2, but resulted in an augmentation of the pressor response to $PGF_{2\alpha}$. The magnitude of the enhanced pressor response following $PGF_{2\alpha}$ was approximately three times greater than before blockade and was larger than the augmented pressor responses observed in pentolinium-treated rats (10). Although elimination of the compensatory reflexes could explain the augmented pressor response to $PGF_{2\alpha}$, a local potentiating action of hexamethonium on the effects of $PGF_{2\alpha}$ cannot be ruled out.

The direct cardiac effects of PGE_2 and $PGF_{2\alpha}$ were still observed in the presence of complete β-adrenergic blockade, suggesting that these agents may not exert their effects through the β-receptors.

The time of onset of the systemic pressure response following AA administration was delayed in comparison to PGE_2 and $PGF_{2\alpha}$ suggesting a requirement for biosynthesis of AA into an active vasodilator. The additional delay noted after ganglionic blockade most likely represents a prolonged circulation time so that arrival of the active compound in the peripheral systemic circulation takes longer.

Summary. Arachidonic acid (AA) 300 μg/kg, and PGE_2, 5 μg/kg consistently produced a decrease in systemic arterial pressure in anesthetized dogs. $PGF_{2\alpha}$, 5 μg/kg, produced a pressor response. All three compounds increased myocardial contractile force, but the magnitude of the change following AA was less prominent. After ganglionic blockade, the depressor response to AA and PGE_2 persisted and the pressor response to $PGF_{2\alpha}$ was augmented. Myocardial contractile force did not increase following AA in ganglion-blocked animals indicating that the cardiac responses observed before hexamethonium were mediated by the baroreceptor reflexes. A much larger dose of AA (900 μg/kg) resulted in a small positive inotropic effect on the heart. This possibly represents a direct cardiac effect of AA, or may be indicative of increased biosynthesis of an intermediate endoperoxide, or PGE_2 and $PGF_{2\alpha}$. Both PGE_2 and $PGF_{2\alpha}$ have a direct positive inotropic effect on the heart. The persistent cardiac effects of PGE_2 and PGF_2 after β-adrenergic blockade suggests that these compounds may not interact with the β-receptors of the myocardium.

1. Nakano, J. and Cole, B., *Amer. J. Physiol.* **217,** 222 (1969).
2. Rose, J. C., Johnson, M., Ramwell, P. W., and Kot, P. A., *Proc. Soc. Exp. Biol. Med.* **147,** 652 (1974).
3. Oakes, G., Mafid, M., Brinkman, C. R., and Assali, N. S., *Proc. Soc. Exp. Biol. Med.* **142,** 194 (1973).
4. Anderson, F. L., Kralios, A. C., Tsagaris, T. J., and Kuida, H., *Proc. Soc. Exp. Biol. Med.* **140,** 1049 (1972).

5. Horton, E. W. and Main, I. H. M., *Brit. J. Pharmacol.* **24,** 470 (1965).
6. Anggard, E. and Bergstrom, S., *Acta Physiol. Scand.* **58,** 1 (1963).
7. Koss, M. C. and Nakano, J., *Proc. Soc. Exp. Biol. Med.* **142,** 383 (1972).
8. Nakano, J., *Mod. Concepts Cardiovasc. Dis.* **40,** 49 (1971).
9. Silver, M. J., Hoch, W., Kocsis, J. J., Ingerman, C. M., and Smith, J. B., *Science* **183,** 1085 (1974).
10. Ducharme, D. W., Weeks, J. R., and Montgomery, R. G., *J. Pharmacol. Exp. Ther.* **160,** 1 (1968).

Received March 27, 1975. P.S.E.B.M., 1975, Vol. 149.