PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 193-197 (1976)

Plasma Prostaglandin $F_{2\alpha}$ and 15-Keto-$F_{2\alpha}$ Concentrations During Splanchnic Artery Occlusion Shock[1] (39172)

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Splanchnic artery occlusion shock (SAO) is a severe form of cardiovascular shock in which ischemia of the splanchnic region is the primary pathophysiological phenomenon initiating the shock state. Humoral factors appear to play a significant role in this form of shock as well as in others such as hemorrhagic, endotoxic, cardiogenic, and burn shock (1). Prostaglandins (PG's) as humoral factors have been shown to accumulate in the blood during hemorrhagic and endotoxic shock (2–7). The primary purposes of this investigation were to quantitate the arterial plasma concentrations of prostaglandin $F_{2\alpha}$ and one of its metabolites 15-keto-prostaglandin $F_{2\alpha}$ (15-keto-$PGF_{2\alpha}$) during SAO shock in the dog as evidence for a role of endogenous prostaglandins in circulatory shock.

Methods. Adult male mongrel dogs (14 to 18 kg) were anesthetized with sodium pentobarbital (30 mg/kg, iv). No additional anesthetic was administered. A patent airway was established with an endotracheal tube, but artificial ventilation was not employed. The right femoral artery and vein were exposed for catheterization, and catheters were positioned for the measurement of mean arterial blood pressure (MABP) and central venous pressure (CVP), respectively. Lead III of the electrocardiogram and vascular pressures were continuously recorded on a Grass Model 7 polygraph. A midline laparotomy was performed in all dogs and the celiac, superior mesenteric, and inferior mesenteric arteries were isolated. Splanchnic artery occlusion shock was produced in nine animals by total occlusion of the splanchnic vessels for a period of 2 hr after which time the clamps were removed and the animals were observed for an additional 90 min. Seven animals comprised a sham group and were treated in an identical manner to the SAO shock group except that the splanchnic vessels were not occluded.

Arterial blood samples were drawn at specified times. Plasma obtained from these samples were used in the determination of amino nitrogen (8) and protein (9) concentrations, cathepsin D activities (10), and MDF activities (11). Additionally, 30-ml arterial blood samples for prostaglandin analysis were drawn into heparinized plastic syringes which contained 150 μg of indomethacin to inhibit *in vitro* prostaglandin synthesis. Prostaglandins were extracted from arterial plasma into chloroform at pH 4.0 to 4.5 by the method of Unger *et al.* (12). The extraction residues were chromatographed by thin layer chromatography (TLC), after which $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ bands were eluted with 100% ethanol. During the TLC procedure employed, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, 13-14-dihydro-$PGF_{2\alpha}$, and 15-keto, 13-14-dihydro-$PGF_{2\alpha}$ migrated with R_f values sufficiently different for their separation. Aliquots of the $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ samples were evaporated to dryness under nitrogen and radioimmunoassayed. The anti-$PGF_{2\alpha}$ antibody used in this study was produced in rabbits and diluted 1:2250 in 0.154 *M* Tris buffer (pH 7.4) prior to use. Methods of antibody production, characterization studies, and cross-reactivity data have previously been published (13). The anti-$PGF_{2\alpha}$ antibody cross-reacted to a sufficient degree with 15-keto-$PGF_{2\alpha}$ to allow for the assay of this metabolite after its separation from $PGF_{2\alpha}$, 15-keto,-13,14,-dihydro-$PGF_{2\alpha}$, and 13,14-dihydro-$PGF_{2\alpha}$ by TLC. Tracer amounts of [^{3}H]PGA_1 (New England Nuclear) were added to each arterial plasma sample just prior to extraction. All

[1] This investigation was supported in part by Research Grant No. HL-17745 from the NIH.

final prostaglandin concentrations were thus corrected for the percentage of PG recovery through the extraction, chromatography, and elution procedures.

Results. The effects of SAO shock on mean arterial blood pressure, heart rate, plasma amino nitrogen, and plasma cathepsin D activity are shown in Table I. The preocclusion MABP of the SAO group was not significantly different from that of the sham group. The MABP of the sham shock group remained relatively constant throughout the 210-min experimental period, in contrast to the MABP of the SAO group which followed a biphasic pattern. At 60 min postocclusion, the MABP of the SAO group increased, and was significantly higher than that of the sham group. The MABP of the SAO group returned to near control values at 120 min postocclusion, at which time the clamps were removed. Within 5 min of release, the MABP of the SAO group dropped precipitously to 45 mm Hg, and remained significantly depressed for the remainder of the experiment. The preocclusion heart rate of the sham group was 161 beats/min, which remained relatively constant for the entire experimental period. Prior to release, the heart rates of the SAO group were not significantly different from those of the sham group. However, within 5 min of release, the heart rate of the SAO group decreased, and remained significantly lower than that of the sham SAO group during all but one postrelease sampling period. This bradycardia occurred at a low MABP, a situation in which a reflex tachycardia would have been expected. Furthermore, bilateral vagotomy in two SAO dogs did not block the decrease in heart rate observed after release of the clamps. The plasma amino nitrogen concentration remained stable in the sham group during the entire experimental period, whereas it significantly increased in the SAO group during the period of occlusion, decreased soon after release, and increased again 60–90 min after release. The activity of the lysosomal enzyme, cathepsin D, remained relatively constant in the sham SAO group during the experimental sampling period. In contrast, plasma cathepsin D activity increased dramatically from the preocclusion value in the SAO shock dogs. The five-min postrelease cathepsin D activity was elevated to about eight times the control value. This increase continued, and the final cathepsin D activity was 22 times

TABLE I. HEMODYNAMIC AND HUMORAL FACTORS DURING SAO SHOCK.[a]

Group	Time (min)	N	MABP (mm Hg)	HR (b/min)	Plasma amino-N (μmoles/ml)	Cathepsin D (units/mg protein)
SAO shock	−10	9	112 ± 4	165 ± 11	3.7 ± 0.5	8 ± 2
	0 (occlude)					
	60	9	145 ± 6**	157 ± 10	6.0 ± 0.8^{+}	11 ± 3
	120 (release)	9	123 ± 6	143 ± 11	7.0 ± 1.1^{+}	32 ± 8*
	125	9	45 ± 11**	120 ± 6^{+}	8.0 ± 1.5^{+}	62 ± 10***
	140	8	48 ± 10***	108 ± 15^{+}	5.5 ± 1.1	84 ± 17***
	160	7	44 ± 10***	127 ± 10	5.7 ± 1.4	109 ± 16***
	180	6	42 ± 10***	126 ± 10*	5.0 ± 0.6*	139 ± 23***
	210	6	39 ± 9***	116 ± 13^{+}	6.1 ± 0.8*	178 ± 33***
Sham shock	−10	7	104 ± 8	161 ± 10	4.0 ± 0.7	6 ± 2
	0 (occlude)					
	60	7	107 ± 7	187 ± 15	3.5 ± 0.7	6 ± 2
	120 (release)	7	110 ± 8	158 ± 16	3.6 ± 0.7	6 ± 1
	125	7	109 ± 10	156 ± 15	3.5 ± 0.7	8 ± 2
	140	7	110 ± 10	160 ± 16	4.0 ± 0.8	9 ± 2
	160	7	111 ± 10	161 ± 17	3.4 ± 0.8	7 ± 2
	180	7	116 ± 10	175 ± 15	2.6 ± 0.5	7 ± 3
	210	7	113 ± 8	168 ± 18	3.5 ± 0.8	11 ± 4

[a] All values are means ± SEM. N = number of dogs studied. The −10-min period is prior to occlusion of the celiac, superior mesenteric, and inferior mesenteric arteries. Cathepsin D units are expressed as mEq tyrosine released × 10^{-6}/hour at 37°. Significance of Student's unpaired t test is as follows: $^{+}$, $P < 0.05$, compared with sham group at same time; *, $P < 0.02$; **, $P < 0.01$; *** $P < 0.001$.

greater than the preocclusion value.

Arterial plasma concentrations (means ± SEM) of prostaglandin $F_{2\alpha}$ in the SAO group were determined to be 0.63 ±0.23, 0.90 ± 0.22, and 0.93 ± 0.26 ng/ml for the preocclusion, 60-min post-, and 120-min postocclusion periods, respectively. In the sham group, the preocclusion, 60-min post-, and 120-min postocclusion concentrations of $PGF_{2\alpha}$ were 0.78 ± 0.41, 0.75 ± 0.47, and 0.89 ± 0.50 ng/ml plasma. A considerable variation in basal circulating $PGF_{2\alpha}$ concentrations among dogs was encountered during this study, and although the arterial concentrations of $PGF_{2\alpha}$ increased in the SAO group after release of the clamps, no statistically significant differences between the SAO and sham groups could be observed with absolute PG concentrations. However, when the arterial $PGF_{2\alpha}$ concentrations are expressed as a percentage of control to normalize the data, significant findings were observed (Fig. 1). The presentation of prostaglandin data on a percentage of control basis is justified in this situation. In animals having widely differing concentration ranges of $PGF_{2\alpha}$, an overlapping of ranges between animals negates analysis of the data in absolute terms. Normalizing the data on a percentage change basis allows proper assessment of changes occurring in the group as a whole. Recent evidence suggests that the observed effects of prostaglandins in an animal are the net result of PG interaction with other humoral and hormonal substances. Depending upon the profile of active substances present in an animal at any one time, what might be a low concentration of PG in one animal might functionally be a high concentration in another. Therefore, the absolute concentration of a prostaglandin in an animal may be misleading, and relative change thus becomes the important parameter. The control reference values in Fig. 1 represent the mean concentrations of $PGF_{2\alpha}$ in each group during the preocclusion, 60-min post-, and 120-min postocclusion sampling periods, since these values are within 10% of each other. In the sham group, postrelease values of $PGF_{2\alpha}$ generally decreased below the control value during the sampling period. In contrast, the percentage of control values for $PGF_{2\alpha}$ of the SAO group increased above the control value for the entire postrelease sampling period. SAO group values were significantly greater than those of the sham group at all times beyond 5 min after release. Arterial concentrations of 15-keto-$PGF_{2\alpha}$ were also measured in these plasma samples. The mean concentrations of 15-keto-$PGF_{2\alpha}$ in the SAO group ranged between a control concentration of 1.6 ng/ml and a shock period concentration of 4.8 ng/ml, with postrelease 15-keto-$PGF_{2\alpha}$ concentrations significantly greater than those of the sham group ($P < 0.05$). In the sham group, the arterial concentration of 15-keto-$PGF_{2\alpha}$ was between 1.0 and 1.4 ng/ml over the entire experimental period.

Figure 2 is a plot of mean arterial plasma concentration of $PGF_{2\alpha}$ versus 15-keto-

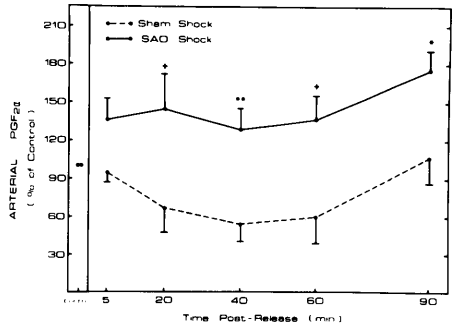

FIG. 1. Arterial $PGF_{2\alpha}$ concentrations expressed as percentage of control. The control values for the SAO and sham groups reflect the combined mean values of the −10-, 60-, and 120-min postocclusion periods in each group. All values are means ± SEM. Significance of Student's unpaired t test is as follows: (+) = $P < 0.05$, (*) = $P < 0.02$, (**) = $P < 0.01$ compared with sham group at same time.

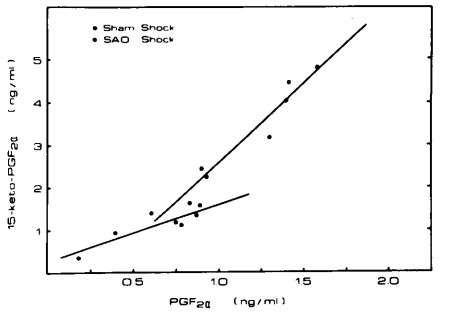

FIG. 2. Correlation between arterial $PGF_{2\alpha}$ and arterial 15-keto-$PGF_{2\alpha}$. Linear regression of mean concentrations of $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ measured at each sampling period. Sham group, $Y = (1.42)(x) + (0.26)$, $r = 0.89$. SAO groups, $Y = (3.27)(x) + (-1.14)$, $r = 0.96$.

$PGF_{2\alpha}$ at each sampling period. Two distinct linear regression lines can be calculated. The line best fitting the sham group data can be described by the equation $Y = (1.42)(x) + 0.26$, $r = 0.89$. The line best fitting the SAO group data is described by the equation $Y = (3.72)(x) + (1.14)$, $r = 0.96$.

At 90 min after release, 60-ml blood samples were collected for MDF determinations. Within the sham group at this time, MDF activity was determined to be 16 ± 2.8 MDF units ($n = 4$). The MDF activity in the blood of SAO animals was determined to be 57 ± 3.5 MDF units ($n = 4$), a value which is significantly greater than that of the sham group ($P < 0.001$). Six of nine SAO dogs survived the 210-min experimental period whereas survival of the sham group dog was 100% during the same period.

Discussion. Although circulating prostaglandins have been shown to increase during a variety of types of shock, the role of prostaglandins in circulatory shock is not well defined. Prostaglandin concentrations increase in arterial plasma during hemorrhagic or endotoxic shock (3–6) and in venous plasma during hemorrhagic or endotoxic shock (2, 7). The present data demonstrate an increase in circulating prostaglandin concentrations in splanchnic artery occlusion shock. One common feature of hemorrhagic, endotoxic, and SAO shock is that mesenteric artery flow is compromised very early in the course of the shock state (1, 14). In the present study, release of clamps occluding the mesenteric vessels resulted in a dramatic fall in the MABP which could not be completely explained by loss of circulating blood volume into the splanchnic region. This fall in MABP plus the observed decrease in heart rate of the SAO dogs even after bilateral vagotomy, strongly suggest humoral factors being washed from the ischemic splanchnic region by the reestablishment of blood flow. The elevated plasma amino nitrogen concentrations and cathepsin D activities in SAO shock strongly support the concept of proteolysis and lysosomal breakdown in the ischemic splanchnic region. Early increases in these substances during the occlusive stage of SAO shock probably represent transport of these materials from the splanchnic region into the general circulation via lymphatic channels (15).

Arterial concentrations of $PGF_{2\alpha}$ increased 40% in the postrelease period in SAO shock dogs. Flynn *et al.* (6) reported a twofold increase in the amount of $PGF_{2\alpha}$ circulating in arterial plasma during hemorrhagic shock in the dog. This difference may be attributable to (a) a more generalized systemic ischemia during hemorrhagic shock versus a regional ischemia during SAO shock, and (b) a difference in the manner in which prostaglandins are metabolized or cleared from the circulation in the two forms of shock. Arterial $PGF_{2\alpha}$ concentrations decrease with time in the sham SAO dogs except for the 210-min value. The exact mechanism of this general decrease in $PGF_{2\alpha}$ concentration in the sham SAO group with time is unknown. The relatively constant $PGF_{2\alpha}$ concentration seen during the preocclusion and the 60-min and 120-min postocclusion periods suggests that the observed decrease in the $PGF_{2\alpha}$ concentration of the sham SAO animals may have resulted from hemodilution due to frequent blood sampling "postrelease" and replacement of the samples with equal volumes of 0.9% NaCl.

Although we observed an increase in the circulating concentration of $PGF_{2\alpha}$ during SAO shock, this finding does not indicate whether the increased concentration is due to increased synthesis and release of $PGF_{2\alpha}$ during SAO shock or to diminished metabolism of this prostaglandin. Simultaneous measurements of 15-keto- and $PGF_{2\alpha}$ in arterial blood provide a partial answer. In the sham group, the arterial concentration of 15-keto-$PGF_{2\alpha}$ remained relatively constant during the experimental period in contrast to the SAO shock groups in which the arterial concentration of 15-keto-$PGF_{2\alpha}$ increased significantly after release. This suggests that $PGF_{2\alpha}$ was being synthesized and released in the SAO animals and was being converted into 15-keto-$PGF_{2\alpha}$ by 15-hydroxyprostaglandin dehydrogenase (PGDH). This is confirmed by the linear regression plots of the mean $PGF_{2\alpha}$ versus 15-keto-$PGF_{2\alpha}$ concentrations. In the sham SAO animals, a slope of 1.42 and a correlation coefficient of 0.89 suggest that $PGF_{2\alpha}$ is efficiently con-

verted to 15-keto-$PGF_{2\alpha}$ and that the circulating 15-keto-$PGF_{2\alpha}$ is being rapidly removed from the general circulation. The slope of 3.72 and a correlation coefficient of 0.96 for the SAO group regression line again suggest an efficient metabolism of $PGF_{2\alpha}$ to 15-keto-$PGF_{2\alpha}$. However, since 15-keto-$PGF_{2\alpha}$ is a dependent variable of $PGF_{2\alpha}$ in a 1:1 relationship, the observed increase in slope from 1.42 to 3.72 suggests that 15-keto-$PGF_{2\alpha}$ has a longer circulating half-time in SAO shock dogs than in sham dogs. Whether this represents an altered ability of the kidneys or other tissues to remove 15-keto-$PGF_{2\alpha}$ from the circulation remains to be determined. Although PGDH activity does not appear to be decreased during SAO shock, Nakano and Prancan (16) have reported a decreased activity of PGDH during endotoxin shock in the rat. Thus, PGDH efficiency is not universally impaired during circulatory shock, although the clearance of prostaglandins and their metabolites from the circulation may be reduced in shock.

Summary. Arterial plasma concentrations of $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ were determined in sham shock and splanchnic artery occlusion shock dogs. Arterial $PGF_{2\alpha}$ concentrations (expressed as percentage of control) increased significantly in the SAO group when compared to the sham group during postrelease sampling periods. Similarly, 15-keto-$PGF_{2\alpha}$, a major metabolite of $PGF_{2\alpha}$ also increased significantly in arterial blood in SAO shock. Comparison of 15-keto-$PGF_{2\alpha}$ and $PGF_{2\alpha}$ at each sampling period suggest that the efficiency of 15-hydroxyprostaglandin dehydrogenase is not impaired during SAO shock in the dog. However, the ability of the kidney and other organs to remove 15-keto-$PGF_{2\alpha}$ from the circulation during SAO shock does appear to be significantly reduced.

Although the changes in circulating concentrations of $PGF_{2\alpha}$ are significant, the role of the increased prostaglandin is not clearly understood. We found no basis for any toxic effect of the $PGF_{2\alpha}$ nor of any beneficial action. Others, however, have found exogenous $PGF_{2\alpha}$ to improve survival in circulatory shock (17).

The authors are grateful to Drs. J. Brian Smith and M. Silver for the anti-$PGF_{2\alpha}$ antibody used in this study. We are also grateful to Dr. John Pike of the Upjohn Company for a generous supply of prostaglandin. Indomethacin was supplied by Dr. Alexander Scriabine, Merck Institute for Therapeutic Research, West Point, Pa.

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Received September 2, 1975. P.S.E.B.M. 1976, Vol. 151.