HUMAN SERUM ALBUMIN (HSA) DECREASES PROSTAGLANDIN A_1 (PGA_1) METABOLISM

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Summary - PGA_1 was incubated with rabbit renal cortical homogenates containing HSA (0-4.5%). The ability of this tissue to readily metabolize PGA_1 progressively decreased with increasing HSA levels in the incubates The rate of disappearance of ^3H-PGA_1 was twice as rapid in rats treated with salicylic acid (S.A.) in comparison to control animals; since only 30% of the injected radioactivity was bound to the plasma of the S.A. treated rats, as compared to 90% bound to control plasma, an association may exist between the degree of binding of ^3H-PGA_1 and its rate of clearance. The studies indicate that PGA_1 interaction with HSA decreases its metabolism in vitro, and slows down its clearance in vivo, implicating HSA as a possible factor in prostaglandins metabolism in vivo.

The original observation of Slaunwhite et al (1) that transcortin bound cortisol is biologically inactive, led to a re-evaluation of the significance of hormone binding to plasma proteins. The importance of binding is now well accepted as a storage phenomenon rather than a phenomenon solely of solubility. The bound hormones, being protected from metabolism and excretion

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by dissociation from their plasma binding sites, become available to tissues as physiologically active substances. The binding of prostaglandins (PG's) to human plasma has recently been studied (2, 3). The affinity of the PG's studied for the plasma proteins was found to decrease with increasing the number of polar groups into the cyclopentane ring. Thus, PGA, the least polar, is bound to a much higher degree than PGE which in turn is significantly higher than PGF. Aspirin-like drugs have recently (4) been shown to inhibit the binding of PG's to HSA, resulting in an increment of the free hormone. Since serum albumin appears to be the only plasma protein that binds prostaglandins measurably, this study was undertaken to test whether the rate of PGA metabolism is influenced by its binding to this plasma protein.

Materials and Methods - I: In vitro studies: Rabbits were stunned by neck blow, exsanguinated and the kidneys removed. Following decapsulation, rabbit renal cortex was separated by scissor dissection. The cortical tissues were then homogenized in 0.1M sodium phosphate buffer pH 8.0 (1g tissue/4ml buffer). 2ML aliquots of the homogenates were then added to 25ml flasks containing labelled plus unlabelled PGA_1 (0.001μc, 10^{-4}M), NAD^+ (10^{-2}M), and various concentrations of HSA (0-4.5%), all in 2ml of the same buffer. The mixtures were then incubated for 20 min. at 37° C with constant shaking. The reactions were terminated by addition of 95% ethanol (20ml); after the removal of the precipitated protein, the aqueous phase was acidified (pH3) and extracted three times with ethyl acetate (15ml). The residue obtained after evaporation was suspended in 1ml of 90:10:2 (benzene: ethyl acetate: ethanol, v/v/v) and was applied to a 1.5g silicic acid column (mesh 325). PGA and two metabolites were separated by elution with increasing amounts of ethyl acetate in benzene. A gradient elution apparatus was utilized

with the first compartment connected to the column which contained 50ml of the least polar solvent system (90:10 benzene: ethyl acetate V/V); the second compartment contained benzene: ethyl acetate 40:60 (50ml). 2ML fractions were collected and counted in a liquid scintillation counter in 10ml of Aquasol.

II: In vivo studies: Rats (200g) were treated with salicylic acid (200 mg/kg). Three to four hours after treatment, the rats were anesthetized with Nembutal and a polyethylene catheter introduced into the carotid artery. PGA_1 (.1μC/1μg PGA_1) was injected into the right femoral vein; at timed intervals (0-180 sec.) blood was simultaneously withdrawn from the carotid artery. The plasma (.1ml) was counted. Control rats were treated with the salicylic acid vehicle (water) and injected with PGA as described. The percentage bound (B) of PGA in the rat plasma in both the control and the treated rats were determined by equilibrium dialysis where 0.2ml of plasma was diluted to 1ml with 0.05M sodium phosphate buffer pH 7.4. The mixture was then placed in a dialysis bag and dialyzed against 5ml of the same buffer at 4° C for 36 hours. 200 μl Aliquot from the contents of the bag and 1ml aliquot of the dialysate were transferred to counting vials containing 10ml of Aquasol (New England Nuclear Corp., Boston, Mass.). The results are expressed as per cent ^{3}H-PGA_1 bound/ml plasma. Statistical analysis was performed by Students t test.

Results - In vitro studies: In the absence of HSA in the incubates, rabbit renal cortical homogenates converted PGA into two less polar metabolites. The yield of Met. I was 7.9% and that of Met. II was 29.6% of all recovered activities. This is illustrated in Fig. 1, where peak 1 is Met. I, peak 2 is Met. II, and the third peak corresponds to that of ^{3}H-PGA_1.

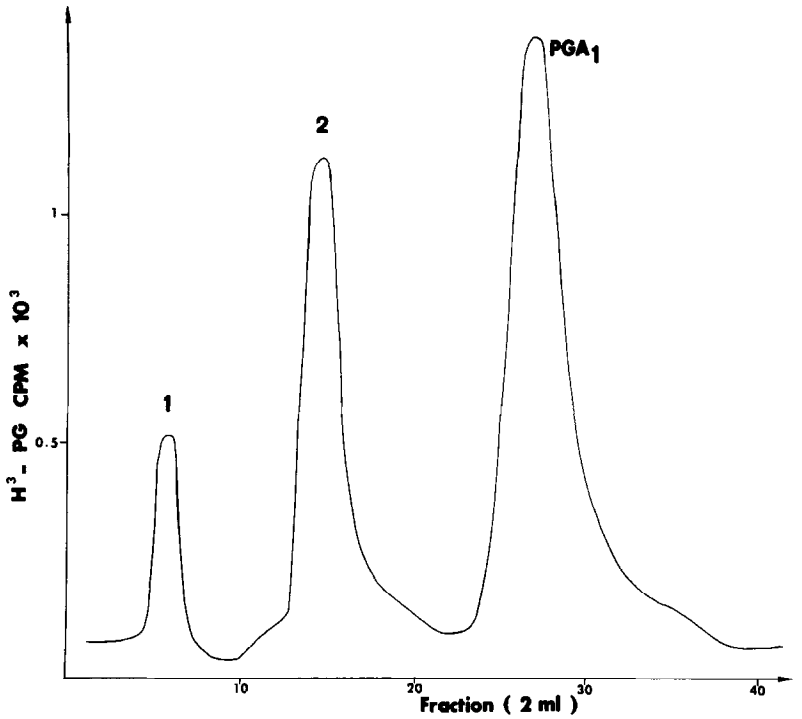

Figure 1: Silicic acid chromatography of acidic lipid extracts of rabbit renal cortical homogenates incubated with ^{3}H-PGA$_1$ (10^{-4}M) in the absence of HSA. Met. 2 formed 29.6% of all recovered activities.

Figure 2 illustrates that in the presence of HSA (4.5%) the yield of Met. I is not significantly changed (6.2%); however; the yield of Met. II was significantly decreased from 29.6% to 5%, while that of ^{3}H-PGA$_1$ increased from 35 to 65%. Table I illustrates the progressive effects of increasing amount of HSA in the incubates on the yield of PGA$_1$ metabolites. It is evident that the disappearance of PGA and the formation of Met. II are inversely related to the concentration of HSA in the incubates. About 20% of the recovered activities was a more polar product(s) of PGA and was unchanged by addition of HSA.

II: In vivo studies: Figure 3 illustrates that more than 90% of PGA is bound to the plasma of the control rats, while only approximately

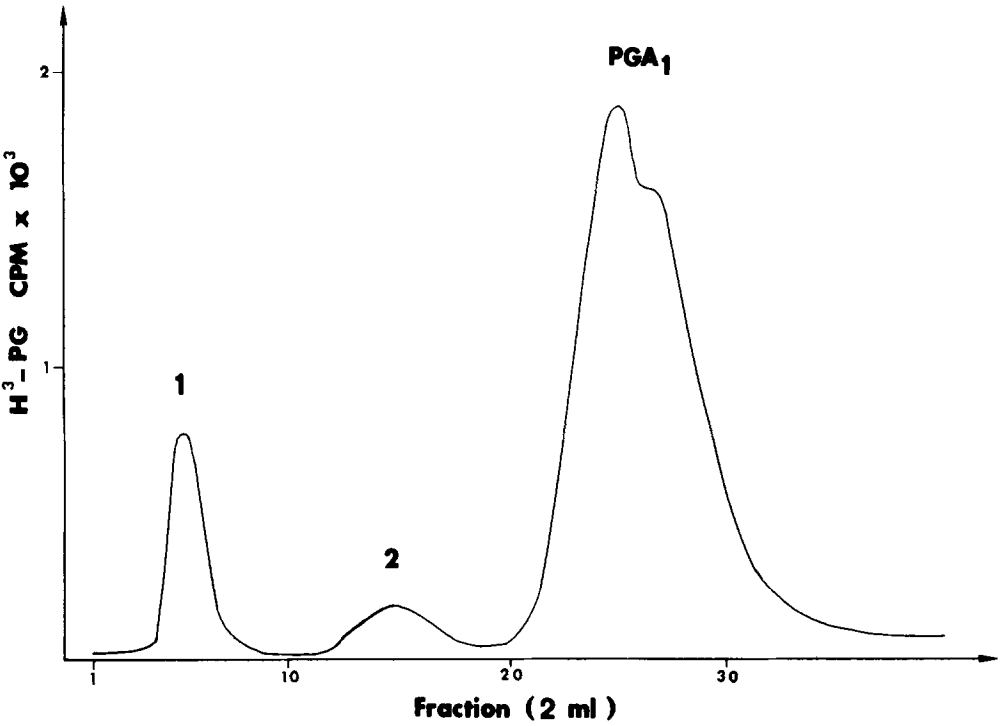

Figure 2: Silicic Acid chromatography of acidic lipid extracts of rabbit renal cortical homogenates incubated with ^{3}H-PGA_1 ($10^{-4}M$) in the presence of HSA (4.5%). Met. 2 formed only 5% of all recovered activities.

30% of PGA_1 is bound to the plasma of the treated rats, indicating a large increment in the free and presumably active hormone.

Figure 4 shows that the rate of disappearance of ^{3}H-PGA_1 in the salicylic acid treated rats is almost twice as rapid as the non-treated rats. Figures 3 and 4 illustrate an association between the degree of binding of PGA and its rate of clearance.

Discussion - It has recently been shown (5) that the interaction of HSA with PGE_2 inhibits the latter's contractile effects on the isolated gerbil colon in vitro, but does not alter its hypotensive potency in the rat blood pressure in vivo. It has also been demonstrated that a PGA_2 like compound is formed in high yield from PGE_2, when injecting the latter into the rat in

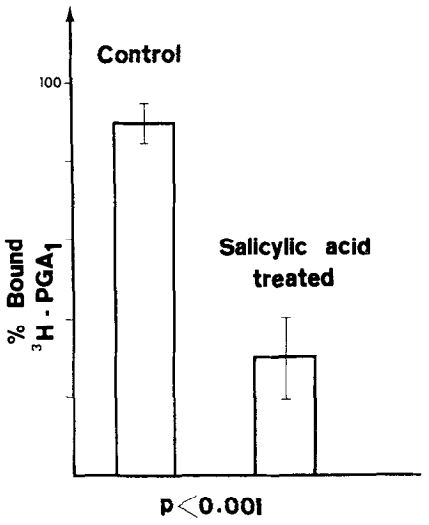

Figure 3: Comparison of per cent ^{3}H-PGA$_1$ bound in plasma of salicylic acid treated and control rats. Each value represents mean ± SEM P<0.001 n=6.

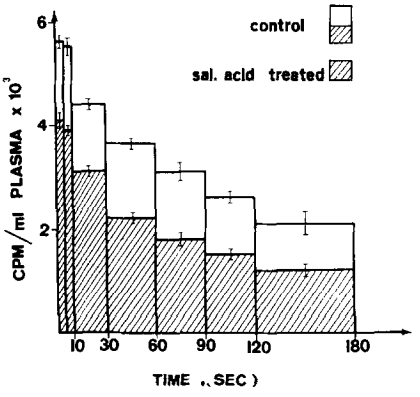

Figure 4: Rate of disappearance of radioactivity in plasma of rats salicylic acid treated and control rats following injection of ^{3}H-PGA$_1$. Each value represents mean ± SEM P<0.005 (0-60 sec.), P<0.05 (60-180 sec.) n=6.

saline (6). However, 15-keto-PGE$_2$ was the major product when injecting PGE$_2$ in HSA solution, indicating that the interaction with HSA might influence the type of metabolites formed. In this study it is demonstrated that the rate of disappearance of radioactive PGA$_1$ is twice as rapid in rats pre-treated with pharmacological doses of salicylic acid in comparison to non-treated

TABLE 1

EFFECTS OF HSA ON THE YIELD OF PGA_1 METABOLITES

%HSA In Incubates	Met. I	Met. II	PGA_1
0	7.9	29.6	35
0.33	7.7	25.0	40
1.32	8.7	15	44
4.5	6.2	5	65

Each value represents the mean of the percentage of 2 observations.

rats. Since a larger portion (90%) of the injected radioactivity is bound to the plasma of the non-treated rats, it is evident that the interaction with HSA decreases PGA_1 rate of clearance. Our studies also reveal that pre-incubating PGA_1 with HSA decreases its metabolism by rabbit renal cortex in vitro. Preliminary studies indicate that the metabolite affected by HSA is 15-keto-PGA_1 (7). These results seem to show that the biological activity of prostaglandins may be partially suppressed by complex formation with human serum albumin, and that the binding of prostaglandins (or metabolites) to HSA in human plasma may be a determinant of their rate of metabolism. Since their degree of binding is inversely related to their polarity, the least polar prostaglandins are thus expected to have a longer half life.

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