Role of Hepatic Blood Flow in Regulating Plasma Concentration of Antidiuretic Hormone after Hemorrhage.* (28439)

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Recently there has been much interest in the mechanisms responsible for the regulation of blood volume(1,2,3). The plasma volume is maintained constant by a balance between fluid intake and output, and one of the most important regulating mechanisms for the latter is the influence of the antidiuretic hormone (ADH) on urine flow. When the blood volume is reduced by hemorrhage, there is indirect evidence that the increase in circulating ADH contributes to the reduction in urine flow(4). Weinstein *et al.* (5) demonstrated that the release of ADH was increased after hemorrhage. However, the extent to which an altered peripheral degradation of ADH can contribute to the post-hemorrhage increase in the circulating ADH concentration has not been investigated, although an auto-regulation theory has been proposed(6) in which alterations in hepatic blood flow are assumed to play a major role in regulating the circulating ADH level. In rats(7) and dogs(8) there is evidence that the liver and the kidneys are the main organs responsible for the normal removal of ADH. In the present experiments the hepatic inactivation rate of ADH was studied in dogs when the hepatic blood flow was altered by hemorrhage. The purpose was to evaluate the role played by the hepatic blood flow in regulating the circulating ADH concentration.

Methods. The experiments were carried out on healthy mongrel dogs weighing from 7.2 to 15.1 kg and splenectomized at least one month prior to the study. Before the experiments, it was ascertained that water and food intake was normal in these animals. On the day of the experiment, the dog was anesthetized by intravenous injection of sodium pentobarbital (33 mg/kg). Under fluoroscopic control, a No. 8 Cournand catheter was introduced *via* an external jugular vein into the left hepatic vein. A catheter was introduced into the abdominal aorta *via* a femoral artery. A femoral vein was catheterized and sulfobromophthalein sodium (BSP) infused at a constant rate. From the BSP infusion rate and from the difference in BSP concentrations between the arterial and the hepatic venous plasma, the hepatic plasma flow (HPF) was estimated from at least 3 pairs of samples(9,10). The arterial cell percentage was determined by centrifuging hematocrit tubes for 30 minutes at 1500 × gravity and correcting the readings for plasma trapping(11). The estimated hepatic blood flow (HBF) was calculated from the HPF and the arterial cell percentage. The plasma volume (PV) was measured by using I^{131} labelled human serum albumin(11). A well-type scintillation counter connected to a pulse-height analyzer and a scaler was used for assaying the radioactivity. Hemorrhage was performed by removing a known amount of blood from the arterial catheter at a rate of 100 to 200 ml/min. The volume of blood removed in different experiments ranged from 11 to 41% of the control blood volume. During the post-hemorrhage period, the HBF and PV were followed. Thirty to 60 minutes after hemorrhage, blood samples were drawn simultaneously from the femoral artery and the hepatic vein. The antidiuretic activity of the plasma was assayed in duplicate by intravenous injection into rats hydrated with an ethanol solution as described elsewhere(12). Duplicate assays agreed within ± 10% of the mean. U.S.P. posterior pituitary standard (U.S.P. Reference Standards, New York City) was used as the standard.

From these measurements, the following can be calculated:

Splanchnic extraction ratio for ADH (E_s)=

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$$\frac{(ADH)_{art.} - (ADH)_{hep.v.}}{(ADH)_{art.}}$$

Splanchnic clearance of ADH (C_S) = HPF $\times$ E_s.

Results. After hemorrhage, the HBF decreased, and the decrease became more marked as the amount of blood loss increased. The experiments were arranged in the order of decreasing HBF (in ml/min/10 kg) and divided into 4 groups (Table I). The ADH concentration in the arterial plasma was higher in the experiments where the volume bled was larger and the HBF was lower. The splanchnic ADH extraction ratio (E_s) in these 4 groups of experiments showed no significant differences and the average value for all 28 experiments was 0.22 (S.E.$_m$ 0.018, S.D. 0.096). The splanchnic ADH clearance (C_s), calculated from HPF and E_s, thus decreased progressively as the HBF was reduced by hemorrhage. The percentage of total plasma ADH that is inactivated in the splanchnic area per minute can be calculated as 100 C_s/PV, where C_s is the clearance in ml plasma per minute and PV is the plasma volume in ml. With increasing amounts of hemorrhage, the post-hemorrhage PV became progressively reduced, but the degree of reduction was not as large as that of C_s. Hence as the HBF was reduced by hemorrhage, the percentage of total plasma ADH inactivated in the splanchnic area became less (Fig. 1).

Discussion. The changes in HBF after hemorrhage agree with the data in the litera-

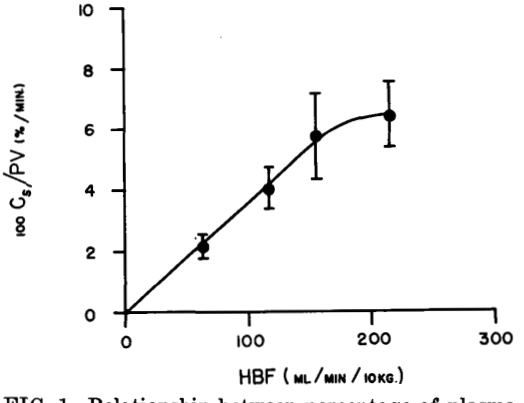

FIG. 1. Relationship between percentage of plasma volume cleared of ADH by splanchnic organs per min (100 C_s/PV) and hepatic blood flow (HBF).

TABLE I. Splanchnic Clearance of ADH After Hemorrhage.

Exp group	No. of exp	Body wt (kg) M ± S.E.	HBF (ml/min/10 kg) M ± S.E. (range)	$(ADH)_{art.}$ (μU/ml plasma) M ± S.E.	E_s M ± S.E.	C_s (ml/min/10 kg) M ± S.E.	PV (ml/10 kg) M ± S.E.	100 C_s/PV (%/min) M ± S.E.	Hemorrhage (ml/10 kg) M ± S.E.
I	(5)	10.2 ± .91	216 ± 12.8 (190-261)	149 ± 23.2	.21 ± .037	3.15 ± .38	49.8 ± 3.7	6.5 ± 1.09	151 ± 46.4
II	(6)	11.7 ± 1.07	156 ± 5.3 (142-171)	188 ± 36.8	.24 ± .058	2.40 ± .55	42.1 ± 2.2	5.8 ± 1.40	220 ± 44.9
III	(9)	10.4 ± .54	117 ± 3.5 (104-130)	247 ± 39.4	.23 ± .030	1.71 ± .24	41.3 ± 1.1	4.1 ± .67	270 ± 22.1
IV	(8)	11.8 ± .85	66 ± 3.5 (54- 78)	312 ± 44.6	.21 ± .033	.85 ± .16	38.8 ± 1.6	2.2 ± .38	308 ± 23.5

M = mean. S.E. = standard error of mean.

ture(13,14,15). With the HBF varying from 54 to 261 ml/min/10 kg, however, the E_s did not alter significantly among the 4 groups of experiments (Table I). Thus the splanchnic organs can remove about 22% of the ADH from each unit volume of plasma flowing through, regardless of flow rate. Nevertheless, as the HBF was reduced by hemorrhage, the absence of an increase of E_s resulted in a decrease of C_s proportionate to that of HBF (Table I). Not only was the *volume* of plasma cleared per unit time decreased, but the *percentage* of total plasma volume cleared per unit time (100 C_s/PV) also was reduced (Fig. 1). Recently Share (16) has reported that the half-life of exogenous ADH in anesthetized normal dogs is independent of the quantity of ADH administered and that the mechanisms involved in removing the circulating ADH are not readily saturated. Hence the decrease of C_s in the present experiments would indicate that after hemorrhage there was some derangement in the splanchnic metabolism of ADH. After severe hemorrhages (Group IV) the decrease of the absolute ADH removal rate [$C_s \times (ADH)_{art.}$, in mU/min/10 kg] as well as C_s offers a stronger evidence for a disturbance in splanchnic metabolism.

When the volume bled was small and the HBF approached normal values (Fig. 1), there was a tendency for 100 C_s/PV to approach a constant value and it seems likely that 100 C_s/PV for dogs with normal HBF is about 7%/min (*i.e.*, 7% of the plasma ADH is removed in the splanchnic area per minute). Furthermore, if one assumes that E_s of 0.22 prevailed in our dogs before hemorrhage, with the HPF averaged 148 ml/min/10 kg(10), then the normal C_s would be about 33 ml/min/10 kg. Since the normal PV averages 485 ml/10 kg(10), 100 C_s/PV would again be about 7%/min. Lauson and Bocanegra(17) found that the total pitressin clearance in pentobarbitalized normal dogs averaged 16.1% of the plasma volume per minute. By comparing the effectiveness of pitressin infusions *via* a femoral vein and *via* the portal vein, Lauson and Bocanegra(18) concluded that the liver was responsible for one-half to one-third of the total plasma clearance (hence about 6 to 8% per minute). The other major organ responsible for the inactivation of ADH under normal conditions is the kidney(18). Since the renal blood flow is also reduced by hemorrhage(13), the renal ADH clearance probably also decreases. Thus, the decrease in the splanchnic and possibly also the renal clearance of ADH contributes to the increase in the circulating ADH concentration after hemorrhage. The normal total plasma ADH clearance is, however, only about 16% per minute(17). Therefore the reduction of inactivation rate cannot account for the early post-hemorrhage increase in plasma ADH concentration, which may be 5 times the control value within 4 minutes after a 20% hemorrhage (Chien, Usami and Peric, unpublished observations). The release of ADH from the hypothalamico-hypophyseal system after hemorrhage(5) is probably the principal reason for this early rise in the circulating ADH concentration. On the other hand, it seems likely that the reduction in ADH clearance plays a part in maintaining a high plasma ADH concentration later (*e.g.*, one hour after hemorrhage).

In our experiments, ADH concentrations in the arterial and hepatic venous plasma were compared, hence the extraction and clearance of ADH calculated were those in the splanchnic organs, including the gastrointestinal tract as well as the liver. In the rat, there is evidence that the intestine does not inactivate ADH(19). Lauson and Bocanegra's data on the dog(8,17,18) also indicate that the liver is the only splanchnic organ removing significant amounts of ADH. Therefore it is reasonable to assume that the E_s and C_s calculated in our experiments represent respectively the extraction and clearance of ADH by the liver.

Summary. Hemorrhage in splenectomized dogs under pentobarbital caused a decrease of hepatic blood flow (HBF) and an increase of antidiuretic hormone (ADH) concentration in the arterial plasma. The splanchnic extraction ratio for ADH remained at about 0.22 with all degrees of hemorrhage. Therefore as the HBF was reduced by hemorrhage, the splanchnic clearance of ADH decreased, and so did the percentage of total plasma ADH cleared by the splanchnic organs (mainly the liver) per unit time. After hem-

orrhage, the decrease in ADH clearance is not a major factor in causing the initial rapid rise in the circulating ADH concentration, but plays a part in maintaining this concentration at an elevated level.

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Effect of Mercaptoethanol on Complement Binding Ability of Human 7 S Gammaglobulin.* (28440)

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It is well known that the $beta_{2M}$ or 19 S γ globulin-antibodies are split into smaller 7-8S units by breaking disulfide bonds with a variety of sulfhydryl reagents with concomitant loss of all antibody activity(1). In contrast, such treatment does not split 7 S gamma immune globulins and does not destroy their antigen combining capacity. This difference in reactivity has been widely used as a presumptive test to distinguish these two classes of antibodies(2). While the ability of 7 S gammaglobulin antibodies to react with antigen is unaffected by such treatment, no data are available regarding the effect on secondary reactions reflecting the interaction between antigen and antibody, such as complement fixation. This question is of importance in view of the suggestion by Grabar (3) and Becker and his collaborators(4) that sulfhydryl structures may be involved in complement fixation, and also because of the wide use of reductive cleavage as a screening test for 19 S antibodies.

Data are reported here showing that mercaptoethanol treatment of human 7 S gammaglobulin and 7 S gammaglobulin antibodies abolishes their capacity to bind complement when they react with antigen or after heat-aggregation, a procedure known to make 7 S gammaglobulins anticomplementary (5).

Materials and methods. Sera from patients suffering from systemic lupus erythematosus (SLE) and from infectious hepatitis, which contained complement binding antibodies against cytoplasmic particulate constituents (mitochondria, lysosomes, microsomes), were used in this study. The 7 S fractions of these sera were obtained by

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