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Research Article

DEPRESSION OF APPARENT p-AMINOHIPPURATE EXTRACTION RATIO BY GLUCOSE [1]

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During an investigation of the diurnal variations of renal function two normal male subjects were found to have depressed *p*-aminohippurate clearances (C_{PAH}) with filtration fractions of 0.45 and 0.44 respectively, a marked deviation from the normal mean value of 0.19 (1). In 11 patients with congestive heart failure, filtration fractions varying from 0.31 to 0.80 were observed, contrary to the experience of Merrill (2) and Mokotoff, Ross and Leiter (3), who described elevated filtration fractions in congestive failure but not of this magnitude. The renal extraction ratio of *p*-aminohippurate (E_{PAH}), determined by renal vein catheterization, in the two normal subjects was 0.63 and 0.73, while in five of the subjects in cardiac failure it ranged from 0.62 to 0.71. Bradley (4) has reported that E_{PAH} averages 0.94 (range 0.87–1.00) in normal subjects while Breed and Maxwell (5) report 0.93 (range 0.88–0.98). That E_{PAH} is maintained in the presence of congestive heart failure has been noted by Merrill (2), Breed and Maxwell (5) and Edelman and his associates (6).

In an effort to explain the low values of E_{PAH} in the above instances the experimental protocols were reviewed to uncover any differences between our technique and that customarily used for the measurement of C_{PAH} and E_{PAH}. Two major differences emerged. Because we were studying diurnal variations in renal function, our subjects were infused with PAH and inulin throughout a 24 hour period, the extraction ratios being performed at the end of this period. In addition, 5 per cent glucose in distilled water, delivered at a constant rate of 0.5 cc./min., was employed as diluent for the test substances in order to avoid the administration of excessive amounts of saline. Experiments were therefore devised to examine the effects of these procedures on E_{PAH}.

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METHODS

All subjects were convalescent males without clinical evidence of cardiovascular-renal disease, selected from the wards of the Third (New York University) Medical Division of Bellevue Hospital.

Glomerular filtration rate was measured by the inulin clearance (C_{IN}), the effective renal plasma flow by the PAH clearance (C_{PAH}). E_{PAH} was determined by renal vein catheterization using the technique of Warren, Brannon and Merrill (7) and Bradley and Bradley (8) and calculated from the equation:

$$E_{PAH} = \frac{A_{PAH} - RV_{PAH}}{A_{PAH}}$$

where A_{PAH} and RV_{PAH} are the concentrations of PAH in simultaneous samples of arterial and renal venous plasma.

Inulin was determined by a modification of Harrison's method as described by Goldring and Chasis (9) and also by a modification of Roe's method (10). PAH was determined by the method of Smith and his associates (11).

Details of the technique of continuous 24 hour clearances are described elsewhere (12).

PHYSIOLOGICAL STUDIES

I. *Effect of duration of infusion on E_{PAH}.* To determine whether E_{PAH} may be depressed as a result of prolonged infusion two subjects were infused with PAH in distilled water for 24 hours. In subject J. F., E_{PAH} at the end of that time was 0.90 and 0.91, while in J.M., E_{PAH} was 0.93 and 0.93.

II. *Effect of infusion of glucose in distilled water on C_{PAH} and E_{PAH}.* To examine the possible physiological effect of glucose on C_{PAH} and E_{PAH}, an infusion of 5 per cent glucose in distilled water was administered at a rate of 0.5 cc./min. into a separate peripheral vein while these measurements were being made in three subjects. In G. M. control values for C_{PAH} averaged 702 cc./min. for three clearance periods. During the administration of glucose into a separate vein over an interval of five hours the average C_{PAH} was 664 cc./min. D. M. was studied in this manner

with infusion of glucose over a period of 4.75 hours, during which time successive determinations of E_{PAH} were 0.91, 0.91, and 0.91. In J. H. control values for E_{PAH} were 0.91 and 0.92; after two hours of administration of glucose E_{PAH} was 0.90 and 0.91.

III. *Effect of mixing 5 per cent glucose in distilled water with PAH* in vitro. To determine the possible effects of 5 per cent glucose in distilled water when used as a diluent for the sustaining infusion of PAH, determinations of C_{PAH} and E_{PAH} were performed during the infusion of such mixtures at a rate of 0.5 cc./min. Mixtures were incubated at room temperature for varying periods of time preceding their administration.

A. Subject L. W. was infused with PAH in normal saline over a four hour period. C_{PAH} during this interval was 665, 669, and 646 cc./min., averaging 660 cc./min. The sustaining infusion was then changed to one containing PAH in 5 per cent glucose which had been incubated for four hours. During the third and fourth hours of this infusion C_{PAH} was depressed to 581 cc./min., a 12 per cent decrease. After re-institution of PAH in normal saline, C_{PAH} remained depressed at 553 cc./min. during the first hour, and then increased to 625 and 645 cc./min. during the third and fourth hours respectively.

B. Subject G. S. was studied similarly, infusion of PAH in distilled water being followed by a mixture of PAH in 5 per cent glucose incubated for three hours, after which PAH in water was re-instituted. In this instance C_{IN} was measured simultaneously with C_{PAH}. C_{PAH} was depressed from 703 to 546 cc./min. during infusion of the PAH glucose mixture, a decrease of 22 per cent. This depression of C_{PAH} was associated with a constant value of C_{IN}, resulting in an increase of the filtration fraction from 0.183 to 0.221. C_{PAH} returned to control levels 2.5 hours after re-institution of PAH in distilled water.

C. In subject J. C. control E_{PAH} determined during infusion of PAH in distilled water was 0.90. PAH in 5 per cent glucose, incubated four hours at room temperature, was then infused. After 3.5 hours E_{PAH} was 0.83, after 4.5 hours 0.84 and after six hours 0.81. Four hours after returning to the infusion of PAH in distilled water E_{PAH} had increased to 0.88. Changes in E_{PAH} were reflected by inverse variations in the plasma concentration of PAH.

D. Control E_{PAH} in subject J. S. was 0.95. After one hour of infusion of PAH in 5 per cent glucose incubated 21 hours, E_{PAH} was 0.80 and fell progressively to a low value of 0. 76 after five hours. Plasma concentration of PAH increased during this interval.

E. In F. Z. control E_{PAH} was 0.94 and 0.94. Infusion of PAH in 5 per cent glucose incubated for 18 hours was associated with a depression of E_{PAH} to 0.74 after one hour, and a further decrease to 0.64 after 3.5 hours. Re-institution of PAH in saline resulted in an increase of E_{PAH} to 0.69 and 0.70 after 0.5 hours. Plasma concentration of PAH again reflected these variations in E_{PAH}.

These observations in normal subjects indicate that depression of C_{PAH} and E_{PAH} is not attributable to prolonged infusion of PAH or to any physiological effect of 5 per cent glucose in distilled water infused at 0.5 cc./min. However, a reduction of E_{PAH} and C_{PAH} could be consistently demonstrated when 5 per cent glucose in distilled water was used as diluent for the PAH infusion. The reduction was greater and effected more rapidly when the infusion mixture had been incubated at room temperature for long periods of time. It was apparent that PAH and glucose react chemically when mixed in the infusion flask, and accordingly the following *in vitro* studies were carried out.

CHEMICAL STUDIES

Approximately 1 gm. per cent solutions of PAH in (*1*) distilled water and (*2*) 5 per cent glucose were prepared. Analysis showed a concentration of PAH of 965 mg. per cent in (*1*) and 980 mg. per cent in (*2*). Both solutions were then incubated at 37° C. After 24 hours of incubation (*2*) had a distinct yellow coloration, while (*1*) was unchanged in appearance. Five per cent glucose alone incubated for the same period underwent no color change. Analyses after incubation showed a concentration of PAH of 985 mg. per cent in (*1*) and 695 mg. per cent in (*2*), a decrease of 29 per cent in PAH concentration of the latter.

The same solutions were further incubated for seven days. PAH concentration at that time was

970 mg. per cent in (*1*) and 675 mg. per cent in (*2*).

The same solutions were kept at room temperature for 13 additional days, when analyses for PAH showed no further change. After heating with 0.48 *N* HCl at 96° C. for 3.5 hours by the technique customarily used for analysis of total PAH (11), the apparent PAH concentration in the glucose solution (*2*) increased to 928 mg. per cent from the previously determined 675 mg. per cent.

The PAH–5 per cent glucose mixture which had been incubated for seven days at 37° C. and a standard aqueous solution of comparable PAH concentration were analyzed using varying normalities of HCl in the acidification step before coupling. Above 0.3 *N* HCl the apparent PAH concentration of the standard aqueous solution remained constant while the apparent concentration of PAH in the glucose mixture (*2*) continued to increase with increasing acidity. The results are summarized in Table I.

TABLE I

Effect of increasing acidification on recovery of PAH

Normality of HCl	Apparent concentration PAH mg./100 cc.	
	PAH incubated in 5 per cent glucose	Aqueous solution of PAH
0.012	85	95
0.30	520	510
0.60	585	515
1.20	680	515

DISCUSSION

These studies show that when PAH is dissolved in 5 per cent glucose in distilled water and the mixture allowed to stand for even a few hours, a reaction occurs between PAH and glucose which results in the formation of some compound which does not produce color in the standard PAH method. When such mixtures are infused into man, E_{PAH} and C_{PAH} are substantially reduced. The simultaneous but separate infusion of PAH and 5 per cent glucose at the rate of 0.5 cc./min. does not reduce either E_{PAH} or C_{PAH}, and reduction of these terms in the first instance is apparently entirely attributable to *in vitro* reaction.

Incubation of PAH at 37° C. with 5 per cent glucose in distilled water results in development of color in the solution, and a marked decrease in the concentration of free or reactive PAH. Hydrolysis with HCl at 96° C. restores the concentration of free PAH to its initial value. It is clear that the *p*-amino radical is involved in the formation of a glucose complex which is capable of being hydrolyzed. It is suggested that this complex is the product of a Schiff base reaction between the *p*-amino group of PAH and the aldehyde group of glucose; however, no detailed chemical analysis has been undertaken of this PAH-glucose reaction product. This reaction product is partially hydrolyzed by the 1.2 *N* HCl used in the standard method (11) of PAH analysis, and yields increasing concentration of free PAH as the HCl used for acidification is increased through 0.012 *N*, 0.30 *N*, 0.60 *N* and finally to 1.2 *N*, while an aqueous solution of PAH develops a maximum and constant color when 0.30 *N* HCl is used.

Two explanations of the reduction of E_{PAH} and C_{PAH} when PAH-glucose mixtures are infused suggest themselves.

The PAH-glucose reaction product, which we will designate as X, may be excreted by the tubules, but its properties may be such as to retard, even when present in very small quantities, the tubular excretion of PAH. Although this explanation cannot be disproved, we consider it improbable that a PAH-glucose reaction product would be excreted by the tubules. From several considerations it would appear more likely that it would have a clearance approximating the filtration rate. This assumption is in keeping with the low extraction ratio and clearance of PAH–X mixtures.

Alternatively, it is to be noted that X is partially hydrolyzed in PAH with the concentration of HCl (1.2 *N*) used in the coupling reaction, and hence the analysis of peripheral and renal venous plasma yields higher PAH concentrations than are actually present. If X has a low clearance there is, relative to PAH, more X in the plasma than in the urine, and hydrolysis during analysis raises the apparent value of P_{PAH} more than U_{PAH}, thus reducing C_{PAH}. Similarly, the hydrolytic formation of PAH from X in both peripheral and renal venous blood reduces the apparent value of E_{PAH}. This is, we believe, the correct explanation.

The duration of time during which the PAH

and 5 per cent glucose are in contact before they are infused influences the extent to which X is formed, and the most rapid and marked reduction of E_{PAH} and C_{PAH} occurs when the PAH-glucose mixture is prepared several hours before infusion.

To what extent X may be formed in the body by reaction of PAH with endogenous glucose is not known. The fact that nearly all investigators report a value of 0.93 for E_{PAH} argues against the presence of any appreciable plasma concentration of X, but X might contribute to the failure of E_{PAH} to reach 1.00, a circumstance which is usually attributed to the moiety of plasma perfusing non-excretory tissue.

Klopp, Young and Taylor (13) have reported that C_{PAH} is reduced at plasma glucose concentrations of 390–500 mg./100 cc. attained during the measurement of Tm_G in man. Depression of C_{PAH} during hyperglycemia of 31.4 per cent in seven women and 52.5 per cent in six men has been reported by Grimelli and his colleagues (14). Neither of these two groups of investigators states whether the PAH and glucose were mixed and administered from the same infusion flask. This reduction of C_{PAH} has also been observed by Earle and Farber (15) during simultaneous determinations of C_{PAH} and Tm_G in man with separate infusion of PAH and glucose. These observations may represent a reduction of renal plasma flow; however, in the absence of observations on E_{PAH} the formation of X either in the PAH-glucose infusion mixture or *in vivo* cannot be excluded.

It has also been observed by Klopp, Young and Taylor (13) and Grimelli and his associates (14) in man, and by Houck (16) and Kelley and McDonald (17) in the dog that Tm_{PAH} is reduced during the simultaneous determination of Tm_G. Kelley and McDonald state clearly that their priming and sustaining infusions were composed of mixtures of glucose, PAH and inulin. Again, these observations may indicate a true reduction in Tm_{PAH} owing to limitations of available energy in tubular cells, as Houck suggests; it may be, however, that in concentrated PAH-glucose infusion mixtures and at high plasma concentrations of glucose the PAH-glucose complex is formed to such an extent that the load of free PAH presented to the tubules does not saturate the tubular mechanism for excretion of free PAH.

Houck (16) found that in the dog Tm_G is consistently depressed during simultaneous determination of Tm_{PAH}, while Kelley and McDonald (17) report a marked reduction of Tm_G in one of three dogs. Although this may likewise be attributable to competition for available energy in the renal tubule, the formation of X in the infusion flask or in the body, when high concentrations of both PAH and glucose are present, may influence the determination of Tm_G by hydrolysis of X to glucose during glucose analysis and elevation of P_G and U_G to erroneous values.

In the light of the present evidence all conclusions drawn from observations made during the infusion of PAH-glucose mixtures or during hyperglycemia should be re-examined. Clearly, glucose solutions should not be used as a vehicle for the infusion of PAH.

CONCLUSIONS

1. Infusion of *p*-aminohippurate (PAH) dissolved in 5 per cent glucose solution in man leads to a reduction in the apparent PAH extraction ratio and to a reduction in the PAH clearance.

2. PAH dissolved in 5 per cent glucose solution forms a reaction product in which the *p*-amino group is covered. The reaction product apparently has a low clearance, as indicated by the low extraction ratios of PAH-glucose mixtures. It is, however, hydrolyzed at the acidity used in the coupling reaction for PAH. Consequently, hydrolysis during analysis of plasma yields higher concentrations of free PAH than are actually present, resulting in an apparent depression of both E_{PAH} and C_{PAH}.

3. Glucose should not be used as a diluent for the infusion of PAH during the measurement of E_{PAH} or C_{PAH}.

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