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Kinetic Studies on the Competition Between Para-aminohippuric Acid (PAH) and Diodrast for Renal Transport in the Dog[1]

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Since Marshall and Vickers(1923) succeeded in demonstrating the fact that phenol red is secreted by the renal tubule, many substances have been shown to be excreted by the kidney in a manner similar to phenol red. It is generally accepted at the present time that certain weak organic acids such as phenol red, para-aminohippuric acid(PAH) and Diodrast are actively secreted by the renal tubule through a common transport system(Smith and Smith, 1938). When the renal tubule is subjected to more than one of these substances, the tubular secretion of each substance is found to be inhibited in a competitive manner. The underlying mechanism for this competition is not clear. It has, however, been shown that Diodrast is a more powerful competitive inhibitor than PAH (Josephson et al.,1952; Josephson et al., 1953; Forster and Hong, 1959).

The present investigation is undertaken in order to make a quantitative analysis on the kinetic aspect of the competitive inhibition between PAH and Diodrast.

METHODS

Experiments were carried out in seven anesthetized female mongrel dogs. After anesthesia with pentobarbital sodium (30 mg/kg), the trachea was intubated, and cannulas were introduced into the femoral artery and vein for the purposes of blood sampling and infusion. The urinary bladder was catheterized for quantitative collections of urine.

When each animal recovered from the effect of surgery, a control experiment was undertaken. Priming doses of inulin(50 mg/kg) and of a substrate(120 mg/kg of PAH or 80 mg/kg of Diodrast) were infused, following which a constant infusion of inulin(10 mg/min./kg) was started. The inulin was dissolved in 0.9% NaCl solution containing 2 M urea in order to maintain a good urine flow. After a 20-minute equilibration period, blood and urine samples were taken at 10-minute intervals for a period of an hour. At the time of each urine collection, the urinary bladder was washed with an aliquot of distilled water once or twice, depending upon the rate of urine flow.

Upon completing the control experiment, the identical procedure was repeated in the presence of a competitive inhibitor. Diodrast was employed as the competitive inhibitor when the PAH secretion was studied in the experiment, and vice versa. While the constant infusion of inulin was being maintained, the original substrate in the same amount as in the control experiment was administered along with the corresponding competitive inhibitor. The priming dose of PAH or Diodrast, when used as a competitive inhibitor, was 120 or 80 mg/kg, respectively. Immediately

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following the administration of this priming dose. Diodrast or PAH as a competitive inhibitor was infused continuously at the rate of 2.76 or 2.03 mg/min./kg, respectively, throughout the period of competition studies.

Both plasma and urine samples were analyzed for inulin (Shreiner, 1950), PAH (Smith et al., 1945) and Diodrast (Alpert, 1941).

In calculating the amount of PAH or Diodrast secreted by the renal tubule, the value of f (i. e. the filtrability) was assumed to be 0.92 for PAH (Smith, 1956) and 1.0 for Diodrast (Elsom et al., 1936). The computations of the various kinetic constants were based on Michaelis-Menten equations as described by Neilands and Stumpf (1958) and will be described in detail in the subsequent section.

RESULTS

A) PAH Secretion in the Presence of Diodrast

The tubular secretion of PAH was first studied in the absence of an inhibitor during the control period, and then in the presence of a competitive inhibitor, Diodrast, in the concentration of 0.8 to 0.9 μM/ml. Results obtained from a typical experiment are shown in Fig. 1.

As the plasma concentration of PAH (P_{PAH}) was gradually lowered, in the absence of the inhibitor, from approximately 0.8 to 0.2 μM/ml, the amount of PAH secreted by the renal tubule (T_{PAH}) was correspondingly reduced from 4.8 to 1.8 μM/min./kg. However, in the presence of Diodrast in the concentration of approximately 0.8 μM/ml, the T_{PAH} was only in the order of 1 μM/min./kg at a P_{PAH} level of approximately 1.0 μM/ml.

There was little change in the inulin clearance throughout the entire experimental period.

In order to calculate the various kinetic constants, $1/T_{PAH}$ was plotted against $1/P_{PAH}$ (Fig. 2). The

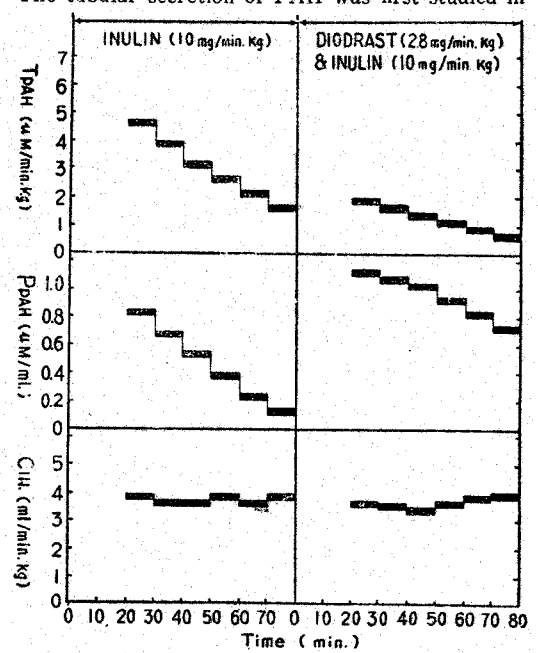

Fig. 1. Changes in the inulin clearance (C_{IN}), the plasma concentration of PAH (P_{PAH}) and the amount of PAH secreted by the renal tubule (T_{PAH}) in the absence of (left half) and in the presence of Diodrast (right half). (At the first zero minute, 50 mg of inulin per kg and 120 mg of PAH per kg were administered intravenously; at the second zero minute, 120 mg of PAH per kg and 80 mg of Diodrast per kg were given).

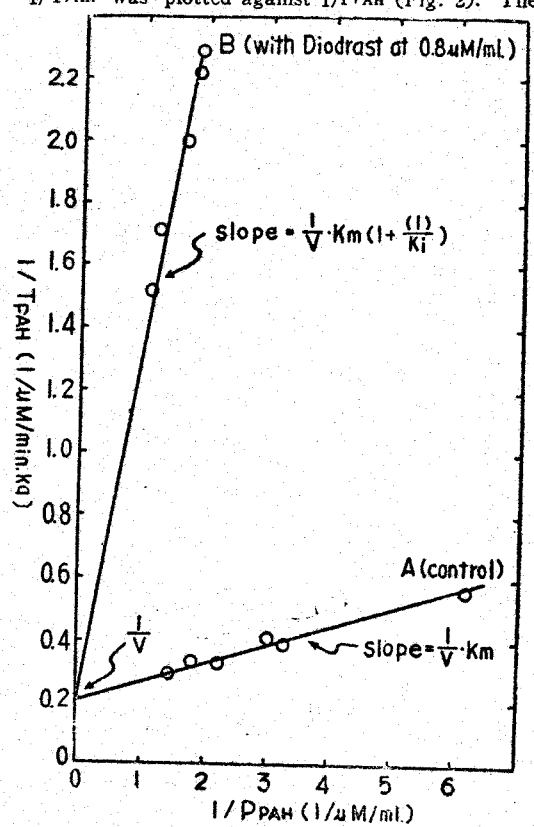

Fig. 2. Relationship between the reciprocal of the amount of PAH secreted ($1/T_{PAH}$) and the reciprocal of plasma concentration of PAH ($1/P_{PAH}$) in the absence of (line A) and in the presence of (line B) Diodrast. Both lines were drawn by visual inspection.

straight lines A and B were drawn by visual inspection. The slope of the line A corresponds to Km/V, where Km denotes the Michaelis constant and V the maximal velocity of tubular secretion (i.e. $T_{m\ PAH}$). Furthermore, the intercept of this line A with the Y-axis corresponds to the reciprocal value of the maximal velocity (1/V) which is equivalent to $1/T_{m\ PAH}$ in the PAH secretory system. In the presence of Diodrast, however, the slope of the corresponding line (i.e. line B in Fig. 2) is equal to $\frac{Km}{V}\left(1+\frac{(I)}{Ki}\right)$, where (I) denotes the plasma concentration of inhibitor (i.e. plasma concentration of Diodrast, P_D) and Ki the dissociation constant of the carrier-inhibitor complex (i.e. the carrier-Diodrast complex, K_D, in this case). Since Diodrast is a competitive inhibitor, the intercept of the line B with the Y-axis corresponds to that of line A.

Once the value of V is determined from the intercept of either line to the Y-axis, Km can be calculated by knowing the slope of line A. The value of Ki is then readily calculated by inserting the value of V, Km and (I) to the slope of line B. The actual numerical figures determined in this manner for each experiment are summarized in Table 1.

Table 1. Various Kinetic Constants for the Competitive Inhibition of PAH Secretion by Diodrast

Dog Number	V(=Tm PAH) μM/min./kg	Km μM/ml	Ki(=KD) μM/ml	Km $(1+\frac{(I)}{Ki})$ μM/ml	(I)(=PD) μM/ml
1	5	0.31	0.037	6.82	0.8
2	5	0.18	0.033	4.97	0.9
3	5	0.16	0.031	4.29	0.8
Mean value	5	0.22	0.033	5.36	0.83

The value of V, as computed from the intercept, was in the order of 5 μM/min./kg. In practice, however, it was taken to be 5 μM/min./kg in all cases on the basis of the literature (Smith, 1956), for it is not easy to determine the value of the intercept very accurately. The average Km value was 0.223 μM/ml, indicating that at this plasma concentration of PAH, the PAH transport system is half-saturated. The value of Ki (i.e. K_D), which indicates the real strength of inhibition, averaged 0.0335 μM/ml at the P_D of 0.83 μM/ml. The PAH secretory system in the presence of Diodrast is

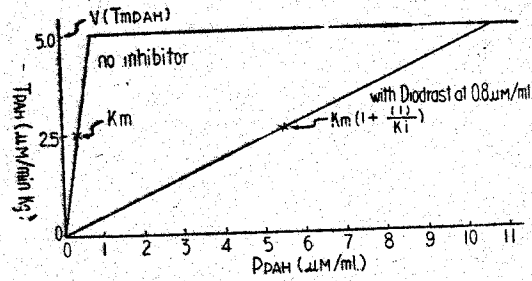

Fig. 3. Hypothetical relationship between the amount of PAH secreted (T_{PAH}) and the plasma concentration of PAH (P_{PAH}) in the absence of (curve on the left) and in the presence of (curve on the right) Diodrast.

half-saturated at a PAH level of 5.36 μM/ml, which corresponds to the value of $Km\left(1+\frac{(I)}{Ki}\right)$. In other words, a considerable fraction of the PAH transport system is occupied by Diodrast so that the T_{PAH} curve is markedly shifted to the right (Fig. 3).

B) Diodrast Secretion in the Presence of PAH

The tubular secretion of Diodrast was studied

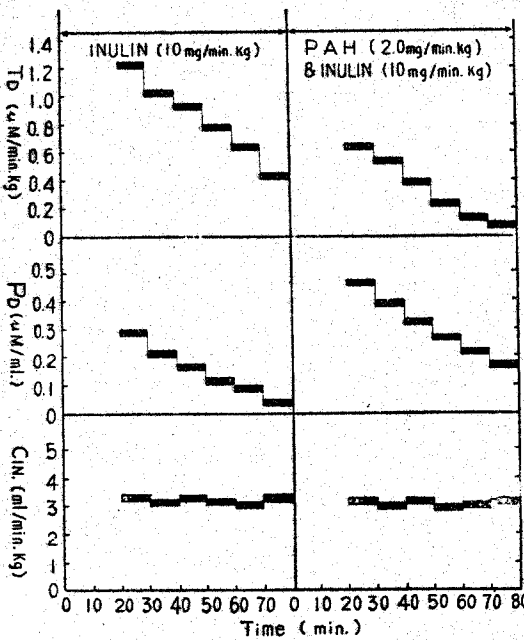

Fig. 4. Changes in the inulin clearance(C_{IN}), the plasma concentration of Diodrast(P_D) and the amount of Diodrast secreted by the renal tubule (T_D) in the absence of (left half) and in the presence of PAH (right half). (At the first zero minute, 50 mg of inulin per kg and 80 mg of Diodrast per kg were administered intravenously; at the second zero minute, 80 mg of Diodrast per kg and 120 mg of PAH per kg were given.)

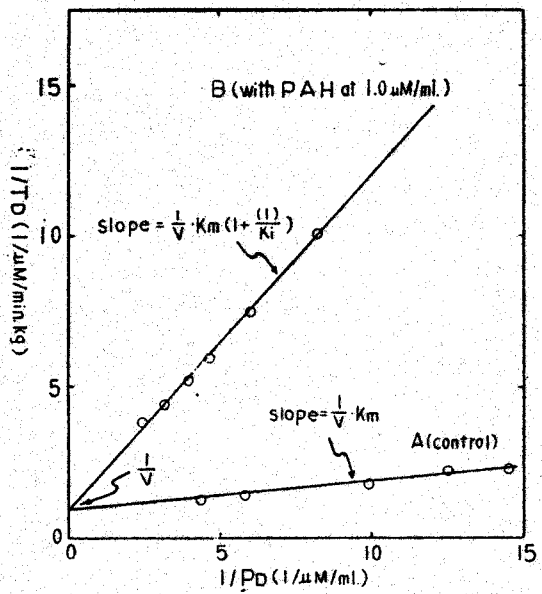

Fig. 5. Relationship between the reciprocal of the amount of Diodrast secreted (1/TD) and the reciprocal of plasma concentration of Diodrast (PD) in the absence of (line A) and in the presence of PAH (line B). Both lines were drawn by visual inspection.

both in the absence and in the presence of its competitive inhibitor, PAH, and the results obtained from this series were analyzed in the same manner as was done for the secretion of PAH.

In the presence of PAH at the concentration of approximately 1.0 μM/ml, the tubular secretion of Diodrast (TD) was markedly diminished, as compared with the control (Fig. 4). By plotting 1/TD as a function of 1/PD (Fig. 5), the values of the various kinetic constants have been calculated and are shown in Table 2.

Table 2. Various Kinetic Constants for the Competitive Inhibition of Diodrast Secretion by PAH

Dog Number	V(=TmD) μM/min/kg	Km μM/ml	Ki(=KPAH) μM/ml	$Km(1+\frac{(I)}{ki})$ μM/ml	(I)(=PPAH) μM/ml
1	1.6	0.16	0.13	1.16	1.2
2	1.6	0.15	0.14	1.24	1.0
3	1.6	0.13	0.15	1.16	1.5
4	1.6	0.16	0.16	1.16	1.0
Mean value	1.6	0.15	0.14	1.41	1.17

The value of V (i.e. TmD), as computed from the intercept, was approximately 1.6 μM/min./kg. In practice, however, it was taken to be 1.6 μM/min./kg in all cases on the basis of the literature (Smith et al., 1938), because of the reason indicated in the preceding section. The PD at which the Diodrast transport system is half-saturated was 0.15 μM/ml (Km) and 1.41 μM/ml ($Km(1+\frac{(I)}{Ki})$) in the absence and in the presence of PAH, respectively (Fig. 6). The value of the dissociation constant of the carrier-PAH complex (Ki=KPAH) was 0.145 μM/ml, which was approximately 4 times greater than that of Diodrast.

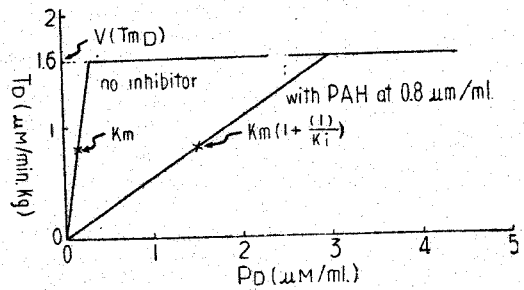

Fig. 6. Hypothetical relationship between the amount of Diodrast secreted (TD) and plasma concentration of Diodrast (PD) in the absence of (curve on the left and in the presence of (curve on the right) Diodrast.

DISCUSSION

It is evident that both PAH and Diodrast are indeed secreted by the renal tubule through a common transport system (Figs. 2 and 5). Although it is not entirely clear what the intermediary steps involved in the system are, there may be not only a certain carrier or carriers at the two membranes (i.e. the luminal and capillary side), but there may also exist a certain trapping substance within the tubular cells (Forster and Hong, 1958). Because of this uncertainty concerning nature of the intermediary steps, the mechanism was considered to involve one carrier as a whole, which has simply been referred to as the carrier in the present investigation.

Although this carrier is able to form a complex with PAH and Diodrast in the process of tubular transport, it has been clearly demonstrated in the present investigation that the carrier-

Diodrast complex is approximately 4 times more firmly bound than the carrier-PAH complex. Hence, it can be readily predicted that the inhibition of PAH secretion by Diodrast is much greater than the inhibition of Diodrast secretion by PAH.

In compliance with this prediction, several groups of investigators have reported that, when both PAH and Diodrast are present in plasma at the equimolar concentration, Diodrast was more readily excreted than PAH in man (Josephson et al., 1952), in rabbits(Josephson et al., 1953) and in the Lophius (Forster and Hong, 1959). Furthermore, Diodrast has also been shown to be a more powerful inhibitor of chlorophenol red transport than PAH in the isolated renal tubules of flounders(Hong and Forster, 1960).

Perhaps it may be worth pointing out that Diodrast, which has a lower Tm than PAH, is a more powerful competitive inhibitor by virtue of its lower dissociation constant than is PAH. Similar results have been reported in the transport of various sulfonphthalein dyes in isolated flounder tubules. Bromphenol blue or bromcresol green, which accumulates very little in the tubular lumen, is shown to be the most potent inhibiors of the phenol red or chlorophenol red transport (Forster, Sperber and Taggart, 1954, Forster and Hong, 1958).

These facts, together with the data presented in this paper, support the earlier notion of Forster and Hong (1959) that the substance with the greatest affinity (or the lowest dissociation constant) to the carrier seems to be secreted at the lowest rate, but inhibits the secretion of other competitive substances most powerfully, by occupying the intermediary carriers at the highest rate.

SUMMARY

The manner in which PAH and Diodrast are secreted competitively by the renal tubules has been studied in anesthetized dogs, with special emphasis on the kinetic aspect. Various kinetic constants have been calculated by the use of Michaelis-Menten equations, and results are briefly summarized as follows.

1. The transfer maxima of PAH and of Diodrast were 5 and 1.6 μM/min./kg, respectively.
2. The values of Km for the PAH and Diodrast transport system were 0.223 and 0.15μM/ml, respectively.
3. The dissociation constants of the carrier-PAH and carrier-Diodrast complex were 0.145 and 0.0335 μM/ml, indicating that Diodrast is 4 times more strongly bound to the carrier than is PAH.
4. The possible interrelation between the the transfer maxima and the strength of competition has been discussed.

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