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ON THE MECHANISM OF ACETATE ENHANCEMENT OF RENAL *p*-AMINOHIPPURATE TRANSPORT

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SUMMARY

The effect of acetate on the ability of renal cortical tissue to accumulate and to synthesize *p*-aminohippurate was determined in young and adult rats. Acetate increased the maximal rate of *p*-aminohippurate uptake in tissue from adult rats with no change in K_m values. The effect of acetate on *p*-aminohippurate accumulation by renal cortical slices from adult rats was smaller than that from rabbits but the stimulatory effect was apparent. The *p*-aminohippurate slice/medium (S/M) ratio was increased at high pH (8.2) and acetate increased further the *p*-aminohippurate S/M ratio in both age groups.

The ability of renal cortical homogenates to synthesize *p*-aminohippurate was used to estimate the activity of the enzyme glycine acyltransferase (EC 2.3.1.13). Enzyme activity was low in renal cortical homogenates from 10-day-old rats when measured under optimal conditions and with glycine rate limiting. Acetate did not change *p*-aminohippurate synthesis in tissues from young or adult rats. These data discount the hypothesis that acetate enhancement of *p*-aminohippurate transport is due to the formation of acetylglycine.

INTRODUCTION

The effect of acetate on renal tubular transport of the organic acid *p*-aminohippurate has been studied by several investigators *in vivo* and *in vitro*[1-4]. Cross and Taggart[1] demonstrated that acetate enhanced *p*-aminohippurate accumulation in rabbit renal cortex slices. Acetate also increased maximal transport capacity for *p*-aminohippurate in the intact dog[2]. Schachter *et al.*[4] observed that long-chain acylglycines inhibited *p*-aminohippurate transport whereas acetylglycine did not. They suggested that in the presence of excess acetate, formation of acetylglycine within the kidney is favored at the expense of long-chain acylglycines. This argument suggests that the "acetate effect" is not truly stimulation of transport but reduction of endogenous inhibitors (acylglycines). This hypothesis was strengthened by the observation that acetate stimulation of *p*-aminohippurate transport occurred only in those species with kidneys capable of conjugating glycine with acetate and longer acyl compounds[4]. The hypothesis, however, has been challenged by Murdaugh and Eliott[5] who observed no inhibition of the acetate effect upon the addition of excess glycine.

It has recently been demonstrated that acetate has no effect on *p*-aminohippurate accumulation in renal cortical slices of very young rats[6]. It was of interest to use the developing animal as a model to elucidate the mechanism involved in acetate stimulation of *p*-aminohippurate transport. According to the hypothesis of Schachter *et al.*[4], if acetate does not stimulate *p*-aminohippurate accumulation in newborn then the ability of this tissue to conjugate glycine should be low as well. Both of these functions, then, should develop in a parallel fashion. Furthermore, if the presence of acetate alters glycine conjugation then the addition of acetate to the appropriate enzymatic reaction should influence the rate of enzyme activity.

The purpose of this investigation was to elucidate the factors involved in acetate stimulation of *p*-aminohippurate transport. The specific objectives were: (1) to determine the relative effects of acetate on accumulation of *p*-aminohippurate by renal cortex of rat, rabbit and chicken, (2) to determine the effect of changes in medium pH on acetate stimulation of *p*-aminohippurate accumulation; (3) to quantitate the activity of glycine acyltransferase in kidney tissue of adult and newborn (10-day) rats, and (4) to determine the effect of acetate on this enzymatic activity.

METHODS

Determination of p-aminohippurate slice/medium (S/M) ratio

Female Sprague–Dawley rats were bred in the departmental animal quarters so that young animals of known age could readily be obtained. Litter size was routinely reduced to 8 pups within 2 days of birth and young rats were left with their mothers until the time of experimentation.

Adult and young rats, adult New Zealand rabbits and White Leghorn chickens were sacrificed by a blow on the head and the kidneys removed immediately, weighed, and placed in ice-cold saline. Renal cortical slices were prepared free hand and briefly kept in cold saline until incubation. All incubations were conducted in duplicate. To obtain sufficient tissue the kidneys of several animals were pooled (*i.e.* at 10 days of age 4 pups were required for 1 duplicated incubation). Approximately 100 mg of slices were placed in 2.7 ml of the medium devised by Cross and Taggart[1], adjusted to pH 7.4. The concentration of *p*-aminohippurate was $7.4 \cdot 10^{-5}$ M.

The slices were incubated for 90 min under flowing 100% oxygen at 25 °C in a Dubnoff metabolic shaker. After incubation the slices were quickly removed from the beakers, blotted on gauze and weighed. A 2-ml aliquot of medium was taken from each beaker. To both tissue and media 3 ml of 10% trichloroacetic acid were added, the tissue macerated, and the samples diluted to 10 ml with water. *p*-Aminohippurate was measured by the method of Smith *et al.*[7]. The transport of *p*-aminohippurate was expressed as the final ratio of concentrations in slice and medium (S/M) where S equals mg/g of tissue and M equals mg/ml of medium.

To determine the effect of acetate, sodium acetate was added to produce final concentrations in the medium of 1, 2 and $4 \cdot 10^{-3}$ and $1 \cdot 10^{-2}$ M.

To increase pH of the medium to 8.2, 0.1 ml of the 0.1 M sodium phosphate Cross and Taggart medium was replaced with 0.1 ml of 0.2 M 2-amino-2-methyl-1,3-propanediol.

p-Aminohippurate uptake

Slices from pooled kidneys were equally divided into 16 beakers. Duplicate

incubations were conducted at 4 *p*-aminohippurate concentrations (1, 2, 4 and $8 \cdot 10^{-4}$ M) and 2 times (2 and 12 min). The uptake of *p*-aminohippurate per min between 2 and 12 min at each concentration was determined and the data were plotted on a Hofstee plot.

p-Aminohippurate synthesis in renal cortical homogenates

The rate of *p*-aminohippurate synthesis, which represents the activity of glycine acyltransferase, was determined in renal cortex from adult and 10-day-old rats and White Leghorn chickens

Renal cortex was homogenized with cold 0.25 M sucrose in a glass homogenizer with a glass pestle. The incubation mixture contained potassium phosphate buffer (pH 7.56), 25 μmoles; $MgCl_2$, 5 μmoles, glycine, 43 μmoles; fumarate, 2.5 μmoles, ATP, 2.5 μmoles, *p*-aminobenzoic acid, 3 μmoles, and 0.3 ml of kidney homogenate. The final volume was 1 ml. All experiments were determined with and without $1 \cdot 10^{-2}$ M sodium acetate in the reaction mixture.

After incubation for 30 min (unless otherwise indicated) at 37 °C in a Dubnoff metabolic shaker, the tubes were placed on ice and 4 ml of 0.2 M trichloroacetic acid were added. The rate of *p*-aminohippurate synthesis was determined at varying glycine concentrations (0, 1, 2, 4 and 5 μmoles in the reaction mixture). The data were plotted on a Hofstee plot.

p-Aminohippurate was determined by the method of Cohen and McGilvery[8] and protein was measured by the method of Lowry *et al.*[9] with bovine albumin fraction V as the standard

p-Aminohippurate recovery

p-Aminohippurate recovery was determined with or without renal cortical homogenate in the reaction mixture in ten separate experiments. *p*-Aminohippurate (0.2 μmole) was added to the reaction mixture instead of *p*-aminobenzoic acid and the normal extraction procedure was followed. When tissue was in the reaction mixture *p*-aminohippurate recovery was 99.80 ± 2.28 %. With no tissue in the reaction mixture, recovery was 97.65 ± 2.37 %.

Statistical analyses

All data are reported as means ± S.E. Differences between means were analyzed statistically using Student's *t* test, group comparison[10] or Duncan's test following analysis of variance[11].

RESULTS

p-Aminohippurate accumulation in renal cortical slices from adult rat and rabbit was determined at varying concentrations of acetate in the medium (0, 1, 2 and $4 \cdot 10^{-3}$ and $1 \cdot 10^{-2}$ M) (Fig. 1). Acetate increased the *p*-aminohippurate ratio more in renal cortical slices from rabbit (98 % increase at 10^{-2} M) than in rat (44 % at 10^{-2} M)

In the presence of 10^{-2} M acetate in the medium, the rate of *p*-aminohippurate uptake by renal cortical slices from adult rats was markedly enhanced (Fig. 2). Acetate nearly doubled the maximal rate of uptake in the adult tissue (control, 14.71; acetate,

25.29 μg/g tissue per min). But the K_m (−slope) was not changed (control, 6.01; acetate, $7.21 \cdot 10^{-4}$ M).

The effect of high pH (8.2) and the effect of acetate at high pH on *p*-aminohippurate accumulation was determined in renal cortical slices from 10-day-old and adult rats (Fig. 3). The *p*-aminohippurate S/M ratio was increased from 10.30 ± 0.67 at pH 7.4 to 14.13 ± 0.37 at pH 8.2 in adult tissue, from 4.87 ± 0.25 to 6.96 ± 0.45 in tissue from 10-day rats. Acetate not only increased the *p*-aminohippurate S/M ratio at physiological pH (7.4) but also at high pH. In adult tissue, the *p*-aminohippurate S/M ratio was significantly enhanced in the presence of 10^{-2} M acetate at high pH (from 14.13 ± 0.37 to 20.79 ± 0.20). Acetate slightly, but consistently, increased the *p*-aminohippurate S/M ratio at high pH in slices from 10-day animals (from 6.96 ± 0.45 to 7.74 ± 0.35).

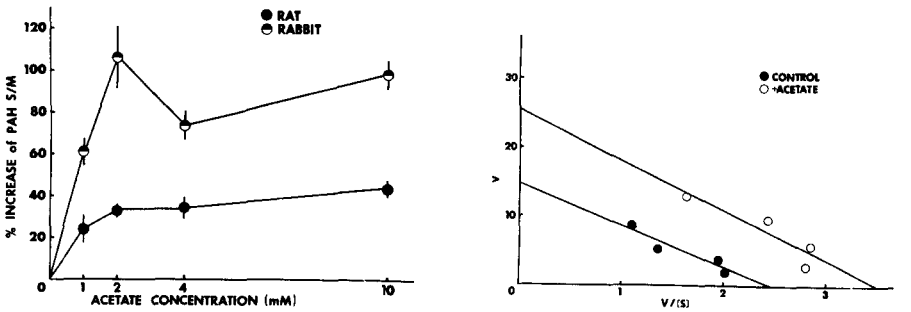

Fig 1 Effect of acetate on *p*-aminohippurate (PAH) accumulation (S/M ratio) in renal cortical slices from adult rabbits and rats. The concentrations of acetate in the medium were 1, 2 and $4 \cdot 10^{-3}$ and $1 \cdot 10^{-2}$ M Values are means ± S E of 4 experiments

Fig 2 Effect of acetate on *p*-aminohippurate uptake in renal cortical slices from adult rats Values are average of 4 experiments at each concentration of *p*-aminohippurate (1, 2, 4 and $8 \cdot 10^{-4}$ M) and each incubation time (2 and 12 min) In this Hofstee plot, V represents *p*-aminohippurate uptake (μg *p*-aminohippurate/g tissue per min) and $[S]$ represents *p*-aminohippurate concentration in the medium The two slopes ($-K_m$) (control, 6 01, acetate, $7\ 21 \cdot 10^{-4}$) were not different Lines are calculated regression lines.

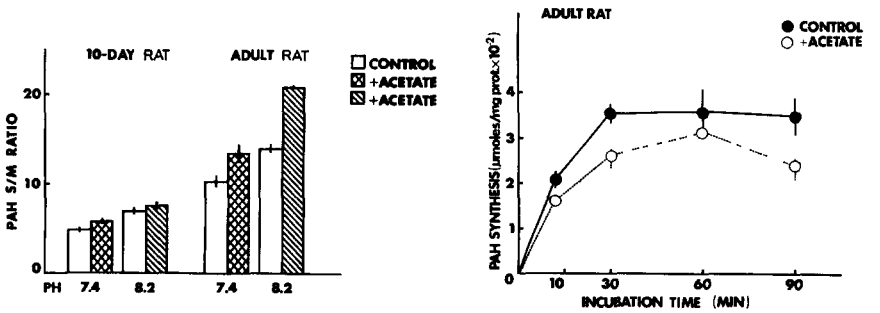

Fig. 3 Effect of acetate (10^{-2} M) and high pH (8 2) on *p*-aminohippurate (PAH) accumulation in renal cortical slices from 10-day-old and adult rats. Each bar represents means ± S E of 4 experiments Acetate significantly enhanced the *p*-aminohippurate S/M ratio at pH 8 2 in both tissues (Duncan's test).

Fig 4. Effect of acetate on *p*-aminohippurate (PAH) synthesizing enzyme system in renal cortical homogenates of adult rats Values are means ± S E of 4 experiments, each experiment was the average of triplicate determinations. The absence of a vertical bar indicates S E is within the radius of the point

p-Aminohippurate synthesis in renal cortical homogenates from 10-day and adult rats was determined in the presence and absence of acetate ($1 \cdot 10^{-2}$ M). Incubation time was varied from 10–90 min. Maximal *p*-aminohippurate synthesis in tissue from 10-day-old rats (2.39 ± 0.47 μmoles/mg protein $\times 10^{-2}$) was lower than that from adult (3.44 ± 0.51 μmoles/mg protein $\times 10^{-2}$) (Figs 4, 5). *p*-Aminohippurate synthesis in adult tissue was maximal at 30 min incubation (Fig. 4) whereas *p*-aminohippurate concentration in tissue from 10-day-old rats reached a maximal value at 60 min of incubation (Fig. 5). Acetate in the reaction mixture did not significantly alter *p*-aminohippurate synthesis in renal homogenates of either age animal.

The rate of *p*-aminohippurate synthesis was determined in renal cortical homogenates from 10-day and adult rat at varying concentrations of glycine (0, 1, 2, 4 or 5 μmoles) in the reaction mixture. Similar incubations were conducted in the presence of 10^{-2} M acetate. The maximal rate of *p*-aminohippurate synthesis for the adult tissue (3.59 μmoles/mg protein per 30 min $\times 10^{-2}$) was markedly higher than for

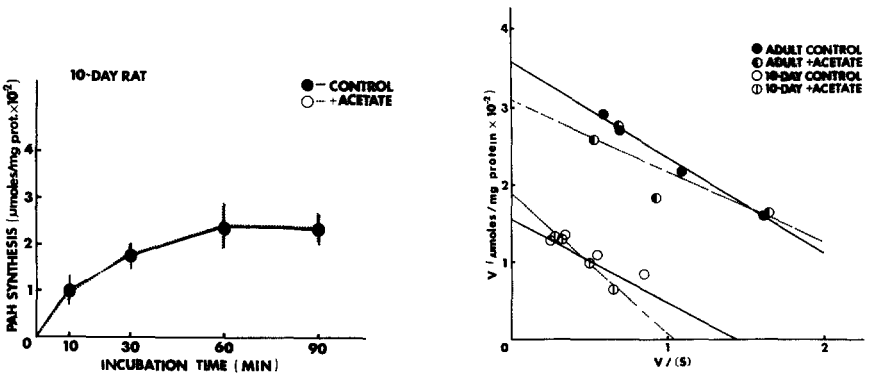

Fig. 5 Effect of acetate on *p*-aminohippurate (PAH) synthesizing enzyme system in renal cortical homogenates of 10-day-old rats. Values are means ± S E of 4 experiments, conducted in triplicate.

Fig 6. Effect of acetate on the rate of *p*-aminohippurate synthesis in renal cortical homogenates of 10-day and adult rats Glycine concentrations in the reaction mixture were 0, 1, 2, 4 and 5 μmoles In this Hofstee plot, V represents the rate of *p*-aminohippurate synthesis, μmoles/mg protein per 30 min $\times 10^{-2}$ in adult, μmoles/mg protein per 60 min $\times 10^{-2}$ in 10-day animals. [S] represents the concentration of glycine in the reaction mixture. Lines are calculated regression lines The slopes were not different between or within age groups (Student's t test).

TABLE I

EFFECT OF ACETATE ON *p*-AMINOHIPPURATE TRANSPORT (S/M RATIO) and *p*-AMINOHIPPURATE SYNTHESIS IN THE CHICKEN KIDNEY

No of Expt	*p-Aminohippurate S/M ratio*		*p-Aminohippurate synthesis (μmoles/mg protein × 10⁻²)*	
	Control	*+Acetate*	*Control*	*+Acetate*
1	12.50	16.10	0.58	0 85
2	5 93	9.20	0.16	0
3	12 53	20 88	0 16	0
$\bar{x}$	10 32	15.39	0 30	0 28
(± S.E.)	(2 19)	(3 39)	(0.14)	(0 28)

the 10-day animals (1.55 μmoles/mg protein per 60 min × 10^{-2}). Acetate had no effect on the maximal rate of *p*-aminohippurate synthesis or K_m (−slope) in either tissue (Fig. 6).

The effect of acetate on *p*-aminohippurate accumulation in renal cortical slices and on *p*-aminohippurate synthesis in renal cortical homogenates from White Leghorn chicken was determined. Acetate increased the *p*-aminohippurate S/M ratio consistently but no *p*-aminohippurate synthesis could be measured in this tissue (Table I).

DISCUSSION

Schachter *et al.*[4] indicated that enhancement of *p*-aminohippurate transport by acetate appeared to occur only in those species capable of glycine conjugation[4]. The exception was the rat kidney which has glycine acyltransferase activity but reportedly little or no increase in *p*-aminohippurate transport in response to acetate[12]. However, in the present work acetate significantly and consistently increased the *p*-aminohippurate S/M ratio (Fig. 1) and the rate of *p*-aminohippurate uptake in slices from adult rat (Fig. 2).

The synthesis of hippuric acid resembles, in several respects, the synthesis of peptide bonds[13]. Cohen and McGilvery[8,14,15] found significant formation of *p*-aminohippurate from *p*-aminobenzoic acid and glycine in homogenates and slices of rat liver and kidney. Synthesis of hippuric acid was low in liver homogenates from newborn rats[16,17] and the activity of directly measured glycine acyltransferase varied with the age of the rat in a pattern similar to hippuric acid synthesis[18], thus suggesting that this enzymic step is rate-limiting in the overall reaction. Measurement of *p*-aminohippurate synthesis from glycine and *p*-aminobenzoic acid then, directly reflects the activity of glycine acyltransferase.

Low *p*-aminohippurate synthesis in the 10-day rat kidney is consistent with the minimal stimulation of *p*-aminohippurate accumulation produced by adding acetate. However, no effect of acetate on *p*-aminohippurate synthesis by renal cortical homogenates was observed in either adult or 10-day animals (Figs 4, 5). Addition of exogenous acetate to the enzyme reaction system should yield acetyl-CoA as the most abundant acyl-CoA available to the glycine acyltransferase system. A relative abundance of acetyl-CoA would be expected to promote the synthesis of acetylglycine at the expense of the longer chain inhibitory acylglycines[4]. If this hypothesis were correct the addition of acetate should decrease acyl-CoA, thereby decreasing *p*-aminohippurate synthesis. This, however, was not the case. Addition of acetate under optimal conditions over a series of incubation times did not influence *p*-aminohippurate synthesis (Figs 4, 5) nor was there an effect of acetate when glycine concentration was made rate-limiting (Fig. 6). In the latter experiments if glycine and acetate did react, the addition of acetate should decrease available glycine and thus decrease the rate of *p*-aminohippurate synthesis. This, however, was not the case. Furthermore, in three experiments utilizing chickens no *p*-aminohippurate synthesis could be measured in homogenates from these animals. However, slices prepared from these kidneys were able to accumulate *p*-aminohippurate and this was enhanced by acetate (Table I). These data clearly show that there is no relationship between the effect of acetate on *p*-aminohippurate transport and the acetylglycine synthesis system.

What, then, is the mechanism of the stimulatory effect of acetate on *p*-amino-

hippurate transport? Apparently acetate does not alter the permeability of the slice to *p*-aminohippurate[5]. The apparent affinity of the transporting system for *p*-aminohippurate was not altered by acetate when the maximal rate of transport was enhanced (Fig. 2). Cross and Taggart[1] suggested that an acetate requiring reaction is rate limiting in the transport of *p*-aminohippurate. This is difficult to reconcile with the minimal effect of acetate in tissue from newborn Nevertheless, the suggestion could be correct for possibly factors other than acetate could be rate limiting in tissue from young animals.

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