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Acetate Influence upon the Transport Kinetics of p-Aminohippurate at 37°C in Rabbit Kidney Slices (39679)

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Acetate has a stimulatory effect on the renal cortical *p*-aminohippurate transport system *in vitro* (1) and *in vivo* (2). This effect was first interpreted as a direct effect on metabolism related to the transport system (1-3). In fact, Cross and Taggart (1) suggested that the stimulation of *p*-aminohippurate accumulation seen in adult kidneys indicates that acetate is one of the rate-limiting cellular components involved in the transport system. On the other hand, Schachter *et al.* (4) suggested that acetate enhances *p*-aminohippurate uptake by its conjugation with glycine to form acetylglycine, which functions to relieve a normal degree of competitive inhibition by long chain acylglycines. The suggested relative specificity rendered by acetate (1-4) as a stimulatory agent was challenged by Koishi (5) who showed that acetate inhibited, as well as stimulated, *p*-aminohippurate uptake, and that pyruvate, succinate, and α-ketoglutarate quantitatively mimicked the effects elicited by acetate. The controversy concerning the role of acetate in the active uptake of weak organic acids by the kidney prompted us to perform comparable studies using *p*-aminohippurate as a model weak organic acid in order to elucidate the specific function of acetate.

Material and methods. Adult New Zealand rabbits weighing from 2.5 to 4.0 kg were used in this investigation. After the rabbits were sacrificed by injecting air through an auricular vein, the kidneys were immediately removed and placed in an ice-cold (0°) low-Na (10 mM) Cross–Taggart medium devoid of acetate. Renal cortical slices, approximately 0.3–0.5 mm thick and weighing 200–300 mg, were cut with a Stadie–Riggs tissue slicer and then were randomly divided into two groups. One group was transferred to a modified low-Na Cross–Taggart preincubation medium containing 10 m*M* acetate, whereas the other was transferred to a similar preincubation medium devoid of acetate. Preincubation was done at 0° for 30 min. The groups of slices were incubated in their appropriate high-Na (100 m*M*) modified Cross–Taggart medium containing *p*-aminohippurate, in either the presence or absence of 10 m*M* acetate, respectively. The compositions of the 10 and 100 m*M* Na media have previously been described (6). In the nonacetate series, sodium acetate was replaced by an equimolar amount of NaCl. All preincubations and incubations were performed under 100% O_2. The incubations were done in a Dubnoff metabolic shaker at either 25 or 37° for 45 min.

Three different types of experiments were performed. In the first series of experiments, the effect of acetate on *p*-aminohippurate accumulation and oxygen consumption at both 25 and 37° was examined. Groups of slices were preincubated and incubated in either the presence or absence of acetate. In the accumulation experiments, the slices were blotted and weighed at the end of the incubations; the calculations were based upon this wet weight. In the oxygen consumption experiments, the slices were blotted and weighed prior to incubation. Oxygen uptake was represented on the basis of this initial wet weight, because some of cell components were thought to disperse into the medium and consume oxygen, though these would be excluded from the final wet weight of the slices.

The second series of experiments was a kinetic study similar to that described by Gerencser *et al.* (6). At the end of incubation, the slices were immediately removed, blotted, and weighed. The slices and the incubation media were then analyzed for *p*-aminohippurate.

In a third series of experiments, we examined the effect of acetate on the energy charge potential (7), focusing on the adeno-

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sine 5′-triphosphate concentrations in the slices. Following preincubation, the slices were blotted and weighed, after which they were incubated in either the presence or absence of 10 m*M* acetate at 37°. After incubation, slices were frozen in liquid nitrogen until the analyses for nucleotides were done.

Calculations were based on tissue wet weight. The concentration of *p*-aminohippurate in the slices and in the medium was determined by the methods of Smith *et al.* (8). The oxygen consumption of the slices was measured using a Warburg manometric respirometer as described by Cross and Taggart (1). Adenosine triphosphate analyses were performed as described by Lamprecht and Trautschold (9). Adenosine 5′-diphosphate and adenosine 5′-monophosphate were determined as described by Adam (10). Energy charge potential was calculated as defined by Atkinson (7).

All data are reported as means ± SE. Differences between means were analyzed statistically using the Student's *t*-test. Where appropriate, paired comparisons were made. The 0.05 level of probability was used as the criterion of significance.

Results. The first series of experiments examined the effects of acetate on the accumulation of *p*-aminohippurate at both 25 and 37° (Table I). The slice to medium ratio (S/M) for *p*-aminohippurate increased significantly, by approximately 50%, at both temperatures. It is also of interest to note that there was no significant difference in the corresponding S/M values between the two temperatures in either the presence or absence of acetate, indicating, in agreement with the results of Cross and Taggart (1), that the uptake of *p*-aminohippurate reaches a maximum at 25°.

In contrast, the next group of experiments showed that acetate induced a significant increase in the oxygen consumption at 25° but not at 37° (Table II). Moreover, the oxygen consumption was significantly higher at 37 than at 25° in both the presence and absence of acetate ($P < 0.05$).

The rate of *p*-aminohippurate uptake at 37° as a function of *p*-aminohippurate concentration in the incubation medium is shown in Fig. 1. The rate of uptake increased curvilinearly as a function of *p*-aminohippurate concentration in both the absence (control) and presence of acetate, although the rate of uptake at a given concentration was significantly higher in the presence of acetate as compared to the control.

In order to determine an effect of acetate on K_m and V_{max} for the transport of *p*-aminohippurate, the data shown in Fig. 1 were kinetically analyzed using a Hofstee plot. A typical experiment shown in Fig. 2 indicates

TABLE I. THE EFFECTS OF ACETATE ON S/M RATIO OF p-AMINOHIPPURATE AT VARIOUS TEMPERATURES

Temperature (°C)	Acetate (10 m*M*/liter)	S/M of *p*-aminohippurate	*P* Value
37	+	13.40 ± 1.09[a]	<0.05
	−	8.89 ± 0.71[a]	
25	+	11.60 ± 1.10[b]	<0.05
	−	8.14 ± 0.31[b]	

[a] Values are means ± SE of four paired experiments.

[b] Values are means ± SE of three paired experiments.

TABLE II. THE EFFECTS OF ACETATE ON OXYGEN CONSUMPTION AT VARIOUS TEMPERATURES.

Temperature (°C)	Acetate (10 m*M*/liter)	Oxygen consumption (μl/hr/mg)	*P* Value
37	+	1.56 ± .05[a]	N.S.
	−	1.52 ± .05	
25	+	0.84 ± .02	<0.05
	−	0.74 ± .03	

[a] Values are means ± SE of nine paired experiments.

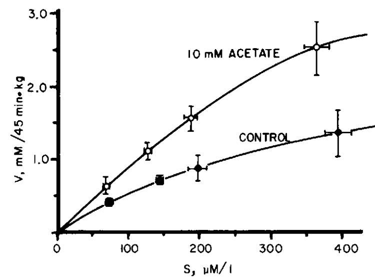

FIG. 1. Rate of accumulation (V) of *p*-aminohippurate as a function of final-medium *p*-aminohippurate concentrations (S). Each point represents the mean of six determinations at either the control or a 10 m*M* acetate concentration.

an increase in V_{max} (the intercept with the ordinate) but no change in K_m (the slope of the line) by acetate. The K_m of slices in the 10 mM acetate and acetate-free media was 0.30 ± 0.08 and 0.27 ± 0.05 mM, respectively, in six paired experiments (not significantly different from one another). The V_{max} of the same slices in the 10 mM acetate and acetate-free media was 2.99 ± 0.52 and 1.94 ± 0.20 mM/45 min·kg, respectively (n = 6). The mean V_{max} of slices bathed in the 10 mM acetate medium was significantly higher than the mean V_{max} of slices bathed in the acetate-free medium ($P < 0.05$ for paired t-test). It is evident that acetate significantly increased V_{max} but had no effect on the K_m.

The next series of experiments examined the effect of acetate on energy charge potential at 37°. The tissue concentrations of various nucleotides and the calculated energy charge potential at 37°C are summarized in Table III. Acetate significantly decreased the level of adenosine triphosphate, whereas it significantly ($P < 0.05$) increased that of adenosine diphosphate. However, acetate had no significant effect on the energy charge potential.

Discussion. The results obtained in the present work indicate that acetate increases the uptake of p-aminohippurate by about 50% at both 25 and 37° (Table I), whereas it increases the oxygen consumption at 25 but not at 37° (Table II). Such a dissociation of acetate on the transport and metabolism at 37° suggests that the stimulatory effect of acetate on organic acid transport is not mediated by the stimulation of metabolic energy (1). However, a possibility that the failure to increase oxygen consumption by acetate at 37° might be due to the limitation in diffusion of oxygen from the medium into the the slice should be considered. According to the Warburg criteria (11),

$$b' = \left(\frac{8D \cdot Uo}{\alpha}\right)$$

where b' = the limiting thickness of the tissue slice into which oxygen can diffuse from both sides of the slice; D = Krogh diffusion coefficient of oxygen in water (1.7×10^{-5} cm^2·min^{-1}·atm^{-1} at 37° (11); Uo = oxygen pressure in incubation medium (1 atm with oxygenation); and α = oxygen consumption. When b' = 0.05 cm, as in the present work, the limiting oxygen consumption of the slice calculated using the above formula is 3.24 μl/hr·mg at 37°. Since the values of oxygen consumption observed in the present work are about 50% of this value (Table II), there is no reason to believe that acetate failed to increase oxygen consumption at 37° because of the diffusion limitation.

Whatever the mechanism of action of acetate is, one may rule out the possibility that acetate alters the affinity of the transport carrier (s) to p-aminohippurate, as indicated by the absence of any change in K_m in the presence of acetate (Fig. 2). The values of K_m measured in the present work at 37° agree well with those measured at 25° in the kidney cortical slice of the rabbit (6) and the rat (5). On the other hand, acetate significantly increased V_{max} in the present work. This finding is also compatible with an increase in $T_{M_{PAH}}$ *in vivo* in the dog kidney, following the administration of acetate (2). These results suggest that acetate increases the turnover rate of the carrier–organic acid complex, possibly by increasing its mobility.

The level of tissue adenosine triphosphate decreased significantly at 37° in the presence of acetate (Table III), although acetate

TABLE III. EFFECTS OF ACETATE ON TISSUE NUCLEOTIDE CONCENTRATIONS AND ENERGY CHARGE POTENTIAL

	Milliosmolar concentration	Concentration of acetate in medium 10 mM	P value*
Adenosine triphosphate (μmol/g wet wt)	1.74 ± 0.13[b]	1.40 ± 0.20	<0.05
Adenosine diphosphate (μmol/g wet wt)	1.09 ± 0.10	1.44 ± 0.20	<0.05
Adenosine monophosphate (μmol/g wet wt)	1.76 ± 0.28	1.88 ± 0.17	N.S.
Energy charge potential[c]	0.49 ± 0.03	0.44 ± 0.03	N.S.

[a] Student's t-test (paired t-test) used for statistical comparisons.
[b] Values are means ± SE of five observations.
[c] ([ATP] + 1/2 [ADP])/([ATP] + [ADP] + [AMP]).

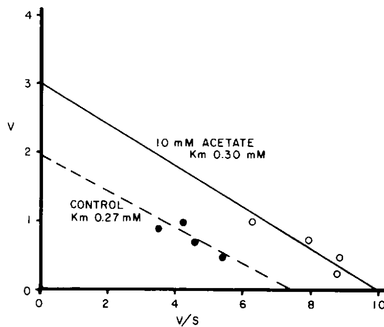

FIG. 2. Hofstee plot of kinetic data. The intercept on the ordinate represents the V_{max} of accumulation for either the control or a 10 m*M* acetate medium. The slope of the line represents the negative K_m. V_{max} and K_m are in millimoles/45 min/Kilogram wet weight and millimoles/liter, respectively.

did not increase the oxygen consumption at this temperature (*vide supra*). It is possible that acetate interacts specifically with adenosine triphosphate to energize the carrier-organic acid complex to increase its mobility. Since the metabolic rate is so high at 37°, there presumably is enough ATP in the tissue to support the action of acetate without increasing the oxygen consumption.

Based on these considerations, we propose that acetate enhances the active transport of *p*-aminohippurate by stimulating the utilization of cellular ATP. However, this utilization of ATP by the action of acetate does not disrupt the overall energy status of the transporting cells, as indicated by the absence of a change in the energy charge potential in the presence of acetate.

Summary. The present investigation involving *p*-aminohippurate uptake by rabbit kidney slices has shown (i) confirmative evidence that *p*-aminohippurate uptake is stimulated by the presence of acetate at both 25 and 37°; (ii) oxygen consumption being stimulated by acetate at 25 but not at 37°; (iii) the K_m for *p*-aminohippurate uptake at 37° is not affected by the presence of acetate in the medium; (iv) the V_{max} for *p*-aminohippurate uptake at 37° is directly related to the presence of acetate in the medium; and (v) acetate stimulates ATP utilization at 37° but does not alter energy charge potential.

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