Biochemical Pharmacology, Vol. 38, No. 20, pp. 3431–3437, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00
Pergamon Press plc

INHIBITION OF RAT LIVER TRANSAMINASES BY LOW LEVELS OF ACETALDEHYDE AND THE PHARMACOLOGIC EFFECTS OF B_6 VITAMERS*

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(*Received* 21 *October* 1988; *accepted* 24 *February* 1989)

Abstract—To better define the significance and mechanism of acetaldehyde-mediated transaminase inhibition, acetaldehyde metabolism was studied in rat liver homogenates and cytosols. When either preparation was incubated at 37° with 1.5 mM acetaldehyde for 4 hr, acetaldehyde levels fell rapidly in the first 30 min and little inhibition of aspartate aminotransferase (GOT) or alanine aminotransferase (GPT) resulted. In contrast, incubation with 50 mM ethanol also resulted in a peak acetaldehyde level of 1.0 to 1.5 mM by 2 hr, but this level was then maintained for the next 2 hr and transaminases were inhibited by 20–35%. Sequential addition of low dose (125–250 μM) pulses of acetaldehyde to rat liver preparations resulted in a progressive decrease in the rate of acetaldehyde disappearance. When the pulsing schedule was adjusted accordingly to maintain acetaldehyde levels between 50 and 250 μM for 8 hr, transaminases were again inhibited by 20–40%. Finally, addition of 1–5 mM pyridoxal and pyridoxal 5′-phosphate, aldehydic B_6 vitamers, to cytosols 2–4 hr after pulsing with acetaldehyde was begun, almost completely prevented further transaminase inhibition. In contrast, the non-aldehydic B_6 vitamers, pyridoxine, pyridoxamine and pyridoxamine 5′-phosphate, did not affect acetaldehyde-mediated transaminase inhibition. These findings suggest that (1) prolonged exposure to low levels of acetaldehyde impairs acetaldehyde metabolism in rat liver homogenates and cytosols; (2) acetaldehyde toxicity may be more dependent on sustained exposure to acetaldehyde than on the peak level of acetaldehyde attained; and (3) aldehydic B_6 vitamers can modify on-going acetaldehyde-mediated transaminase inhibition.

Alcohol abuse in humans is accompanied by decreased aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) activities in liver biopsy specimens [1]. Previous *in vitro* studies in this laboratory have shown that incubation of rat liver cytosols or human RBC lysates with ethanol (EtOH) or acetaldehyde (ACH) leads to inhibition of cytosolic GOT and GPT activities [2–4]. This effect requires further nonoxidative metabolism of ACH since: (1) inhibition is both time and temperature dependent; (2) inhibition is not affected by blocking ACH oxidation with cyanamide or disulfiram but is prevented by preincubation with other metabolic inhibitors (i.e. fluoride and cyanate); (3) incubation with other reducing substrates and/or acetate does not produce transaminase inhibition; and (4) direct addition of ACH to GOT and GPT assay mixes has no effect on either enzyme activity [2–4]. Both GOT and GPT are activated by pyridoxal 5′-phosphate (PLP), the coenzyme form of vitamin B_6, and it has been suggested that ACH can modify B_6 metabolism [5, 6]. However, ACH-mediated transaminase inhibition does not result from either modification of the B_6 coenzyme binding site or from displacement of B_6 coenzymes from transaminase apoenzymes [2–4].

In our previous reports, relatively high initial ACH concentrations were required for transaminase inhibition [2, 3]. *In vivo* experiments suggest that hepatic ACH levels do not exceed 250 μM during EtOH administration [7–12]. Thus, in the present study, the effects of sustained exposure to low levels of ACH on rat liver transaminases were evaluated. Moreover, since aldehydic B_6 vitamers can bind to the same cell components as ACH [5, 6, 13, 14], the effects of pharmacologic levels of these B_6 vitamers on ACH-mediated transaminase inhibition were assessed.

MATERIALS AND METHODS

ACH was obtained from the Fisher Scientific Co. and 3-(*N*-morpholino) propane sulfonic acid (MOPS) was obtained from Calbiochem. All other reagents were obtained from the Sigma Chemical Co.

Whole liver homogenates and mitochondria-depleted cytosols were prepared from six to ten decapitated Sprague–Dawley rats using 1 g wet weight of liver in 3 ml of fresh Tris/sucrose medium as previously described [2, 3]. All samples were frozen immediately at −22°. Experimental results were not affected by storage of homogenates or cytosols at −22° for up to 6 weeks prior to use. Results reported were obtained using a single pooled sample and confirmed using two other pooled preparations.

For each experiment, 0.4-ml aliquots of liver cytosol or homogenate were placed in 15-ml polystyrene centrifuge tubes, capped with parafilm, and incu-

* This study was supported by a grant from the Veterans Administration.

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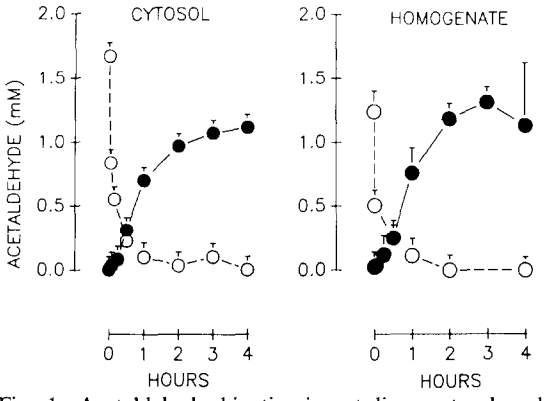

Fig. 1. Acetaldehyde kinetics in rat liver cytosol and homogenate. Cytosol and homogenate were incubated at 37° with either 50 mM ethanol (●) or 1.5 mM acetaldehyde (○). At timed intervals, acetaldehyde levels were determined as described in Materials and Methods.

Table 1. Effects of serial acetaldehyde pulses on acetaldehyde metabolism

Time period (hr)	Rate of acetaldehyde disappearance (μmol/l/min)	
	Homogenate	Cytosol
0–1	11.42	7.17
1–2	8.57	3.92
2–3	2.42	1.25
3–4	2.25	1.17
4–6	1.71	1.04
6–8	1.46	1.01

During incubation at 37°, small volume pulses of 40 mM acetaldehyde, sufficient to increase the concentration of acetaldehyde by 125–250 μM, were added to homogenates or cytosols at timed intervals as shown in Fig. 3, and acetaldehyde levels were determined as described in Materials and Methods.

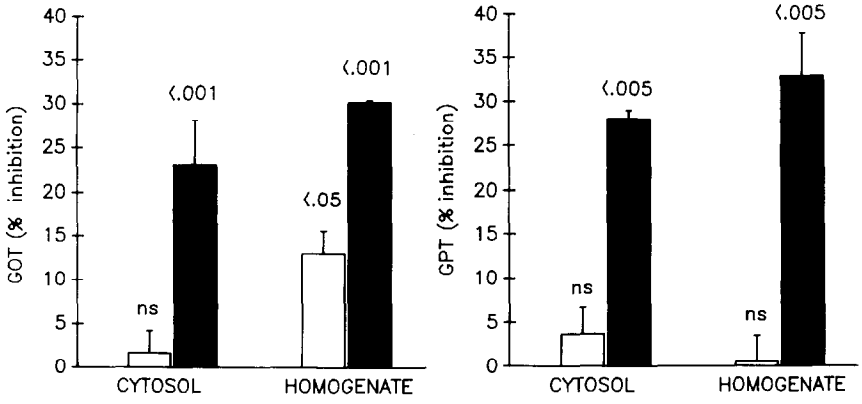

Fig. 2. Effects of acetaldehyde and ethanol on rat liver transaminases. Cytosol and homogenate were incubated at 37° alone (control) or with either 50 mM ethanol or 1.5 mM acetaldehyde. After 4 hr, GOT and GPT activities were determined, and the percent inhibition produced by ethanol (closed bars) or acetaldehyde (open bars) was calculated as described in Materials and Methods.

bated at 37° in a shaking water bath with 7.5 mM MOPS (pH 7.4) and the additives indicated. At timed intervals, 30-μl aliquots were diluted into 0.3 ml of ice-cold Tris/sucrose. GOT and GPT were assayed by coupling their activities to malate dehydrogenase and lactate dehydrogenase, respectively, and following the decrease in NADH spectrophotometrically at 340 nm [2]. To the remaining material, 0.4 ml of ice-cold 8% trichloroacetic acid was added and allowed to stand in ice for 10 min. Supernatant fractions were removed by centrifugation, and their ACH content was determined using yeast alcohol dehydrogenase [15]. This method was sensitive to ACH levels in liver preparations as low as 10 μM. All enzyme assays and ACH determinations were completed within 2 hr of obtaining the sample. Where indicated, B_6 vitamers, which had been dissolved previously in Tris/sucrose medium and adjusted to pH 7.4, were also added to liver cytosol preparations at various times during the incubation period. The percentage inhibition of GOT and GPT was calculated from the ratio of enzyme activities in liver preparations incubated in the presence of ACH to enzyme activities in liver preparations incubated in an identical fashion but in the absence of ACH.

RESULTS

Role of acetaldehyde kinetics in transaminase inhibition. When homogenate or cytosol preparations were incubated at 37° with 1.5 mM ACH, ACH levels fell rapidly during the first 0.5 hr, and little inhibition of GOT or GPT was noted (Figs. 1 and 2). In contrast, incubation with 50 mM EtOH increased ACH levels to the same peak value of 1.0 to 1.5 mM but inhibited GOT and GPT by 25–30%. This suggests that sustained exposure to ACH may be more important than the maximum level of ACH attained.

This possibility was explored further by adding low dose pulses of ACH to homogenate and cytosol systems. The amount and frequency of ACH addition were estimated from the rate of ACH disappearance determined in preliminary experiments. The rate of ACH disappearance was consistently

Table 2. Effect of preincubation with aldehydic B_6 vitamers on acetaldehyde-mediated transaminase inhibition

	Control (units/ml)	Acetaldehyde (units/ml)	(% Inhibition)
I. GOT			
No B_6	11.68 ± 0.13	7.24 ± 0.14	38 ± 1
5 mM PL	10.88 ± 0.07	9.70 ± 0.20	11 ± 2
P	< 0.001	< 0.001	< 0.001
5 mM PLP	12.58 ± 0.20	11.60 ± 0.10	8 ± 1
P	< 0.005	< 0.001	< 0.001
II. GPT			
No B_6	5.13 ± 0.14	4.42 ± 0.06	14 ± 3
5 mM PL	5.38 ± 0.014	4.02 ± 0.08	25 ± 1
P	NS	< 0.005	< 0.005
5 mM PLP	4.49 ± 0.23	4.39 ± 0.33	2 ± 2
P	< 0.02	NS	< 0.01

Cytosol was incubated at 37° for 40 min alone or with either 5 mM PL or 5 mM PLP. Low dose pulses of acetaldehyde were then added as shown in Fig. 3. After an additional 8 hr of incubation, transaminase activities were determined as described in Materials and Methods. Values are means ± one standard deviation for three determinations.

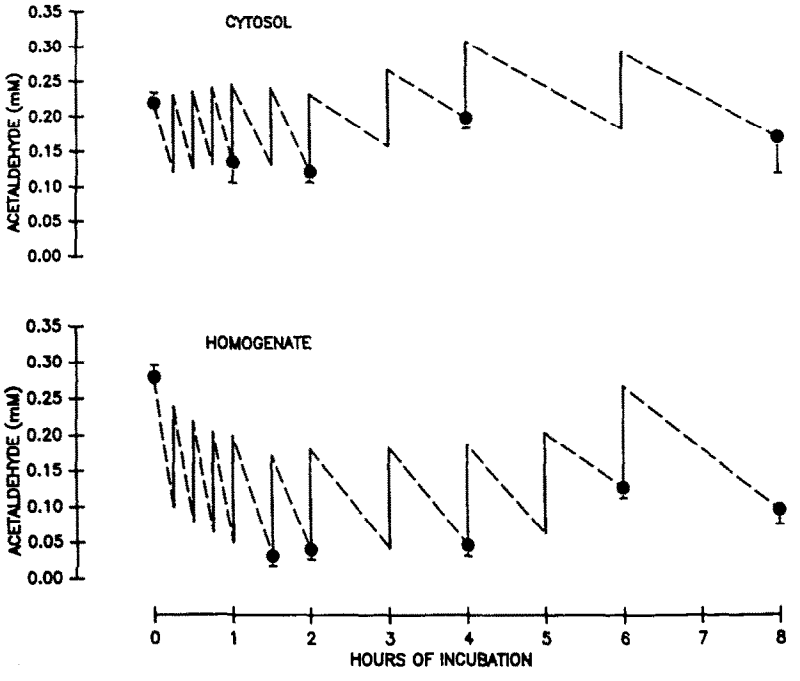

Fig. 3. Effect of low dose pulses of acetaldehyde on acetaldehyde levels in rat liver preparations. Cytosol and homogenate were incubated at 37°. The initial concentration of acetaldehyde was 250 μM. Where indicated by the solid vertical lines, 2 μl of a 40 mM acetaldehyde solution was added to the cytosol so as to increase the concentration of acetaldehyde by 125 μM. Data points shown are the mean ± one standard deviation for acetaldehyde levels measured in triplicate samples. Intermediate values were extrapolated (dashed lines) from the known time and amount of intervening pulses.

faster in homogenates than in cytosols but ACH levels fell more slowly after each successive pulse in both systems (Fig. 3; Table 1). As shown in Fig. 4, when ACH levels were maintained between 50 and 250 μM for 4–8 hr, significant GOT and GPT inhibition was noted by 4 hr and reached 20–40% at 8 hr. In these experiments, the mean ACH level over 8 hr was 213 ± 35 μM in cytosol and 146 ± 41 μM in homogenates, while the total amount of ACH added during this period was 1.09 mM for cytosols and 1.68 mM for homogenates.

Effects of B_6 vitamers on ACH-mediated transaminase inhibition. The effects of aldehydic B_6 vitamers, pyridoxal (PL) and PLP, on rat liver

Table 3. Effects of aldehydic B_6 vitamers (5 mM) on on-going acetaldehyde-mediated transaminase inhibition

	Control (units/ml)	Acetaldehyde (units/ml)	(% Inhibition)
I. GOT			
No B_6	10.13 ± 0.13	5.99 ± 0.54	43 ± 5
5 mM PL	8.41 ± 0.35	7.61 ± 0.08	9 ± 4
P	< 0.005	< 0.01	< 0.005
5 mM PLP	10.16 ± 0.09	8.83 ± 0.18	13 ± 1
P	NS	< 0.005	< 0.005
II. GPT			
No B_6	6.27 ± 0.13	4.48 ± 0.39	30 ± 4
5 mM PL	6.03 ± 0.31	5.40 ± 0.18	10 ± 4
P	NS	< 0.005	< 0.005
5 mM PLP	5.57 ± 0.20	4.95 ± 0.09	13 ± 2
P	< 0.05	NS	< 0.005

Rat liver cytosol was incubated at 37° alone (Control) or with low dose pulses of acetaldehyde (Acetaldehyde) as shown in Fig. 3. After 2 hr, acetaldehyde inhibited GOT by 8 ± 2% ($P < 0.005$) and GPT by 4 ± 3% ($P < 0.05$). At this time, 5 mM PL, 5 mM PLP or the same volume of Tris/sucrose medium (No B_6) was added, and samples were incubated for an additional 4 hr. Aliquots were then assayed for GOT and GPT activities as described in Materials and Methods. Values are means ± one standard deviation for three determinations.

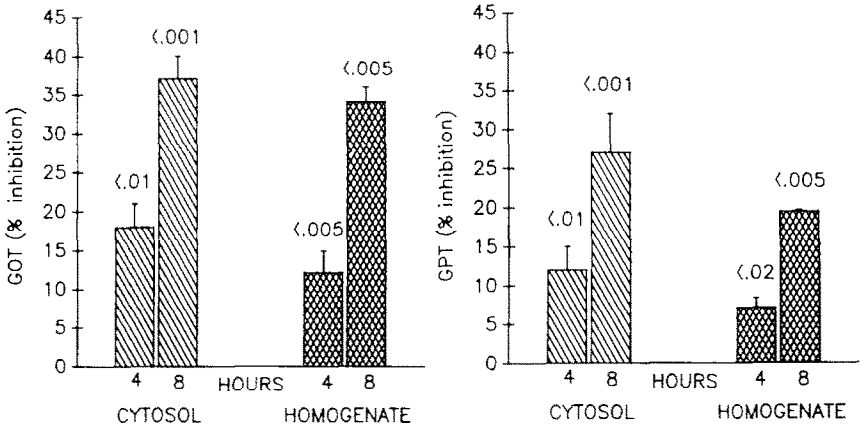

Fig. 4. Effect of low dose acetaldehyde pulses on rat liver transaminases. Cytosol and whole homogenate were incubated as described in the legend of Fig. 3. After 4 and 8 hr, GOT and GPT activities were measured as described in Materials and Methods and compared to the transaminase activities in similarly incubated preparations pulsed only with Tris/sucrose medium.

transaminases and on ACH-mediated transaminase inhibition were then studied. PL (1–5 mM) inhibited GOT by 7–15% but had no significant effect on GPT (Tables 2–4). In contrast, 5 mM PLP had little effect on GOT but inhibited GPT by 10–15% (Tables 2 and 3). However, no inhibition of GPT occurred when the concentration of PLP was decreased to 1 mM (Table 4).

Both PL and PLP also modified ACH-mediated transaminase inhibition. *Preincubation* of cytosol for 40 min with 5 mM PL decreased ACH-mediated GOT inhibition but potentiated ACH-mediated inhibition of GPT (Table 2). In contrast, when 5 mM PL was added 2 hr *after* pulsing with ACH was initiated, further ACH-mediated inhibition of both GOT and GPT was decreased markedly (Table 3). In fact, as little as 1 mM PL added as late as 4 hr after initiating ACH pulsing significantly limited further inhibition of both transaminases (Table 4). Similarly, either 1 or 5 mM PLP added 40 min prior to ACH pulsing or 2–4 hr after pulsing with ACH was begun decreased or prevented ACH-mediated inhibition of GOT (Tables 2–4). At a concentration of 5 mM, PLP also appeared to decrease ACH-mediated GPT inhibition but this was due primarily to a decrease in GPT activity in the control tubes (Tables 2 and 3). However, at a concentration of 1 mM, PLP selectively decreased ACH-mediated GPT inhibition (Table 4).

The effects of non-aldehydic B_6 vitamers on ACH-mediated transaminase inhibition were also evaluated. As shown in Table 5, addition of 5 mM pyri-

Table 4. Effects of aldehydic B_6 vitamers (1 mM) on on-going acetaldehyde-mediated transaminase inhibition

	Control (units/ml)	Acetaldehyde (units/ml)	(% Inhibition)
I. GOT			
No B_6	12.77 ± 0.12	7.11 ± 0.06	44 ± 1
1 mM PL	11.47 ± 0.25	7.83 ± 0.08	32 ± 1
P	< 0.005	< 0.001	< 0.001
1 mM PLP	12.03 ± 0.40	8.13 ± 0.13	32 ± 1
P	< 0.025	< 0.001	< 0.001
II. GPT			
No B_6	5.77 ± 0.10	4.36 ± 0.03	24 ± 2
1 mM PL	5.76 ± 0.05	4.61 ± 0.08	20 ± 1
P	NS	< 0.01	< 0.05
1 mM PLP	5.80 ± 0.10	4.68 ± 0.15	19 ± 1
P	NS	< 0.025	< 0.02

Rat liver cytosol was incubated at 37° alone (Control) or with low dose pulses of acetaldehyde (Acetaldehyde) as shown in Fig. 3. After 4 hr, GOT was inhibited by 25 ± 1% ($P < 0.001$) and GPT was inhibited by 15 ± 3% ($P < 0.001$). At this time, 1 mM PL, 1 mM PLP or an equal volume of Tris/sucrose medium (No B_6) was added, and samples were incubated for an additional 4 hr. Aliquots were then assayed for GOT and GPT activities as described in Materials and Methods. Values are means ± one standard deviation for three determinations.

doxine (PN), pyridoxamine (PM), or pyridoxamine 5'-phosphate (PMP) 2 hr after initiation of low dose ACH pulses did not alter appreciably GOT and GPT activities in control or ACH-treated cytosols.

DISCUSSION

ACH is a highly reactive molecule which can readily bind to many cell components *in vitro* and which may play a significant role in EtOH toxicity *in vivo* [16–18]. However, many of the *in vitro* effects of ACH are only noted when concentrations of ACH much higher than those believed to occur *in vivo* are used. ACH levels in rat liver of 10–250 μM have been reported after administration of EtOH orally or by hepatic perfusion [7–12]. Nonetheless, chronic EtOH consumption may increase both net ACH formation and the sensitivity of metabolic processes to ACH [19–27].

We have reported that incubation of rat liver cytosols with EtOH or ACH leads to inhibition of GOT and GPT, a finding consistent with the low levels of hepatic GOT and GPT which accompany EtOH abuse *in vivo* [1–3]. In these experiments, however, initial ACH levels equal to or greater than 1 mM were required to produce transaminase inhibition. Thus, the current study was undertaken to determine if sustained exposure to lower levels of ACH could also lead to transaminase inhibition in rat liver homogenates and cytosols. Indeed, 1.5 mM ACH disappeared rapidly from both preparations and produced little transaminase inhibition (Figs. 1 and 2). In contrast, when the same peak level of ACH was achieved during incubation with EtOH, striking inhibition of GOT and GPT was noted.

Serial low dose pulses of ACH were then added to both liver preparations. Not surprisingly, ACH disappeared more rapidly from homogenates that contain a mitochondrial low K_m aldehyde dehydrogenase than from cytosols which contain only 6% of the mitochondrial marker enzyme glutamate dehydrogenase [3] (Fig. 3). It is also of note that each serial ACH pulse disappeared more slowly than the preceding one in both homogenates and cytosols, suggesting that prolonged ACH exposure may potentiate ACH accumulation (Table 1). In fact, decreased oxidation of ACH has been described in both hepatic cytosol and mitochondria after chronic EtOH administration [21, 22, 24, 27–29].

Strikingly, when pulses of ACH were delivered so as to maintain ACH levels between 50 and 250 μM, far greater inhibition of GOT and GPT occurred than when the same total dose of ACH was added as a single pulse at the beginning of incubation (Fig. 4). Similarly, we have noted that low dose ACH pulses inhibit GOT and GPT in human RBC lysates when ACH concentrations are kept between 10 and 200 μM [4], a range achievable *in vivo* in chronic alcoholic subjects after EtOH ingestion [25, 30–33]. Taken together, these findings provide evidence for the first time that ACH toxicity may be more dependent on sustained exposure to ACH than on the maximum level of ACH attained.

Although ACH can displace PLP from some protein-binding sites [5, 6], PLP does not correct ACH-mediated transaminase inhibition when added to dilute assay mixes after incubation of undiluted human RBC lysates or rat liver cytosols with ACH [2–4]. Moreover, incubation of undiluted RBC lysates with ACH did not decrease the affinity of endogenous apoGOT for PLP (L. Solomon, unpublished observations). Thus, it is unlikely that ACH-mediated transaminase inhibition is due to displacement of B_6 coenzymes or to modification of the PLP binding site. Nonetheless, since both ACH and PLP can bind to other amino groups on the transaminase proteins and since modification of such groups may impair enzyme activity [34, 35], the

Table 5. Effects of non-aldehydic B_6 vitamers (5 mM) on on-going acetaldehyde-mediated transaminase inhibition

	Control (units/ml)	Acetaldehyde (units/ml)	(% Inhibition)
I. GOT			
No B_6	12.35 ± 0.16	8.55 ± 0.19	31 ± 2
5 mM PM	11.04 ± 0.19	8.15 ± 0.06	26 ± 2
P	< 0.05	NS	NS
5 mM PMP	12.35 ± 0.10	8.61 ± 0.03	30 ± 1
P	NS	NS	NS
5 mM PN	11.29 ± 0.22	8.53 ± 0.04	24 ± 2
P	NS	NS	NS
II. GPT			
No B_6	4.31 ± 0.01	3.40 ± 0.03	21 ± 1
5 mM PM	4.44 ± 0.04	3.45 ± 0.03	22 ± 1
P	NS	NS	NS
5 mM PMP	4.36 ± 0.11	3.43 ± 0.12	21 ± 1
P	NS	NS	NS
5 mM PN	4.42 ± 0.01	3.54 ± 0.09	19 ± 1
P	< 0.05	NS	NS

Rat liver cytosol was incubated at 37° alone (Control) or with low dose pulses of acetaldehyde (Acetaldehyde) as shown in Fig. 3. After 2 hr, GOT was inhibited by 12 ± 4% ($P < 0.02$) and GPT was inhibited by 7 ± 5% ($P = 0.05$). At this time, 5 mM pyridoxamine (PM), 5 mM pyridoxamine 5′-phosphate (PMP), 5 mM pyridoxine (PN) or the same volume of Tris/sucrose medium (No B_6) was added, and samples were incubated for an additional 4 hr. Aliquots were then assayed for GOT and GPT activities as described in Materials and Methods. Values are means ± one standard deviation for three determinations.

possibility that pharmacologic levels of aldehydic B_6 vitamers could modify ACH-mediated transaminase inhibition was considered. Indeed, both PL and PLP decreased or prevented on-going ACH-mediated transaminase inhibition in rat liver cytosol (Tables 3 and 4). In contrast, non-aldehydic B_6 vitamers had no effect on ACH-mediated transaminase inhibition (Table 5).

It was also of note that PL itself inhibited GOT, whereas high levels of PLP inhibited GPT (Table 2). Furthermore, PL potentiated ACH-mediated GPT inhibition when added *prior* to beginning ACH pulses but decreased ACH-mediated GPT inhibition when added *after* pulsing was initiated (Tables 2–4). Thus, both the B_6 vitamer concentration and the sequence of binding of ACH and aldehydic B_6 vitamers to sites on the transaminase apoproteins may alter enzyme activity profoundly.

Finally, the distinct effects of the different B_6 vitamers suggest that significant interconversion of these vitamers did not occur under the conditions of these experiments. Since cytosols were not supplemented with ATP, formation of PLP, PMP and PNP from PL, PM and PN, respectively, by the enzyme pyridoxine kinase may well have been limited. Similarly, both low levels of flavin cofactors in liver cytosols and feedback inhibition of the enzyme PMP oxidase by PLP [36, 37] may prevent sufficient generation of PLP from either PMP or PNP to modify ACH-mediated transaminase inhibition. Further studies are required therefore, to define the potential roles of different B_6 vitamers in modifying ACH toxicity *in vitro* and *in vivo*.

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