Letter to the Editor: Resolution of the Presence of Multiple Forms of the Cyclic Nucleotide Phosphodiesterases (PDE) in Normal and Reye's Syndrome Liver

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The published literature cites the presence of alterations of a number of enzymes in the liver of Reye's syndrome subjects (2, 3, 9, 11, 12). It is, indeed, highly unlikely that each of these alterations is the consequence of a separate defect. Rather, as pointed out by Mitchell (Pediatr. Res., 13: 945 (1979).), these alterations are likely to be mediated through a common regulatory defect. Because the cyclic nucleotides are involved in the regulation of a wide range of metabolic processes, many of which appear to be involved in this disorder as pointed out by us earlier (6), the status of the enzymes which determine their intracellular concentrations is relevant to our understanding of the pathophysiology of this disease.

Table 1. Initial velocity: cyclic AMP PDE of a human liver homogenate

Substrate concentration 10^{-6} M	nmoles converted/mg protein/min
0.153	0.026
0.200	0.035
0.25	0.037
0.33	0.037
0.50	0.058
1	0.095
2	0.188
10^1	0.709
20^1	1.02
50^1	2.73
75^1	3.05
100^1	4.63
150^1	8.22
200^1	6.65
500^1	7.64
1000	
1500	12.50

¹ Values used by Mitchell.

The Michaelis constants were derived by the Lineweaver-Burk method obtaining the intercepts of the slopes by linear regression analysis (8). The figure could not accommodate all the points used in our analysis by virtue of the wide range of concentrations used. Because Mitchell only used the points of the S-V curve, it necessarily incorporated only those points we used for determining the kinetic constants for the high K_m enzyme. We agree that there is only one form of PDE by the data used by Mitchell. The reciprocals of the upper inset figure would have provided values for the other form, the low K_m PDE. The raw S and V values used for the particular study represented by Figure 4 are presented in

Table 1. The technique used by Mitchell to analyze our data magnifies the variables resulting from translation of numerical data into a figurative representation necessarily involving at least two interpreters, the investigator and the artist.

Fig. 1.

Fig. 2.

Thompson and Appelman (13), in the paper cited by Mitchell, found only one PDE in the liver of the rat. Subsequent reports from these (10) and other workers (1) including one on human liver (4), indicate the presence of multiple forms which can be

resolved by kinetic analysis as well as by separation by physical and chemical means (1, 5).

The point made by Mitchell regarding the need to monitor the contribution of one form to the other is well taken, particularly for the low K_m form of PDE where the contributions of the high K_m form could be substantial. However, in our study, K_m differences were found with the high K_m cyclic AMP PDE. With this form, the contributions of the low K_m enzymes would surely be negligible especially because the V_{max} for the low K_m form is two orders of magnitude lower than the high K_m PDE.

It should be emphasized that the difference in cyclic AMP K_m 's found at high substrate concentrations, although statistically significant ($P < 0.025$, > 0.010), may not hold when sample size is increased or where the individual molecular forms are separately analyzed. The data presented were obtained from a $30,000 \times g$ spun homogenate after dialysis to remove endogenous cyclic nucleotides. There are many modulators of PDE activity, not all of which are dialyzable (5, 7).

Recently, we have examined control and Reye's liver after homogenization in water or 20 mM Tris-HCl, centrifugation at $30,000$ or $100,000 \times g$ and separation by DEAE chromatography with and without EGTA. Figures 1 and 2 are representative of our findings on human liver after homogenization in water and centrifugation at $30,000 \times g$, using a linear NaCl gradient (0–0.5 M). At $100 \mu M$ substrate concentration, two peaks of activity are found whereas at $10 \mu M$, two cyclic GMP peaks and four cyclic AMP peaks are found. Centrifugation at $100,000 \times g$ as well as exposure to EGTA result in pattern changes which are currently under analysis. Thus, extension of our studies indicates the presence of multiple forms of PDE in the liver of patients with Reye's syndrome as well as control subjects. Whether the observed statistical difference reported earlier in crude preparations is reflected in these better resolved forms is under investigation currently.

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