J. Enzyme Inhibition, 1997, Vol. 11, pp. 293–298
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INHIBITORY KINETIC STUDIES ON RAT HEPATIC AND RENAL SUCCINATE DEHYDROGENASE WITH GUANIDINE

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(Received 15 March 1996; in final form 15 August 1996)

Guanidine-induced alterations in substrate dependent kinetics of hepatic and renal succinate dehydrogenase (SDH) have been investigated under *in vitro* conditions. Guanidine hydrochloride (GuHCl) induced a mixed type of inhibition by decreasing the maximal velocity (V_{max}) and increasing the Michaelis-Menten constant (K_m). The competitive (K_i) and non-competitive (K_i') inhibitory constants were calculated. The values showed that the inhibitory influence of GuHCl is more due to decreased enzyme substrate affinity rather than reduction in the active site density of the enzyme as revealed by low K_i values.

Keywords: Succinic dehydrogenase; guanidine.

INTRODUCTION

Guanidine compounds have long been implicated as uremic toxins[1] and are known to cause hepatic coma and severe hepatic damage if accumulated in excess.[2,3] These compounds produce epileptic symptoms, violent convulsions and seizures in rats due to hyperammonemia following renal obstruction and failure.[4] Earlier investigation in our laboratory showed that succinate dehydrogenase (SDH), a key enzyme in the TCA cycle, was inhibited by guanidine.[5] In the present study the nature of inhibition caused by guanidine hydrochloride on SDH was characterized by determining the inhibitory constants.

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MATERIALS AND METHODS

Male Wistar rats (150 ± 10 g) were maintained under laboratory conditions and fed on standard diet (Hindustan Lever Ltd., Bangalore) and water *ad libitum*. The mitochondrial fraction was collected by the method of Koch.[6]

The rats were sacrificed, the liver and kidney were excised in ice-cold sucrose solution (0.25 M). The tissues were separately homogenized in sucrose solution and centrifuged at 2500 rpm for 10 min. The residue was discarded and the supernatant was again centrifuged at 15000 rpm for 20 min in a Hitachi refrigerated centrifuge (Model-CR-20B2). The supernatant was discarded and the pellet obtained was suspended in sucrose solution and again centrifuged at 15000 rpm for 20 min. The mitochondrial pellet thus obtained was resuspended in the sucrose solution and used as an enzyme source for SDH. Succinate dehydrogenase activity was assayed by the method of Nachlas *et al.*[7] as modified by Pramilamma and Swami.[8] Determination of the concentration of inhibitor required to cause 50 per cent inhibition of the enzyme activity (IC_{50}) is a widely used method to assess the inhibitory potential of the inhibitor employed. Hence, the activity of the enzyme was assayed at varied concentrations of inhibitor and the IC_{50} for guanidine hydrochloride was calculated by making use of the linear relationship between inhibitor concentration and per cent inhibition by the method of Job *et al.*[9]

SDH activity was assayed over a range of sodium succinate concentration (5–60 mM) in the presence and absence of the IC_{50} concentration (0.6 mM) of GuHCl. The Michaelis-Menten constant (K_m) and Maximal velocities (V_{max}) were determined by employing the least squares for the best fit. The inhibitory constants (K_i and K_i') for the hepatic and renal SDH were calculated as suggested by Dixon and Webb.[10] The protein content was estimated by the method of Lowry *et al.*[11] The results were analyzed statistically and the level of significance was calculated using Student's 't' test.[12]

RESULTS

The effect of GuHCl on SDH activity is presented in Table I. The GuHCl concentration versus SDH activity relationship yielded a characteristic curve with 50 per cent of inhibition of enzyme activity at 0.6 mM of GuHCl. The Lineweaver-Burk double reciprocal plots showed an increase in Michaelis-Menten Constant (K_m) and decrease in V_{max} in both the tissues studied (Table II). The inhibitory constants (K_i) have been derived according to the equation of Dixon and Webb.[10] The non-competitive inhibitory constant (K_i') is the dissociation constant

TABLE I Effect of GuHCl on SDH activity from rat microsomes

Concentration of GuHCl (mM)	*SDH activity**	*Per cent inhibition relative to control*
Control	1.826 ±0.019	—
0.1	1.608 ±0.019	-11.93
0.2	1.382 ±0.02	-24.31
0.3	1.168 ±0.016	-36.03
0.4	1.106 ±0.025	-39.43
0.5	1.002 ±0.016	-45.12
0.6	0.916 ±0.021	-49.83
0.7	0.820 ±0.083	-55.09
0.8	0.608 ±0.023	-66.70
0.9	0.290 ±0.009	-84.11
1.0	0.016 ±0.006	-99.12

*Values are expressed in μmoles of formazan formed/mg protein/h. All the values are mean±SD of six individual observations. The difference of values between control and GuHCl addition are significant at $P<0.001$.

of Enzyme-Inhibitor-Substrate Complex (EIS) while the competitive inhibitory constant (K_i) is that of the Enzyme-Inhibitor Complex (EI). The value of K_i was lower than K_i' (Table II, Figure 1).

DISCUSSION

The inhibitory kinetic studies on hepatic and renal SDH revealed that guanidine hydrochloride influences enzyme-substrate affinity (K_m) to a greater extent than the maximal velocity (V_{max}). Since K_m is altered drastically over V_{max} the type of inhibition caused by GuHCl is of mixed type tending towards the competitive type.

TABLE II Effect of GuHCl (IC_{50}) on the kinetic parameters of SDH from rat liver and kidney microsomes

Tissue	*Sample*	*Kinetic parameters*			
		V^*_{max}	K_m (mM)	K_i (mM)	K'_i (mM)
Liver	Control	2.860 ±0.01	10.0 +0.04		
	Control+ 0.6 mM GuHCl	2.410 ±0.01	12.0 ±0.04	1.414	3.213
	% Change	(−15.73)	(20.0)		
Kidney	Control	2.856 ±0.06	10.0 ±0.02		
	Control+ 0.6 mM GuHCl	2.406 ±0.08	12.0 ±0.04	1.414	3.208
	% Change	(−15.75)	(20.0)		

*V_{max} values are expressed in μmoles of formazan formed/mg protein/h. All the values are mean±SD of six samples. The difference values between control and GuHCl addition are significant at $P<0.001$.

However the kinetic constant values indicated that guanidine-induced inhibition of SDH is due to reduced affinity of E for S rather than reduction in active site density. The observed changes in the kinetic parameters show that GuHCl strongly inhibits SDH activity *in vitro*. Since it is well established that guanidines accumulate in the tissues and serum of uremic patients due to renal failure[13] and hyperguanidinemia is the characteristic feature of such patients[4], it may be speculated that accumulated guanidine may inhibit SDH *in vivo*. In accord with this, Raman Rao *et al.*[5] reported a decreased SDH activity in the tissues of the guanidine-treated rat.

The reduced succinate oxidation in the tissues of guanidine-treated rat indicates that guanidine affects succinate-fumarate interconversions and depresses oxidative metabolism at the level of the mitochondria.[5] This leads to an overall slowing of the citric acid cycle pathway thereby reducing energy production.

It can be concluded that the low K_i values indicate an increased affinity of GuHCl for the enzyme. Both renal and hepatic enzymes are similarly sensitive to GuHCl inhibition as evidenced by the inhibitor kinetic constant values.

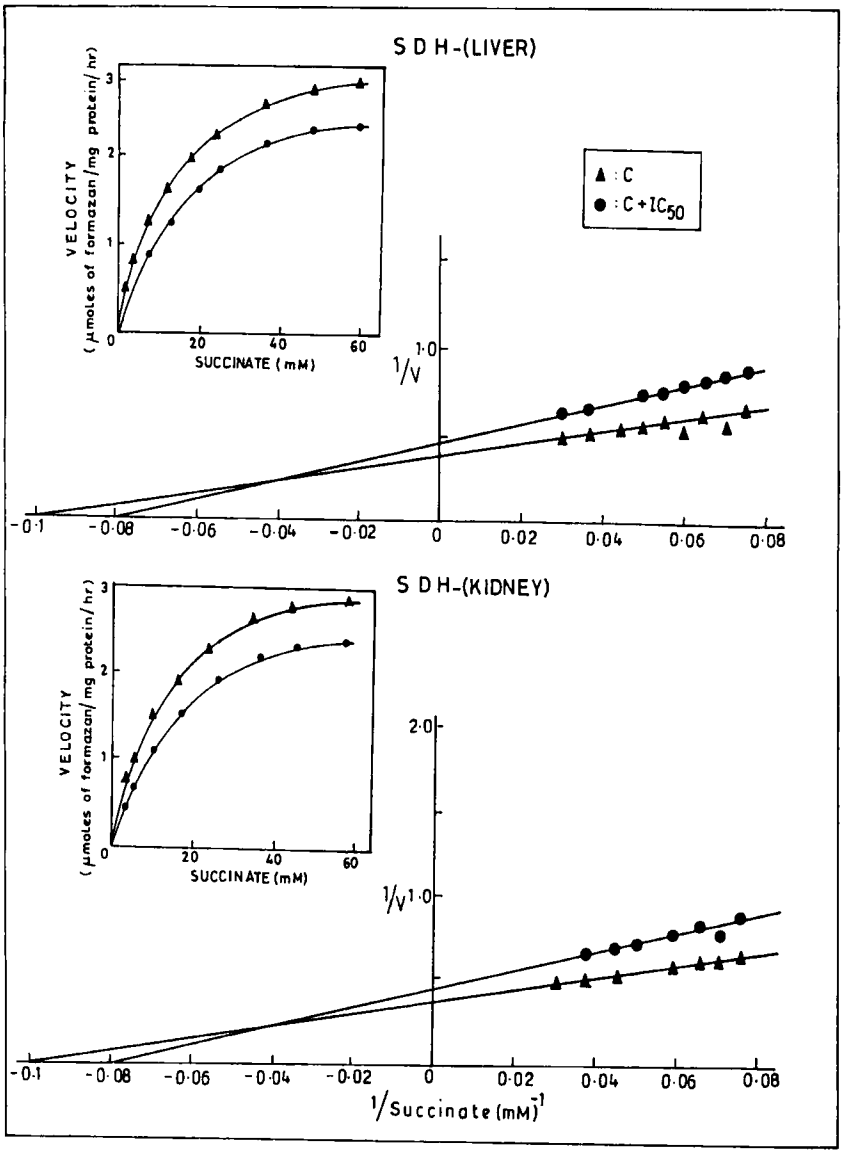

FIGURE 1 Lineweaver-Burk plots for the inhibition of rat (liver and kidney) succinate dehydrogenase by GuHCl.

Acknowledgements

Authors are thankful to University Grants Commission, New Delhi, for the financial assistance through a project sanctioned to KI.

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