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POSSIBLE MECHANISM OF ADVERSE REACTION FOLLOWING LEVODOPA PLUS BENSERAZIDE TREATMENT

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1 Rats treated for seven days with seryl-trihydroxybenzylhydrazine (benserazide), an inhibitor of peripheral aromatic L-amino acid decarboxylase (500 mg/kg, daily, i.p.) alone or in combination with L-DOPA methylester (500 mg/kg, daily, i.p.) for seven days showed a moderate but significant decrease of liver aldehyde dehydrogenase (ALDH), without accompanying change in alcohol dehydrogenase (ADH) activity, compared with saline-treated controls.

2 Administration of L-DOPA methylester (500 mg/kg, daily, i.p.) alone for seven days had little effect on liver ADH or ALDH.

3 The combined treatment might be conducive to the *in vivo* formation of L-DOPA-derived tetrahydroisoquinoline derivatives which might be implicated in L-DOPA produced adverse effects.

Introduction

The clinical efficacy of 3,4-dihydroxyphenylalanine (L-DOPA) in the treatment of Parkinson's disease is well established (Cotzias, Van Woert & Schiffer, 1967). However, L-DOPA produced dose-dependent side-effects often limit the desired therapeutic response. Co-administration of L-DOPA with an inhibitor of peripheral aromatic L-amino acid decarboxylase (ADC), e.g. benserazide (Birkmayer, 1969; Tissot, Gaillard, Guggisberg, Gautier & de Ajuriaguerra, 1969) or carbidopa (Cotzias, Papavasiliou & Gellene, 1969), reduced the therapeutic dose requirements for L-DOPA and decrease some of L-DOPA mediated adverse effects. The search for biochemical explanations for L-DOPA-induced side-effects resulted in the hypothesis (Sandler, 1973) that tetrahydroisoquinoline derivatives formed during L-DOPA therapy (Sandler, Carter, Hunter & Stern, 1973; Davis, Cashaw & McMurtrey, 1975), from the condensation of L-DOPA-derived monoamines with their respective oxidative deaminated metabolites (Holtz, Stock & Westermann, 1964; Collins, Cashaw & Davis, 1973; Turner, Baker, Algeri, Frigerio & Garattini, 1974), might be implicated in L-DOPA-induced adverse reactions. Furthermore, it is known that inhibition of aldehyde dehydrogenase (ALDH) by certain drugs augments the formation of these alkaloid-like compounds which may possess considerable pharmacological activity (Holtz *et al.*, 1964; Baird-Lambert & Cohen, 1975).

Benserazide possesses a terminal alcohol group which can be oxidized to a corresponding aldehyde derivative; subsequent cleavage of the benserazide molecule by hydrolysis of the seryl-hydrazine linkage (Bukard, Gey & Pletscher, 1964) may well produce alcohol and aldehyde intermediates capable of altering the activities of both alcohol dehydrogenase (ADH) and ALDH. To test this possibility, the specific activities of liver ADH and ALDH were determined after acute and short-term administration of benserazide and L-DOPA to rats.

Methods

Adult male Sprague-Dawley rats, 70–85 day old, were maintained on Purina pellet food and water *ad libitum*. They were divided into eight groups, four groups each of the acute and semi-chronic experiments. Drugs were all administered intraperitoneally (i.p.) in 0.9% w/v NaCl solution (saline). In the acute experiments, one group was given benserazide (N^1-DL-seryl-N^2-(2,3,4 trihydroxybenzyl) hydrazine, Ro 4-4602), 500 mg/kg body weight; another received L-DOPA methylester 500 mg/kg; the remaining two groups were injected with a combination of benserazide and either L-DOPA methylester (500 mg/kg each) or saline. In the semi-chronic experiments, the same drugs in identical dosages were administered once daily for seven consecutive days. All animals were killed by

decapitation 16 h after drug administration; their livers were removed, rinsed with 0.1 M phosphate buffer, pH 7.0, weighed individually, homogenized in sufficient ice-cold 0.1 M KCl solution to make a 15% (w/v) homogenate and centrifuged for 90 min at 22,000 *g*. The resulting supernatants were assayed for cytoplasmic ADH (EC, NAD, 1.1.1.1) and ALDH (EC, NAD, 1.2.1.3.) by the methods of Blair & Vallee (1966) and Blair & Bodley (1969), respectively. A portion of each supernatant fluid was utilized for protein determination by the biuret procedure. Findings are expressed in terms of specific activity (nmol min^{-1} mg^{-1} protein) measured at 25°C. The data were evaluated by Student's *t*-test.

Results and Discussion

Figure 1 shows the effects of acute (upper panel) and semi-chronic administration (lower panel) of benserazide and L-DOPA, separately and in combination on specific activities of liver ADH and ALDH, expressed as mean ± s.d. Acute administration of the drugs produced little change in specific activity of either enzyme compared with saline-treated controls. However, benserazide, 500 mg/kg daily for seven consecutive days, brought about a moderate (19.6%) decrease of liver ADH compared with control value although this did not reach significance ($P < 0.1$). At the same time, there was a significant (25.5%) inhibition of liver-ALDH activity compared with saline-treated rats ($P < 0.01$). Semi-chronic administration of L-DOPA alone did not alter liver ADH and liver-ALDH activities. Co-administration of benserazide with L-DOPA for seven days significantly decreased ALDH activity to a mean of 4.13 ± 0.51 nmol min^{-1} mg^{-1} protein compared with 6.04 ± 0.38 nmol min^{-1} mg^{-1} protein for saline controls ($P < 0.05$).

The present results show that semi-chronic administration of benserazide, alone or in combination with L-DOPA, results in some inhibition of liver ADH and significant reduction in liver-ALDH activities. It should be noted that intermediate aldehydes derived from the oxidative deamination of monoamines are reduced to their corresponding alcohol metabolites by the action of ADH or oxidized to acid derivatives by ALDH. These pathways represent the major routes of metabolism for aldehyde intermediates of catecholamines in both extracerebral tissues and brain (Taylor & Laverty, 1969) respectively. It is thus conceivable that inhibition of liver ALDH in a manner noted here by an inhibitor of peripheral ADC accompanying L-DOPA therapy might give rise to condensation products composed of DOPA-derived aldehydes and their amine precursors. These compounds may act as false transmitters (Cohen, 1973) and in this way contribute to the appearance of L-DOPA-induced adverse reactions.

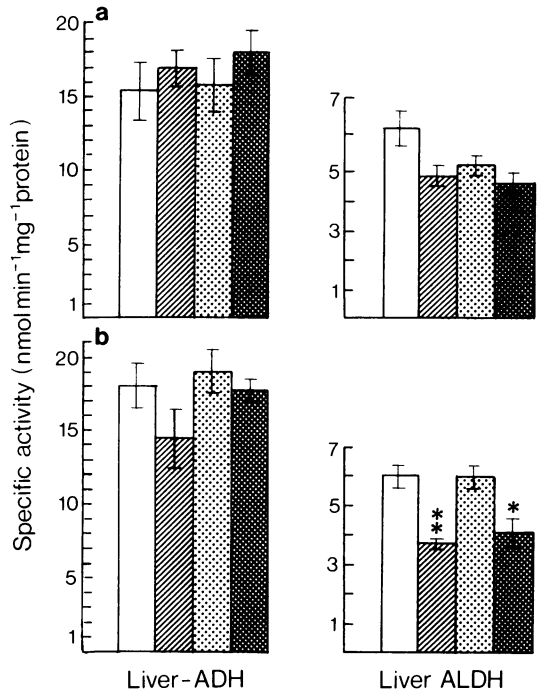

Figure 1 Effect of (a) acute and (b) semi-chronic administration of L-DOPA methylester (500 mg/kg, i.p.), benserazide, an inhibitor of aromatic L-amino acid decarboxylase (ADCI), (500 mg/kg, i.p.) separately and together, on the specific activities (nmol min^{-1} mg^{-1} protein) of rat liver alcohol dehydrogenase (ADH) and liver aldehyde dehydrogenase (ALDH). Each value represents the mean derived from eight rats in each treatment group. Vertical lines show s.d. Open columns = saline-treated controls; hatched columns = ADCI-treated; strippled columns = L-DOPA-treated; cross-hatched columns = ADCI plus L-DOPA-treated. $*P < 0.05$; $**P < 0.01$.

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