The effect of ascorbic acid on the sulphate conjugation of ingested noradrenaline and dopamine

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Changes in the plasma concentration of free and conjugated noradrenaline (NA) and dopamine (DA) following oral administration of these catecholamines were examined in four normal males before and after treatment with ascorbic acid.

The marked increase in plasma conjugated DA and NA was considerably reduced after ascorbic acid treatment, presumably due to competitive inhibition of sulphate conjugation. In one subject where there was a considerable delay in the appearance of sulphates, an early rise in free NA and DA concentration occurred. There were no changes in plasma free NA in the other subjects, although DA increased in two after ascorbic acid treatment.

These findings emphasise the importance of sulphate conjugation in the gut or liver in inactivating endogenous amines in foods and indicate that interference of this protective mechanism may arise from high doses of ascorbic acid and other related compounds which compete for sulphation.

Keywords ascorbic acid sulphate conjugation noradrenaline dopamine

Introduction

After eating a banana, which contains approximately 1 mg of dopamine (DA) and 0.25 mg of noradrenaline (NA) (Waalkes *et al.*, 1958), plasma sulphate conjugated NA and DA increase markedly, with only a small and variable increase in free DA and no change in free NA (Davidson *et al.*, 1981). Thus sulphate conjugation plays an important role in inactivating ingested catecholamines.

Other substances, including oestrogens, paracetamol and ascorbic acid, are also metabolised by sulphate conjugation. Intraduodenal administration of isoprenaline in dogs caused a greater increase in heart rate and pulse pressure when given large amounts of ascorbic acid (Houston *et al.*, 1976) and concomitant ingestion of oestrogen and ascorbic acid in humans increases free plasma oestrogen levels (Baçk *et al.*, 1981). These effects are presumably due to saturation of sulphotransferases by ascorbate. This is more easily achieved because of limited availability of sulphate (Houston & Levy, 1975; Levy & Yamada, 1971).

This study was conducted to determine if large doses of ascorbic acid altered the metabolism or haemodynamic actions of orally administered DA and NA.

Methods

Four healthy non-smoking medical staff, aged 26 to 31 years, participated in this investigation. They were randomly allocated to take either 2 g of ascorbic acid or matched placebo (containing 2 g of lactose) capsules, daily for 7 days. The study was conducted double-blind with subjects and attendants being unaware of whether ascorbic acid or placebo had been taken. Before reporting to the laboratory at the end of this period they avoided amine containing foods and beverages for 24 h and fasted for 12 h. A 2 h urine collection was obtained for ascorbic acid assay before the laboratory visit. Basal blood samples for catecholamine estimation were taken 20 and 30

min after insertion of a forearm venous cannula using a sampling technique and single isotope radio-enzymatic assay for free and sulphate conjugated catecholamines as described previously (Joyce *et al.*, 1982). An additional 3 g of ascorbic acid (or placebo) and a solution containing 1 mg of dopamine hydrochloride (equivalent to 0.77 mg DA, Intropin, Protea Pharmaceuticals) and 0.25 mg noradrenaline acid tartrate (equivalent to 0.13 mg NA, Levophed, Winthrop Laboratories) in water were then taken orally. The solution was prepared immediately before administration. Further blood samples were taken at 5 min intervals for 30 min, 10 min intervals for 60 min and 30 min intervals for 90 min. Blood pressure and pulse rate were measured at regular intervals throughout the procedure with a 'Dinamap' (Critikon, USA) automatic blood pressure recorder. Subjects remained supine throughout the study period.

After an interval of at least 1 week subjects

received the alternative treatment for 7 days and the above protocol was repeated.

Urinary ascorbic acid was determined by a colorimetric method (Harris, 1935). Results are expressed as means $\pm$ s.e. mean.

Results

In three subjects there were no consistent changes in the levels of plasma free NA following catecholamine administration after either placebo or ascorbic acid treatment. There was also no change in plasma free DA after placebo treatment although two subjects showed a small rise in free DA after ascorbic acid.

As shown in Figures 1 and 2 there was a prompt and substantial rise in both sulphate conjugated DA and NA levels in placebo treated subjects. In three subjects (B, C, D) the rise was

Figure 1 The effect of oral administration of dopamine (0.77 mg at 0 min) on plasma sulphated dopamine concentration in four placebo (●—●) and ascorbic acid (▲---▲)-treated subjects.

Figure 2 The effect of oral administration of noradrenaline (0.13 mg at 0 min) on plasma sulphated noradrenaline concentration in four placebo (●—●) and ascorbic acid (▲---▲)-treated subjects.

evident 10 min after administration of catecholamines with levels subsequently declining at 30 to 60 min for DA (Figure 1), and 50 to 90 min for NA. After ascorbic acid treatment these rises were delayed in subjects C and D and the peak levels reached in all three were much smaller. In subject A there was a considerable delay in the rise in plasma conjugated NA and DA which was

not prolonged further by ascorbic acid treatment although levels were appreciably lower. This subject also differed by showing an early and marked rise in both free DA and NA concentration in plasma, which was similar after placebo and ascorbic acid treatment and persisted for 90 min.

There were no changes in pulse rate or blood

pressure in any of the subjects. Urine ascorbic acid levels were $46.1 \pm 23.0 \mu\text{mol}/2 \text{ h}$ after ascorbic acid and $5.3 \pm 2.6 \mu\text{mol}/2 \text{ h}$ after placebo treatment.

Discussion

Sulphate conjugation is an important mechanism for the metabolism of catecholamines (Davidson *et al.*, 1981; George *et al.*, 1974; Joyce *et al.*, 1982) and between two and five times the amount of free NA and A and approximately 250 times the amount of free DA circulate as sulphate conjugates. The enzyme responsible, phenolsulphotransferase, is present in high concentrations in the gut (Conolly *et al.*, 1972), liver (Haggendal, 1963), kidney (Rennick & Quebbemann, 1970) and platelets (Hart *et al.*, 1979). Phenolsulphotransferase activity in the gut, or liver, may contribute to the metabolic inactivation of catecholamines protecting against the possible haemodynamic effects of exogenous catecholamines contained in foods (Davidson *et al.*, 1981).

Ascorbic acid treatment markedly diminished the increase of sulphate conjugated DA and NA in plasma after oral administration. This is consistent with ascorbic acid being a competitive inhibitor of sulphate conjugation (Houston *et al.*, 1976; Back *et al.*, 1981). The variable rise in free DA and lack of an associated rise in free NA in three of the four subjects studied may be explained by metabolism of these catecholamines by other pathways.

In one subject the increases in plasma free NA and DA, observed after both placebo and ascor-

bic acid treatment, were accompanied by a marked delay in the rise of sulphate conjugates. This suggests that as a result of incomplete conjugation more free catecholamines were available for absorption. Considerable variation in the ability to sulphate orally ingested compounds has also been reported for paracetamol and salicylamide (Bonham Carter *et al.*, 1983; Caldwell *et al.*, 1980). Gastric emptying time may also be prolonged by amines (Thompson & de Carle, 1982) and ascorbic acid (Hunt & Knox, 1972). Furthermore the presence of endogenous inhibitors, which may alter the activity of intestinal and liver sulphotransferases (Anderson & Weinshilboum, 1979), could be responsible for some of the variations observed.

Although no significant haemodynamic alterations following oral catecholamines were observed in this study, defects of sulphation have been described in essential hypertension (Kuchel *et al.*, 1981), phaeochromocytoma (Kuchel *et al.*, 1980) and dietary induced migraine (Smith *et al.*, 1971) and in these situations the consequences could be quite different, particularly if ascorbic acid or other competing substrates are also taken. Since little information is available on the inhibitory dose range of ascorbic acid, it is conceivable that saturation of sulphate conjugation causes the hypertensive crises in phaeochromocytoma or intensification of headache in migraine precipitated by orange juice (Kuchel *et al.*, 1982), containing amines and ascorbic acid, or other amine-containing foods taken in conjunction with ascorbic acid and possibly paracetamol.

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