PARACETAMOL (ACETOMINOPHEN) SULPHOCONJUGATION IN MAN: NO CORRELATION WITH TYRAMINE SULPHOCONJUGATION

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SUMMARY

There is considerable evidence that subjects vulnerable to endogenous depression excrete less tyramine sulphate after an oral dose of free tyramine than controls (the tyramine test). In this study, 26 psychiatric inpatients, exhibiting a wide range of responses to the test, and 10 normal controls were challenged with oral doses of paracetamol and tyramine on two separate occasions. Urinary output of paracetamol sulphate and paracetamol glucuronide in all subjects was monitored but there were no significant correlations with tyramine sulphate output. Thus, the output of these metabolites appears to be under complex control, and paracetamol cannot be substituted for tyramine in the "tyramine test". The basic deficit responsible for low values in the tyramine test is unlikely to stem from sulphate depletion or a generalised disturbance of the sulphation system, and remains obscure.

KEY WORDS

tyramine, paracetamol, sulphoconjugation, glucuronidation, depression

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INTRODUCTION

A low value in the tyramine test appears to be a trait marker for endogenous unipolar depression /1-4/. In this test, the subject swallows a capsule of tyramine hydrochloride and the urinary output of tyramine sulphate over the next 3 h is determined. Several independent studies have shown that patients who are either currently depressed or with a history of endogenous depression excrete significantly less tyramine sulphate in a 3 h period than do matched controls /1-4/. First degree family members who have never themselves been depressed have also been found to excrete low levels /3/, raising the possibility that low sulphoconjugation of this kind is a genetic marker for the condition. Reduced tyramine sulphoconjugation has also recently been identified in patients with migraine, tension headache /5/ and idiopathic orofacial pain /6/. Low tyramine sulphoconjugation also seems to be associated with a positive response to tricyclic medication /7/. The biochemical link between low tyramine sulphoconjugation and a predisposition to depression remains obscure /8/. The major route of tyramine metabolism is by oxidative deamination and is catalysed by monoamine oxidase; its sulphoconjugation is catalysed by phenolsulphotranferase M. Platelet levels of both these enzymes have been shown to be similar in low sulphoconjugators and controls /9/. No obvious links between either tyramine sulphation and other biochemical findings in depression have yet been found /8/.

In the present study, we examined paracetamol sulphoconjugation and glucuronidation, in parallel with tyramine sulphoconjugation, in a group of psychiatric patients and controls. Our aim was to investigate subjects with a wide range of values in the tyramine test. Half of the patient group fell into a category previously shown to be abnormally low on the tyramine test, i.e. severe endogenous unipolar depression /1/.

It is already known that paracetamol and tyramine sulphoconjugation are catalysed predominantly by the same isoenzyme, phenolsulphotransferase M /10/. The pharmacokinetics of paracetamol conjugation have been well studied /11-13/. The major urinary metabolite is the glucuronide conjugate (approximately 35-75% of the original dose in healthy controls), although sulphoconjugation is also an important route (approximately 20-30%). Both can be measured simply by high performance liquid chromatography (HPLC).

The aims of the present study were twofold: to investigate whether and to what extent low sulphoconjugation is manifested with other

substrates in suitable subjects, in order to try to understand more about the basic lesion; and to assess whether paracetamol in particular would be suitable as an alternative test compound. Some people are reluctant to undergo the tyramine test, as it involves taking an unfamiliar compound, a tyramine capsule, whereas they would be prepared to take a single tablet of the more familiar paracetamol. In addition those with a history of high blood pressure, cardiac arrythmia or heart disease, or of recent monoamine oxidase inhibitor ingestion, are excluded because of the possible pressor effects of tyramine. It would therefore make the test easier and more widely applicable if tyramine could be replaced by paracetamol.

MATERIALS AND METHODS

Subjects

A group of twenty-six psychiatric in-patients was recruited from the Brook General Hospital, London, suffering from a variety of disorders (unipolar depression, n=13; bipolar depression, n=9; obsessive-compulsive disorder, n=3; phobic disorder, n=1) and taking a variety of medications which, for ethical reasons, continued throughout the study. However, each individual's psychiatric morbidity and medication did not vary throughout the study. Controls (n=10) were recruited from laboratory personnel, with no known personal or family history of mental illness. They were all drug free for at least one week prior to the time of testing.

Ethical permission was obtained for the study, and each subject gave their informed consent.

Biochemical tests

A low tyramine diet was instituted for 24 h before testing, and was followed by an overnight fast. To start the test, each subject emptied their bladder, and then ingested either paracetamol (1 g) or tyramine hydrochloride (125 mg). All urine passed in the next 3 h was collected precisely; after paracetamol, the collection was continued for a further 6 h. A normal diet was resumed after the first 3 h. Three to 7 days later the test was repeated by all subjects, substituting the second compound for the one that they had ingested earlier. The total volume of urine in each collection was recorded and aliquots stored at -20°C until

assayed. The time interval was variable (3-7 days) because of hospital routine. It was chosen to be long enough to allow for recovery of the sulphating system, but short enough to preclude much change in the state of the patient.

Paracetamol conjugates

Separation of free paracetamol and its significant conjugates (sulphate, glucuronide, cysteine, and mercapturic) was achieved by HPLC with UV detection (254 nm), using a modification of the method of Andriaenssens and Prescott /14/. The C18 μBondapak column (15 cm) was fitted with a guard column and injection loop (20 μl) and run isocratically (2 ml/min) with the buffer 0.1 M potassium dihydrogen phosphate: formic acid: isopropanol (100:0.1:1.7). Linear calibration curves were established daily for paracetamol sulphate and glucuronide over the concentration range 8.3-66.7 μg/ml, using pure conjugates mixed with blank urine, and internal standard N propionic-4-aminophenol (final dilution 50 μg/ml). Repeat injections established that intraday variation was 2.6% for paracetamol glucuronide and 1.1% for the sulphate. Interday variation was found to be about 2.5% for both conjugates. Test urine samples were diluted as appropriate, mixed with internal standard and injected directly on to the HPLC system.

Tyramine sulphate

Tyramine excreted as the sulphate conjugate was measured by a modification of the method of Walker and Sandler /15/. Free and sulphated tyramine were separated by passing the urine, adjusted to pH 6.25, through an Amberlite CG-50 ion exchange column. After addition of the internal standard, *p*-hydroxyphenylpropylamine (20 nmol), to an aliquot of the eluant (1 ml), the conjugate was hydrolysed (pH 0.9, 100°C, 30 min). A borate buffer (0.5 M boric acid/potassium chloride/sodium hydroxide, pH 10.2; 2 ml) was added, the pH adjusted to 10.2 and the liberated tyramine extracted into ethyl acetate (2x10 ml). The combined organic phases were evaporated and the residue dissolved in ethanol (400 μl). A portion (100 μl) was dried under nitrogen and derivatised using acetonitrile (20 μl) and heptofluorobutyric anhydride (100 μl) (65°C, 40 min). Derivatising agents were evaporated under nitrogen and the residue dissolved in heptane (100 μl), to be injected into a Carlo Erba HRGC mega series gas chromatograph fitted with a capillary column (50 m, SE-30 Ultraphase, 0.25 μm

thickness) and ^{63}N electron capture detector. 20 µl of reconstituted sample was injection loaded via a 20 µl injection loop. The assembly was run over a temperature gradient: temperature 1 (T1) = 51°C; 2 min; gradient T1/T2 = 20°C/min; T2 = 155°C, 0 min; gradient T2/T3 = 2°C/min; T3 = 185°C, 0 min; gradient T3/T4 = infinity; T4 = 250°C, 2 min (gradient T3/T4 = infinity is the oven setting for maximal rate of increase in temperature). Peaks were measured by a Hewlett Packard 3390A integrator.

No interfering peaks were observed in any of the initial blank urine samples on either HPLC or gas chromatography.

RESULTS

Considerable variation in output of each conjugate was observed among the sample group (Table 1). Patients excreted significantly less of both tyramine sulphate and paracetamol sulphate than controls in the first 0-3 h period (both $p<0.001$). However, because of the drugs the patients were taking these differences obviously have to be treated with caution.

When simple correlations were examined between tyramine sulphate output and that of other metabolites (paracetamol sulphate 0-3 h;

TABLE 1

Output of tyramine and paracetamol conjugates (mg) in the whole group, the patient group and the controls (mean ± SD)

	Total n = 36	patients n =2 6	control n = 10
tyramine-SO_4 (0-3hr)	5.1 ± 2.2	4.5 ± 2.0	6.8 ± 2.0
paracetamol -SO_4 (0-3 hr)	130 ± 72	105 ± 53	195 ± 74
paracetamol -SO_4 (0-9hr)	296 ± 99	351 ± 165	344 ± 73
paracetamol-glucuronide (0-3hr)	232 ± 128	231 ± 145	233 ± 68
paracetamol-glucuronide (0-9hr)	721 ± 220	720 ± 231	725 ± 203

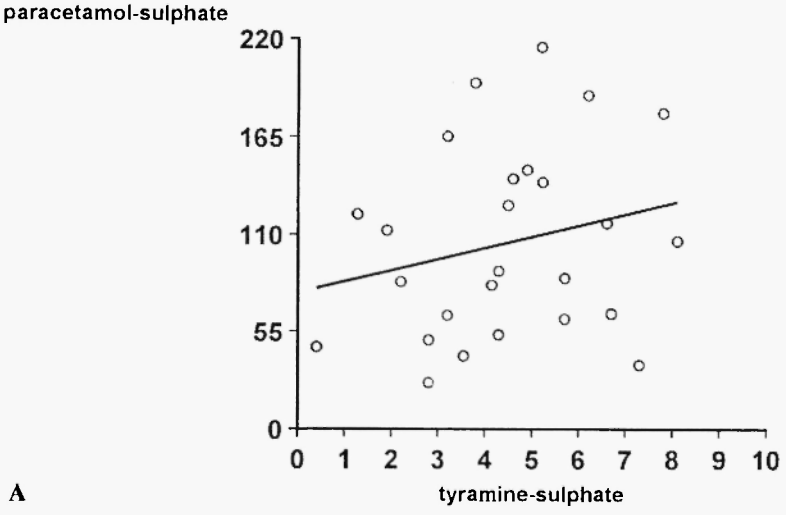
0-9 h; paracetamol glucuronide 0-3 h; 0-9 h) no significant correlations were observed within either the controls or the patient group (Table 2). Figure 1 shows the individual values for tyramine and paracetamol sulphate output in the first 3 hours in the different groups.

As two types of subject were knowingly used (psychiatric patients and laboratory personnel), two separate statistical models were employed, and tests performed to see whether the data could be combined and treated as a single group. Analysis of variance and test for constant

TABLE 2

Pearson correlations between tyramine-sulphate output (0-3 hours) and that of the other metabolites in the patient group and controls

	patient (n =26)	controls (n =10)
paracetamol -SO_4 (0-3 hr)	0.23	-0.46
paracetamol -SO_4 (0-9hr)	0.33	-0.14
paracetamol-glucuronide (0-3hr)	0.11	0.26
paracetamol-glucuronide (0-9hr)	0.21	-0.22



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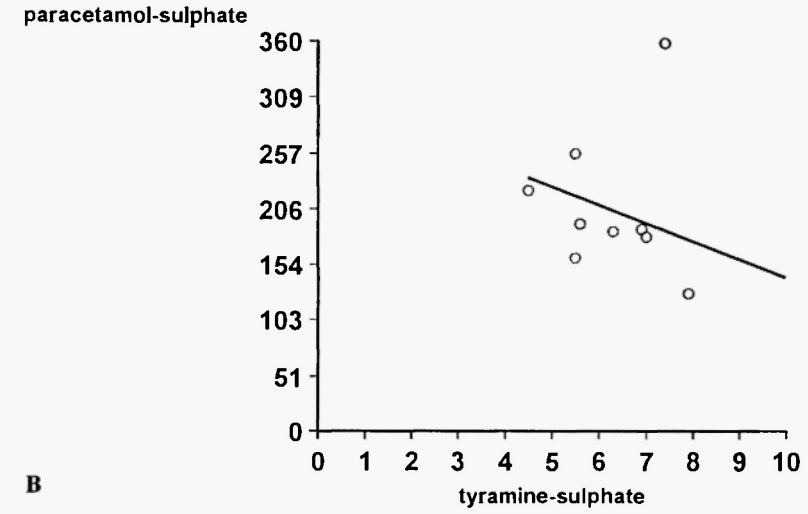

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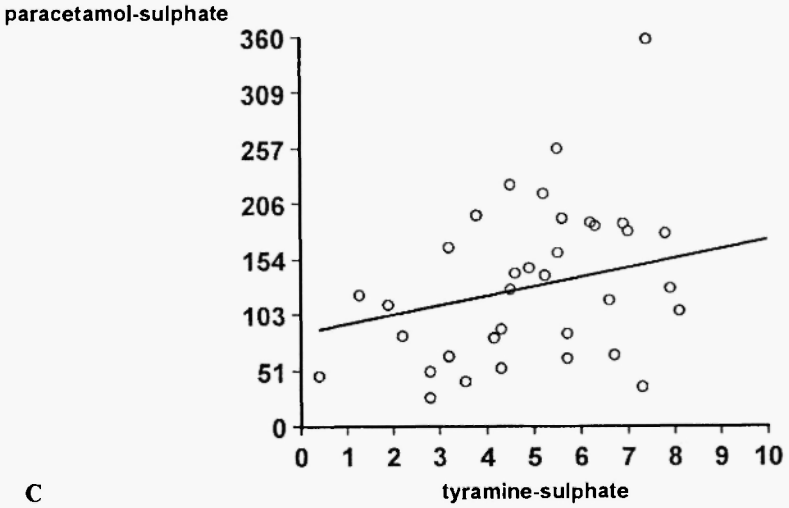

C

Fig. 1: Tyramine sulphate (0-3 h) and paracetamol sulphate output (0-3 h) in: **A:** the patient group (n=26); **B:** the control group (n=10); **C:** the whole group (n= 26).

slopes showed that for each correlation this was possible and this allowed for the legitimate combination of subjects into a single, larger group (n=36). When this was done, and the correlations repeated, no significant correlations were observed between tyramine sulphate output (0-3 h) and any of the six measures of paracetamol metabolism.

DISCUSSION

This study failed to find a significant relationship between the urinary output of tyramine sulphate and paracetamol sulphate after an oral load of the parent compound. The lack of correlation suggests that there is more than one factor controlling the sulphation of these two compounds.

The patients were taking a wide range of medications. It is possible that this interfered with tyramine or paracetamol metabolism. However if paracetamol and tyramine are sulphoconjugated by the same mechanism, and the drugs were the same on the two occasions, there should still have been a high correlation between the rank order of individuals in their sulphoconjugation of the two compounds, and this was not observed.

Some significant differences in metabolite excretion rates between the patient and control groups were observed, but these must be treated with caution due to the possible confounding effects of the patients' medication. If tyramine and paracetamol sulphation were under similar or related control, any drug effects would be negligible once the data had been combined into a single group, as the sulphation of both compounds would be equally affected. However no significant correlations were apparent when the group was considered as a whole.

All subjects were in receipt or a well-balanced diet, capable of supplying sufficient amounts of sulphate, and care was taken to allow sufficient time for the sulphate pool to be replenished before the second compound was administered. Thus, low excretion of tyramine sulphate is unlikely to reflect a generalised depletion of sulphate, supporting the earlier work of Bonham Carter *et al.* /16/. As the correlation between tyramine sulphate sulphoconjugation and that of paracetamol over the longer period (0-9 h) also failed to reach statistical significance, the difference cannot be accounted for by a slower paracetamol absorption rate.

As sulphation and glucuronidation form the major metabolic pathways for paracetamol /11/, it might be expected that a reduction in

sulphation might be compensated for by an increase in glucuronide formation. *This was not observed.* This again points to some complexity in the controlling mechanisms of one or both systems, with different factors assuming prominence in different individuals. There are known to be several isoenzymes for phenolsulphotransferase (PST). Both tyramine and paracetamol are substrates for PST M rather than PST P, although whereas tyramine is highly specific, paracetamol is to some extent a mixed substrate /10/. At saturating doses the relative rate of sulphation of the two compounds is similar /10/. It is possible that other sulphotransferases, such as that for CCK /17/, also play some role.

CONCLUSION

This study shows clearly that the defect which underlies low sulphoconjugation of tyramine in severe depression does not control paracetamol sulphoconjugation in a similar manner. Paracetamol thus cannot be substituted for tyramine in the tyramine test and the basic lesion accounting for low values in the latter in endogenous depression remains obscure.

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