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PRELIMINARY COMMUNICATIONS

A MONOAMINE OXIDASE INHIBITOR IN HUMAN URINE

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Enzyme inhibitors may be useful as drugs [1]. Although some endogenous enzyme inhibitors are well recognised, e.g. of dopamine β-hydroxylase [2], their possible *in vivo* roles as physiological regulators of enzyme activity have been little studied. Following an earlier and as yet unconfirmed report [3] that plasma from schizophrenics with low platelet monoamine oxidase (MAO) (EC 1.4.3.4.) activity contains an MAO inhibitor not demonstrable in normal plasma, we have been able to demonstrate that normal human urine contains an inhibitor or inhibitors of both MAO A and B, and that this inhibition cannot be accounted for by the activity of the quantitatively main urinary constituents or by a large group of known monoamine substrates or metabolites.

The inhibitor was assayed by its effect on rat liver MAO. Except where specified otherwise, the test system contained 100 µl of 0.1 M sodium phosphate buffer, pH 7.4, 20 µl rat liver homogenate (2.5% w/v), 20 µl ^{14}C-tyramine (150 µM, sp.act. 2.5 µCi/µmole) and 100 µl of urine, or potential inhibitory substances diluted in buffer or of buffer alone. The mixture was incubated at 37°C for 30 min, the reaction stopped with 0.1 ml of 2 M citric acid and the reaction products extracted into 3 ml toluene/ethyl acetate (1:1). A similar system was used with ^{14}C-5-hydroxytryptamine (5-HT) (sp.act. 2.5 µCi/µmole) but when ^{14}C-phenylethylamine (PEA) (sp.act. 12.5 µCi/µmole) was used as substrate, 3 ml toluene alone was employed. Radioactive substances were obtained from the Radiochemical Centre, Amersham. Assays were carried out in duplicate, using reagents of analytical grade, from British Drug Houses (Poole, U.K.) or Sigma London Chemical Co.Ltd. (Kingston-upon-Thames. U.K.).

All the human urine samples (random specimens from 14 normal subjects) tested showed varying degrees of inhibition ranging from 25-75%, with a mean of 49% ± 7.1 (mean ± S.D.). The degree of inhibition was similar when the pH of the urine was adjusted to pH 7.4, and whether a flocculent or clear portion of urine was tested. Human platelet (1 mg protein/ml) MAO was inhibited to a similar extent by the various samples, so that the effect does not depend on the generation of an inhibitor by rat liver.

Fig.1 shows the effect of diluting one urine sample serially in buffer using concentrations suitable for selective oxidation of 5-HT, a substrate for MAO A, and PEA, a substrate for MAO B. The sample inhibited both forms of MAO and, in each case, the inhibitory effect was still detectable at a dilution of 1 in 32. The effect on PEA oxidation was somewhat more potent than that on 5-HT oxidation at higher urine dilutions. The inhibitor or inhibitors apparently compete with 5-HT, but the interaction with PEA seems to be more complex. However, because the inhibitor can bind to MAO, as described below, a kinetic analysis which assumes reversibility may not be applicable.

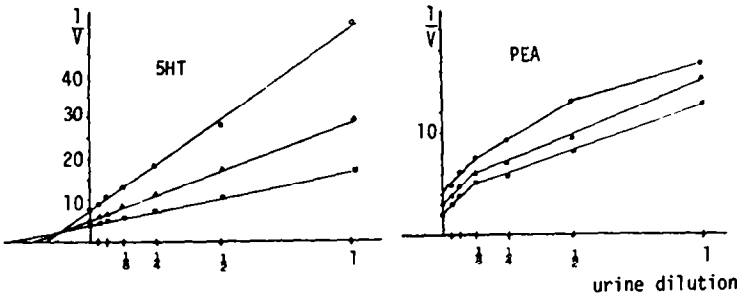

Fig.1. Inhibition of rat liver MAO by urine.
Rat liver homogenates, 2.5% and 0.5%, were used with 5-HT and PEA respectively (see text). The results of a single experiment are shown in the figure, but a second experiment gave similar results.

○ △ □ = 50, 100, 200 µM 5-HT

● ▲ ■ = 2.5, 5, 10 µM PEA

Table I shows the quantitatively major constituents of urine, and the effect of each at pH 7.4 and characteristic concentration [4] on rat liver MAO activity. Most showed no inhibition and some had slight inhibitory effect only. One urine sample was analysed for certain constituents and the effect of an artificial combination of them in buffer at similar concentrations was determined independently. Table I shows that whereas the urine sample produced an inhibition of 57%, the combined constituents resulted in inhibition of only 6%. The authentic urine sample diluted 1 in 4 inhibited by 23%, whereas a similar dilution of artificial mixture of major constituents produced no inhibition. It may be concluded that most of the observed inhibition was not caused by any of the quantitatively major urinary constituents. The addition of EDTA (2% w/v) to the urine sample did not affect its inhibitory potency, which could not, therefore, be due to the action of divalent cations such as copper.

The effects of certain monoamine metabolites were also studied; metadrenaline, 4-hydroxy-3-methoxyphenylglycol, 3,4-dihydroxyphenylacetic acid, DL-normetadrenaline, 4-hydroxy-3-methoxymandelic acid, *m*-hydroxyphenylacetic acid, *p*-hydroxymandelic acid, homovanillyl alcohol, phenylacetic acid, homovanillic acid and 5-hydroxyindoleacetic acid were all tested at a concentration of 4 mg/l, *p*-hydroxyphenylacetic acid at 20 mg/l and phenylacetylglutamine at 100 mg/l. None caused any inhibition.

One possibility to be considered is that the monoamine substrates present in urine cause the effect by direct combination or isotope dilution. Daily output values of *p*-tyramine, dopamine, noradrenaline, adrenaline and 5-HT are of the order of 420 [ref.5], 400 [ref.6], 50 [ref.6], 25 [ref.6], and 100 µg [ref.5] respectively. Such concentrations would in each case represent less than 1% of the Km concentration in the assay mixture. *tele*-Methylhistamine, a substrate for MAO B [7] is excreted in amounts of up to 480 µg per day [8], and this is also only about 1% of its Km (about 130 µM, unpublished observation). It is apparent, therefore, that these particular amines are not present in sufficient concentration to account for the observed inhibition of MAO by urine.

Some of the monoamines and their metabolites are present as conjugates. If these conjugates themselves were responsible for the inhibition, it should decrease after their hydrolysis. To test this possibility, each of a further series of urine samples treated at 37°C overnight with a sulphatase-glucuronidase preparation (*suc d'Helix pomatia*, Industrie Biologique Francaise, Clichy, France) (0.1 ml of extract added to 10 ml urine adjusted to pH 6) was assayed.

TABLE I. INHIBITION OF MONOAMINE OXIDASE BY A VARIETY OF URINARY CONSTITUENTS

Constituent	Conc. (mg/100ml)	% Inhibition	Constituent	Conc. (mg/100ml)	% Inhibition
Urea	2000	4	Glycine	44	0
Uric acid	50	0	L-Histidine	40	0
Creatinine	100	0	Hippuric acid	200	0
Ammonia N	50	1	Citric acid	400	0
Inorganic S	40	0	Formic acid	40	0
Creatine	20	0	Simulated urine sample		
Na	112	0		Undiluted	6
Cl	225	0		½	3
Ca	13	0		¼	0
K	170	3	Authentic urine sample		
Mg	130	0		Undiluted	57
P	300	0		½	38
				¼	23
				⅛	9

The actual concentration of urea, uric acid, creatinine, ammonia N, Na, Cl, Ca, K in the authentic urine sample was the same or less than that tested in the simulated urine sample. The concentrations in the latter were: urea, 1250; uric acid, 25; creatinine, 75; ammonia N, 32.5; inorganic S, 44 (mg/100 ml).

The inhibitory potency of control urine was not changed by 12 h at 37°C. A control sample of *Helix* extract in water was also incubated. The inhibition caused by hydrolysed samples, compared with *Helix* extract control, was 71 ± 3.2% (mean ± S.E.) compared with the inhibition of 57 ± 2.9% brought about by these samples before treatment. Hydrolysis therefore increased rather than decreased the inhibition. This result suggests that the conjugates themselves do not inhibit MAO, and raises the possibility that the inhibitor is conjugated and that treatment causes its release. However the *Helix* preparation is very impure and further experiments would be needed to establish the nature of its effect on the inhibitor.

An estimate of the molecular weight of the inhibitor was obtained by passing a urine sample through a Sephadex G 10 column. In two experiments, 0.5 ml was passed through a 15 cm column (12 ml) in 70 mM phosphate buffer, pH 7.4, and 32 x 0.5 ml fractions collected. In each run, full recovery of the inhibitor or inhibitors was obtained, in a broad band peaking just after dextrose (mol.wt.180), so that the size of the unknown was presumably of a similar order.

Because of indirect evidence suggesting that some degree of MAO inhibition in chicks may derive from gut flora [9], we tested urine samples both from conventional and germ-free rats. All samples were adjusted to pH 7.4. The mean (± S.E.) inhibition by 24 h urine samples from 8 conventional rats was 79 ± 2.1, and from 12 germ-free rats, 80 ± 2%. Thus, an inhibitor or inhibitors are present in rat urine with output values being unaffected by the state of the gut flora.

Inhibitory activity appeared to be neither completely reversible nor irreversible. Dialysis (24 h) of a sample of 1 ml rat liver homogenate which had been incubated with 1 ml urine, showed similar activity to a 1 ml sample of the same rat liver homogenate plus 1 ml buffer. However, some binding of inhibitor to MAO may also occur. After 30 min preincubation of 0.5 ml of 2.5% rat liver homogenate with 2.5 ml urine at 20°C, centrifugation for 1 h at 26,000 x g and resuspension of the precipitate in 2.5 ml buffer, the MAO remained 80% inhibited compared with a control with buffer instead of urine. Repeating the procedure

washed out most of the inhibitory principle, leaving only a 20% inhibition.

If this MAO inhibitor derives from plasma or tissues, the manner in which the homogenate is prepared might affect the proportion bound, and the MAO activity available for assay. It is of particular interest, in this context, that Berrettini and Vogel [3] in their report of a circulating small molecule inhibitor in schizophrenics with low platelet MAO activity found that adding platelet poor plasma from three subjects to normal platelets and centrifuging down the platelet plug caused a reduction in the Vmax and Km of normal platelet MAO. The situation may be complex, however, for Yu *et al.* [10] have detected an activating principle for MAO in human plasma, also a small molecule. Hormones such as oestrogen, progesterone [11], and thyroxine [12] can alter MAO activity in particular organs with a slow time course. It is tempting to speculate that further control may be exerted by small molecule inhibitors of the type discussed in this paper. Further evidence is obviously required to substantiate this hypothesis.

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