J. Pharm. Pharmacol. 1989, 41: 97–100
Received May 26, 1988

The Enhanced Daily Excretion of Urinary Methylamine in Rats Treated with Semicarbazide or Hydralazine May Be Related to the Inhibition of Semicarbazide-sensitive Amine Oxidase Activities

G. A. LYLES AND S. A. MCDOUGALL

Department of Pharmacology and Clinical Pharmacology, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, Scotland, UK

Abstract—The effects of amine oxidase inhibitors upon the daily urinary excretion of monomethylamine (MMA), dimethylamine (DMA), trimethylamine (TMA) and ammonia in the rat have been examined. Administration of hydralazine (5 mg kg^{-1}) or semicarbazide (100 mg kg^{-1}), drugs which irreversibly inhibit semicarbazide-sensitive amine oxidases (SSAO) but not monoamine oxidase (MAO), enhanced MMA excretion by around three- to six-fold above pretreatment levels, whereas no effect of pargyline (25 mg kg^{-1}), a selective irreversible inhibitor of MAO was found. No apparent changes in DMA or TMA excretion in response to drug-treatment were observed. Ammonia excretion also was generally unchanged except for an apparent marked increase (approximately four-fold) over the 24 h following semicarbazide, a result which might be explained if ammonia is a degradation product of semicarbazide metabolism in the rat. With recent evidence that MMA is a substrate in-vitro for SSAO activities, results here may indicate that SSAO or related enzymes are involved in endogenous MMA turnover.

The aliphatic amines monomethylamine (MMA), dimethylamine (DMA) and trimethylamine (TMA) occur in man and other species. They exist in foods (Zeisel & DaCosta 1986), or are absorbed after gut bacterial degradation of dietary creatinine, choline and lecithin (Zeisel et al 1983; Lowis et al 1985). DMA may arise from methylation of MMA, or demethylation of TMA (Simenhoff 1975). MMA is produced endogenously by degradation of creatinine and sarcosine (Davis & De Ropp 1961) and from the deamination of adrenaline by mitochondrial monoamine oxidase (MAO) activities (Schayer et al 1952). Thus, several sources may account for the urinary and faecal aliphatic amine levels determined in some of these studies.

MMA is a substrate in-vitro for a semicarbazide-sensitive amine oxidase (SSAO) in human and rat vasculature (Precious et al 1988). It is not yet clear if this enzyme is a quinoprotein or contains pyridoxal phosphate as cofactor. However, the substrate and inhibitor specificity of SSAO distinguish it from the A and B forms of the flavoenzyme MAO (Lyles 1984). Although present in various tissues, SSAO is particularly active in vascular smooth muscle cells (Ryder et al 1979; Lyles & Singh 1985), possibly as a plasmalemmal constituent (Wibo et al 1980), but its physiological importance is unknown. As our earlier work failed to indicate any in-vitro deamination of MMA by MAO activities, this amine could be an endogenous substrate for SSAO. Consequently, here we have measured urinary levels of MMA in rats, before and after treating them with drugs capable of inhibiting either SSAO or MAO activities. The method used allows the simultaneous determination of MMA along with DMA, TMA and ammonia, so we have obtained data on these other amines also for comparison.

Correspondence to: G. A. Lyles, Department of Pharmacology and Clinical Pharmacology, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, Scotland, UK.

Materials and Methods

Male albino Sprague-Dawley rats (450–550 g) were supplied by the Departmental Breeding Colony, Animal Services Unit, University of Dundee. The hydrochlorides of monomethylamine (MMA), dimethylamine (DMA), trimethylamine (TMA), n-propylamine (PA), hydralazine, pargyline and semicarbazide were from Sigma Chemical Company Ltd., Poole, UK.

Four rats (different groups for each study) housed individually in metabolism cages were fed a standard rodent diet (No. 1: Special Diet Services Ltd., Witham, UK) and given free access to water. Individual urine samples were collected over two consecutive periods of 24 h before i.p. injection (5 mL kg^{-1}), of rats with either pargyline (25 mg kg^{-1}), hydralazine (5 mg kg^{-1}), semi-carbazide (100 mg kg^{-1}), or saline (0·9% NaCl w/v, the inhibitor injection vehicle). Urine was then collected for at least two further 24 h periods. Four metabolism cages only were available to us, so each treatment above was studied in separate longitudinal studies.

Urines were collected into 50 mL tubes containing 0·5 mL 2 M HCl, and centrifuged at 1000 *g* for 10 min, before supernatants were decanted and volumes recorded. Triplicate samples each of 250 μL of supernatant were placed in individual vials containing 250 μL 1 M NaOH and sealed with Teflon-lined caps containing a rubber septum (Chromacol Ltd., London, UK). 250 μL samples of standard aqueous solutions of the hydrochlorides of MMA, DMA and TMA (0·1–2·0 mM) and also of ammonia (10–200 mM) were subjected to the same protocol. Ammonia concentrations obtained from stock solutions (originally 0·88 sp.gr.) were estimated by titrimetric analysis (by neutralization with H_2SO_4). All vials containing either urines or standard amines were injected with 1 μL PA (200 mM) to serve as internal standard before chromatographic analysis, essentially by the method of Zeisel et al (1983), and using a Pye Series 104 GLC

instrument with an ionization amplifier and flame ionization detector. Samples or standards (1 μL) from sealed vials were then injected into a glass-lined injection port heated to 200°C, providing entry to a glass column (6 ft length × 2 mm inside diameter) containing 60/80 mesh Carbopack B/4% Carbowax 20M/0·8% KOH packing (Supelchem, Sawbridgeworth, UK). Oven temperature was maintained at 70°C. Amines were quantitated by calculating resultant peak areas using a Hewlett Packard 3390A electronic integrator.

Standard curves obtained from plotting standard amine sample peak areas/internal standard (PA) peak areas against appropriate standard amine concentrations were used to determine unknown concentrations of urinary amines, and after taking account of individual daily urine volumes, the data were expressed as amine excretion/24 h collection period.

Comparison of mean values for different days within a given treatment was initially carried out by one-way analysis of variance (ANOVA). Where significant differences ($P < 0{\cdot}05$) between means were indicated, comparisons of amine excretion on post-treatment days with that on the immediate 24 h period before treatment were carried out by paired t-test.

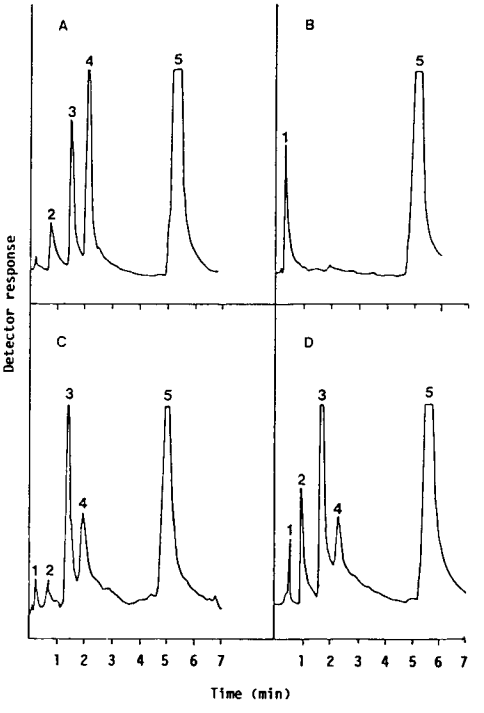

FIG. 1. Tracings of representative chromatograms obtained from GLC analysis of 1 μL aliquots from samples prepared as described in Methods. A. Mixture of standard MMA, DMA and TMA (originally 0·4 mM before sample preparation for GLC). B. Standard ammonia (originally 200 mM). C. Control urine from rat before drug treatment. D. Urine collected during the 24 h following semicarbazide treatment. Peaks identified from their characteristic relative retention times determined when amines were studied individually were 1, ammonia; 2, MMA; 3, DMA; 4, TMA; 5, PA (1 μL of a 200 mM solution added to each sample vial before GLC as an internal standard). Slightly truncated peaks on some of these chromatograms arose from choosing an attenuator setting to display the smaller MMA peaks more clearly on the chart recorder. However, the separate integrator unit was capable of calculating total peak areas accurately, independent of this attenuation.

Results and Discussion

Typical chromatograms obtained from standard amine solutions are shown in Fig. 1A, B whereas Fig. 1C shows a representative chromatogram of urine from an untreated rat. Peaks in the latter corresponding to ammonia and the aliphatic amines were identified on the basis of comparing their retention times on the column with those of the standards. Fig. 1D shows a chromatogram from urine collected during the 24 h following semicarbazide administration to the animal. This illustrates qualitatively the enhanced heights and areas of peaks 1 and 2, corresponding to ammonia and MMA, respectively, which were characteristic features following semicarbazide treatment of all animals studied.

Mean data for the daily excretion of amines in each experimental study group are shown in Table 1. ANOVA failed to indicate any significant daily variation in DMA excretion rates during any of the four longitudinal studies. Thus, there was no evidence that drug (or vehicle) administration at the end of day 2 produced any subsequent change in DMA output. MMA excretion was not significantly altered by either saline or pargyline, whereas significant increases of between about three- and six-fold were found for the two daily periods following semicarbazide or hydralazine, when compared (paired t-test) with the immediate 24 h period preceding drug administration. Two further collections (day 5 and 9) were made in the semicarbazide study, and these suggested that a gradual decline occurs in the elevated MMA excretion rate.

In contrast to the relatively stable normal excretion rates for MMA and DMA, we encountered considerable daily variation in TMA and ammonia excretion even within individual untreated animals from one day to the next. Large variations in TMA excretion were also found by Lowis et al (1985) in longitudinal studies on the influence of normal and precursor-supplemented diets upon aliphatic amine excretion in the rat. As a whole, our mean values for urinary excretion of each aliphatic amine fall within ranges reported by these authors using rats (Wistar) of a similar weight and fed the same maintenance diet as used here. In the current work, no significant variations in TMA excretion rates were indicated by ANOVA within any of the longitudinal studies, nor were there any obvious trends suggestive of consistent changes in TMA excretion occurring after drug administration. With ammonia, significant differences between mean values were found only in the semicarbazide study. Here, ammonia excretion was significantly higher (4·4-fold) during the 24 h period following, compared with that immediately before, semicarbazide administration.

The drugs used here were chosen on the basis of previous ex-vivo studies showing doses which may achieve selective irreversible inhibition of either MAO or SSAO activities (e.g. Lyles & Callingham 1974; Lyles et al 1983; Strolin Benedetti et al 1984). The lack of effect of the MAO-selective agent pargyline upon MMA excretion suggests that MAO is not involved in MMA degradation. In contrast, a marked increase in MMA excretion occurred after treatment with semicarbazide or hydralazine, which are capable of inhibiting various enzymes (including amine oxidases) containing

Table 1. Daily urinary excretion (μmol/24 h) of monomethylamine (MMA), dimethylamine (DMA), trimethylamine (TMA) and ammonia (NH_3). Urines were collected for two 24 h drug-free periods (day 1 and 2) before administration of saline, 25 mg kg^{-1} pargyline, 5 mg kg^{-1} hydralazine or 100 mg kg^{-1} semicarbazide. Each value is the mean (±s.e.) of four rats.

Day of Study	1	2	3	4	5	9
Treatment/Amine						
Saline						
MMA	1·3±0·2	1·6±0·1	1·0±0·2	1·2±0·2	—	—
DMA	5·8±0·5	7·9±1·1	6·1±0·4	5·5±0·6	—	—
TMA	1·5±0·5	1·8±0·1	1·0±0·2	2·2±0·6	—	—
NH_3	179±34	182±24	281±25	359±56	—	—
Pargyline						
MMA	1·0±0·1	1·5±0·4	1·0±0·2	1·3±0·4	—	—
DMA	5·5±0·6	6·8±0·7	3·9±0·5	5·9±1·4	—	—
TMA	2·7±0·9	2·3±0·2	0·7±0·2	1·1±0·2	—	—
NH_3	115±18	156±15	352±94	321±115		
Hydralazine						
MMA	1·5±0·3	1·6±0·2	5·6±0·5**	4·9±0·4*	—	—
DMA	8·6±1·9	7·5±0·7	8·0±1·1	10·1±1·4	—	—
TMA	0·9±0·4	0·5±0·1	1·7±0·3	2·4±0·8	—	—
NH_3	259±56	320±19	243±46	388±129	—	—
Semicarbazide						
MMA	1·0±0·2	0·8±0·1	3·7±0·5*	4·6±0·8*	3·7±1·0	1·9±0·1
DMA	4·6±1·0	4·1±0·3	4·5±0·2	5·4±0·6	7·1±1·4	5·8±0·2
TMA	2·0±0·4	0·6±0·1	0·5±0·2	0·8±0·4	1·7±0·7	1·0±0·2
NH_3	142±51	135±36	600±109*	159±31	102±31	189±15

*$P<0·05$; **$P<0·01$ (paired t-test) compared with day 2.

carbonyl functions in their cofactor(s). MMA is a substrate in-vitro for SSAO in vascular tissue (Precious et al 1988) and also for the related soluble enzyme, plasma amine oxidase (McEwen 1965). Although the current results do not prove unequivocally that decreased degradation is responsible for the enhanced MMA excretion, they are consistent with the possibility that SSAO or related enzyme(s) may be involved in endogenous MMA turnover. Others have previously found increases in urinary MMA excretion in rats treated with certain hydrazine-based MAO inhibitors (Davis & De Ropp 1961; Simenhoff 1975; Dar et al 1985), although the ability of these drugs also to inhibit SSAO (Lyles 1984) and similar amine oxidases, rather than their inhibition of MAO might be a more likely explanation for these findings.

Deamination is unlikely to be directly involved in the endogenous metabolism of DMA or TMA (Simenhoff 1975), and the lack of any obvious effects of the inhibitors used here upon urinary DMA and TMA levels is consistent with this. While Simenhoff proposed that DMA can be formed by endogenous methylation of MMA, he failed (as we did here) to find any increase in DMA in rats excreting enhanced MMA levels after treatment with hydrazides.

The administration of semicarbazide appeared to produce a marked increase in ammonia excretion during the 24 h period after treatment. Although we have no direct proof for the following explanation, we speculate that ammonia may be a major metabolite arising from endogenous breakdown in the rat of the large doses of semicarbazide (NH_2-CO-$NHNH_2$) administered.

Relatively little is known of the biological significance of MMA. Its plasma concentrations are elevated in uraemic patients, and it has been proposed that MMA may be a contributory neurological toxin in such patients (Simenhoff 1975; Baba et al 1984). Cytotoxic actions of MMA upon cultured neurons and fibroblasts have been demonstrated (Gilad & Gilad 1986; Cain & Murphy 1986), and MMA has been used to study lysosomal function and cellular endocytotic activity (e.g. Davies et al 1980; Cooper et al 1987). The interrelationships between endogenous levels of MMA, DMA and TMA have also undergone scrutiny with respect to potentially carcinogenic nitrosoamine formation from these precursors (Zeisel & DaCosta 1986; Zeisel et al 1988). Since the in-vitro inhibition of SSAO is a property which has been identified among a number of drugs including some such as hydralazine in clinical use (Lyles 1984), the possibility that these and other agents may influence the endogenous breakdown of MMA thus modifying any physiological or pathological actions of this amine would seem to warrant further consideration.

References

Baba, S., Watanabe, Y., Gejyo, F., Arakawa, M. (1984) High-performance liquid chromatographic determination of serum aliphatic amines in chronic renal failure. Clin. Chim. Acta 136: 49–56

Cain, C. C., Murphy, R. F. (1986) Growth inhibition of 3T3 fibroblasts by lysosomotropic amines: correlation with effects on intravesicular pH but not vacuolation. J. Cell. Physiol. 129: 65–70

Cooper J. L., Selinfreund, R., Wakshull, E., Wharton, W. (1987) Interaction between monensin and lysosomotropic amines in the regulation of processing of epidermal growth factor by BALB/c 3T3 cells. Mol. Cell. Biochem. 73: 1–9

Dar, M. S., Morselli, P. L., Bowman, E. R. (1985) The enzymatic systems involved in the mammalian metabolism of methylamine. Gen. Pharmacol. 16: 557–560

Davies, P. J. A., Davies, D. R., Levitzki, A., Maxfield, F. R., Milhaud, P., Willingham, M. C., Pastan, I. H. (1980) Transglutaminase is essential in receptor-mediated endocytosis of α_2-macroglobulin and polypeptide hormones. Nature 283: 162–167

Davis, E. J., De Ropp, R. S. (1961) Metabolic origin of urinary methylamine in the rat. Ibid. 190: 636–637

Gilad, G. M., Gilad, V. H. (1986) Cytotoxic effects of monodansylcadaverine and methylamine in primary cultures of rat cerebellar neurones. Int. J. Devl. Neurosci. 4: 401–405

Lowis, S., Eastwood, M. A., Brydon, W. G. (1985) The influence of

creatinine, lecithin and choline feeding on aliphatic amine production and excretion in the rat. Br. J. Nutr. 54: 43–51

Lyles, G. A. (1984) The interaction of semicarbazide-sensitive amine oxidase with MAO inhibitors. In: Tipton, K. F., Dostert, P., Strolin Benedetti, M. (eds). Monoamine Oxidase and Disease. Academic Press, London, pp 547–556

Lyles, G. A., Callingham, B. A. (1974) The effects of thyroid hormones on monoamine oxidase in the rat heart. J. Pharm. Pharmacol. 26: 921–930

Lyles, G. A., Garcia-Rodriguez, J., Callingham, B. A. (1983) Inhibitory actions of hydralazine upon monoamine oxidizing enzymes in the rat. Biochem. Pharmacol. 32: 2515–2521

Lyles, G. A., Singh, I. (1985) Vascular smooth muscle cells: a major source of the semicarbazide-sensitive amine oxidase of the rat aorta. J. Pharm. Pharmacol. 37: 637–643

McEwen, C. M. (1965) Human plasma monoamine oxidase. I. Purification and identification. J. Biol. Chem. 240: 2003–2010

Precious, E., Gunn, C. E., Lyles, G. A. (1988) Deamination of methylamine by semicarbazide-sensitive amine oxidase in human umbilical artery and rat aorta. Biochem. Pharmacol. 37: 707–713

Ryder, T. A., MacKenzie, M. L., Pryse-Davies, J., Glover, V., Lewinsohn, R., Sandler, M. (1979) A coupled peroxidatic technique for the histochemical localization of monoamine oxidase A and B and benzylamine oxidase. Histochemistry 62: 93–100

Schayer, R. W., Smiley, L. R., Kaplan, H. E. (1952) The metabolism of epinephrine containing isotopic carbon. J. Biol. Chem. 198: 545–551

Simenhoff, M. L. (1975) Metabolism and toxicity of aliphatic amines. Kidney Int. (Suppl.) 7: S314–317

Strolin Benedetti, M., Boucher, T., Guffroy, C., Dostert, P. (1984) MD 220661, an "in vivo" substrate of semicarbazide-sensitive amine oxidase. J. Pharm. Pharmacol. (Workshop Suppl). 36: 29W

Wibo, M., Duong, A. T., Godfraind, T. (1980). Subcellular location of semicarbazide-sensitive amine oxidase in rat aorta. Eur. J. Biochem. 112: 87–94

Zeisel, S. H., DaCosta, K.-A. (1986) Increase in human exposure to methylamine precursors of N-nitrosamines after eating fish. Cancer Res. 46: 6136–6138

Zeisel, S. H., Wishnok, J. S., Blusztjan, J. K. (1983) Formation of methylamines from ingested choline and lecithin. J. Pharmacol. Exp. Therap. 225: 320–324

Zeisel, S. H., DaCosta, K.-A., LaMont, J. T. (1988) Mono-, di- and trimethylamine in human gastric fluid: potential substrates for nitrosodimethylamine formation. Carcinogenesis 9: 179–181