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COMMUNICATIONS

Urinary 3-methoxy-4-hydroxyphenylglycol, an index of peripheral rather than central adrenergic activity in the rat

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3-Methoxy-4-hydroxyphenylglycol (MHPG), has been shown to be an important metabolite of noradrenaline in mammalian brain (Schanberg, Breese & others, 1968; Korf, Aghajanian & Roth, 1973; Walter & Eccleston 1973) whilst 3-methoxy-4-hydroxymandelic acid (VMA), occurs at very low concentrations in brain (Chase, Breese & others, 1971; Karoum, Neff & Wyatt, 1976). Both MHPG and VMA, however, are found in large quantities in the urine, 2–5 μg of the metabolites being excreted in a 24 h period in man. Since the metabolism of noradrenaline in the brain appears to be to the glycol rather than the carboxylic acid metabolites, it has been suggested that a greater part of urinary MHPG is derived from the cns and that urinary MHPG concentrations may reflect changes in central noradrenergic activity. (Maas, Dekirmenjian & others, 1972, 1973). It is more probable, however, that the larger proportion of MHPG in the urine originates from peripheral noradrenaline and adrenaline sources (Bareggi, Marc & Morselli, 1974; Karoum, Wyatt & Costa, 1974).

After monoamine oxidase inhibitors, the concentration of MHPG and other deaminated metabolites has been shown to decrease in the urine (Molinoff & Axelrod, 1971). We have attempted to show therefore, in the rat, the impoitance of the urinary MHPG concentration when the central and/or peripheral noradrenaline metabolism is altered with the monoamine oxidase inhibitor nialamide.

Nialamide (Pfizer Ltd) was dissolved in 0·1 M HCl and adjusted to pH 7·0 with 0·1 M NaOH before injection. Control injections consisted of the vehicle alone. Intraperitoneal injections were 100 mg kg^{-1}. Intracerebroventricular injections by the method of Noble, Wurtman & Axelrod, (1967) were in a volume of 20 μl and at a concentration of 8·5 mg ml^{-1}. If the weight of the brain is assumed to be 1·7 g, (180–220 g rat) this gave a nialamide concentration of 100 mg kg^{-1} relative to the rat brain weight.

Rats housed individually in metabolism cages designed for separate collection of urine and faeces, were allowed to acclimatize for 24 h. 24 h urine samples were then obtained on one control day and on two days after dosing. Urine volumes were measured and aliquots removed for creatinine estimation using the Technicon automated method. Samples were frozen at −20° until estimated. For brain MHPG estimation rats were killed by a blow on the head and the brains removed and analysed without storing.

Urinary MHPG was measured using a semi-micro method based on the method of Bond (1972). Urine (0·2 ml) adjusted to pH 5·0 with 20 μl M sodium acetate buffer pH 5·0, was incubated with Helicase solution (Koch Light) (5 μl), at 37° for 17 h. The incubate was twice extracted by shaking for 3 min with 0·8 and 0·5 ml of ethyl acetate. The layers were separated by centrifugation, 0·5 ml of the extract removed each time and combined in small glass tubes. The extract was evaporated to dryness under N_2 at 56° and the residue resuspended in 0·4 ml distilled water. Redistilled acetic anhydride (50 μl) and a 16·5% w/v solution of potassium hydrogen carbonate (0·6 ml) were added to the aqueous sample containing MHPG, and the acetylation reaction allowed to complete over 30 min with occasional shaking. Acetyl MHPG was extracted from the aqueous layer by shaking for 3 min with dichloromethane (1·8 ml). After 2 min centrifugation at 1000 *g*, 1·3 ml of the lower organic phase was removed and placed in (Eppendorf) reaction tubes containing approximately 0·1 g anhydrous sodium sulphate and shaken for 30 s. The tubes were then centrifuged at 9000 *g* (Eppendorf centrifuge) for 4 min, the extract carefully decanted into small test tubes and evaporated to dryness under N_2 at 56°. The residue was resuspended in 0·5 ml ethyl acetate and acetyl MHPG trifluoroacetylated with the addition of 0·1 ml trifluoroacetic anhydride and heated at 56° for 15 min. The reaction mixture was reduced to dryness undeı N_2 at 56° and the residue resuspended in ethyl acetate (1·5 ml) containing α-hexachlorocyclohexane (150 ng ml^{-1}). Gas chromatography was on a 2·1 m column of 3% SE 30 at 160° with oxygen-free nitrogen as carrier gas.

MHPG was extracted from rat brain using the method of Walter & Eccleston (1973) with the following modifications. Ethyl acetate extracts were resuspended in 0·4 ml of distilled water instead of 4 ml, and the chromatography stage using Bio Rad AG1X4 resin was omitted. Acetylation and trifluoroacetylation were as for urinary MHPG above.

Increasing amounts of MHPG added to rat urine or rat brain were recovered with a linear relation to the concentration. The mean (with s.d.) MHPG concentration (free + conjugated) was found to be 5·64 s.d.

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Table 1. *The effect of nialamide intraperitoneally and intracerebroventricularly on urinary and brain MHPG concentrations.* The number of rats used is shown in parentheses.

		MHPG concentration (free + conjugated)	
		Urine $\mu g\ mg^{-1}$ creatine with s.d.	Brain $ng\ g^{-1}$ with s.d.
Experiment 1			
Pre-injection 24 h control		4·93 s.d. 0·99 (4)	
Nialamide (100 mg kg^{-1}, i.p.)	1st 24 h	1·61 s.d. 0·50* (4)	
	2nd 24 h	1·50 s.d. 0·28* (4)	
Saline (i.p.)	+48 h		110 s.d. 8 (6)
Nialamide (100 mg kg^{-1}, i.p.)	+48 h		76 s.d. 14*** (6)
Experiment 2			
Pre-injection 24 h control		6·55 s.d. 1·46 (4)	
Nialamide (170 μg, i.c.v.)	1st 24 h	6·53 s.d. 1·90 NS (4)	
	2nd 24 h	7·96 s.d. 1·92 NS (4)	
Vehicle (20 μl, i.c.v.)	+48 h		177 s.d. 24 (5)
Nialamide (170 μg, i.c.v.)	+48 h		106 s.d. 41** (4)

* $P < 0·01$ *vs* pre-injection 24 h control.
** $P < 0·02$ *vs* vehicle.
*** $P < 0·01$ *vs* saline.
NS not significantly different from control.

2·07 $\mu g\ mg^{-1}$ creatinine (n = 98) in rat urine and 161 s.d. 27 $\mu g\ g^{-1}$ (n = 8) in rat brain.

Both the urinary and brain MHPG concentrations of the intracerebroventricular control group were significantly greater than those of the intraperitoneal control group. This was probably due to the greater stress encountered by the former group since they had a sutured scalp wound from the injection procedure.

In the 24 h after dosing, nialamide (100 mg kg^{-1}, i.p.) caused a 67% ($P < 0·01$) decrease in the urinary concentration of MHPG. The drug effect persisted during the following 24 h when a similar (70% $p < 0·01$) decrease of urinary MHPG occurred. Nialamide at this dose also caused a decrease in the brain concentration of MHPG by 32% ($P < 0·01$) at 48 h (Table 1, Experiment 1).

No effect on urinary MHPG was evident when nialamide was given intracerebroventricularly at a concentration (20 μl of 8·5 mg ml^{-1} solution) calculated to be equivalent to 100 mg kg^{-1} relative to the weight of the rat brain (1·7 g). There was, however, a significant 41% decrease ($P < 0·02$) in the brain MHPG concentration of these animals 48 h after dosing (Table 1, Experiment 2).

Thus, although a significant reduction of brain MHPG was observed 48 h after the intracerebroventricular injection of nialamide, the urinary excretion of MHPG in the 48 h post-drug period was no different from the 24 h pre-drug period. These data therefore support the notion that in the rat, the urinary MHPG concentration does not reflect the noradrenergic activity of the brain (Bareggi & others, 1974), but is an index of total body noradrenaline and adrenaline metabolism.

There are real problems extrapolating such data from rats to man, and in man the fraction of MHPG in the urine that originates from brain may be much larger than in the rat, thus allowing urinary MHPG to be used as an index of central noradrenergic activity. The clinical evidence neither strongly supports or completely refutes this idea (Schildkraut 1973).

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