3-Methoxy-4-hydroxyphenylglycol sulphate (MOPEG-SO_4) as an index of cerebral noradrenaline turnover following depletion of transmitter stores in the rat

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A major metabolite of noradrenaline in rat brain is 3-methoxy-4-hydroxyphenylglycol sulphate (MOPEG-SO_4) (Schanberg, Schildkraut & others, 1968; Meek & Neff, 1973a; Braestrup, Nielsen & Scheel-Kruger, 1974). The concentrations of this metabolite have been proposed as an index of cerebral noradrenaline turnover since the administration of noradrenergic agonists and antagonists and the use of stressful procedures have produced changes in its cerebral concentration which parallelled expected alterations in transmitter utilization (Meek & Neff, 1973b; Braestrup, 1974; Stone, 1975).

We were therefore surprised to find relatively little change in cerebral MOPEG-SO_4 concentrations in rats pretreated with reserpine, alone or in combination with an inhibitor of dopamine-β-hydroxylase FLA-63 (Dolphin, Jenner & Marsden, 1976), for both procedures produced a marked reduction of cerebral noradrenaline concentrations (Holzbauer & Vogt, 1956; Weil-Malherbe & Bone, 1958). However, a recent report has suggested that alterations in the concentration of conjugated metabolites of noradrenaline do not occur until transmitter stores are reduced by about 40% (Stone, 1976). This finding has been interpreted as suggesting that central noradrenergic neurons can release and metabolize noradrenaline at a normal rate despite synthesis blockade, so long as adequate stores of the amine are available.

The present investigation pursues this hypothesis by examining cerebral concentrations of MOPEG-SO_4 as an index of cerebral noradrenaline turnover following a range of procedures designed to cause a variable reduction in cerebral noradrenaline concentrations. This has been achieved by depletion of stored noradrenaline using reserpine and inhibition of its synthesis using the inhibitor of tyrosine hydroxylase α-methyl-*p*-tyrosine (α-MT), and the inhibitor of dopamine-β-hydroxylase (DBH) FLA-63 (bis-1-methyl-4-homopiperazinyl-thiocarbonyl disulphide).

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Table 1. *The effect of various treatments designed to deplete noradrenaline (NA) on the cerebral concentrations of noradrenaline and MOPEG-SO_4.*

	Treatment: Dose (mg kg^{-1}); length of treatment			Whole brain concns*		Ratio
	Reserpine	α-MT	FLA-63	NA (ng g^{-1})	MOPEG SO_4	NA:MOPEG-SO_4
1	—	—	—	423 ± 39	136 ± 3	3·1
2	—	—	25; 3 h	263 ± 97	113 ± 9	2·3
3	—	—	25; 24 h	157 ± 105	82 ± 3	1·9
4	—	—	25; 3 d	116 ± 28	75 ± 7	1·5
5	10; 21 h	—	—	9 ± 1	53 ± 4	0·2
6	10; 21 h	—	25; 3h	9 ± 1	22 ± 2	0·4
7	—	200; 5 h	—	128 ± 10	73 ± 4	1·8
8	—	200; 5 h	25; 3 h	73 ± 3	78 ± 5	0·9
	Significance between results:					
			1 *vs* 2	$P<0·05$	$P<0·01$	
			1 *vs* 3	$P<0·05$	$P<0·001$	
			1 *vs* 4	$P<0·001$	$P<0·001$	
			3 *vs* 4	NS	NS	
			1 *vs* 5	$P<0·001$	$P<0·01$	
			5 *vs* 6	NS	$P<0·05$	
			1 *vs* 7	$P<0·001$	$P<0·001$	
			7 *vs* 8	$P<0·001$	NS	

* Each value represents the mean (±1 s.e.m.) of at least 4 separate determinations.

For treatment 1, rats were given 0·9% saline 3 h before death. For treatments 2 and 3, FLA-63 (25 mg kg^{-1}) was administered 3 h and 24 h before death. For treatment 4, FLA-63 (25 mg kg^{-1}) was administered daily for 3 days and animals killed 3 h after the last dose. For treatments 5 and 6, reserpine (10 mg kg^{-1}) was administered 21 h before and 0·9% saline or FLA-63 (25 mg kg^{-1}) respectively 3 h before death. For treatments 7 and 8, animals received α-MT (200 mg kg^{-1}) 5 h before death and respectively 0·9% saline or FLA-63 (25 mg kg^{-1}) 3 h before being killed.

Significant differences between the results were determined using Student's *t*-test.

Male Wistar rats (125–175 g; Animal Suppliers Ltd.) received reserpine (10 mg kg^{-1}, i.p.; Halewood Chemicals) or α-MT methyl ester HCl (200 mg kg^{-1}, i.p.; Sigma Chemical Co; dissolved in 0·9% w/v sodium chloride soln) alone or in combination with FLA-63 (25 mg kg^{-1}, i.p.; Labkemi AB; dissolved in dilute HCl adjusted to pH 7 with dilute sodium hydroxide), as indicated in the legend to Table 1. Animals were killed by stunning and decapitation and the brains were rapidly removed, cooled to —20° and weighed. One whole brain was used for each estimation of either noradrenaline or MOPEG-SO_4.

In a subsequent experiment, rats received α-MT methyl ester HCl (200 mg kg^{-1}, i.p.) and were killed at intervals up to 8 h following administration. Whole brains were again taken for MOPEG-SO_4 determinations but for noradrenaline determination brains were rapidly dissected on ice and the amine determined in the cortex, hypothalamus, mesolimbic area and mid-brain.

Noradrenaline was determined by the fluorometric technique of Weil-Malherbe & Bigelow (1968) following separation by the ion exchange column chromatographic method of Atack (1973). MOPEG-SO_4 was estimated by the fluorometric method of Meek & Neff (1972). The relative reduction in MOPEG-SO_4 compared with noradrenaline is indicated by the ratio noradrenaline: MOPEG-SO_4.

Pretreatment of animals with the dopamine-β-hydroxylase inhibitor FLA-63 (25 mg kg^{-1}) 3 h before death produced a 38% reduction in whole brain noradrenaline concentrations whereas MOPEG-SO_4 concentrations were reduced by only 17% (ratio noradrenaline/MOPEG-SO_4 2·3 compared with 3·1 in the control animals) (Table 1). This finding is compatible with the need for a substantial reduction (*ca* 40%) in noradrenaline concentrations before large changes in the cerebral concentrations of MOPEG-SO_4 are observed. Indeed, decreasing cerebral noradrenaline even further by pretreatment with FLA-63 (25 mg kg^{-1}) 24 h before death, which resulted in a fall in the amines concentrations to 37% of control values, was associated with a reduction in MOPEG-SO_4 concentrations to only 60% of normal (ratio noradrenaline/MOPEG-SO_4 1·9). Thus, even a considerable reduction in cerebral noradrenaline concentration is associated with only a modest fall in cerebral MOPEG-SO_4 value. The repeated administration of FLA-63 (25 mg kg^{-1}) daily for 3 days did not further decrease the concentration of noradrenaline or that of MOPEG-SO_4 (ratio 1·5).

Reserpine (10 mg kg^{-1}) 21 h before death reduced cerebral noradrenaline to 2% of the control value presumably by disruption of granular storage. This was accompanied by a smaller reduction of MOPEG-SO_4 concentrations, to 39% of normal (ratio noradrenaline/MOPEG-SO_4 0·2). The higher concentration of MOPEG-SO_4 is not surprising since reserpine does not inhibit noradrenaline synthesis and may even increase it (Glowinski, Iversen & Axelrod, 1966; Meek, Krall & Lipton, 1970). This is supported by the ability of FLA-63 (25 mg kg^{-1}) administered 3 h before death to further decrease MOPEG-SO_4 concentrations in reserpine-treated animals.

Both in the studies using FLA-63 alone and those employing reserpine the absolute concentrations of noradrenaline do not correlate with the apparent turnover of the transmitter as assessed by cerebral MOPEG-SO_4 concentrations.

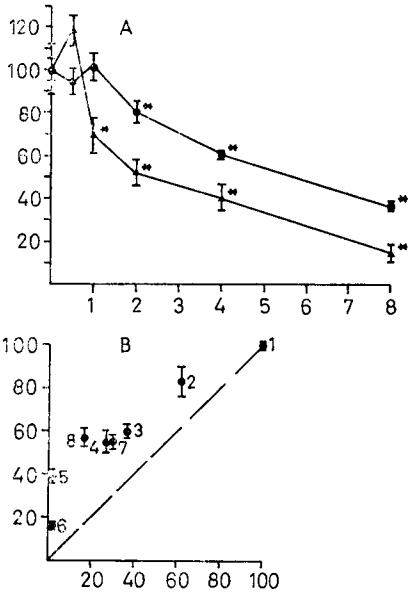

FIG. 1. The relationship between (A) cortical noradrenaline concentrations (—▲—) and whole brain MOPEG-SO_4 concentrations (—●—) at intervals following administration of α-MT (200 mg kg^{-1}) and (B) cerebral noradrenaline and MOPEG-SO_4 concentrations following various treatments designed to lower brain noradrenaline. Before α-MT (200 mg kg^{-1}) administration control values of cortical noradrenaline and whole brain MOPEG-SO_4 were 224 ± 26 ng g^{-1} and 125 ± 6 ng g^{-1} respectively. Each value for the time course of α-MT depletion represents the mean (± 1 s.e.m.) of at least 8 separate determinations. Significant differences between results were determined by Student's *t*-test: *represents $P<0·05$. The relationship between cerebral noradrenaline and MOPEG-SO_4 concentrations following various treatments designed to lower brain noradrenaline values has been constructed from the data in Table 1. The numbers refer to the treatment numbers in this Table. The standard error bars represent variation in the concentration of MOPEG-SO_4. The dotted lined shows a hypothetical unity relationship between these parameters. Ordinates: A: % control values; B: % control MOPEG-SO_4 values. Abscissa: A: Time (h); B: % control noradrenaline values.

This conclusion is supported by further studies using an inhibitor of tyrosine hydroxylase. The administration of α-MT (200 mg kg^{-1}) 5 h before death reduced cerebral noradrenaline by 70% and MOPEG-SO_4 by 45% (ratio noradrenaline/MOPEG-SO_4 1·8). The reduction of noradrenaline produced by α-MT (200 mg kg^{-1}) was enhanced by the administration of FLA-63 (25 mg kg^{-1}) but MOPEG-SO_4 concentrations were not further reduced.

This evidence is compatible with the idea that reduced neuronal stores of noradrenaline can maintain a relatively normal level of neurotransmission as judged by MOPEG-SO_4 concentrations up to a critical point of about 40% depletion as suggested by Stone (1976). This is further substantiated by the time course of the fall in whole brain MOPEG-SO_4 and cortical noradrenaline concentrations seen following a single administration of α-MT (200 mg kg^{-1}). Thus, 1 h following α-MT administration noradrenaline values are reduced by 32% with no fall in MOPEG-SO_4 (Fig. 1a). At 2 h, however, noradrenaline values have fallen by 48% and are accompanied by a 20% fall in MOPEG-SO_4. At 8 h, the amine values are only 14% of the controls while MOPEG-SO_4 concentrations are 36% of normal values. Similar correlations were obtained with noradrenaline concentrations in the hypothalamus, mesolimbic area and mid-brain.

The lack of a unity relationship between noradrenaline and MOPEG-SO_4 is highlighted by comparing the correlation between noradrenaline and MOPEG-SO_4 concentrations as a percentage of control values (Fig. 1b) for all treatments. It is apparent that the data following depletion of noradrenaline indicate MOPEG-SO_4 to be present in amounts which would not be expected from the concentration of noradrenaline.

Several pools of noradrenaline are believed to exist and it would appear from the present data that not only the newly synthesized pool but also stored pools may be mobilized to maintain cerebral noradrenergic transmission. Indeed, recent evidence has suggested that under conditions of heavy demand such as seen following noradrenaline receptor blockade both newly synthesized and stored amine are readily available for release (McMillen & Shore, 1977). If this is so then MOPEG-SO_4 would appear a better index of noradrenaline turnover following synthesis inhibition than the concentration of the transmitter.

Other explanations must also be considered when interpreting these data. Thus, an alternative might be that although the rate of formation of MOPEG-SO_4 from noradrenaline is rapid, its rate of disappearance from the brain is slow. However, the time course of depletion of noradrenaline and MOPEG-SO_4 following α-MT treatment (Fig. 1a) shows the rate of decline of both transmitter and metabolite to be similar once noradrenaline values fall below 60% of normal.

In conclusion, when brain noradrenaline concentration is reduced, MOPEG-SO_4 values are maintained and afford a better index of neuronal activity than measurement of the transmitter itself. Under other circumstances, both the concentration of brain noradrenaline (or its rate of change) and that of brain MOPEG-SO_4 give information on noradrenaline neuronal activity, but when brain noradrenaline stores are depleted, the concentration of its metabolite appears to reflect noradrenaline transmission more accurately.

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