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Intra- and extraneuronal formation of the two major noradrenaline metabolites in the cns of rats

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The two major noradrenaline metabolites in the rat brain, total 3-methoxy-4-hydroxyphenylglycol (MOPEG) and total 3,4-dihydroxyphenylglycol (DOPEG), were measured by a new analytical procedure after intraventricular (i.v.t.) injection of [^{3}H]noradrenaline or [^{3}H]dopamine. I.v.t. injection of [^{3}H]noradrenaline to rats with a 6-hydroxydopamine-induced destruction of nerve terminals in the central nervous system, resulted in an increased accumulation of ^{3}H-MOPEG compared to ^{3}H-DOPEG. On the contrary, reserpine induced a greater increase in ^{3}H-DOPEG than in ^{3}H-MOPEG accumulation when these two metabolites were formed from [^{3}H]noradrenaline synthesized *in vivo* by i.v.t. injection of [^{3}H]dopamine. These results indicate that the formation of DOPEG and MOPEG occur with some preference intra- and extraneuronally, respectively. The results, however, also show that these two formations sites should not be considered as specific.

Conjugated 3-methoxy-4-hydroxyphenylglycol (MOPEG) is a major metabolite of noradrenaline in the central nervous system (cns) of rats. Labelled MOPEG is found in high amounts both after intraventricular (i.v.t.) injection of labelled noradrenaline (Schanberg, Schildkraut & others, 1968; Meek, Krall & Lipton, 1970; Sugden & Eccleston, 1971; Stone, 1973) or after administration of labelled precursors of noradrenaline (Braestrup, Nielsen & Scheel-Krüger, 1974; Nielsen, Eplov & Scheel-Krüger, 1974; Nielsen, unpublished). Also endogenously, appreciable amounts of free and conjugated MOPEG are found in brain tissues (Sharman, 1969; Meek & Neff, 1972; Braestrup, 1973).

Until recently little attention was paid to conjugated 3,4-dihydroxyphenylglycol (DOPEG) which appears also to be a major metabolite of noradrenaline in the cns both *in vivo* (Sugden & Eccleston, 1971; Stone, 1973; Braestrup & others, 1974; Nielsen & others, 1975) and *in vitro* (Rutledge & Jonason, 1967; Schweitzer & Friedhoff, 1969). No method is available for the determination of endogenous conjugated DOPEG, but the endogenous level of free DOPEG almost equals that of free MOPEG in brain tissue (Sharman, 1969; Ceasar & Sharman, 1972). After i.v.t. injection of labelled noradrenaline (Sugden & Eccleston, 1971; Stone, 1973) or its precursors (Braestrup & others, 1974; Nielsen, unpublished), total labelled DOPEG is found in amounts almost equal to total labelled MOPEG.

In the present study, the two major metabolites of noradrenaline, total ^{3}H-MOPEG and total ^{3}H-DOPEG, were efficiently separated from interfering radioactivity and measured after i.v.t. injection of [^{3}H]dopamine (^{3}H-DA) or [^{3}H]noradrenaline (^{3}H-NA). Throughout this communication the terms ^{3}H-MOPEG and ^{3}H-DOPEG denote the sum of free plus conjugated metabolite; according to Schanberg & others (1968) and

Eccleston & Ritchie (1973), MOPEG in the rat brain is mainly conjugated with sulphate. To obtain some information about possible intra- and extraneuronal formation sites of these two metabolites, rats were treated with reserpine or 6-hydroxydopamine (6-OH-DA).

Animals

Male Wistar rats (200–250 g) were kept in individual cages with free access to food and water. For i.v.t. injections, a hole was drilled 1 mm caudal and 1 mm lateral to the bregma under light ether anaesthesia. ^{3}H-NA, ^{3}H-DA or 6-OH-DA were injected slowly in 15–20 μl to a depth of 4·2 mm, mainly according to Noble, Wurtman & Axelrod (1967). The animals were killed after various pretreatment schedules, and the whole brains were homogenized in the cold within 1 min and assayed for their contents of ^{3}H-DA, ^{3}H-HA, ^{3}H-MOPEG and ^{3}H-DOPEG or for endogenous dopamine noradrenaline and total MOPEG.

Biochemical procedures

Endogenous noradrenaline and dopamine were isolated by the alumina-procedure of Anton & Sayre (1962) and estimated fluorimetrically (Weil-Malherbe, 1971).

Endogenous total MOPEG was estimated by g.l.c. with electron capture detection as the pentafluoropropionyl derivative according to Braestrup (1973).

^{3}H-NA, ^{3}H-DA, total ^{3}H-MOPEG and total ^{3}H-DOPEG were estimated by a new analytical procedure (Braestrup & others, 1974). Brains were homogenized in acetic acid and ^{3}H-NA and ^{3}H-DA were isolated and separated by cation-exchange chromatography on Amberlite CG 120. The effluent and washings from the Amberlite column were incubated with Glusulase (final concentration in incubate: 10 000 Roy U ml^{-1} sulphatase and 1,300 Fishman U ml^{-1} glucuronidase, Endolab) for 20 h. Total ^{3}H-MOPEG and total ^{3}H-DOPEG were then extracted into ethyl acetate and separated on t.l.c. with chloroform–acetic acid–water (2:2:1) as the solvent system. In analyses made one h after i.v.t., ^{3}H-DA, MOPEG was further isolated from interfering impurities by a second run in the solvent system chloroform–methanol–NH_3 (12:7:1). Tritium was measured by liquid scintillation counting in Instagel. Results were corrected for efficiency by external standard ratio and calculated as d. min^{-1} g^{-1} brain tissue. Vehicle treated control rats were always analysed along with drug-treated animals and all data for drug treatments in Tables and Fig. 1 are derived from paired observations of a single drug-treated animal compared to a single control rat. Means and standard error of the means are calculated from (n) single paired observations. Statistical analyses were made using Student's *t*-test on the absolute values in the control group versus the drug-treated group or from paired observations of one metabolite compared to paired observations of another metabolite.

Drugs. Reserpine, 7·5 mg kg^{-1} (Serpasil, Ciba) or pargyline, 75 mg kg^{-1} (pargyline-HCl, Abbott) were injected subcutaneously or intraperitoneally, respectively. 6-OH-DA (6-hydroxydopamine hydrochloride, H 88/32 Hässle) was dissolved immediately before use in cold isotonic saline at pH 5, containing 0·1 mg ml^{-1} ascorbic acid. Under light ether anaesthesia 180 μg 6-OH-DA as base in 15 μl was injected in the left lateral ventricle and 48 h later a second dose of 250 μg was applied. These rats were assayed for endogenous noradrenaline, dopamine and total MOPEG or ^{3}H-NA and its metabolites 48 h after the last dose of 6-OH-DA.

After various pretreatment schedules (see Tables and Fig. 1) ^{3}H-NA (1 μCi; (–)-noradrenaline-7-^{3}H (N), 5·7 Ci mmol^{-1}, NEN, Boston) or ^{3}H-DA (10 μCi; 3,4-dihydroxyphenethylamine Ring-^{3}H~(G)~-hydrochloride, 500 mCi mmol^{-1}, Amersham, U.K.), were injected intraventricularly in 15 μl Ringer solution. The rats were killed 1, 2 or 6 h later.

RESULTS

Intraventricularly injected ^{3}H-DA

The formation of ^{3}H-NA, ^{3}H-MOPEG and ^{3}H-DOPEG from ^{3}H-DA in 6-OH-DA- or reserpine-pretreated rats is summarized in Table 1. The accumulation of ^{3}H-NA was reduced to very low levels (less than 7% of control) by either pretreatment and also the accumulation of the two major noradrenaline metabolites, ^{3}H-MOPEG and ^{3}H-DOPEG was strongly reduced. After 6-OH-DA the decrease in ^{3}H-MOPEG (to about 30% of controls), however, was highly significantly ($P<0·001$) less pronounced than the decrease in ^{3}H-NA (to about 7% of controls).

Table 1. *The effects of various pretreatments on the formation of ^{3}H-NA, total ^{3}H-MOPEG and total ^{3}H-DOPEG after intraventricularly injected ^{3}H-DA (10 μCi, 3 μg).* Two doses of 6-OH-DA (180 and 250 μg) were administered 96 and 48 h before ^{3}H-DA, reserpine (7·5 mg kg^{-1}) 18 h before ^{3}H-DA and pargyline (75 mg kg^{-1}) 3/4 h before ^{3}H-DA. Rats were killed 1 or 2 h after ^{3}H-DA. Values are mean ± s.e.m. of (n) determinations as % of control.

		% of control, paired observations			
Pretreatment	h after, ^{3}H-DA	^{3}H-DA	^{3}H-NA	^{3}H-MOPEG	^{3}H-DOPEG
6-OH-DA	1	40·9 ± 4·6 (5)***	6·9 ± 0·9 (4)***	33·2 ± 1·3 (5)***§§	26·3 ± 7·6 (5)**
6-OH-DA	2	27·6 ± 2·3 (4) NT	6·5 ± 1·0 (5)***	28·7 ± 2·0 (5)***§§	30·1 ± 9·3 (5)*§
Reserpine	2	9·8 ± 1·8 (4)***	4·8 ± 0·6 (4)***	23·2 ± 7·4 (4)***§	18·8 ± 8·6 (4)***
Pargyline	1	950 ± 90 (3)**	208 ± 30 (3)*	7·9 ± 1 (3)**	27·8 ± 4·6 (3)*

Note: Throughout ^{3}H-MOPEG or ^{3}H-DOPEG denotes the sum of free + conjugated metabolite.

* $P<0·05$; ** $P<0·01$; *** $P<0·001$ relative to controls. § $P<0·05$; §§ $P<0·001$ relative to noradrenaline.

Control levels in d. min^{-1} g^{-1} × 10^3 ± s.e.m. of (n) values. One hour after ^{3}H-DA: ^{3}H-NA, 58·9 ± 3·1 (7); ^{3}H-DA, 85·5 ± 6·2 (8); ^{3}H-MOPEG, 5·55 ± 0·26 (8); ^{3}H-DOPEG, 2·51 ± 0·35 (8). Two hours after ^{3}H-DA: ^{3}H-NA, 38·7 ± 4·4 (9); ^{3}H-DA, 15·7 ± 2·4 (8); ^{3}H-MOPEG, 5·88 ± 0·35 (9); ^{3}H-DOPEG, 1·29 ± 0·23 (9). No corrections for recovery.

Pretreatment of the rats with 75 mg kg^{-1} pargyline ¾ h before i.v.t. injected ^{3}H-DA resulted in the expected increase in ^{3}H-DA and ^{3}H-NA and a strong decrease in ^{3}H-MOPEG and ^{3}H-DOPEG in spite of the high amounts of radioactivity remaining in the amine fractions (Table 1).

Administration of reserpine (7·5 mg kg^{-1}) to rats, after prior synthesis and accumulation of ^{3}H-NA from i.v.t. ^{3}H-DA, effected an 120% increase in ^{3}H-DOPEG while ^{3}H-MOPEG was only increased by 50% (Fig. 1). ^{3}H-DOPEG was significantly more increased than ^{3}H-MOPEG ($P<0·05$).

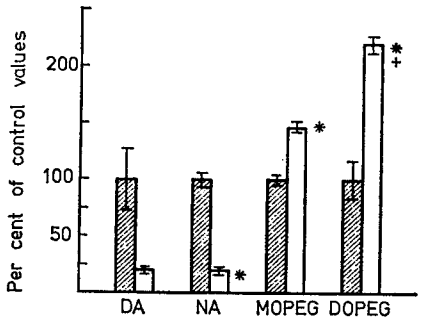

FIG. 1. Effects of reserpine (7·5 mg kg^{-1}) on ^{3}H-NA and its two major metabolites ^{3}H-MOPEG and ^{3}H-DOPEG formed from intraventricularly injected ^{3}H-DA. ^{3}H-DA (10 μCi) was injected 7 h before and reserpine 3 h before decapitation. Hatched columns = control; open columns = reserpine-treated. Shown are the means ± s.e.m. of 4–5 values. *different from control $P<0·001$. † different from MOPEG $P<0·05$.

Control levels in d. min^{-1} g^{-1} × 10^3 ± s.e.m. of (n) values. ^{3}H-NA, 15·9 ± 1·0 (5); ^{3}H-DA, 3·03 ± 0·87 (5); ^{3}H-MOPEG, 3·31 ± 0·13 (4); ^{3}H-DOPEG, 1·21 ± 0·15 (4). No corrections for recovery.

Intraventricularly injected ^{3}H-NA

The decreased accumulation of i.v.t. injected ^{3}H-NA in 6-OH-DA pretreated rats (Table 2) substantiates the impaired storage capacity induced by the degeneration of catecholaminergic neurons. One h after ^{3}H-NA, ^{3}H-MOPEG was greatly increased while the level of ^{3}H-DOPEG was unchanged. Two hours after ^{3}H-NA the increase in ^{3}H-MOPEG induced by 6-OH-DA had levelled off while the level of ^{3}H-DOPEG was now reduced to 40% of controls.

Administration of ^{3}H-NA intraventricularly in reserpine-pretreated rats resulted in the expected decrease in ^{3}H-NA accumulation and an increase in both ^{3}H-MOPEG and ^{3}H-DOPEG (Table 2).

Table 2. *Effect of 6-OH-DA or reserpine on the metabolism of ^{3}H-NA after intraventricular injection of ^{3}H-NA (1 μCi, 0·03 μg).* Two doses of 6-OH-DA (180 μg and 250 μg base) were administered 96 and 48 h before ^{3}H-NA and reserpine (7·5 mg kg^{-1}) 18 h before ^{3}H-NA. Each value is the mean ± s.e.m. of (n) determinations in per cent of control.

		% of control, paired observations		
Pretreatment	h after, ^{3}H-NA	^{3}H-NA	^{3}H-MOPEG	^{3}H-DOPEG
6-OH-DA	1	28·5 ± 2·3 (4)***	200 ± 14·7 (4)***	116 ± 28 (4)
6-OH-DA	2	18·3 ± 3·1 (5)***	117 ± 24 (5)	39·8 ± 4·6 (4)**
Reserpine	1	11·7 ± 0·6 (5)***	161 ± 11 (5)***	198 ± 25 (4)**

**$P<0·01$; ***$P<0·001$ relative to controls.

Control levels in d. min^{-1} g^{-1} × 10^3 ± s.e.m. of (n) values. One hour after ^{3}H-NA: ^{3}H-NA, 179 ± 5·0 (9); ^{3}H-MOPEG, 54·5 ± 1·7 (9); ^{3}H-DOPEG, 19·7 ± 1·7 (8). Two hours after ^{3}H-NA: ^{3}H-NA, 120 ± 6·5 (5); ^{3}H-MOPEG, 37·2 ± 1·2 (5); ^{3}H-DOPEG 15·5 ± 1·0 (4). No corrections for recovery.

Endogenous dopamine, noradrenaline and total MOPEG

The endogenous levels of dopamine, noradrenaline and total MOPEG after pretreatment with 6-OH-DA or reserpine are shown in Table 3. Total MOPEG is not decreased to the same low level as noradrenaline by 6-OH-DA ($P<0.001$) and that reserpine induced an increase in total MOPEG after 3 h ($P<0.001$).

Table 3. *Effects of 6-OH-DA and reserpine on endogenous dopamine, noradrenaline and total MOPEG.* Values are mean ± s.e.m. of (n) determinations as % of control. Endogenous levels in untreated rats: dopamine 832 ± ng g^{-1}; noradrenaline 409 ± 12 ng g^{-1}; total MOPEG 93·6 ± 7·8 ng g^{-1}.

	% of control		
Pretreatment	Dopamine	Noradrenaline	total MOPEG
6-OH-DA, 180 + 250 μg	47 ± 3 (6)***	22 ± 2 (6)***	42 ± 3 (5)***§
Reserpine 7·5 mg kg^{-1}, 18 h	4·0 ± 0·7 (4)***	<5 (4)	17·4 1·4 (4)***
Reserpine 7·5 mg kg^{-1}, 3 h	3·8 ± 1 (6)***	8·5 ± 3 (6)***	136 ± 6 (4)***

* $P<0.05$; ** $P<0.01$; *** $P<0.001$ relative to controls. § $P<0.001$ relative to noradrenaline.

DISCUSSION

Intraventricular injection of ^{3}H-DA is a reliable technique for the investigation of the metabolism of noradrenaline in central noradrenergic neurons (Braestrup & others, 1974).

The present study further validates the use of this technique, showing that, prior destruction of noradrenergic neurons with 6-OH-DA or destruction of the storage granules with reserpine, almost completely inhibit the accumulation of ^{3}H-NA formed from ^{3}H-DA, thus confirming previous findings (Glowinski, Iversen & Axelrod, 1966; Laverty & Taylor, 1970). The levels of the two major noradrenaline metabolites ^{3}H-MOPEG and ^{3}H-DOPEG were also decreased by pretreatment with reserpine or 6-OH-DA (Table 1) indicating a reduced net synthesis of noradrenaline after 6-OH-DA or reserpine. Previous reports have failed to show the currently observed decrease in the two major metabolites of noradrenaline after i.v.t. injection of ^{3}H-DA in 6-OH-DA- or reserpine-pretreated rats because of the lack of separation of MOPEG and DOPEG from metabolites of ^{3}H-DA (Glowinski, & others, 1966; Laverty & Taylor, 1970).

The decrease in ^{3}H-MOPEG after ^{3}H-DA in 6-OH-DA-pretreated rats was expected, but it was a surprise that the concentration of ^{3}H-MOPEG compared to the controls (30%) was greater than that of ^{3}H-NA compared to the controls (7%, Table 1). The concentration of ^{3}H-MOPEG after the MAO inhibitor pargyline (8% control, Table 1) shows that ^{3}H-MOPEG can be reduced to less than 30% of the controls.

Also the endogenous level of noradrenaline was more reduced than the endogenous level of total MOPEG by 6-OH-DA (Table 3). The reason why 6-OH-DA should decrease noradrenaline further than MOPEG is not clear but a compensatory increase in the activity of adrenergic neurons surviving the 6-OH-DA treatment may be indicated (Uretsky, Simmonds & Iversen, 1971; Agid, Javoy & Glowinski, 1973). Other mechanisms such as differential metabolism of noradrenaline in cell bodies and terminals (Bhatnagar & Moore, 1971) should, however, also be considered.

To study the extraneuronal metabolism of noradrenaline, rats were treated with 6-OH-DA intraventricularly. With the applied dosage schedule central noradrenergic terminals are degenerated, especially in the periventricular regions (Uretsky & Iversen, 1970; Ungerstedt, 1971; Bloom, 1971). The vast degeneration of noradrenergic neurons in the present study is indicated by the low concentration of endogenous noradrenaline induced by 6-OH-DA (Table 3).

Intraventricular injection of ^{3}H-NA to rats with marked 6-OH-DA-induced degeneration in noradrenergic neurons and terminals resulted in a shift in the metabolism of noradrenaline. At both time intervals we found the concentration of ^{3}H-MOPEG to be greater than that of ^{3}H-DOPEG compared to controls (Table 2). These results indicate that in a situation where neuronal uptake and neuronal metabolism of noradrenaline is almost eliminated, the metabolism of exogenous noradrenaline preferentially proceeds by the combined influence of COMT and MAO. It should be noted, however, that the formation of DOPEG is not impaired after 6-OH-DA (Table 2) leaving a possibility for extra-neuronal DOPEG formation. The preferential formation of MOPEG extraneuronally found in the present study is in accordance with previous investigations showing an appreciable increase in *O*-methylation (^{3}H-normetanephrine) (Breese & Traylor, 1970; Uretsky & others, 1971) versus a decrease in a "^{3}H-catechol-deaminated" fraction (Breese & Traylor, 1970) after intracerebral injection of ^{3}H-NA in 6-OH-DA-pretreated rats.

To study the intraneuronal metabolism of noradrenaline in the cns, ^{3}H-NA was synthesized in the noradrenergic neurons from ^{3}H-DA and the rats were then treated with reserpine, which is believed to destroy the amine storage granules with a subsequent intraneuronal exposure of the stored amines. This treatment induced a marked increase in ^{3}H-DOPEG and only a minor increase in ^{3}H-MOPEG (Fig. 1), indicating that noradrenaline is preferentially metabolized to the non-methylated deaminated metabolite ^{3}H-DOPEG most probably intraneuronally. Stone (1973) injected ^{3}H-NA intraventricularly in reserpine-pretreated rats and found an almost equal increase in ^{3}H-MOPEG and ^{3}H-DOPEG (see also Table 2), these results, however, are masked by noradrenaline-metabolism taking place before uptake in noradrenergic neurons and by metabolism of noradrenaline in other neuron systems.

The high amounts of ^{3}H-MOPEG found after reserpine (Fig. 1) were unexpected, but also the endogenous level of MOPEG was increased 3 h after reserpine (Table 3). Of total DOPEG in the rat brain, 80–90% is found in conjugated form (Stone, 1973; Braestrup & others, 1974), which may not be *O*-methylated by COMT (Axelrod & Tomchick, 1958; Miyazaki, Yoshizawa & Fishman, 1969), and under normal physiological conditions it seems that DOPEG is transformed to the conjugated form which is not further metabolized. It may be, however, that forced intraneuronal metabolism of noradrenaline after reserpine produces sufficient free DOPEG for the formation of the observed high amounts of MOPEG, presumably by prejunctional COMT (Jarrott, 1971; Langer, Stefano & Enero, 1972) or possibly extra-neuronally after DOPEG-leakage through the neuronal membrane. The formation of MOPEG after i.v.t. injection of free DOPEG has recently been found by Eccleston & Ritchie (1973) and Nielsen & Braestrup, unpublished.

The preferential formation of MOPEG extraneuronally and DOPEG intraneuronally found in the present study is compatible with the idea that the concentration of endogenous MOPEG (mainly as the sulphate ester) is indicative of central noradrenergic activity (Walter & Eccleston, 1972, 1973; Korf, Aghajanian & Roth, 1973; Stone,

1973; Braestrup, 1974; Braestrup & others, 1974). The correlation between the level of MOPEG-SO_4 and noradrenaline release, however, still needs further clarification, since it is possible that released noradrenaline is metabolized to DOPEG by reincorporation into the nerve terminals (Stone, 1973; Dubocovich & Langer, 1973) and because the present data indicate that the level of MOPEG may be increased in a condition of essential intraneuronal metabolism of noradrenaline (reserpine treatment).

From the present results it thus appears that the formation of MOPEG and DOPEG from noradrenaline in the rat brain occurs with some preference extra- and intraneuronally, respectively. The results, however, also show that the two formation sites are a matter of preference rather than a specific phenomenon.

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