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Effects of drugs on the formation of 3-methoxytyramine, a dopamine metabolite, in the substantia nigra, striatum, nucleus accumbens and tuberculum olfactorium of the rat

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3-Methoxytyramine (3-MT) was assayed with a sensitive and rapid liquid chromatographic separation on a Sephadex G 10 column in combination with a fluorimetric detection in a continuous flow system. The method made it possible to measure pargyline-induced 3-MT concentrations in various regions of a single rat brain. After haloperidol, morphine or sulpiride treatment, pargyline-induced 3-MT concentrations in the striatum, nucleus accumbens, tuberculum olfactorium and substantia nigra of the rat were comparable with reported changes of homovanillic acid concentrations. Dose-response curves for 3-MT increase in various brain regions after (+)-amphetamine pretreatment were studied. The effect of (+)-amphetamine on 3-MT formation was much more pronounced in nerve terminal areas, especially in the mesolimbic structures. The influence of apomorphine, γ-hydroxybutyric acid, haloperidol, reserpine or combined treatment of haloperidol and (+)-amphetamine revealed pronounced differences on regional 3-MT concentrations, indicating important differences between the regulation of dopamine metabolism in the substantia nigra and nerve terminal areas.

Recently much attention has been paid to the regional differences in the effects of centrally acting drugs on dopamine metabolism in the striatum, nucleus accumbens, tuberculum olfactorium and substantia nigra of the rat brain (Andén & Stock 1973; Bartholini 1976; Westerink & Korf 1976a,b). These studies were based on the determination of the levels of the two main dopamine metabolites: 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA); less attention was paid, to 3-methoxytyramine (3-MT), a minor metabolite. The results of Kehr (1976), however, suggested that 3-MT concentration measured after monoamine-oxidase (MAO) inhibition are a better reflection of impulse-dependent dopamine release than are DOPAC or HVA concentrations.

The present study describes the effects of various centrally acting drugs on regional 3-MT concentrations during MAO-inhibition. A modification of the recently described automated fluorimetric assay (Westerink & Korf 1977) was used for the determination of 3-MT.

MATERIALS AND METHODS

Reagents

All solutions were made with glass double distilled water and analytical grade reagents. A KOH-formate solution was made by adding carefully 60 ml concentrated formic acid (98%) to 200 ml 10 M KOH. The NH_3-reagent was prepared daily by mixing equal volumes of 25% NH_3-solution and water. The $K_3Fe(CN)_6$ solution contained 40 mg% and was stable at 4 °C for several weeks. The 20 mg% cysteine solution was used on the same day.

Instrumentation

A continuous flow analysis system (Autoanalyzer, Technicon) was used in combination with a Perkin Elmer 1000 fluorimeter, equipped with a Mark I flow cell (600 μl). The primary filter was a 320 nm interference filter and as secondary filter a Corning C.S. 3–73 cut-off filter was used.

3-MT assay

Tissue samples (up to 100 mg) were homogenized in 1 ml 0·4 M perchloric acid. The excess of perchlorate was precipitated by addition of 100 μl of the KOH-formate solution (final pH: 2–3). After centrifugation (20 min, 4000*g*, 4°C) the supernatants were transferred to glass tubes and two drops (0·03–0·10 ml) of 5% perchloric acid added. A tissue extract was put on a Sephadex G 10 column (5 × 70 mm) prepared in a long size Pasteur pipette as described by Westerink & Korf (1976a). The Sephadex G 10 column was connected to the sample tube of an Autoanalyzer flow system, in which a 3-MT specific fluorophore was

continuously recorded. The flow diagram shown in Fig. 1 is similar to the procedure of Westerink & Korf (1977). After the tissue extract had passed through the column, 3·5–4·0 ml 0·01 M formic acid was added, and the chromatogram was recorded. The column could be re-used immediately without regeneration. Homogenized samples (pH 2–3), before addition of perchloric acid can be preserved for at least 8 weeks when kept at −20 °C.

Animals, drug treatments and dissection

Male albino Wistar rats, 175–250 g (T.N.O., Zeist, The Netherlands), were used. Drugs were administered intraperitoneally and the animals were killed by focussed microwave radiation (Litton Systems 70/50; 4 s), following the time schedules given in Results. The following drugs were used: (+)-amphetamine SO_4, apomorphine HCl, benztropine HCl, γ-hydroxybutyric acid (GHB), haloperidol (Serenase, Janssen), morphine HCl, pargyline HCl, reserpine and sulpiride (Dogmatil).

The various dopaminergic areas, including the striatum, nucleus accumbens, tuberculum olfactorium and mesencephalic tissue containing both the substantia nigra and group A10 of the ventral tegmental area, were dissected as described before (Westerink & Korf 1976a; Westerink & Korf 1976b). Striatal 3-MT was assayed as recently described (Westerink & Korf 1977). The concentrations given for the striatum are the mean of the bilateral parts, which were analysed separately.

RESULTS

3-MT assay

The sensitivity of the previously described 3-MT assay could be increased about four-fold (from 10–15 to 2·5–3 ng/sample) when a Sephadex G-10 column was connected to the flow system (Fig. 1) and complete elution profiles (chromatograms) recorded. Fig. 2 shows the chromatograms of standard solutions and brain samples; the standard curve of 3-MT was slightly non-linear. The absence of 3-MT in non-dopaminergic brain structures such as the cerebellum illustrates the specificity of the method. The recovery of 25 ng 3-MT added to cerebellar tissue (40–70 mg) was 92 s.d. 4%, n = 5). 3 ng/sample could be reproducibly measured. With a sample tube size of 0·42 ml min^{-1}, the analysis takes about 10 min. The small Sephadex column can be used for at least one week.

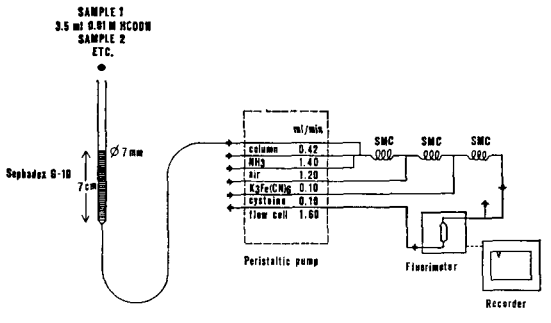

FIG. 1. Schematic representation of the 3-MT assay procedure including the Autoanalyser flow diagram. SMC refers to single mixing coil.

Control values

Control values of striatal 3-MT concentrations after 60, 120 and 180 min pargyline treatment (75 mg kg^{-1}) are given in Table 1. Comparison of values obtained from rats killed by microwave radiation or decapitation revealed a significant post mortem effect, which confirmed the necessity of microwave fixation (Gropetti et al 1977). No change in 3-MT concentrations were observed when the brain was dissected one h after death by microwave radiation.

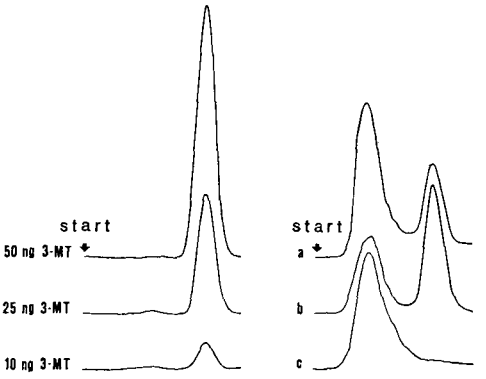

FIG. 2. 3-MT chromatograms of pure standards (10, 25 and 50 ng applied to the column in 1 ml) and brain samples. The brain samples (recorded at increased gain in comparison with the pure standards) represent (a) substantia nigra (20 mg, 60 min pargyline, 75 mg kg^{-1}), (b) substantia nigra (25 mg, 60 min pargyline, 75 mg kg^{-1}; 60 min (+)-amphetamine SO_4) and (c) 45 mg cerebellar tissue (60 min pargyline, 75 mg kg^{-1}).

Table 1. The effect of microwave radiation or decapitation on the striatal 3-MT concentrations of pargyline treated rats.

Pargyline (75 mg kg^{-1}) (min)	Death by: Microwave	Decapitation
0	<0·10 (4)	0·10 ± 0·02 (4)
60	0·42 ± 0·02 (14)	0·51 ± 0·02 (6)[a]
120	0·54 ± 0·01 (4)	0·64 ± 0·08 (6)[a]
120 (+ 60 min at 20° after death)	0·45 ± 0·02 (4)	not determined
180	0·63 ± 0·02 (4)	0·92 ± 0·10 (2)[a]

Different form microwave treated rats: [a]$P < 0·02$ by Student's *t*-test

Influence of drugs

Haloperidol (0·5 mg kg^{-1}) and morphine (10 mg kg^{-1}) were administered 2 h before, and sulpiride (40 mg kg^{-1}) 3 h before, death. Table 2 shows that haloperidol, morphine and sulpiride induced a rise in 3-MT concentrations in all structures studied, however the rise observed after haloperidol in the substantia nigra was not statistically significant. Percentage changes of HVA concentrations, obtained in comparable experiments (Westerink & Korf 1976 a,b), are included in Table 2. Haloperidol caused the most pronounced percentage 3-MT rise in the striatum and nucleus accumbens, while morphine increased the percentage 3-MT concentrations more strongly in the mesolimbic structures. The effect of sulpiride on the 3-MT concentrations was most pronounced in the nucleus accumbens.

Fig. 3 shows the dose-response curves of 3-MT increase for (+)-amphetamine SO_4 in the substantia nigra, striatum, nucleus accumbens and tuberculum olfactorium. (+)-Amphetamine SO_4 was injected together with pargyline (75 mg kg^{-1}) 1 h before death. The percentage 3-MT increased showed pronounced regional differences. The greatest increase was seen in the nucleus accumbens, while the rise in the substantia nigra reached only statistical significance at a dose of 10 mg kg^{-1}. The ED 50% of the effect of amphetamine on 3-MT concentrations seems similar in the structures studied. Table 3 shows the influence of apomorphine (5 mg kg^{-1}), benztropine (10 mg kg^{-1}), GHB (1500 mg kg^{-1}) and reserpine (5 mg kg^{-1}) on 3-MT concentrations after pretreatment with pargyline (75 mg kg^{-1}). Apomorphine, benztropine, GHB, reserpine and pargyline were injected 1 h before death. Apomorphine and GHB decreased 3-MT concentrations in the striatum, while reserpine increased 3-MT concentrations in the striatum and tuberculum olfactorium. Benztropine did not influence 3-MT values in the striatum, nucleus accumbens or tuberculum olfactorium. No change was observed in 3-MT concentrations in the substantia nigra after apomorphine, GHB or reserpine.

The effect of a combined treatment of (+)-amphetamine (10 mg kg^{-1}) and haloperidol (1 mg kg^{-1}) on pargyline-induced 3-MT concentrations in both striatum and substantia nigra were compared. Haloperidol was administered 90 min before, and (+)-amphetamine and pargyline 60 min before, death. Fig. 4 clearly shows that the effects of amphetamine and haloperidol as well as the combination of both drugs were much more pronounced in the striatum.

Table 2. Effect of various drugs on 3-MT and HVA* concentration in the striatum, nucleus accumbens, tuberculum olfactorium and substantia nigra after combined treatment with pargyline (75 mg kg^{-1}, 60 min).

Structure	3-MT (μg g^{-1} ± s.e.m., N)	3-MT (% of controls ± s.e.m., N)	HVA (% of controls ± s.e.m., N)
Controls			
Striatum	0·47 ± 0·05 (6)		
Accumbens	0·29 ± 0·03 (4)		
Tub. olf.	0·23 ± 0·03 (4)		
Sub. nigra	0·16 ± 0·01 (4)		
Haloperidol 0·5 mg kg^{-1} 120 min before death			
Striatum	1·73 ± 0·08 (4)^{c}	368 ± 17 (4)	520 ± 20 (5)
Accumbens	1·12 ± 0·01 (4)^{b}	386 ± 65 (4)	520 ± 15 (5)
Tub. olf.	0·63 ± 0·15 (4)^{a}	273 ± 65 (4)	320 ± 15 (5)
Sub. nigra	0·21 ± 0·02 (4)	131 ± 13 (4)	115 ± 7 (5)
Morphine 10 mg kg^{-1}, 120 min before death			
Striatum	0·77 ± 0·09 (3)^{b}	164 ± 21 (3)	244 ± 9 (12)†
Accumbens	0·92 ± 0·03 (3)^{c}	318 ± 11 (3)	331 ± 35 (12)
Tub. olf.	0·85 ± 0·04 (3)^{c}	367 ± 18 (3)	218 ± 18 (12)
Sub. nigra	0·30 ± 0·03 (3)^{b}	187 ± 18 (3)	255 ± 8 (4)†
Sulpiride 40 mg kg^{-1} 180 min before death			
Striatum	0·76 ± 0·09 (4)^{b}	161 ± 19 (4)	258 ± 13 (5)
Accumbens	1·08 ± 0·01 (4)^{c}	372 ± 3 (4)	309 ± 25 (5)
Tub. olf.	0·45 ± 0·05 (4)^{b}	200 ± 21 (4)	268 ± 10 (5)

* HVA values were taken from previous studies (Westerink & Korf 1976 a,b; Westerink & Korf 1976b; Westerink et al 1977).

†HVA concentrations in the striatum and substantia nigra were measured after a dose of 20 mg kg^{-1} of morphine (Westerink & Korf 1976b).

Different from control: $^{a}P<0·05$, $^{b}P<0·01$, $^{c}P<0·001$ by Student's *t*-test.

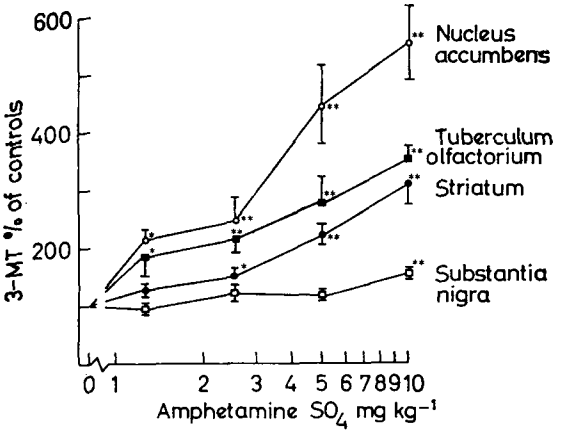

FIG. 3. Dose-response curves for 3-MT in the substantia nigra (□), striatum (●), nucleus accumbens (○) and tuberculum olfactorium (■) for (+)-amphetamine SO_4, (60 min) in combination with pargyline (75 mg kg^{-1}, 60 min). Values are given as percentages of controls ± s.e.m. of 5–6 determinations (ordinate). Control values are given in Table 3. Different from control: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ by Student's *t*-test.

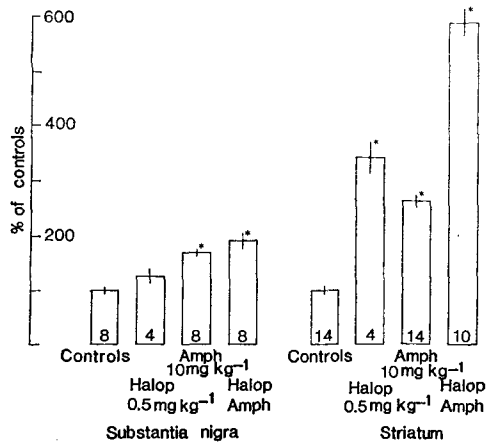

FIG. 4. The effect of haloperidol (0·5 mg kg^{-1}, 90 min), (+)-amphetamine.SO_4 (10 mg kg^{-1}, 60 min) and combined treatment of haloperidol and amphetamine on pargyline induced (75 mg kg^{-1}, 60 min) 3-MT concentrations in the substantia nigra and the striatum. Control values are given in Table 2. Different from control: $*P < 0.001$ by Student's *t*-test. Ordinate: percentage of controls.

Table 3. Effect of various drugs on 3-MT concentrations in the striatum, nucleus accumbens, tuberculum olfactorium and substantia nigra after combined treatment with pargyline (75 mg kg^{-1} 60 min).

Structure	3-MT (μgl g^{-1} ± s.e.m., N)	3-MT (% controls ± s.e.m., N)
Controls		
Striatum	0·37 ± 0·02 (7)	
Accumbens	0·17 ± 0·01 (5)	
Tub. olf.	0·15 ± 0·02 (6)	
Sub. nigra	0·16 ± 0·01 (6)	
Apomorphine 5 mg kg^{-1}, 60 min before death		
Striatum	0·12 ± 0·02 (6)[b]	32 ± 5 (6)
Sub. nigra	0·13 ± 0·01 (6)	81 ± 7 (6)
Benztropine 10 mg kg^{-1}, 60 min before death		
Striatum	0·30 ± 0·02 (4)	81 ± 5 (4)
Accumbens	0·18 ± 0·01 (4)	105 ± 5 (4)
Tub. olf	0·12 ± 0·02 (4)	80 ± 12 (4)
GMB 1500 mg kg^{-1}, 60 min before death		
Striatum	0·11 ± 0·01 (6)[b]	29 ± 3 (6)
Sub. nigra	0·14 ± 0·01 (6)	85 ± 7 (6)
Reserpine 5 mg kg^{-1}, 120 min before death		
Striatum	0·79 ± 0·12 (4)[a]	214 ± 34 (4)
Tub. olf.	0·37 ± 0·04 (4)[b]	247 ± 26 (4)
Sub. nigra	0·19 ± 0·02 (4)	119 ± 13 (4)

Different from control: $^{a}P < 0.01$, $^{b}P < 0.001$ by Student's *t*-test.

DISCUSSION

The 3-MT assay

The 3-MT assay is an adaptation of a semi-automated analysis of which details and specificity were discussed previously (Westerink & Korf 1977). Sensitivity was increased about four fold, as the 3-MT specific fluorescence is recorded as a peak and not diluted in the 'amine' fraction as described in the manual separation. The method enables reliable measurement of pargyline-induced 3-MT concentrations in discrete brain areas of a single rat brain. The method is rapid, as the tissue extract is applied to the column without pretreatment, and the analysis can be performed within 10 min. Dopamine and noradrenaline can be analysed similarly with an appropriate Autoanalyzer system (Westerink & Korf 1977).

Nerve terminal areas

A good correlation was found between the effects of drugs on the firing of dopaminergic neurons and their ability to increase or decrease the concentrations of DOPAC in terminal areas (Roth et al 1973). The results of Kehr (1976) and Kehr et al (1977), however, suggest that 3-MT concentrations are a better reflection of impulse-dependent dopamine release than are DOPAC or HVA values. Unpublished experiments using a COMT-inhibitor revealed that 3-MT (during pargyline treatment), is unable to leave the brain, while 3-MT conjugates could not be detected (ms in preparation). The find-

ing that 3-MT is not able to leave the brain excludes interference with efflux mechanisms during drug treatment, which is a disadvantage when studying drug-induced changes with concentrations of acidic dopamine metabolites DOPAC and HVA.

Studies based on regional DOPAC and HVA concentrations have revealed that classical neuroleptics like haloperidol increase dopamine turnover preferentially in the striatum and nucleus accumbens and to a lesser extent in the tuberculum olfactorium, while atypical neuroleptics such as sulpiride and non-neuroleptics such as morphine increase dopamine turnover more noticeably in the mesolimbic structures (Bartholini 1976; Westerink et al 1977; Scatton et al 1977).

Although complete dose-response curves for the effects of various drugs on regional 3-MT concentrations were not studied, the present data suggest that haloperidol induces a higher percentage increase of 3-MT in the striatum, and nucleus accumbens, whereas non-neuroleptics, including amphetamine, preferentially increase 3-MT in the mesolimbic structures. This result confirms the outcome of studies on the effect of drugs on regional DOPAC and HVA concentrations.

The substantia nigra

Recent studies indicated functional dopamine release from dendrites and/or cell bodies in the substantia nigra (Korf et al 1976; Geffen et al 1976; Nieoullon et al 1977). Data on dopamine, DOPAC and HVA concentrations determined after various drug treatments have revealed similarities as well as differences between dopamine metabolism in the substantia nigra and the terminal areas (Hefti et al 1976; Westerink & Korf 1976b; Periric & Walters 1976; Kebabian et al 1977; Fadda et al 1977). The slight effect of haloperidol on 3-MT concentrations in the substantia nigra (Table 2) contrasts with the pronounced increase seen in terminal areas and is in agreement with reported changes in DOPAC and HVA values (Westerink & Korf 1976b; Kebabian et al 1977). Fadda et al (1977), however, reported a similar percentage increase in DOPAC values in the striatum and substantia nigra. They also reported control DOPAC concentrations in the substantia nigra about 10 times higher than in comparable studies (Westerink & Korf, 1976b; Kebabian et al 1977).

The dose-response curves of 3-MT increase for (+)-amphetamine showed great differences in regional responses. Evidence for dopamine release by (+)-amphetamine SO_4, as measured by 3-MT accumulation, in the substantia nigra could only be obtained using a high dose (10 mg kg^{-1}). The ED50% of the effect of amphetamine on 3-MT values, however, seems similar in the various structures, which suggests that the amphetamine-sensitive dopamine pool is most responsive in the nucleus accumbens and less so in the substantia nigra.

The findings on the different effects of amphetamine and haloperidol on the 3-MT accumulation in the substantia nigra in comparison with the striatum are supported by the small percentage 3-MT increase seen in the substantia nigra after the combined treatment with the two drugs, in contrast with the strong additive increase observed in the striatum (Kehr et al 1977).

The presence of a functional dopamine release in the substantia nigra is questioned by the absence of decreased 3-MT formation in this area after GHB treatment. Other evidence that GHB does not influence dopamine metabolism in the substantia nigra was provided by the reported absence of dopamine increase (Hefti et al 1976; Periric & Walters 1976), which is characteristic for terminal areas (Roth et al 1973). Differences between the regulation of dopamine metabolism in the substantia nigra and the striatum are also indicated by the absence of a 3-MT decrease in the substantia nigra after apomorphine treatment.

The absence of any change in 3-MT values in the substantia nigra after reserpine treatment, indicated that the capacity of the reserpine-sensitive pool in this structure (Björklund & Lindvall 1975) is relatively small. If dopamine is compartmentalized in the brain, it might be possible that pools like the reserpine-sensitive and the amphetamine-sensitive ones are more rapidly exhausted in somatodendritic areas in comparison with terminal areas. This could be an explanation why during certain drug treatments the responsiveness of these dopamine pools is less clearly reflected in dopamine metabolite concentrations in the substantia nigra. Some support for this hypothesis is provided by the findings of relatively low dopamine concentrations in the substantia nigra (Versteeg et al 1976).

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