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Amineptine: its effect on the dopaminergic system of rats

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3-Methoxytyramine formation was enhanced by amineptine (40 mg kg^{-1} i.p.) in the striatum and limbic area, indicating an increase in the concentration of dopamine in the extraneuronal space. Since dopamine turnover, determined as the rate of L-dopa accumulation, is reduced by the drug at short (10 min) times, the enhanced extraneuronal dopamine concentration seems mainly related to amineptine-induced inhibition of its uptake.

Amineptine is an antidepressant drug characterized by a selective effect on the dopaminergic system (Samanin et al 1977; Dankova et al 1977). Although this drug was synthesized several years ago its mechanism of action remains unclear. It has been reported to raise rat striatal homovanillic acid (HVA) in-vivo (Samanin et al 1977; Dankova et al 1977) and, 60 min after administration, to reduce dopamine (DA) turnover (Algeri et al 1978) as assayed by measuring the rate of incorporation of [^{3}H]tyrosine into [^{3}H]DA. However, the levels of the DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3-MT), were not modified (Ponzio et al 1984). This latter finding is surprising since HVA is the end product of the catabolism of DOPAC by catechol-*O*-methyltransferase (COMT) and of 3-MT by monoamine oxidase (MAO) (Westerink & Spaan 1982a, b).

It is possible that amineptine could increase 3-MT formation, in relation to elevated DA release and/or to DA reuptake inhibition, without apparently modifying 3-MT basal levels.

We therefore investigated whether 3-MT formation was affected since although 3-MT levels appear not to be modified, the basal levels of a compound could be the result of a balance between rate of synthesis and rate of catabolism so some change may have occurred.

Materials and methods

Animals and drug treatment. CD-COBS male rats (Charles River, Italy), 150–200 g, were housed under standard, controlled conditions with free access to food and water. The drugs used were: amineptine HCl (Servier Laboratories), pargyline HCL (Aldrich) and *m*-hydroxybenzylhydrazine dihydrochloride (NSD 1015) (Janssen Chimica). All drugs were dissolved in water and administered intraperitoneally (for details, see Results). Control animals received the vehicle only.

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Tissue sampling. Animals were killed at selected times by decapitation or, for 3-MT detection, by microwave irradiation focusing on the head (1·3 kW, 2·45 GHz for 4·25 s), in order to rapidly inactivate the enzyme catechol-*O*-methyltransferase (COMT) (Groppetti et al 1977). Brains were removed and the striata and limbic area (nucleus accumbens and tuberculum olfactorium) were rapidly dissected, frozen on dry ice and kept at −80 °C until assayed biochemically.

Biochemical determinations. 3-MT and L-dopa were determined in rat striata or limbic area as previously described by Ponzio et al (1981a, b) and Benfenati et al (1982). Briefly, tissues were homogenized in 0·4 M perchloric acid centrifuged for 15 min at 10 000*g* and samples then extracted from the clear supernatant. Liquid chromatography with electrochemical detection (LCEC) (Bioanalytical System Inc., West Lafayette, Ind.) was used for all biochemical determinations.

Results

Rate of 3-MT accumulation after amineptine in pargyline-pretreated rats. To see whether the rate of 3-MT formation was modified by amineptine, we performed an experiment in which 3-MT catabolism was inhibited by a MAO inhibitor, pargyline (75 mg kg^{-1} i.p.); 5 min later animals received amineptine (40 mg kg^{-1} i.p.) and were killed 10, 15 or 20 min after.

The rate of 3-MT accumulation after pargyline was linear (Fig. 1) in both the striatum and limbic area. Amineptine alone failed to raise 3-MT levels in both areas. However when it was given with pargyline, the rate of 3-MT accumulation was significantly enhanced in both areas (Fig. 1).

L-Dopa accumulation after amineptine in NSD-treated rats. To see whether the formation of L-dopa was modified by amineptine, we performed an experiment in which L-dopa catabolism was inhibited by the decarboxylase inhibitor NSD (100 mg kg^{-1} i.p.), animals simultaneously received amineptine (40 mg kg^{-1} i.p.) and were killed 10 min later.

After decarboxylase inhibition, the L-dopa content in the striatum rose 23 times above control values and in the limbic area 5·4 times. L-Dopa accumulation was reduced by amineptine, the content being greater than basal values by 16 times in the striatum and 3·5 times in the limbic area. L-Dopa accumulation was therefore

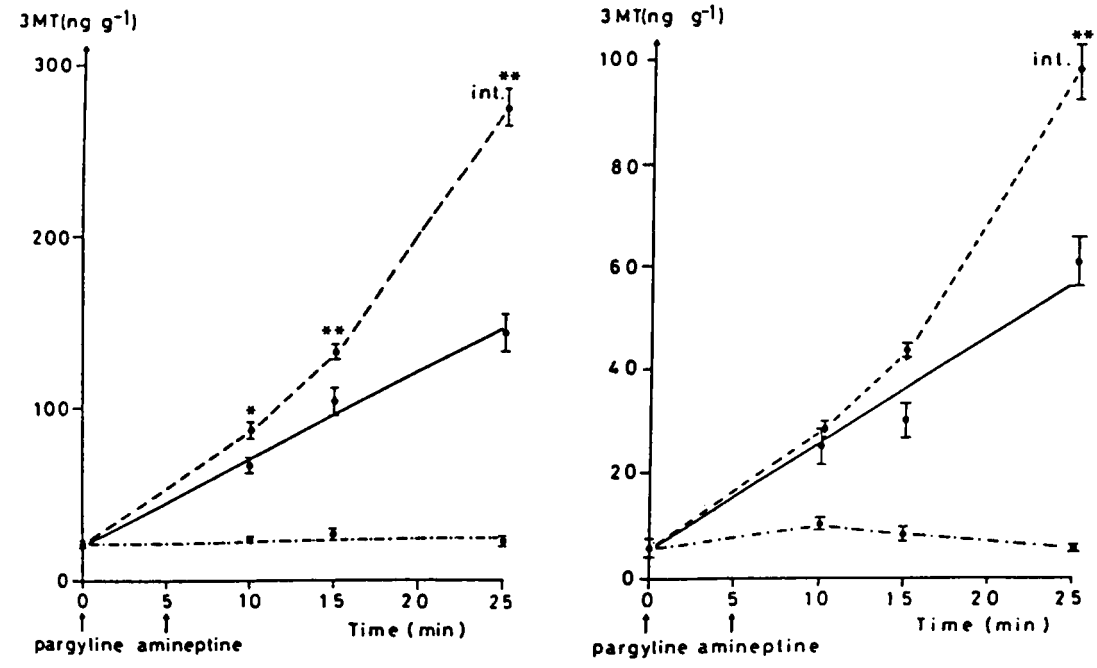

FIG. 1. Rate of 3-MT formation after amineptine in rat striatum (left) and limbic area (right) with and without MAO inhibition. Animals received pargyline (75 mg kg^{-1} i.p.) 5 min before amineptine (40 mg kg^{-1} i.p.) and were killed by microwave irradiation 5, 10 and 20 min after the last treatment. Data are the mean + s.e. of six determinations. Statistical significance was analysed by two-way ANOVA (Kirk 1968) and Tukey's (a) test (Cicchetti 1972) using an SPBS computer program (Rocchetti & Recchia 1982). Key: ---●---, pargyline + amineptine; ——●——, pargyline; -·-·-●-·-·-, amineptine. $*P < 0.05$ vs pargyline, $**P < 0.01$ vs pargyline.

reduced by amineptine by 31 and 35% in striatum and limbic area, respectively.

Discussion

Amineptine, an antidepressant drug acting preferentially on the dopaminergic system, elevates striatal HVA (Samanin et al 1977; Dankova et al 1977) with no significant effect on the other DA metabolites, 3-MT and DOPAC (Ponzio et al 1984). This effect is peculiar, as DA in the brain is metabolized to DOPAC by the enzyme MAO located intraneuronally, and to 3-MT by the enzyme COMT. As COMT is mainly located extraneuronally (Westerink 1979) 3-MT is considered an expression of DA present in the synaptic cleft.

These metabolites 3-MT and DOPAC, are, in turn metabolized to HVA by MAO and COMT, respectively (Westerink 1979). HVA is therefore the end product of DA metabolism, and an increase of HVA levels could be related to (i) a reduction of DA reuptake, (ii) increased DA formation and release, (iii) a reduction of the active transport of acidic metabolites across the blood-brain barrier. The third hypothesis can be excluded because other acidic metabolites, such as DOPAC, 5-HIAA and MHPG-SO_4, that utilize the same pathway of elimination from the brain, were not affected by amineptine (data not reported).

We and others (Ponzio et al 1981a, b, 1985; Westerink & Spaan 1982a, b) have reported, that, since 3-MT turnover is high, its elevated formation could, to some extent, be balanced by its elevated elimination, as with methylphenidate, an indirect DA agonist (Braestrup 1977), that does not affect 3-MT (Waldmeier et al 1981) but increases 3-MT formation (Ponzio et al 1985). Thus, the increased 3-MT formation after MAO inhibition in the striatum and in the limbic area in amineptine-treated rats reflected a rise in extraneuronal DA caused, possibly, either by increased DA release or by a reduction of DA reuptake. The reduction of DA turnover induced by amineptine at 60 min could also be related to these two hypotheses (Algeri et al 1978). However, while DA reuptake inhibitors increase DA in the synaptic cleft, thereby causing hyperstimulation of DA autoreceptors with a consequent drop in DA synthesis, DA release causes a different response. At first, the lowered concentration of DA within the nerve ending resulting from increased DA release, stimulates DA synthesis by disinhibition of tyrosine hydroxylase activity; then the enhanced extraneuronal concentration of DA leads to reduced DA synthesis again through hyperstimulation of DA autoreceptors (Andén et al 1967; Algeri et al 1982). On the basis of these findings, the mechanism of action of amineptine seems more closely related to inhibition of DA reuptake than to increased DA release. In fact DA formation is reduced

Table 1. L-Dopa accumulation after amineptine in NSD-treated rats.

	Striatum		Limbic area	
	L-Dopa ng g^{-1} ± s.e.	NSD + amineptine / NSD	L-Dopa ng g^{-1} ± s.e.	NSD + amineptine / NSD
Controls	17 ± 0·9		38 ± 8	
NSD (100 mg kg^{-1} i.p.)	391 ± 28**††		205 ± 11**††	
NSD + amineptine (100 + 40 mg kg^{-1} i.p.)	272 ± 15**	0·69	133 ± 17**	0·65

Rats were treated simultaneously with amineptine (40 mg kg^{-1} i.p.) and NSD (100 mg kg^{-1} i.p.) to inhibit amino acid decarboxylase, and decapitated 10 min thereafter. Data are the means ± s.e. of 6 animals per group. Statistical significance was analysed by a modification of Duncan's new multiple test (Kramer 1956) using an SPBS computer program (Rocchetti & Recchia 1982).

** $P < 0·01$ vs controls. †† $P < 0·01$ vs NSD + amineptine.

at both short (Table 1) and longer times after drug administration (Algeri et al 1978).

Other biochemical results follow this pattern; in-vivo pretreatment with amineptine counteracts DA depletion induced by 6-OHDA (Ponzio et al 1984) and reduces DA uptake (Manias & Taylor 1983), while in-vitro amineptine inhibits [^{3}H]-DA uptake by rat striatal synaptosomal preparations; with an IC50 of $1·4 \times 10^{-6}$ M (Ceci et al 1986).

In summary, amineptine increases the formation of 3-MT in the striatum and in the limbic area, but only after MAO inhibition. This increased 3-MT formation reflects an elevation of extraneuronal DA that seems mainly related to inhibition of DA uptake.

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