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# Amfonelic acid antagonism of dopamine and norepinephrine depletion by α-methyl-*m*-tyrosine in rat brain

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Amfonelic acid is a potent inhibitor of dopamine uptake. According to Ross and Kelder [1], the order of potencies among inhibitors *in vitro* of [$^3$H]dopamine uptake by rat striatal homogenates is amfonelic acid > mazindol > EXP 561 > benztropine > *d*-amphetamine > pipradol > nomifensine > methylphenidate > cocaine > desipramine. Not only is amfonelic acid one of the most potent inhibitors of dopamine uptake that is available, but it also is one of the most selective. The other compounds mentioned above inhibit the uptake of norepinephrine as well as of dopamine. Wong and Bymaster [2] reported that the order of selectivities as inhibitors *in vitro* of [$^3$H]dopamine uptake as opposed to [$^3$H]norepinephrine uptake by rat brain synaptosomes is amfonelic acid > mazindol ≃ cocaine > benztropine ≃ *d*-amphetamine. Although various *in vivo* effects of amfonelic acid have been described [3–6], few data exist that reveal the selectivity of amfonelic acid as an inhibitor *in vivo* of uptake by dopamine neurons relative to uptake by norepinephrine neurons. The study described herein was done to investigate this selectivity, using an agent (α-methyl-*m*-tyrosine; α-MMT) that causes depletion of both dopamine and norepinephrine in rat brain by a mechanism that permits uptake inhibitors to antagonize the depletion [7]. By measuring dopamine and norepinephrine in the same brains, an index of inhibition of uptake by both types of catecholamine neurons is provided.

Male Wistar rats (130–150 g) from Harlan Industries, Cumberland, IN, were kept in a room at 24° with daily 12 hr light:12 hr dark cycles and given free access to food and water. Drug solutions were injected s.c. or i.p., and rats were killed by decapitation. Cerebral hemispheres were dissected, frozen on dry ice, and stored at −15° prior to analysis. Catecholamines in the cerebral hemispheres were determined by high performance liquid chromatography with electrochemical detection [8]. All data are shown as mean values ± standard errors for five rats per treatment group. Comparisons between groups were made by Student's *t*-test. Amfonelic acid was a gift from the Sterling–Winthrop Research Institute.

Figure 1 shows that the α-MMT-induced depressions of dopamine and norepinephrine concentrations were antagonized in a dose-related manner by amfonelic acid. The depletion of dopamine by α-MMT was 54, 43, 40 and 11 per cent (not significant) after 0, 2.5, 5 and 10 mg/kg doses of amfonelic acid. The depletion of norepinephrine by α-MMT was 78, 70, 70 and 53 per cent after 0, 2.5, 5 and 10 mg/kg doses of amfonelic acid. These data indicate that amfonelic acid inhibited uptake both by dopamine neurons and by norepinephrine neurons *in vivo* but the greater effect was on dopamine neurons. At the highest dose of amfonelic acid, no significant depression of dopamine occurred after α-MMT injection, whereas norepinephrine decreased to less than half of the control value. Amfonelic acid may be as selective an agent as is currently known for inhibition of dopamine uptake *in vivo*. Speciale *et al.* [9] have observed that amfonelic acid can alter levels of norepinephrine and its metabolites in certain brain regions, suggesting direct or indirect effects on norepinephrine neurons. German *et al.* [10] have demonstrated electrophysiological effects of amfonelic acid on norepinephrine neurons and suggested that they were due to norepinephrine uptake inhibition. Those findings and the slight but significant ($P < 0.05$) antagonism of norepinephrine depletion that we observed at the 5 and 10 mg/kg doses of amfonelic acid support the idea that amfonelic acid does inhibit norepinephrine, as well as dopamine uptake, *in vivo*.

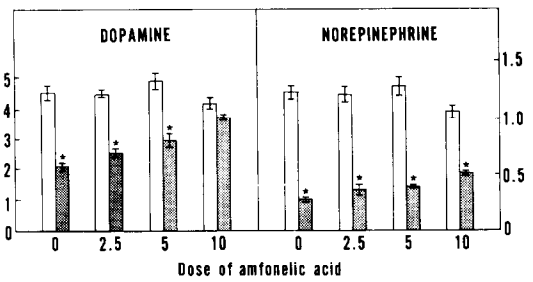


Fig. 1. Amfonelic acid antagonism of the depletion of cerebral dopamine and norepinephrine by α-MMT in rats. DL-α-MMT (Sigma) (50 mg/kg, s.c.) and amfonelic acid (i.p. at the mg/kg doses indicated) were injected 6 hr before the rats were killed. Ordinates show dopamine and norepinephrine concentrations in nmoles/g (wet weight) of tissue (cerebral hemispheres). Open bars show control animals receiving only amfonelic acid at the doses indicated; shaded bars represent animals treated with α-MMT and amfonelic acid. Asterisks indicate significant differences ($P < 0.05$) between values represented by open and shaded bars. Values are means ± S.E.; N = 5.

The effects of amfonelic acid contras with those of several tricyclic antidepressant drugs and related compounds that we have studied as uptake inhibitors *in vivo* based on their abilities to antagonize catecholamine depletion by α-MMT. Drugs like protriptyline, nortriptyline, amitriptyline, desipramine, imipramine and nisoxetine have antagonized norepinephrine (and epinephrine) depletion by α-MMT with little or no effect on dopamine depletion [7, 11–14; R. W. Fuller and K. W. Perry, unpublished data]. These tricyclic drugs are known to be weaker *in vitro* inhibitors of dopamine uptake than of norepinephrine uptake [15]. Although Wong and Bymaster [2] reported that LR 5182, a new, potent inhibitor of dopamine uptake, was more selective than amfonelic acid *in vitro*, LR 5182 was not selective *in vivo* in their experiments. We also observed that LR 5182 antagonizes the depletion of norepinephrine by α-MMT at least as well as it antagonizes the depletion of dopamine [11]. Thus, amfonelic acid seems to be more selective than LR 5182 *in vivo*. Amfonelic acid should be particularly useful for the pharmacologic manipulation of dopaminergic neurons in experiments in which effects on norepinephrine neurons need to be minimized.

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# Inhibition of ribonucleic acid polymerase activity by ellipticine*

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Ellipticine (5,11-dimethyl-6H-pyrido-(4,3-b)-carbazole), isolated as a plant alkaloid from *Ochrosia* species [1], possesses antitumor activity in experimental animals [2]. Ellipticine binds to DNA by intercalation [3], and inhibits cellular DNA and RNA synthesis more efficiently than protein synthesis [4–6]. It kills cultured cells in all phases of the cell cycle [4], and causes chromosome damage of Chinese hamster ovary cells at a drug concentration of 1–4 μg/ml [4]. On interaction of ellipticine with poly d(A-T)·poly d(A-T), changes in the absorption spectra and the melting temperature have been observed in preliminary experiments [4]; with poly d(G-C)·poly d(G-C) no changes were observed, suggesting that there may be a preferential interaction of ellipticine with A:T base pairs of the nucleic acid [4]. This question has been explored in the present study by using an *in vitro* system of highly purified RNA polymerase.

Tritium-labeled nucleotides were obtained from Schwarz/Mann, Orangeburg, NY. Unlabeled nucleotides were purchased from the Worthington Biochemical Corp., Freehold, NJ, or from P-L Biochemicals, Milwaukee, WI. Calf thymus DNA was obtained from Worthington. Poly d(A-T)·poly d(A-T) and poly d(G-C)·poly d(G-C) were obtained from P-L Biochemicals. These templates were dissolved in 0.01 M Tris–HCl (pH 7.0) buffer containing 0.1 mM EDTA and 0.1 M NaCl. Twenty optical density units at 260 nm were taken to be equal to 1.0 mg template/ml. Creatine kinase and phosphocreatinine were obtained from Boehringer Biochemicals, Mannheim, Germany, and crystalline bovine serum albumin from Miles Laboratories, Elkhart, IN. Polymin P was a gift from the BASF-Wyandotte Corp., Parsippany, NJ. Frozen *Escherichia coli*, RNase-minus strain, MRE 600 cells, grown up to 3/4 log in enriched medium, were obtained from the Grain Processing Co., Muscatine, IA. DNA-cellulose was prepared according to the method of Alberts and Herrick [7]. Biogel A 1.5 m was obtained from Bio-Rad Laboratories, Richmond, CA. RNA polymerase activity was purified according to Burgess and Jendrisak [8]. The highly purified enzyme preparation was stored in small aliquots in 50% glycerol at −20°. The protein content of the enzyme preparation was determined by the method of Lowry *et al.* [9], using crystalline bovine serum albumin as a standard. Ellipticine (NSC 71795), daunomycin·HCl (NSC 82151), adriamycin·HCl (NSC 123127), and dactinomycin (NSC 3053) were obtained from Dr. John Douros, Developmental Therapeutics Program, Division of Cancer Treatment, NCI, Bethesda, MD. Stock solutions of these drugs were freshly made in 90% dimethylsulfoxide (DMSO) solution.

The highly purified RNA polymerase preparation had a 280:260 nm absorption ratio of 1.50. In a 7.5% polyacrylamide gel in 0.1% sodium dodecylsulfate, the enzyme preparation gave three major bands corresponding to $\beta\beta'$, $\sigma$ and $\alpha$ subunits of the enzyme [8]. One unit of enzyme

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