Comparative effects of pemoline, amfonelic acid and amphetamine on dopamine uptake and release *in vitro* and on brain 3,4-dihydroxyphenylacetic acid concentration in spiperone-treated rats

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Pemoline, 2-imino-5-phenyl-4-oxazolidinone, has long been known as a cns stimulant (Schimidt, 1956; McColl & Rice, 1962). Tagliamonte & Tagliamonte (1971) suggested that its stimulant action is mediated by brain dopamine. Shore (1976a, b) recently reported that amfonelic acid, another non-amphetamine stimulant, greatly enhanced the elevation of brain DOPAC (3,4-dihydroxyphenylacetic acid) by dopamine receptor blocking agents that increase the rate of firing of dopamine neurons. Shore interpreted that amfonelic acid and certain other non-amphetamine stimulants including methylphenidate and cocaine facilitated the impulse-mediated release of dopamine. Since Everett (1975, 1976) suggested that pemoline acted by "increasing release of dopamine onto central receptors", we thought pemoline and amfonelic acid might act by similar mechanisms. With that in mind, we compared the effects of pemoline, amphetamine and amfonelic acid on [^{3}H]dopamine accumulation by rat striatal synaptosomes and on the spiperone-induced elevation of DOPAC in rat brain.

The *in vitro* uptake of [^{3}H]dopamine into rat striatal synaptosomes was determined as described previously (Wong & Bymaster, 1976). The [^{3}H]dopamine concentration was 0·1 μM. For the determination of dopamine release, striatal synaptosomes were preloaded by incubating for 15 min with 0·2 μM [^{3}H]dopamine. The efflux of [^{3}H]dopamine from washed synaptosomes was measured during 10 min of incubation after addition of inhibitor. For the *in vivo* experiments, male albino rats, 150 g, of the Wistar strain (Harlan Industries, Cumberland, Indiana) were used. All drug injections were given intraperitoneally, spiperone (Janssen Pharmaceutica) at 0·5 mg kg^{-1}, (+)-amphetamine sulphate (Chemical Procurement) at 15 mg kg^{-1}, amfonelic acid (Sterling-Winthrop) at 5 mg kg^{-1}, and pemoline (synthesized in the Lilly Research Laboratories) at 80 mg kg^{-1}. Rats were decapitated, and the DOPAC concentration in whole brain was measured spectrofluorometrically by the method of Murphy, Robinson & Sharman (1969). There were 5 rats per experimental group, and all data are presented as mean values ± standard errors. Comparison between groups were made by the Student's *t*-test.

Fig. 1 shows the effect of the three drugs on dopamine uptake and release by synaptosomes *in vitro*. (+)-Amphetamine and amfonelic acid were about equally effective as uptake inhibitors. Both compounds at higher concentrations released dopamine. 10^{-8} and 10^{-7}M, amfonelic acid was a more effective releaser than amphetamine but the converse was true at 10^{-6} and 10^{-7} M. Pemoline was much less effective both as an uptake inhibitor and as a releaser of dopamine.

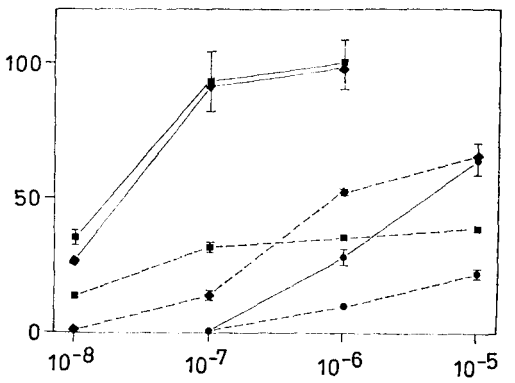

FIG. 1. Inhibition of [^{3}H]dopamine uptake (solid lines) and release of [^{3}H]dopamine (broken lines) in rat striatal synaptosomes by amfonelic acid (■), (+)-amphetamine (◆) and pemoline (●). Ordinate: % inhibition of uptake or % release. Abscissa: Concentration (M).

Table 1. *Influence of pemoline, amfonelic acid and amphetamine on the spiperone-induced elevation of DOPAC concentration in rat brain.*

Treatment group	Brain DOPAC, ng g^{-1}	
	No pretreatment	Spiperone-pretreated
Experiment 1		
Control	78 ± 2	325 ± 18
Amfonelic acid	88 ± 2* (+13%)	858 ± 50* (+164%)
Amphetamine	40 ± 4* (−49%)	250 ± 18* (−23%)
Experiment 2		
Control	99 ± 4	304 ± 9
Pemoline	90 ± 2 (−9%)	235 ± 44* (−23%)

* Significantly different from corresponding control group, $P<0.05$

Experimental drugs were injected 1 h after spiperone and 1 h before the rats were killed.

* Correspondence.

Table 1 shows the results of two experiments in which brain DOPAC concentration was measured in control rats and in spiperone-pretreated rats given the three drugs. In both experiments, spiperone caused a greater than three-fold increase in DOPAC concentration. Amfonelic acid, which alone had only a slight effect on DOPAC, greatly enhanced the spiperone-induced elevation of DOPAC. This is in agreement with the report of Shore (1976a, b), who used haloperidol instead of spiperone. Amphetamine alone markedly decreased DOPAC and attenuated rather than enhanced its spiperone-induced elevation. Brain DOPAC concentration was not significantly changed by pemoline alone in this experiment (in other experiments we have observed a slight but statistically significant decline in DOPAC at 1 h after pemoline at this dose). Pemoline significantly diminished the spiperone-induced increase in DOPAC. Both pemoline and amphetamine lowered DOPAC by 23% in spiperone-pretreated rats whereas amfonelic acid increased DOPAC by 164% in these rats. Thus pemoline resembles amphetamine rather than amfonelic acid.

Amfonelic acid and amphetamine similarly inhibit dopamine uptake into synaptosomes *in vitro* but cause opposite changes in brain DOPAC concentration *in vivo*. This difference might be explained if amphetamine acts *in vivo* primarily by inhibiting dopamine uptake across the outer neuronal membrane, whereas amfonelic acid acts *in vivo* primarily by facilitating the release of vesicular dopamine. DOPAC is primarily formed *in vivo* inside the dopamine neuron (Roffler-Tarlov, Sharman & Tegerdine, 1971). Amfonelic acid facilitates release of granule-bound dopamine, exposing it to metabolic attack by intraneuronal monoamine oxidase.

Our results indicate that pemoline does not affect brain dopamine neurons in the same way as amfonelic acid, rather its action may be qualitatively like that of amphetamine. *In vitro* pemoline is a weaker inhibitor of dopamine uptake than amphetamine, and *in vivo* it lowers brain DOPAC concentration to a lesser extent than does amphetamine.

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Reduction of pyrogens—application of molecular filtration‡

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Although heat or alkali effectively inactivate pyrogens associated with glass or production equipment, such treatment may adversely affect chemical constituents of the dosage form (Avis, 1970). Several methods have been suggested for removing pyrogens associated with the solute: recrystallization, careful heating in the presence of dilute alkali, acid or oxidizing agents (Avis, 1970); adsorption on charcoal (Ferenczi-Szirovicza & Mod, 1975) asbestos (Avis, 1970) or other materials (Hollander & Harding, 1976); anion exchange chromatography (Palmer & Whittet, 1961: Grabner, 1975) or silicic acid thin layer chromatography (Chen, Chang & others, 1975).

Recently, pyrogenic contaminants were encountered in 5-methyltetrahydrohomofolate disodium, an expensive, oxidizable, folate antagonist with a mol. wt of 517. Four bulk lots were tested for pyrogens, by the United States Pharmacopeia (U.S.P.) XIX procedure, in 24 rabbits at 100 mg kg^{-1}. All rabbits exhibited a peak increase in colonic temperature of 0·6° or greater (mean = 1·2° range, 0·6–3·1°). Therefore, these materials did not meet compendial requirements. Since we felt that published methods may not provide optimal removal of pyrogens from an unstable drug on a multi-litre scale, molecular filtration was evaluated. Briefly, solutions containing drug and antioxidant were filtered through a 293 mm, 0·22 μm membrane to remove any

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‡ After this text was submitted a similar report came to our notice (Zimmerman G., Kruger, D. and Woog M. 1976, *Drugs made in Germany* **19**, 123–128). Both reports are in general agreement.