polyphoretin PPP, a PG antagonist, also enhanced the responses about 15 to 30% and did not show any antagonistic effects. The doses of indomethacin and PPP used here markedly inhibited the relaxation induced by 10 μg ml^{-1} ATP in the presence of histamine, 10 μM, as described in our previous paper.

From these results, it is apparent that the non-adrenergic inhibitory response to field stimulation and the response to ATP of guinea-pig tracheal muscle are different with respect to their pharmacological characteristics. We conclude that the transmitter substance of non-adrenergic inhibitory neurons is neither ATP nor PGs. The enhancement of the inhibitory response by indomethacin and PPP may be caused by inhibition of the PG-mediated negative feedback control of adrenergic neurotransmission (Hedqvist, 1973).

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Actions of amfonelic acid and other non-amphetamine stimulants on the dopamine neuron*

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The mechanism of the central actions of amphetamine has been of great interest, in part because the drug can cause effects in man resembling paranoid schizophrenia and because the drug appears to produce its central actions by releasing and blocking re-uptake of brain catecholamines (e.g. Snyder, Banerjee & others, 1974).

The most striking evidence that amphetamine acts through brain catecholamines is the observation that the drug's central effects are blocked by inhibition of tyrosine hydroxylase, the rate-limiting step in catecholamine biosynthesis, suggesting that amphetamine acts centrally largely through newly synthesized catecholamine (Weissman, Koe & Tenen, 1966; Sulser, Owens & others, 1968). Consistent with this interpretation, depletion of brain catecholamine pools by reserpine does not inhibit amphetamine's central actions, although peripheral catecholamine depletion is known to inhibit the cardiovascular actions of amphetamine.

Certain other cns stimulants produce amphetamine-like central effects in laboratory animals and in man, but the central effects of some of these are not inhibited by blockade of tyrosine hydroxylase, but are blocked or greatly attenuated by central catecholamine depletion, suggesting a mechanism different from that of amphetamine. Included in this category are cocaine, methylphenidate, and the highly potent stimulant, amfonelic acid (Snyder & others, 1974; Aceto, Harris & others, 1967; Aceto, Botton & others, 1970). All of these drugs produce in the rat a marked cns stimulation, while in man they produce not only an amphetamine-like cns stimulation, but also hallucinations, paranoid ideation and exacerbation of schizophrenic symptoms (Snyder & others, 1974; Rosenberg, F. J., personal communication).

The present study describes experiments on the action of these non-amphetamine stimulants, especially amfonelic acid (AFA). The results indicate that the non-amphetamines have a unique action on the central dopamine neuron that is quite different from that of amphetamine.

The initial indication of a unique action on the dopaminergic neuron came from observations of the effects of AFA on dopamine turnover in the corpus striatum of the rat as measured by the accumulation of the dopamine metabolites, homovanillic acid (HVA) and dihydroxyphenylacetic acid (Dopac). Subsequent studies utilized the disappearance of striatal dopamine after tyrosine hydroxylase inhibition, a useful estimate of impulse flow in the dopamine neuron (Andén, Corrodi & others, 1971). Dopamine, HVA and Dopac were measured in the corpus striatum of drug-treated rats by established fluorometric techniques (Neff & Costa, 1966; Andén, Roos & Werdinius, 1963; Murphy Robinson & Sharman, 1969).

As shown in Table 1, neither AFA nor haloperidol alone affected significantly striatal dopamine concentra-

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Table 1. *Effect of amfonelic acid, haloperidol or their combination on rat striatal dopamine, HVA and Dopac. Values are mean concentrations, $\mu g\ g^{-1} \pm$ standard error.* Rats were given the drugs s.c. and killed 90 min after drug administration.

	Concentration ($\mu g\ g^{-1} \pm$ s.e.)		
	Dopamine	HVA	Dopac
Control	13·6 ± 0·34	0·67 ± 0·03	1·28 ± 0·10
AFA (2·5 mg kg^{-1})	14·6 ± 0·35	1·65 ± 0·17	1·62 ± 0·12
Haloperidol (1 mg kg^{-1})	13·4 ± 0·53	2·66 ± 0·19	4·75 ± 0·25
AFA + haloperidol	8·68 ± 0·24*	6·14 ± 0·16*	11·4 ± 0·97*

* Differ from haloperidol only $P < 0.001$.

tions, but the combination of these drugs caused a significant lowering. AFA, given alone, slightly but significantly ($P<0.01$) raised striatal HVA levels and slightly but not significantly, levels of Dopac. Haloperidol, as expected, increased the concentration of both dopamine metabolites. When AFA and haloperidol were administered simultaneously, there resulted a very large accumulation of HVA and Dopac in amounts much greater than after haloperidol alone. This augmentation of dopamine metabolite accumulation and lowering of dopamine level by the drug combination suggested that AFA potentiated the release of dopamine to a rate faster than synthesis could replenish the amine store. Since haloperidol is believed to enhance dopamine turnover by reflexly accelerating nigrostriatal impulse flow (Andén & others, 1971), the effects of AFA described above may be explained on the basis that the stimulant acted by facilitation of impulse-induced dopamine release.

This possibility was examined by a second experimental design in which the effect of the drugs on striatal dopamine concentration was measured after blockade of dopamine synthesis. The tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine was administered to rats and 30 min later AFA, haloperidol or their combination was given. The rats were killed after an additional 30 min and striatal dopamine measured. This short time was utilized so as to minimize the lowering action of haloperidol alone in the presence of a synthesis inhibitor. As shown in Table 2, neither haloperidol nor AFA given alone had a significant effect on dopamine levels compared with control rats given only α-methyl-*p*-tyrosine, but the combination of AFA and haloperidol produced a marked lowering of striatal dopamine. Doses of AFA as low as 0·2 mg kg^{-1} had a significant effect in the presence of 0·1 mg kg^{-1} haloperidol.

Other stimulants facilitating the lowering of dopamine in the presence of haloperidol after synthesis blockade included methylphenidate and cocaine (Table 2). These stimulants, like AFA, showed no effect unless given with haloperidol. Neither (+)-amphetamine nor its (−)-isomer or (+)-methamphetamine shared the dopamine-lowering effect of the non-amphetamine stimulants, and, in fact, they inhibited dopamine decline. However, the dopamine-lowering seen with AFA

Table 2. *Effect of stimulants and other drugs on rat striatal dopamine concentration in the presence of haloperidol and synthesis blockade by α-methyl-p-tyrosine (α-MT).* Levels of significance refer to a lowering from values for haloperidol. Rats were given α-MT (50 mg kg^{-1}, i.p.) and 30 min later were given the other drugs s.c. at separate sites in the doses indicated except that γ-butyrolactone was given i.p. Animals were killed 30 min later.

Drug treatment	Dopamine concentration $\mu g\ g^{-1} \pm$ s.e.	
Control (α-MT alone)	9·73 ± 0·53	
Haloperidol (0·1 mg kg^{-1})	9·03 ± 0·36	
AFA (2·5 mg kg^{-1})	9·27 ± 0·31	
Halo. + AFA (2·5 mg kg^{-1})	4·02 ± 0·42	($P < 0.001$)
Halo. + AFA (1·0 mg kg^{-1})	5·00 ± 0·30	($P < 0.001$)
Halo. + AFA (0·2 mg kg^{-1})	7·23 ± 0·23	($P < 0.005$)
Halo. + methylphenidate (5 mg kg^{-1})	6·40 ± 0·46	($P < 0.005$)
Halo. + methylphenidate (1 mg kg^{-1})	7·47 ± 0·40	($P < 0.05$)
Halo. + cocaine (5 mg kg^{-1})	7·69 ± 0·50	($P = 0.05$)
Halo. + cocaine (15 mg kg^{-1})	5·26 ± 0·35	($P < 0.01$)
Halo. + (+)-amphetamine (5 mg kg^{-1})	11·2 ± 0·26	(NS)
Halo. + (−)-amphetamine (5 mg kg^{-1})	10·4 ± 0·49	(NS)
Halo. + (+)-methamphetamine (5 mg kg^{-1})	9·43 ± 0·43	(NS)
Halo. + benztropine mesylate (5 mg kg^{-1})	8·86 ± 0·17	(NS)
Halo. + benztropine mesylate (15 mg kg^{-1})	8·08 ± 0·50	(NS)
Halo. + desipramine (5 mg kg^{-1})	8·22 ± 0·17	(NS)
Halo. + morphine (5 mg kg^{-1})	8·56 ± 0·35	(NS)
Halo. + AFA (2·5 mg kg^{-1}) + apomorphine (5 mg kg^{-1})	9·35 ± 0·16	(NS)*
Halo. + AFA (2·5 mg kg^{-1}) + γ-butyrolactone (750 mg kg^{-1})	10·2 ± 0·42	(NS)*†

* Differ significantly from haloperidol + AFA ($P < 0.001$).
† In this instance α-MT dose was 100 mg kg^{-1}.

plus haloperidol persisted when (+)-amphetamine was added to the combination. Desipramine, morphine, or benztropine mesylate were without effect at the doses used. In other experiments with AFA, not shown, it was found that the stimulant caused a lowering of dopamine in the presence of another neuroleptic, chlorpromazine (5 mg kg^{-1}), as well as with haloperidol. In preliminary experiments, AFA showed a similar enhancement of haloperidol-induced dopamine release from the olfactory tubercle.

To further ascertain that the mechanism of the enhanced dopamine release was by facilitation of impulse-induced release of the amine, still a third type of experiment was performed. It is known that apomorphine and γ-hydroxybutyric acid inhibit nigrostriatal impulse flow, the former by a reflex action following direct stimulation of striatal dopamine receptors and the latter by a different but unknown mechanism (Walters & Roth, 1974). When apomorphine or γ-hydroxybutyric acid (in the form of its lactone, γ-butyrolactone) was given to rats which also received AFA and haloperidol after synthesis blockade, the dopamine lowering was blocked (Table 2).

Thus several lines of evidence indicate that the non-amphetamine stimulants act by facilitation of neurogenic dopamine release. Such an effect is difficult to measure under normal circumstances, but can be readily observed after enhancement of neuronal impulse flow by a neuroleptic such as haloperidol. The inability of the amphetamines to duplicate the effects of the non-

amphetamine stimulants demonstrates that although both classes of stimulants produce similar behavioural effects, their mechanisms of action seem to be quite different. Interestingly, AFA did not produce similar effects on hypothalamic noradrenaline concentrations after synthesis blockade and haloperidol administration, suggesting that at least at that site, noradrenergic neurons are not affected by AFA, a finding which is in accord with the observation that AFA does not share amphetamine's cardiovascular actions (Aceto, Harris & Lesher, 1966).

The precise mechanism by which AFA and the other non-amphetamine stimulants exert their action is not completely clear. Blockade of dopamine uptake after impulse-induced release must be considered, and in experiments examining drug effects on dopamine uptake by rat striatal synaptosomes, we found AFA to be a potent inhibitor of uptake, about 20 times more active than benztropine, a substance previously described as an active uptake inhibitor in his system (Horn, Coyle & Snyder, 1971). Benztropine mesylate, however, in doses up to 15 mg kg^{-1} showed no significant AFA-like effects on striatal dopamine concentrations in the presence of haloperidol and synthesis blockade, and also caused little behavioral activity in normal rats. Furthermore, amphetamine, inactive in enhancing dopamine depletion (Table 2) is also a potent uptake blocker (Horn & others, 1971).

Another possible mechanism may involve an action whereby the non-amphetamines enhance the movement of neuronal dopamine from a relatively non-accessible pool to an impulse-releasable site. In this regard, we have recently presented evidence for the relative unavailability of the large pool of stored dopamine in the release process (Shore & Dorris, 1975; Sears & Shore, 1975). Still a third possbility is that these drugs may act on a dopamine neuron pre-synaptic site which governs dopamine release by a nerve impulse. Regardless of the mechanism, it is clear that AFA has an action on the dopamine neuron quite different from that of amphetamine and that its actions are shared by other non-amphetamines such as cocaine and methylphenidate. These results provide further evidence for a role of brain dopamine in the central manifestations of the various stimulants.

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