Amfonelic acid, a non-amphetamine stimulant, has marked effects on brain dopamine metabolism but not noradrenaline metabolism: association with differences in neuronal storage systems*

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Several non-amphetamine stimulants including cocaine, methylphenidate (Snyder, Banerjee & others, 1974) and amfonelic acid (AFA) (Shore, 1976) mimic the central actions of amphetamine in man and other species. It is now realized from animal experiments that while all of these stimulants exert their effects via a catecholamine, the mechanism of amphetamine's actions differs from those of the non-amphetamines. Thus depletion of brain catecholamine stores with reserpine does not inhibit the central actions of amphetamine but does inhibit those of the non-amphetamines. Conversely, inhibition of tyrosine hydroxylase does not affect the non-amphetamines but blocks the central actions of amphetamine (Weissman, Koe & Tenen, 1966; Chiueh & Moore, 1975ab; Aceto, Harris & others, 1967; Aceto, Botton & others, 1970; Scheel-Krüger, 1971). It is believed that amphetamine acts specifically by releasing newly synthesized catecholamines, while the non-amphetamines mobilize catecholamines from reserpine-sensitive storage pools. The predominantly important catecholamine in amphetamine's central action is thought to be dopamine (e.g. Randrup & Munkvad, 1974; Moore, 1977), although there also exists evidence for a role of noradrenergic influence as well (Mogilnicka & Braestrup, 1976).

Amfonelic acid (AFA) is a highly potent non-amphetamine stimulant whose actions are inhibited by reserpine but not by the tyrosine hydroxylase inhibitor, α-methyltyrosine (Aceto & others, 1967; 1970). We have recently provided evidence that AFA and other non-amphetamine stimulants act on the dopamine neuron to facilitate impulse-induced dopamine overflow and that this is accomplished by an action of the drugs to mobilize the amine from a large relatively inert storage pool in such a manner that the amine becomes more available for impulse-induced release (Shore, 1976).

AFA has been reported to have little cardiovascular or anorectic actions (Aceto, Harris & Lesher, 1966), suggesting that it may not effect the release of noradrenaline from noradrenergic neurons despite its actions on dopamine neurons. To examine this possibility, we performed experiments on brain noradrenaline metabolism analagous to those reported earlier on dopamine metabolism (Shore, 1976). In the previous study it was shown that AFA caused an enhancement of the action of a dopamine receptor blocking drug in raising concentrations of the dopamine metabolites, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC), in the corpus striatum of the rat. Furthermore, AFA, when given after blockade of dopamine synthesis by α-methyltyrosine (α-MT), greatly potentiated the accelerated rate of decline of brain dopamine seen after a dopamine receptor blocking drug (haloperidol). In the present study, the effects on brain noradrenaline metabolism of AFA alone, or in combination with haloperidol or adrenoceptor blocking drugs, were studied by examining whole brain concentrations of the noradrenaline metabolite, 3-methoxy-4-hydroxyphenylglycol sulphate (MOPEG-SO_4). The effect of AFA on the rate of noradrenaline depletion after tyrosine hydroxylase inhibition was also studied. Whole brains were used because of noradrenaline's wide distribution in brain.

Female Sprague-Dawley rats, 200–250 g, were given AFA (2·5 mg kg^{-1}, s.c.) either alone or in combination with haloperidol (1·0 mg kg^{-1}, s.c.), phenoxybenzamine (25 mg kg^{-1}, i.p.) or propranolol (20 mg kg^{-1}, i.p.). All doses refer to the free acid or base. The animals were killed 90 min later. Whole brains were rapidly removed, chilled in cold saline and kept frozen on dry ice until assayed the same day for MOPEG-SO_4 (Meek & Neff, 1972) or for DOPAC (Murphy, Robinson & Sharman, 1969). Subsequent studies were made of drug effects on the depletion of whole brain noradrenaline after inhibition of tyrosine hydroxylase with DL-α-methyl-*p*-tyrosine (α-MT) (50 mg kg^{-1}, i.p.). Rats were treated with AFA and/or phenoxybenzamine 30 min after α-MT and the animals were killed 60 min after injection of α-MT. Similar experiments were carried out substituting the noradrenaline uptake inhibitor, desipramine, for AFA. Whole brain noradrenaline concentration was determined fluorometrically according to Neff & Costa (1966).

The drugs used were: amfonelic acid (Sterling-Winthrop Research Institute, Rensselaer, N.Y.) haloperidol (McNeil Laboratories, Fort Washington, PA); phenoxybenzamine (Smith, Kline & French, Philadelphia); propranolol (Ayerst Laboratories, New York); DL-α-methyl-*p*-tyrosine (Regis Chemical Company, Morton Grove, Il); and desipramine (USV Pharmaceuticals, Tuckahoe, N.Y.).

As reported by Braestrup & Nielsen (1976), phenoxybenzamine produced a significant increase in whole brain MOPEG-SO_4 concentration, while propranolol and haloperidol produced no significant change. Table 1 shows that AFA had no effect when given alone, nor did it modify the actions of the blocking drugs. Thus brain MOPEG-SO_4 concentrations were elevated to the same extent by phenoxybenzamine alone as with the

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combination of phenoxybenzamine and AFA (Student's *t*-test, $P > 0{\cdot}20$). In contrast to this lack of affect on MOPEG-SO_4, there was a marked potentiation by AFA of haloperidol's effects on brain dopamine metabolism as evidenced by the ninefold increase of brain DOPAC concentrations (Table 1). This increase in DOPAC concentrations is similar to that reported to occur in the corpus striatum (Shore, 1976). Thus the action of AFA on brain dopamine metabolism is not seen on noradrenaline metabolism.

To support this conclusion, a second experimental design was used in which the effect of AFA and phenoxybenzamine on brain noradrenaline concentration was measured after inhibition of tyrosine hydroxylase. To minimize the depletion by phenoxybenzamine alone in the presence of α-MT, a short time was used. Table 2 shows that after α-MT, AFA alone did not hasten the rate of depletion of noradrenline, nor did it accelerate significantly the noradrenalic lowering effect of phenoxybenzamine. This is in contrast to previous findings on dopamine where under similar circumstances AFA markedly potentiated the dopamine-lowering affects of haloperidol after blockade of the amine's synthesis. Such effects of AFA on dopamine metabolism were seen over a wide dose range (0·2–2·5 mg kg^{-1}) (Shore, 1976) and it could be deduced that the mechanism involved enhanced dopamine overflow.

The experiments described herein suggest that AFA does not affect the noradrenaline neuron as it does the dopamine neuron. This conclusion is in harmony with earlier reports that AFA does not produce peripheral sympathetic stimulation and that AFA-induced hyperactivity is poorly inhibited by phenoxybenzamine (Aceto & others, 1966; 1967; 1970). The short time course with α-MT may have masked some effects of AFA on the noradrenergic system, but this same time course revealed a striking action of AFA on dopamine metabolism. Therefore, AFA appears to be highly preferential in its effects on dopamine release.

How can one account for the selectivity of AFA on dopamine metabolism? AFA is an inhibitor of both noradrenaline (Aceto & others, 1970) and dopamine (Shore, 1976) neuronal membrane uptake systems. One possibility might be a selective inhibition *in vivo* of the dopamine reuptake system by AFA, but doses of 2·5 mg kg^{-1} AFA are ineffective on noradrenaline metabolism, while as little as 0·2 mg kg^{-1} is effective on dopamine metabolism (Shore, 1976). Furthermore, a large dose (5 mg kg^{-1}) of the potent noradrenaline uptake inhibitor, desipramine, did not enhance the rate of noradrenaline lowering seen when phenoxybenzamine was administered to rats treated with α-MT (Table 2). A reserpine-like action by AFA on the dopamine neuron seems unlikely, as AFA alone does not alter either DOPAC concentrations nor does the drug alone deplete striatal dopamine or enhance the rate of the amine's depletion after α-MT (Shore, 1976). A likely explanation may lie in a difference between dopaminergic and noradrenergic transmitter storage pools. With the dopamine neuron, we have presented behavioural and biochemical evidence that the rate of movement of stored dopamine

Table 1. *Effects of AFA and catecholamine receptor blockers and their combination on whole brain MOPEG-SO_4 and DOPAC content.* Rats were injected with amfonelic acid (2·5 mg kg^{-1}, s.c.), phenoxybenzamine (25 mg kg^{-1}, i.p.), propranolol (20 mg kg^{-1}, i.p.) or haloperidol (1·0 mg kg^{-1}, s.c.) and were killed 90 min later. Numbers in parentheses represent the number of animals in each group.

	MOPEG-SO_4 $\mu g\ g^{-1} \pm$ s.e.m.	DOPAC $\mu g\ g^{-1} \pm$ s.e.m.
Saline	0·157±0·004 (20)	0·107±0·011 (6)
Haloperidol	0·142±0·007 (6)	0·365±0·012 (6)
Phenoxybenzamine	0·225±0·015 (7)[a]	
Propranolol	0·176±0·007 (5)	
AFA	0·158±0·10 (7)	0·112±0·008 (6)
AFA + haloperidol	0·155±0·006 (6)	0·977±0·049 (6)[b]
AFA + phenoxybenzamine	0·211±0·011 (7)[a]	
AFA + propranolol	0·177±0·015 (6)	

[a]Differs from saline control, $P < 0{\cdot}01$ (Dunnett's *t*-test).
[b]Differs from haloperidol alone, $P < 0{\cdot}001$ (Student's *t*-test).

Table 2. *The effects of α-MT alone or in combination with other drugs on whole brain noradrenaline content.* Rats were injected with DL-α-methyl-*p*-tyrosine (50 mg kg^{-1}, i.p.). Some rats were also given either phenoxybenzamine (25 mg kg^{-1}, i.p.) or amfonelic acid (2·5 mg kg^{-1}, s.c.) or their combination 30 min later. Other rats were given desipramine (5 mg kg^{-1}, i.p.) in place of AFA. Animals were killed 60 min after α-MT. Numbers in parentheses represent the number of animals in each group.

	Noradrenaline $\mu g\ g^{-1} \pm$ s.e.m.
Saline	0·492 ± 0·007 (18)
α-MT	0·419 ± 0·012 (18)
α-MT + phenoxybenzamine	0·381 ± 0·015 (10)
α-MT + AFA	0·413 ± 0·018 (6)
α-MT + AFA + phenoxybenzamine	0·420 ± 0·019 (8)
α-MT + desipramine	0·399 ± 0·009 (11)
α-MT + desipramine + phenoxybenzamine	0·401 ± 0·016 (10)

α-MT with or without drugs showed a significant ($P < 0{\cdot}01$) lowering from saline controls. No significant difference was observed within the treated groups. (Dunnett's *t*-test).

to an impulse-releasable site occurs only slowly, such that the haloperidol-induced increase of dopamine overflow is inhibited by α-MT even at a time when 80% of stored dopamine remains in the neuron (Shore & Dorris, 1975). It has been suggested that the action of AFA in enhancing dopamine overflow, even after synthesis blockade, may be by mobilizing the major dopamine storage pool so as to make more dopamine available for neurogenic release (Shore, 1976). With regard to the noradrenaline neuron, we have demonstrated that no functional dichotomy of noradrenaline storage pools appears to exist when examined under analogous experiments on noradrenaline metabolism (McMillen & Shore, 1977). Thus intraneuronal interpool movement of noradrenaline to an impulse-releasable site appears to be normally so rapid that the process cannot be enhanced.

The results reported in the present study demonstrate that the striking effects of AFA on dopamine metabolism are not duplicated on noradrenaline metabolism. The preferential action of this and other non-amphetamine stimulants on the dopamine neuron may be a consequence of a fundamental difference in interpool neurotransmitter relations between the dopamine and noradrenaline neuronal systems.

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