Br. J. Pharmac. (1982), **77**, 511–515

THE EFFECTS OF AMFONELIC ACID AND SOME OTHER CENTRAL STIMULANTS ON MOUSE STRIATAL TYRAMINE, DOPAMINE AND HOMOVANILLIC ACID

A.V. JUORIO

Psychiatric Research Division, University Hospital, Saskatoon, Saskatchewan S7N 0X0, Canada

1 The concentrations of *p*- and *m*-tyramine, dopamine and homovanillic acid were measured in the mouse striatum following the subcutaneous administration of amfonelic acid, (+)-amphetamine or nomifensine.

2 The administration of 2.5–25 mg/kg of amfonelic acid produced a reduction in *p*-tyramine that lasted at least 8 h. *m*-Tyramine was significantly increased and this was observed between 2 and 24 h after drug treatment. The levels of homovanillic acid were increased within 4 h after amfonelic acid administration.

3 (+)-Amphetamine treatment (5 mg/kg) produced a reduction in *p*-tyramine observed up to 4 h after its administration and no significant changes in *m*-tyramine.

4 The administration of 10 mg/kg of nomifensine produced no significant changes in *p*-tyramine, *m*-tyramine or homovanillic acid. By increasing the dose to 20 mg/kg, nomifensine produced an increase in *p*-tyramine and homovanillic acid.

5 The present results support the view that amfonelic acid and (+)-amphetamine would respectively release granular or newly synthesized dopamine, both actions being accompanied by an increase in tyrosine hydroxylase activity and dopamine turnover which in turn reduces *p*-tyramine but produces no change or an increase in *m*-tyramine.

6 The effects of nomifensine were observed after the administration of a relatively high dose (20 mg/kg), that was lethal to some mice (about 20%, at 2 h), and more likely to possess unspecific actions.

Introduction

The highest concentration of the *p*- and *m*-isomers of tyramine were observed in the striatum (Philips, Durden & Boulton, 1974; Philips, Davis, Durden & Boulton, 1975) where they appeared to be stored by reserpine-sensitive granules (Boulton, Juorio, Philips & Wu, 1977; Juorio, 1979). Recent iontophoretic studies have shown that the administration of small amounts of *p*- or *m*-tyramine to cortical or caudate nucleus neurones produced a marked potentiation in the reduction in the rate of firing produced by dopamine (Jones & Boulton, 1980). Some years ago it was shown that the administration of (+)-amphetamine produced a reduction in the concentration of striatal *p*-tyramine and no change or an increase in *m*-tyramine (Danielson, Wishart & Boulton, 1976; Juorio, 1977a), an effect that was potentiated by pretreatment with chlorpromazine or haloperidol (Juorio, 1977a). These effects of (+)-amphetamine or antipsychotics on brain tyramine were observed in conjunction with an increase in dopamine turnover (Andén, Roos & Werdinius, 1964; Jori & Bernardi, 1969) and led to the hypothesis that increases in dopamine turnover occur concomitantly with both reduction in *p*-tyramine and either no change or increases in *m*-tyramine (Juorio, 1979; 1982a). The opposite effects were observed with decreases in dopamine turnover (Juorio, 1979).

Amfonelic acid produced marked increases in locomotor activity (Aceto, Harris, Lesher, Pearl & Brown, 1967), increased dopamine metabolism, and appeared to act by facilitating the transfer of dopamine from the storage pool into a mobile pool easily released by nerve impulses (Shore, 1976). Like (+)-amphetamine, amfonelic acid blocked the reuptake of dopamine and *p*- and *m*-tyramine (Shore, 1976; Dyck, 1981), but the tyramines were preferentially released compared to dopamine (Dyck, 1981).

This investigation examines the effect of amfonelic acid on the induced changes in striatal *p*- and *m*-tyramine concentrations and dopamine turnover; the concentrations of dopamine and homovanillic acid were also measured. For comparison, the effects of

0007-1188/82/110511-05 $01.00

administering two other potent central stimulants, (+)-amphetamine and nomifensine (Braestrup, 1977), were also studied.

Methods

Amfonelic acid was dissolved in 1 ml of 1 N sodium hydroxide, diluted about 10 times with 0.9% w/v NaCl solution (saline) and the pH adjusted to 7.5–8.0 with 1 N HCl. (+)-Amphetamine sulphate was dissolved in saline and nomifensine in 0.05–0.1 ml glacial acetic acid, then diluted with 5–10 ml of saline. All drugs were given by subcutaneous single injections. Amfonelic acid was generously provided by Sterling-Winthrop Research Institute, Rensselaer, New York, U.S.A.; (+)-amphetamine sulphate (as (+)-α-methyl phenylethylamine sulphate) by Health Protection Branch, Health and Welfare, Ottawa, Canada and nomifensine by Hoechst Canada, Inc., Montreal, Quebec, Canada.

Male albino Swiss mice (18–22 g body weight) were killed by decapitation; the brain was removed rapidly and the striatum, consisting mainly of the head of the caudate nucleus and including some of the underlying putamen (approximate weight was between 27 and 35 mg), was dissected out. Striata from three mice were pooled, immediately frozen on dry ice, weighed and homogenized in 0.1 N HCl containing disodium edetate (EDTA, 1 mg/ml). The amines in the tissue homogenate were derivatized with 5-dimethylamino-1-naphthalenesulphonyl (dansyl) chloride and the resultant derivatives extracted and separated by thin layer chromatography as previously described (Juorio, 1982b). The estimations of *p*- and *m*-tyramine were carried out by a high resolution mass spectrometric selected ion monitoring (integrated ion current) technique. Other details concerning this procedure have been described (Philips, *et al.*, 1974; 1975). Dopamine was estimated by the fluorimetric method proposed by Laverty & Sharman (1965), using the pooled striata of two mice. Dopamine was separated on a Dowex 50 × 4 ion exchange chromatography column, after which the acetylated fluorophore developed by condensation with 1,2-diaminoethane was estimated by spectrophotofluorimetry. Checks on the recoveries of 100 ng of added dopamine were carried out in each experiment; the percentage recovery was 85 ± 4 (12) (mean ± s.e.mean; number of experiments in parentheses). The results were corrected for losses. Homovanillic acid was estimated in the pooled striata of five mice. The tissues were homogenized in 0.1 N HCl, deproteinized with 0.4 N perchloric acid, extracted with *n*-butyl acetate and from it into 0.05 M Tris buffer and estimated fluorimetrically (Andén, Roos & Werdinius, 1963). Checks on recoveries of 200 ng of added homovanillic acid were carried out in every experiment; the percentage recovery was 79 ± 2 (21) (mean ± s.e.mean; number of experiments in parentheses) and the results corrected.

Results

Thirty minutes after the subcutaneous injection of 2.5 mg/kg of amfonelic acid, no significant changes were observed in the concentration of the *p*- or *m*-isomers of tyramine but there was a moderate increase (to 134% of controls) in the striatal concentration of homovanillic acid (Table 1); by 2 h after the treatment, there was a reduction in striatal *p*-tyramine (to 69% of controls), an increase in *m*-tyramine (to 130% of controls) and in homovanillic acid (to 149% of controls) (Table 1). A higher dose of amfonelic acid (5 mg/kg) led to reductions in striatal *p*-tyramine (to 31–81% of control values)

Table 1 Effects of the subcutaneous administration of amfonelic acid on mouse striatal *p*-tyramine (*p*-TA), *m*-tyramine (*m*-TA), dopamine (DA) and homovanillic acid (HVA)

Dose (mg/kg)	*Time* (h)	p-*TA* (ng/g)	m-*TA* (ng/g)	*DA* (ng/g)	*HVA* (ng/g)
—	—	21.0 ± 0.8 (21)	6.3 ± 0.4 (21)	10,170 ± 680 (13)	1,130 ± 50 (22)
2.5	0.5	21.7 ± 1.6 (4)	6.0 ± 0.2 (4)	—	1,510 ± 80 (7)[c]
2.5	2	14.4 ± 1.0 (9)[c]	8.2 ± 0.8 (9)[a]	—	1,680 ± 120 (7)[c]
5	1	17.1 ± 1.5 (5)[a]	6.9 ± 1.1 (5)	8,930 ± 950 (8)	1,850 ± 100 (5)[c]
5	2	13.0 ± 0.9 (8)[c]	6.9 ± 1.4 (8)	9,930 ± 600 (12)	2,180 ± 320 (12)[b]
5	4	6.6 ± 0.5 (17)[c]	9.1 ± 0.5 (17)[c]	8,260 ± 790 (9)	1,890 ± 190 (9)[c]
5	8	12.7 ± 0.9 (14)[c]	7.7 ± 1.2 (14)[c]	7,770 ± 670 (11)[a]	1,000 ± 90 (8)
5	24	22.4 ± 2.2 (6)	9.6 ± 1.5 (6)[a]	11,120 ± 510 (8)	1,090 ± 100 (8)
25	2	8.7 ± 1.3 (10)[c]	5.7 ± 0.8 (10)	—	4,260 ± 180 (4)[c]
25	4	4.3 ± 0.5 (8)[c]	11.9 ± 1.2 (8)[c]	—	3,100 ± 210 (4)[c]

Values are means (± s.e. of mean, number of experiments in parentheses) in ng/g of fresh tissue. Student's *t* test: [a] $P < 0.05$; [b] $P < 0.005$; [c] $P < 0.001$ were obtained by comparison with the controls.

Table 2 The effect of the subcutaneous administration of (+)-amphetamine sulphate (5mg/kg) on mouse striatal *p*-tyramine (*p*-TA) or *m*-tyramine (*m*-TA)

Time (h)	p-*TA* (ng/g)	m-*TA* (ng/g)
—	19.7±0.8 (20)	7.5±0.5 (18)
0.5	9.5±0.7 (12)[a]	12.0±3.0 (12)
1	5.1±0.5 (8)[a]	6.0±0.9 (7)
2	6.1±1.0 (10)[a]	7.9±0.8 (8)
4	8.5±0.7 (6)[a]	6.0±0.5 (6)
8	19.9±1.2 (9)	7.9±1.1 (7)

Values are means (±s.e. of mean, number of experiments in parentheses) in ng/g of fresh tissue. Student's *t* test: [a] $P < 0.001$ were obtained by comparison with the controls.

that were maximal at 4 h after drug administration and lasted at least 8 h, returning to control levels by 24 h after drug administration (Table 1). This dose of amfonelic acid produced significant increases (to 144–281% of controls) in striatal *m*-tyramine that were observed between 4 and 24 h after drug administration and were maximal by 8 h (Table 1). The only significant change in striatal dopamine was a moderate reduction (to 76% of controls) that was observed at 8 h after drug administration (Table 1). The administration of this same dose of amfonelic acid produced significant increases in striatal homovanillic acid that were maximal after 2 h of treatment returning to the control concentrations by 8 h (Table 1).

Higher doses of amfonelic acid (25 mg/kg) led to a reduction of striatal *p*-tyramine concentrations (to 20–41% of controls) that was observed at 2 or 4 h after its administration, and a doubling in the concentration of *m*-tyramine (to 189% of controls) observed at 4 h after treatment (Table 1). This dose of amfonelic acid (25 mg/kg) also produced marked increases (to 274–377% of controls) in the striatal homovanillic acid concentrations observed 2 or 4 h following its administration (Table 1).

The administration of (+)-amphetamine sulphate (5 mg/kg) produced a significant reduction in the concentration of striatal *p*-tyramine that was observed 30 min after drug administration, reached a maximal effect at 1 and 2 h and returned to control values at 8 h after drug administration (Table 2). The treatment produced no significant changes in mouse striatal *m*-tyramine levels (Table 2).

No significant changes were observed in striatal *p*- or *m*-tyramine at 2 h after the subcutaneous administration of 10 mg/kg of nomifensine (Table 3); however, by increasing the dose to 20 mg/kg both *p*-tyramine and homovanillic acid were increased (to 136 and 161% of controls respectively) (Table 3); no significant changes were observed in striatal *m*-tyramine (Table 3). The toxicity of this dose was also higher, causing the death of about 20% of the mice.

Discussion

These experiments show that the parenteral administration of amfonelic acid produced a reduction in mouse striatal *p*-tyramine lasting at least 8 h and a significant increase in *m*-tyramine lasting 24 h (Table 1). The treatment with amfonelic acid also increased homovanillic acid, confirming earlier experiments carried out with rat striatum (Shore, 1976; Braestrup, 1977; McMillen & Shore, 1978). The mechanism by which amfonelic acid exerts its action has yet to be explained. However, it has been suggested that this drug enhances the movement of neuronal dopamine from the granular storage pool to

Table 3 Effect of the subcutaneous administration of nomifensine (10 or 20 mg/kg) on mouse striatal *p*-tyramine (*p*-TA), *m*-tyramine (*m*-TA) or homovanillic acid (HVA)

Dose (mg/kg)	p-*TA* (ng/g)	m-*TA* (ng/g)	*HVA* (ng/g)
—	19.2±1.0 (8)	6.5±0.7 (8)	870±90 (8)
10	24.5±2.6 (9)	8.1±0.9 (9)	870±50 (10)
20	26.1±2.4 (9)[a]	7.3±0.9 (9)	1400±130 (10)[a]

The mice were killed 2 h after the injection. Values are means (±s.e. of mean, number of experiments in parentheses) in ng/g of fresh tissue. Student's *t* test: [a] $P < 0.0025$ were obtained by comparison with the controls.

another pool from which it is easily released by the nervous impulse; alternatively it may act on a dopaminergic neurone presynaptic site that controls dopamine release by the nervous impulse (Shore, 1976; Braestrup, 1977) with a resultant increase in dopamine turnover rate. It has been known for some time that increases in dopamine turnover produced by a variety of treatments are accompanied by a reduction in *p*-tyramine and no change or an increase in *m*-tyramine (see review, Juorio, 1982a) and now similar changes have been observed after the administration of amfonelic acid.

A possible explanation for these reciprocal changes between the concentrations of the *p*- and *m*-isomers of tyramine and the turnover rate of dopamine could be that either tyramine isomer could act as a modulator. Which one is acting would depend on whether tyrosine hydroxylase is activated or not; if the enzyme is activated the trend would shift towards the hydroxylation of *p*-tyrosine to form L-DOPA (Bartholini & Pletscher, 1969) which would then be decarboxylated to dopamine. In turn, the availability of *p*-tyrosine for decarboxylation will be reduced and the concentration of *p*-tyramine will be decreased. *m*-Tyramine could be formed by either *m*-hydroxylation of phenylalanine (Ishimitsu, Fujimoto & Ohara, 1980) followed by decarboxylation and/or by dehydroxylation of L-DOPA or dopamine (Boulton & Dyck, 1974). From this it follows that if tyrosine hydroxylase is activated, *m*-tyramine is the one that acts as a modulator. The opposite changes will take place when the activity of tyrosine hydroxylase is reduced and in this situation *p*-tyramine will act as a modulator (Juorio, 1982a).

(+)-Amphetamine causes a marked reduction in striatal *p*-tyramine and no change or a tendency to increase in *m*-tyramine that were observed 30 min after acute administration (Danielson *et al.*, 1976; Juorio, 1977a); the present experiments (Table 2) confirm these initial findings and further show that the reduction in *p*-tyramine lasts at least 4 h.

Nomifensine, an effective blocker of dopamine uptake (Hunt, Kannengiesser & Raynaud, 1974), produced at the higher dose level (20 mg/kg) an increase in both homovanillic acid (Gerhards, Carenzi & Costa, 1974 and Table 3) and *p*-tyramine (Table 3). These results are surprising because they are at variance with those obtained after other treatments that increase dopamine turnover (see review by Juorio, 1982a). However, the effective dose of nomifensine was relatively high (20 mg/kg) and therefore more likely to produce unrelated effects.

The present results obtained after the administration of amfonelic acid support the contention that drugs that increase dopamine turnover reduce *p*-tyramine and cause either no change or an increase in *m*-tyramine, while the expected converse effects are observed after drug-induced reductions in dopamine synthesis (Juorio, 1977a, b; 1979). This pharmacologically defined interdependence may be related to interactions (presumably modulation at the synaptic levels) which have been demonstrated by iontophoretic administration of dopamine in combination with small amounts of *p*- or *m*-tyramine (Jones & Boulton, 1980).

I thank Dr A.A. Boulton for discussions; Drs D.A. Durden and C. Kazakoff for supervising the mass spectrometric analysis; Dr B.A. Davis for synthesizing the deuterated compounds; D. Chou, M. Mizuno, S. Smith, G. Wheatley and E.P. Zarycki for expert technical assistance; Saskatchewan Health and the Medical Research Council of Canada for continuing financial support.

References

ACETO, M.D., HARRIS, L.S., LESHER, G.Y., PEARL, J. & BROWN, JR. T.G. (1967). Pharmacological studies with 7-benzyl-1-ethyl-1,4-dihydro-6-oxo-1,8-naphthyridine-3-carboxylic acid. *J. Pharmac. exp. Ther.*, **158**, 286–293.

ANDÉN, N.-E., ROOS, B.-E. & WERDINIUS, B. (1963). On the occurrence of homovanillic acid in brain cerebrospinal fluid and its determination by a fluorimetric method. *Life Sci. (Oxford)*, **2**, 448–458.

ANDÉN, N.-E., ROOS, B.-E. & WERDINIUS, B. (1964). Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. *Life Sci. (Oxford)*, **3**, 149–158.

BARTHOLINI, G. & PLETSCHER, A. (1969). Enhancement of tyrosine hydroxylation within the brain by chlorpromazine. *Experientia*, **25**, 919–920.

BOULTON, A.A. & DYCK, L.E. (1974). Biosynthesis and excretion of *m- and p*-tyramine in the rat. *Life Sci. Oxford)*, **14**, 2497–2506.

BOULTON, A.A., JUORIO, A.V., PHILIPS, S.R. & WU, P.H. (1977). The effects of reserpine and 6-hydroxydopamine on the levels of some arylalkylamines in the rat brain. *Br. J. Pharmac.*, **59**, 209–214.

BRAESTRUP, C. (1977). Biochemical differentiation of amphetamine vs methylphenidate and nomifensine in rats. *J. Pharm. Pharmac.*, **29**, 463–470.

DANIELSON, T.J., WISHART, T.B. & BOULTON, A.A. (1976). Effect of acute and chronic injection of amphetamine on intracranial self-stimulation (ICS) and some arylalkylamines in the rat brain. *Life Sci. (Oxford)*, **18**, 1237–1244.

DYCK, L.E. (1981). Methylphenidate-like stimulants *in*

vitro release [^{3}H] tyramine but not [^{14}C] dopamine. *Eur. J. Pharmac.*, **69,** 371–374.

GERHARDS, H.J., CARENZI, A. &.COSTA, E. (1974). Effect of nomifensine on motor activity, dopamine turnover rate and cyclic 3',5'-adenosine monophosphate concentrations of rat striatum. *Naunyn Schmiedebergs Arch. Pharmac.*, **286,** 49–63.

HUNT, P., KANNENGIESSER, M.-H. & RAYNAUD, J.-P. (1974). Nomifensine: A new potent inhibitor of dopamine uptake into synaptosomes from rat brain corpus striatum. *J. Pharm. Pharmac.*, **26,** 370–371.

ISHIMITSU, S., FUJIMOTO, S. & OHARA, A. (1980). Formation of *m*-tyrosine and *o*-tyrosine from L-phenylalanine by rat brain homogenates. *Chem. Pharm. Bull.*, **28,** 1653–1655.

JONES, R.S.G. & BOULTON, A.A. (1980). Interaction between *p*-tyramine, *m*-tyramine or β-phenylethylamine and dopamine on single neurons in the cortex and caudate nucleus of the rat. *Can. J. Phys. Pharmac.*, **58,** 222–227.

JORI, A. & BERNARDI, D. (1969). Effect of amphetamine and amphetamine-like drugs on homovanillic acid concentration in the brain. *J. Pharm. Pharmac.*, **21,** 694–697.

JUORIO, A.V. (1977a). Effects of D-amphetamine and antipsychotic drug administration on striatal tyramine levels in the mouse. *Brain Res.*, **126,** 181–184.

JUORIO, A.V. (1977b). Effect of chlorpromazine and other antipsychotic drugs on mouse striatal tyramines. *Life Sci. (Oxford)*, **20,** 1663–1668.

JUORIO, A.V. (1979). Drug-induced changes in the formation, storage and metabolism of tyramine in the mouse. *Br. J. Pharmac.*, **66,** 377–384.

JUORIO, A.V. (1982a). A possible role for tyramines in brain function and some mental disorders. *Gen. Pharmac.*, **13,** 181–183.

JUORIO, A.V. (1982b). The effect of γ-hydroxybutyrate on mouse striatal tyramine, dopamine and homovanillic acid. *Br. J. Pharmac.*, **75,** 447–450.

LAVERTY, R. & SHARMAN, D.F. (1965). The estimation of small quantities of 3,4-dihydroxyphenylethylamine in tissues. *Br. J. Pharmac.*, **24,** 538–548.

McMILLEN, B.A. & SHORE, P.A. (1978). Amfonelic acid, a non-amphetamine stimulant, has marked effects on brain dopamine metabolism but not noradrenaline metabolism: Association with differences in neuronal storage systems. *J. Pharm. Pharmac.*, **30,** 464–466.

PHILIPS, S.R., DAVIS, B.A., DURDEN, D.A. & BOULTON, A.A. (1975). Identification and distribution of *m*-tyramine in the rat. *Can. J. Biochem.*, **53,** 65–69.

PHILIPS, S.R., DURDEN, D.A. & BOULTON, A.A. (1974). Identification and distribution of *p*-tyramine in the rat. *Can. J. Biochem.*, **28,** 366–373.

SHORE, P.A. (1976). Actions of amfonelic acid and other non-amphetamine stimulants on the dopamine neuron. *J. Pharm. Pharmac.*, **28,** 855–857.

(Received May 10, 1982.
Revised June 14, 1982)