"Designer" Amphetamines: Effects on Behavior and Monoamines With or Without Reserpine and/or α-Methyl-para-tyrosine Pretreatment

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Rats were given single injections of vehicle or one of three doses of (+)-amphetamine (AM), 4-methoxyamphetamine (MA) or 4-ethoxyamphetamine (EA) after pretreatment with vehicle or reserpine, and vehicle or α-methyl-para-tyrosine (AMPT). EA is a "designer" drug that was recently seized from an illicit laboratory in Canada. Locomotion of the rats was recorded after treatment with the drugs, and whole brain levels of the drugs as well as monoamine neurotransmitters and their major acidic metabolites were then determined. Neither of the ring-substituted AM analogues influenced locomotion. AM induced locomotion in a dose-dependent manner, and this effect was blocked by AMPT but potentiated by reserpine. Brain concentrations of EA were lower than those of the other two drugs. The brain levels of monoamines and their metabolites indicate that AM releases a newly synthesized pool of dopamine which is transferred to vesicles after re-uptake. A very low dose of AM, but not higher doses, was found to elevate serotonin (5-hydroxytryptamine: 5-HT) levels independently of effects on catecholamines. Both MA and EA affected monoamine metabolites in a manner consistent with actions as reversible inhibitors of monoamine oxidase—an effect which has been previously demonstrated to be true for MA. Both drugs increased 5-HT levels at a very low dose, as did AM, but also increased noradrenaline levels at this dose. It is concluded that EA is not a psychomotor stimulant, but is similar in many of its effects to MA, a potent hallucinogen.

Key Words: amphetamine, designer drugs, 4-methoxyamphetamine, 4-ethoxyamphetamine, monoamines, reserpine, α-methyl-para-tyrosine

INTRODUCTION

4-ethoxyamphetamine (EA) is a compound with potential as a drug of abuse. Quantities of this compound were seized by the Canadian police in 1987 from an illicit laboratory which was found to be synthesizing "designer" amphetamine (AM) compounds. The compound was subsequently determined to be EA by the Drug Identification Division of Health and Welfare, and thereupon synthesized by one of the authors (A.W.B.). Thus, there is an interest in determining the behavioral and neurochemical profile of this drug. It has structural similarity to AM, a potent psychomotor stimulant without direct hallucinogenic effects, and 4-methoxyamphetamine (MA), an AM analogue with virtually no stimulant effects but with major hallucinogenic properties (Shulgin 1978). Little is currently known concerning the properties of EA. The present experiments on EA examined the locomotor stimulant effects of this drug in comparison to those of AM and its 4-methoxylated analogue. The effects of reserpine and/or a catecholamine synthesis inhibitor, α-methyl-para-tyrosine (AMPT), on locomotor activity and on whole brain levels of monoamines and their major acidic metabolites induced with these drugs were also determined.

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The behavioral studies were predicated on the possibility that EA may produce psychomotor stimulant effects, as does AM, or that the potential for such stimulant effects may be uncovered by pretreatment with reserpine, which potentiates the stimulant effects of AM (Ross 1979). AM-induced locomotion has been established to be a function of dopamine (DA) release, primarily in the nucleus accumbens, requiring both intact DA terminals (Kelly and Iversen 1976) and intact DA synthesis (Miller and Shore 1982; Scheel-Kruger 1971). Assessing the effects of EA on locomotion is therefore an indirect assessment of increased DA in the synaptic cleft, either via increased release or inhibition of DA re-uptake. It is also of interest to determine if the neurochemical actions of the ring-substituted AMs follow the pattern of AM-like or of methylphenidate-like psychomotor stimulants. AM-like stimulants release a newly-synthesized pool of DA independent of either impulse activity in the cell (Fischer and Cho 1979; Raiteri et al 1979) or calcium (Arnold et al 1977; Westerink et al 1989) possibly via a carrier-mediated accelerative exchange-diffusion mechanism (Kuczenski 1978; Kuczenski et al 1990). While AM releases DA from a reserpine-insensitive and synthesis-sensitive pool, methylphenidate-like stimulants primarily block re-uptake and/or increase impulse-dependent release of DA, and the behavioral effects of these agents are blocked by reserpine rather than by synthesis inhibition (Clemens and Fuller 1979; Miller and Shore 1982; Reigle et al 1981; Ross 1977; 1979; Ross and Renyi 1978; Scheel-Kruger 1971). The two classes of psychomotor stimulants are also differentiated by their effects on one of the acid metabolites of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), methylphenidate-type stimulants increase while AM-like stimulants decrease DOPAC in brain (Braestrup 1977). The metabolite is thought by some researchers (Kuczenski et al 1990; Kuczenski and Segal 1989) to primarily (but not exclusively) reflect metabolism of cytoplasmic DA.

In the present study rats were treated with vehicle or one of three doses of AM, MA or EA after pretreatment with vehicle or reserpine, a vesicle-disrupting drug that depletes intra-terminal stores of monoamines, and with vehicle or AMPT, a catecholamine synthesis inhibitor. Locomotor activity was measured following these injections. The animals were then killed, their brains removed and brain levels of noradrenaline, DA and its metabolites (homovanillic acid [HVA] and 3,4-dihydroxyphenylacetic acid [DOPAC]), and serotonin (5-hydroxytryptamine, 5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) were assessed, as were the brain levels of the drugs. If EA produces psychomotor effects, then these experiments will determine whether or not such stimulant actions are AM- or methylphenidate-like. In addition, the neurochemical measures will help to elucidate any influences on monoamines that may or may not have behavioral consequences.

METHODS

The effects of vehicle and three doses of EA hydrochloride, MA hydrochloride and AM sulphate (2, 8 and 32 μmol/kg, sc) on locomotor behavior was determined. These doses of AM have been used previously in this laboratory in a variety of experiments, and have been found to cover a wide range of behavioral effects, including a dose with minimal stimulant effects (2 μmol/kg), one (8 μmol/kg) that produces maximal locomotion, and a dose sufficient to induce intense stereotyped behaviors (32 μmol/kg). Subjects of these experiments were male Sprague-Dawley rats (200-300 g at the beginning of the study) with 8-10 rats in each group. They were maintained on a 12:12 h light: dark cycle (lights on from 07:00-19:00), and tested between 09:00 and 15:00. Each experiment utilized drug and experiment naive groups of animals. Two rats from each group were selected randomly and tested on each working day.

Rats were individually placed in cages (25 x 25 x 30 cm) equipped with two infrared photobeams transecting the length of the cages, placed 3 cm from the wire grid floor and spaced 14 cm apart. The sensitivity of the photobeams were set such that only gross body movements were counted (Acadia Instruments) and repetitive counts that may be due to stereotypy, grooming, or tail movements excluded. Interruptions of photobeams were counted by computer in blocks of 10 min. Rats received one injection (vehicle or reserpine, 2.5 mg/kg, i.p.) 24 h before the second injection (vehicle or AMPT-methyl ester, 50 mg/kg, i.p.). Animals were acclimatized to the testing boxes for 1 h after the second injection. Food (Wayne Rodent Blox) and water were available *ad libitum*, except during the two hour tests. The rats received the third and final injections (vehicle, AM, EA or MA), and were then returned to the test boxes for 1 h.

Results were subjected to analysis of variance with three independent factors (reserpine or vehicle pretreatment [2 levels], AMPT or vehicle pretreatment [2 levels] and treatment drug [vehicle, AM, MA and EA — 4 levels]). The treatment groups were therefore as follows, where V = vehicle, R = reserpine, α = AMPT, A = AM, M = MA, E = EA, 0 = vehicle for AM or its analogue, 2, 8 and 32 (as subscripts) = doses of the compounds: VVA_0, VVA_2, VVA_8, VVA_{32}, VVM_0, VVM_2, VVM_8, VVM_{32}, VVE_0, VVE_2, VVE_8, VVE_{32}, $V\alpha A_0$, $V\alpha A_2$, $V\alpha A_8$, $V\alpha A_{32}$, $V\alpha M_0$, $V\alpha M_2$, $V\alpha M_8$, $V\alpha M_{32}$, $V\alpha E_0$, $V\alpha E_2$, $V\alpha E_8$, $V\alpha E_{32}$, RVA_0, RVA_2, RVA_8, RVA_{32}, RVM_0, RVM_2, RVM_8, RVM_{32}, RVE_0, RVE_2, RVE_8, RVE_{32}, $R\alpha A_0$, $R\alpha A_2$, $R\alpha A_8$, $R\alpha A_{32}$, $R\alpha M_0$, $R\alpha M_2$, $R\alpha M_8$, $R\alpha M_{32}$, $R\alpha E_0$, $R\alpha E_2$, $R\alpha E_8$, and $R\alpha E_{32}$. Time, as blocks of 10 minutes, was also included as a dependent factor in preliminary analysis, However, since the block factor did not alter the interpretation of results, only total activity over a 1 h period is included in the Results section.

The rats were sacrificed by guillotine decapitation immediately after behavioral testing. The brains were removed and frozen in isopentane on solid carbon dioxide and then stored at -80°C until the day of analysis. At this point, half of each brain was prepared and neurochemicals were assayed using high-pressure liquid chromatography with electrochemical detection, following the method of Baker et al (1987). The remaining brain hemisphere was analyzed for content of the appropriate AM analogue via gas chromatography with electron-capture detection following extraction and derivatization procedures developed at this laboratory (Baker et al 1986). The results of the chemical analyses were subjected to the appropriate multivariate analysis of variance with Tukey's HSD post-hoc tests.

RESULTS

Analysis of variance of the behavioral results (Figure 1) revealed a significant reserpine x AMPT x AM compound (drug) x dose of AM compound (dose) effect ($F(6/368) = 7.67, p < .001$). Individual comparisons were made between groups treated with a particular stimulant and the vehicle control within a particular pretreatment set (eg. VVA (2, 8 or 32 μmol/kg doses) with VVV, or $R\alpha A_{32}$ with $R\alpha A_0$).

The two higher doses, but not the 2 μmol/kg dose, of AM significantly increased locomotion, and the effect of the middle dose was blocked by AMPT, but not by reserpine.

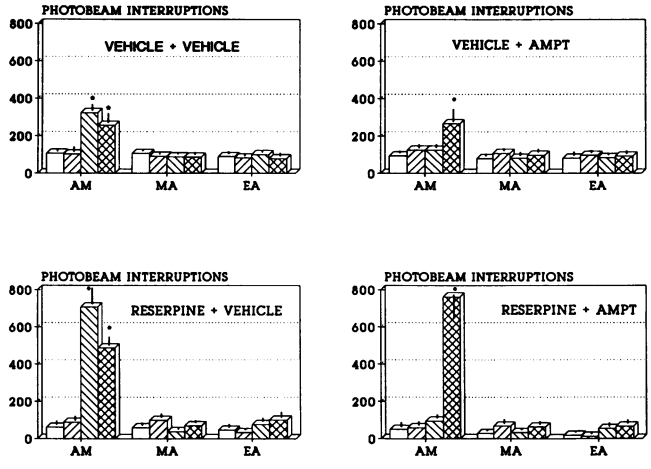

Fig. 1: Locomotor activity of rats measured by photobeam interruptions (means + SEM) for 1 h after injection (sc) with vehicle (open bars), or one of three doses (2 μmol/kg: right-diagonal bars; 8 μmol/kg: left-diagonal bars; 32 μmol/kg: cross-hatched bars) of (+)-amphetamine (AM), 4-methoxyamphetamine (MA) or 4-ethoxyamphetamine (EA) 24 h after injection with vehicle or reserpine (2.5 mg/kg, i.p.), and 1 h after injection with vehicle or AMPT (50 mg/kg, i.p.).
***Significantly different from the appropriate vehicle group, $p < .05$.**

Reserpine potentiated the effects of both doses, and AMPT increased this potentiation for the 32 μmol/kg dose, while blocking locomotion induced with the 8 μmol/kg dose. Neither of the ring-substituted AMs produced significant effects on locomotion in either direction, regardless of pretreatment regimen.

Rats were informally observed after the last drug treatment. All rats treated with RVM_{32}, 3 of 8 rats treated with $R\alpha M_{32}$, and 2 of 8 rats treated with RVE_{32} exhibited myoclonic seizures accompanied by intense salivation.

The brain levels of the drugs 3 h after administration are shown in Figure 2. Analysis of variance indicated that there was a significant drug x dose effect on drug levels ($F(6/404) = 33.3, p < .001$). Higher levels of AM than either MA or EA were found after the low dose, and the intermediate dose of EA resulted in less drug in brain tissue than either AM or MA. Levels of MA after the highest dose were higher than for the other two drugs, while EA levels were consistently lower than those of AM, and significantly so at the two lower doses.

The effects of the drugs on DA levels are illustrated in Figure 3. Reserpine significantly decreased DA levels in all groups ($F(1/368) = 702.6, p < .001$), as did catecholamine synthesis inhibition with AMPT ($F(1/368) = 133.88, p < .001$), with reserpine producing a greater effect than AMPT, and with an additive effect with the two combined. AM significantly increased DA levels at the 2 and 8, but not the 32, μmol/kg doses, and this effect was blocked by either AMPT or reserpine (reserpine x AMPT x drug x dose: $F(6/368) = 2.10, p = .05$). MA had no effect on DA levels, but the 2 μmol/kg dose of EA significantly increased DA levels. The effect of the latter drug was not blocked by AMPT, but was reversed by reserpine.

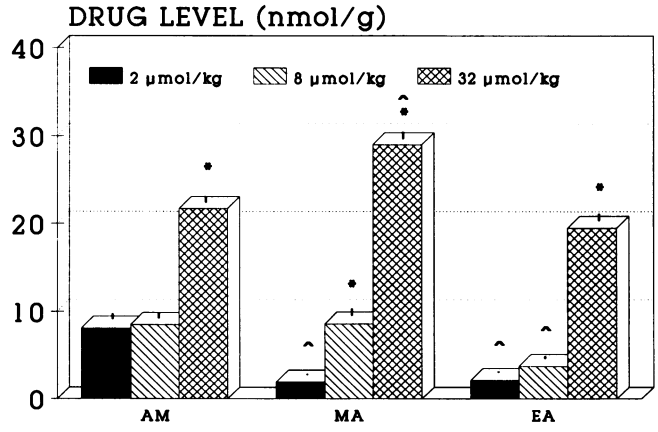

Fig. 2. Whole brain drug levels (means + SEM) from rats 1 h after treatment with one of three indicated doses of (+)-amphetamine (AM), 4-methoxyamphetamine (MA) or 4-ethoxyamphetamine (EA).
***Significantly different from the appropriate vehicle group, $p < .05$.**
^Significantly different from equivalent dose of AM, $p < .05$.

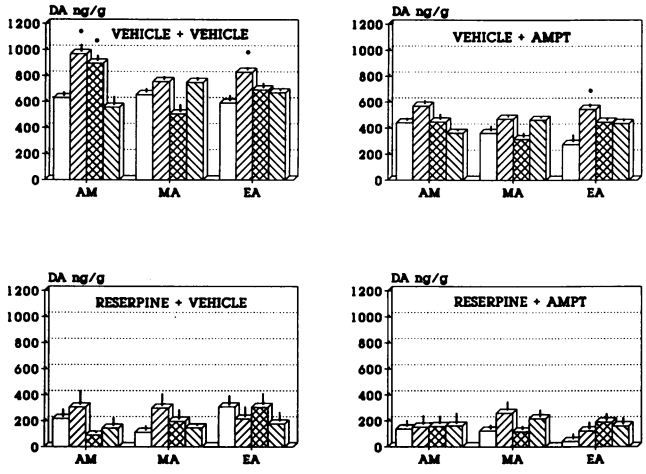

Fig. 3: Whole brain dopamine (DA) levels (mean ng/g tissue + SEM) from rats 1 h after injection (sc) with vehicle (open bars), or one of three doses (2 μmol/kg: right-diagonal bars; 8 μmol/kg: cross-hatched bars; 32 μmol/kg: left-diagonal bars) of (+)-amphetamine (AM), 4-methoxyamphetamine (MA) or 4-ethoxyamphetamine (EA) 24 h after injection with vehicle or reserpine (2.5 mg/kg, i.p.), and 1 h after injection with vehicle or AMPI (50 mg/kg, i.p.). *Significantly different from the appropriate vehicle group, $p < .05$.

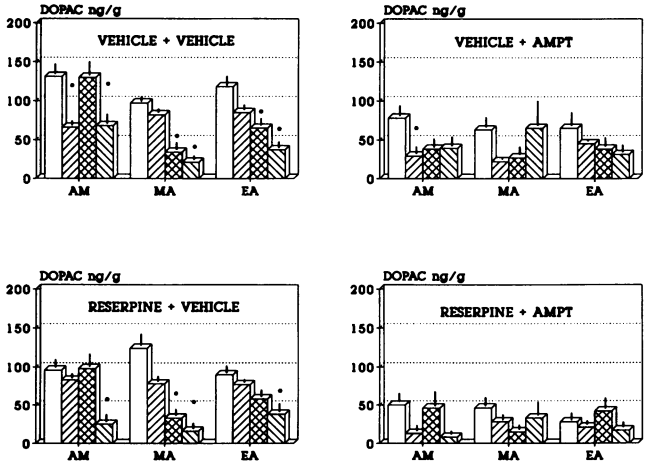

Fig. 4: Whole brain DOPAC levels (mean ng/g tissue + SEM) from rats 1 h after injection (sc) with vehicle (open bars), or one of three doses (2 μmol/kg: right-diagonal bars; 8 μmol/kg: cross-hatched bars; 32 μmol/kg: left-diagonal bars) of (+)-amphetamine (AM), 4-methoxyamphetamine (MA) or 4-ethoxyamphetamine (EA) 24 h after injection with vehicle or reserpine (2.5 mg/kg, i.p.), and 1 h after injection with vehicle or AMPT (50 mg/kg, i.p.). *Significantly different from the appropriate vehicle group, $p < .05$.

The product of metabolism of DA by monoamine oxidase, DOPAC (Figure 4), was also significantly reduced by both reserpine ($F(1/368) = 15.12$), $p < .001$) and AMPT ($F(1/368) = 118.7$, $p < .001$, across all AM compounds and doses, but unlike DA, the effects of AMPT were greater than the effects of reserpine. There was a significant AMPT x drug dose effect ($F(6/368) = 2.99$, $p < .01$). AM reduced DOPAC levels at the 2 and 32, but not the 8 μmol/kg, doses. The effects of the 2 μmo/kg dose were blocked by reserpine but not AMPT, while the effect of the highest dose of AM on DOPAC was blocked by AMPT but not by reserpine. Both of the ring-substituted AM analogues reduced DOPAC in a graded dose-dependent fashion, and this reduction was blocked by AMPT but not by reserpine, paralleling the effects of these pretreatments on the high AM dose effects (but not the low AM dose actions). Homovanillic acid levels are displayed in Figure 5. There was no significant overall effect of reserpine, but AMPT significantly decreased HVA levels ($F(1/368) = 97.2$, $p < .001$). There was a significant drug x dose effect ($F(6/368) = 5.90$, $p < .001$), with the 8 μmol/kg dose of AM and EA and the 32 μmol/kg dose of MA reducing HVA levels. These effects were blocked by AMPT (AMPT x dose effect: $F(3/368) = 12.8$, $p < .001$), and potentiated by reserpine in the absence of AMPT (reserpine x AMPT x dose effect: $F(3/368) = 3.12$, $p < .05$).

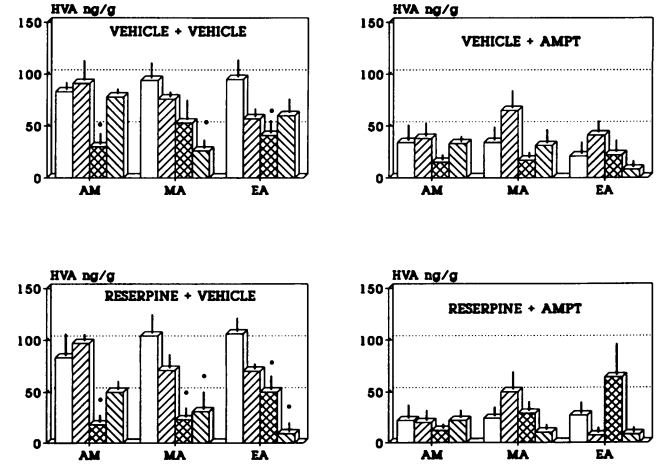

Fig. 5: Whole brain homovanillic acid (HVA) levels (mean ng/g tissue + SEM) from rats 1 h after injection (sc) with vehicle (open bars), or one of three doses (2 μmol/kg: right-diagonal bars; 8 μmol/kg: cross-hatched bars; 32 μmol/kg: left-diagonal bars) of (+)-amphetamine (AM), 4-methoxyamphetamine (MA) or 4-ethoxyamphetamine (EA) 24 h after injection with vehicle or reserpine (2.5 mg/kg, i.p.), and 1 h after injection with vehicle or AMPT (50 mg/kg, i.p.). *Significantly different from the appropriate vehicle group, $p < .05$.

Figure 6 illustrates the effects of the drugs on brain 5-HT levels. Reserpine decreased 5-HT levels over all groups ($F(1/368) = 488.9$, $p < .001$), but AMPT had no significant effect. All three drugs increased 5-HT levels at 2 μmol/kg, the lowest dose, (drug x dose effect: $F(6/368) = 4.55$, $p < .001$), and 8 μmol/kg AM also significantly increased 5-HT levels. Reserpine, but not AMPT, blocked these dose-dependent effects on 5-HT levels (reserpine x dose interaction: $F(3/368) = 18.54$, $p < .001$). DA synthesis inhibition with AMPT did not affect 5-HIAA levels (Figure 7). There was an overall increase in 5-HIAA produced by reserpine ($F(1/368) = 257.8$, $p < .001$). Furthermore, the AM drugs had dose-dependent effects on levels of 5-HIAA (drug x dose effect: $F(6/368) = 23.22$, $p < .001$). The two ring-substituted drugs significantly and equivalently decreased 5-HIAA at the two highest doses, and these effects were not altered by pretreatment. AM did not influence 5-HIAA levels, except for an increase in levels produced by 8 μmol kg after pretreatment with reserpine.

Figure 8 depicts the actions of the drugs on NA levels. Both reserpine ($F(1/368) = 726.4$, $p < .001$) and AMPT ($F(1/368) = 51.7$, $p < .001$) significantly decreased NA levels. The high dose of AM decreased NA levels, but a similar effect was not found with the ring-substituted AM analogues (drug x dose effect: $F(6/368) = 16.47$, $p < .001$). Instead, both designer AMs increased NA levels, with the largest effect produced by the 2 μmol/kg dose. The effect of AM was attenuated by both AMPT and reserpine, but

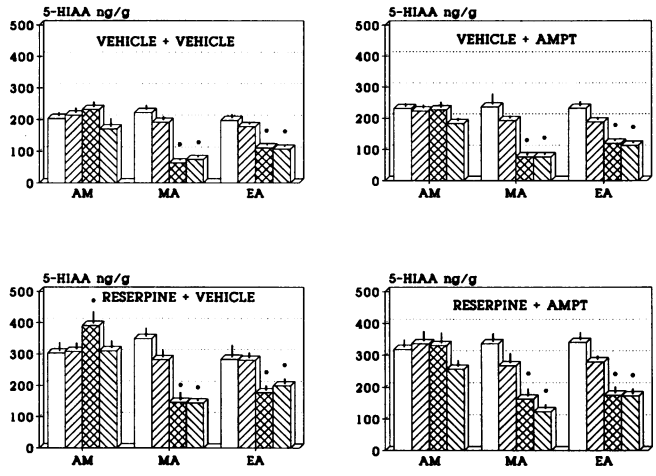

Fig. 7: Whole brain 5-HIAA levels (mean ng/g tissue + SEM) from rats 1 h after injection (sc) with vehicle (open bars), or one of three doses (2 μmol/kg: right-diagonal bars; μ8 mol/kg: cross-hatched bars; 32 μmol/kg: left-diagonal bars) of (+)-amphetamine (AM), 4-methoxyamphetamine (MA) or 4-ethoxyamphetamine (EA) 24 h after injection with vehicle or reserpine (2.5 mg/kg, i.p.), and 1 h after injection with vehicle or AMPT (50 mg/kg, i.p.).
***Significantly different from the appropriate vehicle group, $p < .05$.**

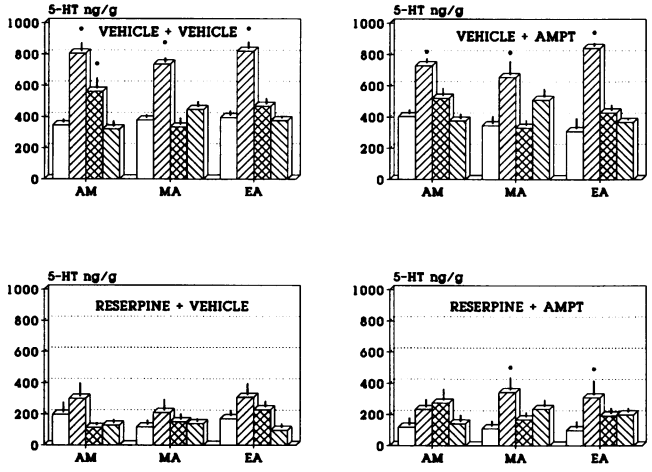

Fig. 6: Whole brain serotonin (5-HT) levels (mean ng/g tissue + SEM) from rats l h after injection (sc) with vehicle (open bars), or one of three doses (2 μmol/kg: right-diagonal bars; 8 μmol/kg: cross-hatched bars; 32 μmol/kg: left-diagonal bars) of (+)-amphetamine (AM), 4-methoxyamphetamine (MA) or 4-ethoxyamphetamine (EA) 24 h after injection with vehicle or reserpine (2.5 mg/kg, i.p.), and l h after injection with vehicle or AMPT (50 mg/kg, i.p.).
***Significantly different from the appropriate vehicle group, $p < .05$.**

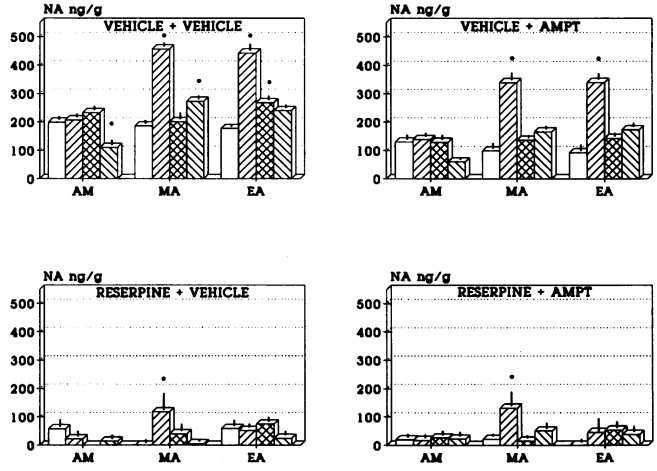

Fig. 8: Whole brain noradrenaline (NA) levels (mean ng/g tissue + SEM) from rats 1 h after injection (sc) with vehicle (open bars), or one of three doses (2 μmol/kg: right-diagonal bars; μ8 mol/kg: cross-hatched bars; μ32 mol/kg: left-diagonal bars) of (+)-amphetamine (AM), 4-methoxyamphetamine (MA) or 4-ethoxyamphetamine (EA) 24 h after injection with vehicle or reserpine (2.5 mg/kg, i.p.), and 1 h after injection with vehicle or AMPT (50 mg/kg, i.p.).
***Significantly different from the appropriate vehicle group, $p < .05$.**

this may be due to a "floor" effect. Neither AMPT nor reserpine blocked the effect of the low dose of MA on increasing NA, while reserpine (but not AMPT) blocked the similar effect of EA (reserpine x drug x dose: $F(6/368) = 6.76$, $p < .001$).

DISCUSSION

AM increased locomotion, as expected, with the greatest effect at the 8 μmol/kg dose (Figure 1), and no effect at the lowest dose (2 μmol/kg). The highest dose (32 μmol/kg) also increased locomotion, but to a lesser extent. This is likely due to the increase in localized focused oral stereotypes that typically occur with this dose. Also as expected, MA failed to influence locomotion at any dose (Biel and Bopp 1978). In this respect, EA was similar to MA, and dissimilar to AM.

The results of the reserpine and/or AMPT treatment on locomotion induced by the 8 μmol/kg dose of AM were similar to those previously reported (see Introduction). AM-induced locomotion at this dose was blocked by AMPT and the effects of the two higher doses were potentiated by reserpine. The failure of AMPT to block the locomotor stimulant effects of 32 μmol/kg AM, and, indeed, the potentiation of the effects of this dose of AM by combined treatment with reserpine and AMPT also fit the current knowledge of AM's actions. The 32 μmol/kg dose is sufficient to produce focused oral stereotypes, which are accompanied by a decrease in the stimulation of locomotion. Thus, blockade of AM's effects would attenuate oral stereotypes and shift the curve to the left, producing greater locomotion.

It is clear from these results that AM-induced locomotion is a function of actions on a newly-synthesized pool of DA, and is enhanced by disruption of vesicles storing monoamines. The potentiation of AM-induced locomotion by reserpine is likely a function of both development of postsynaptic receptor supersensitivity and the re-routing of DA that normally fills vesicles into the cytosol of the presynaptic terminal. In addition, there is likely a conversion of postsynaptic receptors from a normosensitive to an increased agonist sensitivity state (Clark et al 1985a, 1985b) as a function of DA depletion since agonist-sensitivity of DA receptors appears to be a function of history of receptor occupancy over a rather short period of time. The failure of either MA or EA to induce locomotion, even after reserpine pretreatment, argues against an effect of these drugs on the release or blockade of re-uptake of DA in the CNS.

Differences in whole brain levels of the different drugs were observed. Interestingly, EA levels were consistently lower than AM levels. After the highest dose, animals given MA had higher drug levels in the brain than animals treated with either of the other drugs. These findings are somewhat surprising because of a previous observation that after chronic administration of equimolar doses of these drugs, rats sustained higher brain levels of EA than either of the other two drugs (Martin-Iverson and Lodge in press). The present findings suggest that the EA drug may not cross the blood-brain barrier as readily as the other drugs or that it is cleared from the brain or metabolized more quickly than the other drugs. The increased EA levels previously found after chronic administration are likely the result of accumulation after decreased susceptibility to metabolism, due to protection from para-hydroxylation, a major route of metabolism for AM-related drugs in rats (Castagnoli 1978). This suggests that it is unlikely that EA is metabolized more quickly than AM, and indirectly supports the view that EA is slightly less effective at crossing the blood-brain barrier.

AM produced biphasic effects on whole brain levels of DA, with increases in DA at the lower doses, and no significant effects at the highest dose (Figure 3). This finding is consistent with the observation that low doses of AM increase DA synthesis, and as the dose of AM increases, DA synthesis decreases (Kuczenski 1980) and inhibition of metabolism of DA by MAO emerges (Green and Hait 1978). The effects of AM on DA levels were blocked by either synthesis inhibition with AMPT or by vesicle disruption with reserpine. This observation indicates that the increase in DA levels is dependent on both DA synthesis and intact vesicle stores of DA, and has implications for models of compartmentalization of DA in terminals (Arbuthnott et al 1990; Justice et al 1988). One possibility is that newly synthesized DA fills vesicles that are susceptible to reserpine's effects. However, only a very small proportion of newly synthesized DA goes into a long-term vesicular storage pool (Leviel et al 1989); most newly synthesized DA goes into a pool that is releasable by AM and resistant to reserpine's effects (see Introduction). Since newly synthesized DA is released by AM, it then follows that released DA taken into the terminal via the uptake mechanism accumulates in vesicles, and not in the pool(s) available for AM-induced release and metabolism by MAO, an alternate pathway indicated by the model of Justice and his group (1988) and discussed further by Arbuthnott et al (1990). Evidence that DA released by AM can go into a storage pool protected from metabolism has been presented (Miller and Shore 1982). In addition, it is clear that released DA taken up by the uptake system is not available to MAO since uptake inhibitors do not influence decreases in DOPAC produced by AM (Zetterstrom et al 1988). If newly taken-up DA went into a pool susceptible to MAO, then DOPAC levels should be further decreased by co-administration of uptake inhibitors. Newly-taken up DA would normally go into vesicles protected from metabolism (thus the observed decrease in DOPAC with the low dose of AM in Figure 4). In the absence of vesicles after reserpine treatment, this DA brought into the terminal by the uptake mechanism would be available for metabolism. Consistent with this view is the finding that reserpine, but not AMPT, pretreatment blocks the decrease in DOPAC produced by the low dose of AM (Figure 4).

In contrast to the effects of AM on DA levels, MA was without any significant effects on this parameter. On the

other hand, EA produced a significant elevation of DA levels, but only at the lowest dose. Interestingly, this effect of EA was not blocked by synthesis inhibition. It was blocked by reserpine, suggesting that the increase in DA produced by the lowest dose of EA, unlike the similar effect with the lowest dose of AM, was exclusively dependent upon the presence of vesicles.

DOPAC was decreased by AM at both the low and the high doses, but not at the middle dose (Figure 4). The decrease in DOPAC produced by 2 μmol/kg AM is likely due to reduction of a pool of DA available for metabolism by MAO, as DA is released from the terminal (Zetterstrom et al 1988) and as uptake mechanisms re-route DA into vesicles (as discussed above). On the other hand, the decrease in DOPAC found after a high dose is likely a function of direct inhibition of MAO by AM (Green and Hait 1978). That the effects of the low and high doses of AM on DOPAC are a function of separate actions is supported by the observation that the low dose effect is blocked by reserpine but not by synthesis inhibition, while the high dose effect is blocked by synthesis inhibition but not by reserpine. The interpretation of the decrease in DOPAC produced by AM as a result of MAO inhibition is supported by the similar finding that the effects of MA, a relatively potent MAO-A inhibitor (Green and Hait 1980), also produces a straightforward dose-dependent decrease in DOPAC that is blocked by synthesis inhibition but not by reserpine. EA produces effects on DOPAC levels similar to, but less potent than, those of MA.

The susceptibility of the high dose effect of AM, and of both intermediate and high doses of the "designer" AMs, on DOPAC levels to blockade by synthesis inhibition but not by vesicle disruption is indicative of intraterminal compartmentalization of DA and MAO. These observations indicate that DA stored in reserpine-sensitive vesicles is not normally available for metabolism by MAO. However, after reserpine treatment, DA that normally is "earmarked" for vesicle storage must be shunted into a pool that is available to MAO, likely the "free cytosolic" pool. It may be that the pool of DA normally available to MAO is, like the pool that is released by AM, primarily composed of newly synthesized DA.

The lack of a change in DOPAC after the middle dose of AM probably reflects a "mixed bag" of complex actions, as stimulation of DA synthesis decreases and becomes inhibition, as DA release becomes greater and as MAO inhibition and uptake effects become more pronounced. There has been a report of decreases in DOPAC after similar doses of AM (Kuczenski 1980), but these typically occur 60 min post-injection, rather than 180 min as in the present experiments. Recovery of extracellular DOPAC levels after a decrease produced by 2 mg/kg of AM (8 μmol/kg is equal to 1.5 mg/kg) as measured by microdialysis in the striatum is apparent after 180 min (Kuczenski and Segal 1989).

AM also produced a biphasic effect on HVA levels (Figure 5), but in a converse pattern to its effects on DOPAC. That is, both low and high doses were without significant effects on HVA levels, but the middle dose (8 μmol/kg) decreased these levels. MA produced a graded dose-dependent decrease in HVA, but the effect of EA on HVA levels was similar to that of AM: the intermediate dose significantly decreased HVA but the high dose did not. The effects of all three drugs on HVA were attenuated by synthesis inhibition. On the other hand, reserpine potentiated the effects of all three drugs. After reserpine, the intermediate dose of AM produced a greater decrease in HVA, but the decrease produced by the highest dose did not reach statistical significance.

However, the profile of EA's effects after reserpine was similar to that of MA: a graded dose-dependent decrease with the greatest effect at the highest dose. While HVA changes are difficult to interpret, the present findings of a converse relationship between DOPAC and HVA after AM, and the lack of a clear correspondence between DOPAC and HVA after EA, indicates that HVA does not necessarily come primarily from metabolism of DOPAC as has been suggested (Kuczenski and Segal 1989).

Surprisingly, the low dose of all three AM compounds had a large effect on 5-HT levels, doubling 5-HT concentrations in whole brain (Figure 6). The intermediate dose of AM also significantly increased 5-HT levels, but to a much lesser extent. On the other hand, neither the intermediate doses of MA and EA, nor the highest doses of any of the drugs, significantly influenced 5-HT levels. The effect of the low doses was not due to indirect effects mediated by direct actions on DA and NA, since monoamine synthesis inhibition did not block the low dose-induced increase. High doses of AM inhibit tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of 5-HT (Peat et al 1985). Interestingly, AM produced a slight (statistically insignificant) increase in tryptophan hydroxylase activity 3 h after an injection of 1 mg/kg (Peat et al 1985). It seems possible, therefore, that the lower dose of AM used in the present experiment (2 μmol/kg = 0.375 mg/kg) may actually stimulate tryptophan hydroxylase activity. Such an action would explain the increase in 5-HT levels found at this dose, and would parallel the biphasic effect of AM on tyrosine hydroxylase, increasing the activity of this enzyme at low doses and decreasing it at high doses. On the other hand, the increase in 5-HT levels at low doses may result from MAO inhibition in the absence of synthesis inhibition. Higher doses could fail to increase 5-HT levels because of inhibition of synthesis with concomitant MAO inhibition. However, 5-HIAA levels were not found to be decreased at low doses (Figure 7). Furthermore, AM failed to decrease 5-HIAA levels at any dose, while the two ring-substituted AM analogues only decreased 5-HIAA levels at doses that failed to increase 5-HT.

These results indicate the possibility that AM and its ring-substituted analogues have direct effects on 5-HT neurons at a dose much lower than in any previously reported study. The AMs may increase synthesis of 5-HT at the low dose, they may increase vesicular storage of 5-HT, or they may do both. Blockade of the low dose-induced increase in 5-HT by reserpine suggests that the increase is related

to vesicular storage of 5-HT, but whether this is subsequent to increased release or increased storage of 5-HT from some other non-vesicular compartment cannot be determined from the present data.

The finding that reserpine pretreatment increased 5-HIAA levels across all treatments, in spite of reduced 5-HT levels, indicates that the reduced intraneuronal vesicle storage of 5-HT increases metabolism of 5-HT. This is consistent with the view that disruption of vesicles leaves 5-HT in a cytosolic compartment available to MAO.

AM decreased NA concentrations, but only after the highest dose (Figure 8), similar to findings in a prior report (Moore et al 1970). This effect is also similar to a previously reported effect of high doses of AM analogues with potent MAO inhibition activity, tranylcypromine and related prodrugs of tranylcypromine (Coutts et al 1987; Nazarali et al 1987). The decrease in NA by these AM analogues was attributed to their ability to induce release and block reuptake. A similar mode of action may underlie the effect of AM seen in the present case. This is consistent with the attenuation of this effect found after synthesis inhibition and vesicle disruption.

Other than the differences in behavioral effects between the two "designer" amphetamines and AM, the biggest differences were found in their actions on NA. Both MA and EA increased NA levels, with the most marked effects at the lowest doses. The effects of the low dose of MA were not blocked by synthesis inhibition or reserpine. Even concomitant administration of AMPT and reserpine failed to block the increase in NA produced by the lowest dose of MA. Both treatments blocked the effects of higher doses, and reserpine, but not AMPT, blocked the effects of a low dose of EA. This indicates that an increase in synthesis may contribute to the increase in NA, but is not critical to it. The action of EA appears to be related to storage of NA, since its effects are completely blocked by reserpine. Indeed, storage of NA in vesicles likely contributes to the increase in NA produced by MA, since reserpine produces a marked attenuation of the increase. However, some other process must also be implicated in the action of MA. Possibilities include the blockade of uptake mechanisms, or the shunting of NA into a pool that is reserpine-insensitive and is not primarily newly-synthesized.

Regardless of the mechanisms of the effects of the designer AMs on NA, these effects are the most marked differences with AM and cannot be easily explained as MAO inhibition effects. It is tempting to attribute the differences in the behavioral profiles to such an effect. However, there is not strong support in the literature for attributing hallucinogenic potential to effects on NA.

In conclusion, AM induced locomotion in a dose-dependent manner, which was blocked by synthesis inhibition and potentiated by reserpine, while neither of the "designer" AMs exhibited psychomotor stimulant effects. Pretreatment with reserpine enabled MA, and, to a lesser degree EA, to induce myoclonic seizures. AM's effects on whole brain levels of monoamines and acidic metabolites reflect complex actions, including release of DA from a newly synthesized pool, biphasic actions on DA, and possibly 5-HT, synthesis, MAO inhibition at higher doses, and changes in the presynaptic compartmentation of DA. An observed decrease in NA at higher doses is likely due to increased release and blockade of re-uptake. An unexpected increase in 5-HT at low doses indicated the possibility of direct actions on 5-HT, which may suggest a biphasic action on 5-HT synthesis. None of the effects of DA as measured in postmortem tissue was clearly related to the behavioral effects of AM. The effects of MA and EA on the metabolites of DA and 5-HT are most parsimoniously explained by inhibition of metabolism of these monoamines by MAO. However, both drugs had other effects on 5-HT and NA levels that are not readily explicable by MAO inhibition. The similarity of EA's effects to those of MA suggest the possibility that EA could be hallucinogenic; however, the neuropharmacological mechanisms underlying hallucinogenic actions are not known and cannot be safely attributed to any of the neurochemical measures employed in the present study.

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REFERENCES

Arbuthnott GW, Fairbrother IS, Butcher SP (1990) Brain microdialysis studies on the control of dopamine release and metabolism in vivo. *J Neurosci Methods* **34**:73-81.

Arnold EB, Molinoff PB, Rutledge CO (1977) The release of endogenous norepinephrine and DA from cerebral cortex by amphetamine. *J Pharmarcol Exp Ther* **202**:544-557.

Baker GB, Coutts RT, Rao TS (1987) Neuropharmacological and neurochemical properties of N-(2-cyanoethyl)-2-phenylethylamine, a prodrug of 2-phenylethylamine. *Br J Pharmacol* **92**:243-255.

Baker GB, Rao TS, Coutts RT (1986) Electron-capture gas chromatographic analysis of β-phenylethylamine in tissues and body fluids using pentafluorobenzenesulfonyl chloride for derivatization. *J Chromatogr Biomed Appl* **381**:211-217.

Biel JH, Bopp BA (1978) Amphetamines: Structure-activity relationships. In: *Handbook of Psychopharmacology: Stimulants Vol 11.* Iversen LL, Iversen SD, Snyder SH (eds). New York: Plenum Press, pp 1-39.

Braestrup C (1977) Biochemical differentiation of amphetamine vs. methylphenidate and nomifensine in rats. *J Pharm Pharmacol* **29**:463-470.

Castagnoli N (1978) Drug metabolism: review of principles. In: *Handbook of Psychopharmacology: Stimulants Vol 11.* Iversen LL, Iversen SD, Snyder SH (eds). New York: Plenum Press, pp 335-387.

Clark D, Hjorth S, Carlsson A (1985a) Dopamine-receptor agonists: mechanisms underlying autoreceptor selectivity. I. Review of the evidence. *J Neural Transm* **62**:1-52.

Clark D, Hjorth S, Carlsson A (1985b) Dopamine-receptor agonists: mechanisms underlying autoreceptor selectivity. II. Theoretical considerations. *J Neural Transm* **62**:171-207.

Clemens JA, Fuller RW (1979) Differences in the effects of amphetamine and methylphenidate on brain DA turnover and serum prolactin concentration in reserpine-treated rats. *Life Sci* **24**:2077-2082.

Coutts RT, Rao TS, Baker GB, Micetich RG, Hall TWE (1987) Neurochemical and neuropharmacological properties of 4-fluorotranylcypromine. *Cell Mol Neurobiol* 7:271-290.

Fischer JF, Cho AK (1979) Chemical release of DA from striatal homogenates: evidence for an exchange diffusion model. *J Pharmacol Exp Ther* **192**:642-653.

Green AL, Hait MASE (1978) Inhibition of mouse brain monoamine oxidase by (+)-amphetamine in vivo. *J Pharm Pharmacol* **30**:262-263.

Green AL, Hait MASE (1980) p-Methoxyamphetamine, a potent reversible inhibitor of type-A monoamine oxidase in vitro and in vivo. *J Pharm Pharmacol* **32**:262-266.

Justice JB Jr, Nicolaysen LC, Michael AC (1988) Modelling the dopaminergic nerve terminal. *J Neurosci Method* **22**:239-252.

Kelly PH, Iversen SD (1976) Selective 6-OHDA induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant induced locomotor activity in rats. *Eur J Pharmacol* **40**:45-56.

Kuczenski R (1978) Biochemical actions of amphetamine and other stimulants. In: *Stimulants: Neurochemical, Behavioral and Clinical Perspectives.* Creese I (ed). New York: Raven Press, pp 31-61.

Kuczenski R (1980) Amphetamine-haloperidol interactions on striatal and mesolimbic tyrosine hydroxylase activity and DA metabolism. *J Pharmacol Exp Ther* **215**:135-215.

Kuczenski R, Segal D (1989) Concomitant characterization of behavioral and striatal neurotransmitter response to amphetamine using in vivo microdialysis. *J Neurosci* 2:2051-2065.

Kuczenski R, Segal DS, Manley LD (1990) Apomorphine does not alter amphetamine-induced DA release measured in striatal dialysates. *J Neurochem* **54**:1492-1499.

Leviel V, Gobert A, Guibert B (1989) Direct observation of DA compartmentation in striatal nerve terminal by 'in vivo' measurement of the specific activity of released DA. *Brain Res* **499**: 205-213.

Martin-Iverson MT, Lodge BA (in press) Effects of chronic treatment with "designer" amphetamines on brain regional monoamines. *Can J Physiol Pharmacol.*

Miller HH, Shore PA (1982) Effects of amphetamine and amfonelic acid on the disposition of striatal newly synthesized DA. *Eur J Pharmacol* **78**:33-44.

Moore KE, Carr LA, Dominic JA (1970) Functional significance of amphetamine-induced release of brain catecholamines. In: *Amphetamines and Related Compounds.* Costa E, Garattini S (eds). New York: Raven Press, pp 371-384.

Nazarali AJ, Baker GB, Coutts RT, Wong JTF (1987) N-(2-Cyanoethyl) tranylcypromine, a potential prodrug of tranylcypromine: Its disposition and interaction with catecholamine neurotransmitters in brain. *Pharmac Res* 4:16-20.

Peat MA, Warren PF, Bakhit C, Gibb JW (1985) The acute effects of methamphetamine, amphetamine and p-chloroamphetamine on the cortical serotonergic system of the rat brain: evidence for differences in the effects of methamphetamine and amphetamine. *Eur J Pharmacol* **116**:11-16.

Raiteri M, Cerrito F, Cervoni A, Levi G (1979) DA can be released by two mechanisms differentially affected by the DA transport inhibitor nomifensine. *J Pharmacol Exp Ther* **208**:195-202.

Reigle TG, Isaac WL, Isaac W (1981) Behavioral and neurochemical interactions of dextroamphetamine and methylphenidate in rats. *J Pharm Sci* **70**:816-818.

Ross SB (1977) On the mode of action of central stimulatory agents. *Acta Pharmacol Toxicol* **41**:392-396.

Ross SB (1979) The central stimulatory action of inhibitors of the DA uptake. *Life Sci* **24**:159-168.

Ross SB, Renyi AL (1978) Effect of amphetamine on the retention of ^{3}H-catecholamines in slices of normal and reserpinized rat brain and heart. *Acta Pharmacol Toxicol* **42**:328-336.

Scheel-Kruger J (1971) Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of the catecholamines in the brain. *Eur J Pharmacol* **14**:47-59.

Shulgin AT (1978) Psychotomimetic drugs: structure-activity relationships. In: *Handbook of Psychopharmacology: Stimulants Vol 11.* Iversen LL, Iversen SD, Snyder SH (eds). New York: Plenum Press, pp 243-333.

Westerink BHC, Hofsteede RM, Damsma G, Rollema H, de Vries JB (1989) Use of calcium antagonism for the characterization of drug-evoked DA release from the brain of conscious rats determined by microdialysis. *J Neurochem* **52**:722-729.

Zetterstrom T, Sharp T, Collin AK, Ungerstedt U (1988) In vivo measurement of extracellular DA and DOPAC in rat striatum after various DA-releasing drugs; implications for the origin of extracellular DOPAC. *Eur I Pharmacol* **148**:327-334.