Neurochemical and Behavioural Characterisation of Alkoxyamphetamine Derivatives in Rats

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The clinical utility of amphetamine and amphetamine analogues has been jeopardized by a number of side effects and toxicity, partly due to complex mechanisms of action. While some of the analogues have been individually characterised, there is still need for comparative studies, in particular, on their efficacy to release dopamine and 5-hydroxytryptamine, further enlightening some of the synaptic mechanisms conveying their actions. Thus, we have compared four alkoxyamphetamine derivatives, *i.e.*, *p*-methoxyamphetamine; *p*-methoxymethamphetamine; methylenedioxyamphetamine, methylenedioxymethamphetamine, using methamphetamine, and D-amphetamine, as reference substances, on rotational behaviour and releasing mechanisms studied with *in vivo* microdialysis in rats.

All alkoxylated-derivatives produced a long-lasting rotational behaviour at 10 mg/kg s.c., but the reference substances produced a strong rotation already at 2 mg/kg s.c. in 6-hydroxydopamine-lesioned rats. At the concentration of 100 µM, the alkoxylated-derivatives were equipotent to evoke dopamine and 5-hydroxytryptamine release in rat neostriatum, while D-amphetamine and methamphetamine were more efficient on dopamine release. Pre-treatment with methamphetamine or the alkoxylated-derivatives produced a remarkable decrease of the effect of K^+-depolarisation on both dopamine and 5-hydroxytryptamine release.

The insertion of a methoxy or a methylenedioxy group on the benzene ring of D-amphetamine or methamphetamine, or *N*-methylation of the D-amphetamine molecule alters the selectivity of the compounds. The efficacy of the alkoxylated-derivatives on dopamine and 5-hydroxytryptamine release was similar, but stimulated less dopamine release and produced less rotational behaviour than D-amphetamine and methamphetamine. The lower efficacy of K^+-depolarisation following pre-treatments with the derivatives suggests an impairment of releasable monoamine stores. The present observations can enlighten the mechanisms of action of drugs showing a high risk for abuse among young populations.

Keywords: Amphetamine; Ecstasy; Drug abuse; Rotation; Microdialysis; Rat

Abbreviations

5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine (serotonin); 6-OHDA, 6-hydroxydopamine; D-AMPH, D-amphetamine; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HPLC, high-performance liquid chromatography; HVA, homovanillic acid ; MDA, methylenedioxyamphetamine; MDMA, methylenedioxymethamphetamine (Ecstasy); METH, methamphetamine; PMA, para-methoxyamphetamine; PMMA, para-methoxymethamphetamine

INTRODUCTION

Since they were rediscovered in the 1960s and 1970s and classified as "designer drugs", several alkoxyamphetamine derivatives have been widely used for recreational purposes (Buchanan and Brown, 1988), expanding gradually to an illicit market, and becoming today the most popular substances of abuse among the young population, principally associated with "techno" parties and the "rave" culture (see Green *et al.*, 2003).

These alkoxylated-derivatives include methylenedioxymethamphetamine (MDMA), popularly known

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and sold as "Ecstasy"; methylenedioxyamphetamine (MDA); *p*-methoxyamphetamine (PMA); and *p*-methoxymethamphetamine (PMMA), among others (see FIG. 1 for structures). With some differences in their action profile, all of them produce psychomotor stimulation, but also may exhibit some hallucinogenic properties (Kalant, 2001). For instance MDA and PMA are considered to be mainly hallucinogenic, while MDMA and PMMA have been classified as entactogens (Nichols, 1986).

Methamphetamine (METH) itself and other derivatives, such as PMA and PMMA, show greater toxicity than MDMA, the most popular and widely used amphetamine analogue (Kleven and Seiden, 1992). However, due to incomplete/faulty synthesis some more toxic derivatives may be present as impurities in the ecstasy tablets, and other compounds and/or mixtures may be sold as ecstasy (Byard *et al.*, 1998; Pentney, 2001). For this reason, reports on fatalities due to the ingestion of ecstasy tablets that have occurred in the USA, Australia and Europe (Felgate *et al.*, 1998; de la Torre *et al.*, 1999; Kraner *et al.*, 2001), must consider the possibility that the tablets might have been contaminated with PMA and/or PMMA (see Kraner *et al.*, 2001; Martin, 2001; Johansen *et al.*, 2003) or other substances, revealing the real risk of these illegal amphetamines associated with the absolute lack of quality control stemming from their illicit nature.

While the drugs, to some extent, have been characterised individually, we have considered it interesting to compare them regarding neurotransmitter release and rotational behaviour, enlightening on the synaptic mechanisms conveying the action of these drugs. In particular, it was interesting to investigate them on their efficacy to release dopamine (DA) and 5-hydroxytryptamine (5-HT) and on a behaviour supposed to be elicited in the basal ganglia. Therefore, we investigated the drugs with two experimental models in rats: (i) *in vivo* microdialysis (Ungerstedt *et al.*, 1982), and (ii) rotational behaviour in unilaterally 6-hydroxydopamine (6-OHDA) -lesioned animals (Ungerstedt and Arbuthnott, 1970; Herrera-Marschitz and Ungerstedt, 1984a,b).

MATERIALS AND METHODS

6-OHDA Lesion

Male Wistar-UChA rats, weighing 150-170 g, were anaesthetised with a mixture of air and isoflurane and placed in a Kopf stereotaxic frame with the skull oriented according to the atlas of Paxinos and Watson (1982). Similarly to the original description (Herrera-Marschitz and Ungerstedt, 1984a,b), and using a 10 µl Hamilton syringe, 4 µl of 2 µg/ µl of 6-OHDA in sterile 0.9% NaCl solution containing 0.2 mg/ml ascorbate were injected into the ventral tegmental area containing the bundle of axons leaving the mesencephalic DA cell bodies (Ungerstedt, 1971), according to the following coordinates: A -4.4, L -1.3, V -7.8 (Paxinos and Watson, 1982). In all cases, the skin was puffed with a lidocaine solution (Xylocaine, 10 mg/ml; Astra, Södertälje, Sweden) before any surgery wound.

After recovery from anaesthesia the rats were transferred to a local animal department for housing, remaining in Plexiglass cases with food and water *ad libitum*, under a controlled temperature (21ºC) and with a 12:12 h light:dark cycle.

Rotational Behaviour

Two weeks after producing the lesion, the rats were transferred to the laboratory and placed in rotometers equipped with an integration system (Multicounter® model LE3806 by Letica Sci. Instruments), where turns to the left or to the right were recorded at 10 min intervals for a total period of at least 300 min. The device also estimated stereotypes, by counting un-biased motor activity, which was further manually recorded. After a stabilisation period of 20 min, the rotational behaviour was evoked by administering the dopaminergic receptor agonist apomorphine at a dose of 0.05 mg/kg s.c (Herrera-Marschitz and Ungerstedt, 1984a,b). Only rats showing contralateral rotation (at least a maximum of 10 turns/min) following the apomorphine challenge were used for further evaluations with D-amphetamine (D-AMPH), METH, and the (±)-methoxy (PMA, PMMA) or (±)-methylenedioxy (MDA, MDMA) derivatives, allowing at least two week of wash-out between doses. The rats were kept in the housing station before and after pharmacological treatments.

At the end of the experiments, the effect of the lesion was evaluated by implanting two microdialysis probes (see below), one into the left-deafferented neostriatum and the other into the right neostriatum. In the left neostriatum, DA, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) basal levels decreased by ~60%, ~90% and ~95%, respectively, compared to that observed in the right neostriatum. 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) basal levels were, however, similar in both hemispheres (see Table I).

In vivo Microdialysis

Naïve or 6-OHDA-lesioned male Wistar-UChA rats, weighing 300-350 g, were anaesthetised with a mixture of air and isoflurane, and placed in a Kopf stereotaxic frame. The rats were maintained under anaesthesia throughout the experiment by allowing them to breathe

freely into a mask fitted over the nose (1.5-2.0% of isoflurane at an airflow rate of 1.5 l/min). Two CMA 12 microdialysis probes (dialysis membrane length, 4 mm; diameter, 0.5 mm; CMA/Microdialysis AB, Stockholm, Sweden) were implanted vertically into the right and left lateral neostriata with the following coordinates: A 0.7, L ± 3.5, V -7.2 (Paxinos and Watson, 1982) at a 90 degree angle from the coronal plane.

The microdialysis probes were perfused with a Ringer solution at a flow rate of 2 µl/min with a CMA 100 microinjection pump (CMA/Microdialysis AB), collecting 30 min samples with a CMA 140 microfraction collector (CMA/Microdialysis AB). At 180 min after implantation of the microdialysis probes, the drugs were administered locally into the neostriatum via one of the microdialysis probes for 30 min (D-AMPH, METH or their alkoxylated derivatives, diluted in the Ringer solution at 100 µM) (180-210 min). At the end of the experiment a 30 min depolarising pulse of K^+ was given by adding 100 mM KCl to the perfusion medium (270-300 min after the beginning of the microdialysis experiment) through the corresponding microdialysis probe. In other experimental series, K^+-depolarisation was induced without any pre-treatment, but always added at the period 270-300 min. Changes in the perfusion medium were made with a CMA 111 syringe selector (CMA/Microdialysis AB). Body temperature was maintained at 37ºC by using a CMA 150 temperature control system (CMA/Microdialysis AB). Breathing, heart rate and motility were permanently recorded. Dialysates were collected every 30 min (60 µl) and assayed for DA, 5-HT and monoamine metabolites as described below.

Determination of Monoamines

DA and 5-HT and their metabolites (DOPAC, HVA, and 5-HIAA) were assayed by high-performance liquid chromatography (HPLC) coupled to electrochemical detection as previously described (Herrera-Marschitz *et al.*, 1996; Bustamante *et al.*, 2002), using a CMA 250 liquid chromatography pump (CMA/Microdialysis AB), a CMA 200 autoinjector (CMA/Microdialysis AB), a synergy 4-Hydro-RP column (Phenomenex, Torrance, CA), a carbon electrode held at 700 mV and a LC-4C amperometric detector (BAS, Tokyo). Quantification of the substances was achieved by comparison with standard solutions prepared freshly, and integration performed with a PC equipped with an *ad hoc* analogue-digital card and CSW® software (Pronexus, Stockholm). The detection limit was 0.2 nM for DA, DOPAC and 5-HIAA, and 1 nM for HVA. Microdialysis probes showed an *in vitro* recovery of ~15% for each substance.

Drugs and Drug Treatment

The (±)-*p*-methoxy (PMA, PMMA) and (±)-methylenedioxy (MDA, MDMA) derivatives were synthesised by standard procedures as follows: PMA was prepared by lithium aluminium hydride reduction of 1-(4-methoxyphenyl)-2-nitropropene; PMA was converted to its *N*-ethoxycarbonyl derivative by reaction with ethyl chloroformate, and the resulting amide was reduced to PMMA with lithium aluminium hydride. MDA was prepared by reduction of 1-(3,4-methylenedioxyphenyl)-2-nitropropene with lithium aluminium hydride, and MDMA was prepared by reductive amination of 1-(3,4-methylenedioxyphenyl)-2-propanone with methylamine and aluminium amalgam. Finally, the compounds were converted into their hydrochlorides to allow easy dissolution in saline solutions. METH HCl and D-AMPH sulphate (Sigma-Aldrich, St. Louis, MO) were used as reference substances. Standard substances for the analytical determinations (DA, DOPAC, HVA, 5-HT and 5-HIAA), and 6-OHDA HCl, and apomorphine HCl were purchased from Sigma-Aldrich.

For rotational behaviour studies, drugs were dissolved in 0.9% NaCl solution and administered subcutaneously (s.c.) in a volume of 1 ml/kg at the neck level after 20 min habituation in a rotometer. The drugs were administered in the following doses: apomorphine, 0.05 mg/kg s.c. (0.19 µmol/kg); D-AMPH, METH, para-methoxy- and methylenedioxy-amphetamine derivatives, 2 and 10 mg/kg s.c. (14.8 and 74 µmol/kg for D-AMPH; 13.4 and 67 µmol/kg for METH; 12.1 and 61 µmol/kg for PMA; 11.2 and 56 µmol/kg for PMMA; 11.2 and 56 µmol/kg for MDA and 10.4 and 52 µmol/kg for MDMA). For the microdialysis experiments, the drugs were dissolved in pH~7.0 Ringer solution to achieve a 100 µM concentration and perfused for 30 min after a 3 h stabilisation period as previously reported (Bustamante *et al.*, 2002).

Statistics

The rotational behaviour is expressed as the means ± the standard error of the means (SEM) of the number of turns/10 min during a total period of at least 180 min. In the microdialysis model, the levels of the assayed substances are expressed as the concentration found in the perfusates (means ± SEM). Basal values refer to the values obtained before drug (150-180 min) or before 100 mM KCl (240-270 min) administration, and set as 100%.

The effects of individual experimental interventions were analysed with *F*-ANOVA with replication followed by a *post hoc* test (Tukey's) when required. A level of $p < 0.05$ for the one-tailed test was considered to be statistically significant.

The protocols were approved by a Local Ethics Committee for Experimentation with Laboratory Animals at the Faculty level and by an *ad hoc* commission of the Chilean Council for Science and Technology Research (CONICYT), in accordance with the European Communities Council Directive of 24 November (86/609/EEC).

RESULTS

The Alkoxyamphetamine Derivatives

Figure 1 shows the chemical structures of the studied alkoxylated derivatives PMA, PMMA, MDA and MDMA, including D-AMPH and METH. PMA and PMMA are the para-methoxy derivatives of AMPH and METH, respectively. MDA and MDMA are the corresponding 3,4-methylenedioxy derivatives.

Behavioural Evaluation of Lesioned Rats

In the selected rats, D-AMPH (2 and 10 mg/kg, s.c.) produced ipsilateral rotation, with a maximum rate of ~50 turns/10 min, lasting ~150 min after 2 mg/kg, s.c. and ~100 turns/10 min after 10 mg/kg, s.c., lasting >200 min (see FIG. 2A). METH (2 and 10 mg/kg, s.c.) produced a strong ipsilateral rotation, with a maximum rate of ~130 turns/10 min; lasting >200 min after 2 mg/kg, s.c. and ~130 turns/10 min; lasting >240 min after 10 mg/kg, s.c. (see FIG. 2B; Table II).

At 2 mg/kg s.c., the AMPH (PMA and MDA) and METH (PMMA and MDMA) derivatives produced a very short-lasting, low-intensity ipsilateral rotation, followed by hypokinesia, exophthalmus, decreased exploratory activity, rigidity of the anterior limbs and resistance to handling by the experimenters. At 10 mg/kg, s.c., however, all compounds elicited long-lasting ipsilateral rotation. The strongest effect was elicited by the (±)-methylenedioxy derivatives MDA and MDMA, and the weakest by the (±)-*p*-methoxy derivatives PMA and PMMA, considering each analysed parameter (FIGs. 2C-2F; Table II).

Table I Extracellular dopamine (DA), serotonin (5-HT) and metabolites levels (nM, means ± SEM) measured in right (non-lesioned) and left (lesioned) neostriatum of unilaterally 6-hydroxy-dopamine lesioned rats. Comparisons (^{a}p <0.05, bold, underlined; *F*-ANOVA), with the respective levels on the non-lesioned (right) hemisphere.

	Right neostriatum (*n*=12)	Left neostriatum (*n*=12)
DA	6.9 ± 1.2	**3.0 ± 0.7[a]**
DOPAC	394 ± 26	**44 ± 27[a]**
HVA	789 ± 104	**83 ± 36[a]**
5-HT	3.6 ± 0.8	5.5 ± 1.3
5-HIAA	272 ± 34	275 ± 39

In vivo Microdialysis

FIG. 3 shows the time course of DA and 5-HT levels assayed by *in vivo* microdialysis. Each profile comprises: (i) the first six fractions (0-180 min), perfused with Ringer solution only; (ii) the seventh fraction (180-210 min) with drug (100 µM) in Ringer solution; (iii) two further fractions (210-270 min) with Ringer solution only. The effect of K^+-depolarisation induced by including 100 mM KCl into the Ringer solution (270-300 min) is shown in Table IV, compared to the corresponding control estimated at the fraction 240-270 min.

The first column in Table III shows basal levels of striatal DA, 5-HT and metabolites, measured during the 150-180 min period after probe implantation, perfused with Ringer solution only, in non-lesioned rats. DA and 5-HT were detected in the 5-9 and 2-3 nM ranges, respectively. The metabolites DOPAC, HVA

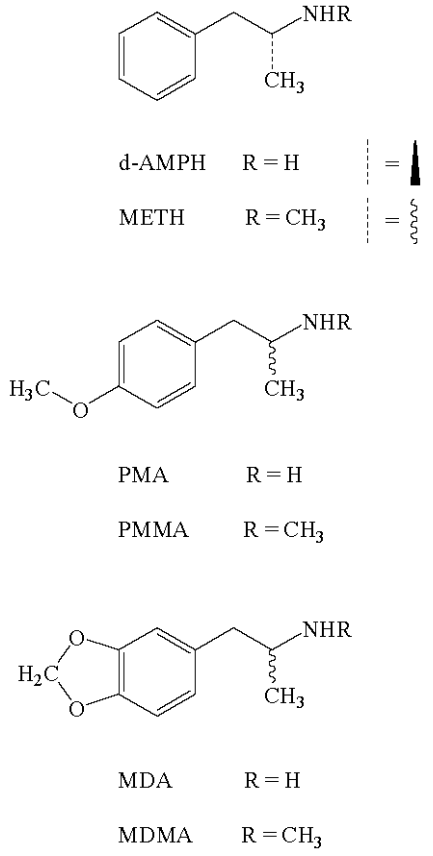

FIGURE 1 Chemical structures of D-amphetamine (D-AMPH), methamphetamine (METH), *p*-methoxyamphetamine (PMA), *p*-methoxymethamphetamine (PMMA), methylenedioxyamphetamine (MDA) and methylenedioxy-methamphetamine (MDMA). Note that D-AMPH is homochiral, whereas all the other compounds are racemic.

and 5-HIAA were detected in the 200-800 nM range.

The effect of 100 µM of the drugs administered locally via the microdialysis probe (180-210 min) is shown in the second column of Table III. All drugs produced a significant increase of DA and 5-HT levels, but the effect of D-AMPH and METH was stronger on DA than on 5-HT release (20- *vs* 3-fold for D-AMPH and 7- *vs* 3-fold for METH respectively). On the contrary, MDA, MDMA PMA and PMMA, showed the same efficacy to increase DA and 5-HT levels (see Table III and FIG. 3A-3F).

The levels of the DA metabolites DOPAC and HVA were decreased by D-AMPH, METH or the alkoxyamphetamines, but that effect was delayed, and only observed at the 210-240 min sample (~25% and 20%, respectively; $p < 0.05$). No significant effect was

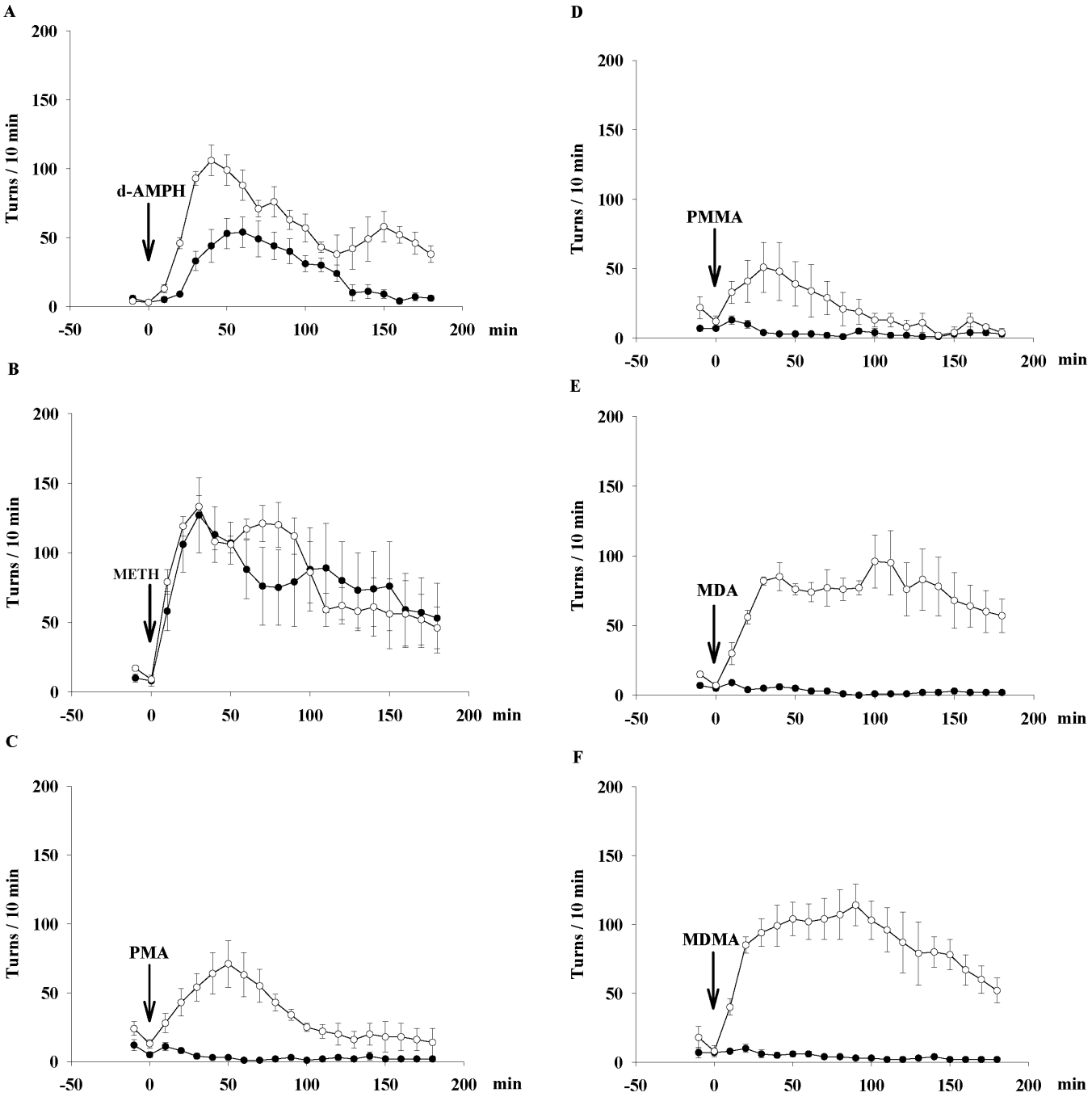

FIGURE 2A-F Ipsilateral rotational behaviour (turns/10 min) elicited by amphetamine derivatives injected s.c. (2 mg/kg [filled circles] and 10 mg/kg [open circles]) at the time marked by the arrows in unilaterally 6-hydroxydopamine-lesioned rats (see Methods). (**A**) D-Amphetamine (D-AMPH); (**B**) methamphetamine (METH); (**C**) *p*-methoxyamphetamine (PMA); (**D**) *p*-methoxymethamphetamine (PMMA); (**E**) methylenedioxyamphetamine (MDA) and (**F**) methylenedioxymethamphetamine (MDMA).

observed on 5-HIAA levels.

The effect of K^+-depolarisation was compared to the perfusion fraction immediately previous (240-270 min) to the fraction when 100 mM KCl was added to the perfusion medium (270-300 min) (Table IV). It was found that K^+-depolarisation produced a significant increase of DA and 5-HT levels (~18-fold and ~3-fold, respectively) when performed without any pre-treatment (Table IVA). After D-AMPH (Table IVB), the effect of K^+ depolarisation on DA levels was decreased (~40%), but not that on 5-HT levels (~100%). Thus, we examined in detail the effect of pre-treatment with the amphetamine derivatives on K^+-depolarisation, assuming that the K^+-depolarisation condition without any previous treatments represented a 100% condition (Table IV, last column). A decrease in the effect of K^+-depolarisation was observed on both DA and 5-HT levels after METH and all alkoxylated derivatives. The effect on K^+-stimulated DA levels was strongly decreased by more than 70% when evaluated after METH or the alkoxy-derivatives. The effect on 5-HT levels was more variable, but decreased by more than 40% after PMA and MDA (Table IV; last columns of B-G *vs* last column of A). The metabolites DOPAC, HVA and 5-HIAA showed a significant decrease following K^+-depolarisation, by ~20% (Table IV, fourth column).

Table II Ipsilateral rotational behaviour evoked by: (**A**) D-amphetamine (D-AMPH); (**B**) methamphetamine (METH); (**C**) *p*-methoxyamphetamine (PMA); (D) *p*-methoxymethamphetamine (PMMA); (E) methylenedioxyamphetamine (MDA), and (F) methylenedioxymethamphetamine (MDMA) in unilaterally 6-hydroxy-dopamine lesioned rats, expressed as maximum/10 min and total (360°) turns observed for approx. 3 hours (mean ± SEM). Comparisons, versus the corresponding dose of D-AMPH ($^a p$ <0.05, versus D-AMPH 2 mg/kg, s.c; $^b p$ <0.05, versus D-AMPH, 10 mg/kg, s.c.; *F*-ANOVA followed by Tukey's *post hoc* test; bold, underlined characters).

Drug (mg/kg, s.c.)	Maximum turns/10 min	Total turns
(A) D-AMPH (n=8)		
2 mg/kg	54±11	463±89
10 mg/kg	106±11	1078±77
(B) METH (n=4)		
2 mg/kg	**127±27**[a]	**1478±336**[a]
10 mg/kg	133±8	**1551±178**[b]
(C) PMA (n=4)		
2 mg/kg	**11±3**[a]	**56±13**[a]
10 mg/kg	71±17	624±142
(D) PMMA (n=4)		
2 mg/kg	**13±3**[a]	**68±15**[a]
10 mg/kg	**51±18**[b]	**391±156**[b]
(E) MDA (n=4)		
2 mg/kg	**9±1**[a]	**52±7**[a]
10 mg/kg	96±19	1310±168
(F) MDMA (n=4)		
2 mg/kg	**10±3**[a]	**74±15**[a]
10 mg/kg	114±15	1438±194

DISCUSSION

The present report shows that the alkoxyamphetamine derivatives PMA, PMMA, MDA and MDMA have a similar pharmacological profile to D-AMPH and METH when studied with the behavioural and neurochemical models reported here, but important differences were observed regarding selectivity. *N*-Methylation (*i.e.*, D-AMPH *vs* METH; PMA *vs* PMMA and MDA *vs* MDMA) is expected to increase

Table III Effect of: (**A**) D-amphetamine (D-AMPH); (**B**) methamphetamine (METH); (**C**) *p*-methoxyamphetamine (PMA); (**D**) *p*-methoxymethamphetamine (PMMA); (**E**) methylenedioxyamphetamine (MDA), or (**F**) methylenedioxymethamphetamine (MDMA) perfusion (100 μM) on striatal extracellular levels of dopamine (DA), DOPAC, HVA, serotonin (5-HT) and 5-HIAA (nM) measured with microdialysis in rats. %, percentage of the effect compared to basal values (150-180) ($^a p$ <0.05; 180-210 min versus 150-180 min; **^{b}p<0.05**; significantly different from the effect produced by D-AMPH [**B-G** versus **A**; last column]; *F*-ANOVA followed by Tukey's *post hoc* test; bold characters, underlined).

Drug (100 μM)	Basal Level (150-180 min)	Evoked by Drugs (180-210 min)	
	nM	nM	%
(A) D-AMPH (n=8)			
DA	6.9±1.2	**113.0±19.2**[a]	**1898±425**[a]
DOPAC	394±26	379±28	96±2
HVA	789±104	760±115	97±7
5-HT	3.0±0.6	**12.2±3.6**[a]	**361±52**[a]
5-HIAA	272±34	273±43	97±5
(B) METH (n=6)			
DA	6.2±1.7	**49.1±9**[a]	**681±131**[a,b]
DOPAC	299±40	312±22	93±9
HVA	297±54	315±49	103±12
5-HT	3.0±0.7	**7.6±1.2**[a]	**258±18**[a]
5-HIAA	201±19	206±15	110±13
(C) PMA (n=6)			
DA	4.8±0.7	**24.1±5.6**[a]	**559±87**[a,b]
DOPAC	351±49	310±63	84±8
HVA	575±105	573±106	100±5
5-HT	2.3±0.9	**9.0±2.3**[a]	**539±110**[a]
5-HIAA	238±34	246±34	104±5
(D) PMMA (n=6)			
DA	8.6±1.4	**34.5±6.0**[a]	**450±89**[a,b]
DOPAC	401±42	374±54	92±8
HVA	848±133	796±134	97±7
5-HT	3.3±1.1	**19.5±7.6**[a]	**462±122**[a]
5-HIAA	273±18	275±19	106±14
(E) MDA (n=6)			
DA	6.2±1.8	**38.6±9.5**[a]	**662±70**[a,b]
DOPAC	435±9	434±9	100±1
HVA	807±63	815±80	100±4
5-HT	3.2±1.0	**10.4±2.5**[a]	**459±132**[a]
5-HIAA	311±20	327±34	104±7
(F) MDMA (n=10)			
DA	6.7±1.6	**42.1±9.5**[a]	**653±136**[a,b]
DOPAC	278±52	247±51	92±6
HVA	580±76	625±92	108±4
5-HT	3.1±0.7	**13.8±2.3**[a]	**554±137**[a]
5-HIAA	268±32	276±31	106±4

Table IV Effect of K^+-depolarization (100 mM KCl) on striatal extracellular dopamine (DA), DOPAC, HVA, serotonin (5-HT) and 5-HIAA levels measured with microdialysis in rats after no pre-treatment (**A**) and after 100 µM of amphetamines (**B-G**). [a]p <0.05, *vs* value (240-270 min) observed immediately before adding 100 mM KCl into the perfusion medium (270-300 min); [b]p <0.05, effect of K^+-depolarization *vs* no pre-treatment (**B-G** *vs* **A**); [c]p <0.05, effect of K^+-depolarization after METH or alkoxylated derivatives *vs* the effect of K^+-depolarization after D-AMPH pre-treatment (C-G *vs* B); *F*-ANOVA followed by Tukey's *post hoc* test; bold, underlined).

Drug (100 µM)	**Basal Level (240-270 min)**	**Evoked by KCl Depolarization (270-300 min)**		
	[nM]	**[nM]**	**% (compared to corresponding basal level; 240-270 min)**	**% (compared to no pre-treatment, A)**
(A) No pre-treatment (n=12)				
DA	6.5±1.1	**114.4±21.6[a]**	**1818±182[a]**	100
DOPAC	427±15	372±26	84±5	100
HVA	700±23	601±33-	85±3	100
5-HT	3.5±0.6	**12.0±1.6[a]**	**361±54[a]**	100
5-HIAA	280±16	**219±11[a]**	**80±3[a]**	100
(B) After D-AMPH (n= 8)				
DA	8.6±1.8	**56.1±12.1[a]**	**1072±166[a,b]**	**59±9[b]**
DOPAC	310±48	303±48	96±4	114± 13
HVA	800±125	633±115	77±4	91±15
5-HT	3.7±1.5	**8.5±1.6[a]**	**309±58[a]**	109±18
5-HIAA	264±38	244±37	94±9	117±12
(C) After METH (n= 6)				
DA	5.8±0.9	**45.9±10.9[a]**	**512±58[a,b,c]**	**28 ± 3[b,c]**
DOPAC	253±19	207±26	**77±8[c]**	92±11
HVA	280±41	210±37	**74±5[c]**	87±13
5-HT	3.9±0.3	**7.8±2.1[a]**	**201±43[a]**	**56±1[b,c]**
5-HIAA	198±19	**147±19[a]**	**79±5[c]**	99±5
(D) After (±)-PMA (n= 6)				
DA	7.1 ±2.1	**33.6±13.0[a]**	**415±70[a,b,c]**	**23±4[b,c]**
DOPAC	284±70	252±85	85±8	101±6
HVA	495±96	425±100	82±7	96±9
5-HT	3.2±0.9	5.0±1.4	**184±36[b,c]**	**55±1[b,c]**
5-HIAA	201±28	175±29	86±6	107±11
(E) After (±)-PMMA (n= 6)				
DA	9.5±2.4	**55.1±10.1[a]**	**459±97[a,b,c]**	**25 ± 5[b,c]**
DOPAC	316±42	279±45	88±7	104±6
HVA	683±137	531±113	80±6 [c]	94±8
5-HT	9.0±3.6	14.1±8.3	**207±36[b,c]**	**62±11[b,c]**
5-HIAA	286±22	252±16	93±13	116±14
(F) After (±)-MDA (n= 6)				
DA	3.2±0.4	**16.3±3.3[a]**	**517±22[a,b,c]**	**28±11[b,c]**
DOPAC	422±16	387±35	93±6	110±15
HVA	775±83	678±114	83±6	98±8
5-HT	2.6±0.7	**5.6±1.8[a]**	**193±8[a,b,c]**	**57 ± 2[b,c]**
5-HIAA	270±35	224±29	74±10	93±12
(G) After (±)-MDMA (n= 10)				
DA	8.6±2.0	**39.5±19.4[a]**	**419±64[a,b,c]**	**23 ± 4[b,c]**
DOPAC	246±56	193±46	**69±7[b,c]**	**82±8[b,c]**
HVA	539±65	**393±48[a]**	**73±3[a]**	**86±3[b]**
5-HT	3.3±0.6	**7.8±1.9[a]**	**272±50[a]**	70 ± 13
5-HIAA	248±30	218±29	87±3	108±13

lipophilicity, and consequently, for these relatively hydrophilic and low-molecular weight compounds, to favour access to the brain. On the other hand, the small alkoxy groups inserted here are not expected to modify this aspect to any great extent. The alkoxy groups and the *N*-methyl group, however, should change the affinities and efficacies of these molecules at the macromolecular targets with which they interact, although the direction of these changes cannot be predicted without detailed structural knowledge of their targets.

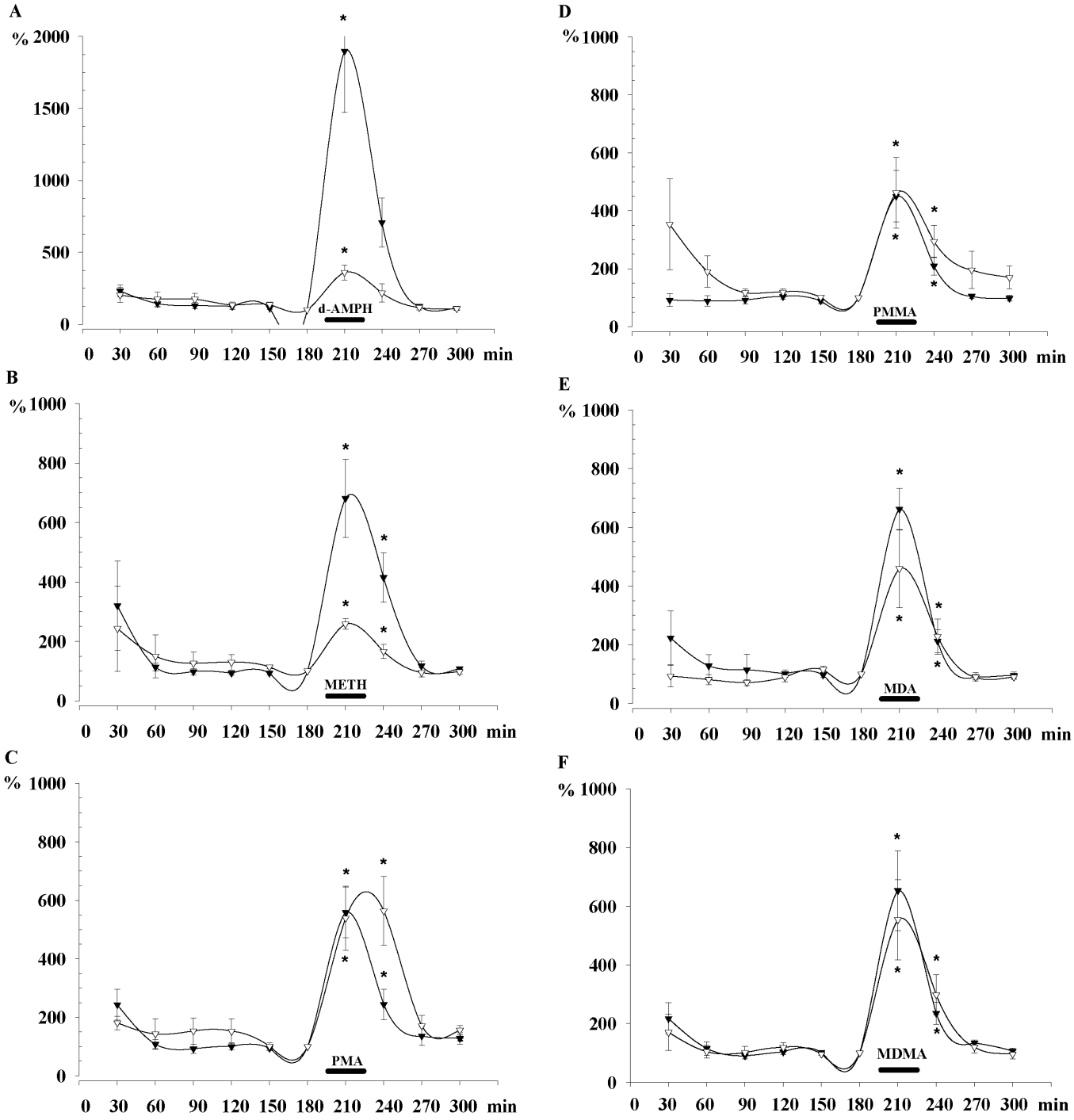

FIGURE 3A-F Effect of local perfusion of 100 µM of amphetamine derivatives on extracellular dopamine (filled columns) and 5-HT (open columns) levels in 60 µl (30 min) samples collected via a microdialysis probe implanted into the lateral neostriatum of non-lesioned rats. Each selected drug was administered for 30 min at the 180-210 min period after the probe implantation. (**A**) D-Amphetamine (D-AMPH); (**B**) methamphetamine (METH); (**C**) *p*-methoxyamphetamine (PMA); (**D**) *p*-methoxymethamphetamine (PMMA); (**E**) methylenedioxyamphetamine (MDA) and (**F**) methylenedioxymethamphetamine (MDMA). Levels are expressed as the percentage (%) (means ± SEM) of the corresponding basal value estimated at the 150-180 min period. $^{*}p < 0.05$ *vs* the corresponding basal value.

Effect of Amphetamine Derivatives on Rotational Behaviour

All drugs elicited ipsilateral rotational behaviour, probably due to an increase of DA release from the intact hemisphere (Ungertedt and Arburthnott, 1970; Ungerstedt, 1971; Zetterström *et al.*, 1986). At the same dose (10 mg/kg, s.c.), the rank-order for inducing a maximum rate of rotational behaviour (turns/min) was METH > D-AMPH >> MDMA ≥ MDA>> PMA≥PMMA. In agreement, D-AMPH and METH induced the strongest increase in DA release, and showed the highest DA/5-HT ratio regarding release stimulation.

The (±)-methoxyamphetamine derivatives, PMA and PMMA, and the (±)-methylenedioxyamphetamine derivatives, MDA and MDMA, evoked rotational behaviour, but that behaviour was robust only at a higher dose (10 mg/kg, s.c.) than that required for D-AMPH and METH (2 mg/kg, s.c.), in agreement with previous studies (Lebsanft *et al.*, 2003). Apart from a short-lasting ipsilateral rotational behaviour, at 2 mg/kg s.c., PMA, PMMA, MDA or MDMA produced hypokinesia, rigidity and hyper-reflexia of the fore-legs, exophthalmus, and resistance to handling by the experimenters. This effect was particularly evident for the AMPH derivatives PMA and MDA.

When compared at the dose of 10 mg/kg s.c., the effect induced by the (±)-methylenedioxyamphetamine derivatives, MDMA and MDA, was stronger than that induced by the (±)-para-methoxyamphetamine derivatives, PMA and PMMA (see FIG. 2), reflecting perhaps pharmacokinetic differences, because the duration of the effect produced by MDMA and MDA was particularly long (>3 h). However, while the peak effect occurred at approximately the same time for all derivatives, MDMA and MDA induced a stronger rate of rotation than PMA and PMMA, suggesting a greater efficacy. In addition, at the 10 mg/kg, s.c. dose all drugs produced marked sympathomimetic signs, such as pilo-erection, exophthalmus and increased respiratory rate, as previously reported (see Bustamante *et al.*, 2004).

Effect of Amphetamine Derivatives on DA and 5-HT Release

The microdialysis experiments showed that the tested drugs increased DA and 5-HT release when perfused at a 100 μM concentration. D-AMPH evoked the strongest effect on DA release (~20-fold), confirming the established knowledge that the mechanism of action of that drug is predominantly related to DA release (Randrup and Munkvad, 1966; Fuxe and Ungerstedt, 1970; Ungerstedt and Arbuthnott, 1970; Ungerstedt, 1971; Zetterström *et al.*, 1983; 1986; Butcher *et al.*, 1988). However, D-AMPH also evoked 5-HT release, although the effect was weaker (~3-fold) than that observed on DA. METH increased DA and 5-HT ~7- and ~3-fold respectively, confirming previous reports on DA release (O'Dell *et al.*, 1991; Kuczenski and Segal, 1992; Abekawa *et al.*, 1994; Holson *et al.*, 1996; Sabol *et al.*, 2001; Bustamante *et al.*, 2002), but also those suggesting an effect on 5-HT neuro-transmission (Tseng *et al.*, 1976; Hotchkiss and Gibb, 1980; Ricaurte *et al.*, 1980; Kuczenski *et al.*, 1995; Haughey *et al.*, 2000; Gough *et al.*, 2002). The effect of D-AMPH was 6-fold stronger on DA than on 5-HT release, but METH was only 2-fold stronger on DA than on 5-HT release. While D-AMPH and METH were more efficient on DA release, the alkoxylated amphetamine derivatives were equally efficient on 5-HT and DA release. In comparative terms, the alk-oxylated amphetamines were ~2-fold more efficient for releasing 5-HT than D-AMPH and METH, thus confirming the idea that the alkoxylated derivatives produce long term effects on brain 5-HT neurons (Peat *et al.*, 1985; Stone *et al.*, 1986; 1987; Schmidt, 1987; Johnson *et al.*, 1988; Steele *et al.*, 1992).

Effect of Amphetamine Derivatives on K^+-stimulated DA and 5-HT Release

We have previously reported (Herrera-Marschitz *et al.*, 1992; 1996) that the peak effect of K^+-depolarisation is observed under the perfusion period, rapidly decreasing after KCl is removed from the perfusion medium. Thus, in the present study, the microdialysis experiment finished after the 270-300 min period perfusing with 100 mM KCl. Local K^+-depolarisation increased DA and 5-HT release >18- and >3-fold, respectively, in rats without any previous treatment. When K^+-depolarisation was induced after D-AMPH, DA levels were only increased 10-fold (*i.e.*, the effect of K^+-depolarisation was decreased by 40%, compared to that observed in animals without any pre-treatment), but the effect on 5-HT was the same (3-fold). The decrease in the effect of K^+-depolarisation on DA release after METH and the alkoxylated amphetamine derivatives (*i.e.*, METH, PMA, PMMA, MDA and MDMA) was even stronger (by more than 70%). Regarding the effect of K^+-depolarisation on 5-HT release, the maximum decrease observed after PMMA (by 38%) and MDMA (by 30%) was more remarkable than that observed after PMA, MDA and METH (by ~45%).

These results are intriguing, and could be related to depletion of a ready-to-release pool of presynaptic monoamines, or to a decrease in the synthesis of monoamines provoked by the amphetamine pre-treatment. However, while D-AMPH elicited the strongest

DA release among all amphetamines (>20 fold for D-AMPH *vs* ~6 fold, for METH and alkoxyamphetamine derivatives), the effect of K^+-depolarisation was still strong after D-AMPH. Thus, the decreased effect of K^+-depolarisation on DA and 5-HT levels after the amphetamine derivatives may suggest rather a long-term depletion, as shown for this class of drugs by several reports (Fibiger and McGeer, 1971; Seiden *et al.*, 1976; Ricaurte *et al.*, 1980; Axt and Molliver, 1991; Pu and Vorhees, 1993). Indeed, as reported for METH (Schmidt and Gibb, 1985; Brunswick *et al.*, 1992; Eisch *et al.*, 1996; Fleckenstein *et al.*, 1997; Frey *et al.*, 1997; Brown *et al.*, 2000), the derivatives may have decreased monoamine uptake (Haughey *et al.*, 2000), thus limiting the bulk of the ready-to-release vesicular and/or non-vesicular pools. In agreement with this, it has been shown that dopamine uptake inhibitors block the neurotoxic effects produced by METH (Marek *et al.*, 1990; Pu *et al.*, 1994). In a previous report (Bustamante *et al.*, 2002), it was demonstrated, however, that while the effect of K^+-depolarisation on DA levels was decreased after METH, the effect on glutamate and aspartate levels was increased, suggesting an enhanced Ca^{2+}-inflow via *N*-methyl-D-aspartate (NMDA) receptor activation, a mechanism of METH excitotoxicity, as proposed by Sonsalla *et al.* (1989; Nash and Yamamoto, 1992; Marshall *et al.*, 1993; Stephans and Yamamoto, 1994). Yamamoto and co-workers (Burrows *et al.*, 2000; Nixdorf *et al.*, 2001) have implied an imbalance in energy metabolism for explaining amphetamine-derivative toxicity, suggesting that striatal dopaminergic terminals are more vulnerable than 5-HT terminals to damage after metabolic stress.

6-OHDA-lesion, Rotational Behaviour and DA and 5-HT Release

The effect of the 6-OHDA injection was verified with the microdialysis experiments, which showed that DA, DOPAC and HVA basal levels decreased by ~ 60%, 90% and 95%, respectively, on the lesioned side (left neostriatum), compared to that observed on the non-lesioned side (right neostriatum). 5-HT and 5-HIAA basal levels were, however, similar in both hemispheres, supporting the idea that the effect of 6-OHDA is specific for DA neurons.

Compared to previous reports (see Herrera-Marschitz *et al.*, 1990; 1992), the effect of the lesion on DA levels was mild, probably suggesting a partial effect of the 6-OHDA treatment, which is in agreement with the rather moderate rate of contralateral rotation observed here when the rats were treated with apomorphine (10 turns/min, compared to 19 turns/min shown by animals with more than 95% depletion of DA levels) (Herrera-Marschitz *et al.*, 1990). Hence, the amphetamines could still evoke DA release from the lesioned neostriatum, but that effect was variable, and significantly less prominent than that observed in the non-lesioned hemisphere and in non-lesioned rats (data not shown).

The strongest rotational behaviour was produced by D-AMPH and METH, the drugs producing the greatest increase in DA release and the highest DA/5-HT ratio, in agreement with the original report, where the ipsilateral rotation and DA release was measured simultaneously (Zetterström *et al.*, 1986). Interestingly, among the alkoxylated derivatives, MDMA and MDA produced the greatest increase of DA release (~6-fold) and the strongest rotational behaviour. No such obvious relationship was observed between rotational behaviour and 5-HT release and, indeed, a negative correlation (Rho-Spearman= -0.8; m,*n*=2.28) was found when comparing the maximum rate of rotational behaviour induced by the drugs at the dose of 2 mg/kg, s.c. and 5-HT release, confirming again the idea that rotational behaviour is mainly depending upon DA, but not 5-HT release. 5-HT release may even elicit cover-behaviours that can interfere with the expression of rotational behaviour.

In conclusion, the present report shows that the introduction of a methoxy or a methylenedioxy group on the benzene ring of D-AMPH or METH, or *N*-methylation of the D-AMPH molecule alters their pharmacological profile, changing the selectivity of the corresponding compounds. METH and the alkoxyamphetamine derivatives PMA, PMMA, MDA and MDMA elicit ipsilateral rotation and increase DA release, as D-AMPH does, when given to unilaterally 6-OHDA lesioned rats, or locally administered by a microdialysis probe. D-AMPH and METH were found to be more efficient than the alkoxylated derivatives in all tested parameters, and among the alkoxylated derivatives, MDMA and MDA produced the greatest increase of DA release and the strongest rotational behaviour. While D-AMPH and METH showed high selectivity for DA release, the alkoxylated derivatives stimulated the release of both DA and 5-HT with similar efficacy.

The present observations can constitute a basis to investigate novel structural modifications leading to the identification of compounds with different mechanistic profiles. Furthermore, this study provides information about the neuropharmacological properties of drugs which, in humans, are commonly abused.

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References

Abekawa T, T Ohmori and T Koyama (1994) Effects of repeated administration of a high dose of methamphetamine on dopamine and glutamate release in rat striatum and nucleus accumbens. *Brain Res.* **643**, 276-281.

Axt KJ and ME Molliver (1991) Immunohistochemical evidence for methamphetamine-induced serotonergic axon loss in the rat brain. *Synapse* **9**, 302-313.

Brown JM, GR Hanson and AE Fleckenstein (2000) Methamphetamine rapidly decreases vesicular dopamine uptake. *J. Neurochem.* **74**, 2221-2223.

Brunswick DJ, S Benmassour, SM Tejami-Butt and M Hauptmann (1992) Effects of high-dose methamphetamine on monoamine uptake sites in rat brain measured by quantitative autoradiography. *Synapse* **11**, 287-293.

Buchanan JF and CR Brown (1988) "Designer drugs" a problem in clinical toxicology. *Med. Toxicol.* **3**, 1-17.

Burrows KB, WL Nixdorf and BK Yamamoto (2000) Central administration of methamphetamine synergizes with metabolic inhibition to deplete striatal monoamines. *J. Pharmacol. Exp. Ther.* **292**, 853-860.

Bustamante D, Y Zhi-Bing, MN Castel, S Johansson, M Goiny, L Terenius, T Hökfelt and M Herrera-Marschitz (2002) Effect of repeated methamphetamine treatment on neurotransmitter release in substantia nigra and neostriatum of the rat. *J. Neurochem.* **83**, 645-654.

Bustamante D, G Díaz-Véliz, C Paeile, G Zapata-Torres and BK Cassels (2004) Analgesic and behavioral effects of amphetamine, *p*-methoxyamphetamine and *N*-alkyl-*p*-methoxyamphetamine derivatives. *Pharmacol. Biochem. Behav.* **79**, 199-212.

Butcher SP, IS Fairbrother, JS Kelly and GW Arbuthnott (1988) Amphetamine-induced release in the rat striatum: an *in vivo* microdialysis study. *J. Neurochem.* **50**, 346-355.

Byard RW, J Gilbert, R James and RJ Lokan (1998) Amphetamine derivate fatalities in South-Australia. Is "ecstasy" the culprit? *Am. J. For. Med. Pathol.* **19**, 261-265.

de la Torre R, J Ortuño, M Mas, M Farre and J Segura (1999) Fatal MDMA intoxication. *Lancet* **353**, 593.

Eisch AJ, SJ O'Dell and JF Marshall (1996) Striatal and cortical NMDA receptors are altered by a neurotoxic regimen of methamphetamine. *Synapse* **22**, 217-225.

Felgate HE, PD Felgate, RA James, DN Sims and DC Vozzo (1998) Recent paramethoxyamphetamine deaths. *J. Anal. Toxicol.* **22**, 169-172.

Fibiger HC and EG McGeer (1971) Effect of acute and chronic methamphetamine treatment on tyrosine hydroxylase activity in brain and adrenal medulla. *Eur. J. Pharmacol.* **16**, 176-180.

Fleckenstein AE, RR Metzger, JW Gibb and GR Hanson (1997) A rapid and reversible change in dopamine transporters induced by methamphetamine. *Eur. J. Pharmacol.* **323**, 9-10.

Frey K, M Kilbourn and T Robinson (1997) Reduced striatal vesicular monoamine transporters after neurotoxic but not after behaviorally-sensitizing doses of methamphetamine. *Eur. J. Pharmacol.* **334**, 273-279.

Fuxe K and U Ungerstedt (1970) Histochemical biochemical and functional studies on central monoamine neurons after acute and chronic amphetamine administration, In: *Amphetamine and Related Compounds* (Costa E, Ed.) (Raven Press:New York), pp 257-288.

Gough B, SZ Imam, B Blough, W Slikker Jr and SF Ali (2002) Comparative effects of substituted amphetamines (PMA, MDMA, and METH) on monoamines in rat caudate: a microdialysis study. *Ann. NY Acad. Sci.* **965**, 410-420.

Green AR, AO Mechan, JM Elliott, E O'Shea and MI Colado (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacol. Rev.* **55**, 463-508.

Haughey JM, AE Fleckenstein, RR Metzger and GR Hanson (2000) The effects of methamphetamine on serotonin transporter activity: role of dopamine and hyperthermia. *J. Neurochem.* **75**, 1608-1617.

Herrera-Marschitz M and U Ungerstedt (1984a) Evidence striatal efferents relate to different dopamine receptors. *Brain Res.* **323**, 269-278.

Herrera-Marschitz M and U Ungerstedt (1984b) Evidence that apomorphine and pergolide induce rotation in rats by different actions on D_1 and D_2 receptor sites. *Eur. J. Pharmacol.* **98**, 165-176.

Herrera-Marschitz M, H Utsumi and U Ungerstedt (1990) Scoliosis in rats with experimentally-induced hemiparkinsonism: dependence upon striatal dopamine denervation. *J. Neurol. Neurosurg. Psychiatr.* **53**, 39-43.

Herrera-Marschitz M, JJ Meana, WT O'Connor, M Goiny, MS Reid and U Ungerstedt (1992) Neuronal dependence of extracellular dopamine, acetylcholine, glutamate, aspartate and gamma-aminobutyric acid (GABA) measured simultaneously from rat neostriatum using *in vivo* microdialysis, reciprocal interactions. *Amino Acids* **2**, 157-179.

Herrera-Marschitz M, Z-B You, M Goiny, JJ Meana, R Silveira, O Godukhin, Y Chen, S Espinoza, E Pettersson, F Loidl, G Lubec, K Andersson, I Nylander, L Terenius and U Ungerstedt (1996) On the origin of extracellular glutamate levels monitored in the basal ganglia by *in vivo* microdialysis. *J. Neurochem.* **66**, 1726-1735.

Holson RR, JF Bowger, P Clausing and B Bough (1996) Methamphetamine-stimulated striatal dopamine release declines rapidly over time following microdialysis probe insertion. *Brain Res.* **739**, 301-307.

Hotchkiss AJ and JW Gibb (1980) Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. *J. Pharmacol. Exp. Ther.* **214**, 257-260.

Johansen SS, AC Hansen, IB Muller, JB Lundemose and MB Franzmann (2003) Three fatal cases of PMA and PMMA poisoning in Denmark. *J. Anal Toxicol.* **27**, 253-256.

Johnson M, AA Letter, K Merchant, GR Hanson and JW Gibb (1988) Effects of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine isomers on central serotonergic, dopaminergic and nigral neurotensin systems of the rat. *J. Pharmacol. Exp. Ther.* **244**, 977-982.

Kalant H (2001) The pharmacology and toxicology of ecstasy (MDMA) and related drugs. *Can. Med. J. Assn.* **165**, 917-928.

Kleven MS and LS Seiden (1992) Methamphetamine-induced neurotoxicity: structure activity relationships. *Ann. NY Acad. Sci.* **654**, 292-301.

Kraner JC, DJ McCoy, MA Evans, LE Evans and BJ Sweeney (2001) Fatalities caused by the MDMA-related drug paramethoxyamphetamine (PMA). *J. Anal. Toxicol.* **25**, 645-648.

Kuczenski R and DS Segal (1992) Differential effects of methamphetamine and dopamine uptake blockers (cocaine, nomifensine) on caudate and accumbens dialysate dopamine and 3-methoxytyramine. *J. Pharmacol. Exp. Ther.* **262**, 1085-1094.

Kuczenski R, D Segal, AK Cho and W Melega (1995) Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioural responses to the stereoisomers of amphetamine and methamphetamine. *J. Neurosci.* **15**, 1308-1317.

Lebsanft HB, A Mayerhofer, KA Kovar and WJ Schmidt (2003) Is the esctasy-induced ipsilateral rotation in 6-OHDA unilaterally lesioned rats dopamine independent? *J. Neural Transm.* **110**, 707-718.

Marek GJ, G Vosmer and LS Seiden (1990) Dopamine uptake inhibitors block long-term neurotoxic effects of methamphetamine upon dopaminergic neurons. *Brain Res.* **513**, 274-279.

Marshall JF, SJ O'Dell and FB Weihmuller (1993) Dopamine-glutamate interactions in methamphetamine-induced neurotoxicity. *J. Neural Transm.* **91**, 241-254.

Martin TL (2001) Three cases of fatal paramethoxyamphetamine overdose. *J. Anal. Toxicol.* **25**, 649-651.

Nash JF and BK Yamamoto (1992) Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4-methylenedioxymethamphetamine. *Brain Res.* **581**, 237-243.

Nichols DE (1986) Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens. *J. Psychoactive Drugs* **18**, 305-313.

Nixdorf WL, KB Burrows, GA Gudelsky and BK Yamamoto (2001) Enhancement of 3,4-methylenedioxymethamphetamine neurotoxicity by energy inhibitor malonate. *J. Neurochem.* **77**, 647-654.

O'Dell SJ, FB Weihmuller and JF Marshall (1991) Multiple methamphetamine injections induce marked increases in extracellular striatal dopamine which correlate with subsequent neurotoxicity. *Brain Res.* **564**, 256-260.

Paxinos G and C Watson (1982) *The Rat Brain in Stereotaxic Coordinates* (Academic Press:San Diego, CA).

Peat MA, PF Warren, C Bakhit and JW Gibb (1985) The acute effects of methamphetamine and *p*-chloro-amphetamine 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.

Pentney AR (2001) An exploration of the history and controversies surrounding MDMA and MDA. *J. Psychoactive Drugs* **33**, 213-221.

Pu C and CV Vorhees (1993) Developmental dissociation of methamphetamine-induced depletion of dopaminergic terminals and astrocytes reaction in rat striatum. *Dev. Brain Res.* **72**, 325-328.

Pu C, EJ Fisher, GP Cappon and CV Vorhees (1994) The effects of amfonelic acid, a dopamine uptake inhibitor, on methamphetamine-induced dopamine terminal degeneration and astrocytic response in rat striatum. *Brain Res.* **649**, 217-224

Randrup AS and I Munkvad (1966) Role of catecholamines in the amphetamine excitatory response. *Nature* **211**, 540.

Ricaurte GA, CR Schuster and LS Seiden (1980) Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: a regional study. *Brain Res.* **193**, 153-163.

Sabol KE, JT Roach, SL Broom, C Ferreira and MM Preau (2001) Long-term effects of a high-dose methamphetamine regimen on subsequent methamphetamine-induced dopamine release *in vivo*. *Brain Res.* **892**, 122-129.

Schmidt CJ (1987) Acute administration of methylenedioxyamphetamine: comparison with the neurochemical effects of its *N*-desmethyl and *N*-ethyl analogues. *Eur. J. Pharmacol.* **136**, 81-88.

Schmidt CJ and JW Gibb (1985) Role of the dopamine uptake carrier in the neurotoxic response to methamphetamine: effect of amfonelic acid. *Eur. J. Pharmacol.* **109**, 73-80.

Seiden LS, CR Fischman and CR Schuster (1976) Long-term methamphetamine-induced changes in brain catecholamines in tolerant rhesus monkeys. *Drug Alcohol Dependend.* **1**, 215-219.

Sonsalla PK, WJ Nicklas and RE Heikkila (1989) Role for excitatory amino acids in methamphetamine-induced nigrostriatal dopaminergic toxicity. *Science* **243**, 398-400.

Steele TD, JC Katz and GA Ricaurte (1992) Evaluation of the neurotoxicity of *N*-methyl-1-(4-methoxyphenyl)-2-aminopropane (para-methoxymethamphetamine, PMMA). *Brain Res.* **589**, 349-352.

Stephans SE and BK Yamamoto (1994) Methamphetamine-induced neurotoxicity: roles for glutamate and dopamine efflux. *Synapse* **17**, 203-209.

Stone DM, DC Stahl, GR Hanson and JW Gibb (1986) The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the brain. *Eur. J. Pharmacol.* **128**, 41-48.

Stone DM, M Johnsson, GR Hanson and JW Gibb (1987) A comparison of the neurotoxic potential of methylenedioxyamphetamine (MDA) and its *N*-methylated and *N*-ethylated derivatives. *Eur. J. Pharmacol.* **134**, 245-248.

Tseng L-F, MK Menon and HH Loh (1976) Comparative actions of the monomethoxyamphetamines on the release and uptake of biogenic amines in brain tissue. *J. Pharmacol. Exper. Ther.* **197**, 263-271.

Ungerstedt U (1971) Striatal dopamine release after amphetamine or nerve degeneration revealed by rotationalbehaviour. *Acta Physiol. Scand.* **367**, 49-68.

Ungerstedt U and GW Arbuthnott (1970) Quantitative recording of rotational behaviour in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system. *Brain Res.* **24**, 485-493.

Ungerstedt U, M Herrera-Marschitz, U Jungnelius, L Ståhle, U Tossman and T Zetterström (1982) Dopamine synaptic mechanisms reflected in studies combining behavioural recordings and brain dialysis. *Adv. Biosci.* **37**, 219-231.

Zetterström T, T Sharp, CA Marsden and U Ungerstedt (1983) *In vivo* measurement of dopamine and its metabolites studied by intracerebral dialysis: changes after D-amphetamine. *J. Neurochem.* **41**, 1769-1773.

Zetterström T, M Herrera-Marschitz and U Ungerstedt (1986) Simultaneous measurement of dopamine release and rotational behaviour in 6-hydroxydopamine denervated rats using intracerebral dialysis. *Brain Res.* **376**, 1-7.