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Behavioral, biochemical and neurotoxicological actions of the α-ethyl homologue of p-chloroamphetamine

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The present set of experiments was designed to examine the effects of extension of the α-methyl of p-chloroamphetamine (PCA) to an α-ethyl. Therefore, the α-ethyl homologue of PCA, 1-(4-chlorophenyl)-2-aminobutane (CAB), was compared to PCA in a number of pharmacological assays. CAB was 2-fold less potent than PCA at inhibiting synaptosomal uptake of [^{3}H]5-hydroxytryptamine ([^{3}H]5-HT), and 5-fold less potent at inhibiting uptake of [^{3}H]dopamine ([^{3}H]DA). In drug discrimination assays, CAB was approximately 3-fold less potent than PCA in animals trained to discriminate 3,4-methylenedioxymethamphetamine (MDMA) or its α-ethyl homologue, S-(+)-N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (S-(+)-MBDB), from saline. Monitoring with in vivo microdialysis, 10 mg/kg of PCA caused a large increase in extracellular DA and a significant decrease in 3,4-dihydroxyphenylacetic acid (DOPAC) in the striatum. In contrast, 11 mg/kg CAB caused no increase and 22 mg/kg CAB caused only a slight increase in extracellular DA. Both doses of CAB caused a decrease in extracellular DOPAC. The potential 5-HT neurotoxicity of CAB was examined by measuring monoamine and metabolite levels and [^{3}H]paroxetine binding at one week following acute doses. A 10 mg/kg dose of PCA caused an 80% decrease in cortical and hippocampal serotonergic markers, while an equimolar dose of CAB decreased only hippocampal 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels. However, 22 mg/kg of CAB produced a 20-40% decrease in all serotonergic markers. Thus, extension of the α-alkyl significantly decreases the dopaminergic effects of PCA. The similar decrease in relative 5-HT neurotoxicity and the decreased ability to alter dopaminergic systems in vivo and in vitro supports the involvement of DA in the neurotoxicity of PCA.

p-Chloroamphetamine; 1-(4-Chlorophenyl)-2-aminobutane (CAB); Drug discrimination; Microdialysis in vivo; Uptake inhibition; 5-HT neurotoxicity

1. Introduction

Extensive work in a number of laboratories over many years has been directed toward understanding the structure-activity relationships of classes of substituted amphetamines which exert various pharmacological actions. Recently, a great deal of that research has focused on the novel psychopharmacology and the apparent selective 5-hydroxytryptamine (5-HT, serotonin) neurotoxicity that is associated with either single or multiple dose administration of 3,4-methylenedioxymethamphetamine (MDMA). It is now well established that MDMA causes a decrease in

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several serotonergic parameters including the levels of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) (Schmidt et al., 1986; Stone et al., 1986), the number of 5-HT uptake sites (Battaglia et al., 1987; 1988), and the V_{max} for [^{3}H]5-HT uptake (Commins et al., 1987; Schmidt et al., 1987). Similarly, p-chloroamphetamine (PCA) induces the same type of decreases in 5-HT and 5-HIAA levels (Fuller and Snoddy, 1974; Harvey et al., 1975), and in the number of 5-HT uptake sites (unpublished results). In fact the neurotoxicity of MDMA is often compared to that of PCA (Stone et al., 1986; Schmidt et al., 1987). More recent evidence suggests that both MDMA and PCA result in a selective degeneration of the fine axon serotonergic projections ascending from the dorsal raphe nucleus (O'Hearn et al., 1988; Mamounas and Molliver, 1988).

In examining the structure-activity relationships of MDMA analogues it was found that extension of the α-methyl to an α-ethyl group altered the pharmacological profile. More specifically, in comparing MDMA with its α-ethyl analogue, N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), it was found that the latter has a decreased effect on dopaminergic systems. For example, in contrast to MDMA, MBDB does not induce the release of dopamine (DA) from superfused rat caudate nucleus slices (Johnson et al., 1986) nor inhibit the uptake of DA into rat striatal synaptosomes (Steele et al., 1987). In vivo data also demonstrate a decreased dopaminergic action for MBDB. Studies using in vivo microdialysis indicate that MDMA, but not MBDB, induces a significant increase in the extracellular concentrations of DA in the striatum (unpublished results). In addition, MDMA but not MBDB causes a substantial (300%) increase in the levels of cortical DA at 3 h post treatment (Johnson and Nichols, 1989). Furthermore, while neither of the dopaminergic agents (+)-amphetamine or cocaine completely substitute in rats trained to discriminate (+)-MBDB from saline, both completely substitute in (±)-MDMA-trained rats (Oberlender and Nichols, 1988; Nichols and Oberlender, 1990).

Despite these differences, MDMA and MBDB share many other pharmacological characteristics. Both are releasers of [^{3}H]5-HT from superfused rat hippocampal slices (Johnson et al., 1986), and are uptake inhibitors of 5-HT and NE into hippocampal and hypothalamic synaptosomes, respectively (Steele et al., 1987). MDMA and MBDB also result in decreased 5-HT and 5-HIAA levels 3 h after dosing (Johnson and Nichols, 1989). Behaviorally, MBDB fully substitutes in rats trained to discriminate (±)-MDMA from saline (Oberlender and Nichols, 1988) and MDMA also substituted in (+)-MBDB-trained rats (Nichols and Oberlender, 1990). Therefore, the discriminative stimulus properties of the two compounds are very similar. Furthermore, both MDMA and MBDB are neurotoxic in rats following multiple doses, although MBDB does seem to be less potent than MDMA in this respect (Johnson and Nichols, 1989).

As mentioned above, MDMA has often been compared to PCA. The purpose of the present study was to determine whether extension of the α-methyl of PCA would result in alterations in the pharmacological profile parallel to those seen with MDMA and MBDB. Therefore, 1-(4-chlorophenyl)-2-aminobutane (CAB) was synthesized, and several of its short- and long-term actions were compared to those of PCA. These included substitution testing in MDMA- and (+)-MBDB-trained rats, [^{3}H]5-HT and [^{3}H]DA uptake inhibition in whole brain synaptosomes, striatal DA release as measured by in vivo microdialysis, and relative 5-HT neurotoxicity following acute administration.

2. Materials and methods

2.1. Materials

Racemic 1-(4-chlorophenyl)-2-aminobutane HCl (CAB) was synthesized by standard procedures. The HCl salt had a melting point of 187-189°C and had spectroscopic properties and an elemental analysis consistent with the expected structure. Chloral hydrate, pargyline HCl, and the HPLC standards were obtained from Sigma Chemical Co. (St. Louis, MO). p-Chloroamphetamine HCl (PCA) was obtained from Regis Chem-

ical Co. (Morton Grove, IL). Fluoxetine HCl was graciously provided by Eli Lilly Laboratories (Indianapolis, IN). [^{3}H]5-HT and [^{3}H]DA were purchased from Amersham (Arlington Heights, IL) at a specific activity of 9.3 Ci/mmol and 10.0 Ci/mmol, respectively. [^{3}H]Paroxetine was purchased, at a specific activity of 28.8 Ci/mmol, from New England Nuclear (Boston, MA). MDMA HCl and (+)-MBDB HCl were synthesized in our laboratory (Nichols et al., 1986). With the exception of drug discrimination experiments, where i.p. injections were employed, all drugs were injected s.c.

2.2. *Animals*

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 175-200 g were used in all experiments. Animals were either group housed six per cage or individually caged in a temperature-controlled room with a 12/12 h lighting schedule. With the exception of rats used in the drug discrimination experiments, food and water were available ad libitum at all times. Rats used in drug discrimination experiments were given water ad libitum and enough food to maintain them at 80% of their free feeding weight. Brains were dissected over ice according to the procedure of Glowinski and Iversen (1966). In neurotoxicity studies, the hemispheres were separated and the frontal cortex and hippocampal regions frozen with liquid nitrogen. The samples were stored separately at $-70\,^{\circ}$C until assay using the [^{3}H]paroxetine binding or HPLC-EC methods described below.

2.3. *Drug discrimination*

Six standard operant chambers (Coulbourn Instruments) equipped with two response levers and a food pellet delivery system were utilized. Food pellets (Bioserve, 45 mg dustless) were used as positive reinforcement during maintenance sessions. The chambers contained a masking white noise and a house light all enclosed in ventilated, sound-attenuated cubicles. The chambers were controlled by solid state logic interfaced through a Coulbourn Instruments Dynaport to an IBM-PC located in an adjunct room.

The complete training procedure utilized has been published previously (Nichols et al., 1986; Oberlender and Nichols, 1988). Briefly, rats were trained to discriminate MDMA HCl (1.75 mg/kg) or (+)-MBDB HCl (1.75 mg/kg) from saline on an FR-50 schedule with 15 min maintenance sessions. All injections were administered i.p. 30 min prior to sessions. To avoid positional preference, half the animals were trained to press DRUG-L and SAL-R, while the other half were trained SAL-L and DRUG-R.

Test sessions were run once or twice a week with at least two maintenance sessions (one with drug and one with saline) between each test session. Test sessions ended with no reinforcement when either 50 responses were made on a lever or 5 min passed, whichever came first. If the rat did not press either lever 50 times he was scored as disrupted and was not included in the calculations. On test days, responding was drug positive if the rats pressed the drug-appropriate lever 50 times. A rat was only given a test drug if he responded correctly in the preceding maintenance sessions. A correct response during maintenance sessions was defined as 85% responding on the correct lever. Also, following the procedure of Colpaert et al. (1982), test data were discarded and the test condition later retested if the rat responded incorrectly in the following two maintenance sessions. At least eight non-disrupted animals were tested at each dose.

2.4. *In vitro [^{3}H]5-HT and [^{3}H]DA uptake inhibition*

A modified procedure of Steele et al. (1987) was employed. Briefly, whole brain minus cerebellum was homogenized in 15 volumes of ice cold 0.32 M sucrose using a glass mortar with a motor driven pestle. The homogenate was centrifuged at $1086 \times g$ for 10 min at $4\,^{\circ}$C. The supernatant was then recentrifuged at $17800 \times g$ for 10 min and the resulting pellet resuspended in the same volume of sucrose solution. Incubations were carried out in a shaking incubator under an atmosphere of 95% O_2-5% CO_2 at $37\,^{\circ}$ or $0\,^{\circ}$C to measure total tissue uptake and non-specific uptake, respectively. A 5 min preincubation was begun by ad-

ding 0.2 ml of the synaptosomal preparation to test tubes containing 1.65 ml of O_2 saturated Krebs-Henseleit buffer (mM: 118 NaCl, 4.8 KCl, 1.3 $CaCl_2$ 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 10 glucose, 0.6 ascorbic acid and 0.03 Na_2EDTA), 50 μl of drug, and 50 μl of pargyline HCl (final concentration of 1 μM). Then either [^{3}H]5-HT or [^{3}H]DA was added in a 50 μl aliquot of the sample mixture (final concentration of 10 nM), and incubations were continued for 5 min more. Incubations were terminated by cooling in ice and were then rapidly filtered through Whatman GF/B filters with a Brandel Cell Harvester (Gaithersburg, MD). The filters were washed with ice cold buffer and allowed to air dry before placing them in plastic scintillation vials. Scintillation cocktail (10 ml of ACS, Amersham) was added and the vials were sealed and allowed to set for 8 h before counting at an efficiency of 54%.

2.5. *In vivo microdialysis*

Under chloral hydrate (400 mg/kg i.v.) anesthesia, each rat was placed in a stereotaxic frame. A U-shaped dialysis probe was implanted in the striatum (A +1.0, L +3.5, V −6.5 from bregma, incisor bar −3.0). The probe was secured using dental cement and a skull screw. The dialysis probe was flushed with a modified Ringer solution (mM: 148 NaCl, 2.7 KCl, 1.2 $CaCl_2$ at pH = 7.4) and the animals were placed in clear plastic cages. Twenty-four hours following surgery, the dialysis probe was connected to an infusion pump (Harvard Apparatus Model 975) set to deliver 2.5 μl/min of Ringer solution. Dialysate samples were collected every 30 min. Baseline samples were collected for at least 3 h and then vehicle or drug was administered i.p. Samples were collected every 30 min for the next 3 h, following which the animals were killed and the location of the probe confirmed.

The dialysis probe construction was similar in design to that described by Korf and Venema (1985). Tungsten wire (50 μm) was inserted into a saponified cellulose ester dialysis fiber (285 μm outer diameter, molecular weight cutoff: 10000 Da) which was folded and inserted into a 23-gauge needle (0.64 mm outer diameter). Two polyethylene tubes (PE 20) were connected to the outlets of the dialysis tube and the probe was 'fixed' with epoxy. The dialysis membrane extended 2 mm beyond the tip of the needle. The percent recovery of DA and DOPAC using these dialysis probes was 14 and 18%, respectively, at a flow rate of 2.5 μl/min.

2.6. *[^{3}H]Paroxetine binding to the serotonin uptake carrier*

The method utilized for [^{3}H]paroxetine binding was a modified procedure of Habert et al. (1985). Brain regions from one hemisphere were thawed, weighed and homogenized in 5 ml of 50 mM Tris HCl with 120 mM NaCl and 5 mM KCl (pH = 7.4) with a Brinkman polytron (setting 6, 2 × 20 s). The homogenates were centrifuged twice at 30000 × *g* for 10 min and were then resuspended in the same volume of buffer.

As described by Battaglia et al. (1988), the ability of a single saturating concentration (1 nM) of [^{3}H]paroxetine to bind to tissue homogenates was examined. Specific binding was defined as that displaceable with 1 μM fluoxetine. Incubations were commenced by the addition of 200 μl of tissue to the buffer described above to give a total volume of 2 ml. The tubes were allowed to equilibrate at 24° C for 1 h before filtering through Whatman GF/C filters presoaked with 0.05% PEI using the Brandel Cell Harvestor. The tubes were then washed twice with ice cold buffer and the filters allowed to air dry before placing them in scintillation vials, adding 10 ml of ACS scintillation cocktail and allowing then to set overnight. The samples were then counted at an efficiency of 54%.

2.7. *HPLC with electrochemical detection*

Two separate HPLC-EC systems were used for analysis of the microdialysis and neurotoxicity samples, respectively. The concentrations of DA and DOPAC were determined in dialysate samples by injection of 80 μl onto an Ultramex 3 μm C18 column (Phenomenex, Torrance, CA) connected to an LC-4B amperometric detector electrode (Bioanalytical Systems, West Lafayette, IN).

The electrochemical detector was equipped with a glassy carbon electrode set at a potential of 650 mV relative to the Ag/AgCl reference electrode. The mobile phase consisted of 35 mM citric acid, 50 mM sodium acetate, 0.67 mM EDTA, 0.23 mM 1-octanesulfonic acid, 1 ml triethylamine and 5% v/v methanol at pH 4.5 delivered at a flow rate of 1.3 ml/min. A Hewlett-Packard (HP 3396A) integrator was used to quantitate the concentrations of DA and 3,4-dihydroxyphenylacetic acid (DOPAC).

In the neurotoxicity studies, cortical and hippocampal samples were prepared by homogenizing the weighed brain areas from one hemisphere in 0.5 ml of 0.4 N $HClO_4$ containing 0.05% Na_2EDTA, and 0.1% $Na_2S_2O_5$ using a motor-driven teflon pestle and Eppendorf 1.5 ml centrifuge tubes. An internal standard of 100 ng/ml of 3-(3,4-dihydroxyphenyl)propionic acid (DHPPA) was used. The samples were centrifuged at 14000 $\times g$ for 4 min with a tabletop centrifuge. The supernatant was assayed for catecholamines, 5-HT and their metabolites by injection of 50 μl onto a Brownlee C18 analytical cartridge column (Anspec, Ann Arbor, MI). The HPLC-EC system consisted of a refrigerated autosampler (TosoHaas, Philadelphia, PA), and a model 400 EG&G Princeton electrochemical detector (Princeton, NJ) with a dual electrode potential set at $E_1 = -200$ mV and $E_2 = 850$ mV versus the Ag/AgCl reference electrode. A mobile phase containing 50 mM NaH_2PO_4, 30 mM citric acid, 0.1 mM Na_2EDTA, 0.034% sodium octyl sulfate and 25% v/v methanol was delivered at a flow rate of 1.0 ml/min. The concentrations of norepinephrine (NE), DA, homovanillic acid (HVA), DOPAC, 5-HT, and 5-HIAA were determined using the Dynamax Methods Manager software (Rainin, Woburn, MA) implemented on an Apple Macintosh SE Computer.

2.8. Statistical analysis

In drug discrimination experiments the ED_{50} values were determined from quantal dose-response curves according to the procedure of Litchfield and Wilcoxon (1949). Percent uptake inhibition was defined as the difference between specific ^{3}H-uptake in control and drug test tubes divided by control uptake times 100% as described in Steele et al. (1987). The IC_{50} values reported are the average of three experiments as determined from graded dose-response curves according to the procedure of Tallarida and Murray (1981). To compare IC_{50} values for [^{3}H]5-HT or [^{3}H]DA uptake inhibition a Student's t-test was employed. All other comparisons utilized an analysis of variance followed by a post hoc comparison as embodied in the computer program EPISTAT (EPISTAT Services, Richardson, TX). The areas under the curve (AUC) from in vivo microdialysis studies were calculated using Simpson's rule.

3. Results

Our results indicate that CAB is significantly less potent than PCA in a number of the assays employed in this study. For example, as presented in table 1, CAB is about 5-fold less potent than PCA at inhibiting [^{3}H]DA uptake. CAB also displays about a 2-fold decrease in potency relative to PCA for [^{3}H]5-HT uptake inhibition. Like PCA, CAB was more potent as a [^{3}H]5-HT uptake inhibitor than as a [^{3}H]DA uptake inhibitor.

In drug discrimination experiments, both PCA and CAB completely substituted for MDMA and (+)-MBDB (table 2). PCA was more potent than CAB in both MDMA- and (+)-MBDB-trained animals but this was statistically significant only in (+)-MBDB animals. This parallels the relative

TABLE 1

The inhibition of [^{3}H]5-HT and [^{3}H]DA uptake was examined in whole brain synaptosomes. The IC_{50} values represent the means ± S.E.M. of three separate experiments. Each experiment utilized six concentrations, run in triplicate on the linear portion of the dose-response curve. The IC_{50} values were calculated as described in Methods section.

	Uptake inhibition	
	[^{3}H]5-HT IC_{50} (nM)	[^{3}H]DA IC_{50} (nM)
PCA	132 ± 7	465 ± 15
CAB	330 ± 18 [a]	2343 ± 12 [a]

[a] Indicates significantly greater than PCA IC_{50} ($P < 0.05$, Student's t-test).

TABLE 2

Animals were trained to discriminate either MDMA HCl or (+)-MBDB HCl from saline using a two lever drug discrimination paradigm. The ED_{50} values for substitution in MDMA-trained (A) and (+)-MBDB-trained (B) animals were calculated according to the method of Litchfield and Wilcoxon (1949).

Drug	Dose		N [a]	D [b]	%SDL [c]	ED_{50} [d]	
	mg/kg	μmol/kg				mg/kg	μmol/kg
(A)							
PCA	0.06	0.30	8	0	13	0.17	0.84
	0.13	0.61	9	1	38	(0.09-0.32)	(0.46-1.55)
	0.25	1.21	11	3	50		
	0.50	2.41	8	0	100		
CAB	0.20	0.91	9	1	13	0.43	1.94
	0.40	1.82	10	1	33	(0.29-0.64)	(1.30-2.91)
	0.60	2.73	9	1	63		
	0.80	3.63	9	1	100		
(B)							
PCA	0.06	0.30	10	2	25	0.17	0.82
	0.13	0.61	9	1	25	(0.10-0.32)	(0.50-1.36)
	0.25	1.21	10	2	38		
	0.50	2.41	9	1	100		
CAB	0.27	1.21	11	3	13	0.62	2.82
	0.53	2.42	8	0	38	(0.41-0.93)	(1.89-4.22)
	0.80	3.64	10	2	50		
	1.07	4.85	10	2	8		

[a] N = total number of rats tested. [b] D = number of animals disrupted (50 presses not completed in 5 min). [c] Percentage of responding (N–D) rats selecting the drug lever. [d] 95% confidence limits in parentheses.

ability of PCA and CAB to inhibit monoamine uptake.

The in vivo microdialysis experiments show striking differences in the ability of CAB and PCA to increase the extracellular concentration of DA and decrease DOPAC levels in the striatum. As illustrated in fig. 1, PCA (10 mg/kg) caused increases in the levels of extracellular DA and decreases in its metabolite, DOPAC. In contrast, an equimolar 11 mg/kg dose of CAB did not change DA levels but did decrease DOPAC levels. CAB, 22 mg/kg (equimolar to 20 mg/kg of PCA), was sufficient to cause a significant decrease in DOPAC and HVA concentrations (fig. 1B and C). It is apparent from fig. 1A that 22 mg/kg of CAB caused an increase in extracellular DA, although this increase is not nearly as marked as seen with 10 mg/kg of PCA. Similarly, the effect of PCA and CAB on DA, DOPAC and HVA concentrations calculated as the AUC indicated that PCA significantly increased extracellular DA and decreased DOPAC (data not shown). When examining the AUC for the CAB treatments, the large variance associated with the substantial DA increase in the PCA group led to a finding of statistical non-significance when comparing the control and 22 mg/kg CAB groups. However, if the PCA group was excluded, the increase in extracellular DA following 22 mg/kg CAB is significantly different from control ($P < 0.001$, ANOVA followed by post hoc comparison).

The acute administration of PCA or CAB had no significant effect on the concentration of NE, DA, DOPAC or HVA in the cortex or hippocampus at one week following administration of PCA or CAB at any dose examined (data not shown). As anticipated, however, PCA produced a selective decrease in all the serotonergic markers examined (fig. 2). In contrast, 11 mg/kg of CAB led to slight but statistically non-significant decreases, with the exception of hippocampal 5-HT and 5-HIAA levels. However, 22 mg/kg of CAB

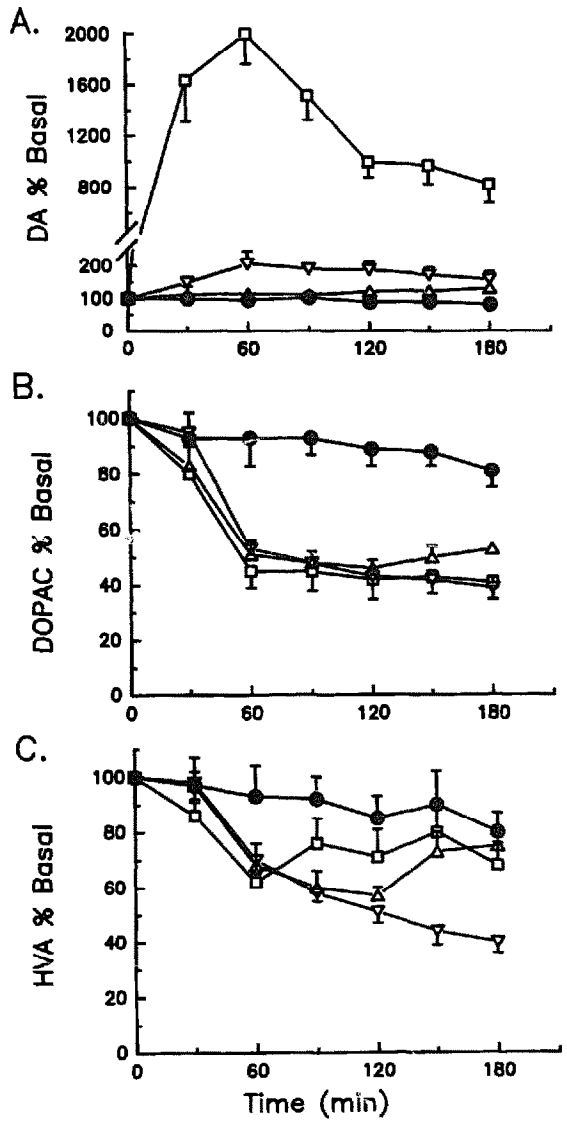

Fig. 1. PCA HCl (10 mg/kg) (□), CAB HCl (11 mg/kg) (△), CAB HCl (22 mg/kg) (▽) or saline (●) was administered i.p. at time 0 and dialysate samples were collected every 30 min for 3 h. The extracellular levels of DA, DOPAC and HVA are presented in (A), (B) and (C), respectively. Values were measured as pg/30 and are expressed as the means ± S.E.M. of four or five rats. Basal concentrations of DA (pg/30 min), DOPAC and HVA (ng/30 min), respectively, were as follows: saline: 31 ± 3, 3.5 ± 0.2, 3.4 ± 0.7; 10 mg/kg PCA: 21 ± 3, 3.9 ± 0.5, 3.3 ± 0.5; 11 mg/kg CAB; 32 ± 1, 4.1 ± 0.2, 3.8 ± 0.3 pg/30 min; and 22 mg/kg CAB: 26 ± 3, 6.2 ± 0.5, 5.3 ± 0.6.

did result in significant decreases in 5-HT, 5-HIAA, and the number of remaining 5-HT uptake sites.

4. Discussion

It is evident from our results that extension of the α-methyl substituent of PCA to an α-ethyl

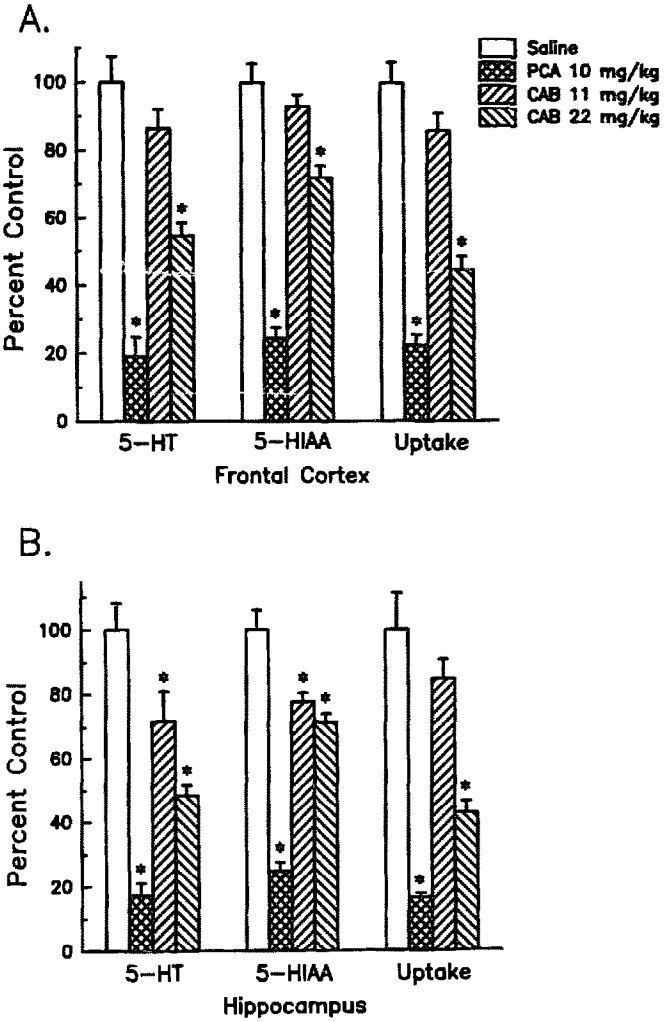

Fig. 2. Saline, PCA 10 mg/kg, CAB 11 mg/kg or 22 mg/kg was injected s.c. and animals were killed one week later. 5-HT and 5-HIAA levels were determined in frontal cortex (A) and hippocampus (B) using HPLC-EC techniques. The number of uptake sites remaining was determined by examining the ability of a saturating concentration of [^{3}H]paroxetine to bind to tissue homogenates as described in the Methods section. Values are represented as the means ± S.E.M. for n = 8. Saline control values were as follows: cortical 5-HT, 390 ± 29 pg/mg wet weight; cortical 5-HIAA, 256 ± 14 pg/mg wet weight; cortical uptake sites 2.45 ± 0.15 fmol/g wet weight; hippocampal 5-HT, 379 ± 31 pg/mg wet weight; hippocampal 5-HIAA, 575 ± 35 pg/mg wet weight; and hippocampal uptake sites 5.83 ± 0.66 fmol/g wet weight. * Indicates significantly decreased from control group ($P < 0.05$, ANOVA followed by post hoc comparison).

attenuated the dopaminergic actions of PCA. For example, substantially higher doses of CAB than PCA were required to significantly alter the in vivo extracellular concentrations of DA (fig. 1). It is also quite apparent that even at twice the dose, the dopaminergic effect of CAB is substantially less than that seen with PCA. While a slight decrease in potency for 5-HT uptake inhibition was seen with CAB, a more pronounced decrease in potency for DA uptake inhibition was observed (table 1). Therefore, it appears that, like the substituted amphetamine MDMA, extension of the α-alkyl group alters the relative ability to affect DA neurons.

The similarities between the substituted amphetamines PCA and MDMA are further evidenced in the drug discrimination results. As seen in table 2, both PCA and CAB substituted in MDMA-trained animals. CAB and PCA also substituted in animals trained to discriminate the α-ethyl analogue (+)-MBDB from saline. Therefore, it appears that PCA, CAB, (+)-MBDB and MDMA all share similar discriminative stimulus properties. It is interesting to note that, when comparing the ED_{50} values for CAB and PCA in either MDMA- or (+)-MBDB-trained animals, PCA is 2.3- and 3.4-fold more potent than CAB, respectively. This more closely resembles the 2.5-fold decrease in 5-HT uptake inhibition than the 5-fold decrease in DA uptake inhibition seen with CAB as compared to PCA. This may be a reflection of the importance of 5-HT release in the stimulus properties of MDMA and (+)-MBDB (Schechter, 1989; Nichols and Oberlender, 1990).

Differences in the pharmacological effects resulting from extending the α-alkyl substituent of MDMA and PCA are also apparent. This is most evident in comparing the dopaminergic effects of MBDB and CAB. It has been previously reported that extension of the α-methyl group to an α-ethyl has little effect on the 5-HT uptake inhibition potency or the non-vesicular 5-HT releasing ability of MDMA (Steele et al., 1987; Johnson et al., 1986). In comparing PCA and CAB there is a small but significant decrease (2.5-fold) in the 5-HT uptake inhibition potency. While extension of MDMA's α-alkyl group appeared to abolish DA releasing and uptake inhibition properties (Steele et al., 1987; Johnson et al., 1986), a similar change to PCA led to a 5-fold decrease in DA uptake inhibition potency but did not destroy this activity altogether. In fact, the CAB IC_{50} values for 5-HT and DA uptake inhibition correspond well to those of MDMA but not MBDB. However, it should be noted that concentrations of only 10 and 5 μM MBDB in DA release and uptake inhibition studies, respectively, were previously examined. Given the potency of MDMA to inhibit DA uptake (an IC_{50} of approximately 4 μM) and the 5-fold decrease in potency of CAB relative to PCA, one might anticipate that the IC_{50} value for [^{3}H]DA uptake inhibition by MBDB would be on the order of 20 μM or more. Therefore, it is possible that MBDB does have DA uptake inhibition and DA non-vesicular releasing properties at concentrations higher than those tested in earlier studies.

Extension of the α-alkyl substituent attenuated but did not abolish the neurotoxic effects of PCA. This appears to be the same type of selective 5-HT neurotoxicity seen with PCA, since CAB resulted in decreases only in serotonergic markers. It should be noted that while a 10 mg/kg dose of PCA was sufficient to decrease serotonergic markers to approximately 20% of control, a 22 mg/kg dose (equimolar to 20 mg/kg of PCA) caused a decrease to only approximately 50% of control. Therefore, CAB is substantially less potent as a 5-HT neurotoxin than is PCA. From the data presented here, one may infer an importance for DA in the expression of the 5-HT neurotoxicity.

The in vivo microdialysis experiments would seem to support this hypothesis. For example, 10 mg/kg of PCA but not CAB significantly increased DA release. Likewise, significant decreases in serotonergic markers are seen one week after a 10 mg/kg dose of PCA but not after an equimolar dose of CAB. However at the higher dose of CAB, significant short-term effects on the dopaminergic system and long-term effects on serotonergic markers were seen. Therefore, it can be hypothesized that, as suggested for MDMA (Stone et al., 1988; Nash et al., 1990; Gibb et al., 1990), dopaminergic effects may play a critical role in the selective neurotoxicity seen with PCA and its analogues. It remains to be determined how much DA

release is too much or 'neurotoxic' and whether activation of serotonergic neurons is necessary for 'excessive' amounts of DA to be taken up by 5-HT axon terminals and produce long-term damage.

To summarize, the initial characterization of the α-ethyl homologue of PCA, CAB, indicates it to be behaviorally active, although somewhat less potent than PCA. Extension of the α-alkyl substituent significantly attenuated the dopaminergic actions of PCA, as indicated in the uptake inhibition and in vivo microdialysis studies. The similarity of the drug discrimination results of PCA and CAB, in MDMA- and (+)-MBDB-trained rats, supports previous conclusions regarding the critical role of 5-HT but not DA in the discriminative properties of MDMA and similar compounds (Oberlender and Nichols, 1988). CAB appears to have retained the selective 5-HT neurotoxicity of PCA but is substantially less potent. And lastly, there appears to be a correlation between the relative dopaminergic and neurotoxic effects of PCA and CAB, supporting suggestions that DA may play a critical role in the 5-HT neurotoxicity of PCA.

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