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Acute Toxicity and Gross Behavioral Effects of Amphetamine, Four Methoxyamphetamines, and Mescaline in Rodents, Dogs, and Monkeys

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Acute Toxicity and Gross Behavioral Effects of Amphetamine, Four Methoxyamphetamines, and Mescaline in Rodents, Dogs, and Monkeys. DAVIS, W. M., BEDFORD, J. A., BUELKE, J. L., GUINN, M. M., HATOUM, H. T., WATERS, I. W., WILSON, M. C., AND BRAUDE, M. C. (1978). *Toxicol. Appl. Pharmacol.* **45**, 49–62. Mescaline (MES) and *d*-amphetamine (AMP) served as reference agents in a study of acute toxic and behavioral effects of four methoxy derivatives of AMP: *dl*-4-methoxyamphetamine (PMA), *dl*-2,5-dimethoxyamphetamine (DMA), *dl*-2,5-dimethoxy-4-bromoamphetamine (DOB), and *dl*-2,5-dimethoxy-4-methylamphetamine (DOM). Acute (24-hr) LD50 values were determined in mice (iv and oral), rats (ip), and dogs (iv). Estimation of the iv lethal dose range for each agent was conducted in a small number of rhesus monkeys. In terms of millimoles per kilogram of the base, MES was consistently the least toxic, whereas DOB or DOM was the most toxic for all species except the mouse. AMP was the most toxic for the mouse and was third in toxicity for all other species. At lethal and sublethal doses of these agents, observations were made for their effects on automatic, motor, and CNS functions and on behaviors possibly correlating with human hallucinogenic effects. According to the resulting profiles, DMA, DOM, and DOB had effects most similar to those of MES, whereas PMA had more in common with AMP. However, PMA, like the other three derivatives and mescaline, caused visual tracking in monkeys and dogs, a sign which may reflect a hallucinatory action.

Mescaline has been known as the hallucinogenic principle of the peyote cactus since the classical work of Heffter in 1897 (Holmstedt, 1967). Because three methoxy groups on the aromatic ring of the phenylethylamine structure of mescaline are important to its psychoactivity, various other methoxylated derivatives of phenylethylamine have been synthesized and their hallucinogenic properties have been clinically evaluated (Smythies and Levy, 1960; Shulgin *et al.*, 1961; Shulgin *et al.*, 1969). The gross behavioral and toxic effects of seven such potential hallucinogens were investigated by Hardman *et al.* (1973) in four laboratory species. The present report presents data on acute toxicity and behavioral responses of four species to several methoxysubstituted derivatives of the phenylisopropylamine structure, using *d*-amphetamine and mescaline as reference compounds. The observable responses to sublethal and lethal doses have been recorded and analyzed for patterns of "amphetamine-like" or "mescaline-like" activity.

METHODS

Compounds tested were as follows: *d*-amphetamine sulfate (AMP; Smith, Kline & French), mescaline hydrochloride (MES; Sigma Chemical Co.), *dl*-4-methoxyamphetamine hydrochloride (PMA), *dl*-2,5-dimethoxyamphetamine hydrochloride (DMA), *dl*-2,5-dimethoxy-4-bromoamphetamine hydrobromide (DOB), and *dl*-2,5-dimethoxy-4-methylamphetamine hydrochloride (DOM). Dosages expressed in milligrams per kilogram refer to the salts of the various agents; those stated in millimoles per kilogram refer to the base compounds. All drugs were dissolved in distilled water shortly before their use.

In mice, rats, and dogs, 24-hr LD50 values were determined with drugs being administered between 0830 and 1030 hr. Calculations of LD50 values and 95% confidence limits were performed by the method of Litchfield and Wilcoxon (1949). Swiss–Webster male mice weighing 18 to 25 g were treated by iv and oral routes, using 10 mice per dose and at least 4 doses of each drug. Male rats of a Sprague–Dawley-derived strain weighing 200 to 250 g were treated by ip injection, eight rats per dose and five dose levels for each drug. Both male and female mongrel dogs were employed in a weight range of 6.5 to 14 kg. Sex and weight factors were distributed as evenly as possible across drugs and doses. Four dogs were treated at each of four doses for every drug. They were housed individually with access to food and water after treatment. Observations on the pattern, severity, and duration of toxic signs and behavioral manifestations were made by trained observers.

An approximation of the iv lethal dose was obtained for each compound by using three to six juvenile male rhesus monkeys (*Macaca mulatta*) weighing 3.5 to 4.7 kg. One animal was treated at each level of an increasing sequence of doses until a fatal response ensued. A second animal was then tested at the highest dose not causing death in the initial trial. If this subject died, the trials were terminated. If the animal survived, a higher dose, intermediate to the highest two previous dosages, was tested with two monkeys. This procedure was repeated as necessary to find a dose which caused death in only one of two treated subjects. The monkeys were placed in primate restraining chairs on the day prior to treatment, and a rectal temperature probe was inserted. Respiratory rate was recorded via a pneumatic cuff, and heart rate was determined by palpation. Water was always available, but food was removed 16 hr prior to drug treatment. Behavioral responses were recorded by trained observers who were unaware of the treatment administered. Such data were recorded during a 5-min period at 30-min intervals until death or return to the predrug state. At least a 10-day interval separated drug treatments in a given subject. No animals were reused unless their normal baseline conditions were restored. Some additional data were collected after sublethal doses by the oral route (for all agents except DOM) to determine if effects seen upon iv treatment would apply also for oral dosing.

For tabulation of observational data, standard, self-explanatory terms for autonomic and somatomotor symptoms were used (Tables 3 through 6) with three possible exceptions. For dogs and monkeys "vocalization" indicates occurrence of what was judged to be an abnormal type of vocalization for the circumstances. For dogs this consisted of howling in a fashion seldom otherwise encountered in the absence of fear or pain stimuli, except that vocalization after amphetamine consisted only of barking. For

TABLE 1

ACUTE LETHALITY DATA FOR AMPHETAMINE, MESCALINE, AND FOUR METHOXYAMPHETAMINES IN FOUR SPECIES OF LABORATORY ANIMALS

Compound	Species	Route	Number of animals per dose	24-Hr LD50 (95% confidence limits)	
				Salt (mg/kg)	Free base (mM/kg)
d-Amphetamine sulfate (AMP)	Mouse	iv	10	52 (47–57)	0.141 (0.128–0.155)
	Mouse	po	10	353 (270–462)	0.958 (0.734–1.255)
	Rat	ip	8	70 (54–94)	0.190 (0.147–0.245)
	Dog	iv	4	8.5 (8.1–8.9)	0.023 (0.022–0.024
	Monkey[a]	iv	2	5.0	0.014
Mescaline hydrochloride (MES)	Mouse	iv	10	110 (96–124)	0.444 (0.387–0.501)
	Mouse	po	10	912 (786–1058)	3.681 (3.173–4.27)
	Rat	ip	8	270 (233–313)	1.090 (0.94–1.263)
	Dog	iv	4	68 (62–74)	0.274 (0.25–0.299)
	Monkey[a]	iv	2	160	0.650
dl-4-Methoxyamphetamine hydrochloride (PMA)	Mouse	iv	10	49 (44–56)	0.243 (0.217–0.280)
	Mouse	po	10	284 (214–370)	1.413 (1.065–1.841)
	Rat	ip	8	46 (35–61)	0.229 (0.172–0.304)
	Dog	iv	4	7.0 (6.5–7.6)	0.035 (0.032–0.038)
	Monkey[a]	iv	2	10	0.050
dl-2,5-Dimethoxyamphetamine hydrochloride (DMA)	Mouse	iv	10	39 (35–43)	0.169 (0.153–0.186)
	Mouse	po	10	>400	>1.72
	Rat	ip	8	63 (49–81)	0.273 (0.211–0.352)
	Dog	iv	4	26 (24.8–27.0)	0.113 (0.107–0.117)
	Monkey[a]	iv	2	32	0.139
dl-2,5-Dimethoxy-4-bromoamphetamine hydrobromide (DOB)	Mouse	iv	10	80 (68–94)	0.225 (0.191–0.266)
	Mouse	po	10	>400	>1.12
	Rat	ip	8	50 (42–60)	0.141 (0.118–0.168)
	Dog	iv	4	6.4 (5.1–8.0)	0.018 (0.014–0.023)
	Monkey[a]	iv	2	2.0	0.003
dl-2,5-Dimethoxy-4-methylamphetamine hydrochloride (DOM)	Mouse	iv	10	36 (31.3–41.4)	0.147 (0.128–0.169)
	Mouse	po	10	330 (258–422)	1.347 (1.052–1.724)
	Rat	ip	8	32.5 (26.9–39.3)	0.133 (0.11–0.161)
	Dog	iv	4	7.2 (5.1–10.0)	0.029 (0.021–0.041)
	Monkey[a]	iv	2	8.0	0.033

[a] Estimated minimal lethal dose, rather than LD50; no fiducial interval available.

monkeys it often consisted of a screeching similar to that usually associated with aggressive interactions between monkeys. The phrase "visual signs" indicates unusual visual behaviors of dogs and monkeys. These especially include visual tracking, a slow visual scanning movement directed toward a neutral field (i.e., apparently following with eyes an object not visible to the observer), as in Siegel *et al.* (1974), but also visual fixation, attentive staring that may often be directed to no apparent stimulus. Finally, "bizarre postures" indicates the occurrence of unusual stances that were maintained for considerable intervals. While there might be similarity to conditions seen otherwise with drugs in a preconvulsive response, these postures are distinguishable by their sustained character and their independence of later occurrence of a convulsive seizure.

RESULTS

Acute LD50 Comparisons

Lethal doses are presented in Table 1, both as milligrams per kilogram of the salts and as millimole per kilogram of the base compounds. Of the three species (mouse, monkey, dog) treated by the iv route, the dog was the most sensitive to four of the six drugs. The monkey was nearly equal to the dog for three of those four and was most sensitive to two drugs. The resistance of monkeys to toxic effects of mescaline was exceptional, making it clearly the least sensitive of these three species. On the other hand, the monkey was much more sensitive to DOB toxicity than were mice. Thus, species differences in toxic responses occurred, as would be expected, and there were deviations from the trend of such species differences for certain drugs.

Table 2 shows the toxicity rankings of all compounds for the four species employed. The rank order was rather similar for mouse (iv), rat (ip), dog (iv), and monkey (iv). However, DMA showed a higher toxicity ranking, and DOB had a lower one in the mouse than in the other species. A consistent finding for all species was that MES clearly is the least toxic of the six drugs tested.

As would be expected, all compounds had considerably higher (ca. 5–10 times) LD50 values orally than by the iv route in mice. Oral LD50 values, however, could not

TABLE 2

COMPARATIVE TOXICITY RANKINGS FOR THE SIX TEST COMPOUNDS IN EACH SPECIES[a]

Species and route	Toxicity ranking (1 = most toxic) based on millimole/kg of free base[b]					
	1	2	3	4	5	6
Mouse, iv	AMP	DOM	DMA	DOB	PMA	MES
Rat, ip	DOM	DOB	AMP	PMA	DMA	MES
Dog, iv	DOB	AMP	DOM	PMA	DMA	MES
Monkey, iv	DOB	AMP	DOM	PMA	DMA	MES

[a] The six compounds tested were *d*-amphetamine (AMP), mescaline (MES), *dl*-4-methoxyamphetamine (PMA), *dl*-2,5-dimethoxyamphetamine (DMA), *dl*-2,5-dimethoxy-4-bromoamphetamine (DOB), and *dl*-2,5-dimethoxy-4-methylamphetamine (DOM).

[b] Drugs not sharing an underline had significantly different ($p < 0.05$) LD50 values, as determined by potency ratios and 95% fiducial limits between LD50 pairs via Litchfield–Wilcoxon calculations, procedures not applicable to the monkey data.

be calculated for DOB and DMA because the incidence of deaths deviated little from 50% between 400 and 900 mg/kg, and higher doses could not be given in a feasible volume.

Toxicological and Behavioral Observations in Mice (Table 3)

After sublethal iv doses of amphetamine, marked hyperactivity and hyperreactivity to auditory stimuli occurred and were followed by onset of stereotyped movements.

TABLE 3

BEHAVIORAL–CNS–AUTONOMIC PROFILES FOR TEST COMPOUNDS IN MICE

	Drugs and dosages (mg/kg)					
Observable effects	AMP[a] (25–79)[b] (159–500)[c]	MES (63–159) (349–1259)	PMA (25–70) (100–500)	DMA (25–63) (200–900)	DOB (25–112) (316–900)	DOM (25–45) (100–600)
Behavioral and CNS[d]						
Inactivity		+				+ + +
Hyperactivity	+ + +		+ +			
Stereotypy	+ + +		+ +	+	+	+ + +
Hyperreactive	+ + +	+ +	+ +	+	+	+
Hyporeactive		+ + +		+ +	+ +	+
Bizarre postures		+		+ + +	+ +	+ + +
Ataxia, weakness		+ +		+	+	+
Tremors		+ +		+ +	+ +	+ + +
Clonic convulsion	+	+	+	+	+	+ +
Tonic convulsion	+		+			
Autonomic[d]						
Mydriasis	+		+	+ +	+ +	
Piloerection	+ + +	+	+ + +	+ + +	+ +	+ + +
Salivation	+ + +	+	+			+ +
Dyspnea		+		+	+	+
Hyperpnea	+ +	+	+ +	+	+	+

[a] Refer to Table 2 for definition of the abbreviations.

[b] Dosages administered iv.

[c] Dosages administered po.

[d] Definition of profiles: +, mild and/or for short duration; + +, moderate and/or for medium duration; and + + +, severe and/or long duration. Graded ratings reflect only relative intensity and/or duration of occurrence. Apparently discrepant observations (e.g., both hyperreactive and hyporeactive) may be related to dose, postinjection interval, or route. These qualifications also apply to Tables 4 through 6.

Intensity and duration of these responses were dose dependent. Lethal doses caused the following sequence: extreme exophthalmos and tremors immediately after injection, mild to marked clonic or tonic convulsions in 30 to 60 sec, and death in less than 3 min.

Both sublethal and lethal iv doses of PMA caused nearly identical behavioral and autonomic effects to those of AMP. Mescaline, however, produced a contrasting behavioral syndrome: inactivity, hyporeactivity, ataxia, tremors, and clonic convulsions. Bizarre postures were present but not prominent; stereotypy was absent.

DOM (iv) induced grooming, exophthalmos, stereotypies, and autonomic signs similar to those obtained with AMP. Running in circles and backward movement were frequently seen. With lethal doses, seizures and early death ensued in a few seconds to a few minutes. DOB (iv) acted less intensely than DOM to produce stereotypies, bizarre

postures, and convulsions. DMA (iv), like DOB, caused low-intensity stereotyped motions which were slower, better controlled, and less jerky and intense than with AMP and PMA.

After oral doses of AMP and PMA, stereotypic behavior was delayed and intensity was reduced. Convulsions preceding death began within 10 min and continued for 5 to 30 min. Bizarre postures but not catalepsy followed oral administration of MES. These did not resemble the ones seen after administration of DOM, DOB, and DMA. Oral doses of DMA, DOM, and DOB evoked behaviors seldom noted with iv doses. Bizarre postures, sometimes seen transiently upon iv treatment, appeared within 10 to 20 min and lasted for 4 hr or more. The predominant behavior was what we have descriptively called "piano-playing." The mice sat on their haunches with forepaws held high; the digits of the forepaws moved individually as the extended paws moved up and down. Mild exophthalmos accompanied strong mydriasis and piloerection. Some DOM-treated mice showed episodic ear-scratching.

Toxicological and Behavioral Observations in Rats (Table 4)

Following ip doses of amphetamine, rats first were extremely hyperactive and then developed intense stereotyped movements and autonomic signs. Mescaline-treated rats were very inactive and hyporeactive. Periodically they exhibited repetitive swimming-like movements but not stereotypies as for AMP. Muscular weakness and ataxia were pronounced. Autonomic signs were limited. The effects of PMA were similar to those of AMP but with less intense arousal. Bizarre posturing was prominent, stereotypy was

TABLE 4

BEHAVIORAL–CNS–AUTONOMIC PROFILES FOR TEST COMPOUNDS IN RATS

	Drugs and dosages (mg/kg, ip)					
Observable effects	AMP[a] (45–178)	MES (224–355)	PMA (28–112)	DMA (40–100)	DOB (32–100)	DOM (24–48)
Behavioral and CNS[b]						
Inactivity		+ + +	+ +	+ +	+ +	+ + +
Hyperactivity	+ + +		+ +			
Stereotypy	+ + +	+	+	+ +	+ +	+ +
Hyperreactive	+ + +	+	+ +			+ + +
Hyporeactive		+ + +	+	+ +	+	+ +
Bizarre postures		+ +	+ + +	+	+	+ + +
Ataxia, weakness		+ + +	+	+	+	+ +
Tremors		+	+	+	+ +	+ + +
Clonic convulsion	+ +	+ +	+ +		+ + +	+ + +
Tonic convulsion	+		+		+ +	+ +
Autonomic[b]						
Mydriasis	+		+	+	+	
Piloerection	+ + +	+	+ + +	+	+	+ + +
Salivation	+ +		+ +	+	+ +	+
Dyspnea		+		+ +	+	+
Hyperpnea	+ + +	+	+	+	+	+

[a] Refer to Table 2 for definition of abbreviations.

[b] Definition of profiles: +, mild and/or for a short duration; + +, moderate and/or for medium duration; and + + +, severe and/or of longer duration.

observed, and initial hyperactivity and hyperreactivity were followed by inactivity. Autonomic signs and convulsions at higher dosages were similar to those of AMP.

DMA also caused stereotypy and mild bizarre postures, but inhibitory rather than excitatory signs predominated. Convulsions were absent even at fatal doses; death apparently resulted from respiratory failure. Both DOB and DOM caused stereotypy equally, but DOM produced more pronounced effects otherwise than did DOB. Moreover, DOB lacked a hyperreactive phase seen with DOM. Also, DOM caused a marked arching of the back, chattering of teeth, and semiclosure of eyes during periods of inactivity. At some doses of DOM and DOB, a forelimb response similar to that of mice treated with DMA, DOM, and DOB was seen. For both drugs, severe clonic–tonic convulsions were the terminal event.

Toxicological and Behavioral Observations in Dogs (Table 5)

Amphetamine (5–9 mg/kg iv) produced stereotypic behaviors for which intensity and duration were dose related unless interrupted by convulsions. The number and/or frequency of components in the stereotypy varied between individuals. Stereotypy sometimes continued uninterruptedly for 1 hr. Initial hyperreactivity to auditory stimuli

TABLE 5

BEHAVIORAL–CNS–AUTONOMIC PROFILES FOR TEST COMPOUNDS IN UNRESTRAINED DOGS

	Drugs and dosages (mg/kg, iv)					
Observable effects	AMP[a] (5–9)	MES (50–100)	PMA (4–15)	DMA (6–30)	DOB (6–8)	DOM (4–8)
Behavioral and CNS[b]						
Hyperactivity	+ + +					
Stereotypy	+ + +					+
Hyperreactive	+		+ + +			
Hyporeactive	+	+ + +	+			+ + +
Bizarre postures		+ +		+ +	+ + +	+ + +
Catalepsy		+				+ + +
Visual signs	+	+	+ + +	+ +	+ +	+
Vocalization	+		+ + +	+	+ +	+ +
Ataxia, weakness		+	+ + +	+ +	+ +	+ +
Tremors	+	+	+ + +	+ +	+ +	+ +
Convulsions						
Myoclonic	+	+	+	+	+ +	
Clonic	+	+	+	+	+ + +	+ + +
Tonic	+	+	+	+	+ + +	+ +
Autonomic[b]						
Mydriasis	+ + +	+	+ + +	+ + +	+ + +	
Piloerection	+	+	+ + +	+	+	
Salivation						
Watery	+ + +		+ +			
Viscous/foamy		+ +		+	+ + +	+ +
Defecation	+		+	+	+	+ +
Urination		+	+	+	+	+ +
Emesis	+			+	+ +	

[a] Refer to Table 2 for definition of abbreviations.

[b] Definition of profiles: +, mild and/or for short duration; + +, moderate and/or for medium duration; and + + +, marked and/or for long duration.

gradually changed to hyporeactivity. Deaths occurred during or immediately after a prolonged tonic–clonic convulsive episode, with cessation of respiration and a weak, irregular heart beat.

The behavior after iv mescaline differed greatly from that after AMP. Responses not seen for AMP were bizarre postures, extreme hyporeactivity, catalepsy, and ataxia. Visual tracking occurred with MES but was no more prominent than for higher doses of AMP. Unresponsiveness to tactile and auditory stimuli began soon after dosing, and within 10 to 30 min dogs were prostrate. After lower doses, stupor lasted for 2 to 8 hr. Near-lethal doses caused mild but nearly continuous clonic–tonic convulsions.

Immediately after iv PMA, dogs displayed extreme ataxia, their legs sliding from under them as if on ice. In the resulting spread-eagle position, they showed intense generalized tremors and a piercing, high-pitched howling. Gentle tactile stimulation or simply approaching the dog increased the intensity of vocalization. Concurrently, there was maximal mydriasis, visual tracking, and staring. After PMA all dogs moved slowly backward, never forward, suggesting an avoidance of some unapparent object. Taken together, these behaviors gave the strongest impression of a hallucinogenic state of any compound tested.

DMA (6 to 30 mg/kg iv) caused bizarre postures, as it did for mice. Dogs stood with forelimbs extended forward and head extended downward. For sublethal doses, this began within 1 min; the duration of 20 to 90 min was directly related to the dose. Also seen with sublethal doses were visual tracking and failure to orient to auditory stimuli. Clonic–tonic convulsions occurred for nearly 5 hr in one dog at 20 mg/kg; however, no other dog showed whole-body seizures. After lethal doses many signs occurred for only 1 to 2 min before hypoxic gasping and respiratory failure intervened, death ensuing while the heart was still beating strongly.

Behavior after DOB (6–8 mg/kg) was similar to that after DMA, including the same peculiar posture, if only briefly. Howling began shortly after injection, often coinciding with the bizarre posture, and the intensity was markedly increased by light tactile stimuli. Within 10 to 20 min all dogs but one had episodic clonic–tonic convulsions. The frequency of such episodes correlated with dosage; lethal doses caused continuous seizures lasting from 2 hr to nearly 36 hr. DOB-induced behaviors suggestive of hallucinations were less dramatic than with PMA.

Responses to DOM (4–8 mg/kg iv) resembled closely those to DMA. All dogs immediately showed vocalization and bizarre postures; the most common one was extension of the neck and forelimb muscles with the head positioned between the forelimbs, followed by a head-rotating motion with concurrent backward movement. Long periods of such behavior occurred with sublethal doses. In all cases this response was followed by loss of righting reflex and intense, high-pitched howling. A feature distinctive to DOM was that no survivors had convulsions, although all had generalized tremors, whereas DOB survivors simply showed a lesser number and frequency of seizures than those that died.

Postmortem examination revealed some changes occurring after all compounds. Hemorrhagic plaques were noted at the lung roots with extension into the lung. Focal hemorrhagic areas were randomly distributed through all lung lobes bilaterally. Hepatic and pulmonary congestion was extensive. Small amounts of serous fluid (2–3 ml) were found in the pericardial sac; in a few dogs serous fluid also was in the thoracic cavity. All showed constriction of the circular muscles of the small intestine. The totally

contracted bladder had varying amounts of apparent venous thrombotic occlusion. Varied degrees of renal cortical hemorrhage were seen.

Some possibly distinctive drug-related changes also were noted. After PMA massive myocardial infarcts extended down the coronary sulcus to and including the apex of both ventricles. Petechial hemorrhages over both atria and the auricular processes occurred to a variable degree. After DOB, there were myocardial infarcts only in the apical area of both ventricles and generalized hemorrhage throughout the submucosal lining of the duodenum, especially severe near the pylorus. Dogs dying after amphetamine regularly had massive endocardial hemorrhages visible in both ventricles. Most DOM-intoxicated dogs had about 10 ml of serosanguineous fluid in the pericardial sac. Their epicardium usually showed apical hemorrhages and punctate infarcts along the coronary sulcus, with several petechial hemorrhages seen on the wall of either ventricle. Profound subendocardial hemorrhages were observed in both ventricles, usually involving the papillary muscles and occasionally affecting the atrioventricular valves. None of the animals subjected to autopsy exhibited any gross anomalies in the brain.

Toxicological and Behavioral Observations in Monkeys (Table 6)

Amphetamine (1–6 mg/kg iv) caused dose-related hyperactivity, autonomic signs, and stereotypic behaviors (e.g., repetitive licking, chewing, arm movements, twisting in

TABLE 6

BEHAVIORAL–CNS–AUTONOMIC PROFILES FOR TEST COMPOUNDS IN CHAIRED MONKEYS

	Drugs and dosages (mg/kg, iv)					
Observable effects	AMP[a] (1–6)	MES (20–160)	PMA (2–16)	DMA (2–48)	DOB (0.125–16)	DOM (0.5–8)
Behavioral and CNS[b]						
Reactivity to stimuli	↑↑↑↓	↑↓↓↓	↑↑↑	↓↓↓	↑↓↓↓	↓↓
Bizarre postures		+		+	+ +	+ +
Spasticity				+ +	+ + +	+
Rigidity		+			+ +	+ +
Tremors	+	+ +	+ + +			+
Convulsions	+	+ + +	+	+	+ + +	+ +
Activity	↑↑↑	↓↓	↑↑↓	↓↓	↓↓	↓
Stereotypy	+ + +		+ +			
Visual signs		+ + +	+ +	+		+ + +
Vocalization	+	+ +		+ + +	+ +	+ +
Fruit refusal	+ + +	+ +	+ + +	+ +	+ +	+ + +
Autonomic[b]						
Respiratory rate	↑↑↑	↑↓	↑↑	↓	↓	↑↑
Heart rate	↑↑		↑↑			↑↑
Body temperature	↑↑↑	↓↓↓	↑↑↑	↓	↓↓	↑↓
Emesis		+			+	
Salivation	+ +	+ + +	+ +	+	+ +	+
Pupil diameter	↑↑↑	↓↑↑	↑↑↑	↑↑↑	↑↑	↑↑↑↓↓↓

[a] Refer to Table 2 for definition of abbreviations.

[b] Definition of profiles: + or ↑, mild and/or for short duration; + + or ↑↑, moderate and/or medium duration; and + + + or ↑↑↑, marked and/or for long duration. The sequence of arrows in opposite directions is related to the dosage and/or time at which responses were seen; i.e., arrows to the left correspond to effects seen at lower dose or earlier time.

chair). All doses produced initial hyperreactivity to tactile or auditory stimuli and refusal of fruit. The latter could be an anorexic effect or a less specific behavioral disruption. When a 4-mg/kg or higher dose was given, the animals became hyporeactive during and after the time of maximal stereotypy (30–60 min postinjection). In contrast to the other compounds, lethal doses of AMP caused convulsions only after large increases in body temperature, suggesting a febrile mechanism.

Mescaline (20–100 mg/kg iv) induced periods of active visual investigation of no apparently novel or interesting object, which to observers suggested visual hallucinations. All subjects treated with 40 to 160 mg/kg refused fruit. Hyperreactivity soon after injection was succeeded by hyporeactivity for 3 to 4 hr. During the latter phase, especially with higher doses, subjects were too depressed to show attentive visual behavior. Lethal and near-lethal doses caused frequent, prolonged convulsive episodes, respiratory depression, and severe hypothermia. Deaths were delayed, i.e., 3 to 4 days after dosing.

A spectrum of effects was produced by PMA (2–16 mg/kg iv). At 2 and 4 mg/kg, and at 3 to 6 hr following 8 mg/kg, overt behavioral changes were nearly identical to those after 20 or 40 mg/kg of MES, including periods of visual investigation and staring. After higher doses overt behavioral and physiological responses more closely resembled effects of AMP, including a similar stereotypy. Ocular signs similar to those at lower doses were also present initially after higher doses. Tremors, fever-associated convulsions, and severe CNS depression were seen with lethal doses, as for AMP.

DOB produced a complex syndrome quite unlike that following AMP. Hyporeactivity, muscle rigidity, and spasticity, hypothermia, and clonic convulsions were noted for 4 to 18 hr. The signs observed before death were similar to those observed with MES, but death occurred much earlier. Doses as low as 0.125 mg/kg induced spasticity; the threshold convulsant dose was about 0.5 mg/kg. With doses above 2 mg/kg, an initial period of 2 to 10 min of marked hyperreactivity and chortling vocalizations occurred. Active visual investigation and fixation were absent. With doses as low as 0.125 mg/kg, however, inappropriate threat behaviors toward no apparent stimuli gave suggestion of possible hallucinogenic action.

Prominent effects of DOM were tachycardia, pupillary changes, hyporeactivity, bizarre postures, fruit refusal, and visual fixation. These were noted in most subjects after doses greater than 2.0 mg/kg. Bizarre posturing was very similar to that with DOB. As with DOB, death was preceded by convulsions, but DOB was a much more potent convulsant. Whereas DOB caused bradypnea and hypothermia, DOM had opposite effects, although the increases were only 20% and 0.5°C, respectively. A marked increase (30–40%) in heart rate with DOM was absent for DOB. By autonomic parameters, DOM resembled AMP more than it did DOB, but in effects on skeletal muscle it matched DOB. Prominent visual fixation after DOM was accompanied by rapidly fluctuating maximal changes in pupil size. The latter sometimes were unilateral rather than bilateral and occurred without alterations in illumination. The pupillary effects and eye movements after DOM are consistent with a possible visual hallucinatory state; they may result from rapid changes in convergence.

A rapid onset of action for DMA was indicated by the consistent occurrence of screeching and/or chortling calls within 1 to 2 min after dosing. Lethal and near-lethal doses resulted in respiratory and/or circulatory crises, with marked facial blanching and

a characteristic grimacing expression, within seconds after iv doses. The posture after DMA resembled that with MES or DOB, limbs being held rigidly in unnatural positions with the neck extended backward. During hyporeactivity (1–8 hr postinjection) following low to moderate doses of DMA, visual investigatory behavior was absent but fixation was prominent.

Oral doses of AMP (2.0, 4.0 mg/kg), MES (20, 40 mg/kg), PMA (2.0, 8.0 mg/kg), DMA (20, 40 mg/kg), and DOB (2.0, 4.0 mg/kg) to restrained monkeys caused behavioral effects that were almost identical to those after low to medium iv doses. Heart and respiration rate and rectal temperature generally showed little change, although DOB and MES caused mild hypothermia, and the highest dose of PMA produced moderate tachycardia. Latencies for behavioral effects of AMP, PMA, and MES were 30 to 45 min, whereas those for the dimethoxy compounds were much shorter (DOB, 10 min; DMA, 2 min). Peak effects occurred 1 to 3 hr after MES, PMA, and AMP but within 5 min for DMA. With either dose of PMA or MES, normal behavior returned in about 6 hr. Monkeys receiving 2.0 and 4.0 mg/kg of AMP appeared normal about 4 and 12 hr later, respectively. The dimethoxy compounds had a duration of effects of 3 to 12 hr.

DISCUSSION

Acute Toxicity in Rodents

In a study of stereoisomers, Benington *et al.* (1973) reported sc LD50 values, in Swiss mice for both DOM and DOB. The corresponding isomers of DOB in each case had slightly lower LD50 values than did the isomers of DOM. Fiducial limits were not reported, however, and the differences seem insufficient to be significant. In contrast, our data show DOM to be significantly more toxic than DOB to mice by both iv and oral routes. Similarly, the ip LD50 for DOM in the rat was significantly lower than that of DOB. In dogs, however, there was no difference between the LD50 values for the two, and in rhesus monkeys DOB appeared more toxic than DOM.

An ip LD50 of 100 mg/kg for DOB in female mice has been reported by Shulgin *et al.* (1971); we found an iv LD50 of 80 (68–94) mg/kg. These fairly high values do not suggest so severe a degree of toxic potential as do the iv lethal doses we found for mongrel dogs (6.4 mg/kg) and for rhesus monkeys (1.0 mg/kg). This is noteworthy in view of the proposed diagnostic application in man of ^{77}Br-labeled DOB as a diagnostic brain scanning agent (Sargent *et al.*, 1975).

An iv LD50 for PMA in mice of 24.9 mg/kg (as the base) has been reported by Paton *et al.* (1975), whereas the comparable value found in the present study was 40.3 (36–46) mg/kg (as the base). A sc LD50 of 60.7 mg/kg for PMA in mice (Nichols *et al.*, 1975), was considerably lower than the oral LD50 of 284 (214–370) mg/kg which we found. Although a number of human fatalities have been reported for PMA (Cimbura, 1974), no estimate of lethal dosage in humans is available.

Behavioral Observation in Rodents

Drug-induced behavioral signs in mice that have been correlated with the action of hallucinogens, especially mescaline, include ear-scratching (Borsy *et al.*, 1964; Kulkarni, 1973) and head twitches (Corne and Pickering, 1967; Siegel and Poole,

1969). In the present case, we noted ear-scratching only with DOM. It seems as if the sign was less prominent than in Kulkarni's observations with DOM. Similarly, head-twitching was not commonly observed after treatment with our test drugs. Another study (Sakurada, 1975) associates head-twitches induced by tyramine and 4-hydroxy-amphetamine given intracerebrally with a serotonergic-mediating mechanism. This supports the hypothesis of Corne and Pickering (1967) that is based on their studies of various hallucinogens and their interactions with methysergide.

A behavioral syndrome which we noted in both mice and rats included hyperreactivity and hyperactivity plus tremors and a stereotypic movement of the fore-paws. This was seen after oral doses of DOM, DOB, and DMA in mice and after ip doses of DOM and DOB in rats. It seems very similar to the behavioral syndrome in rat and mouse after treatment with either L-tryptophan or 5-methoxy-*N*,*N*-dimethyl-tryptamine and a monoamine oxidase inhibitor (Grahame-Smith, 1971; Jacobs and Klemfuss, 1975), which was characterized by tremors, reciprocal treading of the fore-paws, lateral head-weaving and rigidity, hyperreactivity, and hyperactivity. All but the latter components are considered the result of central serotonergic activity. Thus, the behavioral effects of DOB, DOM, and DMA suggest a serotonergic agonist activity or an enhanced release of serotonin, as a central action of these compounds in rodents.

Acute Toxicity and Behavioral Observations in Dogs and Monkeys

Whereas dogs are not commonly employed for behavioral characterization of CNS drugs, Hardman *et al.* (1973) emphasized their value for the purpose of evaluating mescaline-like drugs. The data reported here support that opinion. Their view, however, that dogs were *superior* to monkeys for detecting hallucinogenic actions is not supported by our results, even though the behavioral abnormalities induced in dogs were clearly evident and somewhat more striking than those induced in monkeys. Restraint in primate chairs to permit physiological measures probably reduced considerably the observable inappropriate behavior of our monkeys; this is supported by descriptions of the types of inappropriate behavior scored after hallucinogens by Seigel *et al.* (1974). However, our criteria of a possible hallucinatory state, i.e., visual tracking, visual fixation, or inappropriate nonstereotypic behaviors, were found in monkeys not only for PMA, DMA, DOB, and DOM but also for MES. Failure to detect evidence of hallucinations or bizarre postures after doses up to 200 mg/kg in the study of Hardman *et al.* (1973) contrasts with both our positive findings for MES in monkeys and for MES at dosages of 100 mg/kg or less in dogs.

The rapid behavioral effectiveness of oral as well as iv doses in monkeys suggests that PMA, DMA, or DOB could readily be abused in humans by either oral or parenteral routes. Our observations in self-administration studies on rats indicate, however, that these hallucinogens support self-administration behavior much better via intragastric rather than iv route (Davis *et al.*, 1978).

It is of interest to compare the signs produced by fatal or near-fatal doses of PMA in dogs and monkeys to that for nine cases of human fatalities (Cimbura, 1974). For dogs the initial effects included extreme motor ataxia, generalized tremors, maximal mydriasis, piloerection, salivation, and tachypnea; later clonic–tonic seizures were followed by stupor in which the subjects expired with failing respiration. In monkeys, mydriasis, salivation, and tachypnea were accompanied by tremors and hyperpyrexia.

Clonic (febrile?) convulsions occurred in the last 30 min before death, which came during severe CNS depression. Human fatalities were associated with mydriasis, hyperventilation, hyperthermia, tachycardia, extreme restlessness, and convulsions (Cimbura, 1974). Thus, there is good agreement between these experimental and clinical observations. Data are not available to permit similar comparisons for the other methoxyamphetamines, but this concordance supports the possible extrapolation from dog and monkey to man for these compounds.

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