Pharmacology Biochemistry & Behavior, Vol. 29, pp. 125–128. © Pergamon Journals Ltd., 1988. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Behavioral Effects of Alpha-Benzyl-N-Methylphenethylamine (ABNMP), a Methamphetamine Analog: Inhibition by Ketanserin and Para-Chlorophenylalanine (PCPA)

JEAN LUD CADET, C. RANDALL CLARK* AND STANLEY FAHN

Department of Neurology, Columbia University
College of Physicians and Surgeons, 630 West 168th St., New York, NY 10032
**Auburn University, School of Pharmacy, Auburn, AL 36849*

Received 5 May 1987

CADET, J. L., C. R. CLARK AND S. FAHN. *Behavioral effects of alpha-benzyl-N-methylphenethylamine (ABNMP), a methamphetamine analog: Inhibition by ketanserin and para-chlorophenylalanine (PCPA).* PHARMACOL BIOCHEM BEHAV **29**(1) 125–128, 1988.—Acute administration of alpha-benzyl-N- methylphenethylamine (ABNMP) induces lateral heavings, straub tail, backward locomotion, and hindlimb abduction—which are all components of the serotonin (5-HT) syndrome. Ketanserin, a 5-HT-2 receptor antagonist and pretreatment with the 5-HT neurotoxin parachlorophenylalanine (PCPA) attenuated the manifestation of these behavioral abnormalities. These results suggest that ABNMP may cause an acute release of 5-HT similar to that elicited by parachloroamphetamine.

Alpha-benzyl-N-methylphenethylamine Amphetamines 5-HT Syndrome Ketanserin Parachlorophenylalanine

CHRONIC drug abuse causes very serious medical and neuropsychiatric problems. This is probably related to a number of issues including addiction to multiple drugs and the presence of contaminants resulting from clandestine synthesis of these substances [2, 13, 15].

The amphetamines are among the most frequently synthesized drugs in these laboratories. One of the most popular methods of making methamphetamine (METH) involves the use of phenylacetone also known as phenyl-2-propanone (P-2-P) [2]. Subsequent to the regulation of P-2-P by the Federal Government, illicit chemists began to use phenyl acetic acid as a precursor of the ketone [2,13]. This base-catalyzed reaction also results in the formation of another ketone, namely dibenzylketone. Further reductive amination of the reaction mixture yields both METH and alpha-benzyl-N-methylphenethylamine (ABNMP) [2, 13, 15].

Knowledge of by-products of amphetamine synthesis is important to both clinical and basic neuroscientists for a number of reasons. For example, amphetamine abusers can present clinically with complaints of headache, confusion, psychotic symptoms, movement disorders, intracerebral and/or subarachnoid hemorrhage, or death [6, 12, 19]. While it is possible that these signs and symptoms might be related only to the toxic effects of amphetamine or methamphetamine, the possible presence of impurities must be taken in consideration, since the actual content of METH in samples sold as METH may vary considerably. Such an approach may help to improve the acute as well as the chronic care of the drug abuser since the neuropharmacological profile of the impurities might be different from that of the parent compounds.

Several authors have evaluated the acute and chronic behavioral effects of amphetamine and methamphetamine [3, 4, 10, 14, 16–18]. However, we do not know of any similar report on the effects of ABNMP. The present report provides an initial characterization of the behavioral changes that result from the acute injection of this drug.

METHOD

Male Sprague-Dawley rats, weighing 400–450 g at the beginning of the experiment, were used. All animals were housed 3 per cage in an animal colony room with a 12 hr light-dark cycle. Food and water were provided ad lib.

On the day of testing, each animal was put in an individual cage. They were left undisturbed for 15 minutes before any behavioral observation was done. At that time, the animals were injected with either saline or ketanserin (5 mg/kg). Fifteen minutes later, the animals received another IP injection of either saline or one of the three doses (15, 22.5, and 30

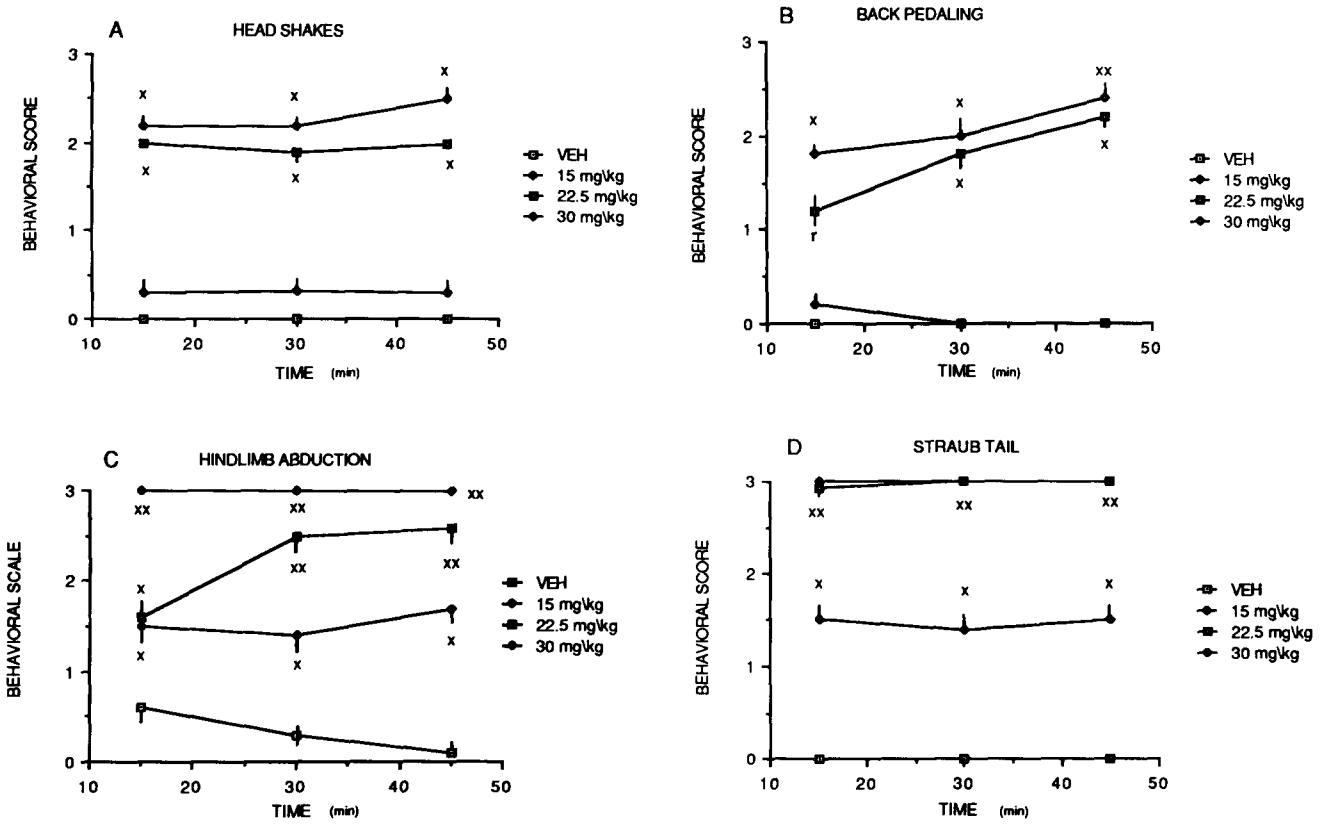

FIG. 1. The effects of ABNMP on (A) Head Shakes, (B) Back Pedaling, (C) Hindlimb Abduction, and (D) Straub Tail. The behaviors were rated as described under the Method section. Values represent means±SEM of 10 animals per group. Significantly different from vehicle: r=$p<0.01$; x=$p<0.0001$; xx=$p<0.00005$ (Scheffe).

mg/kg) of ABNMP (synthesized by C. R. Clark, see [15]). Immediately after that injection, ratings were recorded for 15 min intervals during a 45 min period of continuous observation. Since preliminary experiments had revealed that the drug causes behavioral abnormalities similar to those elicited by serotonergic agonists [8], the following signs were rated by a trained observer who was blind to the treatment given: (1) head shakes, (2) straub tail, (3) back pedaling, (4) hindlimb abduction, (5) random circling, (6) tremor. A 4-point intensity scale (0=absent or normal behavior, 1=mild, 2=moderate, 3=severe) was used [5]. In order to further evaluate the role of the serotonin system in the elicitation of the behaviors caused by ABNMP, another group of nine animals were pretreated with parachlorophenylalanine (PCPA) (300 mg/kg) 72 hr and 16 hr before being the highest dose of the drug (30 mg/kg).

The data were analyzed using an analysis of variance followed by Scheffe's multiple comparisons. The null hypothesis was rejected at the 0.05 level.

RESULTS

The acute administration of ABNMP caused a significant dose effect on head shakes, $F(3,36)=151.8$, $p<0.0001$, straub tail, $F(3,36)=207.1$, $p<0.0001$, back pedaling, $F(3,36)=116.6$, $p<0.0001$, and hindlimb abduction, $F(3,36)=71.03$, $p<0.0001$ (Fig. 1A–D). These changes occur within two minutes after injection of the compound.

Nevertheless, the various aspects of the serotonergic syndrome were differentially affected by the drug (Fig. 1A–D). At 15 mg/kg, hindlimb abduction and straub tail were the most affected, whereas head weavings and backwards walking were minimally influenced. At 22.5 mg/kg, there was significant stimulation of straub tail, head weaving, hindlimb abduction and backward locomotion. The effect on straub tail was maximal at that dose. At 30 mg/kg, both head weaving and backward walking were further stimulated while hindlimb abduction and straub tail were maximally affected using the present intensity scale. The doses of ABNMP used in this study had no stimulatory effect on the other components of the 5-HT syndrome (data not shown). Higher doses of the drug were not used because they caused seizures in the preliminary studies used to characterize the behavioral profile of the compound.

Ketanserin (5 mg/kg) had very significant inhibitory effect on head shakes, $F(1,18)=58.7$, $p<0.0001$, back pedaling, $F(1,18)=36.75$, $p<0.0001$, hindlimb abduction, $F(1,18)=20.22$, $p<0.0003$, and straub tail, $F(1,18)=196.6$, $p<0.0001$, caused by ABNMP (30 mg/kg) (Fig. 2). The percentage reduction of hindlimb abduction was less than that of the aspects of the 5-HT-mediated behaviors.

Pretreatment with PCPA also resulted in significant reduction in head shakes, $F(1,17)=54.16$, $p<0.0001$, back pedaling, $F(1,17)=198.4$, $p<0.0001$, hindlimb abduction, $F(1,17)=25.8$, $p<0.0001$, and straub tail, $F(1,17)=56.2$, $p<0.0001$, caused by ABNMP (30 mg/kg) (Fig. 2). Again, the percentage reduction was less in hindlimb abduction than in the other aspects of the behavioral syndrome.

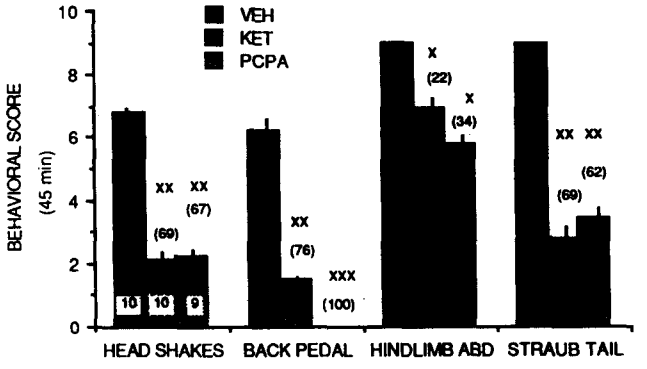

FIG. 2. Inhibitory effects of ketanserin and of PCPA on the behaviors elicited by ABNMP (30 mg/kg). The scores for each sign were summed for the 45 minutes of observation after the injection of ABNMP. The values represent means±SEM of 9 or 10 animals per group (see inserts at the bottom of the bars representing the scores for head shakes). The numbers in parentheses represent percentage inhibition which was calculated as follows: 100 [(mean saline group − mean ketanserin group)/mean saline group]. Significantly different from vehicle pretreated group: x=$p<0.001$; xx=$p<0.0001$; xxx=$p<0.00001$; (Scheffe's test).

DISCUSSION

ABNMP caused significant stimulation of head weavings, straub tail, backward locomotion, and hindlimb abduction which are components of the serotonergic syndrome in rats. All these behavioral abnormalities were also blocked by the 5-HT-2 receptor antagonist ketanserin and the serotonin depleter PCPA. The very significant effect of ketanserin on head weaving is consistent with other studies that have shown that this component of the syndrome is the one most consistently inhibited by 5-HT-2 antagonists [1, 7, 8]. Nevertheless, the relative failure of ketanserin and PCPA to block ABNMP-induced abduction indicates that other neurotransmitters may be involved in its manifestation. It has indeed been reported that the 5-HT-induced syndrome can be attenuated by a number of drugs including alpha-1 adrenergic and dopaminergic antagonists [8, 9, 20]. Moreover, similar behavioral abnormalities can be elicited by some peptides including the enkephalins and thyrotropin-releasing hormone [7,8]. Thus the importance of these neurotransmitter systems in the causation of the ABNMP-induced syndrome needs to be considered.

In any case, the results of the present study point to a role of the serotonin system via stimulation of post-synaptic 5-HT-2 receptors since the behavioral abnormalities were blocked, to varying degrees, by ketanserin. This stimulation could result either from direct stimulation of the receptors, blocking of reuptake, or induction of release of serotonin in a fashion similar to the action of the other amphetamines on monoaminergic neurons [14]. The finding that PCPA also attenuates the effects of ABNMP indicates that the compound probably acts indirectly via an acute release of 5-HT similar to the effects of parachloroamphetamine [21].

The serotonergic syndrome is thought to depend on structures such as the brainstem raphe nuclei and the spinal cord [11]. Our finding that ABNMP causes a 5-HT-like syndrome raises the possibility that this compound may have significant effects on brainstem and spinal cord related processes in humans who abuse METH synthesized in illicit laboratories. It is also possible that some of the casualties from METH injections are related to such phenomena.

The present findings also suggest that the behavioral effects of ABNMP may be somewhat different from those of amphetamine or methamphetamine [3,4] but very similar to those of parachloroamphetamine which also induces the 5-HT syndrome [21]. More studies are needed in order to investigate the effects of the drugs on various monoaminergic systems in order to determine whether there is any correlation between the observed behaviors and possible biochemical changes in rat brain. Further behavioral experiments are required to resolve the issue of the relative importance of other neurotransmitter systems in the manifestation of the ABNMP-induced phenomena.

ACKNOWLEDGEMENTS

The authors wish to thank Alfred Dollison for technical assistance with the behavioral studies.

REFERENCES

1. Arnt, J., J. Hyttel and J-J. Larsen. The citalopram/5-HTP-induced head shake syndrome is correlated to 5-HT receptor affinity and also influenced by other neurotransmitters. *Acta Pharmacol Toxicol* **55:** 363–372, 1984.
2. Barron, R. P., A. V. Kruegel, J. M. Moore and T. C. Kram. Identifications of impurities in illicit methamphetamine samples, *J Assoc Off Anal Chem* **57:** 1147–1158, 1974.
3. Cole, S. O. Brain mechanisms of amphetamine-induced anorexia, locomotion and stereotypy: A review. *Neurosci Biobehav Rev* **2:** 89–100, 1978.
4. Creese, I. and S. D. Iversen. The pharmacological and anatomical substrates of the amphetamine response in the rat. *Brain Res* **83:** 419–436, 1975.
5. Deakin, J. F. K. and A. R. Green. The effects of putative 5-hydroxytryptamine antagonists on the behaviour produced by tranylcypromine and L-tryptophan or tranylcypromine and L-Dopa to rats. *Br J Pharmacol* **64:** 201–209, 1978.
6. Delaney, P. and M. Estes. Intracranial hemorrhage with amphetamine abuse. *Neurology* **30:** 1125–1128, 1980.
7. Drust, E. G. and J. D. Connor. Pharmacological analysis of shaking behavior induced by enkephalins, thyrotropin-releasing hormone or serotonin in rats: evidence for different mechanisms. *J Pharmacol Exp Ther* **224:** 148–154, 1983.
8. Green, A. R. 5-HT-mediated behaviour. *Neuropharmacology* **23:** 1521–1528, 1984.
9. Handley, S. L. and J. Brown. Effects on the 5-hydroxytryptamine-induced head twitch of drugs with selective actions on alpha-1- and alpha-2-adrenoceptors. *Neuropharmacology* **21:** 507–510, 1982.
10. Hotchkiss, A. J. and J. W. Gibb. Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. *J Pharmacol Exp Ther* **214:** 257–262, 1980.
11. Jacobs, B. L. An animal model for studying central serotonergic synapses. *Life Sci* **19:** 777–786, 1976.
12. Kane, F. J., M. H. Keeler and C. B. Reifler. Neurological crises following methamphetamine. *JAMA* **210:** 556–557, 1969.
13. Lambrechts, M. and K. E. Rasmussen. Leuckart-specific impurities in amphetamine and methamphetamine seized in Norway. *Bull Narc* **36:** 47–57, 1984.

14. Moore, K. E., L. A. Carr and J. A. Dominic. Functional significance of amphetamine-induced release of brain catecholamines. In: *Amphetamines and Related Compounds*, edited by E. Costa and S. Garattini. New York: Raven Press, 1970, pp. 371–384.
15. Noggle, E. T., C. R. Clark, T. W. Davenport and S. T. Coker. Synthesis, identification, and acute toxicity of α-benzylphenethylamine and α-benzyl-N-methylphenethylamine. Contaminants in clandestine preparation of amphetamine and methamphetamine. *J Assoc Off Anal Chem* **68:** 1213–1222, 1985.
16. Ricaurte, G., G. Bryan, L. Strauss, L. Seiden and C. Schuster. Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals. *Science* **229:** 986–988, 1985.
17. Ricaurte, G. A., C. R. Schuster and L. S. Seiden. Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: A regional study. *Brain Res* **193:** 153–163, 1980.
18. Ricaurte, G. A., L. S. Seiden and C. R. Schuster. Further evidence that amphetamines produce long-lasting dopamine neurochemical deficits by destroying dopamine nerve fibers. *Brain Res* **303:** 359–364, 1984.
19. Stoessl, A. J., G. B. Young and T. E. Feasb. Intracerebral haemorrhage and angiographic beading following ingestion of catecholaminergic. *Stroke* **16:** 734–736, 1985.
20. Tricklebank, M. D., F. Christian and J. R. Fozard. The involvement of subtypes of the 5-HT_1 receptor and of catecholaminergic systems in the behavioral response to 8-hydroxy-2-(di-n-propylamino)tetralin in the rat. *Eur J Pharmacol* **106:** 271–282, 1985.
21. Trulson, M. E. and B. L. Jacobs. Behavioral evidence for the rapid release of CNS serotonin by PCA and fenfluramine. *Eur J Pharmacol* **36:** 149–154, 1976.