

The Behavioral Effects of the Stereoisomers of 4-Methylaminorex, a Psychostimulant, in the Rat¹

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Accepted for publication February 3, 1994

ABSTRACT

In this study, we examined the effect of the isomers of the psychostimulant 2-amino-4-methyl-5-phenyl- Δ^2 -oxazoline (4-methylaminorex or 4-MAX) on behavior in rats. The s.c. administration of the stereoisomers of 4-MAX, *trans*-4S,5S, *cis*-4R,5S, *cis*-4S,5R and *trans*-4R,5R (0.3–3 mg/kg), produced a dose-dependent increase in locomotor activity. However, at a dose of 10 mg/kg, the isomers of 4-MAX produced an initial increase in locomotor activity followed by the appearance of stereotyped behaviors (continuous sniffing, chewing, head bobbing, etc.) then a subsequent phase of rebound-enhanced locomotor activity all over a time course of 4 hr. The rank order of potency for the stereoisomers of 4-MAX was: 4S,5S > 4R,5S = 4S,5R < 4R,5R. To determine what neurotransmitter systems may mediate the action of 4-MAX, the ability of various receptor antagonists to block or attenuate the effect of *trans*-4S,5S-4-MAX (3 mg/kg s.c.) on locomotor activity was examined. The selective dopamine (DA) D₁ antagonist (–)-*trans*-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl-5H-benzo-[d]naph-thio[2,1b]azepine (SCH 39166) and the D₂ receptor antagonist (–)-eticlopride

significantly attenuated the behavioral action of *trans*-4S,5S-4-MAX, whereas antagonists to serotonergic and adrenergic receptors were ineffective. These results suggest that the ability of 4S,5S-4-MAX to induce locomotor hyperactivity and stereotypical behaviors and to increase rearing durations is primarily mediated by DA receptors. To determine whether the action of 4-MAX is dependent upon 5-hydroxytryptamine or DA concentrations in nerve terminals, rats were pretreated with either reserpine, α -methyl-*p*-tyrosine or *d,l*-*p*-chlorophenylalanine. Both reserpine and α -methyl-*p*-tyrosine pretreatment markedly attenuated the locomotor activity produced by *trans*-4S,5S-4-MAX. Moreover, L-dopa partially reversed the inhibitory action of α -methyl-*p*-tyrosine on 4S,5S-4-MAX-induced behavioral activation. In contrast, the administration of *d,l*-*p*-chlorophenylalanine did not alter the behavioral responses produced by *trans*-4S,5S-4-MAX. Taken together, our results suggest that 4-MAX may increase several locomotor behaviors by inducing neuronal DA release, which subsequently interacts with dopaminergic receptors.

The compound 4-MAX is a phenylisopropylamine derivative with anorectic and psychostimulant properties (Poos *et al.*, 1963; Yelonsky and Katz, 1963). This compound exists as four stereoisomers: *trans*-4R,5R, *cis*-4R,5S, *cis*-4S,5R and *trans*-4S,5S. Recent evidence suggests that 4-MAX may possess a high potential for abuse. For example, *cis*-($\pm$)-4-MAX (street name "U4Euh") has appeared on the clandestine market as a new designer drug and has been misrepresented by drug dealers as cocaine and methamphetamine (Klein *et al.*, 1989; Davis and Brewster, 1988). A recent report has shown that *cis*-($\pm$)-4-MAX is self-administered by monkeys and can substitute for

cocaine in drug discrimination paradigms (Mansbach *et al.*, 1990). Initial reports from humans also indicate that *cis*-($\pm$)-4-MAX is readily self-administered and that its subjective effects are similar to those of cocaine and AMPH (Drug Control Section, 1988). Due to the high abuse potential for 4-MAX, the *cis* isomers were assigned to the Schedule 1 drug category of the Controlled Substances Act in October 1987 (Federal Register, 1989).

So far, relatively few studies have been published regarding the biochemical and pharmacological properties of the isomers of 4-MAX. Glennon and Misenheimer (1990), using the behavioral paradigm of drug discrimination, have shown that the stimulus cues produced by the i.p. administration of *S*-(+)-AMPH in rats generalized to all four isomers of 4-MAX. Bunker *et al.* (1990) have demonstrated that the s.c. administration of ($\pm$)-*cis*-4-MAX (2.5–10 mg/kg) produces a dose-dependent increase in locomotion and stereotyped behavior in

Received for publication September 28, 1993.

¹Supported by United States Public Health Service Grants RO1 DA 07193 and RO1 MH41440 to R.Y.W.

²Supported by National Research Service Award Predoctoral Fellowship F31 DA 05532.

³Supported by United States Public Health Service Grant RO3 DA 0680072.

ABBREVIATIONS: 4-MAX, 2-amino-4-methyl-5-phenyl- Δ^2 -oxazoline, 4-methylaminorex; AMPH, amphetamine; DA, dopamine; NE, norepinephrine; 5-HT, 5-hydroxytryptamine (serotonin); NAc, nucleus accumbens; A9, substantia nigra pars compacta; A10, ventral tegmental area; PCPA, *d,l*-*p*-chlorophenylalanine; AMPT, *D,L*- α -methyl-*p*-tyrosine; CNS, central nervous system; SCH 39166, (–)-*trans*-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl-5H-benzo-[d]naph-thio[2,1b]azepine.

rats, a finding similar to the action of *S*-(+)-AMPH. Further, these authors have shown that 4-MAX produces a substantial increase of DA turnover as well as a reduction of DA levels in neostriatal tissue. In addition, high doses of 4-MAX (20 mg/kg s.c.) decreases concentrations of NE and 5-HT in the hippocampus and neostriatum, respectively. Bunker *et al.* (1990) have also shown that 4-MAX administration induces decreases of tryptophan hydroxylase activity to 38% of controls but has no clear consistent effect on the activity of tyrosine hydroxylase (see also Hanson *et al.*, 1992). This last finding is in contrast with changes in the activities of these enzymes caused by *S*-(+)-AMPH. For instance, the administration of 15 mg/kg of methamphetamine s.c. reduced the activity of both tryptophan hydroxylase and tyrosine hydroxylase in the neostriatum of the rat to 7 and 50% of controls, respectively (Sonsalla *et al.*, 1986). Hanson *et al.* (1992) have also described the potent epileptogenic properties of 4-MAX at doses greater than 15 mg/kg s.c. In contrast, other researchers have reported the administration of much higher doses of *S*-(+)-AMPH without convulsive effects (Trulson and Trulson, 1982).

Using the technique of microdialysis, we have recently reported that the systemic administration of the *cis*-4*R*,5*S* and the *trans*-4*S*,5*S* isomers produces a 4- to 8-fold increase in DA release in the NAc and neostriatum compared to basal levels (Eberle *et al.*, 1992). The ability of 4-MAX to release DA has been inferred by showing that the s.c. administration of 10 mg/kg of 4-MAX increased neostriatal neurotensin and dynorphin levels to the same extent as other AMPH analogs (Merchant *et al.*, 1987; Hanson *et al.*, 1988). However, unlike AMPH, which has been shown to suppress equipotently the firing rate of both A9 and A10 DA cells (Bunney *et al.*, 1975; Wang, 1981), 4*S*,5*S*-4-MAX appears to exert a preferential suppressive action on the firing of A10 DA cells (Vogel *et al.*, 1991; C. R. Ashby *et al.*, unpublished data) indicating a greater action of 4-MAX on the mesolimbocortical over the nigrostriatal DA pathway.

To date, there have been no studies published regarding the behavioral profile of the individual stereoisomers of 4-MAX on rats. Therefore, the aim of the present study was to examine the effect of the stereoisomers of 4-MAX (0.3–10 mg/kg s.c.) on the locomotive and stereotyped behavior of rats and to determine whether the behavioral profile of 4-MAX is significantly different from that previously reported for *S*-(+)-AMPH. We have also determined whether the increase in locomotor activity produced by the systemic administration of the *trans*-4*S*,5*S*-4-MAX is altered by pretreating rats with various dopaminergic, serotonergic and noradrenergic receptors antagonists. The effect of *trans*-4*S*,5*S*-4-MAX on behavior was also examined in animals that were pretreated with various pharmacological agents that deplete monoamine neurotransmitter stores.

Materials and Methods

Subjects. Male Sprague-Dawley (180–250 g) rats were used in this study. These animals were maintained on a 12-hr light/dark schedule with lights on at 7:00 A.M. in a temperature-controlled room. Both water and food were available *ad libitum* except during testing.

Apparatus. The animals were tested in 16.5-inch × 16.5-inch × 12-inch high clear acrylic cages. Locomotor activity in all experiments was measured by an electronic animal monitoring system (ILAM by Omnitech Electronics, Inc., Columbus, OH) which consisted of 16 infrared beams projected across the cages from left to right and 16 infrared beams from front to back all approximately 1 inch from the floor to

measure locomotion. This system also had 16 infrared beams from right to left 5 inches from the floor of the cage to measure rearing durations.

Measurement of locomotor activity and stereotypy. The total distance traveled in millimeters for each animal was integrated over 19 intervals (each 15 min in duration) and recorded onto a PC-AT computer. However, the square root of the total distance traveled was used in the analysis to reduce data spread. Rearing duration, a component of behavioral activation, was also recorded.

The behavior of the animals in all experiments was also monitored visually for 2 min per 15-min interval, and was scored according to the following rating scale (slightly modified from Creese and Iversen, 1973): 0, animal is asleep; 1, animal is awake and stationary with no rearing; 2, animal is moving or rearing sporadically; 3, animal is moving or rearing continuously; 4, animal is exhibiting light stereotypy and 5, animal is exhibiting intense stereotypy. Stereotypy was defined as rapid, purposeless, repetitive motions and included rapid sniffing, head bobbing or weaving, body weaving, rapid backing up while sniffing or gnawing at the animal's own feet and teeth chattering. Raters were naive to the experimental design and to the expected effects of the drug treatments applied.

Drug testing procedures. To avoid residual drug effects or behavioral sensitization caused by previous experience with psychostimulants, each rat received only one dose of one isomer of 4-MAX. Six rats were tested under each condition, exceptions where noted.

Subcutaneous administration of the isomers of 4-MAX and *S*-(+)-AMPH. The four isomers of 4-MAX (*trans*-4*S*,5*S*, *trans*-4*R*,5*R*, *cis*-4*S*,5*R* and *cis*-4*R*,5*S*) were administered subcutaneously at doses of 0.3, 1, 3 and 10 mg/kg with the exception of 0.3 mg/kg of the 4*R*,5*R* isomer. This dose of 4*R*,5*R* was not tested as the behavioral effect produced by this isomer at 1 and 3 mg/kg was not significantly different from that of saline. *S*-(+)-AMPH was administered at 3 mg/kg s.c. Animals were placed into the experimental apparatus for three intervals (45 min) before the administration of the 4-MAX isomers, *S*-(+)-AMPH or the saline control to allow the animals to acclimate to the testing environment.

Application of monoamine receptor antagonists. In a subsequent series of experiments, we examined the effects of various noradrenergic, serotonergic and dopaminergic antagonists on the locomotor activation produced by 3 mg/kg s.c. of *trans*-4*S*,5*S*-4-MAX. This dose and isomer was chosen as it produced behavioral activation that was reliably increased with little stereotypy.

The following 5-HT receptor antagonists were administered: granisetron (5-HT₃, 0.3 and 3 mg/kg), (±)-pindolol (5-HT_{1A,1B,β}, 10 mg/kg), methysergide (5-HT_{1,2}, 10 mg/kg) and ritanserin (5-HT_{2A,2C}, 0.3 and 3 mg/kg). The antagonists to adrenergic and noradrenergic receptors used were: phentolamine (α₁, 10 mg/kg) and idazoxan (α₂, 0.3 and 3 mg/kg). The antagonists to DA receptors used were: SCH 39166 (D₁, 0.03, 0.3 and 3 mg/kg) and (–)-eticlopride (D₂, 0.003, 0.03 and 0.3 mg/kg).

When the effects of an antagonist or saline was tested, it was administered i.p. at the beginning of the experiment (the beginning of interval 1) and the test dose of 4-MAX was given at the end of the third interval (45 min later).

Inhibition of catecholamine and serotonin synthesis and vesicular depletion of monoamines. In a separate series of experiments, animals were pretreated with either reserpine (5 mg/kg s.c. 24 hr before 4-MAX), PCPA (400 mg/kg i.p. 24 hr before 4-MAX) or AMPT (500 mg/kg, administered as 250 mg/kg i.p. 2 and 4 hr before 4-MAX) and the effects of *trans*-4*S*,5*S* at 3 mg/kg s.c. on behavioral responding was examined. It has been shown previously that these doses of these neurotransmitter-depleting agents will reduce the CNS concentration of monoamines 75 to 99% (PCPA; Reader and Gauthier, 1984; AMPT; Weissman and Koe, 1965; reserpine; Hiroi and White, 1990). In addition, to restore CNS DA concentrations, benserazide (50 mg/kg i.p.), which inhibits the peripheral L-aromatic amino acid decarboxylase, and L-dopa (100 mg/kg i.p.), the immediate precursor of DA, were administered to rats pretreated with AMPT 30 min and immediately before 4-MAX, respectively. This treatment has been shown previously

to restore the ability of *S*-(+)-AMPH to inhibit DA cells after a pretreatment with AMPT (Wang, 1981).

Drug sources. 4-MAX was synthesized and provided by Dr. Robert F.X. Klein and the National Institute on Drug Abuse. The following drugs were obtained from Sigma Chemical Co. (St. Louis, MO): L-dopa, PCPA, AMPT and reserpine. The compounds (-)-eticlopride and ritanserin were obtained from Research Biochemicals Incorporated, International (RBI, Natick, MA). Idazoxan, granisetron, benserazide, SCH 39166, methysergide and phentolamine were gifts from Reckitt and Coleman (Hull, UK), Smith Kline and Beecham Pharmaceuticals (Harlow, Essex, UK), Hoffman LaRoche (Nutley, NJ), Schering-Plough Corporation (Bloomfield, NJ), Sandoz Pharmaceuticals (East Hanover, NJ) and Ciba-Geigy (Summit, NJ), respectively.

Analysis of drug effects. For statistical analyses, a general linear regression model with a Tukey test for post-hoc comparisons was used to compare the differences in distance traveled at each of the intervals of interest. The α level for significance was reduced by the number of intervals compared. To provide a more specific test for overall main effects, the values for distance traveled during time intervals 4 to 11 and during 12 to 19 were integrated and the resulting integrations were compared among treatments using the same type of statistical analysis. This provided a more sensitive test of the activation resulting from 4-MAX administration, as the bulk of the treatment effect naturally occurred during the first half of the experiment for most of the

treatments. Also, this provided a more sensitive way of examining those treatments that illustrated their effects through alteration of the time course of behavioral activation. For treatments resulting in neurotransmitter depletions the total distance traveled during the acclimation period (intervals 1–3) was also analyzed using an analysis of variance with Dunnett's post-hoc test with saline pretreatment as the control. Finally, the total distance traveled for the entire duration of the experiment (area under the curve) was calculated and compared using the above methods.

Correlations between the total distance traveled and the behavioral ratings for each interval for those treatments resulting in stereotypy were also calculated. Also, correlations between measures of total distance traveled and rearing duration were made. The SAS (SAS Institute Inc., Carey, NC) statistical package was used for all comparisons.

Results

Effects of the subcutaneous administration of 4-MAX isomers on behavioral activation. Determinations of behavioral activation consisted of measurements of locomotor behavior (total distance traveled) as well as behavioral ratings (see under "Methods"). Overall, 4-MAX produced a dose-dependent increase in behavioral activation for the dose range of

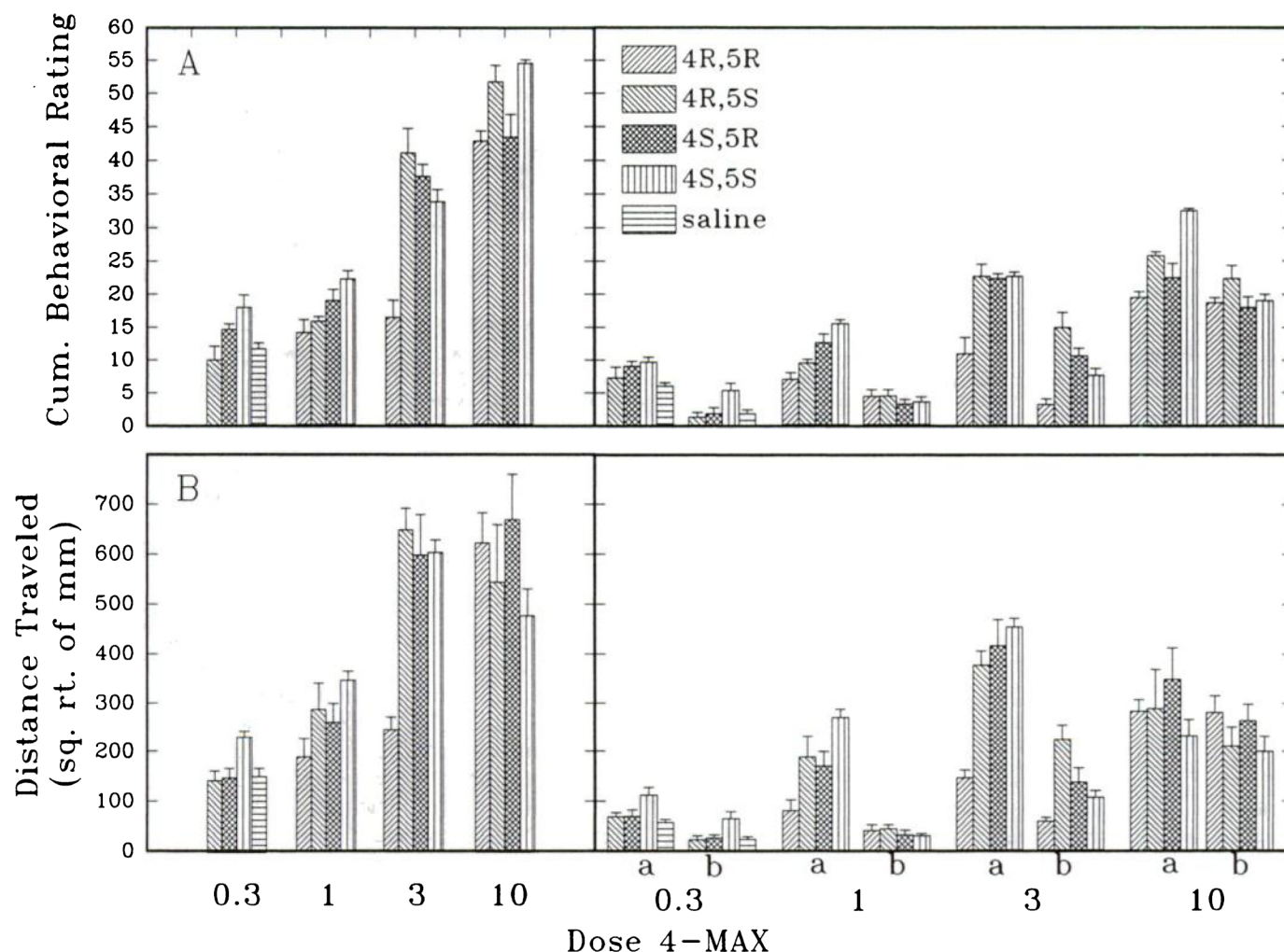


Fig. 1. Comparison of dose response curves for the four isomers of 4-MAX to increase A) behavioral ratings and B) locomotion. Overall, there was a dose-dependent increase in the behavioral rating and locomotor behavior for all isomers except where intense stereotypical behaviors appeared to compete with measures of horizontal locomotion. These reductions in overall locomotion occurred at the highest dose of the more potent isomers (4S,5S and 4R,5S, see also fig. 4). Legend: a, integration of intervals 4 to 11; b, integration of intervals 12 to 19. For 1 mg/kg of the 4R,5S isomer, $n = 8$; for 1 mg/kg of the saline treatment, $n = 10$; for 1 mg/kg of the 4S,5S isomer, $n = 12$; for 3 mg/kg of the 4S,5S isomer, $n = 24$; for all other groups $n = 6$.

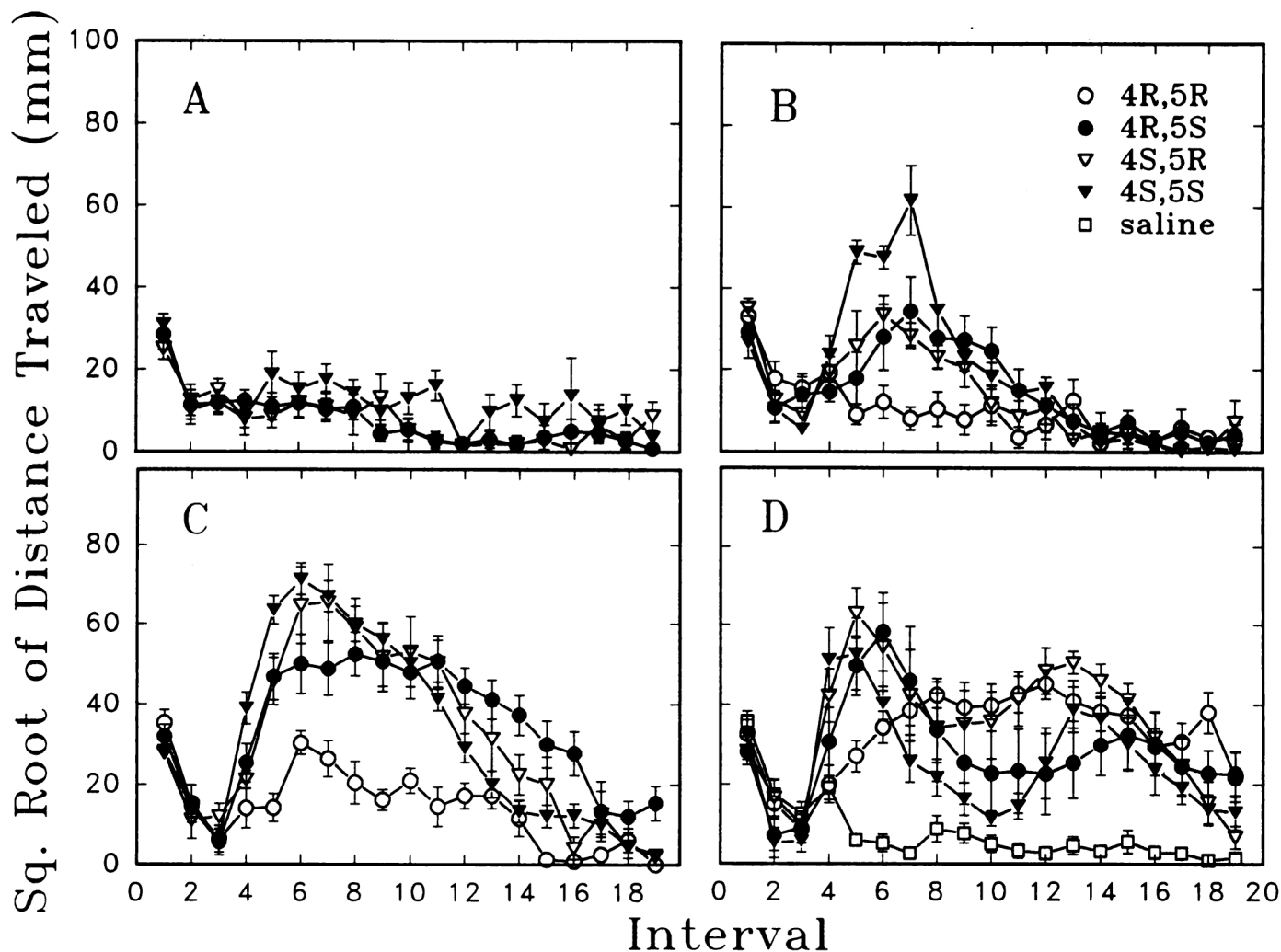


Fig. 2. The complete time course of the locomotor response induced by administration of various doses of the isomers of 4-MAX: A) 0.3 mg/kg, B) 1 mg/kg, C) 3 mg/kg and D) 10 mg/kg. The time course of activation for the saline control is shown in D. 4-MAX was administered at the end of interval 3. The rank of order of potency, determined by the onset and the duration of the peak of locomotion among the four isomers of 4-MAX, was: 4S,5S > 4S,5R = 4R,5S > 4R,5R.

0.3 to 10 mg/kg (figs. 1-3). However, for the 4S,5S and 4R,5S isomers, the greatest increase in total distance traveled occurred at the 3-mg/kg dose with a relative decrease in locomotion at 10 mg/kg. At 10 mg/kg, both 4S,5S and 4R,5S 4-MAX induced high behavioral ratings, indicative of intense stereotypy (see below). For all isomers, the onset of action at the doses of 1, 3 and 10 mg/kg were relatively rapid (within 15-30 min), and peaked about 30 to 45 min after the test drug was administered. At the doses of 3 and 10 mg/kg, the overall duration of activation for most of the isomers was about 4 hr.

The latency for the onset of the behavioral effects produced by 4-MAX administration was quite similar among the isomers of 4-MAX. Therefore, we define the potency of 4-MAX isomers in inducing behavioral activation as a combination of the onset rapidity and amplitude of locomotion and rearing and the intensity of behavioral activation, i.e., the peak and duration of locomotor behavior throughout the experiment and the induction of stereotypy. We were able to integrate these measures by examining plots of the total area under the curve as compared to iterations of intervals 4 to 11 and 12 to 19 (fig. 1B compared to figs. 2 and 3) for both locomotion and behavioral rating. The rank order of potency in behavioral activation for the stereoisomers of 4-MAX was: 4S,5S > 4R,5S = 4S,5R >

4R,5R. Although these isomers often induced similar effects, it was found that the 4R,5R isomer produced the lowest magnitude of behavioral activation. In fact, only at 10 mg/kg was the locomotion behavior produced by this isomer increased significantly over saline.

Comparison of the dose-response curves of 4-MAX isomers. The administration of 0.3 mg/kg of the *trans*-4S,5S isomer resulted in a significant increase in overall total distance traveled as compared to both the *cis* isomers and saline (fig. 1B). At this dose the 4S,5S isomer induced significantly more locomotion than saline during the second half of the experiment. Further, locomotion due to 0.3 mg/kg of the *trans*-4S,5S isomer differed from saline at a few scattered intervals (intervals 7, 11, 14 and 18, fig. 2A; saline is shown in fig. 2D). The *trans*-4R,5R isomer was not tested at this dose. Analysis of the behavioral ratings at 0.3 mg/kg indicated that ratings produced by the application of the 4S,5S isomer were significantly increased over those caused by saline in overall behavioral rating as well as in both halves of the experiment. No other isomer had any behavioral ratings significantly different from that of saline at this dose (figs. 1A and 3A).

At the dose of 1 mg/kg, there were no significant differences among the 4S,5S and the *cis* isomers of 4-MAX for any meas-

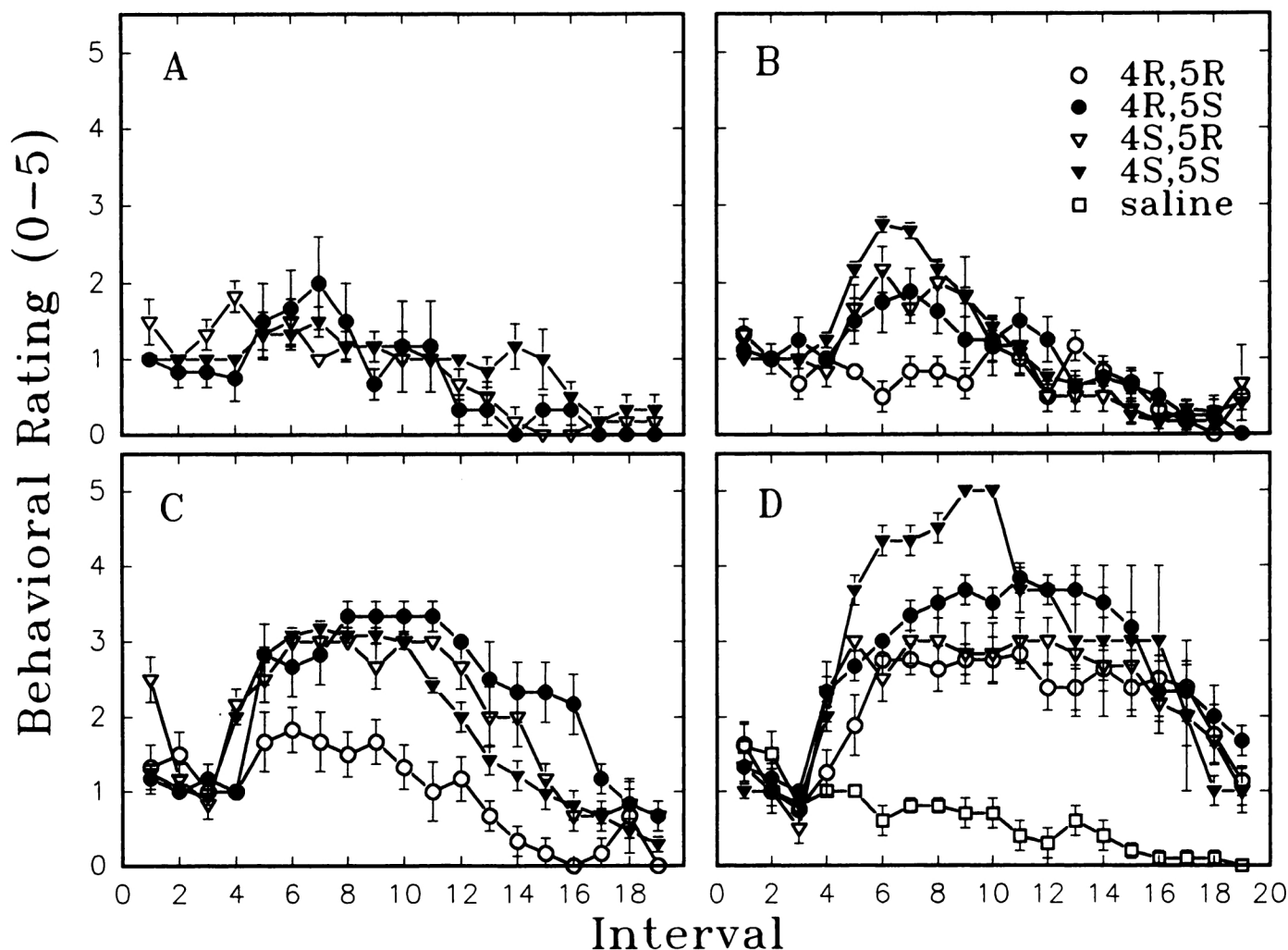


Fig. 3. The behavioral ratings produced by various doses of the four isomers of 4-MAX over all 19 intervals: A) 0.3 mg/kg, B) 1 mg/kg, C) 3 mg/kg and D) 10 mg/kg. At the highest doses, 4R,5S appeared to have higher behavioral ratings than that of 4S,5R, which corresponds to the fact that the 4R,5S isomer produced more stereotypy than did the 4S,5R isomer.

ure. The 4S,5S isomer exhibited significantly increased locomotion over that produced by the 4R,5R isomer and saline, whereas the 4R,5S but not the 4S,5R isomer significantly increased overall locomotion compared to saline. Both the *trans*-4S,5S and the *cis*-4R,5S isomers were significantly different from saline during the first half of the experiment for locomotion, but only the *trans*-4S,5S isomer was significantly different from saline at more than one specific interval (intervals 5-8; fig. 2, B and D). For the second half of the experiment, there were no differences at this dose among the four isomers. There were also no differences between any of the isomers and the saline control during the second half of the experiment. The time course for increases in locomotion produced by the 4S,5S, the 4R,5S and the 4S,5R isomers were nearly identical. They all reached peak activation at intervals 6 and 7 and thereafter slowly declined. Although the 4S,5R isomer at 1 mg/kg did show increases in overall locomotion, this isomer, as well as the 4R,5R isomer, were not significantly different from saline.

At the dose of 1 mg/kg, the overall behavioral ratings indicated that there were no significant differences in behavioral activation among the four isomers of 4-MAX (fig. 1A). However, both the 4S,5S and the 4R,5S isomers induced behavioral activation that was significantly higher than that of saline.

Further, these isomers were also significantly more effective than saline in eliciting behavioral activation during the first but not the second half of the experiment. No other isomer exhibited significant differences in behavioral rating from saline at any portion of the experiment at this dose.

At the dose of 3 mg/kg, the three most potent isomers, 4S,5S, 4S,4R and 4R,5S, were all significantly more potent than the 4R,5R and saline in inducing overall locomotion (fig. 1B). The peak of locomotor activation produced by the 4R,5S isomer was not as high as that of the 4S,5S and the 4S,5R isomers but the duration of activation was more prolonged (fig. 2C). The 4S,5S and the two *cis* isomers showed significantly greater locomotion than that caused by both the 4R,5R isomer and saline during the first half of the experiment. During the second half of the experiment, locomotion produced by the 4S,5S and the two *cis* isomers was significantly greater than that produced by saline but only the 4R,5S isomer increased locomotor behavior greater than the 4R,5R isomer. Furthermore, comparisons of distance traveled at specific intervals between the four isomers and saline indicated that the isomers of 4-MAX produced significantly greater locomotion than that of saline during the following intervals: 4R,5S, 11 intervals (5-16; fig. 2, C and D); 4S,5R, 9 intervals (5-14); 4S,5S, 7 intervals (5-12); 4R,5R, 2 intervals (6, 7).

Similar to the results for total distance traveled, the behavioral ratings for the 4S,5S, 4R,5S and the 4S,5R isomers at 3 mg/kg were significantly greater than that of the 4R,5R and saline during the entirety and both halves of the experiment (fig. 1A). Compared to saline, the 4R,5R isomer had a significantly higher behavioral rating for only the first half of the experiment.

At the dose of 10 mg/kg, the isomers 4S,5S, 4S,5R and 4R,5S all produced multiphasic curves for locomotor activation with a primary peak at approximately 30 min and a secondary peak at approximately 3 hr after drug administration (fig. 2D). The 4R,5R isomer induced a gradual increase in locomotion and plateaued at a lower level compared to the primary peak caused by the other isomers for a relatively long period of time (2.5–3 hr). At this dose, each of the isomers produced total locomotor increases that were significantly greater than that of saline alone (fig. 1B). However, there were no differences among the four isomers for total distance traveled as well as during either half of the experiment. There were also very few specific intervals in which the isomers differed significantly from each other (fig. 2D). Although the locomotion induced by the 4S,5S isomer was significantly greater than that of saline during the second half of the experiment, the other three isomers of 4-MAX produced significantly greater increases in locomotion over saline in both halves of the experiment (fig. 1B). The locomotion produced by the administration of the *trans*-4S,5S and the *cis* isomers differed significantly from that caused by saline at those intervals immediately after drug administration (intervals 4–6) and at those intervals corresponding to the second peak in locomotor activity (intervals 12–16, approximately 3–4 hr after drug administration). The *trans*-4R,5R isomer illustrated significantly enhanced locomotor increases over those induced by saline during intervals 5 to 18 (fig. 2D). All of the isomers at this dose exhibited significantly increased behavioral ratings over saline for overall rating as well as for both halves of the experiment (fig. 1A). As can be seen from this figure the 4S,5S isomer exhibited the greatest increase in overall behavioral rating from 3 to 10 mg/kg and also showed the greatest decrease in overall distance traveled (particularly during the first half of the experiment; see also fig. 3, C and D).

Correlations between locomotion and behavioral rating and between locomotion and rearing. Stereotypy was most often seen during administration of higher doses (3 and 10 mg/kg) of 4-MAX (fig. 3, A–D). Interestingly, at the highest dose of 10 mg/kg, isomers 4S,5S, 4R,5S and 4S,5R produced multiphasic alterations in locomotor activity with a primary peak at approximately 30 min and a secondary peak approximately 3 hr after administration (fig. 2D). The trough between the two peaks in this figure corresponds to the emergence of stereotyped behavior. To illustrate the incompatibility between locomotion and stereotypy, the distance traveled and the behavioral ratings for administration of the 4S,5S isomer at 10 mg/kg are overlaid in figure 4. As is apparent from this figure, we found a strong negative correlation between the values of the behavioral ratings and total distance traveled for those treatments exhibiting stereotypical behaviors (Pearson's correlations were negative from interval 6–12 and ranged from -0.629 to -0.845 for intervals 7–10; $P < .05$).

The locomotion suppression during stereotypy appeared to be nearly identical for the *trans*-4S,5S and the *cis*-4R,5S isomers, whereas the locomotion suppression of animals that received the *cis*-4S,5R was less (fig. 2D). Therefore, the *cis*-

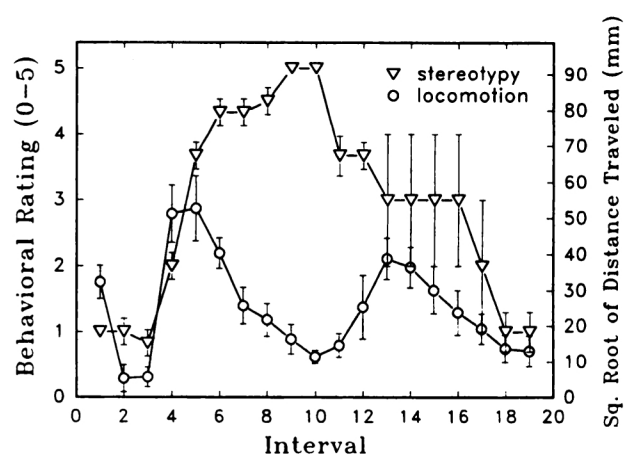


Fig. 4. 4S,5S-4-MAX-induced behavioral ratings and the square root of total distance traveled overlaid to illustrate the incompatibility between locomotion and stereotypy. As described under "Methods," behavioral ratings >3 represent the emergence of stereotypical behaviors and ratings >4 represent intense stereotypy. At the dose of 10 mg/kg, 4S,5S-4-MAX induced the development of intense stereotypy, while at the same time locomotion was markedly suppressed. This is most obvious from intervals 6 to 12. The negative correlation coefficient between behavioral rating and locomotion is highest at interval 10 (Pearson's $r = -0.845$; $P < .05$).

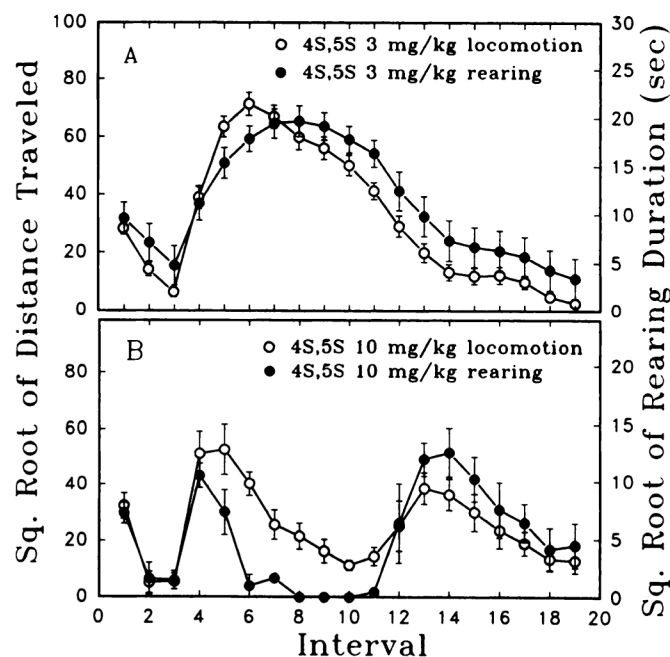


Fig. 5. Representative overlaid diagrams to illustrate the correlation between 4S,5S-4-MAX-induced rearing and locomotion. In A and B the results were obtained with 3 and 10 mg/kg of 4S,5S-4-MAX, respectively. As can be seen from the figure, there was a significant positive correlation between rearing durations and distance traveled throughout most of the experiment (see text).

4R,5S isomer appears to be more effective than 4S,5R in eliciting stereotyped behaviors. In contrast, the *cis*-4S,5R isomer appears to be more effective than 4R,5S in producing directed locomotor activation. Compared to the 4R,5S isomer, the onset of locomotion for 4S,5R was earlier (fig. 2, B and D) and the peak was higher (fig. 2, C and D).

As can be seen in figure 5, rearing durations were highly and positively correlated with measurements of total distance traveled during specific intervals (Pearson's correlations ranged from 0.3 to 0.7 between intervals 5–19; $P < .05$). We therefore

focused our analysis on scores of locomotion (total distance traveled) and behavioral ratings. Analysis of the high correlation between rearing durations and locomotion during periods of stereotypy indicated that increased rearing durations were not a part of stereotypical behaviors. Indeed, during periods of stereotypy induced by 10 mg/kg of the 4S,5S isomer of 4-MAX, rearing was completely inhibited (fig. 5).

Comparison of the behavioral activation produced by 4-MAX and S-(+)-AMPH. S-(+)-AMPH was also applied at 3 mg/kg s.c. which resulted in a behavioral activation similar to that produced by the 4S,5S isomer at the same dose. Figure 6 shows that both the total distance traveled and the behavioral rating scores of these two treatments were quite similar.

Effect of various receptor antagonists on the behavioral effect produced by *trans*-4S,5S-4-MAX. Effect of D_1 and D_2 receptor antagonists on the action of *trans*-4S,5S-4-MAX. The D_2 DA receptor antagonist (-)-eticlopride was administered at 0.003, 0.03 and 0.3 mg/kg i.p. before the 4S,5S isomer of 4-MAX at 3 mg/kg. Overall, locomotor activation at the highest dose of (-)-eticlopride was significantly less than both saline and the other two doses of this antagonist (figs. 7A and 8A). Further, the highest dose of (-)-eticlopride was effective in inhibiting locomotor increases produced by *trans*-4S,5S during intervals both halves of the experiment. For the second half of the experiment, (-)-eticlopride at 0.03 mg/kg was also able to reduce significantly 4S,5S 4-MAX-induced locomotion.

The D_1 DA receptor antagonist SCH 39166, was administered at 0.03, 0.3 and 3 mg/kg i.p. The two highest doses of this compound effectively attenuated 4-MAX-induced locomotor activity (figs. 7B and 8B). However, only at 3 mg/kg was this compound able to attenuate significantly the activation produced by 3 mg/kg of *trans*-4S,5S during the first half of the experiment. In contrast, all of the doses of SCH 39166 used were effective at reducing 4-MAX locomotor increases for the second half of the experiment. At the doses used in this study, (-)-eticlopride did not produce cataleptic effects. However, at the highest dose of SCH 39166 (3.0 mg/kg i.p.), there was a significant suppression of locomotor activity during the acclimation phase of the experiment (analysis of variance followed by Tukey post-hoc test; $P < .05$; figs. 7B and 8B), suggesting

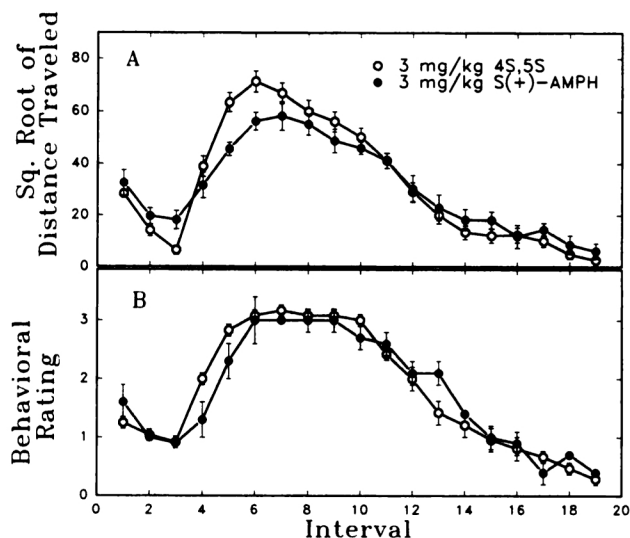


Fig. 6. Comparison of the behaviorally activating effects of equal doses of 4S,5S-4-MAX and S-(+)-AMPH. As can be seen from the figure, these two compounds are equally effective in inducing A) locomotor increases and B) increases in behavioral ratings. For 3 mg/kg of S-(+)-AMPH, $n = 10$.

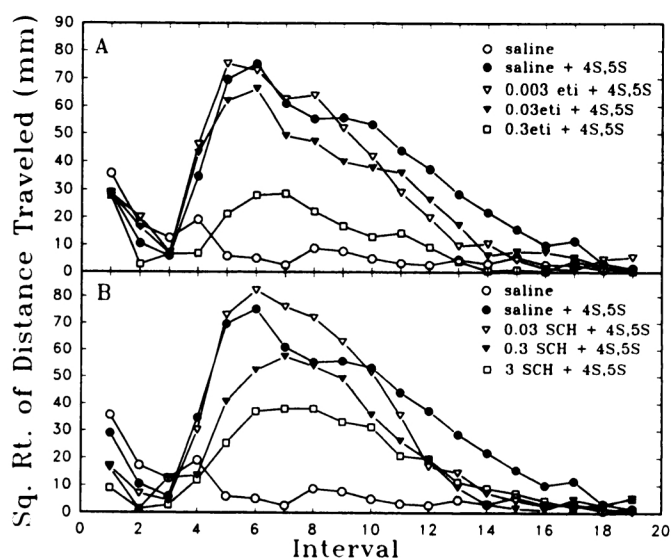


Fig. 7. The attenuation of 4-MAX-induced locomotion by antagonists at D_1 and D_2 DA receptors. The square root of total distance traveled (in mm) is shown by time interval for the locomotion produced by 4S,5S-4-MAX with either A) (-)-eticlopride or B) SCH 39166 pretreatment. The behavioral activation produced by saline alone is included for comparison. As can be seen from figure 7B, the highest dose of SCH 39166 significantly decreased (analysis of variance with Tukey post-hoc test; $P < .05$) behavioral activity during the first three intervals (acclimation period) before the administration of 4S,5S-4-MAX (3 mg/kg), indicating possible cataleptic effects for this compound. Although the attenuation of the behavioral activating effects of 4-MAX by these antagonists was significant at their respective highest doses, neither compound was able to achieve a complete block. Error bars have been removed for clarity. For 3 mg/kg of the 4S,5S isomer with saline pretreatment, $n = 8$; for 0.03 and 3 mg/kg of the SCH 39166 pretreatment, $n = 12$. In figures 7 to 10, 4S,5S-4-MAX was administered at 3 mg/kg.

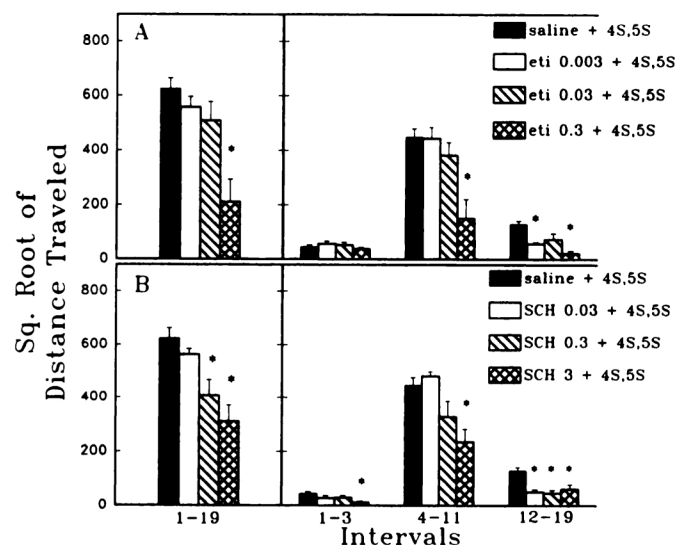


Fig. 8. Prevention or attenuation of 4S,5S-4-MAX-induced behavioral activation by dopaminergic antagonists. The first set of bars indicates total distance traveled over the entire experiment; the second, third and fourth sets of bars indicate distance traveled during the intervals 1 to 3, 4 to 11 and 12 to 19, respectively (same for figs. 9 and 10). *Significantly smaller than saline pretreatment before 3 mg/kg of 4S,5S-4-MAX ($P < .001$, analysis of variance with Tukey post-hoc comparison). All antagonists were administered at the beginning of the experiment, 4-MAX was administered at the end of the third interval.

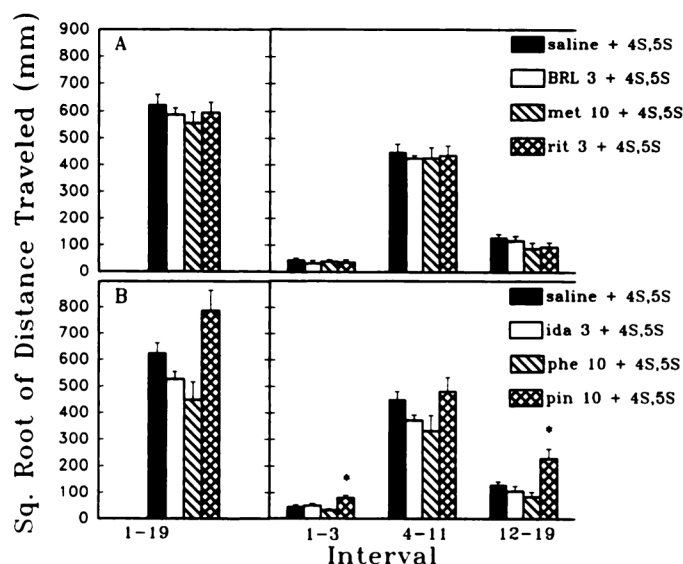


Fig. 9. Failure of A) serotonergic or B) adrenergic antagonists to attenuate the behavioral activation induced by the administration of 3 mg/kg of 4S,5S-4-MAX. ($\pm$)-Pindolol (10 mg/kg i.p.) actually enhanced 4-MAX-induced behavioral activation. *Significantly greater than the saline control ($P < .001$, analysis of variance with Tukey post-hoc comparison). For 3 mg/kg of the ritanserin pretreatment, $n = 10$.

the emergence of catalepsy. Yet, even at this dose of SCH 39166 the attenuation of 4-MAX-induced locomotor behavior was not complete.

Effect of 5-HT receptor antagonists on the action of trans-4S,5S 4-MAX. Overall, antagonists to several 5-HT receptors were ineffective in antagonizing the behavioral effects induced by 3 mg/kg of trans-4S,5S 4-MAX. The pretreatment of the animals with 0.3 and 3 mg/kg of granisetron (a 5-HT₃ receptor antagonist, for 0.3 mg/kg of granisetron, $n = 18$) did not alter the action of trans-4S,5S-4-MAX at 3 mg/kg for overall total distance traveled as well as either half of the experiment. Ritanserin (a 5-HT_{2A/2C} receptor antagonist) administered at 0.3 and 3 mg/kg also failed to block the behavioral effects produced by administration of the trans-4S,5S isomer (only the high doses of granisetron and ritanserin are shown in fig. 9A). ($\pm$)-Pindolol, which blocks 5-HT_{1A/1B} and β adrenoreceptors, administered at 10 mg/kg, induced a significant augmentation of 4-MAX-induced locomotion during the second, but not the first, half of the experiment when compared to a saline pretreatment (fig. 9B). It should be pointed out that animals pretreated with this dose of ($\pm$)-pindolol exhibited increased locomotion during the first three intervals (a 45-min acclimation period just before 4-MAX administration). This action of ($\pm$)-pindolol may account for the overall (nonsignificant) increase of locomotion for the total distance traveled. Methysergide (a nonspecific 5-HT_{1,2} receptor antagonist), at 10 mg/kg, did not block the locomotor activity increase produced by trans-4S,5S administration during any part of the experiment or during any specific interval (fig. 9A).

Effect of adrenergic receptor antagonists on the action of trans-4S,5S-4-MAX. The possible effects of the peripheral α adrenoreceptor antagonist phentolamine (10 mg/kg), the β adrenoreceptor antagonist ($\pm$)-pindolol (10 mg/kg), and the relatively selective α -2 adrenoreceptor antagonist idazoxan (0.3 and 3 mg/kg) on the action of 3 mg/kg trans-4S,5S-4-MAX were also examined. Both phentolamine (10 mg/kg) and idazoxan (3 mg/kg) slightly reduced the locomotor increases

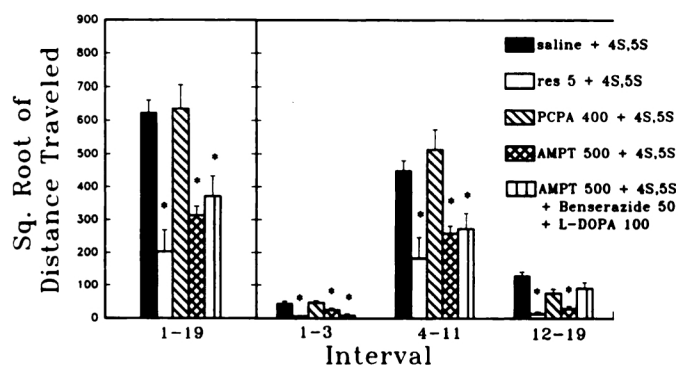


Fig. 10. Effects of pharmacological manipulation of monoamine systems on 4S,5S-4-MAX-induced locomotor behavior. Both reserpine and AMPT but not PCPA pretreatment significantly reduced basal locomotor activity. Compared to a saline pretreatment, reserpine (5 mg/kg s.c. 24 hr before testing), which depletes vesicular monoamine storage, significantly reduced the ability of 4S,5S-4-MAX (3 mg/kg s.c.) to increase locomotor behavior. Similarly, the blockade of dopamine synthesis with AMPT (500 mg/kg total, 250 mg/kg i.p., 4 and 2 hr before testing) significantly attenuated the behavioral activation induced by 4S,5S-4-MAX (3 mg/kg s.c.) during all intervals shown, whereas the inhibition of 5-HT synthesis with PCPA (400 mg/kg i.p. 24 hr before experiment) was without effect. The replenishment of CNS dopamine with administration of the dopamine precursor L-dopa (100 mg/kg immediately before the beginning of the experiment) preceded by the peripheral decarboxylase inhibitor benserazide (50 mg/kg 30 min before L-dopa) restored the locomotor stimulating ability of 4S,5S-4-MAX in the latter half of the experiment (intervals 12–19). *Significantly smaller than that of saline pretreatment before 3 mg/kg of 4S,5S-4-MAX ($P < .001$, analysis of variance with Tukey post-hoc comparison). For AMPT pretreatment alone, $n = 8$.

induced by 4S,5S-4-MAX but these attenuations were not significant. The action of ($\pm$)-pindolol has been mentioned previously. Overall, the pretreatment of rats with these adrenergic antagonists did not significantly alter the behavioral effect produced by the administration of 4S,5S-4-MAX (fig. 9B).

Effects of the administration of serotonin and catecholamine depleting agents on trans-4S,5S-4-MAX-induced behavioral effect. The pretreatment of rats with reserpine (5 mg/kg s.c.) significantly attenuated the action of the trans-4S,5S isomer for overall total distance traveled during the three cumulative intervals examined, 1–3, 4–11 and 12–19 (fig. 10). However, this inhibitory effect did exhibit high within-group variability.

The pretreatment of animals with PCPA (400 mg/kg i.p. 24 hr before the experiment), slightly (but nonsignificantly) enhanced the increases in locomotion induced by trans-4S,5S administration. In contrast, the pretreatment of rats with AMPT (total dose of 500 mg/kg i.p., 250 mg/kg 2 and 4 hr before the experiment) significantly attenuated the effects of trans-4S,5S on locomotor behavior during the entire experiment as well as during all cumulative intervals (fig. 10).

To replenish DA concentrations depleted by AMPT pretreatment, L-dopa (the immediate precursor of DA) was given to a group of AMPT-pretreated rats to determine whether this would reverse the AMPT-induced inhibition of the effect of 4S,5S-4-MAX. The administration of the immediate DA precursor L-dopa (100 mg/kg i.p. immediately before 4-MAX) with the peripheral decarboxylase inhibitor, benserazide (50 mg/kg i.p. 30 min before 4-MAX), which enhances the central effect of L-dopa, appeared to reverse, at least partly, the effects of AMPT on trans-4S,5S-induced behavior (fig. 10). Although AMPT + L-dopa was significantly different from saline for overall activation, it was no longer significant during either

half of the experiment. This reversal was most evident during the second half of the experiment (fig. 10). L-dopa treatment also resulted in vigorous jumping behavior for several of the animals in this group. In fact, it was necessary to place clear plexiglass covers over the cages (with adequate ventilation) to prevent the animals from jumping out of the 12-inch high cages.

Discussion

One of the major findings of this study is that the s.c. administration of the isomers of 4-MAX produced a dose-dependent increase in locomotor activity. The maximal locomotor effect was produced at the dose of 3 mg/kg of 4S,5S-4-MAX. At this dose the effectiveness of the 4S,5S isomer in eliciting locomotive behavior was almost identical to that of *S*-(+)-AMPH. At a higher dose of 10 mg/kg, the stereoisomers 4S,5S, 4R,5S and 4S,5R typically produced multiphasic locomotor behavior characterized by an initial hyperlocomotion followed by the appearance of stereotyped behaviors such as focused sniffing and repetitive head movements. When the stereotypy subsided, a subsequent rebound of enhanced locomotion occurred. This incompatibility between locomotion and stereotyped behavior is similar to that produced by the systemic administration of *S*-(+)-AMPH and other psychostimulants (Schiorring, 1971; Segal, 1975; Kuczenski, 1983; Kolta *et al.*, 1985).

The rank order of potency regarding the increase in behavioral activation produced by the isomers of 4-MAX was: *trans*-4S,5S > *cis*-4S,5R = *cis*-4R,5S > *trans*-4R,5R. This order is identical to that previously reported for the isomers of 4-MAX to substitute for *S*-(+)-AMPH in a drug discrimination paradigm (Glennon and Misenheimer, 1990). Furthermore, we have demonstrated this same rank order of potency for the isomers of 4-MAX to suppress the basal firing rate of spontaneously active A10 DA cells in anesthetized rats (Vogel *et al.*, 1991; C. R. Ashby *et al.*, unpublished data). Using the technique of microdialysis, we have shown that the systemic administration of *trans*-4S,5S-4-MAX is more potent in inducing DA release in the NAc and striatum compared to the *trans*-4R,5R isomer (Eberle *et al.*, 1992). As pointed out by Glennon and Misenheimer (1990), this rank of potency also corresponds to the known structure-activity relationships of phenylisopropylamine psychostimulants such as 4-methylaminorex (Young and Glennon, 1986).

Interestingly, the behavioral profile of the stereoisomers of 4-MAX was not identical. For example, The 4S,5R isomer appeared to be more effective in eliciting locomotor behavior than the 4R,5S, whereas the 4R,5S isomer was more effective in producing stereotyped behavior than the 4S,5R isomer. Moreover, the onset of behavioral activation for the 4R,5R isomer (45–60 min) was markedly delayed compared to that of the *trans*-4S,5S and the *cis* isomers (15 min). This finding is similar to that of Glennon and Misenheimer (1990) who reported that the *trans*-4R,5R isomer produced an increase in behavioral hyperactivity 45 to 50 min after drug administration. In addition, at the dose of 10 mg/kg, the maximal increase in locomotor activity produced by the *trans*-4R,5R isomer plateaued for more than 2 hr (which could be due to the fact that this isomer failed to induce stereotypy), whereas that produced by the other isomers lasted only 30 to 45 min. Whether there is any pharmacokinetic (*e.g.*, absorption, metabolism) or pharmacodynamic differences among the stereoisomers which may

account for the different behavioral pattern is currently unknown.

As a first step toward elucidating the mechanism of action of 4-MAX, we examined the effect of various serotonergic, noradrenergic and dopaminergic receptor antagonists on the locomotor activation produced by the administration of 3 mg/kg of *trans*-4S,5S-4-MAX, a dose which produces a maximal increase in locomotor activity without producing prominent stereotypy. The pretreatment of rats with 0.3 mg/kg i.p. of (–)-eticlopride, a selective D₂ receptor antagonist (Chivers *et al.*, 1988), antagonized the locomotor activation produced by the administration of *trans*-4S,5S-4-MAX. In addition, the administration of 0.3 and 3 mg/kg i.p. of SCH 39166, a selective D₁ receptor antagonist (Chipkin *et al.*, 1990), also significantly attenuated the increase in locomotor activity. These results suggest that the behavioral activating effects of 4-MAX are mediated by DA receptors. However, it should be pointed out that the dose needed for the D₁ DA receptor antagonist SCH 39166 to block the behavioral activation produced by 4S,5S-4-MAX appears to be higher than that needed to antagonize the behavioral effects of *S*-(+)-AMPH. Whereas the ED₅₀ of SCH 39166 to block 4S,5S-4-MAX is 3 mg/kg, the ED₅₀ of SCH 39166 to antagonize the behavioral effect produced by *S*-(+)-AMPH is at least 10-fold less (Callahan *et al.*, 1991; Ouagazzal *et al.*, 1993). Further, even at the dose of 3 mg/kg, SCH 39166 produced only a 50% attenuation of 4-MAX-induced locomotion whereas it also reduced base-line activity suggesting the emergence of cataleptic effects.

Interestingly, the suppressant action of *trans*-4S,5S-4-MAX on A10 DA cell firing in anesthetized rats is reversed by the i.v. administration of eticlopride, but not by SCH 39166 (C. R. Ashby *et al.*, unpublished data). Thus, there is a slight discrepancy between the electrophysiological and behavioral results for this compound. The inhibition of A10 DA cells by 4-MAX isomers probably occurs *via* the action of D₂ DA autoreceptors located on DA cells. However, it is likely that the locus of behavioral activation produced by 4-MAX is not *via* DA receptors in area A10 but due to the action of D₁ and D₂ DA receptors in the NAc or other dopaminergic target areas. It can be speculated that different routes of drug administration (*i.p.* vs. *i.v.*) or the use of chloral hydrate anesthesia as compared to the use of free-moving rats in the two types of experiments may also contribute to the discrepant findings. At present, the precise explanation for the difference in effectiveness of D₁ antagonists in electrophysiological vs. behavioral experiments is unknown.

The i.p. administration of (±)-pindolol (5-HT_{1A,1B}, beta antagonist; Hoyer, 1988), granisetron (5-HT₃ antagonist; Ashby *et al.*, 1991), ritanserin (5-HT_{2A,2C} antagonist; Goodwin and Green 1985) or methysergide (5-HT_{1,2} antagonist; Koek *et al.*, 1992) did not antagonize the increase in locomotor activity produced by *trans*-4S,5S-4-MAX. The administration of several adrenergic antagonists such as phentolamine (*alpha*-1 antagonist; Drew, 1976), idazoxan (*alpha*-2 antagonist; Dettmar *et al.*, 1983) and (±)-pindolol also failed to antagonize the behaviorally activating effects of 4S,5S-4-MAX. Corresponding to these results, we have demonstrated that the suppressant action of the *trans*-4S,5S isomer on A10 DA cells was not reversed by the i.v. administration of ritanserin, granisetron, phentolamine, idazoxan or (±)-pindolol (C. R. Ashby *et al.*, unpublished data). It has also been reported that idazoxan does not antagonize the locomotor hyperactivity elicited by the systemic administration of *S*-(+)-AMPH (Dickinson *et al.*,

1988). Thus, the results of our laboratory and others suggest that the behavioral activating effects produced by *trans*-4S,5S-4-MAX are not mediated by 5-HT_{1A,1B,2A,2C,3} or α -1 or -2 or β adrenoreceptors in the brain. However, the involvement of these receptors cannot be completely ruled out as we have tested many of the antagonists at only one or two doses against 3 mg/kg of 4S,5S-4-MAX.

Combined, our results suggest that the augmentation of the locomotor activity produced by *trans*-4S,5S-4-MAX is related to its interaction with D₁ and D₂ receptors in the brain. However, its direct interaction with D₁ or D₂ receptors is unlikely as phenylisopropylamines and derivatives such as 4-MAX display a very low affinity for these receptors. Rather, 4-MAX may exert its action by releasing DA from DA terminals which, in turn, interacts with DA receptors (Bunker et al., 1990; Eberle et al., 1992), much like the action of S-(+)-AMPH. Recent studies now indicate the presence of D₃, D₄ and D₅ receptors in the brain (Sokoloff et al., 1990; Sunhara et al., 1991; Van Tol et al., 1991). Because, at present, there are no selective antagonists for these receptors, their role in mediating the actions of 4-MAX has not been examined here.

To gain further insight into the neurotransmitter systems that may mediate 4-MAX's behavioral action and to determine whether the effect of 4S,5S-4-MAX is the result of its direct interaction with postsynaptic DA receptors, we pretreated rats with various compounds that deplete the brain of monoamines and examined their effect on the locomotor activation produced by the s.c. administration of *trans*-4S,5S-4-MAX. The pretreatment of animals with reserpine, which depletes neuronal stores of DA, NE and 5-HT by blocking their uptake into storage vesicles (Hiroi and White, 1991), markedly attenuated the increase in locomotor activity produced by the administration of *trans*-4S,5S-4-MAX. Because our results stated above suggest that 5-HT and NE probably do not play a major role in mediating the locomotion enhancing action of 4-MAX, it is likely that reserpine's attenuating effect is related to its depletion of neuronal DA stores. This view is further supported by the fact that AMPT, which significantly depletes the formation of newly synthesized neuronal DA (Weissman and Koe, 1965), also significantly attenuated the effect of *trans*-4S,5S-4-MAX. However, both reserpine and AMPT administration significantly reduced base-line locomotive behavior. Therefore, these results must be interpreted as preliminary. The attenuation of the action of 4-MAX by AMPT was similar to that found previously for S-(+)-AMPH by Weissman et al. (1966). Moreover, this AMPT-induced attenuation of the behavioral effects of 4-MAX was reversed, at least partly, by administering benserazide (a peripheral L-aromatic amino acid decarboxylase inhibitor) and L-dopa, the immediate precursor in the synthesis of dopamine. The incomplete reversal of AMPT's effects on 4-MAX-induced increases in locomotor behavior by benserazide and L-dopa might be related to the emergence of jumping behaviors. Because this new behavior was not expressed as horizontal locomotion, our measurement of locomotor behavior as an index of overall behavioral activation probably underestimated the ability of L-dopa to re-establish the effectiveness of 4-MAX as a psychostimulant in AMPT-pretreated animals. It is also possible that the incomplete reversal of AMPT's effects were due to the fact that 4-MAX, in addition to activating DA receptors, also acts at sites other than those examined here to increase behavioral activation.

As shown by the results obtained with reserpine pretreatment, it is likely that in addition to its reliance on newly

synthesized DA, *trans*-4S,5S-4-MAX also exerts its behavioral actions by releasing vesicular DA. A similar mechanism of action has been reported for S-(+)-AMPH as previous studies have demonstrated that some of the behavioral actions produced by the systemic administration of S-(+)-AMPH are attenuated by pretreating rats with AMPT and reserpine (Kuczenski, 1983). Furthermore, electrophysiological studies indicate that the suppressant action of intravenously administered *trans*-4S,5S-4-MAX and S-(+)-AMPH on spontaneously active A10 DA cells is attenuated by AMPT pretreatment and is completely blocked by the pretreatment of both reserpine and AMPT (Wang, 1981; C. R. Ashby et al., unpublished data). This suppressant action on A10 DA cells is restored by the administration of L-dopa with benserazide.

It appears that manipulations of the 5-HT system do not alter the hyperlocomotion or the stereotypical behaviors produced by 4-MAX. We report here that various 5-HT receptor antagonists have failed to prevent or attenuate the action of 4S,5S-4-MAX. Of particular interest is the 5-HT_{1,2} receptor antagonist methysergide which has been shown to potentiate locomotion produced by methylenedioxymethamphetamine (Gold and Koob, 1988), in which the release of 5-HT is critically involved (Callaway et al., 1991), but has no effect on 4-MAX-induced behavioral activation (present results). Further, the pretreatment of rats with the compound PCPA, which significantly depletes 5-HT content by inhibiting the activity of tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of 5-HT (Koe and Weissman, 1967; Jequier et al., 1967), did not alter the locomotion-producing action of the *trans*-4S,5S isomer. In contrast, it has been reported that the pretreatment of rats with PCPA significantly potentiates the behavioral activation elicited by the systemic administration of low-to-moderate doses of S-(+)-AMPH (Mabry and Campbell, 1973). Along with these results, other major differences between 4-MAX and AMPH may be summarized as follows: 1) whereas AMPH and AMPH analogs act upon both nigrostriatal and mesolimbocortical DA systems, electrophysiological evidence indicates that 4-MAX may be exerting its action preferentially in the mesolimbic DA system (Vogel et al., 1991; C. R. Ashby et al., unpublished data); 2) the D₁ DA receptor antagonist SCH 39166 was much less effective at attenuating the locomotion producing effects of 4-MAX compared to its ability to block the behavioral effects of AMPH (Callahan et al., 1991; Ouagazal et al., 1993) and 3) whereas S-(+)-AMPH decreases the activities of both tyrosine hydroxylase and tryptophan hydroxylase, 4-MAX appears to reduce reliably the activity of tryptophan hydroxylase only (Sonsalla et al., 1986; Bunker et al., 1990; Hanson et al., 1992).

In conclusion, our results indicate that the systemic administration of 4-MAX produces behavioral effects similar, but not identical, to that observed for S-(+)-AMPH. The increase in locomotor activity produced by *trans*-4S,5S-4-MAX is attenuated by the administration of the selective D₁ and D₂ receptor antagonists SCH 39166 (at high doses) and (-)-eticlopride, respectively, and by reserpine and S-(+)-AMPT. Taken together, our results suggest that 4-MAX may increase locomotor activity by releasing neuronal DA, which subsequently interacts with postsynaptic D₁ and D₂ receptors in the brain. However, additional studies are required to substantiate fully this view.

Acknowledgments

We thank the Schering-Plough Corporation, Reckitt and Coleman, Smith Kline and Beecham Pharmaceuticals, Sandoz Pharmaceuticals and Geigy for generous donations of compounds used in the present study.

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