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Comparison of the Action of the Stereoisomers of the Psychostimulant 4-Methylaminorex (4-MAX) on Midbrain Dopamine Cells in the Rat: An Extracellular Single Unit Study

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ABSTRACT In this study, we examined and characterized the action of the stereoisomers of 2-amino-4-methyl-Δ^2-5-phenyl-oxazoline (4-methylaminorex, 4-MAX) on spontaneously active dopamine (DA) neurons in the substantia nigra pars compacta (SNC or A9) and ventral tegmental area (VTA or A10) in anesthetized male rats. This was accomplished using the technique of extracellular single unit recording. The intravenous (i.v.) administration of the stereoisomers of 4-MAX (0.1–6.4 mg/kg) produced a dose-dependent suppression of the basal firing rate of A10 DA cells with the following rank order of potency: trans 4S,5S > cis 4R,5S ≈ cis 4S,5R >> trans 4S,5S 4-MAX. The rank order of potency of the isomers of 4-MAX to suppress the firing of A9 DA cells was trans 4S,5S = cis 4R,5S = cis 4S,5R >> trans 4R,5R. The trans 4S,5S isomer was 5-fold more potent in suppressing DA cell firing in the A10 compared to the A9 area.

The suppressant action of the isomers on A9 and A10 DA cells was reversed by the i.v. administration of haloperidol and the D_2/D_3 receptor antagonists (−)-sulpiride and (−)-eticlopride but not by the D_1 receptor antagonists SCH 23390 and SCH 39166. In addition, the suppressant action of the trans 4S,5S isomer on A10 DA cells was not antagonized or reversed by the i.v. administration of the receptor antagonists granisetron (5-HT_3), ritanserin ($5\text{-HT}_{2A,C}$), idazoxan (α_2), phentolamine (peripheral α_1), (±)-pindolol ($5\text{-HT}_{1A,B}\beta$) or prazosin (α_1). The pretreatment of animals with either α-methyl-p-tyrosine (AMPT) or reserpine, but not p-chlorophenylalanine (PCPA), (±)-fluoxetine or tomoxetine, significantly attenuated the suppression of A10 DA cell firing produced by trans 4S,5S 4-MAX. Overall, our results suggest that the suppressant action of 4-MAX on midbrain DA cell firing may be mediated by the release of DA, which subsequently interacts with D_2/D_3 receptors.

INTRODUCTION

The phenylisopropylamine derivative 2-amino-4-methyl-5-phenyl-Δ^2-oxazoline (4-methylaminorex, 4-MAX) is an indirect acting sympathomimetic agent with appetite suppressant and stimulant properties similar to those of amphetamine (Poos et al., 1963; Rozkowski and Kelley, 1963; Yelnosky and Katz, 1963). There are four stereoisomers of this compound: trans 4R,5R, trans 4S,5S, cis 4R,5S and cis 4S,5R. Recently, the cis racemate of 4-MAX (street name "U4EuH") has appeared on the clandestine market as a new designer drug and has been misrepresented by drug dealers as

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cocaine and methamphetamine (Davis and Brewster, 1987; Klein et al., 1989). Preliminary data from a study using monkeys as subjects indicates that 4-MAX is self-administered and that it can substitute for cocaine in drug discrimination paradigms (Mansbach et al., 1990). Initial reports from human subjects also indicate that the cis racemate of 4-MAX is readily self-administered and its subjective effects are similar to those of cocaine and amphetamine (Drug Control Section, 1988). The high abuse potential of 4-MAX has resulted in the cis isomers being assigned to the Schedule 1 drug category of the Controlled Substances Act in October 1987 (Lawn, 1989).

Several recent studies have examined the effect of 4-MAX on monoaminergic systems in the brain. It has been reported that the subcutaneous administration of 10 mg/kg cis (±)-4-MAX, similar to the effect of amphetamine, produced a substantial reduction in dopamine (DA) levels in neostriatal tissue and a significant increase in homovanillic acid levels (Bunker et al., 1990). The intradialysate administration of trans 4S,5S 4-MAX (10^{-5} M) produces a 6- to 10-fold increase in extracellular DA in the nucleus accumbens and striatum, as determined using microdialysis (Eberle et al., 1992). Glennon and Misenheimer (1990), using the behavioral paradigm of drug discrimination, have shown that the stimulus cues produced by the intraperitoneal administration of S(+)-amphetamine in rats generalized to all four isomers of 4-MAX. The relative potency of the isomers were: trans 4S,5S > cis 4S,5R = cis 4R,5S >> trans 4R,5R 4-MAX. Consistent with these results, we have shown that the subcutaneous administration of the stereoisomers of 4-MAX (2.5–10 mg/kg) produces a dose-dependent increase in locomotor and stereotyped behavior similar to that elicited by S(+)-amphetamine (Batsche et al., 1994). The rank order of potency for the stereoisomers to increase horizontal locomotor activity was: trans 4S,5S > cis 4S,5R = cis 4R,5S >> trans 4R,5R. However, it has been previously shown that 4-MAX can induce seizures following the systemic administration of doses greater than 15 mg/kg s.c. or after intraventricular administration (Hanson et al., 1992; White et al., 1990), an effect that is not generally produced by the systemic administration of similar doses of S(+)-amphetamine. In addition, the systemic administration of 4-MAX decreases tryptophan hydroxylase activity but has no consistent effect on the activity of tyrosine hydroxylase (Bunker et al., 1990). In contrast, S(+)-amphetamine reduces the activity of both tryptophan hydroxylase and tyrosine hydroxylase (Sonsalla et al., 1986).

Thus, 4-MAX appears to have behavioral and biochemical properties similar, though not identical, to amphetamine. Presently, there is still limited data available on this compound, particularly regarding the action of the individual stereoisomers on dopaminergic systems in the brain. Therefore, the aim of this study was to systemically examine the effects of the stereoisomers of 4-MAX on the basal activity of DA cells in the substantia nigra pars compacta (SNC or A9) and ventral tegmental area (VTA or A10) of the rat. In addition, experiments were conducted in order to ascertain what neurotransmitter system(s) may be involved in mediating 4-MAX's action. This was accomplished using the technique of extracellular single unit recording.

MATERIALS AND METHODS

Animal and surgery

Male Sprague-Dawley rats (200–250 g, Taconic Farms, Germantown, NY) were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic instrument. Overall, 200 rats were used in this study. The body temperature was maintained at 37–38°C using an electric heating pad. A lateral tail vein was cannulated with a 25-gauge needle for the administration of additional anesthetic or drug solutions. A burr hole was drilled over the VTA (anterior 3.0–3.5 mm to the lambdoidal suture, lateral 0.5–1.0 mm to the midline and 7.0–8.5 mm ventral to the cortical surface) or SNC (anterior 3.0–3.5 mm, lateral 1.8–2.5 mm and 7.0–8.5 mm ventral to the cortical surface), according to the atlas of Paxinos and Watson (1986). The dura was subsequently retracted for the placement of the recording electrodes.

Criteria for identification of spontaneously active dopamine cells

We and others have antidromically identified DA neurons in the A9 and A10 areas (Guyenet and Aghajanian, 1979; Wang, 1981a; White and Wang, 1984). These dopaminergic neurons exhibit the following characteristics: (1) a wide action potential with a distinct initial segment and late positive component; (2) a characteristic low-pitched sound when monitored through an audio amplifier; (3) a slow, regular or bursting firing pattern; and (4) a spontaneous firing rate of 2–9 Hz (Bunney et al., 1973; Wang, 1981a). Moreover, A9 and A10 DA neurons are very sensitive to DA agonists such as apomorphine and S(+)-amphetamine (Bunney et al., 1973; Wang, 1981b).

Electrophysiological recording techniques

Standard techniques for extracellular single unit recording were used to examine the effects of the isomers of 4-MAX on A9 and A10 DA cells as has been previously described in detail (Bunney et al., 1973; Wang, 1981a). Briefly, single barrel micropipettes were broken under a microscope to a tip diameter of approximately 1 μm and filled with a 2 M NaCl solution saturated with 1% Fast Green dye. The in vitro impedance of the electrodes as 1.5–2 MΩ when measured at 135 Hz. The electrode signal was passed through a high

impedance amplifier and monitored on an oscilloscope. A variable time and voltage window discriminator was used to facilitate the discrimination of single units. The signal-to-noise ratio of recorded cells was at least 3 to 1. The integrated firing rates were recorded in 10 sec intervals from an electronic counter triggered by individual neuronal spikes.

Drug administration

Administration of 4-MAX

After establishing stable basal firing rates (3–5 min) for the isolated A9 and A10 DA cells, the isomers of 4-MAX were given every 60 seconds at cumulative doses of 0.1–6.4 mg/kg, i.v., in increments of 0.02–0.32 ml. The experiments were conducted such that on each day, one stereoisomer was tested on at least two A9 and two A10 DA cells in separate animals.

Reversal studies

In another set of experiments, we examined the ability of idazoxan, prazosin, ritanserin, SCH 39166 and granisetron to reverse the depressant action of the isomers of 4-MAX on spontaneously active cells. The antagonists were administered every 60 seconds at cumulative doses of 0.05–0.8 mg/kg. Only one cell was tested in each rat to obviate the residual drug effect.

Pretreatment studies

In a separate series of experiments, we pretreated animals with various pharmacological agents that produce a significant depletion of catecholamine and indoleamine stores and blocked monoamine uptake. Subsequently, we examined the effect of trans 4S,5S on A10 DA cell firing. Animals were pretreated with either saline (1 ml/kg, i.p.), para-chlorophenylalanine (PCPA, 400 mg/kg, i.p., 24 hours before experiment), α-methyl-para-tyrosine (AMPT, 200 mg/kg, i.p., 2 and 4 hours before the experiment) or reserpine (5 mg/kg, s.c., 24 hours before experiment). In a separate set of experiments, animals were treated with either saline (1 ml/kg, i.p.), (±)-fluoxetine (5 mg/kg, i.p., 2 hours before experiment) or tomoxetine (5 mg/kg, i.p., 2 hours before experiment). Subsequently, an A10 DA cell was isolated, a stable baseline recorded for 3–5 minutes and the response to trans 4S,5S 4-MAX (0.1–6.4 mg/kg, i.v.) examined.

In addition, animals were also pretreated with either saline (1 ml/kg, i.p.), phentolamine (2.5 mg/kg, i.v.) or (±)-pindolol (5 mg/kg, i.p.) and 2 hours later, the response of spontaneously active A10 DA cells to intravenously administered trans 4S,5S 4-MAX was examined. It should be pointed out that separate control groups were used for the fluoxetine and tomoxetine groups, the depletion studies with PCPA, AMPT and reserpine and the phentolamine and pindolol experiments.

Drugs and chemicals

The stereoisomers of 4-MAX (free base form) were supplied by Dr. R.F.X. Klein, DEA Special Testing Laboratories, McLean, VA. and the National Institute of Drug Abuse (NIDA, Rockville, MD). (−)-Eticlopride hydrochloride, idazoxan, prazosin and (−)-SCH 23390 hydrochloride were obtained from Research Biochemicals, Inc. (Natick, MA). Haloperidol (free base) was supplied by McNeil Pharmaceuticals (Fort Washington, PA). Granisetron and (±)-pindolol were supplied by Smith-Kline and Beecham (Ware, U.K.) and Sandoz (Basle, Switzerland), respectively. SCH 39166 was supplied by Schering-Plough (Bloomfield, NJ).

The isomers of 4-MAX (pH 7.0), phentolamine (pH 6.0), ritanserin (pH 5.5), and haloperidol (pH 5.5) were dissolved in a small volume of 2% lactic acid and diluted to the proper concentration with distilled water. (−)-Sulpiride (pH 5.5) and (±)-propranolol (pH 5.0) were dissolved in a few drops of 0.1 N HCl and distilled water. (−)-Eticlopride, granisetron and idazoxan (pH 7.0) were dissolved in distilled water. All drug solutions were freshly prepared or stored refrigerated in the dark for 1–3 days, except the 4-MAX solutions, which were prepared fresh each day.

Histological analysis

At the end of each experiment, the final position of the electrode tip was marked by passing a −30 μA current through the single barrel micropipette for 15 min. This resulted in the deposition of a Fast Green spot around the electrode tip. The rats were then deeply anesthetized and perfused transcardially with 10% buffered formalin. The recording sites, marked by a Fast Green spot, were subsequently verified on 50 μm coronal sections, stained with cresyl violet and counterstained with neutral red, with the aid of a light microscope.

Data analysis

To determine the effect of each cumulative dose on the cell firing rate, basal rate was calculated as a percent of the baseline firing rate averaged over two or three 1-min epochs immediately before the drug injection regimen. The cumulative dose of the stereoisomers of 4-MAX to suppress the baseline firing rate by 25% (ID_{25}) was determined using the graphical method as previously described (Pitts et al., 1990). The ID_{25} was determined, as opposed to the ID_{50} value, because the percent suppression of DA cell firing elicited by the cis isomers and trans 4R,5R 4-MAX was less than 50% in a large number of DA cells at the highest dose tested (see Results). However, the ID_{50} value was calculated for trans 4S,5S 4-MAX isomer in the pretreatment experiments as this isomer reliably produced a 50% suppression of A10 DA cell firing. Differences in the mean fir-

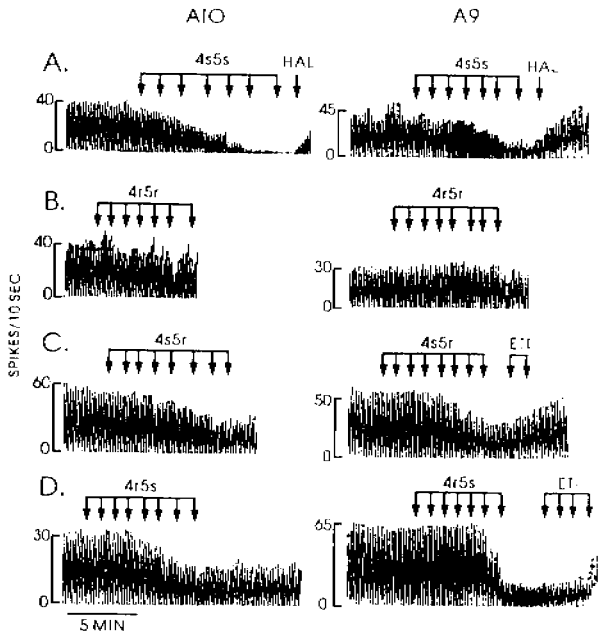

Fig. 1. Cumulative rate histograms illustrating the effects of the intravenous administration of (A) trans 4S,5S, (B) trans 4R,5R, (C) cis 4S,5R, and (D) cis 4R,5S 4-methylaminorex (4-MAX) on the basal firing rate of spontaneously active A9 (right) and A10 (left) DA cells. Note that in A, the i.v. administration of haloperidol (HAL, 0.1 mg/kg) rapidly reverses the suppression of A9 and A10 DA cell firing produced by the i.v. administration of trans 4S,5S 4-methylaminorex. In addition, the i.v. administration of eticlopride (ETI) reverses the suppression of A9 and A10 DA cell firing produced by the cis 4R,5S and cis 4S,5R 4-methylaminorex (see B and D). The starting dose for the isomers of 4-methylaminorex, eticlopride, and haloperidol was 0.1 mg/kg and subsequent doses administered were 0.1, 0.2, 0.4 mg/kg, etc.

ing rates in the different treatment groups were analyzed by an analysis of covariance.

RESULTS

The effects of the stereoisomers of 4-MAX on the basal firing rate of spontaneously active A10 DA cells

The intravenous administration of the trans 4S,5S isomer produced a dose-dependent suppression of the basal firing rate of spontaneously active A10 DA cells [ANCOVA, $F(6,95) = 182.8$, $P < 0.0001$, Figs. 1 and 2], with an ID_{25} value of 0.28 ± 0.03 mg/kg (n = 15, Table I). A dose-dependent suppression of A10 DA cell firing was also observed after the i.v. administration of cis 4S,5R [ANCOVA, $F(6,69) = 30.6$, $P < 0.001$], cis 4R,5S [ANCOVA, $F(6,62) = 31.7$, $P < 0.01$; Figs. 1 and 2] and trans 4R,5R 4-MAX [ANCOVA, $F(6,67) = 2.47$, $P < 0.05$); Figs. 1 and 2]. However, these isomers were significantly less potent than the trans 4S,5S isomer, with ID_{25} values of 1.66 ± 0.33 (n = 10), 2.01 ± 0.3 (n = 10), and 4.82 ± 0.65 mg/kg (n = 11), respectively (ANCOVA followed by Student-Newman-Keuls test). Statistical analysis indicated that there was no difference between the ID_{25} values of the cis isomers (t = 0.9, df = 18, NS). The ID_{25} of trans 4R,5R was significantly greater than that of the cis 4R,5S and cis 4S,4R isomers. Based on the ID_{25} values, the rank order of potency of the isomers to suppress A10 DA cell baseline firing was: trans 4S,5S > cis 4R,5S = cis 4S,5R >> trans 4R,5R.

The effects of the stereoisomers of 4-MAX on the basal firing rate of A9 DA cells

The i.v. administration of trans 4S,5S (ANCOVA, $F(6,95) = 35.2$, $P < 0.00001$), cis 4R,5S (ANCOVA, $F(6,64) = 8.8$, $P < 0.001$), cis 4S,5R (ANCOVA, $F(6,63) = 13.6$, $P < 0.0001$) and trans 4R,5R 4-MAX (ANCOVA, $F(6,63) = 3.1$, $P < 0.05$) all produced a dose-dependent suppression of the basal firing rate of spontaneously active A9 DA cells (Figs. 1 and 2). However, the trans 4S,5S isomer was much less potent in suppressing the firing of A9 DA cells compared to A10 DA cells (Figs. 1 and 2). The ID_{25} value of the trans 4S,5S isomer for A9 DA cells was 1.38 ± 0.35 mg/kg (n = 14), which was significantly different from the ID_{25} value determined for A10 DA cells (t = 3.26, df = 27, $P < 0.01$). The ID_{25} values for cis 4S,5R and cis 4R,5S 4-MAX were 2.41 ± 0.34 (n = 10) and 1.56 ± 0.35 mg/kg (n = 9), respectively, which were not significantly different from that of trans 4S,5S isomer (ANCOVA, Table I). Similar to the results obtained with A10 DA cells, the trans 4R,5R isomer only produced a maximal suppression of 25–50% at a cumulative dose of 6.4 mg/kg (Figs. 1 and 2). The ID_{25} value for trans 4R,5R 4-MAX was 4.30 ± 0.55 mg/kg (n = 11) and this was significantly greater than the ID_{25} values obtained for the other isomers on A9 DA cells (ANCOVA, $F(3,35) = 9.1$, $P < 0.001$, plus Student-Newman-Keuls test). There was no difference between the A9 and A10 areas regarding the potency of the cis isomers or trans 4R,5R 4-MAX to suppress DA cell firing (t = 1.73, df = 17, NS). Based on the above results, the rank order of potency for the stereoisomers of 4-MAX to suppress the basal firing rate of A9 DA cells was: trans 4S,5S ≈ cis 4R,5S ≈ cis 4S,5S >> trans 4R,5R.

It was surprising that the trans 4S,5S isomer was significantly less potent in suppressing the basal firing rate of A9, compared to A10 DA cells. Previously, except for cocaine, it has not been shown that the i.v. administration of direct or indirect DA agonists have a differential effect on the basal firing rate of A9 and A10 DA cells. Therefore, we decided to compare the action of trans 4S,5S 4-MAX with S(+)-amphetamine on the basal firing rate of A9 and A10 DA cells. In contrast, as previously reported (Wang, 1981b), the i.v. administration of S(+)-amphetamine (0.05–3.2 mg/kg) was equipotent in suppressing the basal firing of A9 (ID_{50} = 1.6 ± 0.2 mg/kg, n = 6) and A10 (ID_{50} 1.4 ± 0.1 mg/kg, n = 5) DA cells (Fig. 3).

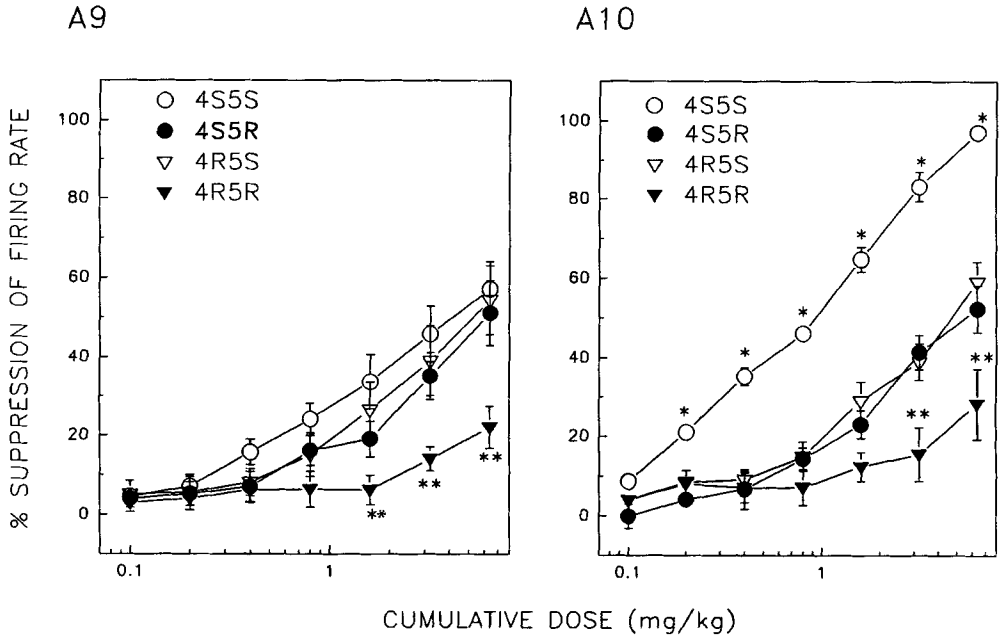

Fig. 2. Dose-response curves for the isomers of 4-methylaminorex to suppress the basal firing rate of spontaneously active A9 (left) and A10 (right) DA cells. Each point represents the mean percent suppression of the basal firing rate ± S.E.M. The number of DA cells examined at each dose of the stereoisomers was 10–15. *Significantly greater than cis 4R,5S, cis 4S,5R and trans 4R,5R; **significantly less than the cis 4R,5S and cis 4S,5R, $P < 0.01$, ANCOVA and Student-Newman-Keuls test.

TABLE I. Comparison of the ID_{25} values of the stereoisomers of 4-methylaminorex on spontaneously active A9 and A10 DA cells

Drug	Brain area	
	A9	A10
trans 4S, 5S	1.4 ± 0.3[1] (14)[2]	0.3 ± 0.03 (15)[4]
cis 4R, 5S	1.6 ± 0.3 (9)	1.7 ± 0.3 (10)[5]
cis 4S, 5R	2.4 ± 0.3 (10)	2.0 ± 0.3 (10)[5]
trans 4R, 5R	4.3 ± 0.5 (11)[3]	4.8 ± 0.6 (11)[3]

[1]Each value represents the ID_{25} (mean dose in mg/kg ± S.E.M.).
[2]The values in parentheses represent the number of cells examined.
[3]Significantly greater than that of trans 4S, 5S, cis 4R, 5S and cis 4S, 5R; $P < 0.01$, ANOVA and Student-Newman-Keuls.
[4]Significantly less than that of trans 4S, 5S in A9, $P < 0.01$. Student's t-test.
[5]Significantly greater than trans 4S, 5S: $P < 0.01$, ANOVA and Student-Newman-Keuls.

The effects of various dopaminergic, adrenergic, and serotonergic receptor antagonists on the suppressant action of trans 4S,5S 4-methylaminorex on spontaneously active A10 DA cells

In these experiments, we examined the efficacy of various adrenergic and serotonergic receptor antagonists to reverse the action produced by the sytemic administration of trans 4S,5S 4-MAX on spontaneously active A10 DA cells. The decision to examine the trans 4S,5S isomer was based on the fact that it produced the most consistent and greatest degree of suppression of DA cell firing, particularly those in the A10 area, compared to the other isomers (see above). The suppressant action of the trans 4S,5S isomer was reversed by the

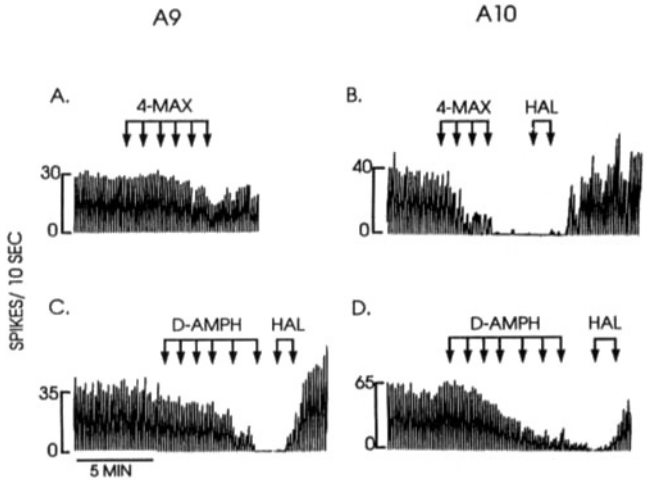

Fig. 3. Cumulative rate histograms illustrating that the intravenous administration of trans 4S,5S 4-MAX (4-MAX) is significantly more potent in suppressing the basal firing rate of a DA cell in the A9 (A) compared to the A10 (B). In contrast, S(+)-amphetamine (AMPH) is equipotent in suppressing the basal firing rate of spontaneously active A9 (C) and A10 (D) DA cells. Note that the intravenous administration of haloperidol (HAL) rapidly reverses the suppressant action of d-amphetamine and 4-MAX. In A and B, the first set of arrows represents the i.v. administration of 0.1, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/kg and 0.1, 0.1, 0.2, and 0.4 mg/kg of trans 4S,5S 4-MAX, respectively. The first set of arrows in C and D represents the i.v. administration of 0.05, 0.05, 0.1, 0.2, 0.4, and 0.8 mg/kg and 0.05, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/kg of d-amphetamine (D-AMPH), respectively. In B–D, the second set of arrows represents the i.v. administration of 0.05 and 0.05 mg/kg of HAL.

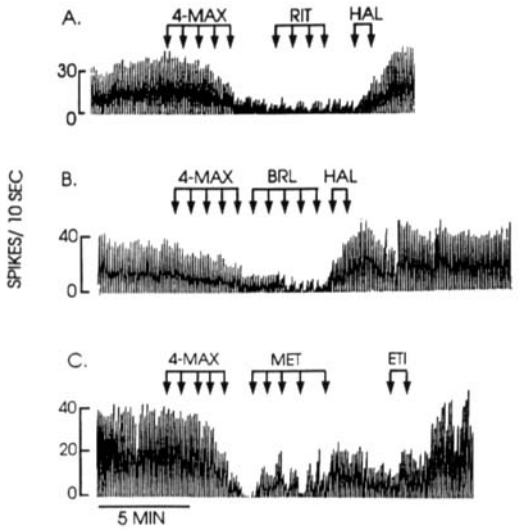

Fig. 4. Cumulative rate histograms demonstrating that the intravenous administration of (A) ritanserin (RIT) or (B) granisetron (BRL) did not reverse the suppression of A10 DA cell firing produced by the i.v. administration of trans 4S,5S 4-methylaminorex (4-MAX). However, it should be noted that the i.v. administration of (C) metergoline (MET) produced a partial reversal of the suppressant action of 4-MAX. In addition, the i.v. administration of either haloperidol (HAL) or eticlopride (ETI) completely reversed the suppression of A10 DA cell firing produced by 4-MAX. In the histograms, the first set of arrows represents the i.v. administration of 0.1, 0.1, 0.2, 0.4, and 0.8 mg/kg of 4-MAX, respectively. The starting dose for ritanserin, granisetron and metergoline was 0.05 mg/kg and subsequent doses administered were 0.05, 0.1, 0.2 mg/kg, etc. In A and B, the third set of arrows represents the i.v. administration of 0.05 and 0.05 mg/kg of HAL, respectively. In C, the third set of arrows represents the i.v. administration of 0.1 and 0.1 mg/kg of ETI, respectively.

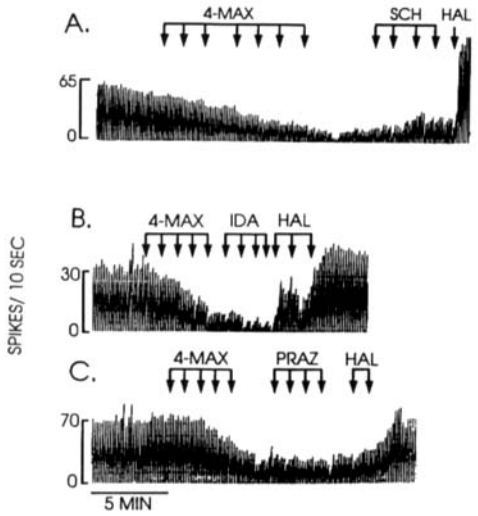

Fig. 5. Cumulative rate histograms illustrating that the i.v. administration of either (A) SCH 39166 (SCH), (B) idazoxan (IDA), or (C) prazosin (PRAZ) did not reverse the suppression of A10 DA cell firing produced by the i.v. administration of trans 4S,5S 4-methylaminorex (4-MAX). The first set of arrows in A represents the i.v. administration of 0.05, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/kg, respectively, of 4-MAX and the second set of arrows represents the i.v. administration of 0.1, 0.1, 0.2, and 0.4 mg/kg of SCH 39166, respectively. The last arrow in A represents the i.v. injection of 0.05 mg/kg i.p. of haloperidol (HAL). The first set of arrows in B and C represents the i.v. administration of 0.1, 0.1, 0.2, 0.4, and 0.8 mg/kg, respectively of 4-MAX. The second set of arrows in B and C represents the i.v. injection of 0.1, 0.1, 0.2, and 0.4 mg/kg of idazoxan (IDA) and prazosin (PRAZ), respectively. The third set of arrows in B represents the i.v. administration of 0.05, 0.05, and 0.1 mg/kg, respectively, of HAL, whereas the third set of arrows in C represents the i.v. administration of 0.05 and 0.05 mg/kg of HAL.

intravenous administration of haloperidol (Fig. 1). In addition, after the i.v. administration of haloperidol, the baseline firing of A10 DA cells was not only reversed to the predrug level, but the basal firing rates were actually increased above the original rate (i.e., overshoot in 8 of 10 cells). The inhibitory effect of the cis 4R,5S and cis 4S,5R isomer was reversed by the i.v. administration of the D_2/D_3 receptor antagonists (−)-eticlopride (n = 7, see Fig. 1) and (−)-sulpiride (10–30 mg/kg, n = 4, not shown).

In contrast, the suppressant action of the trans 4S,5S isomer on A10 DA cells was not reversed by the i.v. administration of either ritanserin ($5\text{-}HT_{2A,C}$ receptor antagonist, 0.05–0.8 mg/kg, n = 8) or granisetron ($5\text{-}HT_3$ receptor antagonist, 0.05–0.8 mg/kg, n = 8, Figs. 4A,B). Interestingly, the i.v. administration of 0.5–2 mg/kg of metergoline ($5\text{-}HT_1/5\text{-}HT_{2A,C},D_2$ receptor antagonist) partially reversed the suppressant action of trans 4S,5S 4-MAX on A10 DA cells (Fig. 4C). Neither the i.v. administration of SCH 23390, a nonselective D_1 receptor antagonist (n = 7, 1–7 mg/kg, not shown), nor SCH 39166, a selective D_1 receptor antagonist (0.1–0.8 mg/kg, n = 7), reversed the suppressant action of the cis isomers on A10 DA cell firing (Fig. 5A). Similarly, the i.v. administration of 0.05–0.2 mg/kg of prazosin (α_1 receptor antagonist, n = 8) or idazoxan (α_2 receptor antagonist, n = 8) did not reverse the suppressant action produced by the trans 4S,5S isomer (Figs. 5B,C).

The effects of (±)-pindolol and phentolamine on the suppressant action of trans 4S,5S 4-methylaminorex on A10 DA cells

Previously, it has been reported that the systemic administration of cis (±)-4-MAX produces hypertensive effects (Yelnosky and Katz, 1963). Consequently, in order to ensure that the suppressant action of 4-MAX is not the result of its sympathomimetic effects, animals were pretreated with phentolamine (peripheral α adrenergic antagonist) or (±)-pindolol (nonselective β adrenergic antagonist). The pretreatment of rats with either phentolamine (2.5 mg/kg, i.v., n = 8) or (±)-pindolol (5 mg/kg, i.p., n = 8) did not alter the suppressant action of trans 4S,5S on A10 DA cells (Fig. 6). The ID_{50} values for the trans 4S,5S after pretreatment with phentolamine and (±)-pindolol were (mean ± S.E.M.) 1.4 ± 0.1 (n = 8) and 1.5 ± 0.12 mg/kg (n = 8), respectively, which were not significantly different from trans 4S,5S alone (1.3 ± 0.1 mg/kg, n = 8).

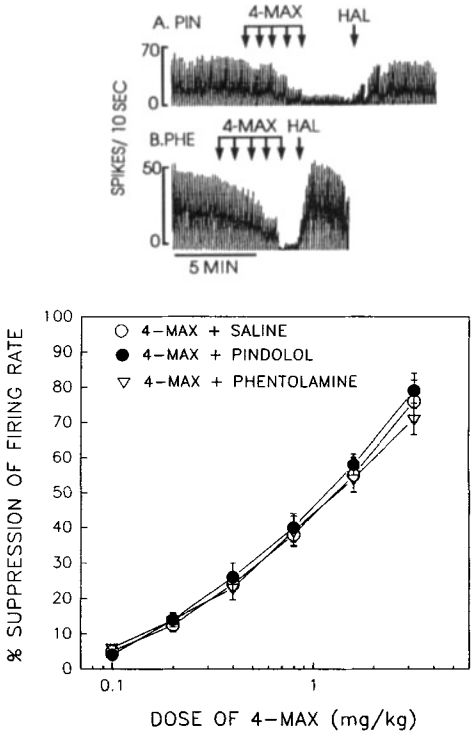

Fig. 6. **Top:** Cumulative rate histograms illustrating that the pretreatment of rats with either (±)-pindolol (PIN, 5 mg/kg, i.p.) or phentolamine (PHE, 2.5 mg/kg, i.v.) two hours prior to the administration of trans 4S,5S 4-MAX did not alter the suppressant action of trans 4S,5S 4-MAX on A10 DA cell firing. The second set of arrows represents the i.v. administration of 0.1, 0.1, 0.2, 0.4, and 0.8 mg/kg of trans 4S,5S 4-MAX, respectively. Note that the suppressant action of trans 4S,5S 4-MAX is reversed by the i.v. administration of haloperidol (HAL, 0.05 mg/kg, last arrow in each histogram). **Bottom:** Dose-response curves illustrating the effect of phentolamine and pindolol pretreatment on the response of spontaneously active A10 DA cells to i.v. trans 4S,5S 4-MAX. Each point represents the mean percent suppression ± S.E.M. Eight DA cells were examined at each dose of trans 4S,5R 4-MAX.

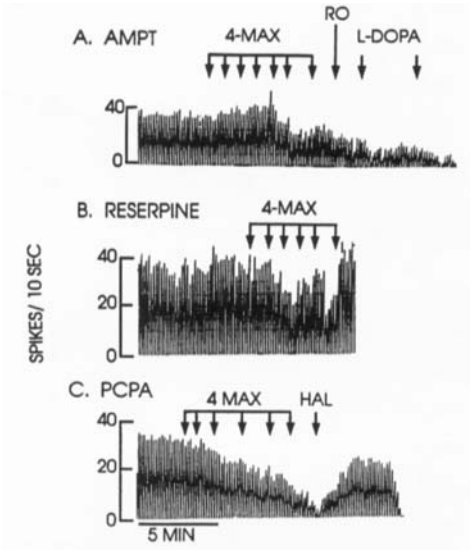

Fig. 7. Cumulative rate histograms demonstrating the effect of intravenously administered trans 4S,5S 4-methylaminorex (4-MAX) on the basal firing rate of spontaneously active DA cells in the A10 area after pretreatment with (A) 200 mg/kg i.p. of α-methyl-p-tyrosine (AMPT, 2 and 4 hours before the experiment), (B) 5 mg/kg i.p. of reserpine (24 hours before the experiment), or (C) 400 mg/kg i.p. of para-chlorophenylalanine (PCPA, 24 hours before the experiment). Note that the pretreatment of animals with AMPT (n = 8) and reserpine (n = 8), but not PCPA (n = 8), significantly attenuated the suppressant action of 4-MAX on A10 DA cell firing. In addition, the firing of the A10 DA cell in A was significantly decreased after the administration of the peripheral aromatic amino acid decarboxylase inhibitor, RO 04-4602 (RO) and the immediate precursor of DA, L-DOPA. In each histogram, the arrows represent the i.v. administration of 0.1, 0.1, 0.2, 0.4, 0.8 mg/kg, etc., of 4-MAX. In A, the arrow following the last injection of 4-MAX represents the i.v. administration of 10 mg/kg of RO and the arrows immediately following the injection of RO represents the i.v. administration of 5 mg/kg of dihydroxyphenylalanine (L-DOPA), respectively. In C, the arrow after the last injection of 4-MAX represents the i.v. administration of 0.05 mg/kg of haloperidol (HAL).

The effect of monoamine depletion on the depressant action of trans 4S,5S 4-MAX on spontaneously active A10 DA cells

In a series of experiments, we pretreated animals with various pharmacological compounds that are known to deplete brain monoamines and determined the response of A10 DA cells to the i.v. administration of trans 4S,5S 4-MAX. The pretreatment of rats with 400 mg/kg i.p. of p-chlorophenyalanine (PCPA, 24 hours before the experiment) produced a 94% decrease in brain 5-HT levels compared to controls but did not significantly alter brain levels of DA (data not shown). Overall, PCPA administration did not significantly alter the suppressant action of trans 4S,5S 4-MAX on A10 DA cells (Figs. 7 and 8). The ID_{50} value for rats treated with PCPA was 1.3 ± 0.1 mg/kg (n = 8), which was not significantly different from control (1.1 ± 0.1 mg/kg, n = 8).

The pretreatment of animals with α-methyl-p-tyrosine (AMPT, 200 mg/kg, i.p., two and four hours before the experiment) produced a 93% decrease in brain DA levels compared to controls (data not shown). In contrast to the results obtained with PCPA, AMPT pretreatment markedly attenuated the depressant action of the trans 4S,5S isomer on the basal firing rate of A10 DA cells (Figs. 7 and 8). Thus, the ID_{50} value of trans 4S,5S after the administration of AMPT was 5 ± 0.4 mg/kg (n = 9), which was significantly greater than control ($t = 6.4$, $P < 0.01$). The administration of benserazide (5 mg/kg, i.v.) and 3,4-β-dihydroxyphenylalanine (L-DOPA, 20–25 mg/kg, i.v.), which presumably restore brain DA levels, reversed the attenuation of the depressant action produced by AMPT on A10 DA cells (Fig. 7). In contrast, the administration of 5 mg/kg i.v. of benserazide and 20 mg/kg of L-DOPA to animals that did not receive AMPT did not alter the effect of trans 4S,5S 4-MAX on A10 DA neurons compared to controls (n = 4, unpublished results).

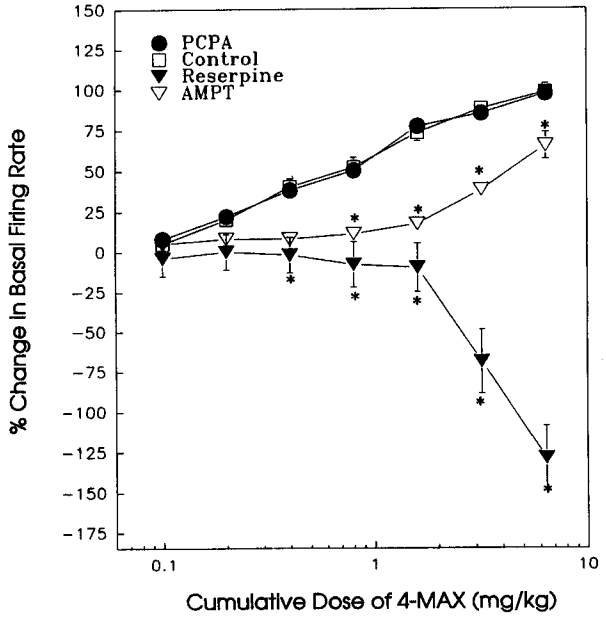

Fig. 8. Dose-response curves for trans 4S,5S 4-methylaminorex to suppress the basal firing rate of spontaneously active cells in animals pretreated with either saline, α-methyl-p-tyrosine (AMPT), p-chlorophenylalanine (PCPA) or reserpine. Each point represents the mean percent suppression ± S.E.M. Eight animals were examined at each dose of trans 4S,5S-4-MAX. *Significantly less than $P < 0.01$, ANOVA and Student-Newman-Keuls test.

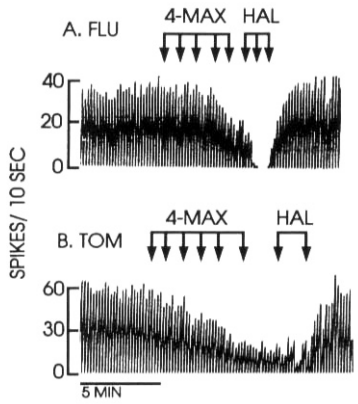

Fig. 9. Cumulative rate histograms illustrating that the pretreatment of animals with either (A) 5 mg/kg i.p. of fluoxetine (FLU) or (B) 5 mg/kg i.p. of tomoxetine (TOM) two hours prior to the administration of trans 4S,5S 4-MAX does not alter the suppressant action of intravenously administered trans 4S,5S 4-MAX on the basal firing rate of spontaneously active A10 DA cells. The arrows in A and B represent the i.v. administration of 0.1, 0.1, 0.2, 0.4, and 0.8 and 0.1, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/kg of trans 4S,5S 4-MAX (4-MAX), respectively. The last set of arrows in A and B represents the i.v. administration of 0.05, 0.05 and 0.1 and 0.05 and 0.05 mg/kg of haloperidol (HAL), respectively.

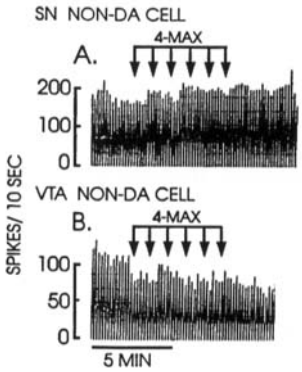

Fig. 10. Cumulative rate histograms showing that the intravenously administration of trans 4S,5S 4-methylaminores (4-MAX) does not alter the basal firing rate of nondopaminergic cells in the (A) A9 or (B) A10 areas. The arrows in A and B represent the i.v. injection of 0.1, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/kg of trans 4S,5S 4-methylaminorex, respectively.

The pretreatment of animals with 5 mg/kg s.c. of reserpine (24 hours before experiment), significantly attenuated the suppressant action of trans 4S,5S 4-MAX on A10 DA cells (Fig. 7). In fact, a number of A10 DA cells now displayed an excitatory response to trans 4S,5S 4-MAX. As a result of this effect, an ID_{50} could not be determined.

The effect of monoamine uptake blockers on the depressant action of trans 4S,5S 4-MAX on A10 DA neurons

In additional experiments, animals were pretreated with 5 mg/kg, i.p., of either (±)-fluoxetine, a selective 5-HT uptake blocker, or tomoxetine, a selective norepinephrine uptake inhibitor, two hours prior to the isolation of A10 DA cells and subsequent i.v. administration of trans 4S,5S 4-MAX. These experiments were conducted in order to ascertain if the suppressant action of intravenously administered trans 4S,5S 4-MAX was related to its interaction with the 5-HT and NE transporters. Overall, the pretreatment of animals with these drugs did not alter the suppressant action of trans 4S,5S 4-MAX on A10 DA cells (Fig. 9). Thus, the ID_{50} values for fluoxetine and tomoxetine were 1.3 ± 0.08 (n = 8) and 1.35 ± 0.1 mg/kg (n = 8), respectively, which were not significantly different from the ID_{50} value (1.25 ± 0.1, n = 8) for saline-pretreated animals.

We did not examine the effect of GBR 12909 (or other GBR analogues) because it has been shown that the administration of GBR 12909 produces a suppression of A10 DA cell firing (Einhorn et al., 1988).

The effect of trans 4S,5S 4-MAX on nondopaminergic cells in the SNC and VTA

The i.v. administration of the trans 4S,5S isomer did not significantly alter the basal firing rate of nondopaminergic cells in either the SNC (n = 8) or VTA (n = 8) areas (Fig. 10).

DISCUSSION

One of the major findings of this study was that the systemic administration of the stereoisomers of 4-MAX

suppressed the basal firing rate of spontaneously active A9 and A10 DA cells in chloral hydrate-anesthetized rats. The rank order of potency of the isomers to suppress the basal firing rate of spontaneously active A10 DA cells was: trans 4S,5S > cis 4R,5S ≈ cis 4S,5R > trans 4R,5R. A similar rank order of potency was shown in a drug discrimination paradigm comparing the potency of the stereoisomers to substitute for S(+)-amphetamine (Glennon and Misenheimer, 1990) and in a study measuring locomotor activity (Batsche et al., 1994). In addition, these results are consistent with structure-activity relationship data for phenylisopropylamine compounds. Thus, as pointed out by Glennon and Misenheimer (1990), the alpha carbon and benzylic positions of the phenylisopropylamines correspond to the 4 and 5 positions of 4-methylaminorex, respectively. Since the structure-activity relationship studies indicate that phenylisopropylamines possessing the S-configuration at the alpha carbon and benzylic position are the most active in a number of experimental paradigms, it might be predicted that the 4S,5S isomer of 4-MAX would be the most potent, as both carbons are in the appropriate configuration.

However, this prediction did not hold true for A9 DA cells as the rank order of potency for the isomers to suppress the basal firing rate of spontaneously active A9 DA cells was: trans 4S,5S = cis 4R,5S = cis 4R,5S >> trans 4R,5R, which is distinctly different from that for the A10 area. Furthermore, the trans 4S,5S isomer was approximately 5-fold more potent in suppressing the firing of DA cells in the A10 area compared to those in the A9 region. The greater potency of trans 4S,5S 4-MAX in suppressing A10, compared to A9, DA cell firing is in contrast to the compound S(+)-amphetamine, which is equipotent in suppressing the basal firing of DA cells in the A9 and A10 areas after sytemic administration in anesthetized rats (Bunney et al., 1975; Wang, 1981b).

Currently, the explanation for trans 4S,5S 4-MAX's greater potency to suppress the baseline firing of A10 compared to spontaneously active A9 DA cells is unknown. It has been shown that the activation of forebrain feedback pathways in the caudate nucleus plays a critical role in mediating the depressant action of S(+)-amphetamine on spontaneously active A9 DA cells (Bunney and Aghajanian, 1976, 1978; Groves et al., 1975), although the activation of forebrain feedback pathways is less critical for S(+)-amphetamine to inhibit the firing of A10 DA cells (Wang, 1981b). Therefore, one possible explanation for the diminished action of trans 4S,5S 4-MAX on A9 DA cells is its ineffectiveness in activating forebrain feedback pathways projecting to the A9 area. Indeed, our preliminary results indicate that the systemic administration of the trans 4S,5S isomer was significantly less potent in suppressing the firing of spontaneously active cells in the striatum compared to cells in the medial prefrontal cortex and nucleus accumbens (Ashby et al., unpublished observations). Finally, using the technique of in vivo microdialysis, it has been shown that the systemic administration of low doses of trans 4S,5S 4-MAX produces a significantly greater increase in extracellular DA levels in the nucleus accumbens compared to the striatum (Eberle et al., 1992). However, further studies must be conducted in order to elucidate the precise role of the forebrain target neurons and their feedback pathways in mediating the action of the isomers of 4-MAX on A9 and A10 DA cells.

The differential effect of the trans 4S,5S isomer on A9 and A10 DA cells is probably not due to a difference in the sensitivity of somatodendritic autoreceptors between the areas, as it has been reported that various direct and indirect autoreceptor agonists are equipotent in suppressing the basal firing of A9 and A10 DA cells (Clarke and Chiodo, 1988; White and Wang, 1984). In the present study, we again demonstrated that S(+)-amphetamine was equipotent in suppressing the firing rate of both A9 and A10 DA cells.

Previously, it has been shown that there is a significant positive correlation between the basal firing rates of A9 and A10 DA cells and the ID_{50} values for apomorphine and S(+)-amphetamine (White and Wang, 1984). Therefore, it is possible that the differential effect of trans 4S,5S 4-MAX on A9 and A10 DA cells may have been related to differences between the baseline firing rates of the neurons examined. However, this is unlikely, as the data in this study were analyzed using ANCOVA, thereby taking into account the contributions of the different firing rates of various DA cell groups and ruling out this possibility.

Finally, it is possible that trans 4S,5S 4-MAX could interact with other monoaminergic systems (e.g., serotonergic, noradrenergic), which could subsequently alter A10, but not A9 DA cell activity. However, the fact that various adrenergic and serotonergic receptor antagonists failed to alter the suppressant action of trans 4S,5S 4-MAX on the firing of A10 DA cells does not support this hypothesis.

The suppressant action of the cis isomers and trans 4S,5S isomer on A9 and A10 DA cell firing was reversed by the i.v. administration of haloperidol and the D_2/D_3 receptor antagonists (−)-eticlopride and (−)-sulpiride (Chivers et al., 1988; Tang et al., 1994). The nonselective D_1 receptor antagonist, SCH 23390, which also binds to 5-HT_{2A} and 5-HT_{2C} receptors, and the selective D_1 receptor antagonist, SCH 39166 (Chipkin et al., 1990), did not reverse the suppressant action of the stereoisomers of 4-MAX on A9 and A10 DA cells. In addition, the suppressant action of trans 4S,5S 4-MAX was not reversed by the systemic administration of various adrenergic or serotonergic receptor antagonists. The i.v. administration of metergoline partially reversed the action of trans 4S,5S 4-MAX on A10 DA cells. Radioligand binding data indicate that meter-

goline, similar to various ergoline derivatives, has moderate affinity at the D_2 receptor (Robertson and Fuller, 1988). Thus, it is possible that metergoline's partial reversal of the suppressant action of trans 4S,5S-4-MAX on A10 DA cells may be explained by this property. Although metergoline is an antagonist at 5-$HT_{1A,B}$ and 5-$HT_{2A,C}$ receptors (Hoyer, 1988), its interaction at these receptors is unlikely to explain its action, as ritanserin (5-$HT_{2A,C}$ antagonist) and (±)-pindolol (5-$HT_{1A,B}$,β antagonist) did not significantly alter the effect of trans 4S,5S 4-MAX on A10 DA cells. Overall, our findings indicate that the central D_2 receptor mediates the suppressant action of stereoisomers of 4-MAX on A9 and A10 DA cells. However, the effect of 4-MAX on A9 and A10 DA cells is unlikely to be due to its direct interaction with D_1 and D_2 receptors as structure-activity relationship studies indicate that phenylisopropylamine derivatives such as 4-MAX display low affinity for DA receptors (Seeman et al., 1985).

This view is further supported by our finding that the suppressant action of the trans 4S,5S isomer on A10 DA cells is significantly attenuated or completely abolished by pretreating animals with AMPT or reserpine, respectively. The administration of AMPT, an inhibitor of tyrosine hydroxylase (Weissman and Koe, 1965; Weissman et al., 1966), produces a depletion of newly synthesized DA stores (Kuczenski, 1983; McMillien et al., 1980), whereas reserpine depletes vesicular stores of DA, 5-HT and norepinephrine (Carlsson et al., 1957). Interestingly, the presumed restoration of DA levels by pretreating rats with the peripheral aromatic decarboxylase inhibitor, benserazide (which blocks the conversion of L-DOPA to DA in the periphery and allows L-DOPA to enter the brain in sufficient amounts) and L-DOPA (Maj et al., 1971) restored the suppressant action of trans 4S,5S 4-MAX. Similarly, it has been reported that the pretreatment of rats with AMPT and/or reserpine also significantly attenuates the suppression of A10 DA cell firing produced by the i.v. administration of the psychostimulants, cocaine (Einhorn et al., 1988) and d-amphetamine (Wang, 1981b). In contrast, pretreatment with PCPA, which significantly depletes brain stores of 5-HT (Koe and Weissman, 1967), did not alter the action of trans 4S,5S 4-MAX, suggesting that the presence of intact 5-HT stores is not involved in mediating its action on A10 DA cells. Overall, these findings suggest that the suppressant action of trans 4S,5S 4-MAX on A10 DA cells is mediated by the release of DA from newly synthesized and vesicular stores, which subsequently interacts with D_2/D_3 receptors.

Interestingly, the i.v. administration of trans 4S,5S 4-MAX produced an increase in the basal firing rate of some spontaneously active A10 DA cells following reserpine pretreatment. Currently, the exact explanation for this phenomenon is unknown. It is possible that the depletion of monoamine levels may allow for the expression of 4-MAX's action on other neurotransmitter systems (i.e., excitatory amino acids) to occur, which could produce an excitation of A10 DA cell firing. However, this hypothesis remains to be experimentally verified.

The pretreatment of animals with the nonselective β-adrenergic blocker, (±)-pindolol and the peripheral α adrenergic antagonist, phentolamine, did not alter the action of trans 4S,5S 4-MAX on A10 DA cells. This suggests that the central action of 4-MAX is not the result of peripheral sympathomimetic effects (i.e., indirect action) as cis (±)-4-MAX has been shown to elevate blood pressure and heart rate (Yelnosky and Katz, 1963) by indirectly releasing noradrenaline. It has been reported that the systemic administration of cocaine, which produces sympathomimetic effects, suppresses the firing of spontaneously active locus coeruleus cells in rats (Pitts and Marwah, 1988). However, there was no correlation between the suppression of cell firing and changes in blood pressure and the intravenous administration of phentolamine blocks the cardiovascular but not the central effects of cocaine. Overall, our results suggest that it is unlikely that 4-methylaminorex, which induces sympathomimetic effects similar to that of cocaine, suppresses midbrain DA cell firing by altering peripheral actions.

Finally, the suppressant action of trans 4S,5S 4-MAX on A10 DA cells is not altered by the pretreatment of animals with the selective 5-HT and norepinephrine uptake blockers, fluoxetine (Fuller et al., 1991) and tomoxetine (Bolden-Watson and Richelson, 1993), respectively. This suggests that trans 4S,5S 4-MAX's action is not mediated via its interaction with either 5-HT or NE uptake sites. The action of 4-MAX is probably different from (±)-methylenedioxymethamphetamine in that the suppressant action of the latter in the medial prefrontal cortex and nucleus accumbens could be prevented by 5-HT and DA uptake blockers, respectively (Pan and Wang, 1991).

In conclusion, our results indicate that there was a significant difference in the potency of the various isomers of 4-MAX to suppress midbrain DA cell firing. Furthermore, the trans 4S,5S isomer was significantly more potent in suppressing A10, compared to A9 DA cell activity. The D_2/D_3 receptors appear to play a significant role in the mediation of 4-methylaminorex's action, as the D_2/D_3 receptor antagonists (−)-eticlopride and (−)-sulpiride, but not various adrenergic and serotonergic receptor antagonists, reverse the suppressant action on A10 DA cells. Finally, it appears that the presence of newly synthesized DA and vesicular DA is important in mediating the suppressant action of trans 4S,5S 4-MAX.

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