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Multiple 5-HT Receptors in Passive Avoidance: Comparative Studies of p-Chloroamphetamine and 8-OH-DPAT

Ilga Misane, Ph.D., and Sven Ove Ögren, Dr. Med.Sc.

The aim of this study was to examine the involvement of multiple 5-HT receptors in passive avoidance (PA) with a focus on 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors. Because increases in 5-HT transmission result in concomitant multiple 5-HT receptor activation, the effects of the 5-HT releasing compound p-chloroamphetamine (PCA) were compared with those of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT in the rat. In addition, some results with the nonselective 5-$HT_{2C/2B/1B}$ receptor agonist mCPP are presented. When injected before PA training, 8-OH-DPAT, mCPP, and PCA produced a dose-related impairment of the 24-hour retention. The crucial involvement of the postsynaptic 5-HT_{1A} receptors in the action of 8-OH-DPAT was confirmed. Thus, the 5-HT_{1A} receptor antagonists WAY 100635 and (-)-pindolol blocked the PA deficit by 8-OH-DPAT. The impairment of PA caused by PCA was attenuated by WAY 100635 and (-)-pindolol, suggesting an involvement of the 5-HT_{1A} receptor. In contrast, the 5-HT_{2A} and 5-HT_{2C} receptors were of negligible importance in the 24-hour retention deficit induced by PCA. However, the ability of the 5-HT_{2C} receptor antagonist Ro 60-0491 to block the inhibitory effects of mCPP indicated an important regulatory role of the 5-HT_{2C} receptor in PA. The nonselective 5-HT receptor antagonist methiothepin attenuated the PA deficit by PCA but lacked activity versus 8-OH-DPAT. These data provide evidence for the hypothesis that, in addition to the 5-HT_{1A} receptor, other 5-HT receptor subtypes are involved in the inhibitory actions of PCA. Importantly, changes in dopamine transmission seemed not to contribute to the PA impairment by PCA. The behavioral alterations caused by the drug treatments at the time of PA training could not be related to the subsequent retention performance. In conclusion, multiple 5-HT receptors are involved in PA with roles that probably differ at various stages of information processing. These findings also suggest that there probably exists a functional distinction between 5-HT receptor subtypes in different types of aversive learning. ***[Neuropsychopharmacology 22:168–190, 2000]*** *© 1999 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

KEY WORDS: *Passive avoidance; 5-HT receptors; 8-OH-DPAT; p-chloroamphetamine (PCA); m-chlorophenylpiperazine (mCPP); dopamine*

From the Division of Cellular and Molecular Neurochemistry, Department of Neuroscience, Karolinska Institute, Stockholm, Sweden.

Address correspondence to: I. Misane, Ph.D., Division of Cellular and Molecular Neurochemistry, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden. Tel.: +46 8 728 7395. Fax: +46 8 302875. E-mail: Ilga.Misane@neuro.ki.se

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Serotonergic (5-HT) projections, arising from the midbrain raphe nuclei (Jacobs and Azmitia 1992; Vertes 1991) innervate limbic (amygdala and hippocampus) and cortical areas known to be involved in the cognition and processing of emotional events (Ambrogi Lorenzini et al. 1998; Gallagher and Chiba 1996; Heilman and Gilmore 1998; Lavond et al. 1993; Ledoux and Müller 1997; Pezzone et al. 1992).

To investigate the role of the limbic and cortical 5-HT in behavior, different appproaches have been employed ranging from manipulations that result in multiple re-

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ceptor stimulation to the selective activation of 5-HT receptor subtypes. The former approach is of particular importance, because 5-HT neurotransmission seems to operate largely via non- or extrasynaptic mode of communication, also known as volume transmission (Agnati et al. 1995; Bunin and Wightman 1998; Descarries et al. 1990; Descarries et al. 1975; Dewar et al. 1991; Jansson et al. 1998). Thus, released 5-HT can act at a distance at multiple 5-HT receptors far away from the synaptic cleft, whereas selective 5-HT agonists act on all receptors of a specific subtype.

Earlier studies have shown that treatments that increase 5-HT activity in the brain, such as 5-HT-releasing compounds [p-chloroamphetamine (PCA), MDMA, and MMAI] (Marona-Lewicka et al. 1996; McNamara et al. 1995; Ögren 1985b; Romano and Harvey 1994; Santucci et al. 1996) as well as the selective 5-HT reuptake inhibitors (SSRIs) (Altman et al. 1984; Lalonde and Vikis-Freibergs 1985; Lucki and Nobler 1985; McElroy et al. 1982; Meneses and Hong 1995; Ögren 1985b) can both enhance and impair performance in aversive learning tasks. Pretraining administration of PCA has been found to produce a marked impairment of both one- and two-way active avoidance acquisition and retention in the rat (Ögren 1982).

Although PCA also causes an acute release of dopamine (DA) (Crespi et al. 1997; Henderson et al. 1993; Johnson et al. 1990; Ögren 1985b; Sharp et al. 1986) as well noradrenaline (NA) (Ögren 1982; Ögren 1985a) in the rat brain, its behavioral effects are mediated primarily via serotonergic mechanisms (Adell et al. 1989; Geyer 1996; Hutson and Curzon 1989; Trulson and Jacobs 1976). In support of this, the one-way active avoidance deficit by PCA was completely blocked when the rats were pretreated with 5-HT reuptake inhibitors zimeldine and fluoxetine but not by the NA uptake inhibitor desipramine (Ögren 1982). Zimeldine also blocked the 5-HT release induced by PCA (Ögren et al. 1982). Several nonselective 5-HT_2 antagonists, which, by themselves, did not impair avoidance learning, also produced a dose-dependent blockade of the PCA-induced deficit (Ögren 1986b). This finding suggested that the impairment of active avoidance acquisition induced by PCA was mediated via stimulation of 5-HT_2 receptors (Ögren 1986b). PCA-treatment also produced a marked impairment of passive avoidance (PA) learning. Thus, PA retention was disrupted when the rats were trained under PCA-induced (30 min before training, 2.5 mg/kg) serotonin release and tested 24 h later (Ögren 1985b; Ögren 1986a). The impairment of PA retention induced by PCA was completely antagonized by the 5-HT uptake inhibitor zimeldine but not by the NA uptake inhibitor desipramine (Ögren 1985b), again showing the primary role of the serotonin. The PCA-induced impairment of PA retention was not blocked by nonselective 5-HT_2 antagonists, such as metergoline or danitracen (Ögren 1985b), indicating the possible involvement of postsynaptic 5-HT_1-like receptors but not 5-HT_2 receptors (Ögren 1985b), because, at that time, the 5-HT receptor classification involved only these two receptor families.

During the past decade, the development in 5-HT receptor pharmacology has resulted in characterization of 15 subtypes of mammalian 5-HT receptors (Hoyer et al. 1994; Hoyer and Martin 1997; Saxena et al. 1998). In addition, new and selective ligands for the 5-HT receptor subtypes have become available. In view of this development, the important role of 5-HT_{1A} receptors in learning and memory tasks including PA was revealed. The selective 5-HT_{1A} agonist 8-OH-DPAT given subcutaneously (SC) before PA training has consistently been found to produce a dose-dependent impairment of retention in the rat when examined 24 h later (Carli et al. 1992; Jackson et al. 1994; Johansson et al. 1988; Misane et al. 1998a; Riekkinen 1994). This PA deficit is mainly attributable to stimulation of postsynaptic 5-HT_{1A} receptors in the brain (Misane et al. 1998a).

A number of aversive conditioning procedures in the rat have been used to study memory for emotional experiences (Davis 1990; Gallagher and Chiba 1996; Gewirtz and Davis 1998; Lavond et al. 1993; Ledoux and Müller 1997; Ögren 1985b). Among them, the PA procedure has been demonstrated to be a valid and reliable tool for assessment of serotonergic manipulations in vivo (Ögren and Misane 1998). Methodologically, this task has several advantages compared with multisession tasks, particularly in terms of the exact timing of drug treatment in relation to training as well as the clarity of experimental design. The training procedure comprises a single trial and is based on the innate preference of rodents for the dark chamber of the apparatus and the "safe" and "aversive" compartments of the test box are clearly defined. The suppression of this innate preference following exposure to unescapable shock is an adaptive response that serves as a measure of learning (retention). The area in which the rat receives shock provides the cues for the contextual reference memory via classical fear-conditioning (Pavlovian conditioning).

The objective of the present study was to examine the involvement of multiple 5-HT receptors in PA with the focus on the 5-HT_1 and 5-HT_2 receptor families; that is, 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors. Because 5-HT transmission operates via multiple 5-HT receptor subtypes, the effects of PCA on PA were compared with those of 8-OH-DPAT. Several selective and nonselective 5-HT receptor antagonists were examined in combination with PCA and 8-OH-DPAT using analogous design. To analyze the role of the 5-HT_{2C} receptors in PA, the relatively selective 5-HT_{2C} receptor antagonist Ro 60-0491 (Martin et al. 1998) was used in the combination studies with m-chlorophenylpiperazine (mCPP). Finally, to confirm the role of serotonergic mechanisms

versus DAergic and NAergic mechanisms in the inhibitory actions of PCA, combination studies with the selective 5-HT reuptake inhibitor paroxetine were performed. Because previous studies did not allow for the exclusion of the involvement of DA in the PCA-induced effects on PA , experiments using the "DA agonist" d-amphetamine, the selective DA D_2 receptor antagonist remoxipride, and the DA D_2/D_3 receptor antagonist raclopride were performed.

METHODS

Animals

Adult male Sprague–Dawley rats (B & K UNIVERSAL AB, Sollentuna, Sweden) weighing 300 to 360 g were used in the passive avoidance studies. The animals were allowed at least a 5-day adaptation period at the standard maintenance facilities of the department before the beginning of the experiments. The animals were housed in plastic type IV *Macrolon*® cages (57 × 35 × 19 cm, with 2l wood-cuttings as bedding), each cage containing four to five (n = 4–5) rats. They were maintained at an ambient room temperature of 20 ± 0.5 °C with 40 to 50% relative humidity. A 12-h light/dark schedule (lights on at 06.00 hours) was used throughout the experiment, and the animals had free access to standard lab chow (Ewos R36, Ewos AB, Sweden) and tap water up to the time of the experiments. The cages were changed twice a week because of the cleaning procedure during the adaptation period. To avoid the influence of additional stress factors on performance, the cages were not cleaned on the passive avoidance days 1 and 2. On the experimental days, the animals were brought to the experimental room and allowed to habituate to the environmental conditions for a period of approximately 60 min before the start of the experiment. Animal housing and all experimental procedures followed the provisions and general recommendations of the Swedish animal protection legislation. The experimental procedures were approved by the local Animal Ethics Committee (ethical N 80/96).

Passive Avoidance (PA) and Behavioral Observations

PA was conducted as described in detail earlier (Misane et al. 1998a; Misane et al. 1998b). A modified shuttle box (Ugo Basile, Comerio-Varese, Italy) with two communicating (7 × 7 cm sliding door built into the separating wall) compartments of equal size and a stainless steel bar floor was used. The right-hand compartment (shock compartment) was painted black to obtain a dark chamber. The left-hand compartment was illuminated by a bulb (24 V; 5 W) installed on the top *Plexiglas* cover. The entire experiment was carried out by the same experimenter.

PA training was conducted in a single session (day 1) during the light phase (09:00– 16:00 h) of a 12-h day/night cycle. The animals (n = 7–40, for details, see figure legends) were treated with the test compounds as described below. After the selected time interval following injection (day 1), rats were placed into the light compartment (with no access to the dark compartment) and allowed to explore for 2 minutes.

During the exploration phase in the PA apparatus, the behavior of the animals was observed, and the presence or absence of the components of the serotonin (5-HT) syndrome (flat body posture, lower lip retraction, reciprocal forepaw treading, head weaving and twitches, wet-dog shakes, hind limb abduction, penile erection, and tremor) (Berendsen et al. 1989; Grahame-Smith 1971; Jacobs 1976; Tricklebank et al. 1984; Trulson and Jacobs 1976) and also the rearing frequency were noted.

When 2 min expired, the sliding door was automatically opened by pressing a pedal, and the rats were allowed to cross over into the dark compartment. Once the rats had entered the dark compartment with all four feet, the sliding door was automatically shut, and an inescapable, constant current, scrambled shock (5 s duration, 0.6 mA) was delivered through the grid floor. Latency to cross into the dark compartment (training latency) was recorded. Cut-off latency was set at 300 s. Because of the drug treatment, some rats failed to move into the dark compartment within 300 s. In this case, the door was reopened, and the rats were gently moved by the experimenter into the dark compartment, where they received the foot shock. Following training, rats were immediately removed from the PA apparatus.

Performance during the retention phase (retention) was tested 24 h after training (day 2). The animals were placed into the light (safe) compartment, with access to the dark one (within 15 s) for a period of 300 s. The latency to cross into the dark compartment with all four feet was automatically measured (retention latency). In addition to the "routine" 24-hour retention, the effects of 8-OH-DPAT were also examined in a 5-min retention test.

The present studies focused on the acquisitional processes; that is; all the drug treatments were made before PA training. The rationale for pretraining administration of 8-OH-DPAT and PCA were based on our previous findings that consistently showed that PCA and selective 5-HT_{1A} agonists impaired 24-hour PA retention when administered before training and/or before retention; whereas, no impairment was seen in the case of immediate post-training administration (Misane et al. 1998a; Ögren 1985b; Ögren 1986a).

Drugs

The following compounds (see Table 1) were used in this study: 8-hydroxy-2-(di-n-propylamino)tetralin hydro-

bromide, (+/−)-8-OH-DPAT; 1-(3-chlorophenyl)piperazine dihydrochloride, mCPP; methiothepin mesylate and (-)-pindolol (all obtained from RBI, Natick, MA, USA); p-chloroamphetamine hydrochloride, PCA; d-amphetamine sulfate (both purchased from Sigma Chemical Co., St. Louis, MO, USA); (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexane carboxamide trihydrochloride (WAY 100635) (Wyeth Research, Taplow, UK); ketanserin tartrate, and spiperone (both obtained from Janssen, Beerse, Belgium); ritanserin; paroxetine; raclopride tartrate; remoxipride hydrochloride monohydrate (all obtained from Astra Arcus AB, Södertalje, Sweden), and N-(2-naphtyl)-N′-(3-pyridyl)-urea hydrochloride, Ro 60-0491 (kindly supplied by Dr. James R. Martin, Hoffman-La Roche Ltd., Basel, Switzerland). All drugs, with the exception of (-)-pindolol, ritanserin, Ro 60-0491, and spiperone, were dissolved in saline (NaCl 0.9%). Ritanserin and spiperone were dissolved in a few drops of acetic acid and distilled water, and the pH was adjusted to 5.5. (-)-Pindolol was dissolved in 0.1 N HCl. Ro 60-0491 was suspended in 0.8% methylcellulose. The test drugs were administered subcuteneusly (SC) or intraperitoneally (IP) (as indicated in the Results and the figure legends) in volumes of 2 ml/kg or 5 ml/kg, respectively. 8-OH-DPAT was administered 15 min, mCPP 30 min and PCA 60 min before PA training. The different 5-HT receptor antagonists were administered at the following times before PA training: ketanserin was injected at 10 min; WAY 100635, methiothepin, and spiperone at 30 min; Ro 60-0491 at 40 min; (-)-pindolol at 45 min, and spiperone at 60 min. Paroxetine was administered 90 min before PA training. The DAergic drugs were injected at the following times before PA training: raclopride at 20 min; d-amphetamine at 30 min; and remoxipride at 60 min. All the control rats received saline (NaCl 0.9%) or the respective solvent injections, and they were run concurrently with drug-treated groups. The doses of the drugs tested refer to the base or salt of the respective drug. All the chemicals used were of analytical grade.

Statistical Analysis

The over-all treatment effects in the passive avoidance studies were examined using one-way analysis of variance (ANOVA). For each significant F-ratio, Fisher's protected least significant difference test (Fisher's PLSD test) was used to analyze the statistical significance of appropriate multiple comparisons (Kirk 1968). A probability level of $p < .05$ was accepted as statistically significant in all the studies and all the post hoc tests were two-tailed. When neither the control nor the respective 5-HT agonist (8-OH-DPAT or PCA)-treated groups differed from each other with respect to both, training and retention latencies, the results obtained in the combination studies with different doses of the 5-HT antagonists were pooled.

Table 1. In Vitro Pharmacological Profile of the Serotonergic Ligands Used in the Present Study

Ligand	5-HT Receptors	Other Receptors
Agonists		
8-OH-DPAT	$5\text{-HT}_{1A} > 5\text{-HT}_7 > 5\text{-HT}_{5A}$	
PCA	5-HT transporter	DA transporter; NE transporter
mCPP	$5\text{-HT}_{2C} > 5\text{-HT}_{2B} > 5\text{-HT}_{2A}$ (antagonist)> >5-HT_{1A}> $5\text{-HT}_{1B} = 5\text{HT}_{1D}$> 5-HT_7	
Antagonists		
WAY 100635	5-HT_{1A}	
(-)-Pindolol	5-HT_{1A}> 5-HT_{1B}	β-adrenoceptors
Methiothepin	5-HT_{2A}> 5-HT_7> 5-HT_6> 5-HT_{1D}> 5-HT_{2C}> .5-HT_{1B}> 5-HT_{1A}> 5-HT_{5A}> 5-HT_{1E}	α_1- and α_2-adrenoceptors; D_1, D_2, and D_3 DA receptors; histamine H_1 receptors
Spiperone	5-HT_{2A}> 5-HT_7> 5-HT_{1A}	D_2 and D_3 DA receptors; α_1-adrenoceptors
Ritanserin	5-HT_{2A}> $5\text{-HT}_{2B} > 5\text{-HT}_{2C} = 5\text{-HT}_7$	
Ketanserin	5-HT_{2A}> 5-HT_7> 5-HT_{2C}	α_2-adrenoceptors
Ro 60-0491	5-HT_{2C}> 5-HT_{2A}	
Selective 5-HT reuptake inhibitor		
Paroxetine	5-HT transporter	

Based on Refs. (Baxter et al. 1995; Beique et al. 1998; Forster et al. 1995; Hoffman et al. 1991; Hoyer 1989; Hoyer et al. 1994; Kennett 1993; Leysen et al. 1993; Martin et al. 1998; Middlemiss 1986; Ruffolo et al. 1995; Schuldiner et al. 1993; Wall et al. 1995).

RESULTS

Dose-Related Effects of "5-HT Agonists" on PA Training and 24-Hour Retention

Figure 1 shows that when tested 24 h after training, the retention latencies in the saline-treated control groups were close to 300 s, indicating that the animals had acquired the task. In contrast, rats treated with 8-OH-DPAT, PCA, or mCPP before training displayed a dose-related decrease in retention latencies: $F_{4,35} = 20.70$; $p < .01$ for 8-OH-DPAT, $F_{3,28} = 14.48$; $p < .01$ for PCA, and $F_{3,27} = 5.07$; $p < .01$ for mCPP, respectively). 8-OH-DPAT produced a significant effect from the 0.1 mg/kg dose ($p < .05$, Fisher's PLSD test); whereas, PCA and mCPP were effective from the 3.0 mg/kg dose.

No significant over-all treatment effect on training

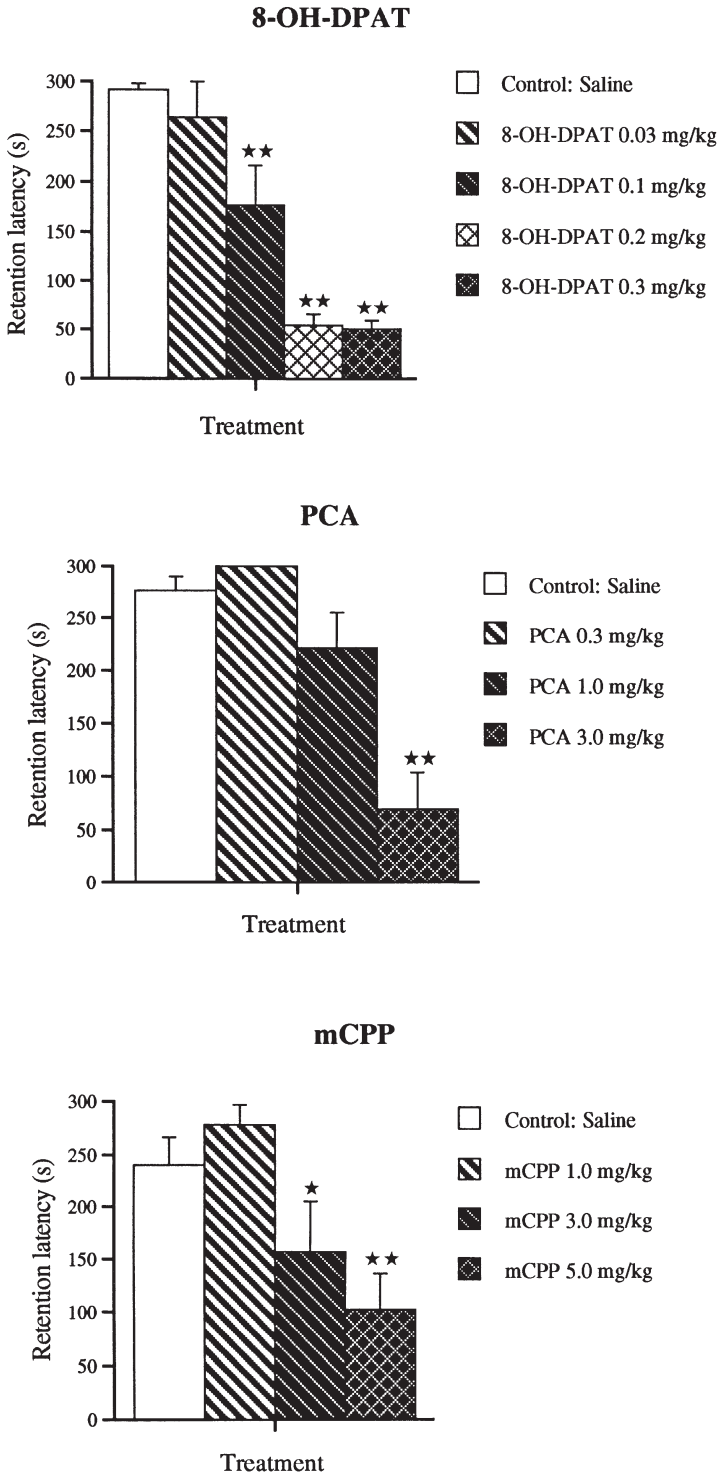

Figure 1. The dose-related effects of the 5-HT agonists on passive avoidance (PA) retention in the rat. 8-OH-DPAT (0.03–0.3 mg/kg SC), p-chloroamphetamine (PCA; 0.3–3.0 mg/kg IP), and mCPP (1.0–5.0 mg/kg SC) were administered 15 min, 60 min, and 30 min before the training session (exposure to inescapable foot shock), respectively. The saline control groups were run concurrently with the respective 5-HT agonist-treated groups. The retention test was performed 24 h later. Vertical bars represent means (±SEM) of retention latencies. Maximal time of latency was set at 300 s (cut-off time). The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Fisher's PLSD test (★$p < .05$ and ★★$p < .01$ versus corresponding saline control group, $n = 7$–8); for details, see Methods.

latencies was found in the 8-OH-DPAT dose-response studies ($F_{4,35} = 2.11$, $p = .10$) (Table 2). However, a decrease in training latency was seen at the 0.03, 0.2, and 0.3 mg/kg doses ($p < .05$ vs. saline control) but not at the 0.1 mg/kg dose ($p > .09$ vs. saline control). Unlike 8-OH-DPAT, mCPP induced a dose-dependent increase in training latencies ($F_{3,27} = 3.56$, $p < .05$) with a significant effect from the 3.0 mg/kg dose ($p < .05$ vs. saline control). In contrast, PCA-treatment did not alter training latencies (Table 2). Based on the dose-response experiments, the 0.2 mg/kg dose of 8-OH-DPAT, the 3.0 mg/kg dose of PCA and the 5.0 mg/kg dose of mCPP were chosen for all subsequent interaction studies in the PA.

Dose-Related Effects of 8-OH-DPAT on PA Training and 5-Min Retention

This experiment using short-term 5-min retention was performed to examine whether the activation of the 5-HT_{1A} receptors would affect the "encoding" of aversive experience. Figure 2 shows that when tested 5 min after training, the retention latencies in the saline-treated control group were 300 s, indicating that the animals had fully "encoded" the aversive experience; whereas, rats treated with 8-OH-DPAT displayed a dose-related decrease in retention latencies ($F_{4,35} = 106.89$; $p < .01$). Unlike 24-hour retention, 8-OH-DPAT produced a significant effect only from the 0.2 mg/kg dose ($p < .01$ vs. saline control) but not at the 0.1 mg/kg dose. A significant over-all treatment effect on training latencies was found in this experiment ($F_{4,35} = 5.85$, $p < .01$), and the decrease in training latency was seen from the 0.03 mg/kg dose ($p < .05$ vs. saline control) (Table 2).

Effects of Different 5-HT Antagonists on PA Retention

Table 3 shows that none of the 5-HT antagonists tested (for the pharmacological profile, see Table 1) had an inhibitory effect on PA retention. The only exception was spiperone, which caused an impairment of PA retention at the 0.1 mg/kg dose ($p < .05$ vs. control). In addition, none of the 5-HT antagonist treatments caused significant alterations in PA training latencies (Table 2).

Table 2. Effects of the 5-HTergic Drugs on Passive Avoidance (PA) Training in the Rat

Compound	Dose (mg/kg) Training Latency (s)				
Agonists					
8-OH-DPAT[a]	0	0.03	0.1	0.2	0.3
	49.1 ± 16.5	21.8 ± 3.3*	22.0 ± 4.1*	28.0 ± 6.7	17.7 ± 5.0*
8-OH-DPAT[b]	0	0.03	0.1	0.2	0.3
	58.7 ± 12.5	33.8 ± 6.6*	19.7 ± 7.1**	19.7 ± 4.1**	14.4 ± 2.8**
PCA	0	0.3	1.0	3.0	
	49.9 ± 14.9	13.8 ± 4.0	28.7 ± 8.6	92.6 ± 38.4	
mCPP	0	1.0	3.0	5.0	
	27.8 ± 6.2	131.7 ± 44.6	151.8 ± 48.0*	197.7 ± 41.9**	
Antagonists					
WAY 100635	0	0.03	0.1	0.3	1.0
	58.3 ± 23.9	49.6 ± 20.4	58.0 ± 21.5	78.0 ± 34.2	57.4 ± 21.3
(-)-Pindolol	0	0.3	1.0	3.0	
	40.0 ± 10.0	58.1 ± 16.6	43.4 ± 16.5	31.6 ± 14.2	
Spiperone	0	0.01	0.03	0.1	
	85.2 ± 36.8	100.1 ± 32.0	71.4 ± 34.0	123.3 ± 45.6	
Methiothepin	0	0.03	0.1	0.3	
	40.2 ± 12.1	48.4 ± 14.6	25.4 ± 6.4	34.8 ± 5.9	
Ritanserin	0	0.3	1.0		
	18.0 ± 4.2	36.8 ± 15.7	51.5 ± 28.3		
Ketanserin	0	1.0			
	65.4 ± 17.7	41.9 ± 6.5			
Ro 60-0491	0	3.0			
	47.9 ± 12.8	36.4 ± 8.5			
Selective 5-HT reuptake inhibitor					
Paroxetine	0	0.3	1.0	3.0	
	74.6 ± 25.4	96.4 ± 51.3	51.1 ± 14.5	98.7 ± 36.8	

All the test drugs were administered before PA training at the times and injection routes as follows: 8-OH-DPAT (SC) 15 min, PCA (IP) 60 min, and mCPP (SC) 30 min, WAY 100635 (SC) 30 min, (-)-pindolol (SC) 45 min, spiperone (IP) 60 min, methiothepin (IP) 30 min, ritanserin (IP) 30 min, ketanserin (IP) 10 min, Ro 60-0491 (IP) 40 min, and paroxetine (IP) 90 min. The values shown are mean durations (± SEM). The statistical analysis was performed by one-way ANOVA followed by Fisher's PLSD test (*$p < .05$ and **$p < .01$ versus corresponding control group, $n = 7$-8); for details, see Methods.

[a]Data with 8-OH-DPAT represent two independent experiments with subsequent use of 24-hour (a) and 5-min retention (b) tests, respectively.

[b]Data with 8-OH-DPAT represent two independent experiments with subsequent use of 24-hour (a) and 5-min retention (b) tests, respectively.

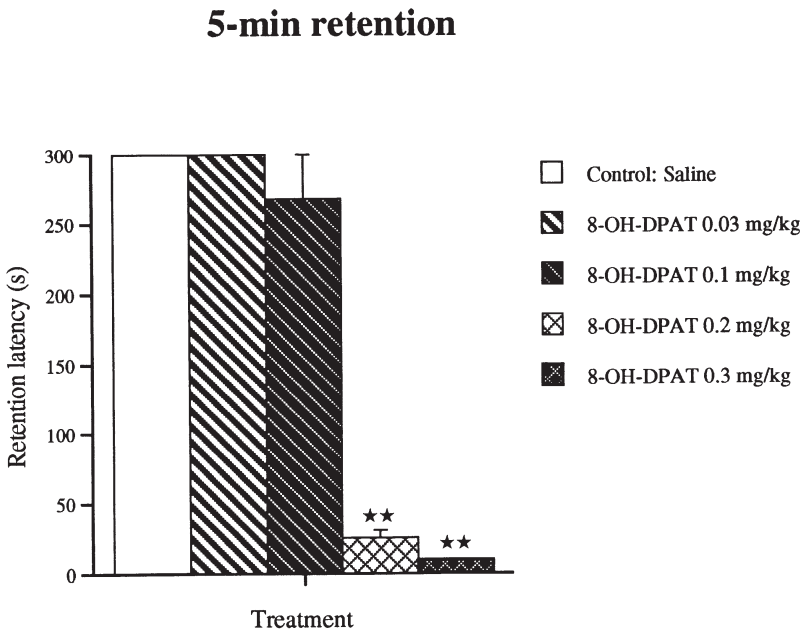

Figure 2. The dose-related effects of 8-OH-DPAT on short-term passive avoidance (PA) retention in the rat. 8-OH-DPAT (0.03–0.3 mg/kg SC) was administered 15 min before the training session. The saline control group was run concurrently with the 8-OH-DPAT-treated groups. The retention test was performed 5 min later. Vertical bars represent means (±SEM) of retention latencies. ★★$p <$ .01 versus saline control group, $n = 8$; for details of statistical analysis and general information, see legend to Figure 1 and Methods.

Effects of the Selective 5-HT$_{1A}$ Antagonist WAY 100635 on Impairment of PA Induced by 8-OH-DPAT and PCA

Figure 3A shows that pretreatment with WAY 100635 (0.03–1.0 mg/kg SC) fully blocked the deficit of PA caused by 8-OH-DPAT (0.2 mg/kg SC); whereas, the lowest dose of WAY 100635 tested (0.003 mg/kg) failed to produce any significant attenuation.

A significant over-all treatment effect in the retention test ($F_{4,67} = 43.91$, $p < .01$) was found when WAY 100635 (0.03–0.3 mg/kg) was combined with PCA (3.0 mg/kg IP). Under these conditions an inverse "U-shape" type of activity of WAY 100635 was found. At the 0.03 and 0.1 mg/kg doses, WAY 100635 attenuated the impairment of PA retention caused by PCA; whereas, no effect was found at the highest 0.3 mg/kg dose tested (Figure 3B).

Effects of the 5-HT$_{1A/1B}$ and β-adrenoceptor Antagonist (−)-Pindolol on Impairment of PA Induced by 8-OH-DPAT and PCA

Similarly to WAY 100635, (-)-pindolol antagonized the impairment of PA by 8-OH-DPAT (0.2 mg/kg SC) (Fig-

Table 3. Effects of the Selected 5-HT Antagonists and the 5-HT Reuptake Blocker Paroxetine on Passive Avoidance (PA) Retention in the Rat

Compound	Dose (mg/kg) Retention Latency (s)				
WAY 100635	0	0.03	0.1	0.3	1.0
	293.0 ± 7.0	287.5 ± 12.5	300.0 ± 0.0	300.0 ± 0.0	282.4 ± 17.6
(-)-Pindolol	0	0.3	1.0	3.0	
	277.1 ± 22.9	294.7 ± 5.3	287.2 ± 12.8	252.0 ± 33.9	
Spiperone	0	0.01	0.03	0.1	
	281.7 ± 12.2	269.7 ± 30.3	262.1 ± 24.9	178.6 ± 39.3*	
Methiothepin	0	0.03	0.1	0.3	
	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	
Ritanserin	0	0.3	1.0		
	292.0 ± 5.3	245.5 ± 36.6	233.7 ± 41.5		
Ketanserin	0	1.0			
	272.4 ± 15.6	280.3 ± 12.8			
Ro 60-0491	0	3.0			
	280.5 ± 19.5	300.0 ± 0.0			
Paroxetine	0	0.3	1.0	3.0	
	278.9 ± 21.1	269.1 ± 20.4	297.6 ± 2.4	232.5 ± 32.0	

For details, see Table 2 and Methods.

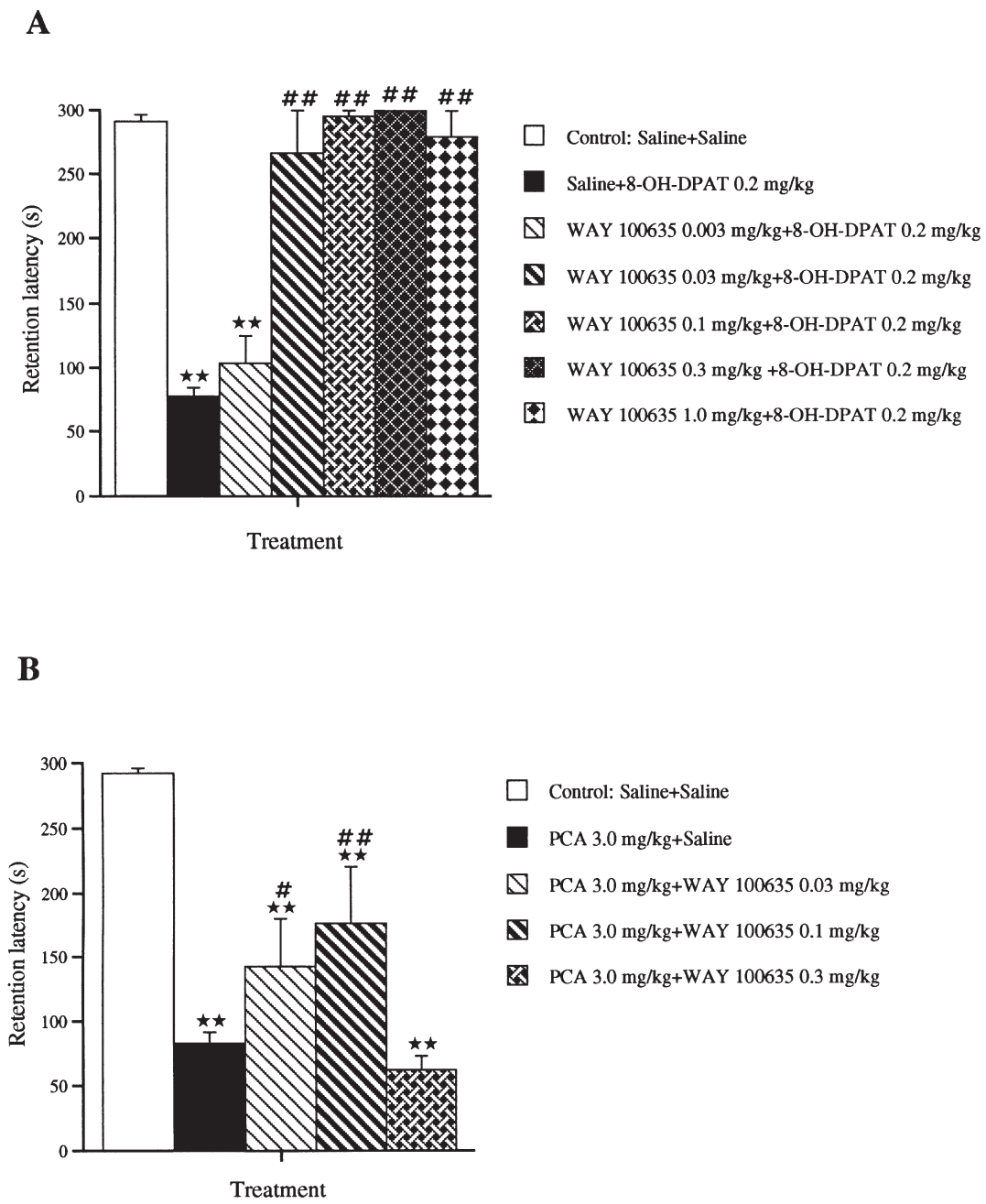

Figure 3. The combined effects of WAY 100635 and 8-OH-DPAT or PCA on PA retention in the rat. **(A)** Rats were injected with WAY 100635 (0.003–1.0 mg/kg SC) and 8-OH-DPAT (0.2 mg/kg SC) 30 min and 15 min before the training session, respectively. **(B)** Rats were injected with PCA (3.0 mg/kg IP) and WAY 100635 (0.03–0.3 mg/kg SC) 60 min and 30 min before the training session, respectively. The saline + saline control groups were run concurrently with WAY 100635- and 8-OH-DPAT- or PCA-treated groups. The retention test was performed 24 h later. Vertical bars represent means (±SEM) of retention latencies. ★★$p < .01$ versus corresponding saline + saline control group; #$p < .05$ and ##$p < .01$ versus corresponding saline + 8-OH-DPAT- or PCA + saline-treated group, n = 8–40; for details of statistical analysis and general information, see legend to Figure 1 and Methods.

ure 4A). A "partial" blockade was seen at the 0.3 to 1.0 mg/kg doses; whereas, the 3.0 mg/kg dose of (-)-pindolol fully antagonized the inhibitory effects of 8-OH-DPAT ($p > .45$ vs. saline + saline control group and $p < .01$ vs. saline + 8-OH-DPAT group). (-)-Pindolol (3.0 mg/kg) also prevented ($p = .51$ vs. saline + saline control group and $p = .05$ vs. saline + 8-OH-DPAT group) the decrease in training latencies seen in the saline + 8-OH-DPAT-treated group ($p < .01$ vs. saline + saline control group) (data not shown).

A significant over-all treatment effect on the retention test ($F = 16.27$, $p < .01$) was found when (-)-pin-

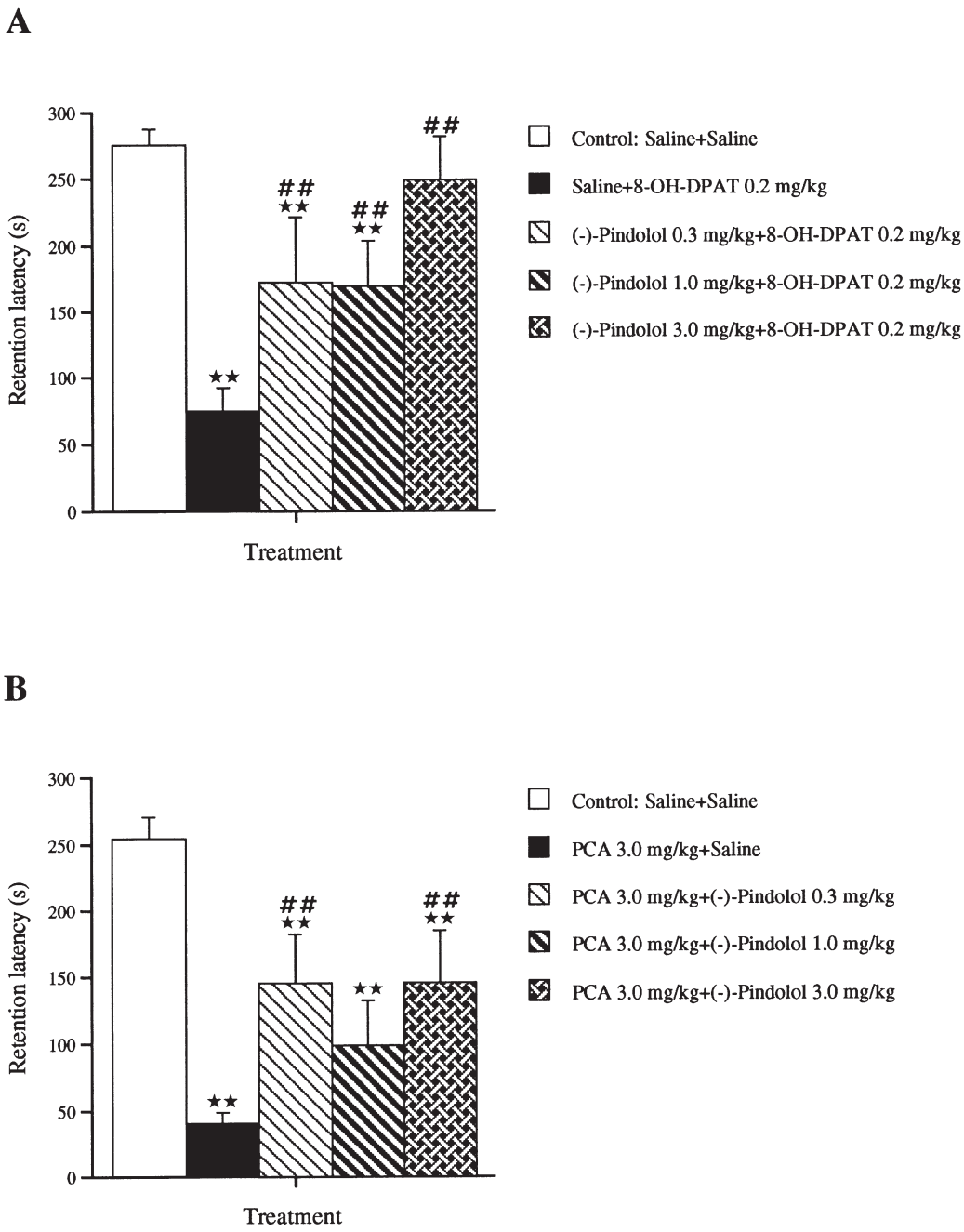

Figure 4. The combined effects of (-)-pindolol and 8-OH-DPAT or PCA on PA retention in the rat. **(A)** Rats were injected with (-)-pindolol (0.3–3.0 mg/kg SC) and 8-OH-DPAT (0.2 mg/kg SC) 45 min and 15 min before the training session, respectively. **(B)** Rats were injected with PCA (3.0 mg/kg IP) and (-)-pindolol (0.3–3.0 mg/kg SC) 60 min and 45 min before the training session, respectively. The saline + saline control groups were run concurrently with (-)-pindolol- and 8-OH-DPAT- or PCA-treated groups. The retention test was performed 24 h later. Vertical bars represent means (±SEM) of retention latencies. ★★$p < .01$ versus corresponding saline + saline control group; ##$p < .01$ versus corresponding saline + 8-OH-DPAT or PCA + saline-treated group, $n = 8$–24; for details of statistical analysis and general information, see legend to Figure 1 and Methods.

dolol (0.3–3.0 mg/kg SC) was combined with the 3.0 mg/kg dose of PCA (Figure 4B). The inhibitory effects of PCA were attenuated by (-)-pindolol at the dose-range tested, although no clear dose-dependent effect was demonstrated.

Effects of the Nonselective 5-HT Receptor Antagonist Methiothepin on Impairment of PA Induced by 8-OH-DPAT and PCA

Figure 5A shows that methiothepin (0.03–0.3 mg/kg IP) failed to block the impairment of PA retention caused

by 8-OH-DPAT (0.2 mg/kg). In contrast, methiothepin (0.1–0.3 mg/kg) attenuated the impairment of PA retention induced by the 3.0 mg/kg dose of PCA ($p < .01$ vs. PCA + saline group and $p < .01$ vs. saline + saline control group); whereas, no effect was found at the lowest 0.03 mg/kg dose (Figure 5B).

Effects of the 5-HT$_{2A/1A}$ Receptor Antagonist Spiperone and the 5-HT$_{2A/2C}$ Receptor Antagonists Ritanserin and Ketanserin on the Impairment of PA Induced by 8-OH-DPAT and PCA

Figure 6A shows that spiperone (0.03 mg/kg IP) attenuated the impairment of PA caused by the 0.1 mg/kg

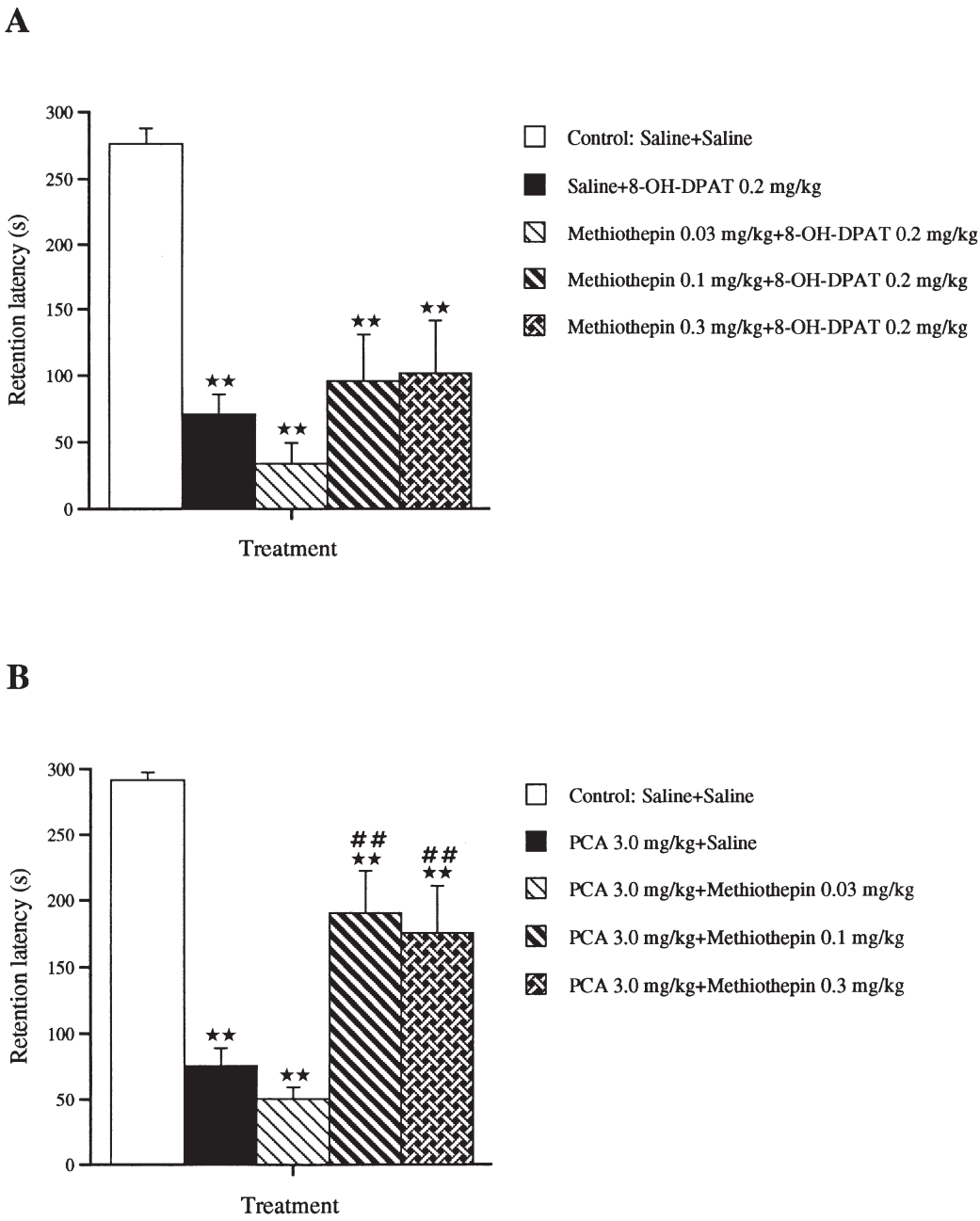

Figure 5. The combined effects of methiothepin and 8-OH-DPAT or PCA on PA retention in the rat. **(A)** Rats were injected with methiothepin (0.03–0.3 mg/kg IP) and 8-OH-DPAT (0.2 mg/kg SC) 30 min and 15 min before the training session, respectively. **(B)** Rats were injected with PCA (3.0 mg/kg IP) and methiothepin (0.03–0.3 mg/kg IP) 60 min and 30 min before the training session, respectively. The saline + saline control groups were run concurrently with (-)-pindolol- and 8-OH-DPAT- or PCA-treated groups. The retention test was performed 24 h later. Vertical bars represent means (±SEM) of retention latencies. ★★$p < .01$ versus corresponding saline + saline control group; ##$p < .01$ versus PCA + saline-treated group, n = 8–24; for details of statistical analysis and general information, see legend to Figure 1 and Methods.

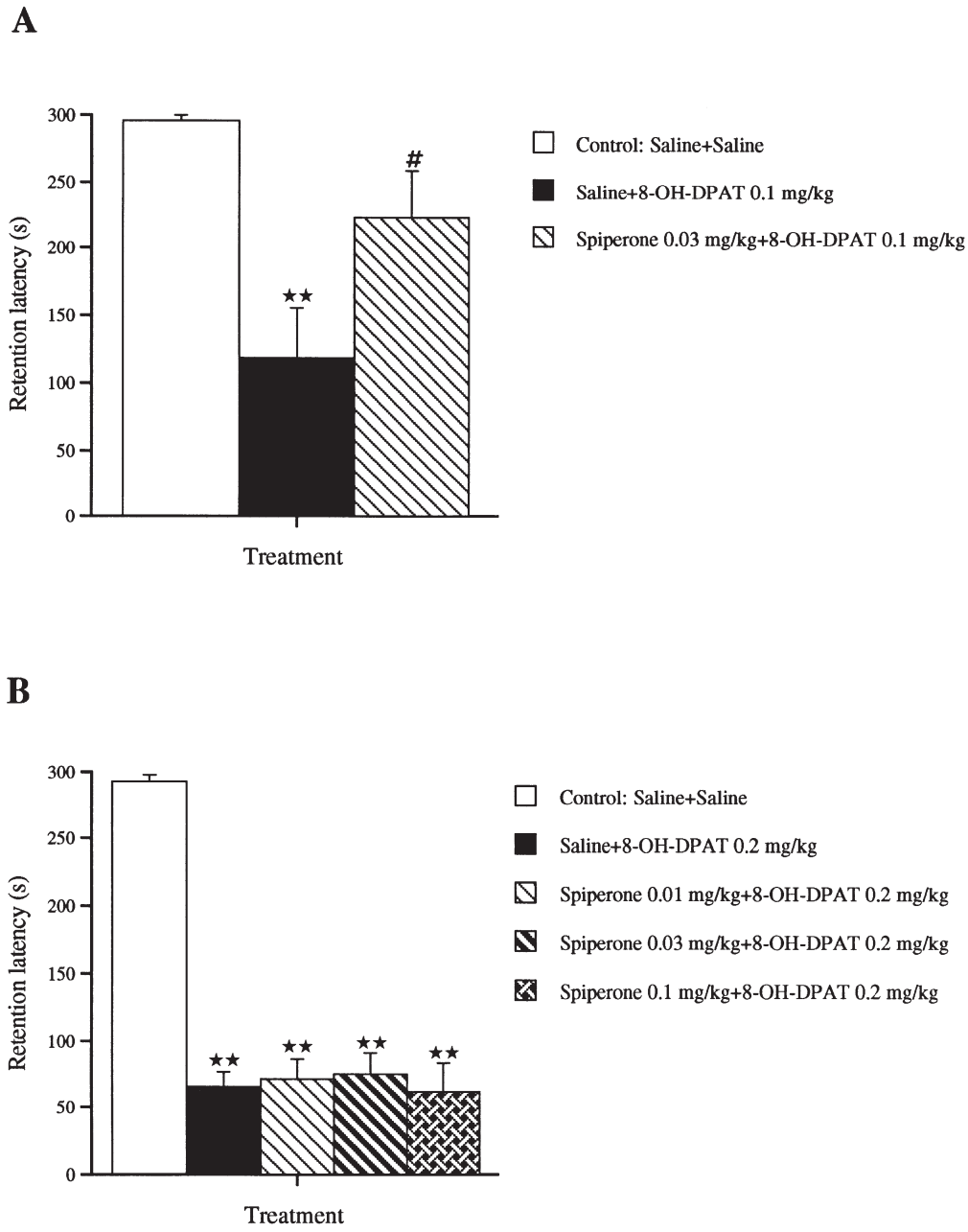

Figure 6. The combined effects of spiperone and 8-OH-DPAT on PA retention in the rat. **(A)** Rats were injected with spiperone (0.03 mg/kg IP) and 8-OH-DPAT (0.1 mg/kg SC) 60 min and 15 min before the training session, respectively. **(B)** Rats were injected with spiperone (0.01–0.1 mg/kg IP) and 8-OH-DPAT (0.2 mg/kg SC) 60 min and 15 min before the training session, respectively. The saline + saline control groups were run concurrently with spiperone- and 8-OH-DPAT-treated groups. The retention test was performed 24 h later. Vertical bars represent means (±SEM) of retention latencies. ★★$p < .01$ versus corresponding saline + saline control group; #$p < .05$ versus corresponding saline + 8-OH-DPAT-treated group, n = 8–24; for details of statistical analysis and general information, see legend to Figure 1 and Methods.

dose of 8-OH-DPAT. However, the antagonism by spiperone seemed to be "competitive," because the drug (0.01–0.1 mg/kg) was not able to attenuate the inhibitory effect of the higher 0.2 mg/kg dose of 8-OH-DPAT (Figure 6B). Unlike spiperone, neither ritanserin (1.0 mg/kg IP) nor ketanserin (1.0 mg/kg IP) modified the impairment of PA retention caused by the lower 0.1 mg/kg dose of 8-OH-DPAT. Spiperone (0.03 mg/kg), ritanserin (1.0 mg/kg), and ketanserin (1.0 mg/kg) did not have any significant effect on the impairment of PA retention caused by PCA (3.0 mg/kg) (data not shown).

Effects of the 5-HT_{2C} Receptor Antagonist Ro 60-0491 on the Impairment of PA Induced by mCPP, 8-OH-DPAT and PCA

These experiments were designed to clarify the possible involvement of 5-HT_{2C} receptors in the inhibition of PA retention by PCA and the possible interplay between the postsynaptic 5-HT_{1A} receptors and 5-HT_{2C} receptors in the mediation of the PA impairment by 8-OH-DPAT. For this reason, the selective 5-HT_{2C} receptor antagonist Ro 60-0491 (3.0 mg/kg IP) was first examined in combination studies with mCPP (5.0 mg/kg SC). ANOVA indicated a highly significant main treatment effect for the retention test ($F_{3,27} = 22.82$, $p < .01$). Ro 60-0491, which by itself at the 3.0 mg/kg dose failed to exert any influence on PA retention, completely blocked the inhibitory effect of mCPP ($p > .22$ vs. vehicle + saline control and $p < .01$ vs. vehicle + mCPP group) (Figure 7A).

Importantly, Ro 60-0491 also attenuated the profound increase in training latencies caused by mCPP administration ($p < .01$ vs. vehicle + saline control and $p < .01$ vs. vehicle + mCPP group) (data not shown). Unlike the combination studies with mCPP, Ro 60-0491 (3.0 mg/kg) failed to antagonize the impairment of PA retention caused by 8-OH-DPAT (0.2 mg/kg) or PCA (3.0 mg/kg) (Figure 7B).

Effects of the Selective 5-HT Reuptake Inhibitor Paroxetine on Impairment of PA Induced by 8-OH-DPAT and PCA

Figure 8 shows that paroxetine (1.0 mg/kg IP), which, by itself, altered neither PA retention (Table 3) nor training latencies (Table 2), displayed differential effects on the impairment of PA retention caused by 8-OH-DPAT and PCA. In contrast to the combination study with 8-OH-DPAT (0.2 mg/kg) in which paroxetine was found to be ineffective (Figure 8A), the selective 5-HT reuptake inhibitor (1.0 mg/kg) completely reversed the disruptive effects of PCA (3.0 mg/kg) on the PA retention ($p > .08$ vs. saline + saline control group and $p < .01$ vs. saline + PCA group) (Figure 8B).

Dose-Related Effects of DAergic Drugs d-Amphetamine, Remoxipride, and Raclopride on PA Training and Retention

Table 4 shows that neither d-amphetamine (0.5–1.5 mg/kg IP) nor remoxipride (1–10 μmol/kg) or raclopride (0.03–0.3 μmol/kg) altered PA retention. Remoxipride, but not raclopride, caused a dose-dependent increase in training latencies ($F_{3,28} = 30.71$; $p < .01$) with a significant effect from the 3 μmol/kg dose ($p < .05$ vs. saline control) (Table 4). No over-all treatment effect on training latencies was found following d-amphetamine ($F_{3,28} = 1.98$; $p = .14$), with the exception of the highest 1.5 mg/kg dose, which caused an increase in training latency ($p < .05$ vs. saline control). No significant differences were found between control and raclopride-treated rats with regard to training latencies (Table 4).

Effects of Remoxipride and Raclopride on Impairment of PA Caused by PCA

These experiments were designed to analyze the possible involvement of DAergic mechanisms (mainly, DA D_2 receptors) in the inhibitory effects of PCA on PA retention. Figure 9A shows that remoxipride (3 μmol/kg) failed to antagonize the impairment of PA retention caused by PCA (3.0 mg/kg). The tendency of remoxipride to increase training latency ($p = .06$ vs. saline + saline control group) was augmented in the PCA + remoxipride treatment group ($p = .01$ vs. saline + saline control and PCA + saline group) (data not shown). Similarly to remoxipride, raclopride (0.1 and 0.3 μmol/kg) did not block the impairment of PA retention caused by PCA (3.0 mg/kg) (Figure 9B).

Behavioral Observations in PA

The behavioral observations in PA apparatus (2-min exploration time) showed that 8-OH-DPAT (0.03–0.3 mg/kg SC) induced a dose-related development of the 5-HT syndrome. The first signs of the 5-HT syndrome (lower lip retraction, flat body posture) were noted already at the 0.1 mg/kg dose. Higher doses resulted in aggravation of the 5-HT syndrome and all the components of the 5-HT syndrome (lower lip retraction, reciprocal forepaw treading, head weaving, flat body posture, and hind limb abduction) were present at the 0.3 mg/kg dose of 8-OH-DPAT. A progressive decrease in rearing (up to complete abolishment) and increase in forward locomotion correlated with the severity of the 5-HT syndrome. The lowest 0.03 mg/kg dose of 8-OH-DPAT failed to produce visually detectable signs of the 5-HT syndrome, and it did not alter rearing. Neither salivation nor tremor was seen in the 8-OH-DPAT-treated animals. Similar to 8-OH-DPAT, PCA also induced the 5-HT syndrome, but symptomology was less specific. Flat body posture, hind limb abduction, and salivation as well as head-weaving and penile erection were the most profound signs in the PCA-treated animals at the 3.0 mg/kg dose accompanied with a marked decrease in rearing and increase in forward locomotion in some animals. Neither lower lip retraction nor tremor was seen in PCA-treated animals.

Unlike 8-OH-DPAT or PCA, mCPP (3.0 and 5.0 mg/kg doses) caused a dose-related decrease in locomotor activity together with an increase in "freezing" behavior and abolishment of rearing. In addition, weakened muscle tone, rigidity, and unilateral flat body posture (lying more on one side of the body), hind limb abduc-

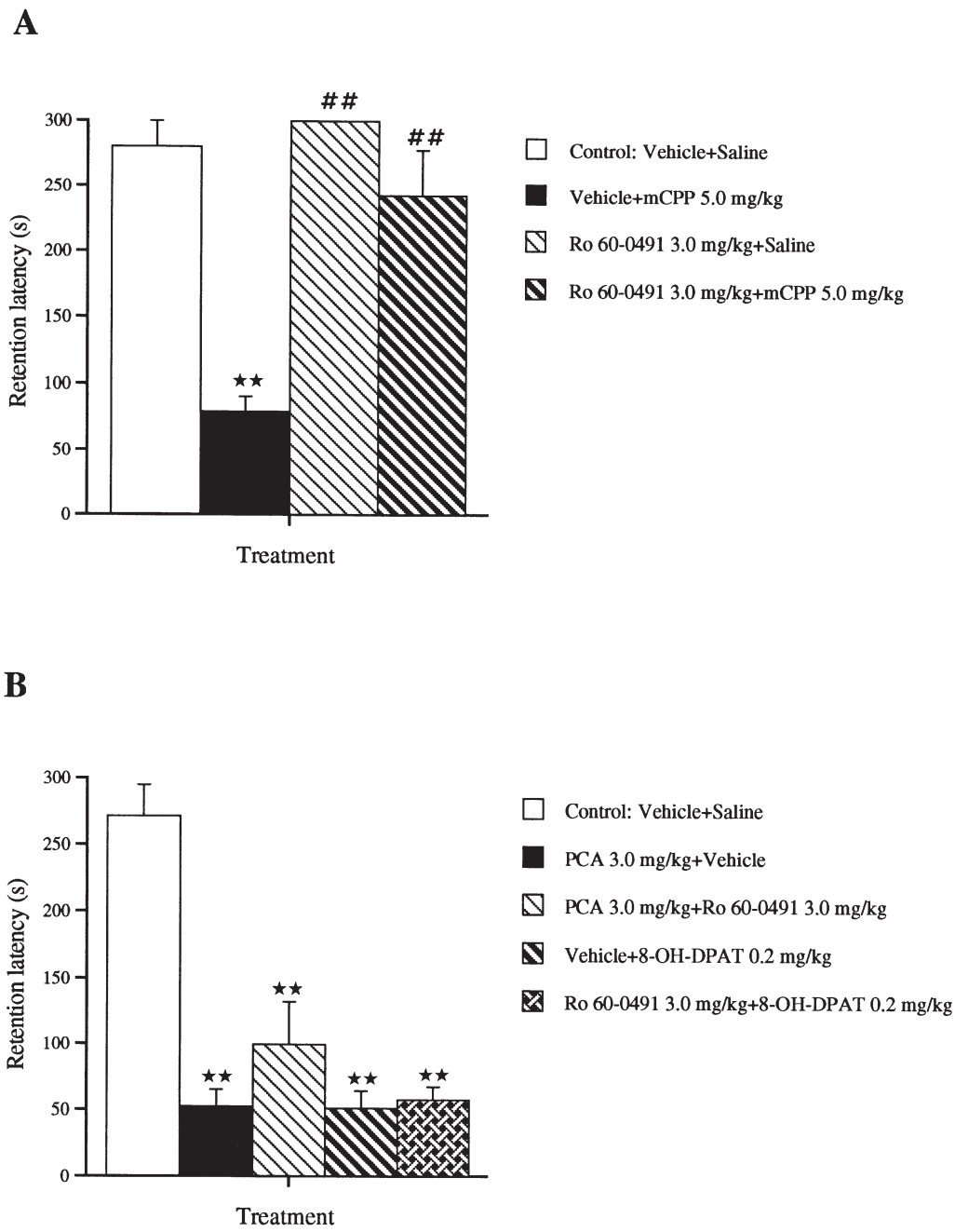

Figure 7. The combined effects of Ro 60-0491 and mCPP or 8-OH-DPAT and PCA on PA retention in the rat. **(A)** Rats were injected with Ro 60-0491 (3.0 mg/kg IP) and mCPP (5.0 mg/kg SC) 40 min and 30 min before the training session, respectively. **(B)** Rats were injected with Ro 60-0491 (3.0 mg/kg IP) and PCA (3.0 mg/kg IP) or 8-OH-DPAT (0.2 mg/kg SC) 40 min, 60 min, and 15 min before the training session, respectively. The vehicle + saline control groups were run concurrently with Ro 60-0491- and mCPP- or 8-OH-DPAT- and PCA-treated groups. The retention test was performed 24 h later. Vertical bars represent means (± SEM) of retention latencies. ★★ $p < .01$ versus vehicle + saline control group; ##$p < .01$ versus vehicle + mCPP-treated group, $n = 7$–8; for details of statistical analysis and general information, see legend to Figure 1 and Methods.

tion, occasional head twitches, as well as nonspecific orofacial movements (tardive dyskinesia) was observed in mCPP-treated animals. Importantly, some of these animals also showed repeated exploration of the entrance into the dark compartment without entering, indicating possible "neophobia."

Both PCA and mCPP caused a dose-related decrease in shock-reactivity; whereas, 8-OH-DPAT rather enhanced it. An increase in tactile response was seen in PCA- and 8-OH-DPAT-treated animals.

In the drug combination studies, WAY 100635 (0.03–1.0 mg/kg SC) abolished all symptoms of the 5-HT syn-

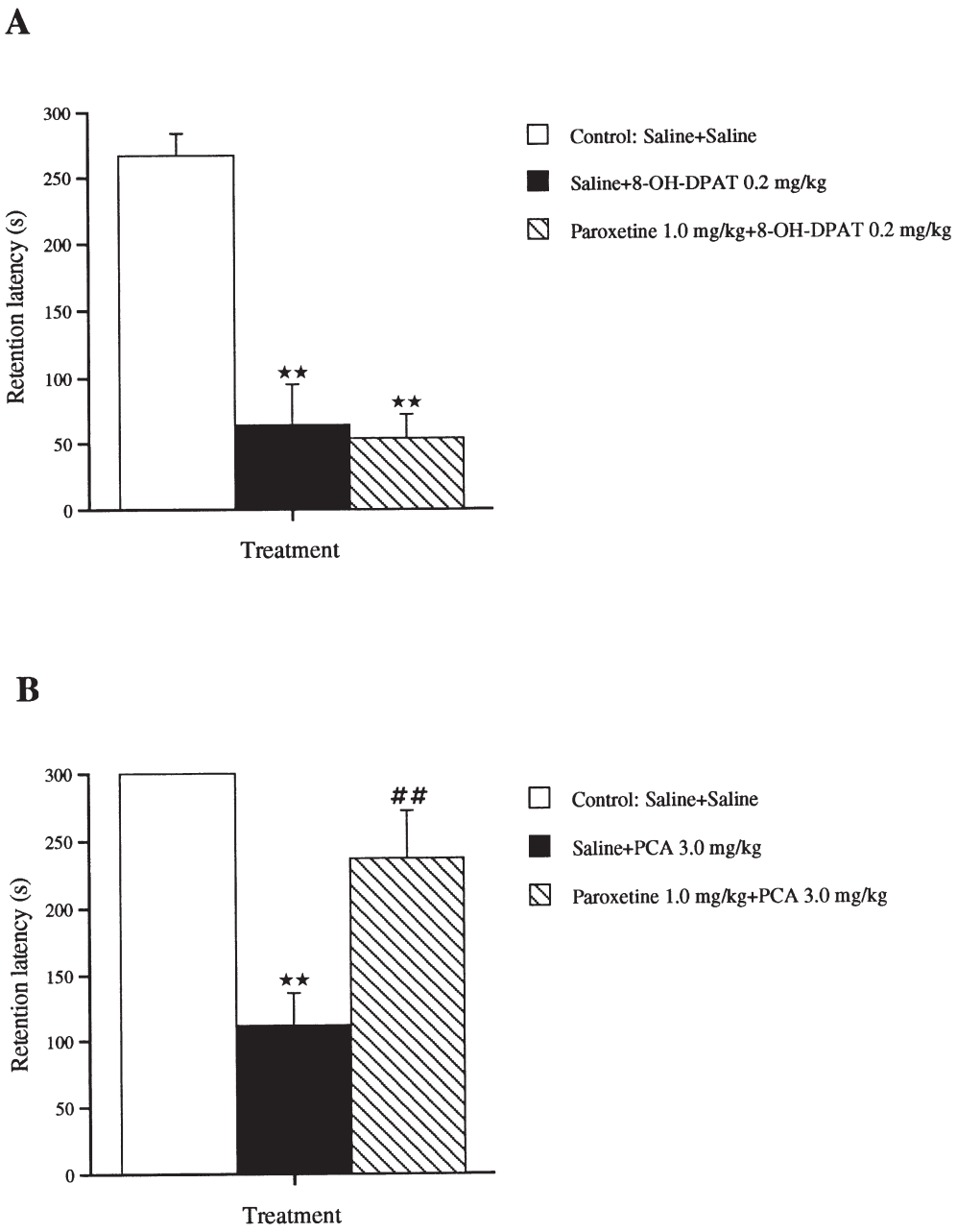

Figure 8. The combined effects of paroxetine and 8-OH-DPAT or PCA on PA retention in the rat. **(A)** Rats were injected with paroxetine (1.0 mg/kg IP) and 8-OH-DPAT (0.2 mg/kg SC) 90 min and 15 min before the training session, respectively. **(B)** Rats were injected with paroxetine (1.0 mg/kg IP) and PCA (3.0 mg/kg IP) 90 min and 60 min before the training session, respectively. The saline + saline control groups were run concurrently with paroxetine- and 8-OH-DPAT- or PCA-treated groups. The retention test was performed 24 h later. Vertical bars represent means (±SEM) of retention latencies. ★★$p < .01$ versus corresponding saline + saline control group; ##$p < .01$ versus saline + PCA-treated group, $n = 8$; for details of statistical analysis and general information, see legend to Figure 1 and Methods.

drome; that is, flat body posture, lower lip retraction, and hind limb abduction induced by the 0.2 mg/kg dose of 8-OH-DPAT. (-)-Pindolol had a dose-related effect on 8-OH-DPAT-induced 5-HT syndrome, with attenuating effect at the 0.3 to 1.0 mg/kg (SC) and full antagonism at the 3.0 mg/kg dose. In addition, WAY 100635 (0.03–1.0 mg/kg) and (-)-pindolol (3.0 mg/kg) normalized rearing in the PA apparatus, which was nearly abolished because of the 8-OH-DPAT-treatment (0.2 mg/kg).

The other compounds tested; that is, methiothepin (0.03–0.3 mg/kg IP), spiperone (0.01–0.1 mg/kg IP), Ro 60-0491 (3.0 mg/kg IP), and paroxetine (1.0 mg/kg IP) attenuated neither the 5-HT syndrome nor the decrease in rearing caused by the 0.2 mg/kg dose of 8-OH-

Table 4. Effects of DAergic Drugs on Passive Avoidance (PA) Training and Retention

Compound (Dose)	Training Latency (s)	Retention Latency (s)
d-Amphetamine (mg/kg)		
0	28.1 ± 8.8	269.6 ± 30.4
0.5	34.5 ± 5.7	300.0 ± 0.0
1.0	61.0 ± 18.2	286.1 ± 12.2
1.5	81.9 ± 28.3*	237.7 ± 41.3
Remoxipride (μmol/kg)§		
0	36.0 ± 7.3	269.6 ± 18.4
1	42.4 ± 8.3	293.1 ± 6.9
3	103.9 ± 38.2*	268.1 ± 30.0
10	283.3 ± 16.7**	271.8 ± 20.0
Raclopride (μmol/kg)§		
0	50.8 ± 13.3	247.6 ± 30.5
0.03	45.1 ± 17.6	226.8 ± 31.9
0.1	31.2 ± 9.4	294.4 ± 5.6
0.3	78.9 ± 16.8	263.5 ± 36.5

The test drugs were administered before PA training at the times and injection routes as follows: d-amphetamine (IP) 30 min, remoxipride (SC) 60 min, raclopride (IP) 20 min. The values shown are mean durations (± SEM). The statistical analysis was performed by one-way ANOVA followed by Fisher's PLSD test (*$p < .05$ and **$p < .01$ versus corresponding control group, $n = 8$).

§Doses of remoxipride (1, 3 and 10) and raclopride (0.03, 0.1 and 0.3) given in μmol/kg correspond to 0.43, 1.29, and 4.3 mg/kg for remoxipride and 0.015, 0.05, and 0.15 mg/kg for raclopride, respectively.

For further details, see Methods.

DPAT. Ritanserin (1.0 mg/kg IP) and ketanserin (1.0 mg/kg IP) did not alter the 5-HT syndrome by the 0.1 mg/kg dose of 8-OH-DPAT; whereas, spiperone (0.03 mg/kg) showed a tendency for reversal.

In the combination studies with PCA (3.0 mg/kg), none of the drugs tested, except for paroxetine, had a normalizing influence on either the 5-HT syndrome or decrease in rearing. It is noteworthy that the highest dose of WAY 100635 (0.3 mg/kg) rather tended to aggravate the PCA-induced 5-HT syndrome (flat body posture, hind limb abduction, and salivation). The complementary signs of the 5-HT syndrome in the WAY 100635 (0.3 mg/kg) + PCA (3.0 mg/kg)-treated animals consisted of occasional head weavings, head twitches, and wet-dog shakes.

Ro 60-0491 (3.0 mg/kg IP) attenuated some of the mCPP-induced behaviors, including a reduction in "freezing" behavior and oral dyskinesias, as well as partial normalization of locomotion, albeit without any detectable effect on suppressed rearing.

The DA agonist d-amphetamine (0.5–1.5 mg/kg IP) induced an increase in locomotor activity, rearing, and sniffing behavior in the PA apparatus. In addition, d-amphetamine-treated animals were more reactive to both tactile stimulation and foot shock. The D_2 antagonist remoxipride (1.0–10 μmol/kg SC) caused a dose-related decrease in exploratory behavior in PA apparatus; whereas, raclopride (0.03–0.3 μmol/kg IP) had no marked effect. Neither remoxipride (3.0 μmol/kg) nor raclopride (0.1–0.3 μmol/kg) attenuated the 5-HT syndrome or the decrease in rearing caused by PCA (3.0 mg/kg).

DISCUSSION

In agreement with earlier reports, increases in serotonergic transmission caused either by pretraining administration of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT (Carli et al. 1992; Jackson et al. 1994; Misane et al. 1998a; Riekkinen 1994) or by the 5-HT releasing compound PCA (Ögren 1985b; Ögren 1986a; Santucci et al. 1996) produced a dose-dependent impairment of PA retention when tested 24 h later.

8-OH-DPAT and 5-HT_{1A} Receptors in PA

The present data extend the evidence for the predominant involvement of the postsynatic 5-HT_{1A} receptors in the deficit of PA retention by 8-OH-DPAT (Misane et al. 1998a). Both, WAY 100635 and (-)-pindolol antagonized the impairment of PA retention and the 5-HT syndrome induced by 8-OH-DPAT. However, WAY 100635 was clearly a more potent postsynaptic 5-HT_{1A} antagonist than (-)-pindolol. The lack of effect by paroxetine shows that inhibitory effects of 8-OH-DPAT in PA are not mediated via modulation of classical 5-HT uptake/transporter sites (Sprouse et al. 1993).

Unlike WAY 100635 and (-)-pindolol, methiothepin failed to block the impairment of PA retention caused by 8-OH-DPAT. This finding may seem paradoxical, because previous results have shown that methiothepin can block parts of the 5-HT syndrome induced by 8-OH-DPAT (0.125 mg/kg) (Tricklebank et al. 1984). However, in our study, methiothepin failed to block the behavioral effect of 8-OH-DPAT (0.2 mg/kg), suggesting that the compound has a limited efficacy as 5-HT_{1A} receptor antagonist in vivo at the doses used (Aulakh et al. 1988; Stenfors et al. 1998).

Spiperone blocked the impairment of PA retention induced by the 0.1 mg/kg dose of 8-OH-DPAT but not the 0.2 mg/kg dose. Therefore, the "surmountable" antagonism by spiperone might reflect its ability to target a limited population(s) of the postsynaptic 5-HT_{1A} receptors. This interpretation is in line with the fact that in vivo spiperone is a more potent antagonist at the 5-HT_{1A} autoreceptors than at the postsynaptic 5-HT_{1A} receptors (Blier et al. 1993; Lum and Piercey 1988; Marrosu et al. 1996; Millan et al. 1993; Tricklebank et al. 1984).

These findings further support the view for the lack of involvement of 5-HT_7 receptors in the action of 8-OH-DPAT. Thus, in addition to ketanserin (Misane et al. 1998a), none of the mixed 5-HT antagonists with affinity to the 5-HT_7 receptor but with substantially

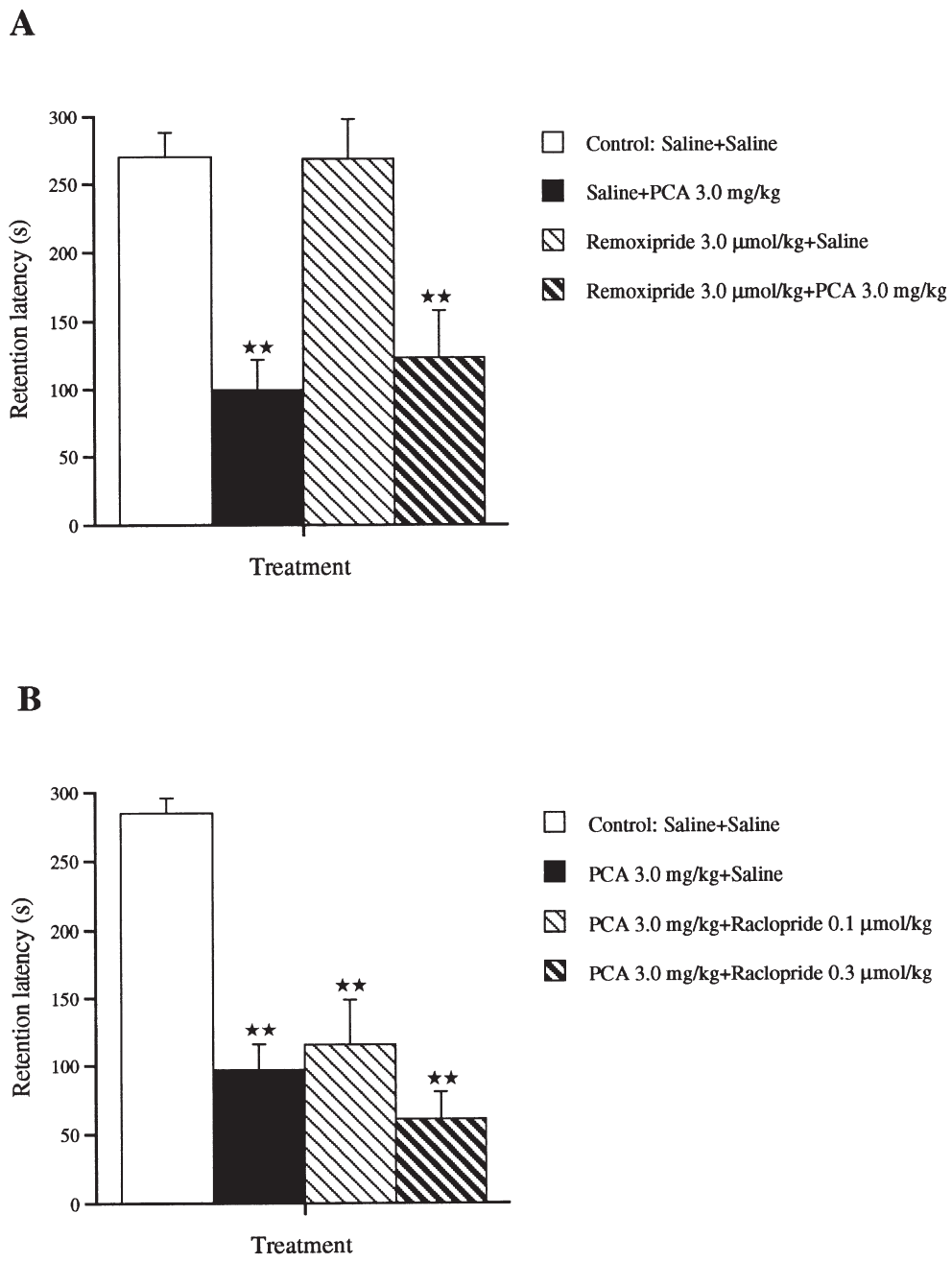

Figure 9. The combined effects of PCA and remoxipride or raclopride on PA retention in the rat. **(A)** Rats were injected with remoxipride (3.0 μmol/kg SC) and PCA (3.0 mg/kg IP) 60 min before the training session. **(B)** Rats were injected with PCA (3.0 mg/kg IP) and raclopride (0.1 and 0.3 μmol/kg IP) 60 min and 20 min before the training session, respectively. The saline + saline control groups were run concurrently with PCA- and remoxipride- or raclopride-treated groups. The retention test was performed 24 h later. Vertical bars represent means (±SEM) of retention latencies. ★★$p < .01$ versus corresponding saline + saline control group, n = 8–16; for details of statistical analysis and general information, see legend to Figure 1, Table 4, and Methods.

lower (methiothepin, spiperone) or no (ritanserin) affinity for the 5-HT_{1A} receptor (Table 1) attenuated the impairment of the PA induced by the 0.2 mg/kg dose of 8-OH-DPAT. However, only studies using the recently developed 5-HT_7 antagonists; for example, SB-258719 (Forbes et al. 1998; Thomas et al. 1998) could resolve this issue.

PCA and Multiple 5-HT Receptors in PA

The present data confirm the proposal (Ögren 1985b) that 5-HT_1, but not 5-HT_{2A} or 5-HT_{2C}, receptors play a major role in the impairment of PA retention caused by PCA. Thus, the antagonists at the 5-HT_{1A} receptors; that is, WAY 100635 and (-)-pindolol, attenuated the inhibi-

tory effects of PCA. It is noteworthy that WAY 100635 showed a biphasic activity, and at the highest dose tested, aggravated both the serotonin syndrome (wet-dog shakes and head twitches) and the deficit of the PA retention by PCA. This may be attributable to the fact that WAY 100635 at higher doses increases 5-HT synthesis (Johansson et al. 1997) and possibly induced 5-HT release, as indicated by our behavioral observations. (-)-Pindolol, however, did not show this "inverse" type of activity, which might be because of its mixed agonist–antagonist properties at the somatodendritic 5-HT_{1A} receptor (Aulakh et al. 1988; Hjorth and Carlsson 1986; Sanchez et al. 1996) probably resulting into a relative decrease in PCA-induced 5-HT release. In contrast, all the compounds with affinities to 5-HT_{2A} or 5-HT_{2C} receptors (Table 1) (spiperone, ritanserin, ketanserin, and Ro 60-0491), with the exception of methiothepin, failed to attenuate the inhibitory effects of PCA.

The present results imply that, in addition to the 5-HT_{1A} receptor, another as-yet unexplored receptor(s), such as the 5-HT_{1B}, 5-HT_4, 5-HT_6, and 5-HT_7 receptors or an even yet-unknown 5-HT binding site(s) could be involved in the inhibitory actions of PCA in PA. This possibility receives support from the combination studies with methiothepin. Methiothepin did not modulate the 8-OH-DPAT-induced impairment of PA retention, although the compound markedly attenuated the inhibitory actions of PCA. The 5-HT_4 receptor is also a possible "candidate," because none of the 5-HT antagonists tested bind to this receptor, which mediates some in vivo effects of PCA, such as acetylcholine release in the rat frontal cortex (Yamaguchi et al. 1997). The 5-HT_4 receptors also seem to have a modulatory role in PA (Meneses and Hong 1997).

The PA retention deficit induced by PCA seems to be largely attributable to postsynaptic events. However, the possible contribution of presynaptic 5-HT receptor mechanisms in the action of PCA requires further analysis. The ability of the "inverse agonist" methiothepin and the selective 5-HT reuptake inhibitors; for example, fluoxetine and paroxetine, to regulate the terminal 5-HT (1B/1D) autoreceptor function should also be considered (Gobert et al. 1997; Moret and Briley 1993; Pauwels 1997). These properties might, at least in part, contribute to the counteracting effects of methiothepin versus PCA, because it was administered after PCA, which means under the release of 5-HT and increased "5-HT tone." Because of pharmacokinetic considerations, paroxetine was administered 90 min before PA training; that is, before PCA. This design might, at least in part, explain "discrepancies" between present and previous results. In a previous study, the nonselective 5-HT antagonists methiothepin and metergoline, when given before PCA, failed to antagonize the impairment of PA retention caused by the 5-HT releasing compound (Ögren 1985b). However, metergoline was also ineffective in blocking the effects of PCA using the design of the present study (to be published), suggesting that the "5-HT tone" at the time of training is important for the action of methiothepin.

5-HT_{2C} Receptors in PA

Although 5-HT_{2C} receptors do not seem to mediate the impairment of PA either by 8-OH-DPAT or PCA, the present study showed that this receptor is of importance in the regulation of PA in view of results with mCPP. The nonselective 5-$HT_{2C/2B/1B}$ receptor agonist mCPP is an important pharmacological tool for characterizing 5-HT_{2C}-mediated responses in vivo, because of its higher in vitro affinity for 5-HT_{2C}/5-HT_{2B} receptors than for 5-HT_{1A} or 5-HT_{1B} receptors (Hoyer et al. 1994; Martin et al. 1998). An increasing body of literature indicates also that most of the diverse in vivo effects of mCPP are attributable to stimulation of 5-HT_{2C} receptors (Curzon and Kennett 1990; Martin et al. 1998; Murphy et al. 1991). Consistent with this notion, its behavioral profile is very similar to that of the selective 5-HT_{2C} agonists Ro 60-0175 and Ro 60-0332 (Martin et al. 1998). In support of this, the impairment of PA retention induced by mCPP was fully blocked by the 5-HT_{2C} antagonist Ro 60-0491, which has clearly higher affinity for the 5-HT_{2C} receptor than for the 5-HT_{2A} receptor (Martin et al. 1998). A possible contribution of 5-HT_{2B} receptors in the actions of both mCPP and Ro 60-0491 remains unclear, because highly selective 5-HT_{2B} antagonists; for example, SB-204741 (Forbes et al. 1995) were not used in the present study.

The increased training latency by mCPP is difficult to interpret. However, behavioral observations indicate that it might, in part, reflect an increase in specific "anxiogenic" or phobic behaviors for example, neophobia as shown both in animal and human studies (Bilkei-Gorzo et al. 1998; Charney et al. 1987; Griebel et al. 1991; Kennett et al. 1989; Whitton and Curzon 1990; Zuardi 1990). If so, blockade of the 5-HT_{2C} receptors by Ro 60-0491 could result into reduction in fear-related behavior ("anxiolytic" effects) and possibly, impairment of PA retention, which was not found in the present study. Therefore, it is obvious that alterations in PA retention cannot be simply interpreted as a result of "anxiolytic" or "anxiogenic" drug actions at the time of training.

Role of Nonspecific Factors in PA

Several additional considerations must be taken into account in the analysis of the present data. First, because drug treatments were made before training, state-dependency at both training and retention tests might

influence subsequent retention in the aversive learning task (Overton 1978). However, state-dependency does not explain the present results, because neither the selective 5-HT_{1A} agonists, including 8-OH-DPAT (Carli et al. 1992; Misane et al. 1998a), nor PCA (Ögren 1985b; Ögren 1986a; Santucci et al. 1996) when administered before both training and retention restored the retention performance to the control levels. In addition, in our previous studies, post-training administration of the 5-HT_{1A} agonists (Misane et al. 1998a) or PCA (Ögren 1985b; Ögren 1986a) did not influence the 24-hour PA retention, showing that there is no long-term carry-over behavioral effect by the drug treatment.

Second, a major problem in studying the role of 5-HT in aversive learning and memory is that changes in 5-HT neurotransmission caused by the selective 5-HT_{1A} agonists (Rigdon and Weatherspoon 1992; Sipes and Geyer 1995) and the 5-HT releasing amphetamines (Davis and Sheard 1976; Geyer 1996; Kehne et al. 1992; Kehne et al. 1996) could alter sensorimotor reactivity at the time of training, which could subsequently influence the PA retention. In addition, 8-OH-DPAT and PCA also induce various behavioral effects; for example, modulate both general locomotor activity (Curzon 1990; Dourish et al. 1985; Evenden and Angeby-Möller 1990; Hutson and Curzon 1989; Ögren and Johansson 1985), nociceptive thresholds (Hamon et al. 1990; Ögren et al. 1985; Ögren and Johansson 1985), and elicit a characteristic behavioral syndrome (5-HT syndrome) (Berendsen et al. 1989; Jacobs 1976; Tricklebank et al. 1984; Trulson and Jacobs 1976). However, the present and previous analysis allow us to conclude that the inhibitory effects of 8-OH-DPAT (Carli et al. 1992; Misane et al. 1998a; Riekkinen 1994) or PCA (Ögren 1985b; Santucci et al. 1996) on PA retention cannot be simply attributed to these "non-learning" factors. Furthermore, the ability of PCA to affect aversive learning was completely dissociated from its motor stimulant and "hypoalgesic" action (Ögren and Johansson 1985). It is clear from the present data that the observed PA deficit caused by the pretraining administration of the "5-HT agonists" occured following doses that engendered behavioral changes; for example, the induction of the 5-HT syndrome or increase/decrease in motor function. Interestingly, the same pattern was seen with d-amphetamine (1.5 mg/kg IP), which increased locomotor activity and shock-reactivity (similar to 8-OH-DPAT), while tending to increase training latencies. However, behavioral observations reflect complex drug effects in the context of the task, and even if there are marked changes in exploratory activity and/or training latencies, these factors neither can serve as a direct measurement of locomotor activity nor seem to be a predictive measure of subsequent 24-hour retention performance. These issues were also elaborated in the studies with mCPP and DA antagonists (see above and below, respectively).

Role of DA in the Inhibitory Actions of PCA in PA

PCA causes an acute release of DA and NA in rat brain in addition to 5-HT. However, its behavioral effects, at least in PA, are primarily mediated by serotonergic mechanisms. In support of previous findings with zimeldine (Ögren 1985b), the pretreatment with the more selective 5-HT reuptake inhibitor paroxetine completely antagonized the PA retention deficit by PCA and also attenuated the PCA-induced serotonin syndrome. It is also notable that d-amphetamine, a potent DA releaser and psychomotor stimulant did not cause an impairment of the PA retention. This is consistent with most studies indicating that d-amphetamine either does not alter or rather enhances PA retention (Banfi et al. 1982; Kovacs and de Wied 1978; Seliger 1975; Seliger 1977). Remoxipride (3–10 μmol/kg) produced a decrease in general motor activity and a profound increase in training latencies without any effect on PA retention; whereas, raclopride affected neither PA training nor retention performance.

Unlike remoxipride and raclopride, spiperone (two times higher affinity for DA D_2 receptors than for the 5-HT_{2A} receptors) (Leysen et al. 1993; Metwally et al. 1998) produced an impairment of PA retention at the 0.1 mg/kg dose, which is at the threshold for induction of catalepsy (Hess et al. 1988). Interestingly, the doses of spiperone (0.03 mg/kg IP), remoxipride (3 μmol/kg SC), and raclopride (0.1–0.3 μmol/kg IP) used in the combination studies with PCA or 8-OH-DPAT have been reported to block DA agonist-induced hyperactivity (Magnusson et al. 1986; Ögren 1996; Ögren and Archer 1994; Ögren et al. 1990; Ögren et al. 1994), thus indicating DA (predominantly, D_2) receptor antagonism in vivo, albeit devoid of apparent catalepsy. However, none of these drugs attenuated the impairment of PA retention or the 5-HT syndrome induced by PCA, indicating no direct involvement of DA in the impairment of PA retention induced by PCA.

Differential Involvement of 5-HT Receptor Subtypes and DA in Aversive Learning

In addition to "purely" pharmacological mechanisms, the differences between mCPP, 8-OH-DPAT, and PCA in PA implicate the possibility for a differential role of 5-HT receptors at the various stages of information processing in PA. Both 8-OH-DPAT- and PCA-treated animals were markedly impaired at the 24-hour retention, which might be attributable to two main factors: inability to encode the information and/or the disruption of information processing from short- to long-term memory. The observation that 8-OH-DPAT-treated animals were impaired already at the 5-min retention suggests that activation of the 5-HT_{1A} receptors might result in disruption of the encoding of aversive experience. It is

also plausible that 5-HT_{1A} receptors might be involved in the processing of the information from short- to long-term memory. Thus, the 0.1 mg/kg dose of 8-OH-DPAT caused a significant deficit in the 24-hour retention, but it did not affect the 5-min retention. Although the animals treated with this "threshold" dose of 8-OH-DPAT displayed both signs of the 5-HT syndrome and a decrease in training latencies, they clearly encoded the aversive experience. Previous studies have found that, unlike 8-OH-DPAT, PCA-treated animals (2.5 mg/kg) displayed very high 5-min retention latencies, indicating good encoding of information (Ögren 1986a); whereas, it was a progressive time-dependent loss of PA retention up to 24 hours. This suggests that PCA-effects are mainly attributable to the disruption of information processing from short- to long-term memory. In view of multiple postsynaptic 5-HT receptor stimulation by PCA, it is possible that there is a 5-HT receptor "opposing" the 5-HT_{1A} receptor in the encoding of aversive experience. In view of the "partial" effect of the 5-HT_{1A} antagonists and methiothepin in the reversal of the 24-hour PA retention deficit induced by PCA, it is likely that another 5-HT receptor(s) is also involved in the information processing from short- to long-term memory.

The present data provide further evidence for the view that the effects of PCA-induced 5-HT release on PA and active avoidance are differently mediated (Ögren 1985b), suggesting that 5-HT receptors play differential roles in aversive learning tasks; 5-HT_{2A} receptors are important in one-way active avoidance; whereas, 5-HT_{1A} receptors are essential in passive avoidance. Both tasks are based on fear conditioning, and the avoidance response is either *an escape* from fear *(one-way active avoidance)* or *an avoidance* of the area in which the animal has been exposed to fear conditioning *(passive avoidance).* In this context, the marked functional separation between 5-HT_{1A} and 5-HT_{2A} receptors probably reflects differences in the neuroanatomical receptor localization, which might reflect divergent neuronal circuitries involved in different aversive learning paradigms. In addition to 5-HT receptors, the differential involvement of DAergic systems in aversive learning tasks should be considered. It is well documented that DA and DA receptors (particularly DA D_2 receptors) play an important role in the performance of the active avoidance (Ögren 1996), which is essential in view of sensorimotor and/or motivational factors that are strongly regulated by DAergic systems. In contrast, DA and DA D_2 and/or DA D_3 receptors seem to have a negligible role in PA task. This distinction is of interest, because, in the case of PCA, the released 5-HT has been shown to have a tonic regulation on striatal DA transmission and to increase DA synthesis via 5-HT_{2A} receptors (Huang and Nichols 1993; Schmidt et al. 1994). However, this mainly concerns locomotor regulation, which seems to be important in active avoidance but not in PA.

CONCLUSION

Multiple 5-HT receptors are involved in PA. However, their roles probably differ at various stages of information processing. Unlike the selective 5-HT_{1A} receptor agonist 8-OH-DPAT, the inhibitory effects of PCA are because of the 5-HT release that results in concomitant multiple receptor activation and extrasynaptic transmission. In addition to the 5-HT_{1A} receptor, another as-yet unexplored 5-HT binding site seems to be involved in the actions of PCA. The present findings provide evidence for the view that there probably exists a functional distinction between 5-HT receptor subtypes in different types of aversive learning, which might be of relevance for human psychopathologies.

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