***S*(–)Propranolol as a discriminative stimulus and its comparison to the stimulus effects of cocaine in rats**

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Abstract

Rationale Racemic propranolol (PRO), a β -adrenoceptor antagonist, has been evaluated as a test agent but not as a discriminative stimulus. Its *S*(–) stereoisomer is thought to subserve the effects of ($\pm$)PRO.

Materials and methods Rats were trained to discriminate *S*(–)PRO (5 mg/kg) from saline in a two-lever food-reinforced operant conditioning task.

Results The *S*(–)PRO stimulus was shown to be centrally mediated, dose-related, time dependent, and stereoselective: *S*(–)PRO (ED_{50} =2.2 mg/kg) was twice as potent as ($\pm$)PRO and approximately four times as potent as *R*(+)PRO. The *S*(–)PRO stimulus generalized fully to the β -adrenoceptor agent pindolol, the α_1 -adrenoceptor agonist methoxamine, cocaine, and the serotonergic agents TFMPP and RU 24969; partial generalization occurred to (–)ephedrine and nisoxetine but not to fenfluramine or 5-OMe DMT. The *S*(–)PRO stimulus was blocked completely (and competitively) when prazosin, an α_1 -adrenoceptor antagonist, was given in combination with the training dose of *S*(–)PRO. Moreover, prazosin exerted antagonism of the *S*(–)PRO-like effect of ($\pm$)PRO or *R*(+)PRO but produced only partial antagonism of the *S*(–)PRO-like effect of cocaine. In a second study, rats were trained to discriminate 8 mg/kg of cocaine from saline. The cocaine stimulus generalized to *S*(–)PRO, ($\pm$)PRO, and *R*(+)PRO. Prazosin partially

attenuated the stimulus effect of cocaine (8 mg/kg) but completely blocked the cocaine-like effects of ($\pm$), *S*(–), and *R*(+)PRO.

Conclusions PRO and cocaine exhibited cross-substitution, but their stimulus effects were antagonized differentially by prazosin. PRO (and its optical isomers) can exert a stimulus effect that is based, at least in part, on increased α_1 -adrenoceptor activity. PRO might be better characterized as an adrenoceptor partial agonist.

Keywords Adrenergic · Serotonin · Cocaine · Drug abuse · Stereoisomers · Agonist · Antagonist · Behavior · Neurotransmitter release · Drug discrimination · Noradrenergic · Psychostimulant · Serotonin receptor · Rat

Introduction

Propranolol (PRO) is a nonselective β_1/β_2 -adrenoceptor antagonist that is used therapeutically for the treatment of certain types of cardiovascular conditions (arrhythmias, angina pectoris, and hypertension) and anxiety (e.g., Simpson 1974; Fitzgerald 1980). In some patients, PRO also can produce CNS side effects such as insomnia and depression; such actions are thought to be associated with certain β -adrenoceptor blockers (e.g., propranolol) that are more lipophilic (i.e., more likely to penetrate the blood–brain barrier) than other β -adrenoceptor blockers that are more hydrophilic (e.g., atenolol; Agon et al. 1991; Frishman et al. 1981; Middlemiss et al. 1981; Neil-Dwyer et al. 1981; Woods and Robinson 1981; Petrie et al. 1982). In radioligand binding studies, PRO displays high affinity for β_1 - and β_2 -adrenoceptors (e.g., Fraundorfer et al. 1994; Schotte et al. 1996). It also displays moderate affinity for serotonin 5-HT_{1A} and 5-HT_{1B} receptors but binds with

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much lower, or no, affinity at 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, α_2 -adrenoceptors, histamine H₁, and dopamine D₁ and D₂ receptors (e.g., Kanba and Richelson 1984; Engel et al. 1986; Boyajian and Leslie 1987; Schlicker et al. 1989; Hamon et al. 1990; Toll et al. 1998). Furthermore, the chemical structure of PRO contains an asymmetric center and, thus, PRO exists as a pair of optical isomers: *S*(–) propranolol (*S*(–)PRO) and *R*(+)propranolol (*R*(+)PRO). The enantiomers bind at β_1/β_2 -adrenoceptors, 5-HT_{1A}, and 5-HT_{1B} receptors in a stereoselective manner with relatively large differences in affinity: *S*(–)PRO is ~100 times more potent than *R*(+)PRO at β -adrenoceptors or 5-HT_{1B} receptors and ~30-fold more potent at 5-HT_{1A} receptors (e.g., Middlemiss 1984; Hoyer et al. 1985a, b; Engel et al. 1986; Pierson et al. 1989; Tsuchihashi et al. 1990).

In addition to its β -adrenoceptor antagonist effects, PRO has been proposed to function in some instances as a central 5-HT receptor antagonist, releaser of norepinephrine (NE), and/or inhibitor of NE reuptake. For example, PRO can antagonize the (a) hypothermia induced by the purported 5-HT_{1A/7} agent 8-hydroxy-2-(di-*n*-propylamino)tertralin (8-OH DPAT) in rats (but not in mice; e.g., Goodwin and Green 1985; To et al. 1995; Toll et al. 1998), (b) suppressant effect of 8-OH DPAT applied microiontophoretically to 5-HT dorsal raphe neurons (Sprouse and Aghajanian 1986), and (c) reciprocal forepaw treading (“serotonin syndrome”) in rats induced by 8-OH DPAT or the 5-HT_{1A/2A/2C/6/7} agent 5-methoxy-*N,N*-dimethyltryptamine (5-OMe DMT; e.g., Tricklebank 1985; Tricklebank et al. 1985; Hoyer et al. 1989; Sadzot et al. 1989; To et al. 1995; Glennon et al. 2000). PRO also may interact with adrenoceptors in ways other than, or in addition to, antagonism of β -adrenoceptors. For instance, PRO can raise central α -adrenoceptor (primarily α_1 -adrenoceptor) activity via the release of NE and/or an inhibition of NE reuptake (e.g., Kelliher and Buckley 1970; Day and Roach 1974; Davies and Reid 1975; Kobinger 1978; Privitera et al. 1979; Tackett et al. 1981; Tuross and Patrick 1986). Interestingly, however, *R*(+)PRO is nearly as potent or slightly less potent (i.e., 1.5- to ~3-fold) than *S*(–)PRO with respect to those indirect agonist actions on adrenoceptors (Daniell et al. 1976; Saelens et al. 1977; Tackett et al. 1981; Tuross and Patrick 1986).

PRO also has been widely employed as a test agent in drug discrimination (DD) studies, a useful procedure to examine the central effect(s) of drugs (e.g., Young and Glennon 1986). For example, PRO [antagonism dose 50% (AD₅₀)=0.005 mg/kg] can block the discriminative stimulus effect of clenbuterol (0.1 mg/kg), a selective β_2 -adrenoceptor agonist, in rats (O'Donnell 1997; Ordway et al. 1987). In contrast, the stimulus effect of agents such as ethanol, *S*(+)amphetamine, *S*(+)methamphetamine, Δ^9 -THC, morphine, or yohimbine is not blocked by propranolol, and, thus,

β -adrenoceptor involvement in their stimulus action has been discounted (e.g., Schechter 1974; Schechter and Cook 1975; Winter 1975; Colpaert et al. 1977; Jarbe and Ohlin 1977; Munzar and Goldberg 1999). PRO also has been evaluated in DD studies that used training agents that might be mediated primarily via serotonergic receptors. In rats trained to distinguish 8-OH DPAT from vehicle, for example, the stimulus was not antagonized by (and did not generalize to) propranolol (Glennon 1986; Cunningham et al. 1987), a finding that questions the robustness of PRO as a 5-HT (5-HT_{1A}?) receptor antagonist. In comparison, investigators have examined the effects of PRO in animals trained to discriminate the nonselective serotonergic (i.e., moderate to high affinity for 5-HT_{1A/1B/1D/2A/2B} and/or 2C receptors) agents TFMPP [1-(3-trifluoromethylphenyl)piperazine] or RU 24969 [5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridyl)-1H-indole] from saline vehicle (e.g., Peroutka 1986; Schlegel and Peroutka 1986; Boess and Martin 1994; Wainscott et al. 1996; Herrick-Davis et al. 1998). In each instance, stimulus generalization (but not stimulus antagonism) occurred to PRO, and the results suggested that propranolol might behave, at least in part, as an agonist at a 5-HT (5-HT_{1B}?) receptor(s) (Glennon et al. 1988; Gardner 1989). Lastly, PRO does not block the stimulus effect of cocaine but has been reported to enhance, generalize partially, or substitute completely in cocaine-trained animals, suggesting an adrenoceptor component of action in the stimulus effect of cocaine (Ho and McKenna 1978; Colpaert et al. 1979; Kleven and Koek 1997). The latter results also are consistent with findings that PRO dose-dependently enhances other behavioral and neurochemical effects mediated by cocaine (e.g., Goldberg and Gonzalez 1976; Harris et al. 1996).

Taken together, the drug discrimination data above appear to indicate that PRO exerts effects that involve multiple mechanisms: a (primary) β -adrenoceptor antagonist component, enhancement of adrenoceptor activity, and/or an agonist effect at a 5-HT receptor(s). To date, however, propranolol (itself) has not been employed as a training drug. Thus, the major goal of the present study was to determine whether *S*(–)PRO, the more potent and (presumed) pharmacologically salient enantiomer, could serve as a discriminative stimulus in rats and to evaluate the effects of various agents that alter monoamine neurotransmission. The drugs chosen for tests of stimulus generalization and/or antagonism included the racemic mixture and *R*(+)PRO isomer of propranolol, cocaine (nonselective increase in monoamine activities through reuptake inhibition), several standard adrenoceptor agents (methoxamine, pindolol, atenolol, nisoxetine, prazosin, and (–)ephedrine), and several “standard 5-HT agents” (fenfluramine, 5-OMe DMT, TFMPP, and RU 24969). It also was of interest to explore more fully the observation that animals trained to discriminate cocaine would generalize to propranolol.

Therefore, a second group of rats was trained to discriminate cocaine from saline vehicle, and tests of stimulus generalization were performed to examine the effects of racemic propranolol and its stereoisomers.

Materials and methods

Subjects The animals were 14 male Sprague–Dawley rats (Charles River Laboratories) that weighed 280–320 g (i.e., 80% food-restricted weights) at the beginning of the study. The animals were divided into two groups and trained to discriminate either *S*(–)PRO (5.0 mg/kg; $n=8$) or cocaine (8.0 mg/kg; $n=6$) from saline vehicle. The rats were housed individually, had unlimited access to drinking water, and were maintained at 80% of their free-feeding body weights by partial food restriction. The protocol to conduct these animal studies was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

Discrimination training Operant conditioning chambers (Coulbourn Instruments, Whitehall, PA, USA, Rat Model E10-10), housed within sound- and light-attenuated outer chambers, were used. Each chamber contained two (response) levers that were mounted at opposite ends of one wall and were separated by a liquid dipper (Model H14-05RE). When activated, the dipper dispensed 0.01 ml of sweetened condensed milk (diluted 2:1 with tap water) for ~2.5 s as reinforcement; furthermore, a magazine mini-bulb (40 mA at 28 V) was lighted for ~2.5 s. Rats were trained to lever respond to a variable interval 15-s schedule of reinforcement on both levers. A session (conducted 5 days/week) was started by the activation of the house-light (28 V) in each chamber, and session duration was 15 min. A pre-session injection interval (PSII) of 15 min was employed. The rats were injected intraperitoneally (i.p.) with either drug (i.e., 5.0 mg/kg of *S*(–)PRO or 8.0 mg/kg of cocaine) or saline on a random schedule, with the constraint that no more than two consecutive sessions with the training drug or vehicle could occur. For half of the rats in each group, responses on the right-side lever were reinforced when drug was given, and responses on the left-side lever were reinforced after saline administration; these conditions were reversed for the remaining rats in each group. On every fifth day of training, discrimination learning was assessed during an initial 2.5-min non-reinforced (extinction) period followed by a 12.5-min training session. Data collected during the extinction periods included percent appropriate responding on the drug (*S*(–)PRO or cocaine) lever (number of responses on *S*(–)PRO- or cocaine-designated lever/total number of responses) and total responses (mean responses/minute).

Individual animals were not used in the stimulus generalization or antagonism studies until they met a stringent criteria of $\geq 80\%$ of their responses on the drug-appropriate lever after administration of their assigned training drug and $\leq 20\%$ of their responses on the same drug-appropriate lever after administration of saline for at least three consecutive weeks.

Generalization and Antagonism tests During these phases of the study, maintenance of the training drug/saline discriminations was insured by continuation of training sessions on a daily basis (except on a generalization or antagonism test day; see below). On one of the 2 days before a test, approximately half of the animals in each group would receive their training dose of the training drug, and the other half of the rats in each group would receive saline; after a 2.5-min extinction session, training was continued for 12.5 min. Animals not meeting the original criteria (i.e., $\geq 80\%$ of total responses on the drug-appropriate lever after administration of training drug and $\leq 20\%$ of total responses on the drug lever after administration of saline) during the extinction session were excluded from the next generalization or antagonism test session. During investigations of stimulus generalization or antagonism, test sessions were interposed among training sessions. During these tests, the rats were allowed 2.5 min of non-reinforced lever responding and were then removed from the operant conditioning chambers. An odd number of training sessions (usually three or five) separated two test sessions.

***S*(–)Propranolol Stimulus: Generalization studies** The first generalization tests investigated the time-course and dose-response effects of the *S*(–)PRO stimulus. The time-course study investigated the effects of decreasing or increasing the PSII between the injection of the *S*(–)PRO training dose and the beginning of a test session. In addition to the standard 15-min delay, the effects of PSII of 5, 60, 90, 120, and 150 min were studied. The dose-response study assessed the *S*(–)PRO-appropriate responding of the rats following the administration of various doses of *S*(–)PRO (1.25, 2.5, and 5.0 mg/kg). Doses of *S*(–)PRO were administered in a random sequence with the standard 15-min PSII before behavioral testing. Stimulus generalization tests in the *S*(–)PRO-trained rats evaluated the following agents: in order, ($\pm$)PRO (2.5, 5.0, 7.5, and 10 mg/kg), *R*(+)PRO (5.0, 10, 15, and 20 mg/kg), cocaine (1.5, 2.5, 3.0, 6.0, 7.0, and 8.0 mg/kg), TFMPP (0.3, 0.5, and 0.75 mg/kg), RU 24969 (0.25, 0.35, and 0.50), 5-OMe DMT (0.5, 0.75, and 1.0 mg/kg), fenfluramine (1.0, 1.5, 2.0, and 3.0 mg/kg), (–)ephedrine (0.3, 1.0, 2.0, 3.0, 3.5, and 4.0 mg/kg), methoxamine (0.3, 0.6, 1.0, 2.0, and 2.5 mg/kg), nisoxetine (5.0, 7.5, 8.5, 9.0, and 10 mg/kg)

and pindolol (5.0, 10, 15, and 20 mg/kg). The substitution tests assessed the percent *S*(-)PRO-like response produced by the administration of the agents. Doses of the compounds were administered in a random sequence, with a 15-min PSII prior to the 2.5-min extinction test period.

***S*(-)Propranolol Stimulus: Antagonism studies** In the first antagonism tests, rats were administered the α_1 -adrenoceptor antagonist prazosin (0.1, 0.3, 1.0, 2.0, 3.0, and/or 4.0 mg/kg) 30 min prior to an injection of the training dose of *S*(-)PRO (5.0 mg/kg), 10 mg/kg of ($\pm$)PRO, 20 mg/kg of *R*(+)PRO, or 8.0 mg/kg of cocaine; each dose of the latter three agents produced a *S*(-)PRO-like response that met criteria for complete stimulus substitution. A subsequent 15 min elapsed before the animals were exposed to the extinction test session as described above. An initial dose of 0.1 mg/kg of prazosin was used. A second antagonism test evaluated the effect of the β_1 -adrenoceptor antagonist atenolol (3.0, 10, and 20 mg/kg) on the *S*(-)PRO stimulus. Doses of atenolol were administered 30 min prior to an injection of the 5 mg/kg training dose of *S*(-)PRO. An initial dose of 3.0 mg/kg of atenolol was used. The degree of antagonism produced by initial doses of each agent was used to determine subsequent doses of the antagonists. Moreover, control tests of prazosin (0.1, 0.3, 1.0, 2.0, and 3.0 mg/kg) and atenolol (1.0, 3.0, 10, 15, 20, and 30 mg/kg), given alone, were conducted to evaluate their potential to produce a *S*(-)PRO-like response; a 45-min PSII was used to evaluate each agent. In a third set of antagonism tests, dose-response functions of *S*(-)PRO were determined in the presence of 1.0 mg/kg or 2.0 mg/kg of prazosin. In these tests, prazosin was administered 30 min before an injection of *S*(-)PRO, which was followed by an elapsed time of 15 min. Eight rats were used in each antagonism test (including control tests of antagonists).

Cocaine Stimulus: Generalization studies Stimulus generalization tests assessed the rats' percentage of cocaine-appropriate responding following administration of various doses of cocaine. Doses of cocaine were administered in a random sequence with the standard 15-min PSII before behavioral testing. Further stimulus generalization tests in these rats evaluated the following agents: in order, *S*(-)PRO (2.5, 5.0, 10, 12.5, 15, and 20 mg/kg), ($\pm$)PRO (12.5, 15, and 20 mg/kg), and *R*(+)PRO (10, 15, 20, and 25 mg/kg). Substitution tests with these agents assessed the percent cocaine-like response produced by the administration of the agents. Doses of the compounds were administered in a random sequence with a 15-min PSII prior to 2.5-min extinction test periods.

Cocaine Stimulus: Antagonism studies In antagonism tests, rats were administered the α_1 -adrenergic receptor antagonist prazosin (0.3, 1.0, 3.0, 4.0, and/or 4.5 mg/kg) 30 min prior

to an injection of the training dose of cocaine (8.0 mg/kg), 15 mg/kg of *S*(-)PRO, 20 mg/kg of ($\pm$)PRO, or 25 mg/kg of *R*(+)PRO; each dose of the latter three agents produced a cocaine-like response that met criteria for complete stimulus substitution. A subsequent 15-min PSII elapsed before the animals were exposed to the extinction test session as described above. An initial dose of 0.3 mg/kg of prazosin was used. In addition, control tests of prazosin (0.3, 0.6, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/kg), given alone, were conducted to evaluate its potential to produce a cocaine-like response; a 45-min PSII was used to evaluate the antagonist. Six rats were used in each antagonism test (including control tests of prazosin).

Data analysis A determination of complete, partial, or no generalization (or antagonism) was based on previously described criteria (e.g., Khorana et al. 2008; Young and Glennon 1986). In this scheme, the results of stimulus generalization (or antagonism) tests are characterized as one of three possible results: (a) *complete stimulus generalization* resulted when the animals, following a given dose of drug or drug combination, made $\geq 80\%$ (i.e., group mean) of their responses on the drug-appropriate lever; (b) *no generalization* (i.e., saline-like responding) occurred when the test agent produced 0–20% drug-appropriate responding; and (c) *partial generalization* occurred when a test drug produced an intermediate level of responding (i.e., 21% to 79% but usually between 40% and 70%) on the drug-appropriate lever. Animals that made fewer than five total responses during the 2.5-min extinction session were characterized as being disrupted because they failed to meet the testing criteria. Thus, percent drug-appropriate responding and response rate data refer only to animals making ≥ 5 responses during the extinction session (Young and Glennon 1986). Data for a particular drug dose were not plotted; however, if $>50\%$ of the animals were behaviorally disrupted following administration of that dose. Where complete stimulus generalization (or antagonism) occurred, potency comparisons were made between the training drug and the test agent via calculation of the effective dose 50% (ED_{50}) or antagonism dose 50% (AD_{50}) as determined from the dose-response data by the method of Finney (1952). The ED_{50} or AD_{50} value represents the calculated drug dose where animals would be expected to make 50% of their responses on the drug-appropriate lever. The response rate data (i.e., responses/min) of each training group were evaluated by repeated measures ANOVA and followed, when appropriate, by Dunnett's *t* test (statistical significance set at $p < 0.05$) for comparison of a control group (i.e., mean response rate after saline) versus doses(s) of training drug, experimental dose groups, or dose combinations. In the *S*(-)PRO versus saline group, statistical evaluation of response rate data was found to be significant ($F(22, 720) =$

8.76, $p < 0.01$), whereas in the cocaine versus saline group, it was not ($F(9, 249) = 0.85$, $p > 0.05$).

Drugs Cocaine HCl, *S*(-)-propranolol HCl, ($\pm$)-propranolol HCl, *R*(+)-propranolol HCl, methoxamine HCl, prazosin HCl, (-)-ephedrine HCl, atenolol, and pindolol were purchased from Sigma Chemical Company (St. Louis, MO, USA). Fenfluramine HCl, nisoxetine HCl, and 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridyl)-1*H*-indole (RU 24969) were gifts from A.H. Robins Company (Richmond, VA, USA), Eli Lilly and Company (Indianapolis, IN, USA), and Roussel-Uclaf (Romainville, France), respectively. 5-Methoxy-*N,N*-dimethyltryptamine hydrogen oxalate (5-OMe DMT) was synthesized in our laboratory, and TFMPP (1-(3-trifluoromethylphenyl)piperazine HCl) was obtained from Research Biochemicals (Natick, MA, USA). Pindolol and atenolol (free bases) were first dissolved in one equivalent of 0.01 N hydrochloric acid before dilution with saline. The other drugs were dissolved in 0.9% sterile saline, and doses of each compound were based on the weight of the stated salt or base. Drugs were administered i.p. in a 1-ml/kg injection volume, except for doses ≥ 10 mg/kg of ($\pm$), *S*(-), or *R*(+) propranolol, which were administered in a 2-ml/kg injection volume.

Results

Figure 1 depicts the rats' learning of the *S*(-)-PRO (5.0 mg/kg) versus saline vehicle discrimination; approximately 55 training sessions [range = 30 to 40 sessions to (initially) meet criteria] were required to reach criteria. Once

Fig. 1 Rats acquisition of discriminative control of responding by 5.0 mg/kg of *S*(-)-propranolol (*S*(-)-PRO) versus saline vehicle (1.0 ml/kg of 0.9% saline). Ordinate Mean ($\pm$ SEM; $n=8$) percentage of responses made on the *S*(-)-PRO-appropriate lever after the i.p. administration of *S*(-)-PRO (solid squares) or saline (open squares). Abscissa Test sessions plotted on a linear scale

achieved, five additional training sessions were conducted to ensure stable performance. Thus, after 60 training sessions, the rats' discrimination performance was stable (i.e., *S*(-)-PRO, $\sim 90\%$; saline, $< 10\%$); response rates were similar under the treatment conditions.

Rats' learning of cocaine (8.0 mg/kg) versus saline discrimination has been described previously (Khorana et al. 2004; Young and Glennon 1997).

The time course of stimulus effect produced by 5.0 mg/kg of *S*(-)-PRO is shown in Fig. 2. When the PSII was shortened to 5 min, the rats responded 40% on the *S*(-)-PRO-designated lever. After a PSII of 60 min, *S*(-)-PRO-appropriate responding (91%) was similar to that observed following the standard PSII of 15 min. PSIs subsequent to 60 min resulted in decreased percentages of total responses on the *S*(-)-PRO-appropriate lever; with PSIs of 120 and 150 min, drug-designated lever responding was lowered to 39% and 11%, respectively. The rats' response rates were not statistically different (Dunnett's t ; $p > 0.05$) from saline treatment(s) in the time-course study of *S*(-)-PRO (Fig. 2, lower graph).

Fig. 2 Time course of the *S*(-)-PRO (5.0 mg/kg) discriminative stimulus in rats ($n=8$) trained to discriminate 5.0 mg/kg of *S*(-)-PRO from saline vehicle. Ordinate Mean ($\pm$ SEM) percentage of responses made on the *S*(-)-PRO-appropriate lever after the i.p. administration of 5.0 mg/kg of *S*(-)-PRO followed by various pre-session injection intervals (top panel). The animals' response rates are shown in the lower panel. Abscissa Pre-session injection interval (minutes) plotted on a linear scale

S(-)Propranolol Stimulus: generalization studies As shown in Fig. 3, the *S(-)PRO* ($ED_{50}=2.2$ mg/kg; 95% CL=1.3–3.6 mg/kg) stimulus generalized to ($\pm$)*PRO* ($ED_{50}=4.3$ mg/kg; 95% CL=3.0–6.4 mg/kg) and *R(+)* *PRO* ($ED_{50}=8.4$ mg/kg; 95% CL=5.8–12.2 mg/kg). A comparison of ED_{50} values revealed that *S(-)PRO* is approximately twice as potent as the racemic mixture and approximately four times more potent than *R(+)**PRO*. Figure 4 shows that the *S(-)PRO* stimulus generalized to the following agents: TFMPP ($ED_{50}=0.5$ mg/kg; 95% CL=0.32–0.74 mg/kg), RU 24969 ($ED_{50}=0.35$ mg/kg; 95% CL=0.28–0.43 mg/kg), methoxamine ($ED_{50}=1.2$ mg/kg; 95% CL=0.65–2.1 mg/kg), pindolol ($ED_{50}=9.0$ mg/kg; 95% CL=5.6–14.4 mg/kg), and cocaine ($ED_{50}=2.3$ mg/kg; 95% CL=1.3–3.8 mg/kg); eight out of eight rats responded/tested at each dose except six out of eight at 2.0 or 2.5 mg/kg of methoxamine. Figures 3 and 4 also indicate that the animals' response rates were not appreciably altered, in comparison to that after administration of saline, following injections of *S(-)PRO*,

Fig. 3 *S(-)PRO* stimulus generalization to ($\pm$), *S(-)*, and *R(+)**PRO* in rats ($n=8$) trained to discriminate 5.0 mg/kg of *S(-)PRO* from saline vehicle. Ordinate Mean ($\pm$ SEM) percentage of responses made on the *S(-)PRO*-appropriate lever after the i.p. administration of various doses of each drug (top figure). The animals' response rates are shown in the lower figure. D Responses following 5.0 mg/kg of *S(-)PRO* and S responses following administration of saline vehicle. Abscissa Drug doses plotted on a logarithmic scale

Fig. 4 *S(-)PRO* stimulus generalization to cocaine, methoxamine, pindolol, TFMPP, and RU 24969 in rats ($n=8$) trained to discriminate 5.0 mg/kg of *S(-)propranolol* from saline vehicle. Ordinate Mean ($\pm$ SEM) percentage of responses made on the *S(-)PRO*-appropriate lever after the i.p. administration of various doses of each drug (top figure). The animals' response rates are shown in the lower figure. D Responses following 5.0 mg/kg of *S(-)PRO* and S responses following administration of saline vehicle. Response rate was significantly ($*p<0.05$) different from the saline vehicle control response rate. Abscissa Drug doses plotted on a logarithmic scale

($\pm$)*PRO*, *R(+)**PRO*, pindolol, TFMPP, or RU 24969, but were significantly reduced (by 50 to 75%) after administration of methoxamine (2.5 mg/kg) or cocaine (8.0 mg/kg).

Figure 5 depicts *S(-)PRO* partial stimulus generalization to (-)ephedrine (maximum 56% *S(-)PRO*-appropriate responding at 3.5 mg/kg by five out of eight rats that responded) and disruption of behavior (i.e., zero out of eight rats responded) at 4.0 mg/kg and nioxetine (maximum 68% *S(-)PRO*-appropriate responding at 8.5 mg/kg by eight out of eight rats responded) and disruption of behavior (i.e., <50% of rats responded) at 9.0 mg/kg (three out of eight rats responded) or 10 mg/kg (one out of eight rats responded). In addition, the animals' response rates were significantly reduced following injection of nioxetine (8.5 mg/kg) or (-)ephedrine (3.0 mg/kg and 3.5 mg/kg). The *S(-)PRO* stimulus did not generalize to the other drugs: 5-OMeDMT and fenfluramine produced a maximum of 18% and 30% *S(-)PRO*-appropriate responding at 0.75 mg/kg (five out of eight rats responded) and 1.5 mg/kg (eight out of eight rats responded), respectively; a higher dose(s) of each agent resulted in disruption of behavior (<50% of rats

Fig. 5 Results of stimulus generalization tests conducted with (–) ephedrine, nisoxetine, fenfluramine, and 5-OMeDMT in rats ($n=8$) trained to discriminate 5.0 mg/kg of *S*(–)PRO from saline vehicle. Ordinate Mean ($\pm$ SEM) percentage of responses made on the *S*(–)PRO-appropriate lever after the i.p. administration of various doses of each drug (top figure). The animals' response rates are shown in the lower figure. *D* Responses following 5.0 mg/kg of *S*(–)PRO and *S* responses following administration of saline vehicle. Response rate was significantly ($*p<0.05$) different from the saline vehicle control response rate. Abscissa Drug doses plotted on a logarithmic scale. See “Results” for further descriptions of data

responded), i.e., two out of eight rats responded at 1.0 mg/kg of 5-OMeDMT or 2.0 mg/kg of fenfluramine and, one out of eight rats responded at 3.0 mg/kg of fenfluramine.

***S*(–)Propranolol Stimulus: antagonism studies** Control tests of prazosin (0.1, 0.3, 1.0, 2.0, and 3.0 mg/kg) or atenolol (1.0, 3.0, 10, 15, 20, and 30 mg/kg), given alone, indicated that neither antagonist produced a *S*(–)PRO-like response (Fig. 6); seven out of eight rats responded at 3.0 mg/kg of prazosin; four and one out of eight rats responded at 20.0 and 30.0 mg/kg of atenolol, respectively. It also was noted that the animals' response rates were significantly reduced (20 mg/kg) or completely disrupted (30 mg/kg) following injection of atenolol. Figure 6 also shows that the administration of prazosin prior to injection of the (a) training dose of *S*(–)PRO (i.e., 5.0 mg/kg) or (b) dose of ($\pm$)PRO (i.e., 10 mg/kg) or *R*(+)PRO (i.e., 20 mg/kg) that produced complete stimulus generalization resulted in dose-related antagonism of the *S*(–)PRO or *S*(–)PRO-like response: Eight out of eight rats responded/tested at each dose combination. A comparison of AD_{50} values revealed that prazosin displayed approximately equal potency to block

Fig. 6 Results of stimulus antagonism tests with various doses of prazosin or atenolol given alone or in combination with a dose of *S*(–)PRO (5.0 mg/kg), ($\pm$)PRO (10 mg/kg), *R*(+)PRO (20 mg/kg), or cocaine (8 mg/kg) in rats ($n=8$) trained to discriminate 5.0 mg/kg of *S*(–)propranolol from saline vehicle. Ordinate Mean ($\pm$ SEM) percentage of responses (top figure) made on the *S*(–)PRO-appropriate lever after the i.p. administration of the various doses of each antagonist given before the dose of each test drug. The animals' response rates are shown in the lower figure. *D* Responses following 5.0 mg/kg of *S*(–)PRO and *S* responses following administration of saline vehicle. Response rate was significantly ($*p<0.05$) different from the saline vehicle control response rate. Abscissa Drug doses plotted on a logarithmic scale. See “Results” for further descriptions of data

the three agents: *S*(–)PRO (prazosin $AD_{50}=0.47$ mg/kg; 95% CL=0.17–0.82 mg/kg), ($\pm$)PRO (prazosin $AD_{50}=0.49$ mg/kg; 95% CL=0.17–0.82 mg/kg), or *R*(+)PRO (prazosin $AD_{50}=0.71$ mg/kg; 95% CL=0.17–2.1 mg/kg). Figure 6 also shows that the animals' response rates were not appreciably altered, in comparison to that after administration of saline, following injections of doses of prazosin in combination with the test dose of *S*(–), ($\pm$), or *R*(+)PRO. In comparison, prazosin produced only partial stimulus antagonism of the *S*(–)PRO-like response exerted by 8.0 mg/kg of cocaine (the maximum decrease was to only 63% *S*(–)PRO-appropriate responding at 3.0 mg/kg of prazosin and 88% *S*(–)PRO-appropriate responding at 4.0 mg/kg of prazosin):

Eight out of eight rats responded at each dose combination. The animals' response rates, however, were significantly reduced following injection of prazosin (3.0 or 4.0 mg/kg) in combination with cocaine (8.0 mg/kg). In contrast, the administration of atenolol prior to injection of the training dose of *S*(-)-PRO (i.e., 5.0 mg/kg) produced no antagonism of the *S*(-)-PRO stimulus effect (Fig. 6). The combination of 3.0 or 10 mg/kg of atenolol and 5.0 mg/kg of *S*(-)-PRO produced 95% and 98% *S*(-)-PRO-appropriate responding, respectively; a higher dose (i.e., 20 mg/kg) of atenolol resulted in disruption of behavior (i.e., zero out of eight rats responded).

Lastly, Fig. 7 shows that pretreatment of the *S*(-)-PRO-trained animals with 1.0 or 2.0 mg/kg of prazosin in combination with various doses of *S*(-)-PRO resulted in parallel shifts of the dose-response functions and, consequently, changes in the ED_{50} dose of *S*(-)-PRO: *S*(-)-PRO given alone (ED_{50} =2.2 mg/kg; 95% CL=1.3–3.6 mg/kg),

Fig. 7 Results of stimulus antagonism tests with various doses of *S*(-)-PRO given alone or in combination with doses of prazosin (1.0 or 2.0 mg/kg) in rats ($n=8$) trained to discriminate 5.0 mg/kg of *S*(-)-propranolol from saline vehicle. Ordinate Mean ($\pm$ SEM) percentage of responses (top figure) made on the *S*(-)-PRO-appropriate lever after the i.p. administration of the various doses of each antagonist before the dose of *S*(-)-PRO. The animals' response rates are shown in the lower figure. D Responses following 5.0 mg/kg of *S*(-)-PRO and S responses following administration of saline vehicle. Abscissa Drug doses plotted on a logarithmic scale

1.0 mg/kg of prazosin and doses of *S*(-)-PRO (ED_{50} =5.4 mg/kg; 95% CL=2.8–10.5 mg/kg), 2.0 mg/kg of prazosin and doses of *S*(-)-PRO (ED_{50} =10.4 mg/kg; 95% CL=6.0–18.0 mg/kg). In the latter tests, the animals' response rates were not markedly different, in comparison to that after administration of saline, following injections of those doses of prazosin in combination with doses of *S*(-)-PRO (Dunnett's t ; $p>0.05$).

Cocaine Stimulus: generalization studies As shown in Fig. 8, the cocaine (ED_{50} =2.7 mg/kg; 95% CL=1.5–4.7 mg/kg) stimulus generalized to *S*(-)-PRO (ED_{50} =12.2 mg/kg; 95% CL=10.4–14.2 mg/kg), ($\pm$)-PRO (ED_{50} =15.9 mg/kg; 95% CL=12.6–20.1 mg/kg), and *R*(+)-PRO (ED_{50} =17.8 mg/kg; CL=13.7–23.1 mg/kg). A comparison of ED_{50} values revealed that the cocaine-like response of *S*(-)-PRO was <1.5 times more potent than the racemic mixture and 1.5 times more potent than the *R*(+)-PRO enantiomer. The animals' response rates were not appreciably altered, in comparison to that after administration of saline, following injections of cocaine, *S*(-), ($\pm$), or *R*(+)-PRO.

Fig. 8 Cocaine-stimulus generalization to cocaine, ($\pm$), *S*(-), and *R*(+)-PRO in rats ($n=6$) trained to discriminate 8.0 mg/kg of cocaine from saline vehicle. Ordinate Mean ($\pm$ SEM) percentage of responses made on the cocaine-appropriate lever after the i.p. administration of various doses of each drug (top figure). The animals' response rates are shown in the lower figure. D Responses following 8.0 mg/kg of cocaine and S responses following administration of saline vehicle. Abscissa Drug doses plotted on a logarithmic scale

Cocaine Stimulus: antagonism studies Control tests of prazosin (0.3, 0.6, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/kg), given alone, indicated that it did not produce a cocaine-like effect (Fig. 9); five out of six rats responded at 2.0, 3.0, and 5.0 mg/kg of prazosin. Furthermore, the animals' response rates were not markedly changed, in comparison to that after administration of saline, following injection of doses of prazosin. Figure 9 also indicates that the administration of prazosin prior to the injection of *S*(-)-PRO (15 mg/kg), ($\pm$)PRO (20 mg/kg), or *R*(+)-PRO (25 mg/kg), doses that produced complete stimulus generalization in the cocaine-trained animals, resulted in a dose-related antagonism of each cocaine-like response: Six out of six rats responded/ tested at each dose combination. A comparison of AD₅₀ values revealed that prazosin displayed nearly equal

potency to block the three agents: *S*(-)-PRO (prazosin AD₅₀=1.7 mg/kg; 95% CL=0.8–3.7 mg/kg), ($\pm$)PRO (prazosin AD₅₀=1.5 mg/kg; 95% CL=0.7–3.1 mg/kg), or *R*(+)-PRO (prazosin AD₅₀=1.2 mg/kg; 95% CL=0.5–2.7 mg/kg). The animals' response rates were not appreciably altered, in comparison to that after administration of saline, following injections of doses of prazosin in combination with the test dose of *S*(-), ($\pm$), or *R*(+)-PRO. In comparison, prazosin produced only partial stimulus antagonism of the stimulus produced by 8.0 mg/kg of cocaine (the maximum decrease was to only 64% cocaine-appropriate responding at 4.0 mg/kg of prazosin and 89% cocaine-appropriate responding at 5.0 mg/kg of prazosin); four and five out of six rats responded at the combination of 3.0 or 5 mg/kg of prazosin and cocaine, respectively. In addition, the animals' response rates were not changed appreciably following injection of prazosin (0.3 to 5.0 mg/kg) in combination with 8.0 mg/kg of cocaine.

Fig. 9 Results of stimulus antagonism tests with various doses of prazosin given alone or in combination with a dose of cocaine (8.0 mg/kg), *S*(-)-PRO (15 mg/kg), ($\pm$)PRO (20 mg/kg), or *R*(+)-PRO (25 mg/kg) in rats ($n=6$) trained to discriminate 8.0 mg/kg of cocaine from saline vehicle (1 ml/kg). Ordinate Mean ($\pm$ SEM) percentage of responses (*top figure*) made on the cocaine-appropriate lever after the i.p. administration of the various doses of prazosin before the dose of each test drug. The animals' response rates are shown in the *lower figure*. *D* Responses following 8.0 mg/kg of cocaine and *S* responses following administration of saline vehicle. *Abscissa* Drug doses plotted on a logarithmic scale. See “Results” for further descriptions of data

Discussion

This study demonstrates quite clearly that *S*(-)-PRO, at 5.0 mg/kg, can serve as a discriminative stimulus in rats. The time-course investigation of the *S*(-)-PRO stimulus revealed a relatively fast onset to action and a relatively short duration of effect. Indeed, the latter results parallel pharmacokinetic studies of propranolol in rats that indicate PRO is rapidly and completely absorbed after intraperitoneal administration and that its peak plasma level is observed within 1 h and steadily declines thereafter (Fitzgerald 1980; Hayes and Cooper 1971). In addition, *S*(-)-PRO- or cocaine-stimulus generalization tests indicated that *S*(-)-PRO is <1.5 to two times more potent than ($\pm$)PRO and 1.5 to four times more potent than *R*(+)-PRO. Thus, the discriminative stimulus effect(s) of PRO appears to reside primarily, albeit not exclusively, with the *S*(-)-isomer. More importantly, however, both groups of animals produced potency relationships between *S*(-), ($\pm$), and *R*(+)-PRO that strongly suggest, but do not conclusively prove, that the stimulus effect of PRO (at the doses tested) is unlikely to be mediated through β -adrenoceptors. Specifically, the *S*(-)-PRO- and cocaine-like stimulus effectiveness of *R*(+)-PRO, which is approximately 100 times less potent than *S*(-)-PRO in blocking cardiac β -adrenoceptors and in radioligand β -adrenoceptor binding studies in brain (Barrett and Cullum 1968; Tsuchihashi et al. 1990), is similar to that of the *S*(-)-isomer. The relatively slight difference in potency between the stereoisomers is much more consistent with their potency to release NE or inhibit NE reuptake and, consequently, increase adrenoceptor activity (Daniell et al. 1976; Saelens et al. 1977; Tackett et al.

1981; Tuross and Patrick 1986; also see *Adrenergic involvement* below).

Serotonergic involvement The *S*(-)-PRO stimulus did not generalize to the nonselective 5-HT agents 5-OMe DMT or fenfluramine. 5-OMe DMT has been shown to bind to 5-HT_{1A/1B/2A/2C/6} and 7 receptors (Glennon et al. 2000; Gozlan et al. 1983; Lyon and Titeler 1988; Offord et al. 1988; Peroutka 1986; Sadzot et al. 1989; To et al. 1995). Fenfluramine is known to release endogenous stores of 5-HT, an effect that should allow released 5-HT to interact at 5-HT receptors in a nonselective manner. If the *S*(-)-PRO stimulus is mediated to some extent by 5-HT receptors, then it might be anticipated that 5-OMe DMT and fenfluramine would exert some degree of partial, if not complete, stimulus generalization. Such was not the case because both drugs produced saline-like responding at the highest non-disruptive doses tested. Thus, the results suggest a negligible contribution of 5-HT in the stimulus effect of *S*(-)-PRO. However, it should be noted that 5-OMe DMT and fenfluramine have sometimes produced stimulus results that could be termed inconsistent when compared with other “serotonergically mediated” agents. For example, in 5-OMe DMT-trained animals, stimulus generalization does not occur to fenfluramine but does occur to 8-OH DPAT (Spencer et al. 1987). In contrast, fenfluramine-trained animals generalize to 5-OMe DMT but not to the 5-HT_{1A} receptor agonist 8-OH DPAT (Young and Glennon 1988). Lastly, an 8-OH DPAT stimulus does not generalize to fenfluramine (Glennon 1986) and, reportedly, does not generalize (Cunningham and Appel 1986; Glennon 1986) or only partially generalizes to 5-OMe DMT (Tricklebank et al. 1987).

In contrast to the results obtained with 5-OMe DMT and fenfluramine, *S*(-)-PRO stimulus generalization occurred to TFMPP and RU 24969. Early studies of the latter two agents as discriminative stimuli emphasized the possible role of 5-HT_{1B} receptors in the mediation of their effects. That conclusion was based on the absence of stimulus generalization to compounds “selective” for other subpopulations of 5-HT receptors as well as upon generalization to other purported “5-HT_{1B} receptor drugs”. For example, in animals trained to discriminate either TFMPP or RU 24969 from vehicle, stimulus generalization occurred to the purported “5-HT_{1B} agents” 1-(*m*-chlorophenyl)piperazine, TFMPP, and RU 24969, but not to 8-OH DPAT (Cunningham and Appel 1986; Gardner 1989; Glennon et al. 1984; McKenney and Glennon 1986). Furthermore, both a TFMPP and a RU 24969 stimulus generalized to PRO, which seemed to suggest an agonist effect of PRO at 5-HT_{1B} receptors (Gardner 1989; Glennon et al. 1988). Later studies with TFMPP and RU 24969, however, determined that these

agents were nonselective 5-HT agonists. For example, TFMPP and RU 24969 are now known to exhibit moderate to high affinity for 5-HT_{1A/1B/1D/2A/2B} and/or 2C receptors (e.g., Peroutka 1986; Schlegel and Peroutka 1986; Boess and Martin 1994; Wainscott et al. 1996; Herrick-Davis et al. 1998). Lastly, it has been reported that a TFMPP stimulus is not blocked by the α_1 -adrenoceptor antagonist prazosin but, curiously, is blocked by the nonselective β -adrenoceptor antagonist (-)-alprenolol (Arnt 1989; Herndon et al. 1992). Nevertheless, even though the present results with 5-OMe DMT and fenfluramine might not suggest a role for 5-HT in the stimulus effect of *S*(-)-PRO, the current findings with TFMPP and RU 24969 still hint that a role for 5-HT cannot be assumed to have been definitively eliminated as a factor in the stimulus mechanism of action of PRO.

Investigations with cocaine The *S*(-)-PRO stimulus generalized to cocaine, and the cocaine stimulus generalized to racemic propranolol and its enantiomers with the following order of potency: *S*(-)-PRO > ($\pm$)-PRO > *R*(+)-PRO. Cocaine interacts with multiple neurotransmitter systems such that it blocks neuronal reuptake of the monoamines dopamine, norepinephrine, and serotonin (e.g., Koe 1976; Rothman et al. 2001). Drug discrimination studies also provide several lines of evidence that indicate the stimulus actions of cocaine are mediated, at least in part, through modulatory actions by dopamine, 5-HT, and NE systems (e.g., Cunningham and Callahan 1991; Kleven and Koek 1997; Terry et al. 1994). In the present tests of stimulus antagonism in the cocaine- or *S*(-)-PRO-trained animals, the administration of prazosin before cocaine produced only partial antagonism. In comparison, the administration of prazosin prior to racemic propranolol (or its stereoisomers) in the cocaine-trained rats produced dose-related antagonism of cocaine-like appropriate responding; prazosin is almost equipotent as an antagonist of the cocaine-like effect of each agent. The latter results are in agreement and consistent with a previous report that prazosin produced partial antagonism of a cocaine stimulus and that prazosin antagonized fully the cocaine-enhancing stimulus effects of PRO in rats (Kleven and Koek 1998). On the other hand, prazosin has been reported to produce either no antagonism (at all) of the stimulus effect of cocaine in rats (Kleven et al. 1999) or full antagonism (or a shift of the dose response) of the stimulus effect of cocaine in pigeons (Johanson and Barrett 1993) and squirrel monkeys (Rowlett et al. 2004; Spealman 1995). Taken together, the latter results raise the likely possibility of a species (and/or other methodological) difference(s) in the behavioral evaluation of the interactive effects between prazosin and cocaine. Nonetheless, the current data obtained in the cocaine- and

S(-)-PRO-trained animals strongly re-emphasize the idea that stimulus generalization between a training drug and a test drug is only evidence that both agents can produce a similar stimulus effect and that it is not necessarily accurate to conclude that they do so via an identical mechanism of action (e.g., Young and Glennon 1986).

Adrenergic involvement In this initial study, *S*(-)-PRO stimulus generalization and antagonism tests were conducted to gauge the degree of involvement of adrenoceptors. The *S*(-)-PRO stimulus was not influenced (in substitution or antagonism tests) by atenolol. The latter results could be due to the hydrophilic character of atenolol, an attribute that should restrict its entry into the brain as compared to the highly lipophilic nature of PRO (e.g., Agon et al. 1991). Moreover, the results with atenolol promote the idea of a (primary) central site of stimulus action for *S*(-)-PRO. It should be noted, however, that atenolol is a relatively selective peripheral β_1 -adrenoceptor agent and, therefore, it would not account for the possible involvement of peripheral β_2 -adrenoceptors in the stimulus effect of *S*(-)-PRO. In other tests of stimulus substitution, the *S*(-)-PRO stimulus generalized partially to the selective NE reuptake inhibitor nisoxetine and the nonselective adrenoceptor agonist (-)-ephedrine. In as much as *S*(-)-PRO stimulus generalization might have occurred at higher doses had animals' behavior not been disrupted, the possibility exists that those partial (although incomplete) generalizations may indicate an increase in adrenergic activity as a component of the stimulus effect of *S*(-)-PRO. That notion was confirmed by determinations of *S*(-)-PRO stimulus generalization to pindolol, a nonselective β -adrenoceptor antagonist that also (at relatively high doses) exerts intrinsic sympathomimetic (agonist) activity (ISA; e.g., Frishman 1988; Gonasun and Langrall 1982), and methoxamine, an α_1 -adrenoceptor agonist. Indeed, a series of antagonism tests appear to confirm that the *S*(-)-PRO stimulus is comprised of an α_1 -adrenoceptor agonist effect. First, the administration of prazosin prior to the injection of ($\pm$)-PRO or its stereoisomers produced dose-related antagonism of *S*(-)-PRO (or *S*(-)-PRO-like) appropriate responding; prazosin is almost equipotent as an antagonist of their effects. The *S*(-)-PRO-trained animals also were subjected to a more extensive series of antagonism tests with pretreatment of 1 or 2 mg/kg of prazosin prior to the administration of various doses of *S*(-)-PRO. Those treatments produced (parallel) rightward shifts of the dose-response curves and, consequently, marked changes in the ED₅₀ dose of *S*(-)-PRO; ED₅₀ values of *S*(-)-PRO shifted 2.5- and five-fold when *S*(-)-PRO was given alone versus when it was given in the presence of 1 and 2 mg/kg of prazosin, respectively. Thus, it is possible that PRO and its stereoisomers might exert a central effect(s)

that arises from an increased release or a decreased reuptake of NE into central adrenergic nerve terminals (e.g., Kelliher and Buckley 1970; Day and Roach 1974; Davies and Reid 1975; Kobinger 1978; Privitera et al. 1979; Tackett et al. 1981; Tuross and Patrick 1986). However, the present experiments cannot discern inhibition of reuptake and/or stimulation of release of NE by PRO, but in either, or both, case(s), the result would be an increase in adrenergic action in the synapse. That prior treatment with prazosin prevents the *S*(-)-PRO stimulus effect suggests that the action may be mediated (primarily) through stimulation of central α_1 -adrenoceptors. In fact, the data demonstrate the competitive relationship between the seemingly α_1 -adrenoceptor agonist effect of *S*(-)-PRO and the α_1 -adrenoceptor antagonist effect of prazosin.

Summary The present study provides evidence that *S*(-)-PRO can serve as a discriminative stimulus in rats. The *S*(-)-PRO stimulus appears to be mediated centrally and is dose-related, time dependent, and stereoselective. The potency relationships between the stimulus effects of ($\pm$)-PRO and its enantiomers emphasize the importance of steric factors and also suggest an action(s) of PRO in the CNS that may not depend upon β -adrenoceptor antagonism. The *S*(-)-PRO stimulus generalized to the nonselective 5-HT agents TFMPP and RU 24969, which suggests the possibility, to some degree, of a serotonergic component in its mechanism of action. The *S*(-)-PRO stimulus also generalized to the nonselective β -adrenoceptor partial agonist pindolol and the α_1 -adrenoceptor agonist methoxamine. Consistent with the proposal of PRO-induced activation of α_1 -adrenoceptors are the findings that the administration of the α_1 -adrenoceptor antagonist prazosin can block the stimulus effect of *S*(-)-PRO (completely and competitively) and the *S*(-)-PRO-like effect of ($\pm$)-PRO and *R*(+)-PRO. Thus, even though PRO (at low doses) has been demonstrated to block a β -adrenoceptor agonist stimulus (i.e., clenbuterol), the present study of PRO (at a higher dose) implicates an increase in α_1 -adrenoceptor activity. Finally, *S*(-)-PRO and cocaine display cross generalization regardless of which agent is used as the training stimulus, but the α_1 -adrenoceptor antagonist effect of prazosin can reveal differences in their mechanisms of action. The latter results re-confirm the concept that stimulus generalization between a training drug and a test compound is simply evidence that both agents can produce a similar stimulus effect but it is not necessarily accurate to conclude that they do so through an identical mechanism of action. Overall, the present results provide support for the idea that PRO can increase central α_1 -adrenoceptor activity. Future studies of PRO should consider its dose-dependent effects and not necessarily assume that every dose of racemic PRO or its stereoisomers will exert

characteristics of a β -adrenoceptor antagonist effect. In fact, PRO might be described more appropriately as a partial adrenoceptor agonist.

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