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Neuropsychopharmacology 1998;19(4):322-32

Role of Endogenous Neurotensin in the Behavioral and Neuroendocrine Effects of Cocaine

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The present experiments were designed to assess the role of endogenous neurotensin (NT) in the behavioral response to acute and daily cocaine, after administration of the NT receptor antagonist, SR 48692. Given that glucocorticoids increase the sensitivity to the psychomotor effects of drugs of abuse, we also investigated the effects of SR 48692 on basal and cocaine-induced corticosterone secretion. Acute administration of SR 48692 (1 mg/kg IP) reduced the number of rearings induced by cocaine (15 mg/kg IP), without modifying horizontal activity. Repeated pretreatment with SR 48692 (1 mg/kg x 5 days) markedly reduced locomotion and rearings after an acute cocaine challenge (day 1), whereas the lower dose of SR 48692 (0.1 mg/kg) had no effect. SR 48692 (1 mg/kg), given daily before cocaine, also decreased cocaine-induced rearing on day 2, but had no effect on the following drug challenges (days 3–10). One week after discontinuing repeated cocaine injections, SR 48692 blocked vertical, but not horizontal, activity induced by an acute cocaine challenge. Rats treated repeatedly with cocaine showed an enhanced behavioral response characterized by the development of stereotypies, which were unaffected by SR 48692. Finally, treatment with SR 48692 did not alter corticosterone circadian secretion nor cocaine-stimulated corticosterone levels, indicating that the attenuation of the behavioral effects of cocaine after NT receptor blockade is not associated with blunted glucocorticoid secretion. These results indicate that administration of SR 48692 attenuates the locomotion and rearing response to cocaine but fails to modify stereotyped behavior, suggesting that SR 48692 modulates the behavioral effects of psychostimulant drugs by acting selectively on the mesolimbic dopaminergic system.

KEY WORDS: *Neurotensin; SR 48692; Cocaine; Locomotion; Rearing; Corticosterone*

INTRODUCTION

Cocaine is a potent psychostimulant drug that binds to the dopamine transporter and blocks dopamine re-uptake, leading to increased extracellular levels of dopamine (Kuhar et al. 1991). The mesolimbic dopaminergic system is considered the main substrate for the motor stimulant and reinforcing effects of cocaine and other drugs of abuse (Koob 1992). Several lines of evidence suggest that neurotensin (NT), a neuropeptide closely associated with dopaminergic systems, both anatomically and functionally (Kasckow and Nemeroff 1991), may be involved in the behavioral effects of cocaine.

First, cocaine and other psychostimulants such as amphetamine induce a pronounced increase in NT peptide content (Cain et al. 1993; Gygi et al. 1994) and NT mRNA expression (Castel et al. 1994; Merchant et al. 1994; Betancur et al. 1997) in the rat striatum and nucleus accumbens. In addition, chronic cocaine modifies NT receptor binding in regions associated with dopaminergic pathways (Pilotte et al. 1991).

Second, the behavioral and neurochemical effects of NT injection into the ventral tegmental area (VTA) resemble those induced by peripheral injection of cocaine. The microinjection of NT into the VTA increases locomotion and rearing (Kalivas et al. 1983) and enhances dopamine release in the nucleus accumbens (Kalivas and Duffy 1990a). NT also has reinforcing properties, as indicated by studies showing that rats self-administer NT into the VTA (Glimcher et al. 1987) and that administration of NT in the same region induces a conditioned place preference (Glimcher et al. 1984).

Third, NT seems to be involved in cocaine-induced sensitization. Following daily injection of NT into the VTA, the acute motor-stimulating action of the neuropeptide is augmented (Kalivas and Taylor 1985), an effect analogous to the sensitization induced by repeated administration of cocaine (Kalivas et al. 1988). The behavioral sensitization induced by either NT or cocaine is associated with an increased level of extracellular dopamine in the nucleus accumbens (Kalivas and Duffy 1990a,b). Furthermore, administration of the NT antagonist SR 48692 delayed the development of behavioral sensitization induced by repeated cocaine (Horger et al. 1994).

In the present series of studies, we further investigated the role of endogenous NT in the behavioral effects of cocaine by administering a nonpeptide antagonist of NT receptors, SR 48692 (Gully et al. 1993), in single and repeated administration schedules. The effects of SR 48692 on locomotion, rearing, and stereotypies were monitored after acute and daily cocaine injections in order to evaluate the development of behavioral sensitization. In addition, we studied whether these effects were associated with a possible dysregulation of the hypothalamic- pituitary-adrenocortical (HPA) axis induced by blockade of NT receptors. For this purpose, we examined the effects of systemic SR 48692 administration on the circadian fluctuation

of corticosterone plasma levels, and the corticosterone secretion induced by acute cocaine.

Corticosterone secretion was studied because this hormone plays an important role in the behavioral effects of cocaine. Suppression of corticosterone secretion by adrenalectomy reduces the locomotor (Marinelli et al. 1997) and reinforcing (Deroche et al. 1997) effects of cocaine, through a dopamine-dependent mechanism (Marinelli et al. 1994; Piazza et al. 1996). An interaction between glucocorticoids and NT in modulating the behavioral response to cocaine is suggested by the effects of this peptide on the HPA axis. Central injection of NT stimulates the release of ACTH and corticosterone (Gudelsky et al. 1989; Nicot et al. 1994). Moreover, chronic administration of SR 48692 at the level of the paraventricular nucleus of the hypothalamus decreases the circadian rise of ACTH and corticosterone plasma levels during the evening, as well as the increase in both hormones after exposure to stress (Nicot et al. 1997; Rowe et al. 1997).

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Charles River, Germany), weighing 200 to 230 g at the beginning of the experiments, were housed three per cage in a temperature (22°C) and humidity (60%) controlled environment under a 12-h light/dark cycle (lights on at 8 A.M.), with *ad libitum* access to food and water. Rats were allowed to habituate 1 week to the animal room prior to their use and were handled daily to reduce handling stress during experiments.

Drugs

SR 48692 (Sanofi Recherche, Montpellier, France) was solubilized in tween 80 (0.1%), dissolved in sterile 0.9% NaCl solution and injected IP at the dose of 0.1 or 1 mg/kg. These doses are based on published data showing that they block most of the effects induced by centrally administered NT or by the endogenous peptide (Steinberg et al. 1994; Brun et al. 1995; Santucci et al. 1997). Control rats were injected with vehicle (saline with 0.1% tween 80). Cocaine hydrochloride (Coopération Pharmaceutique Française, Melun, France) was dissolved in saline and injected IP at the dose of 15 mg/ kg (calculated as the free base).

Behavioral Measurements

Rats were transported each morning from the animal colony to the behavioral room in their own cages and allowed to habituate for 1 h. Animals were then injected with SR 48692 or vehicle and placed in Plexiglas testing cages (area: 25 x 25 cm), where the behavioral response to the novel environment was recorded for 1 h, using a

videocamera attached to the ceiling. After habituation, rats were injected with cocaine, and their behavior was monitored for 2 h. Horizontal (locomotor) activity (distance moved expressed in cm) and vertical activity (number of rearings) were measured automatically from the videotapes with a motion analysis system, Ethovision (Noldus Information Technology, Wageningen, The Netherlands) and expressed as total values for each 10-min interval.

Stereotypies induced by cocaine were evaluated using a behavioral scale described by MacLennan and Maier (1983), which provides an estimate of increasing behavioral intensity from exploratory behaviors to stereotypy. Behavior was analyzed by an investigator unaware of the drug treatment. Rats were rated for 30-s periods starting 10 min after cocaine injection, and continuing every 10 min for 2 h using the following scale: 0, inactive; 1, intermittent activity; 2, continuous activity; 3, rearing; 4, intermittent stereotypic sniffing, repetitive head movements, or both, with periods of nonstereotypic behavior longer than 2 s; 5, intermittent stereotypic sniffing, repetitive head movements, or both, with periods of nonstereotypic behavior shorter than 2 s; 6, continuous stereotypic sniffing; repetitive head movements, or both; and 7, continuous and restricted stereotypic sniffing, repetitive head movements, or both. It should be emphasized that these scores represent the intensity of the behavioral response to cocaine, whereas the automated measures of locomotion and rearing provide quantitative estimates of these behaviors.

Surgery

To obtain repeated blood samples without disturbing the animals, rats were implanted with an intracardiac catheter, under Hypnorm (fentanyl citrate 0.315 mg/ml, and fluanisine 10 mg/ml, dose: 0.5 ml/kg IM; Janssen Pharmaceutica, Tilburg, The Netherlands) and Dormicum (midazolam, 2.5 mg/kg SC; Hoffman-La Roche, Mijdrecht, The Netherlands) anesthesia. After surgery, rats were housed individually and allowed to recuperate for 1 week before the beginning of the experiment.

Corticosterone Assay

Blood samples (300 μl) were collected in chilled tubes coated with EDTA, centrifuged, and the plasma stored at –20°C until assayed. Plasma corticosterone was measured by radioimmunoassay, using a highly specific corticosterone antiserum with a detection threshold of 0.1 μg/100 ml.

Experiment 1: Effect of an Acute Injection of SR 48692 on Cocaine-Induced Locomotion and Rearing

Animals were injected with SR 48692 (1 mg/kg IP) or vehicle (n = 8 rats per group), 1 h before an injection of cocaine (15 mg/kg IP), and locomotor activity and number of rearings were monitored.

Experiment 2: Effect of Repeated Administration of SR 48692 on the Behavioral Response to Acute and Daily Cocaine

Subjects were pretreated with SR 48692 (0.1 or 1 mg/kg IP) or vehicle once daily for 5 days (n = 8 rats per group), followed by daily coadministration of cocaine (15 mg/kg IP) and SR 48692 or vehicle (given 1 h before each injection of cocaine) for 10 days. Horizontal and vertical activities were recorded daily for 1 h before and 2 h after each cocaine injection. After 10 days, the administration of cocaine was interrupted, and the animals continued to receive daily injections of SR 48692 or vehicle for 1 week. Eight days after the last cocaine injection (day 18), rats were injected with the NT antagonist or vehicle as described above, challenged 1 h after with a cocaine injection (15 mg/kg), and their behavior was recorded. Stereotypies were evaluated after the first (day 1) and the last (day 18) cocaine injections.

Experiment 3: Effect of SR 48692 on Basal and Cocaine-Stimulated Plasma Corticosterone Levels

Rats implanted with intracardiac catheters were divided into three groups and injected once daily with SR 48692 (0.1 or 1 mg/kg IP) or vehicle for 5 days (n = 9 per group). To determine the effects of NT receptor blockade on the circadian fluctuation of corticosterone levels, animals were bled after the first SR 48692 injection, between 7 and 7:30 P.M. (nocturnal peak of corticosterone), and the following day, between 9 and 9:30 A.M. (diurnal trough of corticosterone). This procedure was repeated after the fifth daily injection of SR 48692. We also examined the effects of repeated SR 48692 administration on corticosterone secretion induced by cocaine. Because we wanted to compare the behavioral and neuroendocrine responses to cocaine after NT receptor blockade, rats in this experiment were exposed to the same conditions as the rats whose behavioral response was evaluated in experiment 2. Thus, after 5 days of pretreatment with SR 48692, the rats were bled in the animal room as described before, between 9 and 9:30 A.M. (basal corticosterone values), and transported to the behavioral testing room. After 1 h of habituation, animals were injected with vehicle or SR 48692 (0.1 or 1 mg/kg) and placed in the behavioral cages, followed 1 h later by the administration of cocaine (15 mg/kg IP), and blood samples were obtained 30, 60, and 120 min after the injection.

Statistical Analysis

Behavioral data were analyzed using a two-way analysis of variance (ANOVA) for repeated measures. Individual ANOVAs were performed for each cocaine challenge, with one between subjects factor (Treatment) and one within factor (Time). A two-way ANOVA with repeated measures over day was used to evaluate the development of behavioral sensitization. Corticosterone plasma levels were

compared using a two-way ANOVA with repeated measures over time, followed by a Tukey test for multiple comparisons.

RESULTS

Experiment 1: Effect of an Acute Injection of SR 48692 on Cocaine-Induced Locomotion and Rearing

As can be seen in Figure 1, after the animals were injected with SR 48692 or vehicle and introduced in the behavioral testing cages, there was an increase in horizontal activity and rearing behavior, corresponding to the exploration of the novel environment. Acute treatment with the NT receptor antagonist (1 mg/kg) did not alter the behavioral response to novelty. The injection of cocaine induced a rapid increase in horizontal and vertical activity. Administration of SR 48692 did not modify the locomotor activity elicited by cocaine (Figure 1, top), but significantly reduced the number of rearings in the first 40 min after the injection of cocaine [Treatment effect: $F(1,15) = 4.23$, $p < .05$; Figure 1, bottom].

Experiment 2: Effect of Repeated Administration of SR 48692 on the Behavioral Response to Acute and Daily Cocaine

To check whether chronic blockade of NT receptors modified the locomotor response to cocaine, SR 48692 (0.1 or 1 mg/kg) was given once daily for 5 days before starting cocaine administration (pretreatment) and 1 h before each injection of cocaine (cotreatment).

Horizontal Activity. Figure 2 shows the time course of the effects of SR 48692 on horizontal activity on the first (day 1) and the last (day 18) cocaine challenges, as well as the total distance moved after each daily injection of cocaine. On the first behavioral test, pretreatment with SR 48692 for 5 days did not alter horizontal activity induced by exposure to a novel environment. After the first injection of cocaine (day 1), locomotor activity was increased similarly in controls and rats treated with the low dose of SR 48692 (0.1 mg/kg). In contrast, animals injected with the higher dose of SR 48692 (1 mg/kg) exhibited a marked reduction in cocaine-induced locomotion when compared to controls [Treatment effect: $F(1,15) = 5.84$, $p < .03$]. Figure 2 (bottom) shows the cumulative distance moved on the first 2 h after each daily cocaine challenge. No significant differences in horizontal locomotion were observed among the treatment groups on the following cocaine challenges (days 2 to 10). Daily cocaine injections did not induce a progressive increase (sensitization) in the locomotor stimulant effects of cocaine in control subjects. Because behavioral sensitization can be masked by a transient tolerance to the effects of cocaine during repeated exposure and has been reported to be greater at 1 week

than at 24 h after discontinuing repeated drug treatment (Kalivas et al. 1993), we performed a delayed cocaine challenge (15 mg/kg), 8 days after the last cocaine injection. Daily treatment with the NT antagonist was continued during the week of cocaine withdrawal. As shown in Figure 2 (day 18), control animals did not exhibit a sensitized locomotor response after the last cocaine injection, as compared to day 1. In addition, neither dose of SR 48692 modified the horizontal activity elicited by the delayed drug challenge.

Rearing. Figure 3 shows the effects of repeated treatment with SR 48692 on cocaine-induced rearing. On day 1, pretreatment with the higher dose of SR 48692 (1 mg/kg) decreased vertical activity induced by exposure to novelty [Treatment effect: $F(1,15) = 5.24$, $p < .03$]. SR 48692 (1 mg/kg) also suppressed rearing behavior elicited by cocaine on day 1 [Treatment effect: $F(1,15) = 4.26$, $p < .05$], consistent with the decrease in locomotor activity. The lower dose of SR 48692 (0.1 mg/kg) did not affect rearing behavior in response to novelty or cocaine. Figure 3 (bottom) shows the cumulative vertical activity during the first 2 h after each daily cocaine injection. On subsequent cocaine tests (days 2 to 10), the number of rearings induced by cocaine remained lower in rats treated with SR 48692 1 mg/kg as compared to controls, but reached statistical significance only on day 2 [Treatment effect: $F(1,15) = 5.98$, $p < .03$]. After the last cocaine challenge, performed 8 days after discontinuing daily cocaine (Figure 3, day 18), the number of rearings was significantly reduced in animals treated with SR 48692 1 mg/kg [$F(1,15) = 8.22$, $p < .01$]. Rats treated with SR 48692 0.1 mg/kg also showed decreased vertical activity after the last cocaine injection as compared to control animals, although the difference did not reach statistical significance [$F(1,15) = 3.86$, $p < .06$]. Figure 3 (bottom) also shows that repeated exposure to cocaine did not induce a progressive augmentation of the stimulant effects of acute cocaine on rearing behavior in control animals.

Stereotypies. Figure 4 shows the effect of repeated NT receptor blockade on the behavioral rating of the motor stimulant response to cocaine on the first and the last cocaine challenges. On day 1, rats had a low rating in the behavioral scale, consisting mainly of intermittent or continuous horizontal activity and rearing (i.e., a behavioral score of 1–3; see Methods), and no stereotyped behaviors were observed. Pretreatment with SR 48692 (1 mg/kg) resulted in a lower score when compared to vehicle-treated subjects [Treatment effect: $F(1,15) = 6.97$, $p < .01$], in accordance with the reduction in locomotion and rearing observed in this group using the automated system (Figures 2 and 3, day 1), Administration of the lower dose of SR 48692 (0.1 mg/ kg) had no significant effect on the behavioral score of day 1, confirming the lack of effect of this dose on horizontal and vertical activity. When rats were challenged with cocaine 8 days after withdrawal from repeated injections (Figure 4, day 18), the behavioral rating was significantly increased when compared to the

scores observed the first day [Day effect: $F(1,24) = 20.43$, $p < .001$], indicating the development of behavioral sensitization due to of the appearance of stereotypies. In sensitized rats, cocaine-induced stereotypies were intermittent or continuous stereotypic sniffing and repetitive head movements (i.e., a behavioral score of 4–7). No other stereotyped behaviors, such as licking of gnawing, were observed at the dose of cocaine used in this study (15 mg/kg IP), in agreement with previous results. The absence of intense stereotypies could explain why the enhanced levels of stereotyped behavior measured on day 18 were not accompanied by a corresponding decrease in overall locomotor activity (displacement phenomenon). No differences were observed in the behavioral score on day 18 between vehicle- and SR 48692-treated subjects, indicating that neither dose of SR 48692 affected the stereotypies induced by repeated cocaine exposure.

Experiment 3: Effect of SR 48692 on Basal and Cocaine-Stimulated Plasma Corticosterone Levels

The effects of single or repeated SR 48692 administration on the physiological circadian fluctuation of plasma corticosterone levels are illustrated in Figure 5 (top). Treatment with SR 48692 (0.1 or 1 mg/kg) for 1 or 5 days did not alter diurnal or nocturnal corticosterone levels as compared to control subjects. After the 5-day pretreatment with the NT antagonist, the animals were given SR 48692 or vehicle and injected 1 h after with cocaine (15 mg/kg). The acute injection of cocaine induced a marked elevation of corticosterone levels [Time effect: $F(3,81) = 47.32$, $p < .0001$], which peaked at 30 min and approached basal values 120 min after injection (Figure 5, bottom). Pre-exposure to SR 48692 did not modify cocaine-induced activation of the HPA axis.

DISCUSSION

These experiments demonstrate that blockade of NT receptors reduces the behavioral response to acute cocaine. The effects of the NT receptor antagonist SR 48692 were more pronounced after repeated, than after acute, administration. Thus, a single administration of SR 48692 reduced the number of rearings elicited by cocaine, without affecting locomotion. Repeated pretreatment with SR 48692 considerably reduced both horizontal locomotion and rearing induced by acute cocaine administration. After daily cocaine injections, rats developed an increased behavioral response characterized by the appearance of stereotypies, which were not modified by chronic administration of SR 48692. Furthermore, SR 48692 failed to influence the corticosterone secretion induced by cocaine, indicating a dissociation between the effects of the NT antagonist on the behavioral and neuroendocrine responses to acute cocaine.

Locomotion and rearing are frequently used as measures of the psychomotor

activating effects of cocaine and are known to depend upon dopamine release in the nucleus accumbens (Kalivas et al. 1993). Horizontal and vertical activity are highly correlated, so treatments that affect locomotion usually produce parallel modifications of rearing. However, our results reveal a more pronounced effect of SR 48692 on rearing than on locomotion. Acute administration of SR 48692 induced a selective reduction of cocaine-induced rearing. Following repeated pretreatment with the NT antagonist, rearing behavior was significantly reduced after the first two cocaine injections and remained lower on subsequent cocaine challenges, whereas horizontal locomotion was reduced only after the first injection of cocaine. Finally, on the last drug challenge (day 18), performed 1 week after cocaine withdrawal, SR 48692 suppressed cocaine-induced rearing, without altering locomotion. These results are in agreement with previous observations indicating that administration of exogenous NT produces a preferential alteration of vertical activity. For instance, NT injection into the VTA induced a strong increase in rearing accompanied by a small increase in locomotion (Cador et al. 1985). Moreover, administration of NT into the nucleus accumbens, which has been shown to exert an inhibitory effect on dopaminergic transmission, selectively blocked the rearing component of the behavioral response to amphetamine (Haubrich et al. 1982).

The regimen of daily cocaine used in this study induced a behavioral sensitization characterized by the appearance of stereotyped behaviors, similar to that observed by others (Kalivas et al. 1988; Borowsky and Kuhn 1991b; Baumann and Rothman 1993). In contrast to the inhibitory effect of SR 48692 on cocaine-induced locomotion and rearing, the same treatment did not alter the stereotypies observed in sensitized animals. This is particularly interesting, because these behaviors seem to be mediated by different dopamine systems. Psychostimulant-induced locomotor hyperactivity is primarily mediated by mesolimbic dopamine fibers projecting to the nucleus accumbens (Kelly and Iversen 1976; Delfs et al. 1990), whereas stereotypies have been related to the activity of the nigrostriatal pathway (Kelly et al. 1975; Bordi and Meller 1989). Consequently, our findings suggest that SR 48692 selectively modulates the mesolimbic system. This is consistent with behavioral and biochemical studies showing that exogenous NT preferentially modulates the mesolimbic dopaminergic system when compared to the nigrostriatal system. For example, NT injection into the nucleus accumbens blocks locomotion and rearing induced by dopamine agonists, whereas intrastriatal injection of NT does not modify stereotypies induced by the same drugs (Ervin et al. 1981; Ford and Marsden 1990). Likewise, when injected into the cerebral ventricles, VTA, or substantia nigra, NT enhances dopamine efflux and metabolism to a greater extent in the nucleus accumbens than in the striatum (Blaha et al. 1990; Rivest et al. 1991).

A study by Horger and co-workers (1994) reported that pretreatment with SR 48692 for 5 days delayed the development of sensitization of locomotor activity induced by repeated cocaine administration 1 week later. In our study, cocaine induced

a behavioral sensitization characterized by increased stereotyped sniffing, but no enhancement of locomotion and rearing was observed over the course of repeated drug administration. Because we administered cocaine daily, whereas Horger et al. injected cocaine every other day, it is possible that differences in the administration schedule could explain the different behavioral sensitization profiles induced by the psychostimulant. The differential effects exerted by SR 48692 on the sensitization profiles observed in our study and in the study of Horger et al. (delay of locomotor sensitization but no effect on the stereotypic component of behavioral sensitization) are in agreement with our finding of a selective action of this antagonist in modulating the response of the mesolimbic system to cocaine.

Concerning the effects of SR 48692 on the HPA axis, our results indicated that systemic administration of the NT antagonist for 5 days did not modify the circadian rhythm of corticosterone secretion. In contrast, results previously obtained by our group showed that rats chronically implanted with cannulas filled with SR 48692 crystals near the paraventricular nucleus of the hypothalamus had a decreased nocturnal peak of corticosterone and ACTH, as well as a reduced release of both hormones after exposure to restraint or novelty stress (Nicot et al. 1997; Rowe et al. 1997). Our results indicate that the effects of centrally administered SR 48692 on the circadian fluctuation of corticosterone levels are not observed when the same drug is administered systemically. It is likely that the concentration of SR 48692 reaching the hypothalamus after parenteral injection is considerably lower than after direct central administration and might not be high enough to modify the activity of the HPA axis. Furthermore, we observed that the acute injection of cocaine induced a marked elevation in plasma levels of corticosterone, in accordance with previous findings (Rivier and Vale 1987; Borowsky and Kuhn 1991a). Pretreatment with SR 48692 did not affect cocaine-induced elevation of corticosterone levels, indicating that the attenuation of locomotion and rearing in response to an acute injection of cocaine observed in SR 48692-treated animals is not associated with decreased corticosterone secretion. These results suggest that the HPA axis is not involved in the modulation of the behavioral effects of cocaine after blockade of endogenous NT.

The effects of SR 48692 on dopaminergic activity and dopamine-mediated behaviors seem to be variable, since both facilitatory and inhibitory effects of this compound have been reported. For example, acute administration of SR 48692 together with a subeffective dose of methamphetamine resulted in a significant increase in locomotion and rearing as well as in the release of dopamine in the nucleus accumbens (Wagstaff et al. 1994). In another study, acute SR 48692 was shown to potentiate dopamine efflux in the nucleus accumbens evoked by electrical stimulation of the medial forebrain bundle, but only when this release was facilitated by the concomitant administration of haloperidol (Brun et al. 1995). Similarly, Santucci et al. (1997) showed that acute systemic SR 48692 increased the number of spontaneously active cells in the VTA. In contrast, SR 48692 has also been reported to

significantly reduce yawning induced by apomorphine or bromocriptine in rats, as well as turning behavior induced by intrastriatal injection of a number of dopaminergic agonists in mice (Poncelet et al. 1994). In addition, our results and those of Horger et al. (1994) also show a decrease in cocaine-induced behavior after administration of SR 48692.

These seemingly contradictory effects of SR 48692 are not surprising if the complex actions of NT on dopaminergic activity are taken into account. Previous studies have shown that exogenous NT can exert opposite effects, depending on whether it is administered in the cell body region or in the projection areas of dopaminergic neurons. Injection of NT into the VTA increases locomotor activity as well as dopamine metabolism and release in the nucleus accumbens (Kalivas et al. 1983; Ford and Marsden 1990; Kalivas and Duffy 1990a; Rivest et al. 1991). Conversely, when injected into the nucleus accumbens or the cerebral ventricles, NT reduces the hyperactivity elicited by cocaine and amphetamine (Ervin et al. 1981; Robledo et al. 1993). Intra-accumbens injection of NT also inhibits dopamine release in the nucleus accumbens (Tanganelli et al. 1994). Consequently, the dual effects observed after systemic administration of SR 48692 could depend upon a preferential action of this compound on regions associated with either dopamine perikaya or terminal fields.

Although the evidence presented in this study indicates that endogenous NT facilitates the behavioral hyperactivity induced by cocaine, most likely by activating the mesolimbic dopaminergic system, the mechanism by which SR 48692 alters the activity of dopaminergic neurons remains to be elucidated. We recently showed that chronic administration of SR 48692 for 15 days (at the same dose found to be effective in the present study, 1 mg/kg IP) significantly decreased basal dopamine release in the shell division of the nucleus accumbens (Azzi et al., in press), suggesting that the behavioral effects observed in this study could be secondary to decreased dopamine release in the nucleus accumbens. The inhibitory effect of SR 48692 on cocaine-induced motor activity and dopamine release could be mediated via NT receptors located either presynaptically on dopaminergic terminals in the nucleus accumbens or on dopaminergic cell bodies in the VTA. The ability of exogenous NT to stimulate dopaminergic transmission when applied directly on dopamine neurons in the VTA (Kalivas et al. 1983; Kalivas and Duffy 1990a) favors the latter possibility. Consistent with this hypothesis, the VTA of the rat brain contains a high density of NT- and dopamine-containing neuronal perikarya, as well as a high density of NT receptors, located predominantly on dopaminergic neurons (Hökfelt et al. 1984; Szigethy and Beaudet 1989).

Alternatively, SR 48692 could decrease mesolimbic dopaminergic activity indirectly, by blocking NT receptors on nondopaminergic neurons. For example, GABA, excitatory amino acids and serotonin have been shown to influence the behavioral response to psychostimulants by modulating dopaminergic transmission

in the mesolimbic pathway (Kalivas 1993), and NT has been shown to interact with these neurotransmitter systems (Tanganelli et al. 1994; Ferraro et al. 1995; Jolas and Aghajanian 1996).

In conclusion, the present findings indicate that the specific NT receptor antagonists SR 48692 selectively attenuates locomotor activity and rearing after administration of cocaine. SR 48692 did not affect stereotyped behavior induced by the same drug treatment. These findings strongly support a facilitatory role for endogenous NT in the modulation of dopamine neurotransmission in the mesoaccumbens projection, and suggest that NT receptor antagonists might be useful in reducing certain behavioral effects of psychostimulants.

ACKNOWLEDGMENTS

This research was supported by the BIOMED program from the Commission of the European Community, Project "Stress and Depression" (BMH1-CT94-1108). We thank Onno Meijer and Marcel Schaaf for helpful discussions and Dr. Danielle Gully (Sanofi Recherche) for providing SR 48692. We are particularly grateful to Dr. P. V. Piazza for critical reading of the manuscript.

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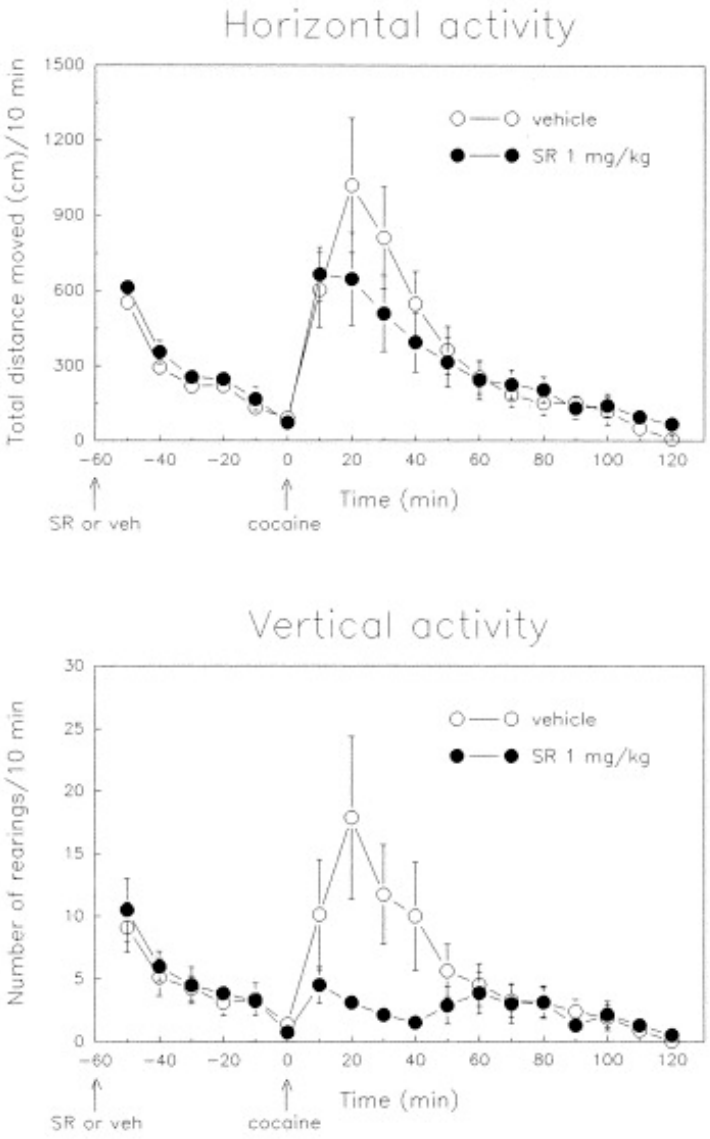

Figure 1. Experiment 1. Effect of an acute injection of SR 48692 on cocaine-induced horizontal and vertical activity. Data are shown as mean ± SEM. SR 48692 (1 mg/kg IP), injected 1 h before cocaine (15 mg/kg IP), decreased the number of rearings induced by the psychostimulant (ANOVA, $p < .05$), without affecting locomotion.

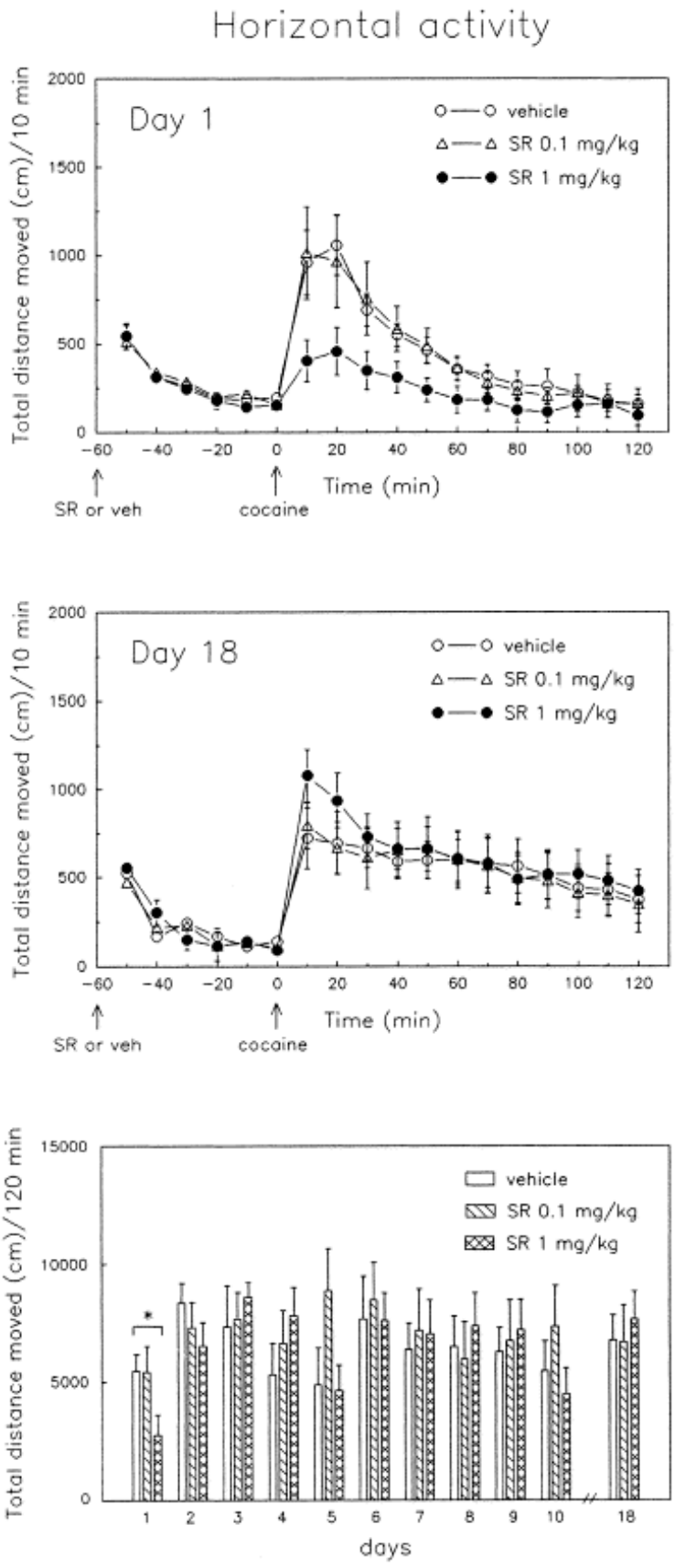

Figure 2. Experiment 2. Effect of repeated SR 48692 administration on horizontal locomotion during daily cocaine challenges. Data are shown as mean ± SEM. The top and middle panels show the time course of horizontal activity on the first (day 1) and the last cocaine challenge (day 18), performed 8 days after cocaine withdrawal. The bottom panel shows the cumulative distance moved on the first 120 min after cocaine administration on each daily cocaine test. Pretreatment with SR 48692 (1 mg/kg) reduced the horizontal activity elicited by cocaine on day 1 (ANOVA, $p < .03$), but did not modify cocaine-induced locomotion on the following cocaine challenges (days 2–10 and 18). Repeated cocaine administration did not induce a progressive increase in horizontal activity. *$p < .05$, comparing SR 48692- to vehicle-treated subjects on each cocaine challenge (ANOVA).

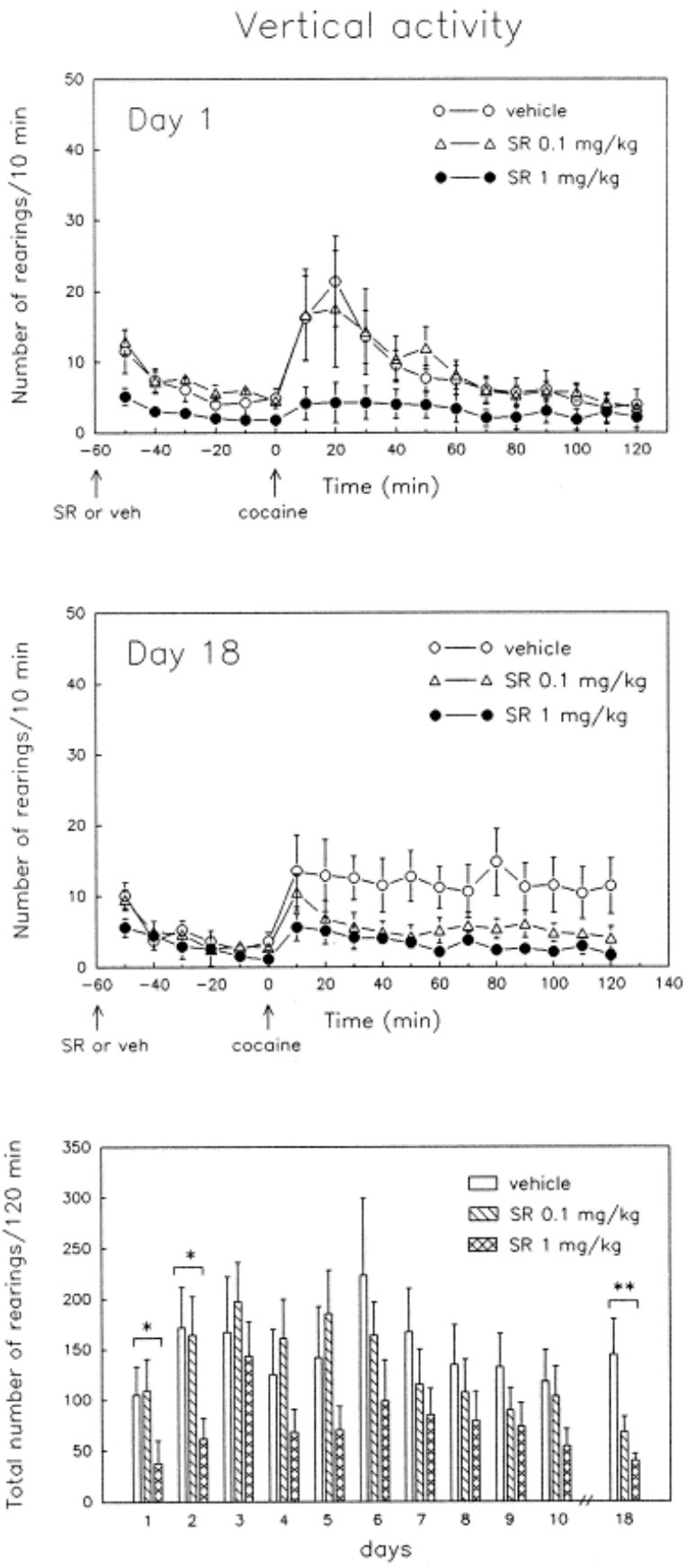

Figure 3. Experiment 2. Effect of repeated SR 48692 administration on vertical activity during daily cocaine challenges. These data were obtained simultaneously with those shown in Figure 2 and correspond to the number of rearings (mean ± SEM). The top and middle panels show the time course of vertical activity on the first (day 1) and the last cocaine challenges (day 18). Bottom panel: Cumulative number of rearings on the first 120 min after cocaine administration on daily cocaine challenges. On day 1, pretreatment with SR 48692 (1 mg/kg) reduced the vertical activity elicited by a novel environment (ANOVA, $p < .03$) and by cocaine ($p < .05$). SR 48692 (1 mg/kg) also decreased rearing in response to cocaine on day 2 ($p < .03$) but had no effect on subsequent cocaine challenges (days 3–10). On day 18 (8 days after cocaine withdrawal), rearing behavior elicited by cocaine was reduced after treatment with SR 48692 1 mg/kg ($p < .01$) and 0.1 mg/kg ($p < .06$). Repeated cocaine administration did not induce a progressive increase in rearing behavior. *$p < .05$, **$p < .01$, comparing SR 48692- to vehicle-treated subjects on each cocaine challenge (ANOVA).

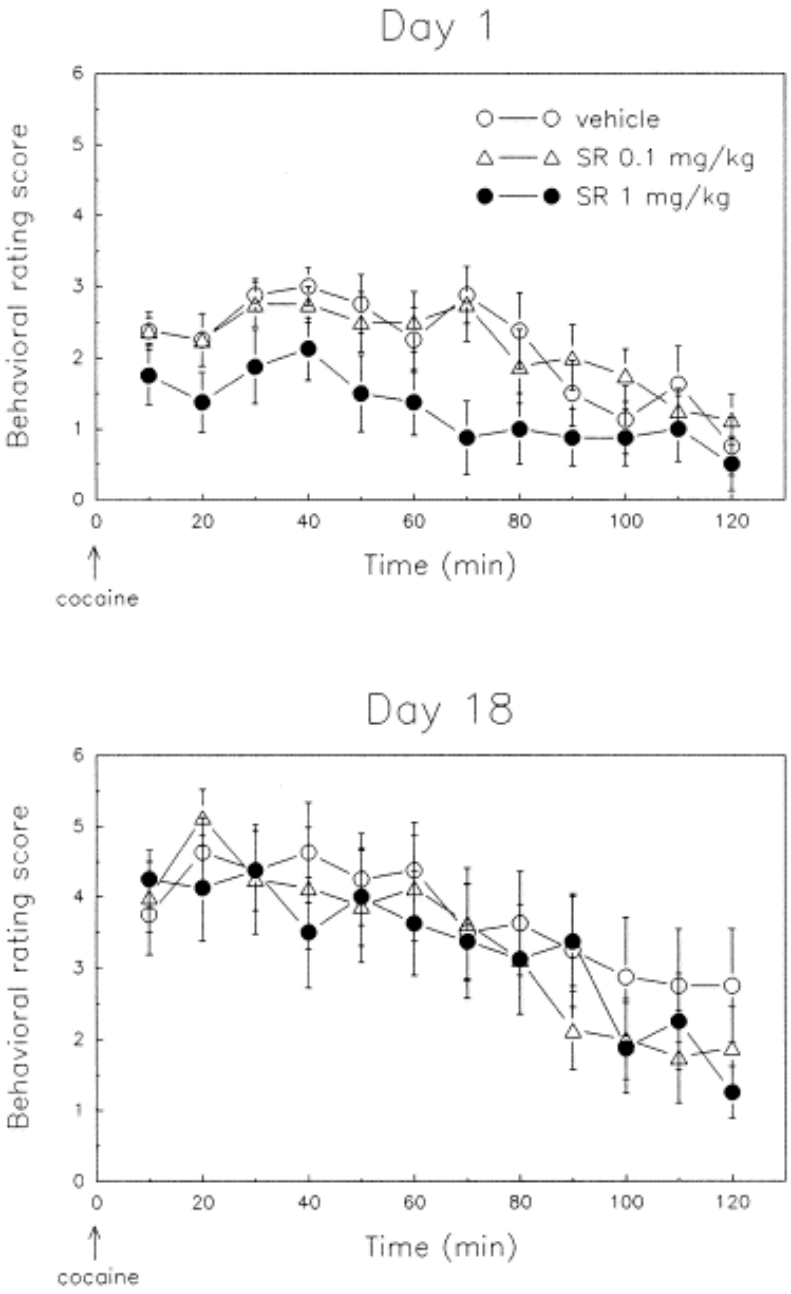

Figure 4. Experiment 2. Effect of repeated treatment with SR 48692 on the behavioral rating after the first cocaine injection (day 1) and after the last cocaine challenge, performed 8 days after discontinuing daily cocaine injections (day 18). The data are shown as the mean ± SEM. On day 1, the low behavioral rating indicated an increase in locomotion and rearing after acute cocaine, which were inhibited by treatment with SR 48692 1 mg/kg (ANOVA, $p < .01$), and no stereotypies were observed. On day 18, there was a clear increase in the behavioral rating (behavioral sensitization) as compared to day 1 (ANOVA, day effect, $p < .001$), indicating the development of stereotypies, which were not affected by treatment with the NT antagonist.

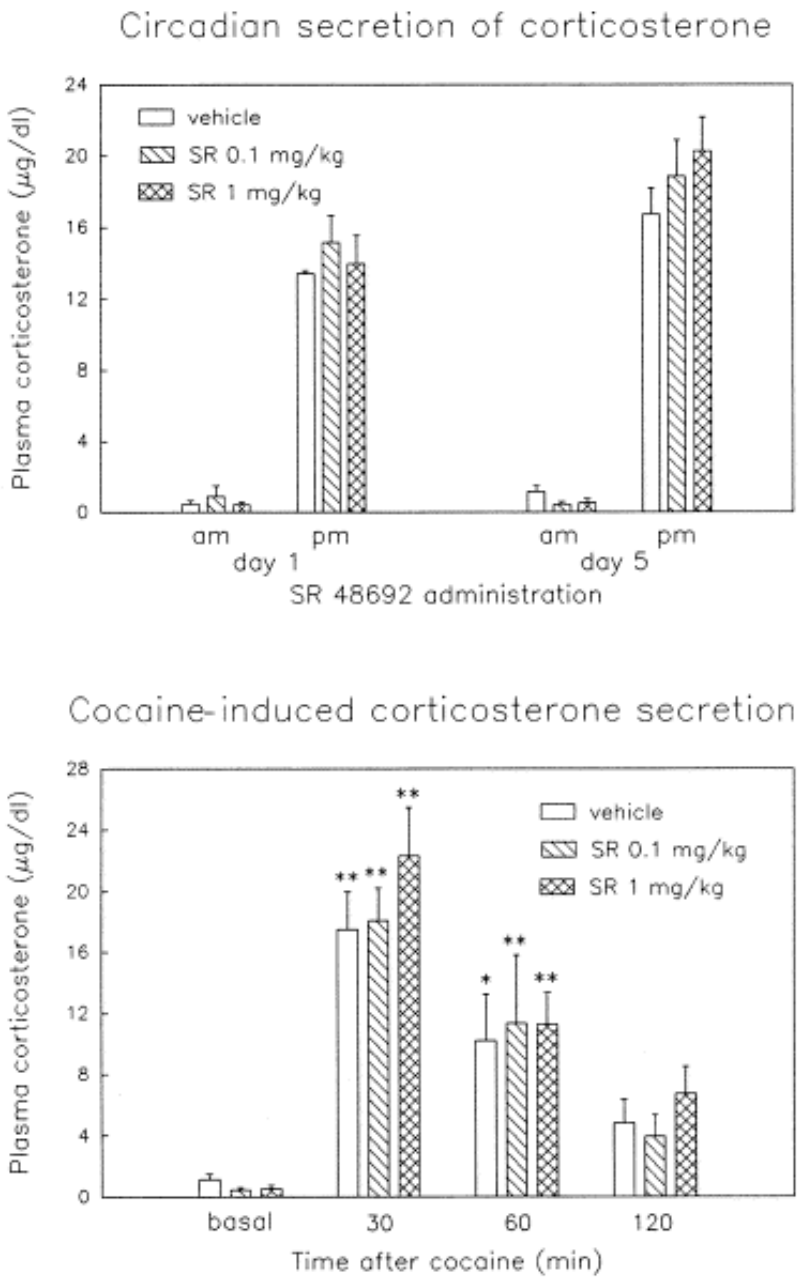

Figure 5. Experiment 3. Effect of SR 48692 pre-exposure and cotreatment on basal and cocaine-stimulated plasma corticosterone levels. Top panel shows the effects of 1 and 5 daily injections of SR 48692 (0.1 or 1 mg/kg IP) or vehicle on the circadian fluctuation of corticosterone plasma levels, on blood samples obtained in the morning (am) and in the evening (pm). Bottom panel shows the corticosterone response to a cocaine challenge (15 mg/kg IP) in rats pretreated with SR 48692 or vehicle for 5 days. Results are expressed as mean ± SEM of plasma corticosterone levels (mg/dl). SR 48692 did not modify the circadian secretion of corticosterone, nor the cocaine-induced release of the hormone. $^{*}p < .05$, $^{**}p < .01$, compared to basal levels within the same treatment group, using ANOVA followed by Tukey test.