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Selective antagonist at D_3 receptors, but not non-selective partial agonists, influences the expression of cocaine-induced conditioned place preference in free-feeding rats

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Abstract

The non-selective dopamine (DA) D_3 partial agonist BP 897 influenced rats' seeking behavior induced by cocaine-associated cues but there are contradictions about its ability to modulate cocaine-induced conditioned place preference (CPP), and mechanisms involved. We therefore re-evaluated its activity on both acquisition and expression of these behaviors, taking into consideration the actual brain concentrations of unchanged drug and its potential active metabolite 1-(2-methoxyphenyl)-piperazine (*o*OCH_3PP), as well as its negative motivational properties. BP 897 induced conditioned place aversion (CPA) at 3 mg/kg, but not at 0.3 and 1 mg/kg. However, in this range of amply spaced doses BP 897 did not affect the acquisition and expression of cocaine (10 mg/kg i.p.) CPP in rats, although its brain concentrations were well above those affecting in vitro D_3 receptors. Concentrations of *o*OCH_3PP were below the limits of quantification of the analytical procedure. As concerns the expression behavior, its structurally and pharmacologically related derivative *N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl]benzo[*b*]furan-2-carboxamide (1 and 3 mg/kg, i.p.) also had no such effect. By contrast, the selective D_3 receptor antagonist SB-277011-A (3 mg/kg, i.p.) antagonized the expression of cocaine-induced CPP, supporting the suggestion that "full" antagonist activity at D_3 receptors is necessary to prevent 10 mg/kg cocaine-induced place conditioning in free-feeding rats.

Keywords: Substance abuse; Cocaine; D_3 receptor; BP 897; Brain concentrations; Conditioned place preference

1. Introduction

Cocaine addiction has been described as a chronic relapsing disease in which periods of intense drug use alternate with periods of abstinence (Gawin and Kleber, 1986; O'Brien et al., 1998). Through repeated association with the use of the drug, environmental stimuli associated with and/or predictive of drugs of abuse acquire strong motivational properties that can trigger drug craving, often leading to relapse to drug taking (Childress et al., 1999; Everitt et al., 2001; Ehrman et al., 1992; Stewart et al., 1984).

Pre-clinical studies show that the non-selective dopamine (DA) D_3 partial agonist BP 897 influenced drug-seeking behavior induced by cocaine-associated stimuli (Pilla et al., 1999; Cervo et al., 2003a). Since BP 897 and its derivative *N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl]benzo[*b*]-furan-2-carboxamide (compound 5g; Campiani et al., 2003) did not alter the response for cocaine self-administration (Pilla et al., 1999; Campiani et al., 2003) it was suggested that D_3 partial agonists may act selectively, reducing the motivational impact of cocaine-associated cues. The interpretation of these findings, however, was confounded by the fact that BP 897 has a complex receptor-binding profile, with antagonistic high affinity for D_2 and D_4 receptors (Pilla et al., 1999; Cussac et al., 2000), and serotoninergic (5-HT_{1A}) and noradrenergic (α_1 and α_2) receptors, whose role in drug-seeking behavior elicited by cocaine-associated stimuli remains to be elucidated.

The conditioned place preference (CPP) paradigm is another model used in drug addiction studies. It consists of an acquisition phase during which the rats receive the drug under study in a distinctive environment (and vehicle in

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another), and an expression phase in which drug-free animals' preference of the environment previously paired with the test compound is evaluated (Bardo and Bevins, 2000; Tzschentke, 1998). Using this paradigm, Duarte et al. (2003) challenged the hypothetical preferential action of non-selective D_3 partial agonist on the motivational impact of cocaine-associated cues. In fact, they reported that BP 897, at doses presumed to be selective for D_3 receptors (0.5–1 mg/kg, i.p.), blocked both acquisition and expression of cocaine (2 mg/kg, s.c.)-induced CPP in food-restricted rats. In contrast to this observation Gyertyán and Gál (2003), using free-feeding animals, found BP 897 aversive, with no effects on acquisition of cocaine (10 mg/kg, i.p.)-induced CPP in rats given similar doses of BP 897.

Because the ability of cues associated with drugs of abuse to elicit CPP may have important implications related to drug "craving" and relapse (Bardo and Bevins, 2000; Hand et al., 1989; Neisewander et al., 1990), considering the inconsistent findings of previous studies (Duarte et al., 2003; Gyertyán and Gál, 2003) we decided to re-examine the effects of BP 897 on the cocaine-induced CPP in free-feeding rats, taking in consideration its negative motivational properties. As far as the expression phase concerns, the effects of BP 897 and its derivative 5g, were compared to those of the selective D_3 antagonist SB-277011-A (Reavill et al., 2000). Finally, to evaluate further the relative importance of DA and other receptors possibly involved in the inhibition of cocaine-seeking behavior by BP 897 and compound 5g, the actual brain concentrations of the compounds were measured. The concentrations of their putative metabolite 1-(2-orthomethoxyphenyl)-piperazine (*o*OCH_3PP) were also preliminarily evaluated since this phenyl-piperazine derivative is known to possess characteristic central pharmacological effects (Minard et al., 1979; Martin et al., 1988).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley CD-COBS rats (Charles River, Italy) weighing 125–150 g (6–7 weeks old) at the beginning of the experiment were used. They were housed two per cage at constant room temperature (21±1 °C) and relative humidity (60%) under a regular light/dark schedule (light 07:30 a.m.–07:30 p.m.) with food and water ad libitum. Animals were allowed to adapt to laboratory conditions for at least a week and were handled for 5 min per day during this period. Testing and training were conducted between 9:30 a.m. and 4:30 p.m. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, 18 Febbraio 1992, Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJL 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

2.2. Drugs

BP 897 and SB-277011-A were generously provided by Bioproject (Paris, France) and Glaxo-Smith-Kline Pharmaceuticals (Verona, Italy), respectively. Compound 5g was synthesized by Prof. G. Campiani as previously described (Campiani et al., 2003). *o*OCH_3PP hydrochloride and cocaine hydrochloride were purchased from Sigma-Aldrich (Milan, Italy) and MacFarlan-Smith (Edinburgh, UK), respectively.

Cocaine, BP 897 and compound 5g were dissolved in 0.9% sterile saline and injected i.p. (2 mL/kg). SB-277011-A was dissolved in 10% of 2-hydroxypropyl-β-cyclodextrin and given in a volume of 2 mL/kg i.p.

2.3. Place conditioning apparatus

The apparatus consisted of four rectangular boxes (80×40×30 cm) with three sides made of wood, one long Plexiglas observation wall, and a wooden lid (Cervo et al., 2002). For the conditioning phase, each box was divided into two equal-sized compartments by a sliding wall. One compartment was painted gray and the other black, with vertical white stripes 3 cm wide; the lids were painted to match their compartment. The two compartments were fitted with distinctive metal floors, one a loose mesh and the other a much closer grid.

For pre-conditioning and test sessions the partition between the two compartments was raised 12 cm off the floor and a 5×3 cm smooth aluminium platform was inserted along the line between them. Two infrared sensors, one for each compartment, set to detect any heat change due to a moving rat of 200 g or more, were assembled on the partition. The sensors were operated by a Paul Fray Ltd computer recording the time the animal spent in the different compartments (by activation of a single sensor), and in the central zone (activation of both sensors) as well as the number of transition between compartments.

The testing room was closed and ventilated, with dim indirect lighting provided by one 15 W incandescent white bulb hanging about 50 cm above the boxes. A loudspeaker about 1 m above the boxes delivered white noise.

2.4. Place conditioning procedure

The procedure consisted of three phases (Cervo et al., 2002). On the first day, before any drug treatment (pre-conditioning period, phase I), each rat was allowed to explore the two compartments of the box for 15 min. The time each animal spent in the two compartments was recorded. No difference was found in the unconditioned preference for gray or black/white compartments [mean times (*s*)±S.E.M. spent in the gray and black/white sections for all animals ($n=344$) were, respectively, 399±12 and 392±12, $P>0.05$, with mean times spent in the gray and black/white compartments in individual experiments ranging from 371.6±15.0 to 432.9±12.8 and from 370.8±18.6 to 427.2±10.1, $P>0.05$, paired Student's *t*-test]. Rats were therefore randomly assigned to

treatment groups and conditioning compartments. Care was taken to ensure that all treatments were matched as closely as possible between compartments.

The schedule during the conditioning phase (phase II) consisted of four injections of drug or vehicle on alternate days. The interval between conditioning days was no less than 24 h and no more than 72 h, so rats receiving eight conditioning sessions were not conditioned during the week-end. This procedure has been commonly used in our laboratory and found not to influence cocaine-induced CPP (Cervo et al., 1996, 1997, 2002). In particular, on Monday rats had the pre-conditioning session, and from Tuesday through Friday they received the first four conditioning sessions, two with cocaine and two with saline. Saturday and Sunday they were left undisturbed in the home cage. The following week, Monday through Thursday, rats received the second four conditioning sessions and on Friday the test session was held.

On odd conditioned days rats were injected with the study drug immediately before being confined in the randomly designated drug side. On the in-between days, each rat was injected with vehicle and confined to the opposite side. This daily order of exposure to drug and vehicle was matched for the rats in each group. Control animals received saline in both compartments.

On the test day (phase III) neither drug nor vehicle was injected. Each rat was placed in the center of the smooth aluminium platform separating the two compartments, with free access to both sides of the box, and the time spent in each compartment was recorded over a 15-min period. The difference in time spent in the drug-paired and the vehicle-associated compartments in the final test session was taken as a measure of place conditioning.

2.5. Evaluation of BP 897 motivational properties

To assess whether BP 897 possess motivational properties 48 rats underwent the place conditioning procedure, receiving four injections of drug or vehicle on alternate days. Separate groups of 12 rats received intraperitoneally (i.p.) 0.3, 1.0 or 3 mg/kg BP 897 immediately before being confined to the randomly designated drug side for 40 min. On alternate days, rats received the BP 897 vehicle. Control animals received vehicle in both the randomly assigned drug and vehicle compartments.

2.6. Influence of BP 897 on the acquisition and expression of cocaine-induced CPP

To evaluate the ability of BP 897 to modify the cocaine-induced CPP, separate groups of 12 rats received eight injections of 10 mg/kg i.p. cocaine (as free base) or vehicle on alternate days immediately before exposure to the designated drug-associated side for 40 min. The dose of cocaine and the conditioning time were selected on the basis of previous experiments in which it induced a significant reliable CPP in control animals (Cervo et al., 1996, 1997, 2002). The study of the effects of BP 897 on a dose–response curve was limited by the fact that in our experimental set-up lower doses of cocaine (2.5 and 5 mg/kg) are threshold doses that have been used to be enhanced by particular treatment (Cervo et al., 2002).

Since BP 897 showed aversive properties (see Results; Duarte et al., 2003; Gyertyán and Gál, 2003) its effect on the acquisition of cocaine-induced CPP was studied in separate groups of rats which received 0.3, 1 and 3 mg/kg BP 897 or vehicle, 30 min before the cocaine pairing and the vehicle pairing. This procedure is commonly used when the net motivational properties of a drug is studied in combination with a compound having aversive properties (Shippenberg and Herz, 1988; Cervo and Samanin, 1995; Cervo et al., 1997, 2002). By conducting all conditioning sessions in the presence of BP 897 its negative motivational properties should be associated to both compartments of the CPP apparatus, thus permitting an assessment of the BP 897-induced changes in the motivational properties of cocaine. Control animals received vehicle in both the randomly assigned drug and vehicle compartments.

To see how the various compounds influenced the expression of cocaine-induced CPP, different groups of 10 rats each underwent cocaine conditioning and received the various compounds only 30 min before testing. Thus, separate groups of rats received 0.3, 1 and 3 mg/kg BP 897 or vehicle. Doses and pre-treatment time were chosen on the basis of previous findings that BP 897 reduced various behaviors induced by cocaine-associated cues (Pilla et al., 1999; Cervo et al., 2003a; Duarte et al., 2003; Gyertyán and Gál, 2003; Le Foll et al., 2002).

2.7. Effects of compound 5g and SB-277011-A on the expression of cocaine-induced CPP

In an attempt to further verify the role of dopamine D_3 receptors in the expression of cocaine-induced CPP, in separate experiments the effects of compound 5g and SB-277011-A or respective vehicles was evaluated. Twelve groups of ten rats each underwent cocaine-conditioning and received 1 or 3 mg/kg compound 5g, 0.3 or 3 mg/kg i.p; SB-277011-A or respective vehicle, 30 min before the test session. The doses, route of administration and pre-treatment time were chosen on the basis of previous findings showing their activity in reducing environmental stimuli-induced cocaine-seeking behavior (Campiani et al., 2003; Di Ciano et al., 2003; Cervo et al., 2003b; Vorel et al., 2002).

2.8. Chemical analysis

Thirty minutes after the end of the pharmacological studies, some rats injected i.p. with 0.3, 1 and 3 mg/kg BP 897 or 1 and 3 mg/kg compound 5g were killed by decapitation under light anesthesia (45 min after the drug administration) and trunk blood was collected in heparinized tubes and frozen. Brains and blood from animals receiving 10 mg/kg of either compounds, which were treated together with the others but not behaviorally tested (see Results), were also collected.

The brain was rapidly excised from the skull and immediately frozen. Plasma and tissue samples were stored at −80 °C until analysis. Plasma and brain concentrations of unchanged compounds and *o*OCH_3PP were determined by high-performance liquid chromatography with UV detection (220 nm). Briefly, 0.5 mL of plasma was adjusted to pH 8–9 and then extracted with diethylether, after adding the internal standard (WAY100,635). Brain tissue was homogenized (5 mL g^{-1}) in HCl 0.1 M containing 10% of CH_3CN and a volume containing approximately 200 mg of tissue was centrifuged. The precipitate was re-dissolved in 0.5 mL of the HCl–CH_3CN solution and re-centrifuged. The supernatants were combined and after adjusting the pH to 8–9 were extracted twice with diethylether. The organic phase was dried, the residue was dissolved in the mobile phase and injected into the chromatographic column (CS-5A Cyano, spheri-5 column; 250 × 4.6 mm). The mobile phase was $CH_3CN:CH_3OH:0.01M(NH_4)H_2PO_4$; 55:5:40 v/v, brought to pH 4.5 with H_3PO_4, delivered isocratically at a flow-rate of 1 mL min^{-1}. The retention times were 13 min for *o*OCH_3PP, 14.9 min for BP 897 (14.1 min for compound 5g) and 18.5 min for the internal standard. The limit of quantification was about 50 ng/mL or 125 ng/g. At these concentrations, the coefficients of variation (C.V.) were between 10–20%, and higher concentrations gave C.V. less than 10% for all compounds in both tissues.

2.9. Statistical analysis

To exclude any unconditioned preference for one side or the other of the apparatus, we compared the time spent by rats in the black/white and gray compartments during the pre-conditioning session using the paired Student's *t*-test. One-way analysis of variance followed by Dunnett's test was used to assess whether BP 897 had motivational properties when administered alone. The effects of BP 897, compound 5g and SB-277011-A on cocaine place conditioning were analyzed by two-way ANOVA followed by Tukey's test. The same test was used to determine any change induced by the different treatments on the time the animals spent on the central zone of the CPP apparatus.

3. Results

3.1. BP 897 motivational properties

Fig. 1 shows the effects of 0.3, 1 and 3 mg/kg BP 897 on place conditioning in rats. One-way ANOVA showed a significant effect of treatment ($F(3,44)=3.0$, $P<0.05$). Post hoc comparisons indicated that 3 mg/kg BP 897 induced a clear CPA, treated animals spending significantly less time in the drug-associated compartment ($P<0.05$ compared to vehicle-treated group, Dunnett's test).

In agreement with previous studies (Pilla et al., 1999) higher doses of BP 897 (10 mg/kg) induced catalepsy in rats and were therefore not used in this conditioning paradigm.

3.2. Influence of BP 897 on the acquisition and expression of cocaine-induced CPP

Fig. 2 shows the effects of BP 897 (0.3, 1 or 3 mg/kg i.p.) on the acquisition (A) and expression (B) of cocaine (10 mg/kg i.p.) CPP. In both experiments two-way ANOVA indicates a significant effect of cocaine ($F_{\text{acquisition}}(1,88)=27.5$, $P<0.01$ and $F_{\text{expression}}(1,88)=42.8$, $P<0.01$) but not of BP 897 ($F_{\text{acquisition}}(3,88)=0.3$, $P<0.05$ and $F_{\text{expression}}(3,88)=0.2$, $P<0.05$). No significant interaction BP 897 × cocaine were found ($F_{\text{int acquisition}}(3,88)=0.2$, $P>0.05$ and $F_{\text{int expression}}(3,88)=0.1$, $P>0.05$, two-way ANOVA). Post hoc comparisons by Tukey's test revealed a significant increase in the time the animals spent in the cocaine-associated compartment ($P<0.01$ compared to vehicle+vehicle-treated groups, Tukey's test). None of the doses of BP 897 showed any motivational properties ($P>0.05$ compared to vehicle+vehicle-treated groups), or modified the effects of cocaine ($P>0.05$).

3.3. Effects of compound 5g and SB-277011-A on the expression of cocaine-induced CPP

Fig. 3 shows the effects of compound 5g (1 or 3 mg/kg i.p.) (A) and SB-277011-A (0.3 or 3 mg/kg i.p.) (B) on the expression of cocaine CPP. In both experiments two-way ANOVA found a significant effect of cocaine ($F_{\text{cocaine}}(1,54)=17.6$, $P<0.01$ and $F_{\text{cocaine}}(1,54)=7.6$, $P<0.01$ in compound 5g and SB-277011-A experiments, respectively) but not of the pre-treatment ($F_{\text{compound 5g}}(2,54)=0.6$, $P>0.05$ and $F_{\text{SB-277011-A}}(2,54)=1.4$, $P>0.05$). A significant interaction between SB-277011-A and cocaine ($F_{\text{SB-277011-A}}(2,54)=3.6$, $P<0.05$) but not between compound 5g and cocaine ($F_{\text{compound 5g}}(2,54)=0.1$, $P>0.05$) was found. Post hoc comparison indicated that 3 mg/kg SB-277011-A completely antagonized the expression of cocaine CPP ($P<0.05$ compared to vehicle+cocaine treated group, Tukey's test).

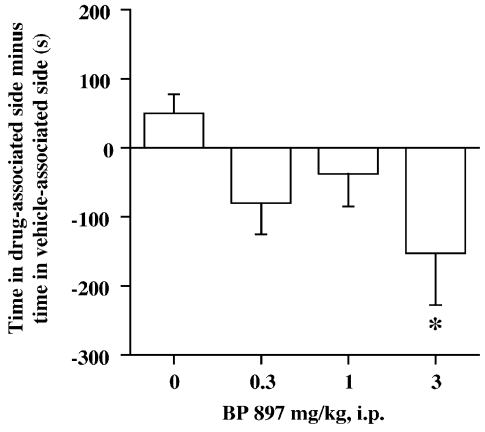

Fig. 1. Effects of BP 897 on place conditioning in rats. Histograms show the mean ± S.E.M of the time spent by twelve rats in the drug-paired side minus time in the vehicle paired side. Data were analyzed by one-way ANOVA followed by Dunnett's test. *$P<0.05$ vs. vehicle-treated group, Dunnett's test.

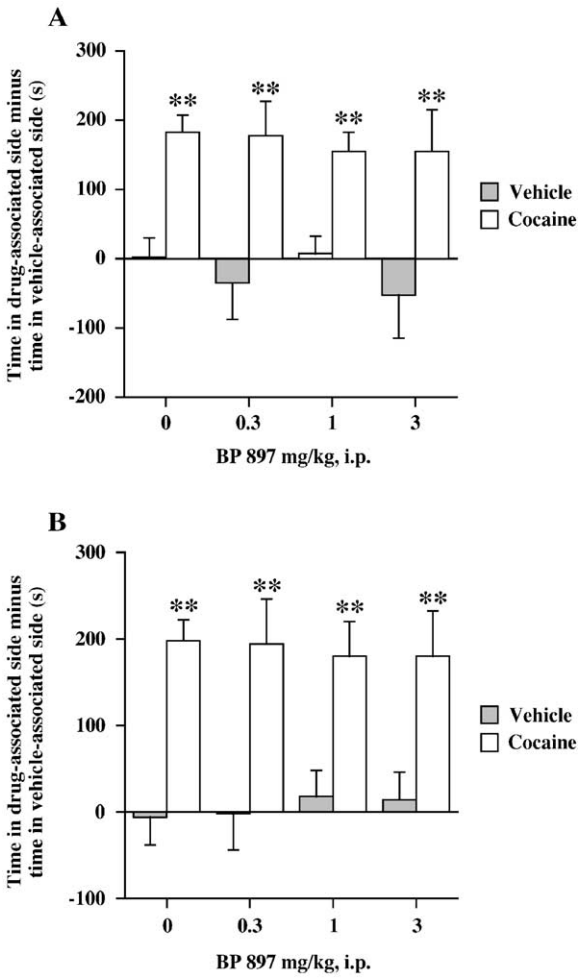

Fig. 2. Effects of BP 897 on the acquisition (A) and expression (B) of cocaine-induced conditioned place preference. Histograms show the mean±S.E.M of the time spent by at least ten rats in the drug-paired side minus time in the vehicle-paired side. Data were analyzed by two-way ANOVA followed by Tukey's test. $^{**}P<0.01$ compared to respective vehicle-treated group, Tukey's test.

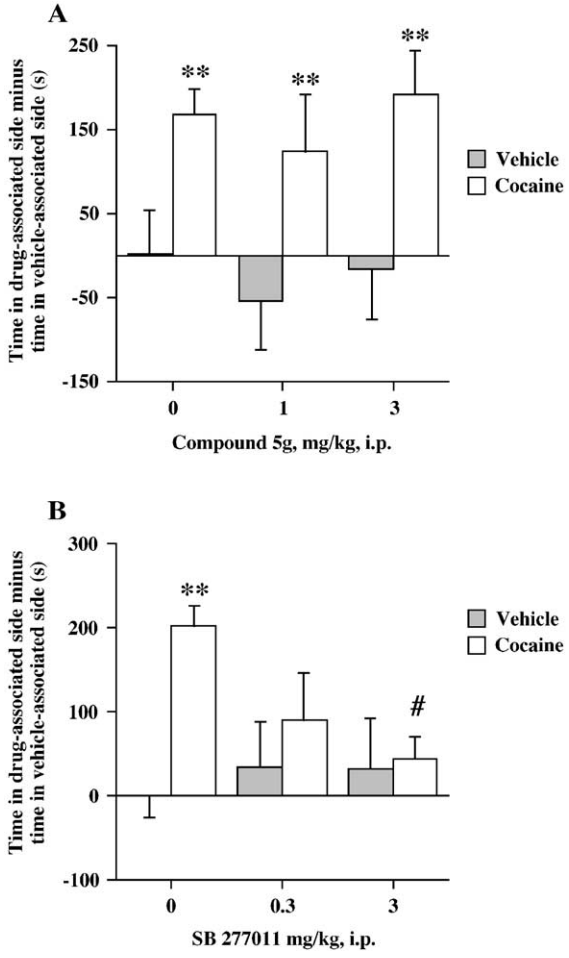

Fig. 3. Effects of compound 5g (A) or SB-277011-A (B) on expression of cocaine-induced conditioned place preference. Histograms show the mean± S.E.M of the time spent by ten rats in the drug-paired side minus time in the vehicle-paired side. Data were analyzed by two-way ANOVA followed by Tukey's test. $^{**}P<0.01$ compared to respective vehicle-treated group, Tukey's test. $^{\#}P<0.05$ compared to cocaine conditioned+vehicle-treated group, Tukey's test.

In all studies the animals spent from 58.0±11.6 to 92.4±15.5 s in the central zone (platform). This time was not statistically different in the various experimental groups ($P>0.05$, Dunnett's or Tukey's test).

Plasma and brain concentrations of BP 897 and its structurally related compound 45 min after dosing, i.e. approximately at the end of the place conditioning paradigm, are shown in Table 1. Plasma concentrations of compound BP 897 were essentially dose-related, ranging from less than 0.1 at the lowest doses to 0.8 μM at 10 mg/kg. Brain concentrations reflected plasma concentrations with mean brain-to-plasma distribution ratios consistently around 4 at the higher doses.

The metabolite oOCH$_3$PP was undetectable in plasma and brain after the doses used in the CPA and CPP paradigms. However, it became detectable after 10 mg/kg BP 897 in rat brain, where it approached 20% of the parent drug concentrations on a molar basis. Plasma concentrations were below the limit of quantification, in agreement with previous reports of a marked concentration of phenyl-piperazine derivatives in rodent brain (Caccia and Garattini, 1990).

Mean plasma concentrations of compound 5g were also dose-related within the range of doses tested in the CPP paradigm. Mean brain concentrations were about three times than those in plasma at the end of the test. Brain concentrations

Table 1
Plasma and brain concentrations of compounds BP 897 and its derivative 5g after intraperitoneal doses in rat

Compound	Dose (mg/kg)	Concentrations (nmol mL^{-1} or g^{-1})	
		Plasma (mean±S.D.)	Brain (mean±S.D.)
BP 897	0.3	<0.1	<0.2
	1	<0.1	0.27±0.02
	3	0.21±0.05	0.84±0.08
	10	0.81±0.16	3.53±0.61
			(0.74±0.18)[a]
Compound 5g	1	<0.1	<0.2
	3	0.15±0.01	0.50±0.05
	10	0.58±0.39	1.59±1.19

Results are mean±S.D. of three rats.
[a] Brain concentrations of 1-(2-methoxyphenyl)piperazine (oOCH$_3$PP).

of *o*OCH_3PP were always below the limit of quantification at these doses of compound 5g, and at the high dose too.

These results suggested that *o*OCH_3PP does not significantly contribute to the CPA induced by BP 897 in rat.

4. Discussion

A main aim of this study was to verify whether BP 897 influences the rewarding effects of cocaine in place conditioning. In view of its aversive properties the compound was given before cocaine pairing and saline pairing sessions to study the net motivational properties (acquisition of CPP) of cocaine (Shippenberg and Herz, 1988; Cervo and Samanin, 1995; Cervo et al., 1996, 1997, 2002). Initial studies confirmed that this non-selective partial agonist of D_3 receptors induced CPA in rats (Duarte et al., 2003; Gyertyán and Gál, 2003) although, possibly because of experimental differences, the effective dose-range is still not clear. We found this effect at 3 mg/kg in Sprague–Dawley rats whereas Duarte et al. (Duarte et al., 2003) found the compound produced CPA at 1 mg/kg only in a dose range from 0.05 to 2 mg/kg in Wistar AF rats. Gyertyán and Gál (Gyertyán and Gál, 2003) observed a dose-dependent CPA after conditioning Sprague–Dawley rats with BP 897 at 0.5–1 mg/kg but did not evaluate the effect of higher doses.

The "nature" as well as the neurochemical mechanism(s) underlying the negative motivational activity of BP 897 is still not clear. The fact that this action is shared by low, possibly pre-synaptic doses of preferential D_3 agonists (e.g. 7-OH-DPAT and the moderately selective PD-128907) (Khroyan et al., 1995, 1997; Gyertyán and Gál, 2003) but not by D_3 antagonists (e.g. SB-277011-A and the less selective L-nafadotride) (Vorel et al., 2002; Gyertyán and Gál, 2003; Chaperon and Thiebot, 1996) supports the assumption that it could be caused by dopamine D_3 agonism. However, BP 897 has nanomolar in vitro affinity for D2 recombinant human receptors (Pilla et al., 1999) and in vivo in rats it behaves as a D2 antagonist (Pilla et al., 1999; Wood et al., 2000; Wicke and Garcia-Ladona, 2001) although at doses 3–10 times those causing CPA, as also underlined by other authors (Gyertyán and Gál, 2003; Duarte et al., 2003). Moreover, it has nanomolar affinities in vitro for 5-HT_{1A} receptors (where it acts as a partial agonist) and for α_{1A}- and α_{2A}-adrenoceptors (as an antagonist) (Cussac et al., 2000).

According to the pharmacokinetic studies BP 897 concentrates in brain tissue achieving concentrations (0.3–0.8 μM, assuming 1 g of tissue equivalent to 1 mL of water) after doses causing CPA (1–3 mg/kg; Gyertyán and Gál, 2003; Duarte et al., 2003, and present results) theoretically more than sufficient for action on all receptors bound in vitro (Ki about 0.9 nM for D_3 receptors and 60–80 nM for D_2 and 5-HT_{1A} receptors and α_{1A}- and α_{2A}-adrenoceptors) (Garcia-Ladona and Cox, 2003). However, the free fraction of the compound in brain tissue is still unknown.

Although an antagonistic action at D_2 receptors is improbable, because preferential D_2 antagonists such as sulpiride, haloperidol, raclopride, α-flupentixol, pimozide do not induce CPA in the rat (see Tzschentke, 1998), there is still too little, often contradictory data to exclude the involvement of other receptors. In fact, high doses of the selective 5-HT_{1A} agonist 8-OH-DPAT induced CPA (Papp and Willner, 1991; Shippenberg, 1991), even though at low doses it induced CPP, but there is no information on the motivational properties of 5-HT_{1A} antagonists as measured in the place conditioning paradigm. Information is also confusing on the motivational properties of selective α_{1A} or α_{2A} antagonists; the non-selective α_1 antagonist prazosin did not induce CPA (Cervo et al., 1993) but the selective α_2 antagonist yohimbine did (File, 1986) while idazoxan, another non-selective α_2 antagonist, induced CPP (Cervo et al., 1993).

At the dose tested BP 897 had no effect on the acquisition of cocaine-induced CPP. This was not surprising because it was already known that doses of 0.5 to 1 mg/kg BP 897 do not interfere with the acquisition of 10 mg/kg i.p. cocaine-induced CPP in Sprague–Dawley rats (Gyertyán and Gál, 2003). Surprisingly, however, neither BP 897 (0.3–3 mg/kg) nor its structurally and pharmacologically related compound 5g (1–3 mg/kg) modified the expression of cocaine CPP although, as mentioned, both compounds reached brain concentrations more than sufficient for an action on D_3 receptors (Pilla et al., 1999). In contrast to this observation, Duarte et al. (2003) found dose-dependent reduction of both cocaine acquisition and expression with BP 897 over a dose range of 0.25 to 1 mg/kg. However, they tested BP 897 in Wistar AF rats at a cocaine dose of 2 mg/kg s.c., whereas our studies were in Sprague–Dawley rats given 10 mg/kg cocaine i.p. Gyertyán and Gál (2003) found no effects on acquisition of cocaine-induced CPP (10 mg/kg, i.p.) over the same range of BP 897 doses in Sprague–Dawley rats, further suggesting that some procedural differences may account for the different responses of BP 897 in the place conditioning paradigm, including rat strain and cocaine dose and route of administration.

Also, Duarte et al. (2003) used animals that were food-restricted and the enhancement of drug reward by chronic food restriction has long been known (Carr, 2002). Cocaine differently affects the behavior of rats with restricted food access, making them more sensitive to the induction of sensitization as well as to CPP (Bell et al., 1997). Food-restricted rats show an enhanced response to cocaine-induced increase of dialysate DA in the core, but not in the shell of the nucleus accumbens (NAc; Cadoni et al., 2003). In view of the hypothesized importance of DA mechanisms in the NAc core sub-region in seeking behavior induced by cocaine-associated cues (Fuchs et al., 2004; Ito et al., 2004; McFarland and Kalivas, 2001; McFarland et al., 2004) it is possible that BP 897 acts as a D_3 antagonist in abolishing cocaine-induced CPP in food-restricted rats because of the high availability of DA, assuming that D_3 receptors mediate this effect. This last interpretation seems to be supported by the studies with the selective D_3 antagonist SB-277011-A. This compound dose-dependently abolished the acquisition and expression of cocaine-induced CPP (Vorel et al., 2002), a finding reproduced in our paradigm. Its effects seem to be specific for drugs of abuse since it was found to antagonize heroin-(Ashby et al., 2003) but not food-induced CPP (Vorel

et al., 2002). This, together with evidences of its effectiveness in animal models of cocaine-(Di Ciano et al., 2003; Cervo et al., 2003b), nicotine-(Andreoli et al., 2003a), and alcohol-seeking (Andreoli et al., 2003b) strongly suggest that full blockade of D_3 receptors modulate drug-seeking behaviors in rats. Accordingly, an effect of SB-277011-A on stress-induced reinstatement of cocaine self-administration has been recently reported (Xi et al., 2004).

In conclusion, despite the scarce and contradictory information in the literature, the present observation suggests that the non-selective partial D_3 agonist BP 897 and, as concerns the expression of CPP, its derivative 5g do not affect the activity of 10 mg/kg cocaine-induced CPP in free-feeding rats. By contrast, SB-277011-A completely prevents the expression of cocaine-induced CPP, supporting the suggestion that "full" antagonist activity at D_3 receptors is necessary to modify the rewarding properties of cocaine as measured in the CPP paradigm in rat.

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