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RESEARCH PAPER

Teneurin C-terminal associated peptide-1 blocks the effects of corticotropin-releasing factor on reinstatement of cocaine seeking and on cocaine-induced behavioural sensitization

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BACKGROUND AND PURPOSE

The stress-related neuropeptide, corticotropin-releasing factor (CRF), has become an important focus of studies of cocaine addiction, and in particular, the effects of stress on cocaine-related behaviours. A recently discovered peptide system, the teneurin C-terminal associated peptides (TCAP), has been implicated in the regulation of the stress response, via a CRF-related mechanism. Here we have determined whether treatment with TCAP-1, a synthetic analogue of TCAP, modulated two cocaine-related behaviours induced by CRF: reinstatement of cocaine seeking, and expression of cocaine-induced behavioural sensitization.

EXPERIMENTAL APPROACH

In Experiment 1, rats trained to self-administer cocaine were given acute or repeated (once daily for 5 days) i.c.v. injections of TCAP-1 before tests for reinstatement in response to CRF (105 pmol, i.c.v.), intermittent footshock stress (0.9 mA), or cocaine (15 mg·kg^{-1}, i.p.). In Experiment 2, rats pre-exposed to cocaine (15–30 mg·kg^{-1}, i.p.) or saline for 7 days were treated with TCAP-1 (once daily for 5 days; i.c.v.) and subsequently tested for locomotor responses to CRF (105 pmol, i.c.v.) or cocaine (15 mg·kg^{-1}, i.p.).

KEY RESULTS

Five day pre-exposure with TCAP-1 blocked CRF-, but not footshock- or cocaine-induced reinstatement of cocaine seeking; acute pretreatment with TCAP-1 was without effect in all test conditions. Similarly, repeated TCAP-1 pre-exposure blocked the cocaine-sensitized locomotor response to CRF, but not to cocaine.

CONCLUSIONS AND IMPLICATIONS

Repeated TCAP-1 exposure induced robust and selective inhibition of cocaine-related behavioural responses to CRF, suggesting that TCAP-1 may normalize signalling within CRF systems dysregulated by cocaine exposure.

Abbreviations

BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; CRF, corticotropin-releasing factor; EXT, extinction; TCAP, teneurin C-terminal associated peptides; VTA, ventral tegmental area

Introduction

The teneurin C-terminal associated peptides (TCAP) are a recently discovered family of endogenous neuropeptides (Qian *et al.*, 2004; Wang *et al.*, 2005a). The TCAP family consists of four highly conserved 40 or 41 amino acid bioactive peptides (TCAP-1-4), each found at the carboxy terminus of one of the four teneurin transmembrane proteins (Tucker and Chiquet-Ehrismann, 2006). The primary structures of the TCAPs share about 20% sequence identity with corticotropin-releasing factor (CRF) (Lovejoy *et al.*, 2006), an important neuropeptide in the regulation of stress responses (Owens and Nemeroff, 1991; Sawchenko *et al.*, 1993; Heinrichs and Koob, 2004). TCAP-1 mRNA is widely expressed in the adult rat brain, including within forebrain and limbic regions critical in the regulation of stress and anxiety (Wang *et al.*, 2005a). Moreover, TCAP-1 is found in high concentrations in brain regions rich in CRF neurons and/or receptors, such as the central and basolateral nuclei of the amygdala, ventromedial nucleus of the hypothalamus, hippocampus and piriform cortex (Aguilera *et al.*, 2004; Wang *et al.*, 2005a)

Recently, synthetic TCAP-1 (serial #: PT00114) has been found to modulate several behavioural and neuronal effects of CRF in a primarily, though not exclusively (Tan *et al.*, 2008), inhibitory manner. For example, 5 days of intravenous TCAP-1 injections decreased CRF-induced anxiety in the open field and elevated plus maze (Al Chawaf *et al.*, 2007), and 5 days of i.c.v. TCAP-1 injections blocked CRF-induced increases in acoustic startle responses (Tan *et al.*, 2008). Furthermore, acute i.c.v. injections of TCAP-1 attenuated CRF-induced expression of c-fos, a widely used marker of neuronal activation (Curran and Morgan, 1995), in the hippocampus, amygdala and other limbic brain regions (Tan *et al.*, 2009). Based on these and similar findings, it has been proposed that TCAP in the CNS may serve as an endogenous neuropeptide to attenuate the actions of CRF and, thereby, influence behaviour (Lovejoy *et al.*, 2009).

Over the past two decades, the role of stress-related neuropeptides in substance abuse, particularly in the neurobiological mechanisms mediating relapse to drug use, has been a major focus of investigation (Shalev *et al.*, 2010). Most notably, the role of CRF in cocaine dependence has been extensively examined. Indeed, central actions of CRF have been shown to be critically involved in some of the long-lasting behavioural effects of prior cocaine experience. For example, in an animal model of relapse, known as the reinstatement procedure, CRF has been found to mediate the reinstatement of cocaine seeking in response to acute footshock stress after extensive extinction training and prolonged drug-free periods (Erb *et al.*, 1998). The role of CRF in footshock-induced reinstatement has been localized to the bed nucleus of the stria terminalis (BNST; Erb and Stewart, 1999), central nucleus of the amygdala (CeA; Erb *et al.*, 2001) and ventral tegmental area (VTA; Wang *et al.*, 2005b). Moreover, central injections of CRF itself into the lateral ventricles (Erb *et al.*, 2006), BNST (Erb and Stewart, 1999), or VTA (Wang *et al.*, 2007), reinstate cocaine seeking.

As in the reinstatement of cocaine seeking, CRF has also been implicated in the expression of long-term behavioural sensitization to cocaine. For example, repeated injections of cocaine, according to a regimen known to produce long-lasting enhancement of locomotion in response to a subsequent cocaine challenge, have been found to induce a potentiated locomotor response to subsequent i.c.v. injections of CRF (Erb *et al.*, 2003). Moreover, in cocaine-sensitized rats, i.c.v. pretreatment with the non-selective CRF receptor antagonist, D-Phe $CRF_{12\text{-}41,}$ or the selective CRF-1 receptor antagonist, CP 154 526, blocks the expression of behavioural sensitization to a subsequent cocaine challenge (Erb and Brown, 2006; Przegaliński *et al.*, 2005). Although the relationship between reinstatement of drug seeking and drug-induced behavioural sensitization is unclear (e.g. De Vries *et al.*, 1998; 2002; Deroche *et al.*, 1999; Vezina, 2004; Knackstedt and Kalivas, 2007; Lenoir and Ahmed, 2007), the argument has been made that the latter phenomenon reflects a progressive enhancement in the rewarding and incentive motivational properties of the drug that may in fact contribute to relapse potential (Robinson and Becker, 1986; Robinson and Berridge, 1993). From this perspective, it is of interest to study common features of the mechanisms contributing to reinstatement of cocaine seeking and to the expression of cocaine-induced behavioural sensitization.

The rationale for the present series of experiments was based on the demonstrated role of CRF in mediating some long-lasting behavioural effects of cocaine, the known functional and neuroanatomical convergence of central CRF and TCAP-1 systems, and evidence that pretreatment with TCAP-1 can act to alter behavioural responses to CRF. In a first set of experiments, we determined the effects of acute and repeated TCAP-1 pre-exposure on the reinstatement of cocaine seeking induced by i.c.v. injections of CRF, exposure to intermittent footshock stress and priming injections of cocaine. In a second set of experiments, we tested the effects of repeated TCAP-1 pre-exposure on the expression of cocaine-induced behavioural sensitization to challenge injections of CRF and cocaine. Overall, our findings demonstrate robust and selective inhibitory effects of repeated TCAP-1 exposure on cocaine-related behavioural responses to CRF.

Methods

Animals

All animal care and experimental procedures were in accordance with Canadian Council of Animal Care guidelines and approved by the University of Toronto animal care committee.

Experiment 1: acute (Exp. 1A) and repeated (Exp. 1B) TCAP-1 pre-exposure on reinstatement of cocaine seeking

A total of 62 male Long–Evans rats (Charles River, Montreal, QC; 275–300 g initial weight) were used in Experiments 1A and B. Rats were individually housed in plastic cages in a temperature- (21 ± 1°C) and humidity-controlled vivarium, and maintained on a reverse light–dark schedule (lights on 1900–0700) with free access to water and standard laboratory rat chow.

Surgery. Under isoflurane anaesthesia (3–5% in O2; Benson Medical, Markham ON), rats were implanted with a 22-gauge cannula (Plastics One, Roanoke, VA, USA). The cannula was

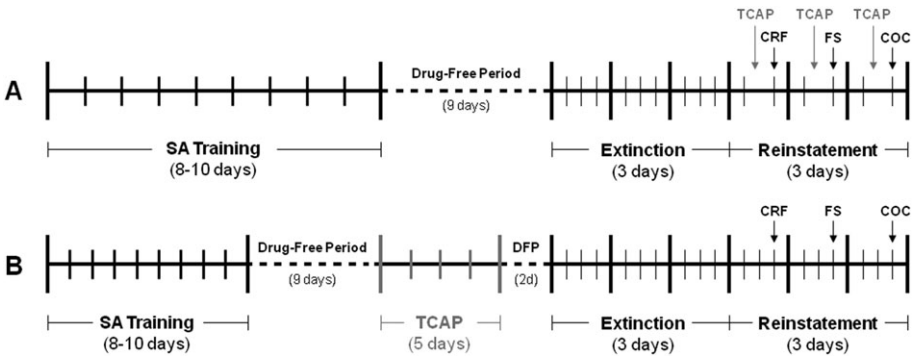

Figure 1

Experiment 1A (A) and 1B (B): Procedural Timeline. Training for self-administration (SA training) followed habituation to the SA chambers.

aimed 1 mm above the right lateral ventricle, according to the following stereotaxic coordinates: A/P: –1.0 mm from bregma; M/L: –1.4 mm from bregma; D/V: –2.7 mm from dura (Paxinos and Watson, 1997). Rats were also implanted with a silastic intravenous catheter (Dow Corning, Midland, MI; inner diameter: 0.51 mm; outer diameter: 0.94 mm) into the right jugular vein. The catheter was a total length of 12 cm, with 3 cm inserted into and secured to the vein with silk sutures. The remaining 9 cm was passed subcutaneously to the skull surface, where it exited into a modified 22-gauge cannula (Plastics One) that, along with the i.c.v. cannula, was embedded in dental cement and anchored to the skull with jeweler's screws. At the end of the surgery, the catheter was flushed with 0.2 mL of a solution containing 50% heparin (1000 IU) and 50% dextrose (25 g/50 mL), and a plastic blocker was placed over the opening of the cannula to protect the catheter from external debris and maintain catheter patency. Likewise, a stainless steel dummy cannula was placed in the i.c.v. guide cannula. Animals were given a 7 day recovery period before commencing any behavioural procedures.

Apparatus. The self-administration (SA) chambers were equipped with two retractable levers (Medical Associates, St. Albans, VT, USA) located 6 cm above a stainless steel rod floor. An infusion pump (Razel Scientific Instruments, Stamford, CN, USA) was activated by responses on one lever (active lever). Each active lever response was automatically recorded using a computer interface operating Medical-IV software (Medical Associates). Responses on the second lever (inactive lever) were recorded but did not result in activation of the pump. Each chamber was equipped with a white stimulus light, located directly above the active lever, and a white house light. Each chamber was also fitted to deliver constant-current, intermittent, inescapable, electric footshock through a scrambler to the metal rod floor (Medical Associates). Footshock was delivered according to a variable time schedule at a mean interval of 40 s (10–70 s range). Each shock (0.9 mA) was 0.5 s in duration.

Procedures. Experiments 1A and B were conducted in five phases: (i) SA training; (ii) drug-free period; (iii) TCAP-1 pre-exposure (Exp. 1B only); (iv) extinction; and (v) testing for reinstatement. A time-line of these procedures is included in Figure 1.

SA training. Before the start of training, rats were habituated to the SA chambers during one 2 h session. Twenty-four to 48 h later, rats were trained to self-administer cocaine (0.35 mg/infusion, i.v.) on a fixed ratio 1 schedule of reinforcement, during once daily 3 h sessions, as described previously (Kupferschmidt *et al.*, 2009). Briefly, the availability of cocaine was signalled by the introduction of the active lever into the chamber, illumination of the white house light (which remained lit throughout the session) and illumination of the white stimulus light above the active lever for 20 s. During the SA session, responses on the active lever resulted in a 3 s infusion of cocaine (in 65 µL saline) and 20 s illumination of the stimulus light, which signalled a 'timeout' period in which additional responses were recorded but not reinforced. SA training was conducted for 10 days. Animals exhibiting stable SA (less than 20% variance in number of infusions between the last 2 days of training) proceeded to subsequent phases of the experiment.

Drug-free period. In Experiment 1A, animals were given a drug-free period of 9 days, such that extinction and testing for reinstatement occurred outside of the initial cocaine withdrawal period. In Experiment 1B, this period was extended to 16 days, during which daily TCAP-1 injections were given (see 'TCAP-1 pre-exposure' below) outside the initial withdrawal period. Animals were left undisturbed in their home cages during this time, with the exception of routine cleaning, feeding and monitoring of weight and health.

TCAP-1 pre-exposure (Exp. 1B only). In Experiment 1B, beginning 9 days into the 16 day drug-free period, rats were given five daily injections of TCAP-1 (0, 30, 300 or 600 pmol, i.c.v.) in their home cage. This regimen of TCAP-1 pre-exposure was chosen based on the parameters of TCAP-1 pre-exposure previously found to alter CRF-induced behavioural anxiety (Al Chawaf *et al.*, 2007; Tan *et al.*, 2008). Initially, animals were pre-exposed to either 0 or 300 pmol TCAP-1, and tested for reinstatement under three different conditions (see 'Testing for reinstatement' below). Based on the effects of these doses, higher (600 pmol) and lower (30 pmol) dose groups were added in subsequent replications to achieve meaningful dose–response profiles for each test condition (*note*: additional animals in the original dose groups were included in each replication).

Extinction. Extinction conditions were identical in Experiment 1A and B. Animals were given three consecutive days of extinction training. Days 1 and 2 consisted of four 60 min extinction sessions, during which all conditions present during SA training were maintained, except that lever presses were not reinforced by cocaine. Each 60 min session was initiated by the same events that occurred at the start of SA training sessions, and extinction sessions were separated by 30 min intervals, during which the active lever was retracted. On Day 3 of extinction, conditions were the same as on Days 1 and 2, with two exceptions: (i) animals were given one sham i.c.v. and one i.p. saline injection, separated by 30 min, between the third and fourth extinction sessions; and (ii) the fourth session began 10 min after the i.p. saline injection. Sham injections were given to familiarize animals with the testing manipulations that occurred in subsequent tests for reinstatement.

Testing for reinstatement. In the 3 days following extinction, animals were given tests for reinstatement. At the start of each test day, animals were given three 60 min extinction sessions. Animals that exhibited 20 or fewer responses on the active lever during the second and third sessions (combined) were subsequently tested for reinstatement. Animals that did not reach this criterion were given an additional extinction session and testing was delayed for 1 day. Immediately following the third session, animals in Exp. 1A were pretreated acutely with TCAP-1 (0 or 300 pmol, i.c.v.); different groups of animals were tested with different doses. Thirty minutes later, animals were given an i.c.v. injection of CRF (105 pmol), exposed to footshock stress (20 min, 0.9 mA), or given a priming injection of cocaine (15 mg·kg^{-1}, i.p.). After an additional 30 or 10 min in the CRF and cocaine conditions, respectively, or immediately after the 20 min of intermittent footshock, animals were presented with the active lever, and non-reinforced lever presses were recorded for 60 min. Each animal was tested in all three conditions on consecutive days and in a counterbalanced order. In each condition, responses on the active lever in the 60 min test session were compared with responses in the extinction session preceding the test. In Experiment 1B, the same general test procedures applied as in Experiment 1A, except that animals were tested on 1–3 of the different conditions, and were not pretreated acutely with TCAP-1 or its vehicle at the time of testing for reinstatement.

Experiment 2: repeated TCAP-1 pre-exposure on cocaine-induced locomotor sensitization

A total of 60 male Wistar rats (Charles River; 275–300 g initial weight) were used. Rats were maintained under conditions identical to those described in Experiment 1.

Surgery. Under isoflurane anaesthesia (3–5% in O2; Benson Medical, Markham ON), rats were implanted with a 22-gauge cannula in the right lateral ventricle, as described in Experiment 1. Rats were given a 7 day recovery period before commencing the experimental procedures.

Apparatus. Locomotor testing was carried out in clear Plexiglas chambers (40 × 25 × 20 cm) with wire mesh lids. An infrared camera positioned above the chambers recorded distanced travelled (cm) using EthoVision software (Version 3, Noldus, the Netherlands).

Procedures. The experiment was carried out in five phases: (i) habituation; (ii) cocaine pre-exposure; (iii) drug-free period; (iv) TCAP-1 pre-exposure; and (v) testing for sensitization. A timeline of the experimental procedures is provided in Figure 2.

Habituation. Seven days after surgery, animals were transported to the room housing the behavioural testing apparatus, placed in the activity chambers for 60 min, administered an injection of saline (i.p.), and returned to the chambers for an additional 120 min. Activity was monitored both before and after the saline injection. Animals were assigned to cocaine or saline pre-exposure conditions such that each group was equated based on their average level of activity in the post-saline injection period.

Cocaine pre-exposure. Starting 48 h after habituation, animals were given once daily injections of cocaine or saline for 7 days. The first and last injections (15 mg·kg^{-1}, i.p.) were administered in the activity chambers, immediately after a 60 min habituation period. The five intervening injections were given in the home cages (30 mg·kg^{-1}, i.p.). This dosing regimen has been found previously to produce behavioural sensitization (Churchill *et al.*, 1999).

Drug-free period. To parallel the drug-free period in Experiment 1B (in which a TCAP-1 pre-exposure phase was also included), animals were given a drug-free period of 19 days between cocaine exposure and testing).

TCAP-1 pre-exposure. Beginning 9 days into the drug-free period, and under the conditions described for Experiment 1B, animals were given five daily injections of TCAP-1 (0 or 300 pmol, i.c.v.) in their home cage.

Locomotor testing. Five days after the last TCAP-1 injection, rats were given two tests for sensitization: one after an i.c.v. injection of CRF (105 pmol) and one after an i.p. injection of

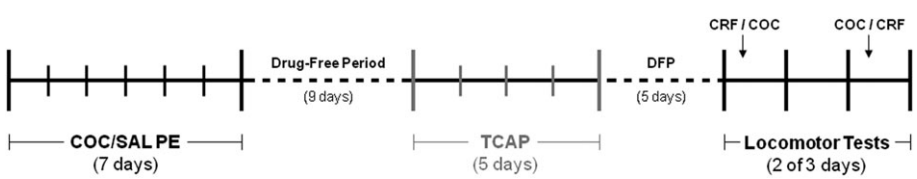

Figure 2

Experiment 2: Procedural Timeline. Habituation to the activity chamber preceded the pre-exposure to cocaine or saline (COC/SAL PE).

cocaine (15 mg·kg^{-1}). The tests were separated by 48 h and given in a counterbalanced order. For each test, animals were initially placed in the activity chamber for 60 min. Subsequently, they were injected with CRF or cocaine and returned to the chamber for an additional 120 min. Activity was recorded both before and after injection. Although, based on our previous work, it was expected that the expression of sensitization would be most evident in the early part of the test sessions (first 30 min), we tested animals for 120 min to maximize the opportunity for detecting potentially subtle effects of the TCAP-1 manipulation.

Data analyses. In Experiments 1A and B, the main dependent variable was the number of responses on the active lever during the extinction session preceding (EXT) and test session following (TEST) the test challenge (i.e. CRF, footshock or cocaine). Separate analyses were carried out for each test condition, using repeated measures ANOVAs for the between-subjects factor of TCAP-1 [0, 300 pmol (Exp. 1A); 0–600 pmol (Exp. 1B)], and the within-subjects factor of Test (EXT, TEST). Significant interactions were followed by repeated measures comparisons or Fisher's LSD *post hoc* comparisons ($P < 0.05$), as appropriate.

In Experiment 1B, the number of responses on the active lever during each extinction day was also analysed using repeated measures ANOVAs for the between-subjects factor of TCAP-1 (0–600 pmol) and the repeated measures factor of Day (1–3), followed by Fisher's LSD *post hoc* comparisons ($P < 0.05$), as appropriate.

In Experiment 2, the distanced travelled (cm) during the 60 min before the first locomotor test, and in the 120 min after the CRF or cocaine test challenge were the main dependent variables. Separate analyses were carried out for each condition, using 2X2 between-subjects ANOVAs for the factors of Cocaine Pre-exposure (Cocaine, Saline) and TCAP-1 Pre-exposure (0, 300 pmol). Significant interactions were followed by Fisher's LSD *post hoc* comparisons ($P < 0.05$), as appropriate.

Materials. Cocaine HCl (Medisca Pharmaceuticals, St. Laurent, QC) was dissolved in saline at concentrations of 3.5 mg·mL^{-1} (i.v.), 15 and 30 mg·mL^{-1} (i.p.). TCAP-1, synthesized by American Peptide Company (Sunnyvale, CA) to be identical to the rat form of TCAP-1, contains a pyroglutamyl acid residue in the first amino terminal position and an amidated carboxy terminus (pEQLLGTGRVQGYDGYFVL SVEQYLELSDSANNIHFMRQSEI-NH_2), as previously described (Wang *et al.*, 2005a). TCAP-1 was dissolved in saline (10^{-5} M, 10^{-4} M and 2×10^{-4} M), and injected in a volume of 3 µL (30, 300, 600 pmol, i.c.v.). CRF (Sigma–Aldrich, Oakville, ON) was dissolved in saline (2.63×10^{-5} M) and injected in a volume of 4 µL [105 pmol (0.5 µg), i.c.v.]. TCAP-1 and CRF were infused using 28-gauge stainless steel injectors that extended 1 mm below the cannula guide tip to the site of injection. Infusions took place over a 2 min period; injectors were left in place for an additional 2 min following infusion to prevent backflow up the cannulae. All drug target nomenclature follows Alexander *et al.* (2009).

Results

Experiment 1A: acute TCAP-1 pretreatment on reinstatement of cocaine seeking

Figure 3 shows the mean (±SEM) number of responses on the active lever during the 60 min tests for CRF (A)-, footshock (B)- and cocaine (C)-induced reinstatement of cocaine seeking, after pretreatment with vehicle or TCAP-1. All test challenges were effective in inducing the reinstatement of cocaine seeking, regardless of TCAP-1 pretreatment; that is, in all tests animals exhibited a higher number of responses on the active lever in the TEST relative to EXT session, and these effects were not altered by TCAP-1. These observations were confirmed by a main effect of Test for each condition ($F_{1,20}$ = 21.14, $P < 0.001$, $F_{1,20}$ = 26.85 $P < 0.001$ and $F_{1,20}$ = 57.51,

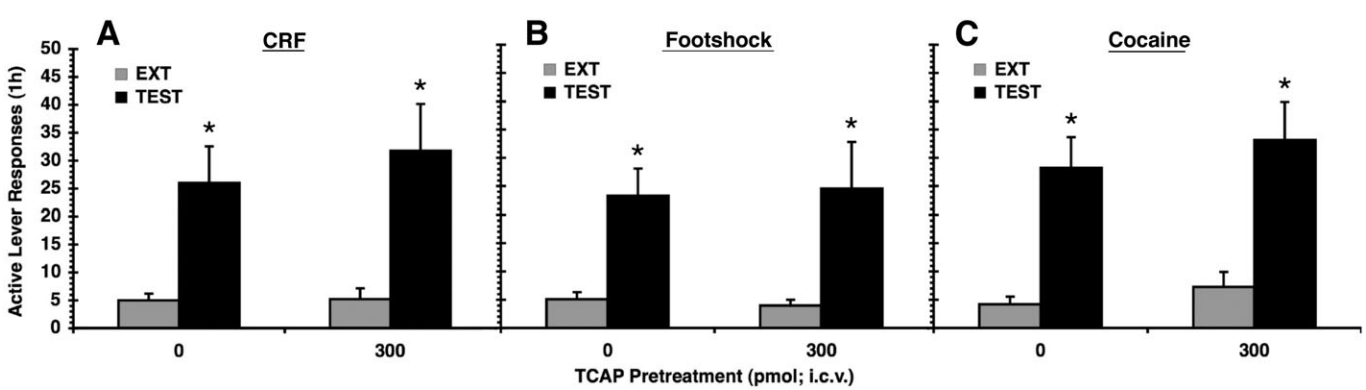

Figure 3

Experiment 1A: Mean (±SEM) number of responses on the active lever during 60 min tests for reinstatement of cocaine seeking in response to CRF (A; 105 pmol, i.c.v.), footshock (B; 20 min, 0.9 mA) or cocaine (C; 15 mg·kg^{-1}, i.p.) in animals pretreated with TCAP-1 (n = 11, 9). *Different from extinction (EXT) condition, $P < 0.05$.

$P < 0.001$ for the CRF, Footshock, and Cocaine tests, respectively), and no main effect or interaction of TCAP-1.

Experiment 1B: repeated TCAP-1 pre-exposure on extinction and reinstatement of cocaine seeking

Because TCAP-1 pre-exposure occurred before both extinction training and testing for reinstatement, it was of interest to look at the effects of this treatment on responding during both experimental phases. Figure 4 shows the mean (±SEM) total number of responses on the active lever during each of three consecutive days of extinction (averaged across four sessions) following pre-exposure to vehicle or TCAP-1. Pre-exposure to TCAP-1 did not alter the time-course of extinction responding and the repeated measures ANOVA was significant only for the main effect of Day ($F_{2,72} = 118.89$, $P < 0.001$).

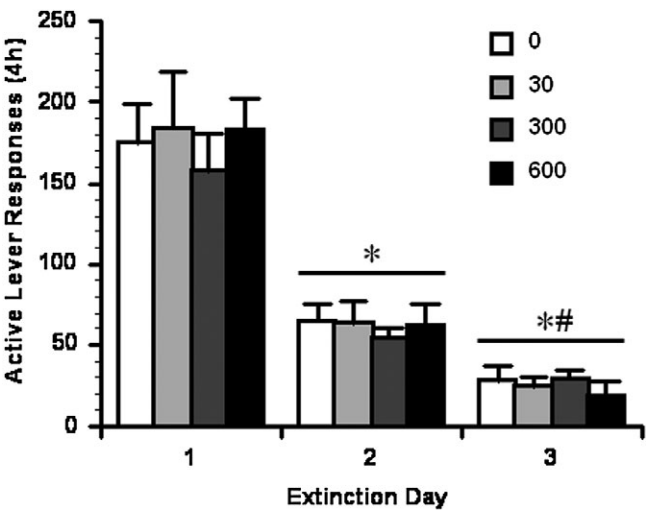

Figure 4

Experiment 1B: Mean (±SEM) total number of responses on the active lever during the four 60 min extinction sessions on each of the three extinction days in animals with or without TCAP-1 (pmol; i.c.v.) pre-exposure ($n = 9$–11). *Different from extinction day 1, $P < 0.05$; #Different from extinction day 2, $P < 0.05$.

Figure 5 shows the mean (±SEM) number of responses on the active lever during the three 60 min tests for reinstatement [CRF (A), footshock (B) and cocaine (C)], after pre-exposure to vehicle or TCAP-1. As in experiment 1A, all three test challenges reinstated cocaine-seeking behaviour, as revealed by main effects of Test ($F_{1,25} = 5.78$, $P < 0.05$; $F_{1,27} = 45.33$, $P < 0.001$; $F_{1,27} = 38.61$, $P < 0.001$). However, in the CRF test condition, repeated TCAP-1 pre-exposure blocked reinstatement of cocaine seeking (Test x TCAP-1: $F_{2,25} = 5.25$, $P < 0.05$; 0 vs. 30 and 0 vs. 300: $P < 0.01$). In contrast, repeated TCAP-1 was without effect in the footshock and cocaine test conditions.

Experiment 2: repeated TCAP-1 pre-exposure on cocaine-sensitized locomotion

Distance travelled during the 60 min before the first test for locomotion was not altered by a prior history of cocaine or TCAP-1 pre-exposure (data not shown). Figure 6 shows the mean (±SEM) distance travelled in response to a CRF (A) or cocaine (B) challenge 19 days after repeated cocaine/saline pre-exposure and 5 days after repeated TCAP-1/vehicle pre-exposure. Although the test sessions were 120 min in duration, we analysed (and present) data obtained in the first 30 min of testing, when the greatest activational effects of the challenges occur. In the CRF condition, repeated measures ANOVA revealed a significant interaction of Cocaine x TCAP-1 ($F_{1,56} = 7.07$, $P < 0.01$). Subsequent *post hoc* analyses showed that the interaction can be attributed to greater distance travelled in cocaine relative to saline pre-exposed animals in the 0 TCAP-1 pre-exposure condition, but not in the 300 pmol TCAP-1 condition. In fact, CRF-induced activity of cocaine pre-exposed animals in the 300 pmol TCAP-1 condition did not differ from that of saline pre-exposed animals in both the 0 and 300 TCAP-1 conditions. Moreover, activity in this cocaine group was reduced relative to cocaine pre-exposed animals in the 0 TCAP-1 condition to a degree that approached significance ($P = 0.06$). Thus, the CRF challenge was associated with the expression of a sensitized locomotor

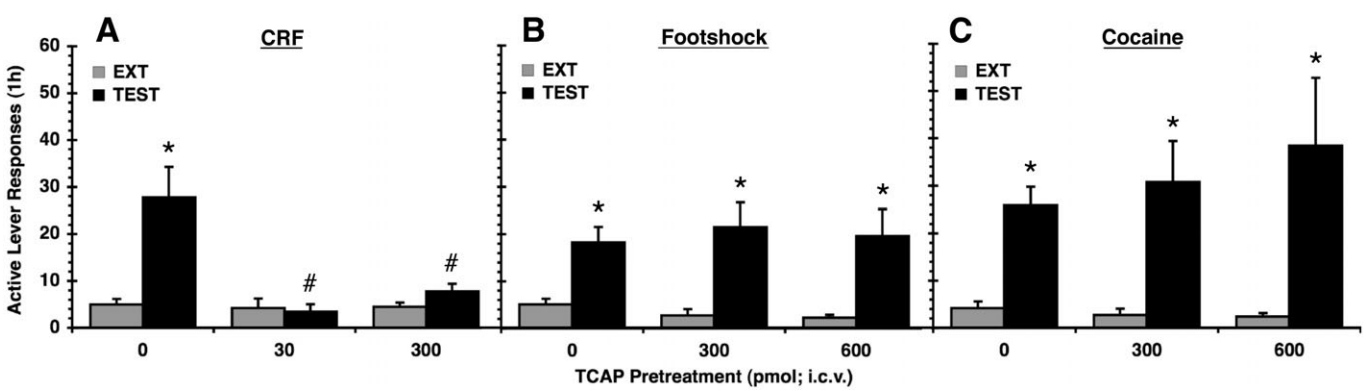

Figure 5

Experiment 1B: Mean (±SEM) number of responses on the active lever during 60 min tests for reinstatement of cocaine seeking in response to CRF (A; 105 pmol, i.c.v.), footshock (B; 20 min, 0.9 mA) or cocaine (C; 15 mg·kg^{-1}, i.p.) in animals previously exposed to 5 days of TCAP-1 ($n = 9$–11). *Different from extinction (EXT) condition, $P < 0.05$; #Different from 0 pmol TCAP-1 condition, $P < 0.05$.

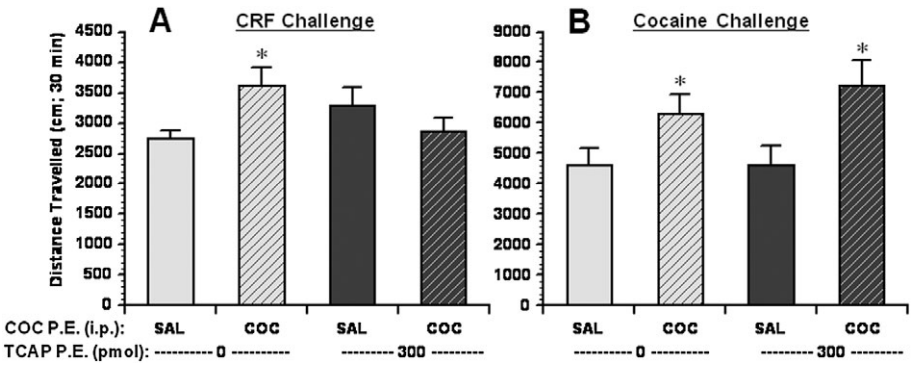

Figure 6

Experiment 2: Mean (±SEM) distance travelled (cm) during 30 min tests for locomotion in response to CRF (A; 105 pmol, i.c.v.) or cocaine (B; 15 mg·kg^{-1}, i.p.) in animals pre-exposed (P.E.) to saline (SAL) or cocaine (COC) (n = 14–15). *Different from SAL condition, $P < 0.05$.

response in cocaine pre-exposed animals, and this effect of cocaine was reversed by TCAP-1 pre-exposure.

In contrast to CRF, cocaine at the time of test induced a greater locomotor response in cocaine relative to saline pre-exposed rats ($F_{1,58} = 11.17$, $P < 0.001$), independent of TCAP-1 pre-exposure (Cocaine x TCAP-1 Pre-exposure interaction: $F_{1,58} = 0.483$, $P = 0.49$, n.s.). Thus, the cocaine challenge induced a sensitized locomotor response in cocaine pre-exposed animals, and this effect was not altered by TCAP-1 pre-exposure.

Discussion

In this paper, we provide the first evidence that repeated exposure to the novel neuropeptide, TCAP-1, blocked the effects of CRF on the reinstatement of cocaine seeking, and on the expression of cocaine-induced behavioural sensitization. TCAP-1 was found to selectively interfere in the expression of CRF-induced behaviours; that is, TCAP-1 was without effect on either footshock- or cocaine-induced reinstatement of cocaine seeking, or the expression of cocaine-induced behavioural sensitization to a cocaine challenge. Overall, our findings are consistent with previous reports that TCAP-1 inhibits CRF-induced behavioural anxiety (Al Chawaf *et al.*, 2007) and CRF-potentiated startle (Tan *et al.*, 2008), and provide support for the hypothesis that TCAP-1 serves to attenuate the physiological actions of CRF (Lovejoy *et al.*, 2009).

At present, the brain loci where TCAP-1 and CRF interact to produce behavioural change, and the mechanism of their interaction, are not known. However, it has recently been shown that the central distribution of TCAP-1 uptake is widespread, and particularly pronounced in limbic and prefrontal brain regions (D Chand *et al.*, in preparation). From this perspective it is of interest that TCAP-1 attenuates CRF-induced increases in c-fos expression in limbic regions – including the amygdala (Tan *et al.*, 2009) – which have been implicated in the effects of CRF on stress-induced reinstatement of cocaine seeking (Erb *et al.*, 2001) and the expression of cocaine-induced behavioural sensitization (Erb *et al.*, 2005). Although the TCAP-1 receptor has yet to be identified, TCAP-1 has been shown in cell culture to act independently of CRF receptors to suppress c-fos and AP-1 reporter activity (Wang *et al.*, 2005a; TG Nock *et al.*, unpubl. obs.). Further *in vitro* evidence indicates that prolonged treatment with TCAP-1 induces structural changes in cytoskeletal elements, as well as neurite and axon formation (Al Chawaf *et al.*, 2007). Collectively, these findings suggest that TCAP-1 may act downstream of CRF receptor activation in a manner that, through repeated exposure to TCAP-1, may induce long-lasting structural alterations that serve to inhibit certain cellular and behavioural effects of CRF.

Without first knowing the mechanism by which TCAP-1 and CRF interact to alter behaviour, it is difficult to speculate on how a history of cocaine exposure might serve to alter the nature of this interaction. One possibility is that TCAP-1 acts to normalize signalling within CRF receptor systems that have become dysregulated as a consequence of cocaine exposure. Indeed, there is considerable evidence that neuronal and behavioural responses to CRF change as a consequence of prior cocaine exposure, and that this change may involve alterations in CRF receptor affinity and intracellular pathways (Corominas *et al.*, 2010). For example, it has been shown that central CRF injections selectively enhance c-fos mRNA expression in the CeA of animals pre-exposed to cocaine, relative to saline (Erb *et al.*, 2005). It has also been shown that augmented CRF-induced long-term potentiation in the CeA, observed following withdrawal from repeated cocaine administration, is mediated by enhancements in CRF_1 receptor function and signalling through the second messenger, protein kinase A (Pollandt *et al.*, 2006). Finally, exposure to cocaine has been shown to sensitize glutamate and dopamine release in the VTA in response to CRF, alterations known to play a critical role in the effects of stress on the reinstatement of cocaine seeking (Wang *et al.*, 2005b; 2007). Thus, it is conceivable that TCAP-1 interferes in CRF-induced reinstatement and CRF-induced expression of behavioural sensitization by acting to normalize changes in CRF receptor transmission.

One striking aspect of our findings was the highly selective nature of the effects of TCAP-1 on behavioural responses to CRF. Particularly surprising was the observation that TCAP-1 completely blocked CRF-induced reinstatement of

cocaine seeking, but failed to alter reinstatement by footshock stress, an effect known to be mediated by CRF (Erb *et al.*, 1998; 2001; Erb and Stewart, 1999; Wang *et al.*, 2005b). In fact, a dose of TCAP-1 two to 20 times that associated with a complete blockade of CRF-induced reinstatement (i.e. 600 pmol) was without effect on reinstatement by footshock stress. Less surprising were the observed dissociations between the effects of TCAP-1 on the expression of CRF- and cocaine-induced reinstatement and behavioural sensitization. Indeed, CRF has been shown *not* to mediate the effects of drug priming on reinstatement (Erb *et al.*, 1998), and there are several reported examples of differences in the neurochemical cross-sensitization of stressors and cocaine, depending on the nature of the sensitizing regimen (e.g. Sorg and Kalivas, 1991; Sorg, 1992; Prasad *et al.*, 1995).

Several factors may help explain the differences in the effects of TCAP-1 on CRF- and footshock-induced reinstatement of cocaine seeking. These include the nature of the stressor (pharmacological vs. physical/environmental), the brain systems they engage (Imaki *et al.*, 1993), and the onset and duration of their neurochemical sequelae (Matsuzaki *et al.*, 1989; Kalivas and Duffy, 1995; Galvez *et al.*, 1996; Erb *et al.*, 2000; de Groote *et al.*, 2005). For example, noradrenaline has been found to play a critical role in footshock-induced reinstatement of cocaine seeking via enhanced neurotransmission within the ventral pathway originating in the lateral tegmental nuclei (Shaham *et al.*, 2000), and subsequent activation of β_1 and β_2 adrenoceptors in the CeA and BNST (Leri *et al.*, 2002). CRF-induced reinstatement of cocaine seeking, on the other hand, is unaltered when noradrenaline transmission is inhibited by systemic injections of the α_2 adrenoceptor agonist, clonidine, at a dose known to block footshock-induced reinstatement of cocaine seeking (Erb *et al.*, 2000; Brown *et al.*, 2009). Thus, it appears that CRF-induced reinstatement of cocaine seeking is less reliant on an intact noradrenaline system than is footshock-induced reinstatement.

The effects of repeated TCAP-1 on reinstatement of cocaine seeking were not only highly selective to reinstatement by CRF, but were also highly effective. Indeed, doses of 30 and 300 pmol TCAP-1 were equally effective in completely blocking the effect of CRF on the reinstatement of cocaine seeking. Similarly high potency has been observed in previous behavioural studies. For example, repeated i.v. exposures to 300 pmol TCAP-1, which would yield considerably lower effective brain concentrations of TCAP-1 relative to i.c.v. exposures to this same dose, are associated with reliable anxiolytic effects in the elevated plus maze and open field tests (Al Chawaf *et al.*, 2007). Likewise, repeated i.c.v. exposures to doses of TCAP-1 ranging from 60 to 300 pmol are anxiolytic in the acoustic startle procedure (Wang *et al.*, 2005a; Tan *et al.*, 2008).

Finally, an alternative explanation for our effects of repeated TCAP-1 exposure on CRF-induced behaviour is that TCAP-1 produces cross-tolerance to the subsequent effects of CRF. Although we cannot completely discount this possibility, we believe it an unlikely explanation given that in Experiment 2, CRF-induced activity in our non-sensitized animals was not reduced by a history of repeated TCAP-1 exposures.

In summary, the present findings demonstrate a robust effect of repeated TCAP-1 administration on two cocaine-related behavioural responses to CRF; namely, CRF-induced reinstatement of cocaine seeking and CRF-induced expression of behavioural sensitization to cocaine. These experiments represent the first exploration of a role for the novel neuropeptide system, TCAP, in the behavioural effects of cocaine, or any drug of abuse. Importantly, the findings extend previous work showing a consistent effect of repeated TCAP-1 exposure on measures of CRF-induced behavioural anxiety. However, given that the effects of TCAP-1 on CRF-induced reinstatement of cocaine seeking did not generalize to reinstatement induced by footshock stress, determination of the physiological relevance of TCAP-CRF interactions in the control of reinstatement responding remains an important question for future research.

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Conflict of interest

Dr D.A. Lovejoy is currently the Chief Scientific Officer of Protagenic Therapeutics, Inc. Dr S. Rotzinger has been a paid consultant to Protagenic Therapeutics, Inc., and holds shares in the company.

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