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ORIGINAL INVESTIGATION

Assessing the antidepressant-like effects of carbetocin, an oxytocin agonist, using a modification of the forced swimming test

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Abstract
Rationale The distribution of oxytocin receptors in limbic regions, as well as evidence that exogenous oxytocin modulates affect and fear processing, suggests that this neuropeptide may have a role to play in the treatment of mood disorders.
Objectives This study compared the effects of acute treatment with the oxytocin receptor agonist, carbetocin with the tricyclic antidepressant, imipramine, using male Sprague–Dawley rats.
Methods Intracerebroventricular (i.c.v.; 1, 10, 100 μg/rat), intravenous (i.v.; 2.5, 5 mg/kg), and intraperitoneal (i.p.; 2, 6.4, 20 mg/kg) carbetocin and imipramine (1.8, 5.6, 10 mg/kg, i.p.) were examined in the modified forced swim and open field tests. The mechanism of action of carbetocin was investigated by co-administering it with the oxytocin antagonist, atosiban, either centrally (5 μg/rat, i.c.v.) or systemically (1 mg/kg, i.v.).
Results Imipramine and carbetocin (all three routes of administration) both significantly reduced immobility and increased swimming and/or climbing behavior in the forced swim test. The systemic effects of carbetocin were blocked by central and systemic atosiban co-administration. Only amphetamine (2 mg/kg, i.p.), included as a false positive in order to distinguish whether antidepressant-like effects were due to psychomotor stimulation, increased locomotor activity in the open field test.
Conclusions Carbetocin produced antidepressant-like changes in behavior via activation of oxytocin receptors in the CNS. The similarities between imipramine and carbetocin in the forced swim test suggest that drugs which target the oxytocinergic system may aid both the understanding and pharmacological treatment of depressive illness.

Keywords Oxytocin · Carbetocin · Forced swim test · Rat · Imipramine · Atosiban · Desmopressin · Antidepressant

Abbreviations

i.p.	intraperitoneal
i.c.v.	intracerebroventricular
i.v.	intravenous
f.s.t.	forced swimming test

Introduction

The neurohypophyseal peptide oxytocin is best known for its actions in the periphery, which include stimulating milk ejection and uterine contractions (Benoussaidh et al. 2005; Sala et al. 1974). However, there is growing interest in oxytocin's role as a neuromodulator in the central nervous system (Sofroniew 1980; Argiolas and Gessa 1991; Landgraf and Neumann 2004), as it is becoming evident that oxytocin is an important modulator of certain behavioral processes such as those which contribute to the expression of mood (Heinrichs et al. 2003; Domes et al. 2007).

Aspects of mood for which oxytocin appears to play a modulatory role include the expression of antidepressant- and anxiolytic-like behaviors, as evaluated in both human (Zetzsche et al. 1996; Scantamburlo et al. 2007) and animal studies (Arletti et al. 1995; Arletti and Bertolini 1987; Meisenberg 1982; Nowakowska et al. 2002; Klenerova et al. 2009) and of stress (reviewed in Neumann 2008). Some of oxytocin's neuromodulatory effects are thought to be mediated via the serotonergic and noradrenergic systems,

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both of which are implicated in the expression of symptoms of depressive disorders (Maas 1975). For instance, there is evidence that oxytocin neurons are innervated by noradrenergic neurons projecting from brainstem nuclei (Cunningham and Sawchenko 1988), acting via a positive feedback mechanism to facilitate one another's release from neuronal projections within the hypothalamus (Bealer and Crowley 1998; Onaka et al. 2003). In addition, examination of oxytocin and noradrenergic projections between the hypothalamus and limbic structures (including the amygdala and septal area) suggests that modulation of noradrenergic transmission by oxytocin receptor activation in limbic areas may contribute to behavioral responses (Gimpl and Fahrenholz 2001; Onaka et al. 2003). Evidence for the interaction between serotonin and oxytocin includes the observation that serotonergic nerve fibers and receptors are located in the supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus where oxytocin is synthesized, and are implicated in regulation of the release of both adeno- and neurohypophyseal hormones (Sawchenko et al. 1983). Central infusion of exogenous serotonin or serotonin agonists stimulates the secretion of oxytocin into venous blood in the rat (Jorgensen et al. 2003); a positive relationship between oxytocin release and the pharmacological actions of SSRIs has also been demonstrated in the rat (Uvnas-Moberg et al. 1999). Evidence for reciprocity between oxytocin and serotonin was published recently by Yoshida et al. (2009). Their work showed that administration of oxytocin using microdialysis in close proximity to serotonergic neurons in the median raphe nuclei (MnR), which express oxytocin receptors, increased serotonin release in the MnR, as well as producing anxiolytic behavioral effects that were able to be blocked by serotonin 2a/c receptor antagonists (Yoshida et al. 2009).

A well-documented animal model that can be used to assess the antidepressant-like activity of drugs is the forced swimming test (FST) (Detke et al. 1995; Porsolt et al. 1978). Previous studies using the FST have suggested that oxytocin has an antidepressant-like effect, namely to increase active swimming behaviors and decrease the time spent immobile (Arletti et al. 1995; Arletti and Bertolini 1987; Meisenberg 1981; Nowakowska et al. 2002). Despite concern that oxytocin may have poor penetrance of the blood-brain barrier, it has been demonstrated that intraperitoneal (i.p.) administration of oxytocin also induces antidepressant-like behaviors in both the rat and mouse FST (Arletti et al. 1995; Arletti and Bertolini 1987; Nowakowska et al. 2002). The mediation of these behavioral effects by central oxytocin receptors has not yet been confirmed, however, and as with any drug suspected of having antidepressant-like activity in the FST, the increase in active behaviors (swimming and/or climbing) that contributes to the reduction in immobility could be a consequence of oxytocin having a stimulatory effect on locomotor activity. Locomotor stimulants, such as amphetamine and caffeine, are known to decrease immobility in the FST despite having little if any clinical antidepressant utility (Porsolt et al. 1978). The locomotor effects of oxytocin receptor activation have not yet been evaluated; indeed, there has been very little research into the antidepressant effects of oxytocin with the exception of the few studies mentioned.

Vasopressin, which is closely related in structure to oxytocin, often mediates effects that are complementary to oxytocin (Landgraf and Neumann 2004). Despite the overlap in their biological targets, the effects of oxytocin and vasopressin are mediated via distinct receptor systems, as has been demonstrated by the development of more selective agonists (Chan et al. 2000). TGOT ([Thr4,Gly7]-oxytocin), also known as carbetocin (Durotocin®), is an oxytocin receptor agonist with a binding profile similar to oxytocin. Like oxytocin, carbetocin is a selective and competitive agonist for the N-terminus of the oxytocin receptor (Gimpl et al. 2005). Carbetocin, currently indicated for the treatment of postpartum hemorrhage, is highly stable with a longer elimination half-life than oxytocin (85–100 versus 3–4 min; Engstrom et al. 1998). Desmopressin (1-deamino-8-D-arginine vasopressin; Minirin®), a vasopressin receptor agonist and derivative of vasopressin (AVP), non-selectively targets the AVP1A and AVP2 receptor subtypes (Méchaly et al. 1999). Atosiban (1-deamino-2-D-Tyr(OEt)-4-Thr-8-Orn-oxytocin; Tractocile®) is an oxytocin and vasopressin 1a receptor antagonist (Manning et al. 1995). The tricyclic antidepressant imipramine (Tofranil®), owes its clinical efficacy to its inhibition of the uptake of noradrenaline and serotonin following their release into the synaptic cleft (Wong et al. 2005).

The aim of the present study was to investigate whether carbetocin has antidepressant-like activity in the rat, similar to systemic administration of the tricyclic antidepressant, imipramine (Tofranil®), when injected via the i.p., i.v., and i.c.v. routes, as measured using the modified FST following acute drug administration. It was hypothesized that both compounds would show an antidepressant-like reduction in immobility, accompanied by increases in active behaviors. It was also predicted that the antidepressant-like actions of these drugs would not be due to any stimulatory locomotor effects, as determined in open field test. To assess this, amphetamine was administered as a false positive control in the FST and open field test. The mechanism by which carbetocin produced antidepressant-like changes in behavior following systemic administration was evaluated using intracerebroventricular and intravenous injection of atosiban. The oxytocin receptor selectivity of the oxytocin/AVP1a receptor antagonist atosiban in this paradigm was explored using intravenous desmopressin.

Materials and methods

Subjects

Two hundred forty male Sprague–Dawley rats weighing between 300 and 500 g were used in this study (Monash University Animal Services, Clayton, Australia). Upon arrival, rats were housed in groups of 4–5 in opaque plastic cages (58 cm long×35 cm wide) with sawdust bedding; food pellets (Specialty Feeds; Western Australia) and tap water were freely available. Rats were maintained in a temperature (19.4–21.8°C) and humidity (36–57%) controlled room under a reversed 12-h light/dark cycle (lights on 2100-0900) for at least 1 week prior to commencing experimentation to enable them to habituate to the new environment. Use of a reversed light/dark cycle was to ensure that testing times coincided with when rats were most behaviorally active. Rats were randomly assigned to receive either saline vehicle or drug on an acute (single day) treatment schedule. All testing was carried out between 0930 and 1430 h. The study protocols were approved by the Animal Ethics Committee of the School of Psychology and Psychiatry, Monash University, in accordance with guidelines from the Bureau of Animal Welfare (Australia), specifically the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Procedure

ICV cannulation surgery

Isoflurane mixed with carbogen gas (95% oxygen, 5% carbon dioxide) was used to anesthetize each rat. Morphine (3.2 mg/kg, i.p.) at a volume of 1 ml/kg was administered preoperatively. The top of the rat's head was shaved, and the rat was then placed into a stereotaxic device to stabilize the head during surgery. The shaved skin was wiped with absolute ethanol, and a 1–2 cm mid-sagittal incision was made. The coordinates for the placement of the guide cannula (Plastics 1, Roanoke, VA) in the lateral ventricle, using bregma as a reference, were 0.8 mm posterior, 1.5 mm lateral to a depth of 3.5 mm; cannula placement was randomized between the left and right ventricles. Two bone screws (one placed on the opposite side of the midline to the cannula; the other placed just posterior to the cannula) were used to provide structural support for the cannula-dental cement assembly. The guide cannula was held in place using Paladur® dental cement (Heraeus Kulzer GmbH, Wehrheim, Germany). The rats were placed under a heat lamp during their recovery from surgery.

The patency of each cannula was evaluated 1–2 days prior to testing using i.c.v. administration of 5 μl Angiotensin II (Sigma-Aldrich, Australia). Administration of angiotensin II triggers vasoconstriction and fluid retention, which is perceived as physiological dehydration. Cannula patency was indicated by the rapid initiation of drinking behavior. After completing the experiments, the placement of the cannula in the lateral ventricle was confirmed post-mortem using an i.c.v. injection of 10 μl of methylene blue (2.5%). The brain was removed from the skull intact and sectioned coronally; the distribution of the blue dye allowed visualization of the ventricular system when the cannula was placed accurately.

Forced swimming test

Antidepressant-like activity was measured using a single 15-min swim as previously described (Broom et al. 2002a,b). Testing commenced 30 min after i.p. injection or 15 min after i.v. or i.c.v. injection of drug or saline and was conducted under low lighting conditions (11 lux). Atosiban pretreatments were co-administered with carbetocin or saline. The rats were then placed in a cylindrical polycarbonate vessel (50.5 cm tall×24 cm diameter) filled with 37 cm of 25°C (±1°C) water. Swim sessions were videotaped and scored using manual and automated (Ethovision XT© Noldus, Netherlands) methods. Cross-scoring by a blind observer showed that there was a stable, positive correlation between scoring methodologies. The water in the tank was replaced after every rat. Following the swim, each rat was towel dried and placed under a heat lamp until dry.

The behaviors that were scored were climbing, swimming, and immobility (Detke et al. 1995). Climbing was defined as the rat making vigorous active movements with all four paws while parallel to the wall of the swimming vessel, as well as having its head and shoulders above the water. Swimming was defined as moving all four paws in an active swimming motion that was more vigorous than was necessary to merely maintain the head above water. Immobility was noted when the rat remained floating in the water without struggling, only making movements that were necessary to keep its head or nose above water. The total counts for climbing, swimming, and immobility during the 15-min swim period were totaled and averaged for each treatment group. The percent time spent performing each behavior (measured in seconds) during the swim session (15 min; 900 s) was calculated for statistical analysis.

Open field test

Locomotor activity was measured using an open field apparatus to determine whether drug treatments that resulted in behavioral changes in the forced swimming test were attributable to changes in spontaneous motor

activity. The apparatus consisted of a square opaque acrylic box (100×100 cm) with 40 cm high walls. Treatment routes and pretreatment times, as well as the level of illumination in the testing room, were identical to what was used in the forced swimming test. Rats were placed in the center of the apparatus, and the total distance traveled (cm) was measured over a 5-min period using Ethovision XT.

Drugs

Imipramine hydrochloride (Tofranil®) was purchased from Sigma-Aldrich (Australia); carbetocin was purchased, and atosiban and desmopressin were gifts, from Ferring Pharmaceuticals (Denmark); amphetamine was purchased from the National Measurement Institute (Australia). Imipramine (1.8, 5.6, 10 mg/kg), carbetocin (1, 10, 100 μg/rat, i.c.v.; 2.5, 5.0 mg/kg i.v.; 2, 6.4, 20 mg/kg, i.p.), atosiban (5 μg/rat i.c.v.; 1 mg/kg i.v.), and amphetamine (2 mg/kg) were solubilized in sterile saline (sodium chloride, 0.9%) and administered at a volume of 1 ml/kg via the i.p. route at 30 min (15 or 30 min for carbetocin) prior to behavioral testing. Carbetocin, desmopressin, atosiban, and saline were administered via i.v. injections with a 15-min pretreatment. Carbetocin, atosiban, and saline were given via i.c.v. infusion in a volume of 5 μl over 60 s 15 min prior to behavioral testing.

Statistics

Raw data for each experiment were analyzed using SPSS for Windows, version 17. Where data satisfied the assumptions of normality and homogeneity of variance, parametric analyses (one-way independent measure ANOVAs and *t*-tests) were performed, along with Tukey's HSD post-hoc test where appropriate. Conversely, non-parametric alternatives (Kruskal–Wallis, with subsequent Mann–Whitney *U* or Dunnett's T3 where indicated) were used when assumptions were violated. Statistical significance was set at $p<0.05$. All data are presented as mean± standard deviation (SD) or standard error of the mean (SEM).

Results

Forced Swimming Test (FST) One-way independent measures ANOVA was conducted for each of the three behavioral measures following saline, 1.8, 5.6, and 10 mg/kg imipramine treatment (i.p.). The results revealed a significant effect of treatment in the percent time spent climbing ($F(3, 30)=60.7$, $p<0.05$, $\eta^2=0.86$), swimming ($F(3,30)=6.80$, $p<0.05$, $\eta^2=0.41$), and immobile ($F(3,30)=23.11$, $p<0.05$, $\eta^2=0.70$); see Fig. 1. Post-hoc analysis showed that the 10 mg/kg dose of imipramine increased climbing relative to treatment with either saline or the lower imipramine doses ($p<0.05$). The 5.6 and 10 mg/kg doses of imipramine both increased swimming relative to saline or the 1.8 mg/kg imipramine treatments ($p<0.05$). The 10 mg/kg dose of imipramine decreased immobility relative to all other treatments; the 5.6 mg/kg dose of imipramine decreased immobility relative to saline treatment ($p<0.05$; Fig. 1).

The outcome of carbetocin treatment was examined following administration via the i.c.v., i.v., or i.p. routes. One-way independent measures ANOVA revealed that there was a significant effect of carbetocin (1, 10, 100 μg/rat, i.c.v.) treatment in the percent time spent swimming ($F(3, 30)=4.13$, $p<0.05$, $\eta^2=0.53$) and immobile ($F(3, 30)=4.14$, $p<0.05$, $\eta^2=0.48$) between treatment groups (see Fig. 2). Post-hoc analysis revealed a dose-dependent increase in the percent time spent swimming ($p<0.05$) and a corresponding reduction in immobility ($p<0.05$), following acute administration of 100 μg/rat carbetocin compared to saline and 1 μg/rat carbetocin (Fig. 2).

When carbetocin (2.5, 5.0 mg/kg) was administered via the intravenous route, there was a significant difference between drug treatments with respect to immobility ($F(4,45)=7.97$, $p<0.05$, $\eta^2=0.42$) and climbing ($F(4,45)=7.26$, $p<0.05$, $\eta^2=0.39$; see Fig. 3A). Subsequent post-hoc testing showed that 2.5 mg/kg carbetocin decreased percent time spent immobile and increased percent time spent climbing relative to all other treatments ($p<0.05$). When atosiban (1 mg/kg i.v.) was co-administered with carbetocin

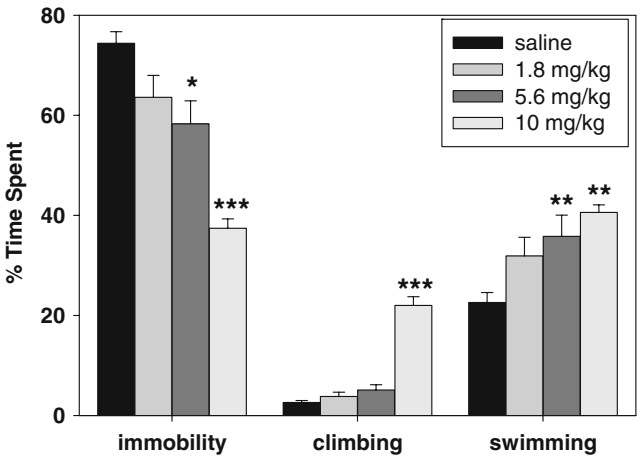

Fig. 1 Effect of acute treatment with imipramine on climbing, swimming, and immobility behaviors in the 15-min forced swimming test. Male Sprague–Dawley rats ($n=8$ or 10/group) were treated with 1.8, 5.6, or 10 mg/kg imipramine or saline via the i.p. route 30 min prior to behavioral testing. The mean for each behavioral measure is expressed as the percent time spent climbing, swimming, or immobile±standard error of the mean (SEM). *differs from saline only; **differs from saline and 1.8 mg/kg imipramine, ***differs from saline, 1.8, and 5.6 mg/kg imipramine ($p<0.05$)

(2.5 mg/kg i.v.), the increase in climbing and decrease in immobility seen following carbetocin alone was completely attenuated (Fig. 3A).

Intravenous administration of the vasopressin receptor agonist, desmopressin (1.0, 5.0 mg/kg), did not affect swimming, climbing or immobility relative to saline treatment. Co-administration of desmopressin (5 mg/kg) with atosiban (1 mg/kg) produced saline-like effects on FST behaviors (Fig. 3B).

When carbetocin (2, 6.4, 20 mg/kg) was administered via intraperitoneal injection, there was a significant effect of treatment on the percent time spent climbing ($F(3, 27)=8.88$, $p<0.05$, $\eta^2=0.50$), swimming ($F(3, 27)=15.26$, $p<0.05$, $\eta^2=0.63$) and immobile ($F(3, 27)=15.56$, $p<0.05$, $\eta^2=0.65$; see Fig. 4). Post-hoc analysis revealed that climbing was increased by treatment with 6.4 mg/kg carbetocin relative to either saline or 2 mg/kg carbetocin treatments ($p<0.05$); 20 mg/kg carbetocin resulted in a significantly greater proportion of time spent swimming compared with saline, 2 or 6.4 mg/kg carbetocin treatments ($p<0.05$); while 6.4 and 20 mg/kg carbetocin resulted in a significantly lower proportion of time spent immobile compared with saline or 2 mg/kg carbetocin treatments ($p<0.05$; Fig. 4).

Carbetocin (6.4 mg/kg, i.p.) was administered at both 15 and 30 min prior to testing in order for outcomes to be comparable with both i.c.v. carbetocin (15 min ptt) and the comparator antidepressant drug treatment, imipramine (30 min ptt). No differences in the efficacy of carbetocin were observed at these two pretreatment times (data not shown). The results from the saline treatments (i.p. 15 and 30 min; i.c.v.

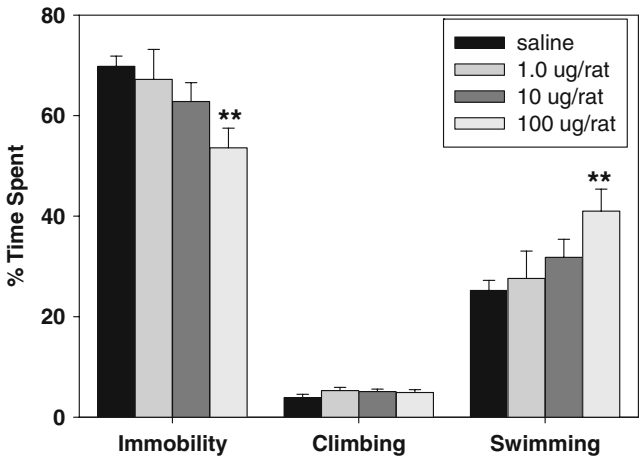

Fig. 2 Effect of acute i.c.v. treatment with carbetocin (1, 10, or 100 μg/rat, $n=8$) compared to saline ($n=9$) on immobility, climbing, and swimming behaviors in the forced swimming test. Male Sprague–Dawley rats were treated with drug or saline via the i.c.v. route 15 min prior to testing. The mean for each behavioral measure is expressed as the percent time spent immobile, climbing, or swimming±standard error of the mean (SEM). **differs from saline and 1.0 μg/rat carbetocin ($p<0.05$)

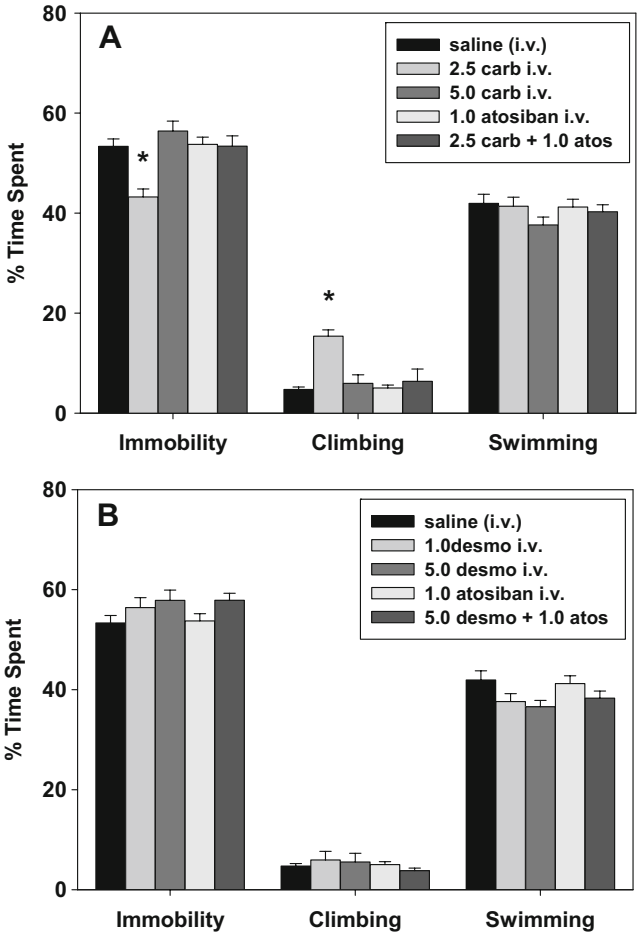

Fig. 3 The effect of intravenous carbetocin (**A**) or desmopressin (B), with or without atosiban, on percent time spent immobile, climbing, and swimming in the forced swimming test. Male Sprague–Dawley rats were administered saline, carbetocin (2.5 and 5 mg/kg), desmopressin (1 and 5 mg/kg), atosiban (1 mg/kg), or a combined treatment. Each value represents the mean±standard error ($n=10$ for all treatments). *significantly different from all other treatments ($p<0.05$)

15 min) were combined as there were no statistically significant differences in behavioral outcomes due to route of administration or pretreatment time (data not shown). When atosiban (5 μg/rat i.c.v.) was co-administered with carbetocin (6.4 mg/kg i.p.), the increase in climbing and decrease in immobility seen following carbetocin alone were completely attenuated (Fig. 5).

The psychomotor stimulant drug amphetamine (2 mg/kg) was examined in the FST and open field apparatus to help exclude a false positive effect of any of the drugs tested. Acute treatment with 2 mg/kg amphetamine significantly reduced the percent time spent climbing and immobile ($p<0.001$) while significantly increasing the percent time spent swimming ($p<0.001$) relative to saline (data not shown).

Locomotor behavior The total distance traveled in an open field apparatus was used as a measure of locomotor activity. A

one-way ANOVA followed by post-hoc analyses revealed that there were two statistically significant differences in locomotor activity between the test drugs and the saline controls. Carbetocin (20 mg/kg, i.p.) treatment resulted in a significant reduction in the total distance traveled (cm±SD) compared to saline (1,551±369 cm versus 2,006±291 cm; $p<0.05$); the only increase in locomotor activity was measured after treatment with amphetamine (2 mg/kg), which significantly increased locomotor activity relative to saline (2,609± 225 cm versus 2,006±291 cm; $p<0.01$) (Table 1).

Discussion

This is the first study to evaluate the oxytocin analogue, carbetocin, in the FST, establishing its efficacy and comparing its potency across three different routes of administration. It is also the first study to demonstrate that carbetocin's antidepressant-like effects are mediated by oxytocin receptors in the central nervous system. Carbetocin has not previously been evaluated for its effects on behavior in the FST, and the original studies using oxytocin did not exclude locomotor stimulant effects in oxytocin's antidepressant-like activity. As hypothesized, treatment with carbetocin (i.p., i.v., and i.c.v.), imipramine, and amphetamine each resulted in antidepressant-like behavioral changes in the FST relative to saline treatment. With the exception of amphetamine, which was included as a false positive, none of these drug treatments increased locomotor activity relative to saline in the open field test, suggesting that decreased immobility measured in the FST was not due to a stimulatory effect of drug treatment on motor activity but rather to an antidepressant-like effect.

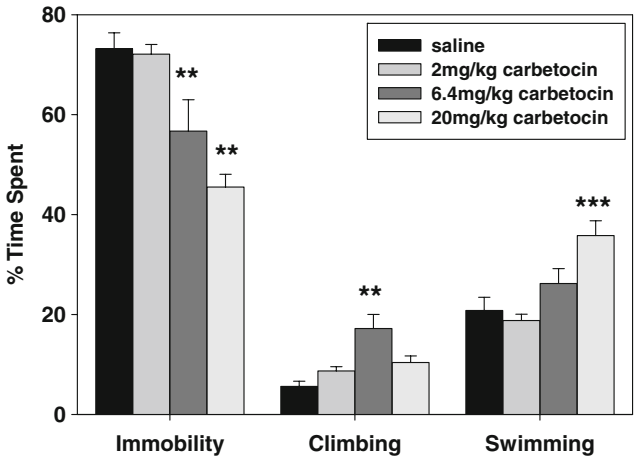

Fig. 4 Effect of acute treatment with carbetocin (2, 6.4, or 20 mg/kg) compared to saline on climbing, swimming, and immobility behaviors in the forced swimming test ($n=8$/group). Male Sprague–Dawley rats were treated with drug or saline via the i.p. route 15 min prior to testing. The mean for each behavioral measure is expressed as the percent time spent climbing, swimming, or immobile±standard error of the mean (SEM). ** differs from saline and 2 mg/kg carbetocin; *** differs from all other treatments; ($p<0.05$)

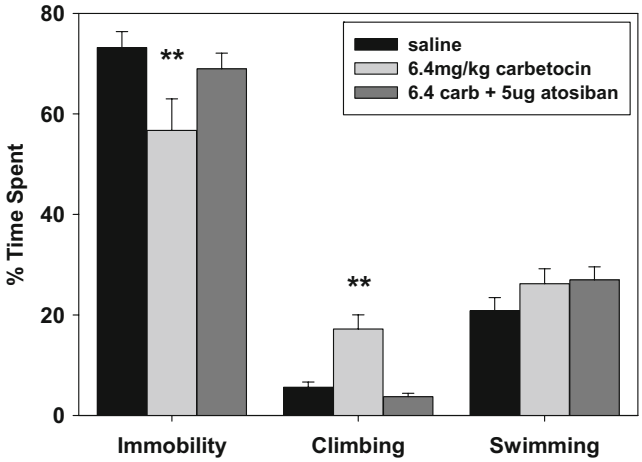

Fig. 5 Effect of acute treatment with carbetocin (6.4 mg/kg, i.p.; $n=8$), with or without atosiban co-administration (5 μg/rat, i.c.v.; $n=7$) compared to saline in the forced swimming test ($n=8$). Detail is same as in Fig. 1. ** differs from saline and carbetocin+5 μg atosiban treatment groups; ($p<0.05$)

Carbetocin significantly reduced the duration of immobility in the FST, with corresponding increases in the duration of swimming and/or climbing behaviors. Carbetocin administered by the i.c.v. and i.p. routes showed evidence of a dose-dependent effect on swimming and immobility behaviors, with 100 μg/rat (i.c.v.) and 20 mg/kg (i.p.) carbetocin significantly increasing the duration of swimming with a corresponding reduction in the duration of immobility relative to saline and a lower carbetocin dose. Intravenously administered carbetocin (2.5 mg/kg) also showed antidepressant-like activity, increasing climbing while decreasing immobility. The apparent non-dose dependency of the antidepressant-like effects of carbetocin via the intravenous route was unexpected. Any future work will involve examining the effects of lower doses of carbetocin because although there were no overt behavioral (locomotor) changes observed, there does seem to be a loss of effect at this higher dose. Since it was observed that injection of 20 mg/kg carbetocin i.p. resulted in reduced locomotor activity in the open field test, a similar effect might have contributed to the lack of efficacy of 5 mg/kg i.v. carbetocin. The relative potency of carbetocin in producing behaviorally similar outcomes via different routes of administration is consistent with its behavioral effects being centrally mediated. This can be seen by comparing similarly effective doses following central (100 μg; approximately 0.2 mg/kg), intravenous (2.5 mg/kg, a 12.5-fold increase in dose), and intraperitoneally administered carbetocin (6.4 mg/kg, a 32-fold increase).

Table 1 Acute treatment with imipramine, amphetamine, and carbetocin (administered via the i.c.v., i.v., or i.p. route) on locomotor activity, measured as distance traveled (cm)±standard deviation of the mean (SD) in an open field apparatus

Treatment group (measurement)	Dose	Sample size (*N*)	Mean distance traveled (cm)±SD
Saline, i.p.	–	8	2,006±291
Saline i.p.		10	3,782±550[a]
Saline, i.v.	–	10	3,684±760[a]
Saline, i.c.v.	–	9	2,040±330
Saline i.c.v. + Saline i.p.	–	6	2,352±320
Imipramine (mg/kg), i.p.	1.8	8	2,197±351
	5.6	8	2,147±335
	10	10	3,820±666[a]
Carbetocin (mg/kg), i.p.	6.4	8	1,612±287
	20	8	1,551±369[c]
Carbetocin (mg/kg), i.v.	2.5	10	3,922±581[a]
	5	10	3,933±672[a]
Carbetocin (μg/rat), i.c.v.	1	8	1,670±318
	10	8	1,700±631
	100	9	1,774±595
Atosiban (mg/kg), i.v.	1	10	3,848±579[a]
Carbetocin (mg/kg) i.p. + atosiban (μg/rat) i.c.v.	6.4+5	8	2,199±520
Carbetocin (mg/kg) i.v. + atosiban (μg/kg) i.v.	2.5+1	10	3,764±653[a]
Amphetamine (mg/kg), i.p.	2	8	2,609±225[b]

[a] Experiments carried out at a different time of year (higher baseline locomotor activity). All statistical analyses were done within same time-of-year groups

[b] significantly different from saline, i.p. ($p<0.001$)

[c] significantly different from saline, i.p. ($p<0.05$)

Co-administration of carbetocin with atosiban completely blocked carbetocin's antidepressant-like effect, producing a saline-like behavioral profile. The similarity in atosiban's blockade of systemically administered carbetocin (i.p. and i.v.) after both central (i.c.v.) and systemic (i.v.) administration of atosiban, and carbetocin's increased potency when administered directly into the lateral ventricles, suggests that carbetocin produces its antidepressant-like effects via a central mechanism. The reported lack of specificity of atosiban for oxytocin receptors (Pettibone et al. 1992) was examined using the vasopressin receptor agonist, desmopressin. Similar to a previous study by Nowakowska et al. (2002) in which subcutaneously injected vasopressin was evaluated in the FST, we found no effect of intravenous desmopressin at doses we have found to be systemically active at central and peripheral receptor targets (unpublished findings). The lack of effect of desmopressin and of atosiban alone or in combination with desmopressin suggests that atosiban's affinity for AVP1a receptors is not contributing to its antagonism of carbetocin's antidepressant-like activity.

It has been demonstrated in the present study that treatment with carbetocin can produce an antidepressant-like effect in the FST via multiple routes of administration. The similarity in behavioral effects arising from central and systemic drug administration concurs with the proposition that at high enough plasma concentrations, systemically administered peptides may reach significant concentrations in the CNS (Landgraf and Neumann 2004). This is in agreement with earlier findings using oxytocin, for example Meisenberg (1981), who demonstrated an antidepressant-like effect in mice following acute i.c.v. administration of oxytocin. Although i.p. administration of oxytocin was not examined in the Meisenberg (1981) study, the author suggested that oxytocin would be less efficacious at producing effects in the CNS via this route due to poor penetration of the blood-brain barrier. Indeed, the present study showed that there was a 32-fold difference in the potency of carbetocin when comparing doses that produced similar behavioral changes via different routes of administration. The findings of the present study suggest that the mechanism underlying earlier reports of oxytocin-induced antidepressant-like activity in mice and rats following i.p. administration (Arletti et al. 1995; Arletti and Bertolini 1987; Nowakowska et al. 2002) was activation of central oxytocin receptors. Although there are some procedural differences between these and the present study, collectively they demonstrate convincingly that oxytocin receptor

activation via either the i.p., i.v., or i.c.v. routes of administration is effective at inducing antidepressant-like activity in two rodent species as measured using the FST.

From the perspective of the simplified swim procedure used in the present study, the demonstration of antidepressant-like effects of imipramine and carbetocin provides independent support for the efficacy of methodological changes to the FST proposed by Broom et al. (2002a, b), whose work provided the first evidence that one swim session is sufficient for the measurement of antidepressant-like behaviors in the rat.

The findings of the present study support the hypothesis that oxytocin receptor activation is likely to play a significant role in the regulation of mood, one consequence of which is to produce antidepressant-like behavioral effects in the rat. Indeed, a doubling of oxytocin release in the central amygdala following forced swimming has been measured using microdialysis in the rat, which is thought to reflect facilitation of coping behaviors during stress (Ebner et al. 2005). Although the involvement of noradrenaline and serotonin in carbetocin's mechanism of action was not specifically investigated in the present study, it can be speculated that carbetocin's antidepressant-like effect may be mediated via a mixed noradrenergic and serotonergic mechanism of action, given the similarities between the behavioral profiles of carbetocin and imipramine in the FST. Imipramine and carbetocin both increased swimming behavior in the FST; an effect that Detke et al. (1995) has associated with a serotonergic mechanism of action. Similarly, the increased climbing behavior measured following systemic administration of either carbetocin or imipramine has been linked with a noradrenergic mechanism of action in the FST (Detke et al. 1995). In this context it is important to reflect on the close anatomical and functional relationships between the serotonergic and noradrenergic pathways. Noradrenergic neurons excite serotonergic neurons in the raphe nucleus via activation of alpha$_1$ adrenergic receptors (Aghajanian 1985). Therefore the effects of antidepressants such as imipramine (and possibly oxytocin and the oxytocin agonist, carbetocin) are unlikely to be solely reliant on activation of one class of neuron, arising instead from interrelated connections between serotonin and noradrenaline (Nutt 2002), as well as oxytocinergic neurons.

The present study demonstrated that carbetocin, an oxytocin receptor ligand, has antidepressant-like activity in the FST via multiple routes of administration. The increased potency of carbetocin following i.c.v. administration, as well as the evidence that its effects were blocked by the oxytocin antagonist atosiban following either central or systemic administration, supports the conclusion that the antidepressant-like effects of carbetocin are a consequence of oxytocin receptor activation in the CNS. The oxytocin receptor specificity of this effect of atosiban was examined using desmopressin (both ligands share affinity for AVP1a receptors). Neither drug produced any behavioral effects in either the FST or open field, suggesting that the antagonism of carbetocin's effects by atosiban was mediated via central oxytocin receptors. This suggests that following systemic administration, carbetocin crosses the blood-brain barrier in sufficient concentrations to produce its antidepressant-like effects. Apart from amphetamine (included as a false positive), none of the drugs screened for antidepressant activity increased locomotor activity. These findings suggest that modulation of central oxytocin receptors is well worth investigating as a pharmacological target for the treatment of depressive disorders. This will be facilitated by the evaluation of more selective and non-peptidic oxytocin and vasopressin receptor ligands. A better understanding of how oxytocin may act synergistically to enhance noradrenergic and serotonergic functions is likely to provide valuable insights into both the underlying neurobiology and pharmacological treatment of depressive disorders.

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The animal experiments reported in this manuscript comply with the regulations of the Bureau of Animal Welfare, Department of Primary Industries, Australia.

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