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RESEARCH PAPER

Body temperature and cardiac changes induced by peripherally administered oxytocin, vasopressin and the non-peptide oxytocin receptor agonist WAY 267,464: a biotelemetry study in rats

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BACKGROUND AND PURPOSE

There is current interest in oxytocin (OT) as a possible therapeutic in psychiatric disorders. However, the usefulness of OT may be constrained by peripheral autonomic effects, which may involve an action at both OT and vasopressin V_{1A} receptors. Here, we characterized the cardiovascular and thermoregulatory effects of OT, vasopressin (AVP) and the non-peptide OT receptor agonist WAY 267,464 in rats, and assessed the relative involvement of the OT and V_{1A} receptors in these effects.

EXPERIMENTAL APPROACH

Biotelemetry in freely moving male Wistar rats was used to examine body temperature and heart rate after OT (0.01 – 1 mg kg^{-1}; i.p.), AVP (0.001 – 0.1 mg kg^{-1}; i.p.) or WAY 267,464 (10 and 100 mg kg^{-1}; i.p.). The actions of the OT receptor antagonist Compound 25 (C25, 5 and 10 mg kg^{-1}) and V_{1A} receptor antagonist SR49059 (1 and 10 mg kg^{-1}) were studied, as well as possible V_{1A} receptor antagonist effects of WAY 267,464.

KEY RESULTS

OT and AVP dose-dependently reduced body temperature and heart rate. WAY 267,464 had similar, but more modest, effects. SR49059, but not C25, prevented the hypothermia and bradycardia induced by OT and AVP. WAY 267,464 (100 mg·kg^{-1}) prevented the effects of OT, and to some extent AVP.

CONCLUSIONS AND IMPLICATIONS

Peripherally administered OT and AVP have profound cardiovascular and thermoregulatory effects that appear to principally involve the V_{1A} receptor rather than the OT receptor. Additionally, WAY 267,464 is not a simple OT receptor agonist, as it has functionally relevant V_{1A} antagonist actions.

Abbreviations

AVP, vasopressin; BAT, brown adipose tissue; C25, Compound 25; NTS, nucleus of the solitary tract; OT, oxytocin

Introduction

Oxytocin (OT) and vasopressin (AVP) are two structurally related neuropeptides with a well-documented role in social behaviour, learning and memory, emotion, and motivation (for review see Benarroch, 2013). Both neuropeptides also have major physiological functions with OT involved in milk ejection, uterine contraction (Borrow and Cameron, 2011), energy balance (Olszewski *et al.*, 2010) and cardiovascular homeostasis (Gutkowska and Jankowski, 2012), and AVP playing important roles in regulating water retention and blood pressure (Frank and Landgraf, 2008). There is current widespread interest in the use of peripherally administered OT in treating human psychopathology characterized by impaired social behaviour, including autism (Domes *et al.*, 2013), addictions (McGregor and Bowen, 2012) and schizophrenia (Feifel *et al.*, 2012).

OT and AVP, like most peptides, are large molecules with physiochemical properties that impede entry into the brain (Mens *et al.*, 1983). While there is emerging evidence of brain penetration of OT in rodents (Neumann *et al.*, 2013), only a small proportion appears to pass, necessitating substantial peripheral doses to obtain clear behavioural effects (Uvnäs-Moberg *et al.*, 1994; Klenerova *et al.*, 2009; Hicks *et al.*, 2012). Such high peripheral doses may result in poor selectivity for their cognate receptors as well as significant cardiovascular and other off-target effects that may influence behaviour (Ring, 2011; Carson *et al.*, 2013). For example, OT influences body temperature (Lundeberg *et al.*, 1994; Ring *et al.*, 2006), and can cause a strong bradycardic response (Costa-e-Sousa *et al.*, 2005). Similarly, peripherally delivered AVP can cause dose-dependent hypothermia (Okuno *et al.*, 1965) and cardiovascular effects (Chernoff and Grabowski, 1971).

OT has affinity for both OT and AVP receptors and demonstrates surprisingly strong binding and functional activity at the AVP V_{1A} receptor (receptor nomenclature conforms to Alexander *et al.*, 2013) (Manning *et al.*, 2012). Indeed, OT-induced proconvulsive effects (Loyens *et al.*, 2011), smooth muscle contractions (Gupta *et al.*, 2009), analgesia (Schorscher-Petcu *et al.*, 2010) and even social behaviour (Sala *et al.*, 2011; Ramos *et al.*, 2013), may be primarily mediated by the V_{1A} receptor rather than the OT receptor. However, the extent to which the V_{1A} receptor mediates thermoregulatory and cardiac effects of OT relative to the OT receptor requires further investigation.

The poor brain penetration of OT has stimulated interest in the development of 'small-molecule' non-peptide ligands that selectively target the OT and/or V_{1A} receptor, with improved oral bioavailability and brain penetration relative to OT (Manning *et al.*, 2012). The first-generation non-peptide OT receptor agonist WAY 267,464, similar to OT, produces anxiolytic- and antipsychotic-like effects in rodents (Ring *et al.*, 2010). We recently showed that WAY 267,464 and OT caused similar, although slightly divergent patterns of c-Fos expression in rat brain, and subtly different behavioural effects in rodents (Hicks *et al.*, 2012). *In vitro* receptor binding and functional assays indicated unanticipated antagonist properties of WAY 267,464 at the V_{1A} receptor, in addition to primary OT receptor agonist effects. This would presumably distinguish WAY 267,464 from OT itself, which is both a V_{1A} and OT receptor agonist (Hicks *et al.*, 2012).

Here, we used biotelemetry to characterize the effects of peripherally administered OT, AVP and WAY 267,464 on body temperature and heart rate in freely moving rats. To determine the exact role of the OT and V_{1A} receptors in the observed changes in body temperature and heart rate, we examined the antagonist effects of the selective non-peptide OT receptor antagonist Compound 25 (C25) (Brown *et al.*, 2010; Ramos *et al.*, 2013), and the selective non-peptide V_{1A} receptor antagonist SR49059 (Serradeil-Le Gal *et al.*, 1993). Finally, given the possibility that WAY 267,464 may have V_{1A} receptor antagonist properties, we examined whether this compound might also antagonize some of the observed effects of OT and AVP.

Methods

Drugs and drug preparation

OT and AVP were purchased from AusPep, Ltd. (Parkville, VIC, Australia). The V_{1A} receptor antagonist SR49059 ((S)-1-[(2R,3S)-5-chloro-3-(2-chloro-phenyl)-1-(3,4-dimethoxy-benzenesulfonyl)-3-hydroxy-2,3-dihydro-1H-indole-2-carbonyl]-pyrrolidine-2-carboxylic acid amide) was obtained from Axon Medchem BV (Groningen, The Netherlands). C25 (5-(3-(3-(2-chloro-4-fluorophenoxy)azetidin-1-yl)-5-(methoxymethyl)-4H-1,2,4-triazol-4-yl)-2-methoxypyridine) and WAY 267,464 (4-(3,5-dihydroxybenzyl)-N-(2-methyl-4-(1-methyl-1,4,5,10-tetrahydrobenzo[β]pyrrolo[2,3-e][1,4]diazepine-5-carbonyl)benzyl)piperazine-1-carboxamide) were synthesized according to the procedures of Brown *et al.* (2010) and Hudson *et al.* (2005), respectively, and were considered to be of >95% purity according to proton, carbon nuclear magnetic resonance spectroscopy and mass spectrometry. All drugs were dissolved in a 15% DMSO, 2% Tween-80 and 83% physiological saline vehicle (Hicks *et al.*, 2012) and administered to rats via i.p. injection at a volume of 2 mL·kg^{-1}, or 4 mL·kg^{-1} for WAY 267,464.

Animals and housing

All experiments were performed in male Wistar rats (total n = 27; Animal Resources Centre, Perth, Australia) weighing 259–309 g at the start of testing. For 7 days before surgery, rats were housed in groups of three to four in large plastic tubs (640 × 400 × 220 mm) with corn cob bedding in a temperature-controlled colony room (23 ± 0.5°C) maintained on a reverse 12 h light/dark cycle (lights off at 09:00 h). Rats were provided with environmental enrichment that consisted of a perspex box, a chew stick and nesting material.

After surgery, rats were single housed in translucent Plexiglas tubs (420 × 260 × 180 mm) to allow for recording of individual animals using the biotelemetry system. These individual tubs had corn cob bedding and a chew stick and nesting material for environmental enrichment, and were kept on racks in a separate test room that was maintained under identical temperature and light conditions to the main colony room. Food and water were available *ad libitum* during all experiments. All experimental procedures were conducted under the approval of the University of Sydney Animal Ethics Committee in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (7th Edition, 2004), and are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

Surgery

Rats were anaesthetized with isoflurane gas (induction: 3% isoflurane, 3 L·min^{-1} O_2; maintenance: 2% isoflurane, 3 L·min^{-1} O_2), placed on a heating pad and the abdominal area was shaved and cleaned with ethanol. After testing of withdrawal reflexes to ensure adequate depth of anaesthesia, a rostro-caudal incision of approximately 2 cm was made along the midline of the abdomen, and the radiotelemetry transmitter was inserted into the peritoneal cavity. Transmitters were secured to the abdominal wall with non-absorbable sutures during closure of the peritoneal cavity. The biopotential leads from the transmitter were arranged in an Einthoven bipolar-lead II configuration with one lead placed near the right foreleg, and the other at the left hind leg. Animals were administered an analgesic (Ilium Flunixil, Troy Laboratories, Glendenning, Australia; 0.02 mg·kg^{-1}, i.p.), an antibiotic (Ilium Oxytet-200 L.A., Troy Laboratories; 0.1 mg·kg^{-1}, i.p.) and sterile physiological saline (0.9%; 2.5 mL·kg^{-1}, i.p.) post-operatively for 3 days, and allowed to recover for 7 days before testing commenced.

Measurement of body temperature and heart rate

Surgically implanted radiotelemetry transmitters [TA11CTA-F40, Data Sciences International (DSI), St. Paul, MN, USA] were used to measure the body temperature (°C) and ECG of freely moving rats. Transmitters were turned on by means of a magnet held close to the animal's abdomen that activated a magnetic switch within the transmitter. Radio signals from the transmitters were detected by a total of eight RPC-1 receivers connected to two Data Exchange Matrices (Dataquest A.R.T., DSI). The temperature and ECG for each implanted transmitter was calibrated according to the manufacturer's configuration settings. A personal computer (Dell, Precision T3500) inside the room running the Dataquest A.R.T. software (version 4.3, DSI) was used for configuration, control, acquisition and storage of body temperature and ECG data. Heart rate (beats min^{-1}) was derived from the ECG data.

Experimental procedures

All experiments were conducted at an ambient temperature of 23°C (±0.5). For each experiment described later, body temperature and heart rate were averaged over 5 min intervals from 60 min before, to 180 min after drug treatment. This post-injection period was sufficiently long for the majority of the observed drug effects to return to baseline levels. Rats remained in their individual home cages throughout the experiments, with the exception of when they were briefly removed to be injected with their allocated treatment. All experiments were performed during the dark phase and commenced 3 h after lights were switched off. Rats show a major activity burst immediately after light offset (Sei *et al.*, 1997), so this time point was chosen to ensure the animal's body temperature and heart rate had stabilized following the transition into the dark phase.

Each drug treatment was separated by a 48 h washout period to ensure adequate drug clearance, and each rat received a maximum of six specific drug treatments (excluding vehicle treatment). To minimize any changes in body temperature and heart rate resulting from handling or injection stress, rats were handled for 5 min each for 5 days prior to testing, and were given a dummy injection of vehicle (2 mL·kg^{-1}) on the last 2 days of the handling period.

OT dose–response (Experiment 1). An initial exploratory experiment characterized the dose-dependent effects of OT on body temperature and heart rate after peripheral administration. Three experimentally naïve rats were each tested with vehicle before three doses of OT (0.01, 0.1 and 1 mg·kg^{-1}) were given in ascending order.

C25 and OT (Experiments 2A and 2B). To determine whether the body temperature and heart rate effects of OT are mediated by the OT receptor, four experimentally naïve rats were administered OT with or without the selective OT receptor antagonist C25 at two dose levels (5 and 10 mg·kg^{-1}). Drug combinations included (i) vehicle + vehicle; (ii) C25 (5 mg·kg^{-1}) + OT (1 mg·kg^{-1}); (iii) C25 (10 mg·kg^{-1}) + OT (1 mg·kg^{-1}); and (iv) vehicle + OT (1 mg·kg^{-1}), and were administered in this sequence. Experiment 2B was performed 1 week after the conclusion of Experiment 2A to determine whether C25 by itself has intrinsic effects on body temperature and heart rate. The same cohort of rats (n = 4) from Experiment 2A were administered (i) vehicle + vehicle; (ii) C25 (5 mg·kg^{-1}) + vehicle; and (iii) C25 (10 mg·kg^{-1}) + vehicle, in this sequence. The first and second injections were separated by 15 min in both experiments.

SR49059 and OT (Experiments 3A and 3B). This experiment employed the selective V_{1A} receptor antagonist SR49059 to examine the extent to which OT acts on the V_{1A} receptor to affect body temperature and heart rate. SR49059 was administered at a dose of 1 mg·kg^{-1}, which produces significant inhibitory effects in a rat model of AVP-induced hypertension (Serradeil-Le Gal *et al.*, 1993) and prevents OT-induced prosocial effects (Ramos *et al.*, 2013). A maximal dose of 10 mg·kg^{-1} was also tested. Four experimentally naïve rats were given the following treatments in the order of: (i) vehicle + vehicle; (ii) SR49059 (1 mg·kg^{-1}) + OT (1 mg·kg^{-1}); (iii) SR49059 (10 mg·kg^{-1}) + OT (1 mg·kg^{-1}); and (iv) vehicle + OT (1 mg·kg^{-1}). Experiment 3B, performed 1 week after the conclusion of Experiment 3A, determined any intrinsic effects of SR49059 alone at 1 and 10 mg·kg^{-1} on body temperature and

heart rate. The same cohort of rats (n = 4) from Experiment 3A were administered (i) vehicle + vehicle; (ii) SR49059 (1 mg·kg^{-1}) + vehicle; and (iii) SR49059 (10 mg·kg^{-1}) + vehicle, in this sequence. The first and second injections were separated by 15 min in both experiments.

AVP dose–response (Experiment 4). Experiment 4 explored the dose-dependent effects of peripheral AVP on body temperature and heart rate. Four experimentally naïve rats were treated with vehicle and then three doses of AVP (0.001, 0.01 and 0.1 mg·kg^{-1}) in ascending order.

SR49059 and AVP (Experiment 5). Four experimentally naïve rats were given the following treatments in the order of: (i) vehicle + vehicle; (ii) SR49059 (1 mg·kg^{-1}) + AVP (0.1 mg·kg^{-1}); (iii) SR49059 (10 mg·kg^{-1}) + AVP (0.1 mg·kg^{-1}); and (iv) vehicle + AVP (0.1 mg·kg^{-1}). The first and second injections were separated by 15 min.

WAY 267,464 dose–response (Experiment 6). Experiment 6 examined whether WAY 267,464 has OT-like effects on body temperature and heart rate, given their previously observed similarities (Ring *et al.*, 2010; Hicks *et al.*, 2012). Four experimentally naïve rats were first tested with vehicle followed by consecutive tests with WAY 267,464 (10 and 100 mg·kg^{-1}) in ascending order, with these doses based on our previous study (Hicks *et al.*, 2012).

C25 and WAY 267,464 (Experiment 7). Experiment 7 determined whether WAY 267,464 acts on the OT receptor to affect body temperature and heart rate. One week after Experiment 6, the same cohort of rats in Experiment 6 (n = 4) received in the following order: (i) vehicle + vehicle; (ii) C25 (10 mg·kg^{-1}) + WAY 267,464 (100 mg·kg^{-1}); and (iii) vehicle + WAY 267,464 (100 mg·kg^{-1}). The first and second injections were separated by 15 min. One rat was removed from the analysis because of a transmitter failure.

WAY 267,464 and OT (Experiment 8). Our recent research has raised the possibility that WAY 267,464 might have a V_{1A} receptor antagonist action (Hicks *et al.*, 2012). Accordingly, we assessed whether the hypothermia and bradycardia produced by OT might be prevented by pretreatment with WAY 267,464. Four experimentally naïve rats were given in the following order: (i) vehicle + vehicle; (ii) WAY 267,464 (10 mg·kg^{-1}) + OT (1 mg·kg^{-1}); (iii) WAY 267,464 (100 mg·kg^{-1}) + OT (1 mg·kg^{-1}); and (iv) vehicle + OT (1 mg·kg^{-1}). The first and second injections were separated by 20 min.

WAY 267,464 and AVP (Experiment 9). In the final experiment, WAY 267,464 was given in combination with AVP as a further test of its putative V_{1A} receptor antagonist action. One week after the AVP dose–response experiment (Experiment 4), the same group of rats used in Experiment 4 (n = 4) received treatments as follows: (i) vehicle + vehicle; (ii) WAY 267,464 (10 mg·kg^{-1}) + AVP (0.1 mg·kg^{-1}); (iii) WAY 267,464 (100 mg·kg^{-1}) + AVP (0.1 mg·kg^{-1}); and (iv) vehicle + AVP (0.1 mg·kg^{-1}). The first and second injections were separated by 20 min.

Statistical analysis

For Experiments 1, 4 and 6 (the dose–response studies of OT, AVP and WAY 267,464), orthogonal polynomial contrasts were used to examine changes in body temperature and heart rate across ascending doses of each drug treatment averaged over the 180 min post-injection period. For the remaining experiments, planned contrasts (two-way repeated measures ANOVA) examined treatment × time effects on body temperature and heart rate with each treatment compared with the relevant control condition. Any baseline (pretreatment) differences in average body temperature and heart rate between treatment conditions were identified during the 60 min before drug treatment using *a priori* contrasts (one-way repeated measures ANOVA) and are noted in the results section. The Bonferroni correction was used to adjust for non-orthogonal contrasts, and problems with sphericity of the data (Mauchly's test) were addressed using the Greenhouse–Geisser correction. Corrected degrees of freedom are reported where relevant. All analyses were conducted using Statistical Package for the Social Sciences (SPSS) version 19 (SPSS Inc., IBM, Chicago, IL, USA) with significance set at 0.05 or where corrected using Bonferroni ($P < 0.05/2 = 0.025$ or $P < 0.05/3 = 0.017$).

Results

Table 1 shows the average body temperature and heart rate during the 60 min baseline period for each treatment in each experiment. Summary statistical results for body temperature and heart rate during the post-injection period for each experiment are presented in Tables 2 and 3 respectively.

Dose-dependent effects of OT on body temperature and heart rate

The effects of OT on body temperature and heart rate are shown in Figure 1A and B respectively. Polynomial contrast analysis showed a significant linear and quadratic trend (both $P < 0.05$) in body temperature across ascending doses of OT, with the highest dose (1 mg·kg^{-1}) causing a strong hypothermic effect over the post-injection period. For heart rate, there was a strong tendency towards a linear trend ($P = 0.053$), and a significant quadratic trend ($P < 0.05$), over ascending doses of OT, which again reflected the highest dose strongly reducing heart rate in the post-treatment period.

Effects of C25 on OT-induced hypothermia and bradycardia

The effects of C25 on the hypothermia and bradycardia induced by OT (1 mg·kg^{-1}) are shown in Figure 2A and B. Consistent with Experiment 1, OT produced a strong hypothermic and bradycardic response relative to vehicle (both $P < 0.01$) that recovered to baseline levels after 2–3 h (treatment × time interaction, body temperature: $P < 0.01$; heart rate: $P < 0.017$). Pretreatment with either dose of C25 (i.e. 5 and 10 mg·kg^{-1}) did not significantly affect these actions of OT (1 mg·kg^{-1}) (both doses $P > 0.017$; Bonferroni-corrected).

When given alone, C25 at 5 and 10 mg·kg^{-1} did not significantly affect body temperature relative to vehicle

Table 1

Average body temperature and heart rate during the baseline period

Experiment	Body temperature (°C)	Heart rate (beats min^{-1})
1. OT dose–response		
(i) VEH	38.11 ± 0.05	438.52 ± 42.89
(ii) 0.01 mg·kg^{-1}	38.18 ± 0.04	453.82 ± 24.08
(iii) 0.1 mg·kg^{-1}	38.10 ± 0.05	435.48 ± 26.88
(iv) 1 mg·kg^{-1}	38.11 ± 0.10	419.73 ± 19.18
2A. C25 (5 and 10 mg·kg^{-1}) and OT (1 mg·kg^{-1})		
(i) VEH + VEH	37.78 ± 0.10	395.26 ± 18.30
(ii) C25 (5) + OT	37.71 ± 0.02	414.00 ± 7.12
(iii) C25 (10) + OT	37.64 ± 0.09	403.84 ± 3.25
(iv) VEH + OT	37.75 ± 0.10	399.29 ± 14.64
2B. C25 (5 and 10 mg·kg^{-1}) and VEH		
(i) VEH + VEH	37.77 ± 0.10	418.65 ± 8.50
(ii) C25 (5) + VEH	37.79 ± 0.07	428.77 ± 8.69
(iii) C25 (10) + VEH	37.78 ± 0.05	413.70 ± 10.02
3A. SR (1 and 10 mg·kg^{-1}) and OT (1 mg·kg^{-1})		
(i) VEH + VEH	38.22 ± 0.06	396.62 ± 10.53
(ii) SR (1) + OT	38.19 ± 0.11	432.36 ± 10.33
(iii) SR (10) + OT	38.26 ± 0.12	445.15 ± 4.20
(iv) VEH + OT	38.34 ± 0.16	431.16 ± 6.84
3B. SR (1 and 10 mg·kg^{-1}) and VEH		
(i) VEH + VEH	37.91 ± 0.12	393.91 ± 6.24
(ii) SR (1) + VEH	37.97 ± 0.04	395.66 ± 8.28
(iii) SR (10) + VEH	38.08 ± 0.08	427.40 ± 9.69
4. AVP dose–response		
(i) VEH	38.19 ± 0.02	444.52 ± 12.01
(ii) 0.001 mg·kg^{-1}	38.05 ± 0.10	429.50 ± 7.58
(iii) 0.01 mg·kg^{-1}	38.19 ± 0.10	441.09 ± 9.73
(iv) 0.1 mg·kg^{-1}	38.17 ± 0.09	428.73 ± 9.67
5. SR (1 and 10 mg·kg^{-1}) and AVP (0.1 mg·kg^{-1})		
(i) VEH + VEH	38.04 ± 0.09	397.61 ± 9.68
(ii) SR (1) + AVP	38.07 ± 0.10	411.80 ± 5.44
(iii) SR (10) + AVP	38.24 ± 0.04	409.37 ± 15.57
(iv) VEH + AVP	38.23 ± 0.10	402.63 ± 7.22
6. WAY dose–response		
(i) VEH	37.98 ± 0.12	414.54 ± 10.73
(ii) 10 mg·kg^{-1}	38.03 ± 0.12	425.14 ± 13.99
(iii) 100 mg·kg^{-1}	38.04 ± 0.15	424.45 ± 11.63
7. C25 (10 mg·kg^{-1}) and WAY (100 mg·kg^{-1})		
(i) VEH + VEH	37.88 ± 0.21	389.29 ± 14.19
(ii) C25 + WAY	37.90 ± 0.09	432.70 ± 15.72*
(iii) VEH + WAY	38.05 ± 0.17	417.44 ± 14.40
8. WAY (10 and 100 mg·kg^{-1}) and OT (1 mg·kg^{-1})		
(i) VEH + VEH	37.82 ± 0.12	396.01 ± 9.39
(ii) WAY (10) + OT	37.98 ± 0.10	418.04 ± 11.14
(iii) WAY (100) + OT	37.90 ± 0.09	403.36 ± 11.39
(iv) VEH + OT	37.71 ± 0.07	398.29 ± 9.86
9. WAY (10 and 100 mg·kg^{-1}) and AVP (0.1 mg·kg^{-1})		
(i) VEH + VEH	37.86 ± 0.12	402.79 ± 15.02
(ii) WAY (10) + AVP	37.92 ± 0.13	395.87 ± 18.54
(iii) WAY (100) + AVP	38.04 ± 0.09	422.58 ± 10.87
(iv) VEH + AVP	37.93 ± 0.05	381.51 ± 4.58

Values represent the mean ± SEM.
Asterisk represents a significant difference in heart rate: *$P < 0.025$.
C25, Compound 25; SR, SR49059; VEH, vehicle; WAY, WAY 267,464.

Table 2

Treatment effects on body temperature during the 180 min post-injection period

Experiment	Treatment	Treatment × Time
1. OT dose–response		
(i) Linear trend	$F(1,2) = 21.15$*	–
(ii) Quadratic trend	$F(1,2) = 23.64$*	–
2A. C25 (5 and 10 mg·kg^{-1}) and OT (1 mg·kg^{-1})		
(i) VEH versus OT	$F(1,3) = 33.93$**	$F(1.49,4.46) = 28.52$**
(ii) OT versus C25 (5) + OT	$F(1,3) = 1.00$	$F(1.84,5.52) = 0.40$
(iii) OT versus C25 (10) + OT	$F(1,3) = 0.01$	$F(1.82,5.47) = 1.29$
2B. C25 (5 and 10 mg·kg^{-1}) and VEH		
(i) VEH versus C25 (5) + VEH	$F(1,3) = 3.16$	$F(2.25,6.75) = 0.62$
(ii) VEH versus C25 (10) + VEH	$F(1,3) = 1.11$	$F(2.35,7.05) = 1.67$
3A. SR (1 and 10 mg·kg^{-1}) and OT (1 mg·kg^{-1})		
(i) VEH versus OT	$F(1,3) = 32.71$*	$F(1.56,4.67) = 17.22$**
(ii) OT versus SR (1) + OT	$F(1,3) = 22.56$	$F(1.68,5.03) = 14.81$**
(iii) OT versus SR (10) + OT	$F(1,3) = 26.76$*	$F(1.91,5.72) = 30.34$***
3B. SR (1 and 10 mg·kg^{-1}) and VEH		
(i) VEH versus SR (1) + VEH	$F(1,3) = 0.91$	$F(1.90,5.69) = 0.41$
(ii) VEH versus SR (10) + VEH	$F(1,3) = 9.19$	$F(2.29,6.86) = 2.51$
4. AVP dose–response		
(i) Linear trend	$F(1,3) = 56.08$**	–
(ii) Quadratic trend	$F(1,3) = 15.07$*	–
5. SR (1 and 10 mg·kg^{-1}) and AVP (0.1 mg·kg^{-1})		
(i) VEH versus AVP	$F(1,3) = 132.56$***	$F(2.27,6.81) = 16.44$**
(ii) AVP versus SR (1) + AVP	$F(1,3) = 33.18$**	$F(1.92,5.77) = 32.63$***
(iii) AVP versus SR (10) + AVP	$F(1,3) = 102.27$**	$F(2.14,6.41) = 22.11$***
6. WAY dose–response		
(i) Linear trend	$F(1,3) = 22.15$*	–
(ii) Quadratic trend	$F(1,3) = 10.56$*	–
7. C25 (10 mg·kg^{-1}) and WAY (100 mg·kg^{-1})		
(i) VEH versus WAY	$F(1,2) = 59.67$*	$F(1.27,2.54) = 14.21$
(ii) WAY versus C25 + WAY	$F(1,2) = 0.34$	$F(1.30,2.59) = 0.23$
8. WAY (10 and 100 mg·kg^{-1}) and OT (1 mg·kg^{-1})		
(i) VEH versus OT	$F(1,3) = 104.03$**	$F(2.22,6.67) = 47.58$***
(ii) OT versus WAY (10) + OT	$F(1,3) = 44.30$**	$F(2.17,6.49) = 19.26$**
(iii) OT versus WAY (100) + OT	$F(1,3) = 27.22$*	$F(2.56,7.69) = 50.66$***
9. WAY (10 and 100 mg·kg^{-1}) and AVP (0.1 mg·kg^{-1})		
(i) VEH versus AVP	$F(1,3) = 32.19$*	$F(1.72,5.17) = 7.00$
(ii) AVP versus WAY (10) + AVP	$F(1,3) = 4.77$	$F(1.33,4.00) = 1.56$
(iii) AVP versus WAY (100) + AVP	$F(1,3) = 0.99$	$F(1.48,4.44) = 1.87$

Asterisks represent significant differences in body temperature: *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.
C25, Compound 25; SR, SR49059; VEH, vehicle; WAY, WAY 267,464.

(both doses $P > 0.025$; Bonferroni-corrected) (Figure 2C). However, both doses caused a subtle increase in heart rate over the 180 min post-injection period relative to vehicle (5 mg·kg^{-1}: $P < 0.025$; 10 mg·kg^{-1}: $P < 0.01$) (Figure 2D).

Effects of SR49059 on OT-induced hypothermia and bradycardia

The effects of SR49059 on OT-induced hypothermia and bradycardia are illustrated in Figure 3A and B respectively. A high dose of OT (1 mg·kg^{-1}) again induced potent

Table 3

Treatment effects on heart rate during the 180 min post-injection period

Experiment	Treatment	Treatment × Time
1. OT dose–response		
(i) Linear trend	$F(1,2) = 17.26$	–
(ii) Quadratic trend	$F(1,2) = 18.45$*	–
2A. C25 (5 and 10 mg·kg^{-1}) and OT (1 mg·kg^{-1})		
(i) VEH versus OT	$F(1,3) = 57.03$**	$F(2.38,7.15) = 7.84$*
(ii) OT versus C25 (5) + OT	$F(1,3) = 4.24$	$F(2.67,8.00) = 1.16$
(iii) OT versus C25 (10) + OT	$F(1,3) = 19.40$	$F(2.39,7.16) = 1.17$
2B. C25 (5 and 10 mg·kg^{-1}) and VEH		
(i) VEH versus C25 (5) + VEH	$F(1,3) = 20.66$*	$F(2.90,8.69) = 1.41$
(ii) VEH versus C25 (10) + VEH	$F(1,3) = 47.06$**	$F(2.73,8.18) = 1.51$
3A. SR (1 and 10 mg·kg^{-1}) and OT (1 mg·kg^{-1})		
(i) VEH versus OT	$F(1,3) = 126.43$**	$F(2.27,6.80) = 7.56$*
(ii) OT versus SR (1) + OT	$F(1,3) = 21.78$	$F(2.52,7.55) = 10.22$**
(iii) OT versus SR (10) + OT	$F(1,3) = 59.30$**	$F(2.62,7.86) = 13.03$**
3B. SR (1 and 10 mg·kg^{-1}) and VEH		
(i) VEH versus SR (1) + VEH	$F(1,3) = 0.32$	$F(2.15,6.45) = 0.86$
(ii) VEH versus SR (10) + VEH	$F(1,3) = 0.09$	$F(2.36,7.08) = 1.67$
4. AVP dose–response		
(i) Linear trend	$F(1,3) = 84.17$**	–
(ii) Quadratic trend	$F(1,3) = 13.67$*	–
5. SR (1 and 10 mg·kg^{-1}) and AVP (0.1 mg·kg^{-1})		
(i) VEH versus AVP	$F(1,3) = 571.38$***	$F(2.21,6.63) = 2.98$
(ii) AVP versus SR (1) + AVP	$F(1,3) = 41.28$**	$F(2.49,7.46) = 2.22$
(iii) AVP versus SR (10) + AVP	$F(1,3) = 92.61$**	$F(2.25,6.76) = 4.72$
6. WAY dose–response		
(i) Linear trend	$F(1,3) = 5.23$	–
(ii) Quadratic trend	$F(1,3) = 12.63$*	–
7. C25 (10 mg·kg^{-1}) and WAY (100 mg·kg^{-1})		
(i) VEH versus WAY	$F(1,2) = 19.16$	$F(1.96,3.92) = 3.98$
(ii) WAY versus C25 + WAY	$F(1,2) = 0.09$	$F(1.66,3.33) = 0.57$
8. WAY (10 and 100 mg·kg^{-1}) and OT (1 mg·kg^{-1})		
(i) VEH versus OT	$F(1,3) = 217.31$***	$F(2.62,7.85) = 6.64$*
(ii) OT versus WAY (10) + OT	$F(1,3) = 58.17$**	$F(2.54,7.61) = 3.43$
(iii) OT versus WAY (100) + OT	$F(1,3) = 31.52$*	$F(2.11,6.34) = 8.49$*
9. WAY (10 and 100 mg·kg^{-1}) and AVP (0.1 mg·kg^{-1})		
(i) VEH versus AVP	$F(1,3) = 108.58$**	$F(2.57,7.70) = 4.63$
(ii) AVP versus WAY (10) + AVP	$F(1,3) = 2.28$	$F(2.54,7.62) = 3.01$
(iii) AVP versus WAY (100) + AVP	$F(1,3) = 18.28$	$F(1.85,5.54) = 2.92$

Asterisks represent significant differences in heart rate: *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.
C25, Compound 25; SR, SR49059; VEH, vehicle; WAY, WAY 267,464.

hypothermia ($P < 0.017$) and bradycardia ($P < 0.01$) relative to vehicle. SR49059 at 1 and 10 mg·kg^{-1} prevented the hypothermia (treatment effect, 1 mg·kg^{-1}: $P = 0.018$; 10 mg·kg^{-1}: $P < 0.017$; treatment × time interaction, 1 mg·kg^{-1}: $P < 0.01$; 10 mg·kg^{-1}: $P < 0.001$) and bradycardia (treatment effect, 10 mg·kg^{-1}: $P < 0.01$; treatment × time interaction, both $P < 0.01$) caused by OT.

SR49059 given alone produced no significant effects on body temperature or heart rate, all $P > 0.025$ (Bonferroni-corrected; Figure 3C and D respectively). There was however,

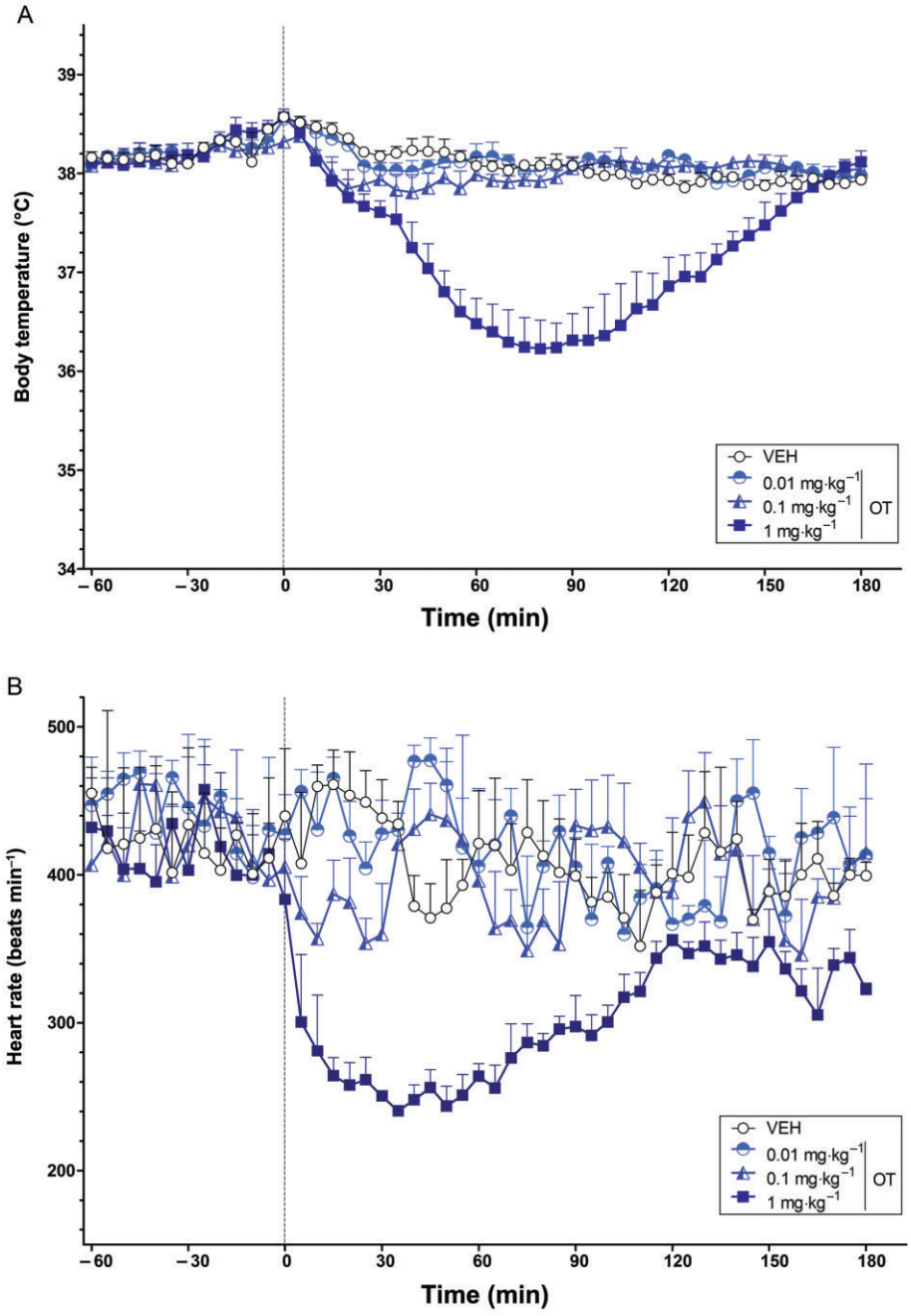

Figure 1

The dose-dependent effects of OT on body temperature (°C) (A) and heart rate (beats min^{-1}) (B) over time (min). The vertical grey hashed line on the *X*-axis at 0 indicates the time of vehicle or OT administration. Data are the means + SEM. VEH, vehicle.

a trend towards a hypothermic effect with the highest dose (10 $mg{\cdot}kg^{-1}$), $P = 0.056$.

Dose-dependent effects of AVP on body temperature and heart rate

The effects of AVP on body temperature and heart rate are illustrated in Figure 4A and B respectively. Polynomial contrast analysis identified a significant linear ($P < 0.01$) and quadratic ($P < 0.05$) trend in body temperature and heart rate across ascending doses of AVP. All doses induced a drop in body temperature and heart rate, with the highest dose (0.1 $mg{\cdot}kg^{-1}$) producing sustained hypothermia and bradycardia across the entire post-injection period.

Effects of SR49059 on AVP-induced hypothermia and bradycardia

Consistent with Experiment 4, AVP at a high dose (0.1 $mg{\cdot}kg^{-1}$) induced potent and prolonged hypothermia

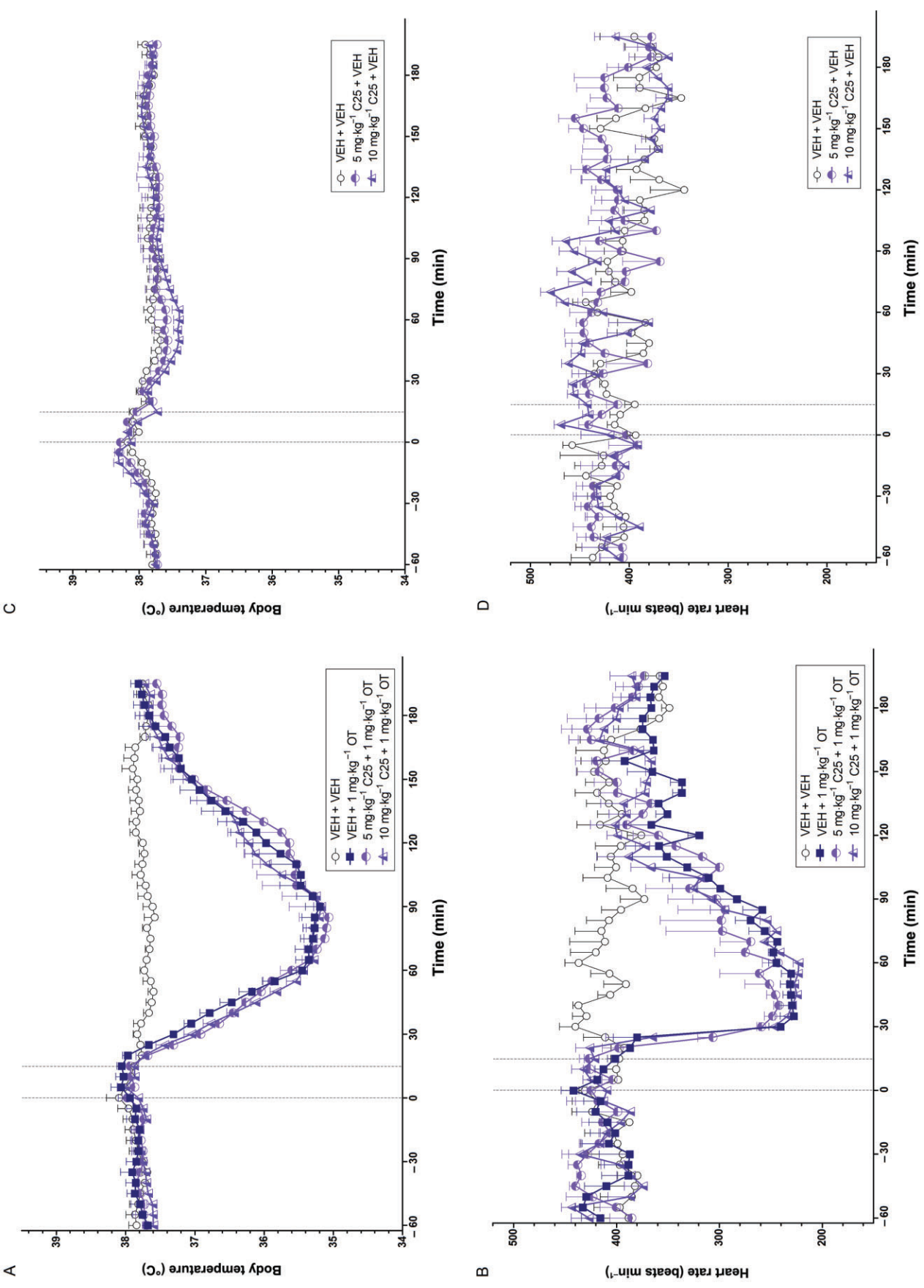

Figure 2

The body temperature (°C) (A, C) and heart rate (beats min^{-1}) (B, D) effects of the non-peptide OT receptor antagonist C25 in combination with OT (A, B) and vehicle (C, D) over time (min). The vertical grey hashed line on the *X*-axis at 0 indicates the time of vehicle or C25 administration, while the second line at $X = 15$ indicates the time of vehicle or OT injection. Data are the means + SEM. VEH, vehicle.

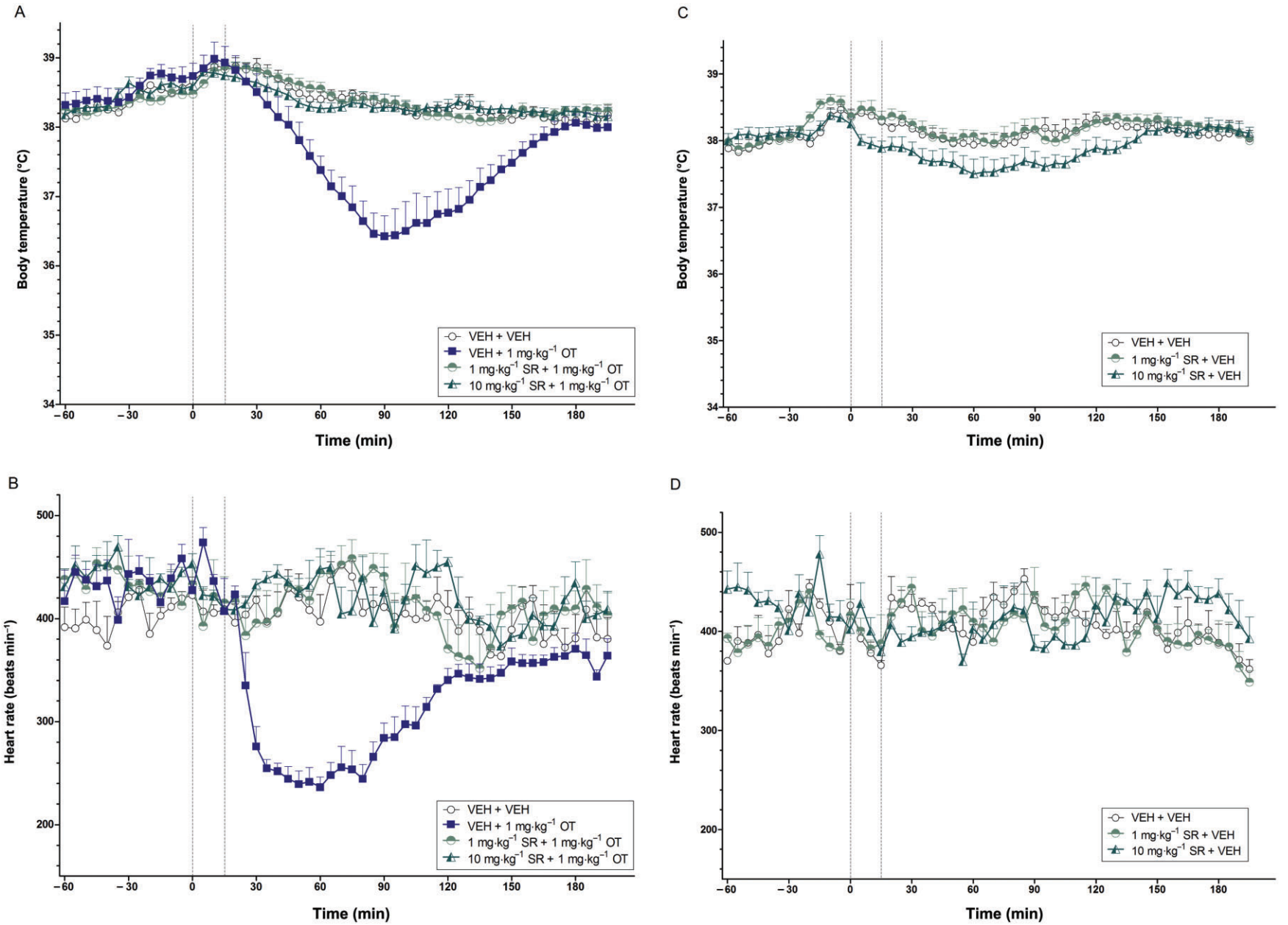

Figure 3

The body temperature (°C) (A, C) and heart rate (beats min^{-1}) (B, D) effects of the non-peptide V_{1A} receptor antagonist SR49059 in combination with OT (A, B) and vehicle (C, D) over time (min). The vertical grey hashed line on the *X*-axis at 0 indicates the time of vehicle or SR49059 administration, while the second line at $X = 15$ indicates the time of vehicle or OT injection. Data are the means + SEM. VEH, vehicle; SR, SR49059.

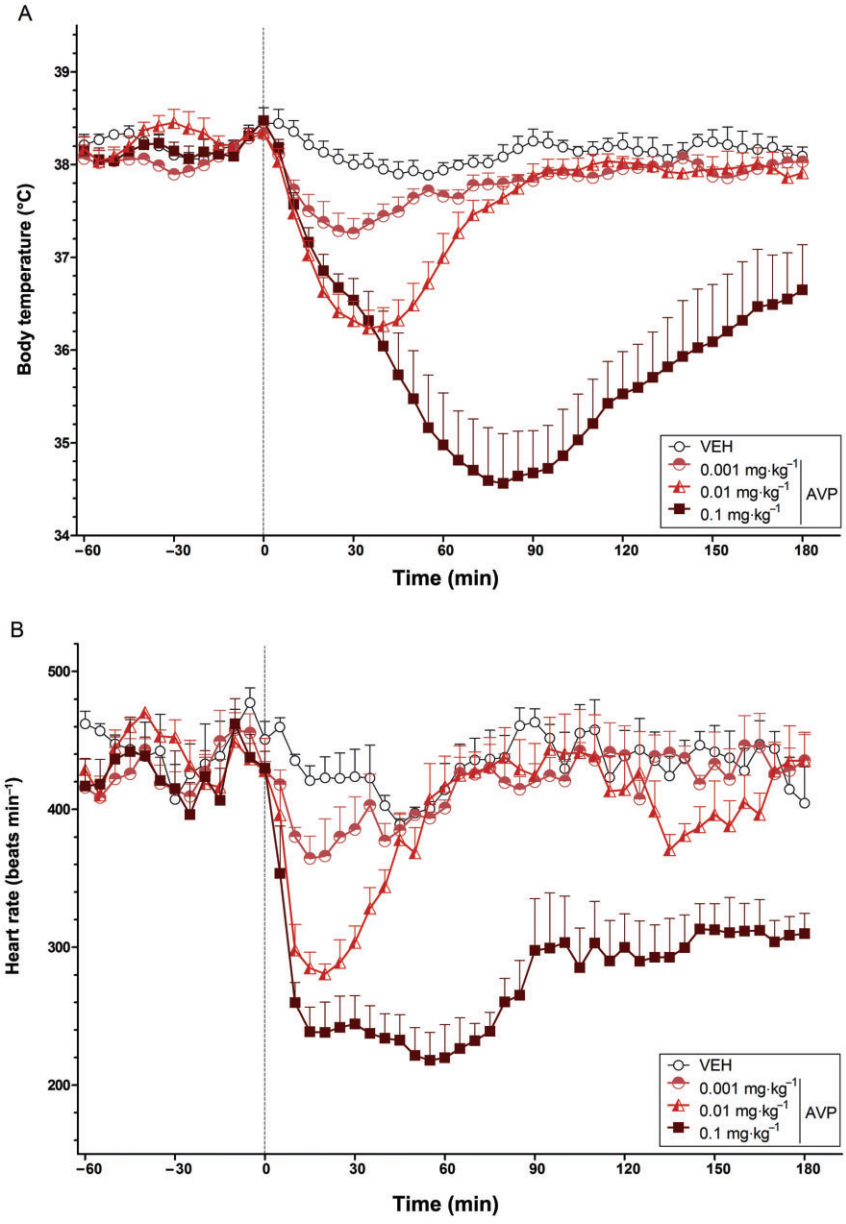

Figure 4

The dose-dependent effects of AVP on body temperature (°C) (A) and heart rate (beats min^{-1}) (B) over time (min). The vertical grey hashed line on the *X*-axis at 0 indicates the time of vehicle or AVP administration. Data are the means + SEM. VEH, vehicle.

and bradycardia (both $P < 0.001$). Pretreatment with either dose of SR49059 (i.e. 1 and 10 mg·kg^{-1}) reduced the hypothermic (treatment effect, both $P < 0.01$; treatment × time interaction, both $P < 0.001$) and bradycardic (treatment effect, both $P < 0.01$) effects of AVP (Figure 5A and B respectively). The higher dose of SR49059 (10 mg·kg^{-1}) was required to completely prevent the body temperature and heart rate effects of AVP (0.1 mg·kg^{-1}).

Dose-dependent effects of WAY 267,464 on body temperature and heart rate

Polynomial contrast analysis identified a significant linear and quadratic trend (both $P < 0.05$) in body temperature over the two doses of WAY 267,464, with the higher dose (100 mg·kg^{-1}) producing hypothermia that lasted across much of the test period (Figure 6A). There was also a significant quadratic trend ($P < 0.05$) in heart rate with WAY 267,464 reflecting a transient reduction in heart rate with the higher dose (Figure 6B).

Effects of C25 on WAY 267,464-induced hypothermia and bradycardia

There were no significant differences in pretreatment body temperature (all $P > 0.025$; Bonferroni-corrected). However, heart rate during the baseline before the C25 +

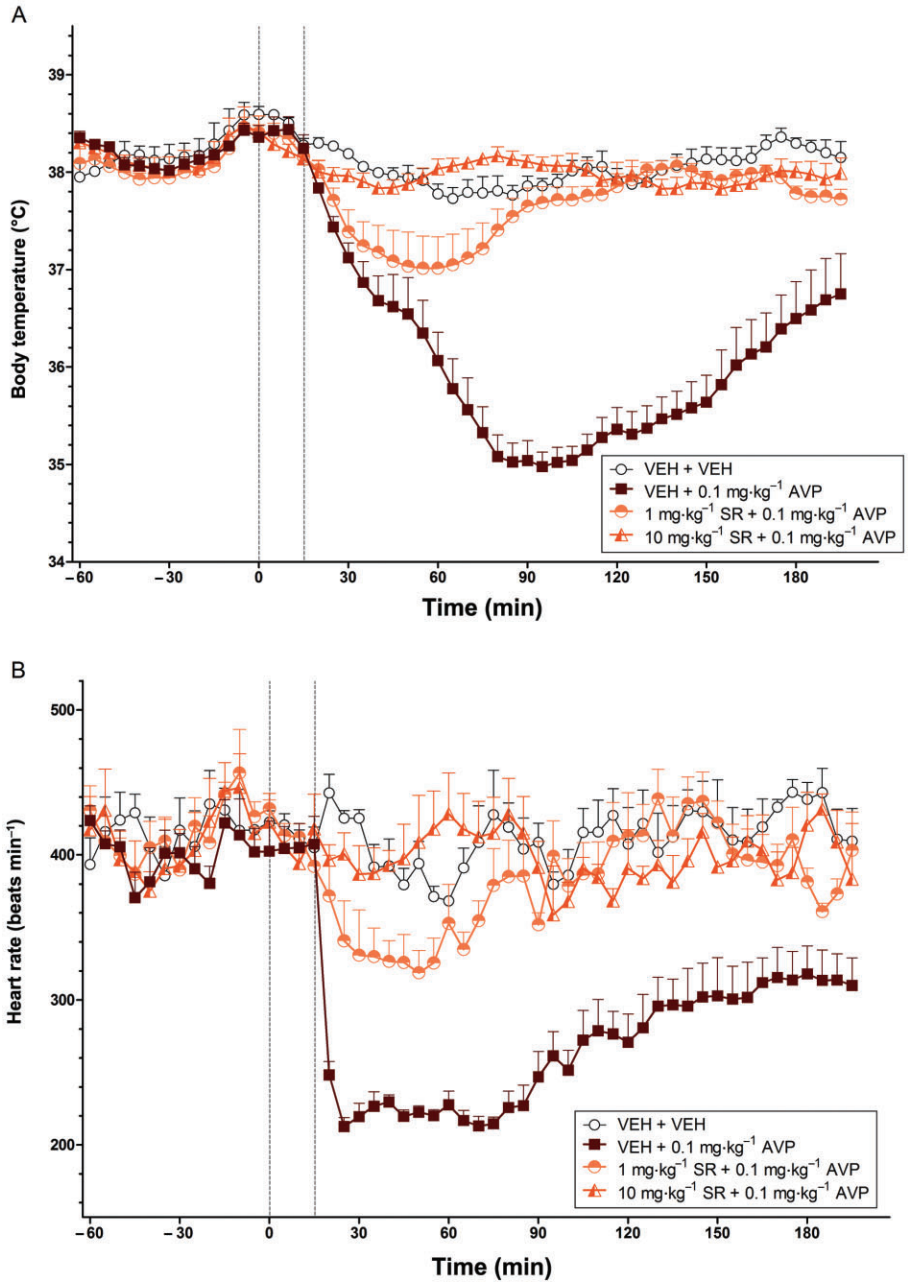

Figure 5

The effects of SR49059 on the body temperature (°C) (A) and heart rate (beats min^{-1}) (B) changes induced by AVP over time (min). The vertical grey hashed line on the *X*-axis at 0 indicates the time of vehicle or SR49059 administration, while the second line at *X* = 15 indicates the time of vehicle or AVP injection. Data are the means + SEM. VEH, vehicle; SR, SR49059.

WAY 267,464 treatment was slightly elevated relative to the baseline for the vehicle + WAY 267,464 condition [$F(1,2) = 54.91$, $P < 0.025$]. A high dose of WAY 267,464 (100 $mg{\cdot}kg^{-1}$) again caused prolonged hypothermia relative to vehicle ($P < 0.025$) (Figure 7A), while the transient bradycardic effect approached significance ($P = 0.048$) (Figure 7B). Pretreatment with C25 at 10 $mg{\cdot}kg^{-1}$ failed to attenuate the hypothermic and bradycardic effects of WAY 267,464 (all $P > 0.025$; Bonferroni-corrected).

Effects of WAY 267,464 on OT-induced hypothermia and bradycardia

OT (1 $mg{\cdot}kg^{-1}$) again produced a robust hypothermic ($P < 0.01$) and bradycardic ($P < 0.001$) effect compared with vehicle that followed a characteristic U-shaped response over time. Pretreatment with 10 or 100 $mg{\cdot}kg^{-1}$ WAY 267,464 significantly attenuated the body temperature (treatment effect, 10 $mg{\cdot}kg^{-1}$: $P < 0.01$; 100 $mg{\cdot}kg^{-1}$: $P < 0.017$; treatment × time interaction, 10 $mg{\cdot}kg^{-1}$: $P < 0.01$; 100 $mg{\cdot}kg^{-1}$: $P < 0.001$) and heart rate (treatment effect, 10 $mg{\cdot}kg^{-1}$: $P < 0.01$; 100 $mg{\cdot}kg^{-1}$:

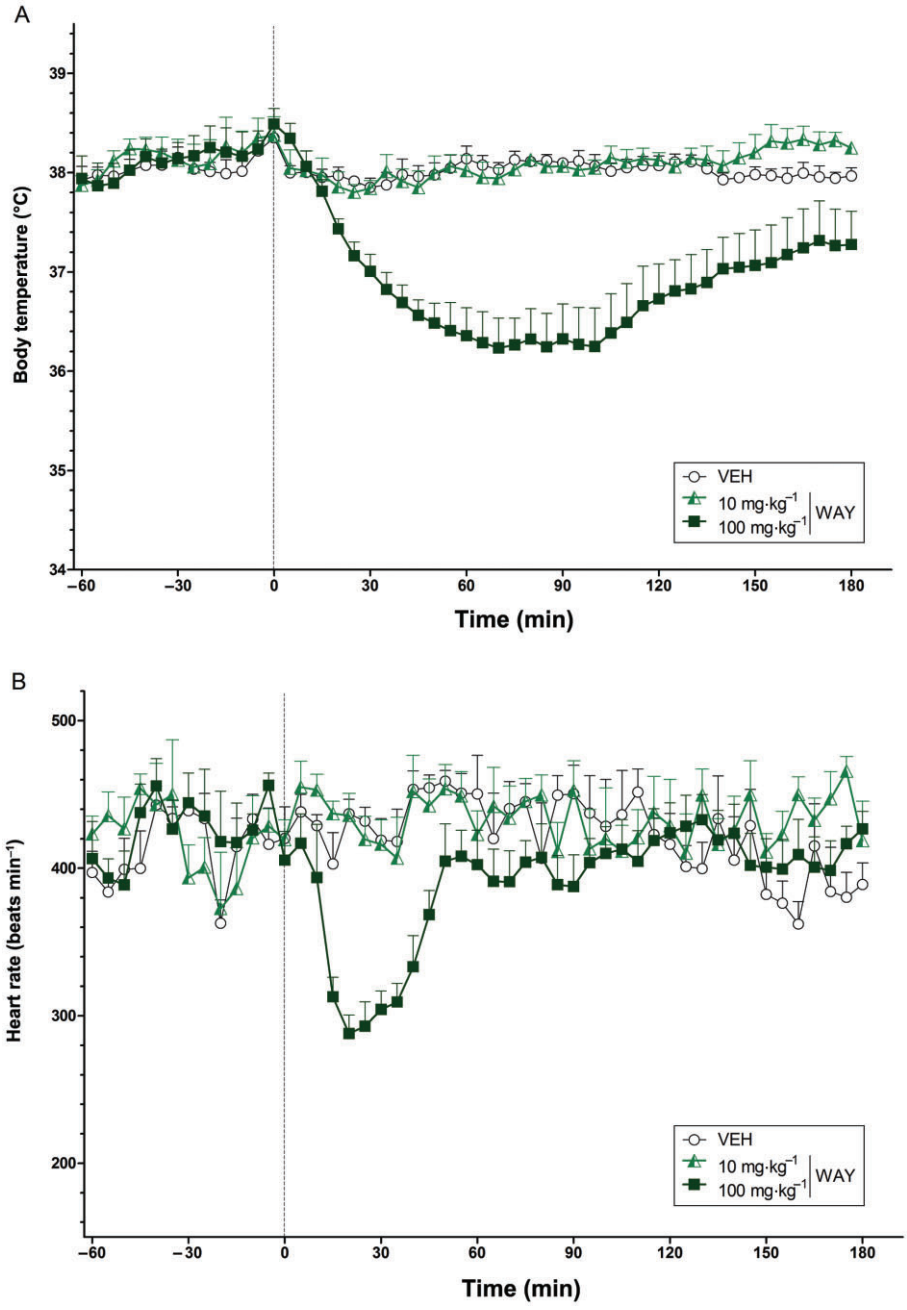

Figure 6

The dose-dependent effects of WAY 267,464 on body temperature (°C) (A) and heart rate (beats min^{-1}) (B) over time (min). The vertical grey hashed line on the *X*-axis at 0 indicates the time of vehicle or WAY 267,464 administration. Data are the means + SEM. VEH, vehicle; WAY, WAY 267,464.

$P < 0.017$; treatment × time interaction, 100 $mg{\cdot}kg^{-1}$: $P < 0.017$) effects induced by OT (Figure 8A and B respectively).

Effects of WAY 267,464 on AVP-induced hypothermia and bradycardia

A high dose of AVP (0.1 $mg{\cdot}kg^{-1}$) again caused a robust decrease in body temperature ($P < 0.017$) and heart rate ($P < 0.01$) relative to vehicle that remained across the test period (Figure 9A and B, respectively). Pretreatment with 10 or 100 $mg{\cdot}kg^{-1}$ WAY 267,464 did not significantly reduce the strong hypothermic and bradycardic effects of AVP (all $P > 0.017$; Bonferroni-corrected), although an apparent antagonist effect on heart rate was close to significance ($P = 0.023$).

Discussion and conclusions

The present study used biotelemetry in freely moving rats to characterize the effects of peripherally administered OT, AVP

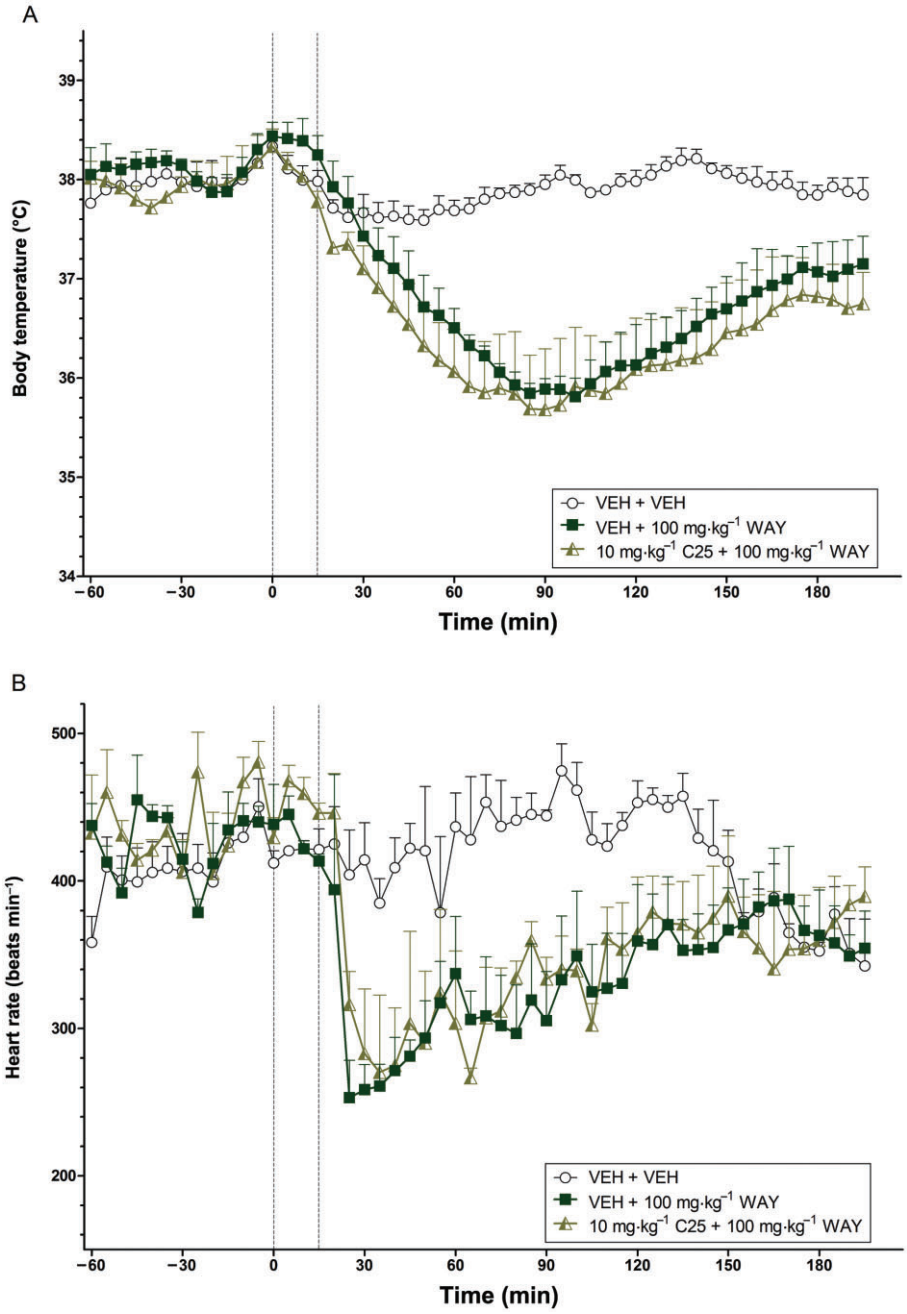

Figure 7

The effects of C25 on the body temperature (°C) (A) and heart rate (beats min^{-1}) (B) changes induced by WAY 267,464 over time (min). The vertical grey hashed line on the *X*-axis at 0 indicates the time of vehicle or C25 administration, while the second line at *X* = 15 indicates the time of vehicle or WAY 267,464 injection. Data are the means + SEM. VEH, vehicle; WAY, WAY 267,464.

and WAY 267,464 on body temperature and heart rate, and to assess the relative involvement of the OT and V_{1A} receptors in their effects. Results show that OT and AVP cause a dose-dependent reduction of body temperature and heart rate, with the highest doses (i.e. 1 and 0.1 $mg{\cdot}kg^{-1}$, respectively) producing effects for up to 3 h after injection. WAY 267,464 (100 $mg{\cdot}kg^{-1}$) also induces hypothermia, but with a more transient bradycardic effect than OT or AVP. Remarkably, pretreatment with a V_{1A} receptor antagonist, but not an OT receptor antagonist, prevented the body temperature and cardiovascular effects of OT (and AVP), suggesting these effects are V_{1A} receptor mediated. Moreover, pretreatment with WAY 267,464 partially prevented the effects of OT, and to a certain extent AVP, providing some functional evidence of V_{1A} receptor antagonist actions of this compound.

Our primary findings of hypothermia and bradycardia with OT and AVP are in general agreement with prior studies of cardiovascular and thermoregulatory function that have

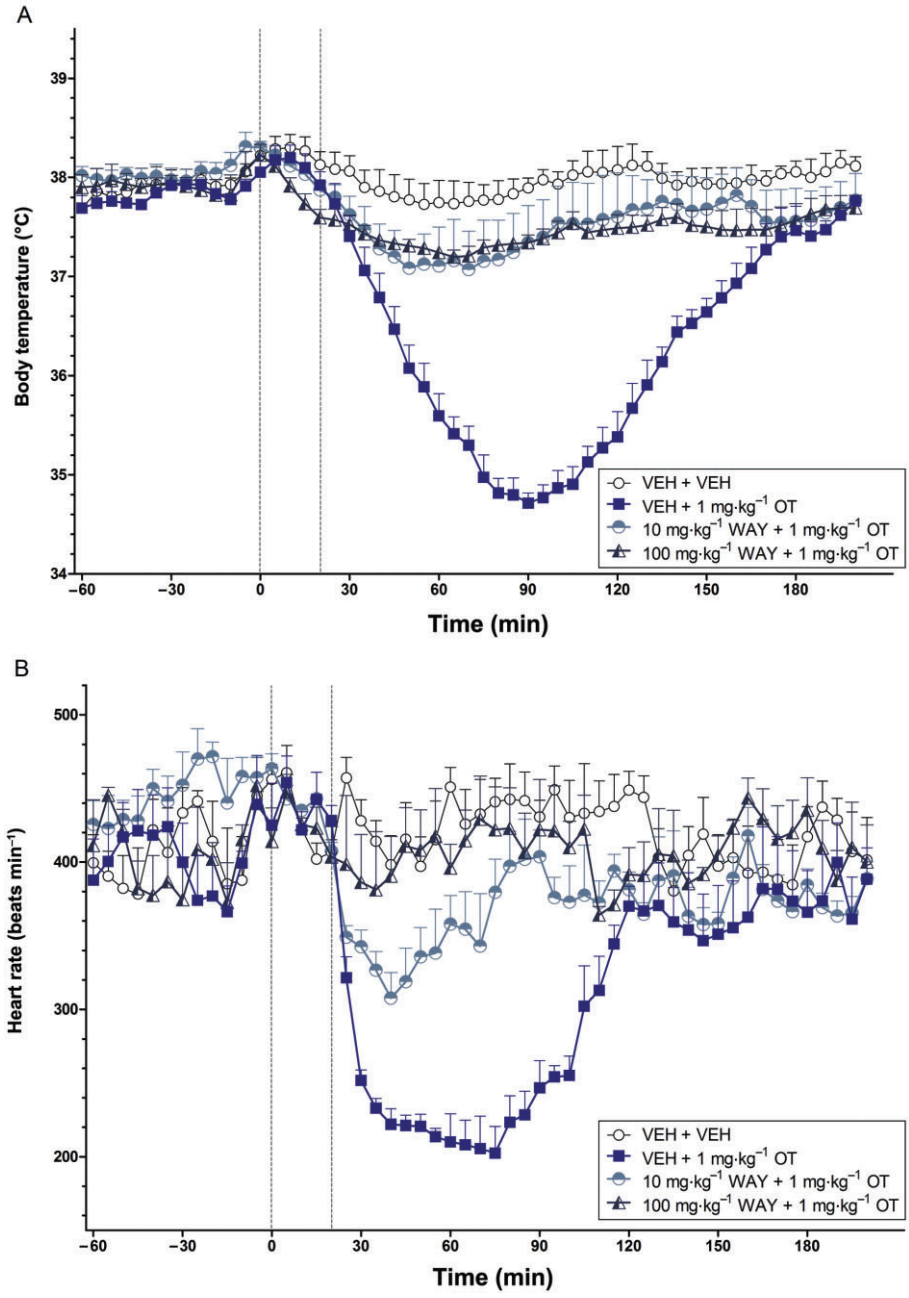

Figure 8

The effects of WAY 267,464 on the body temperature (°C) (A) and heart rate (beats min^{-1}) (B) changes induced by OT over time (min). The vertical grey hashed line on the *X*-axis at 0 indicates the time of vehicle or WAY 267,464 administration, while the second line at $X = 20$ indicates the time of vehicle or OT injection. Data are the means + SEM. VEH, vehicle; WAY, WAY 267,464.

involved peripheral injection of these neuropeptides (Okuno *et al.*, 1965; Chernoff and Grabowski, 1971; Jodogne *et al.*, 1991; Lundeberg *et al.*, 1994; Xiao-Jun and Wiesenfeld-Hallin, 1994; Ring *et al.*, 2006). However, most of these previous studies utilized traditional procedures that do not allow for continuous monitoring of physiological parameters, or involve stressful interventions (e.g. rectal probes) that can themselves affect body temperature and heart rate (Bouwknecht *et al.*, 2000). In addition, previous studies were often conducted within the context of thermoregulatory and cardiovascular responses to stress, injury or illness-related challenges. For example, Grippo *et al.* (2009) showed that chronic subcutaneous injection of OT reduces the tachycardia induced by chronic social isolation in prairie voles. Peripherally injected OT also reduced the hyperthermia induced by successive rectal temperature measurements in mice (Ring *et al.*, 2006). Peripheral OT also produces cardioprotective effects in myocardial ischaemia (Houshmand

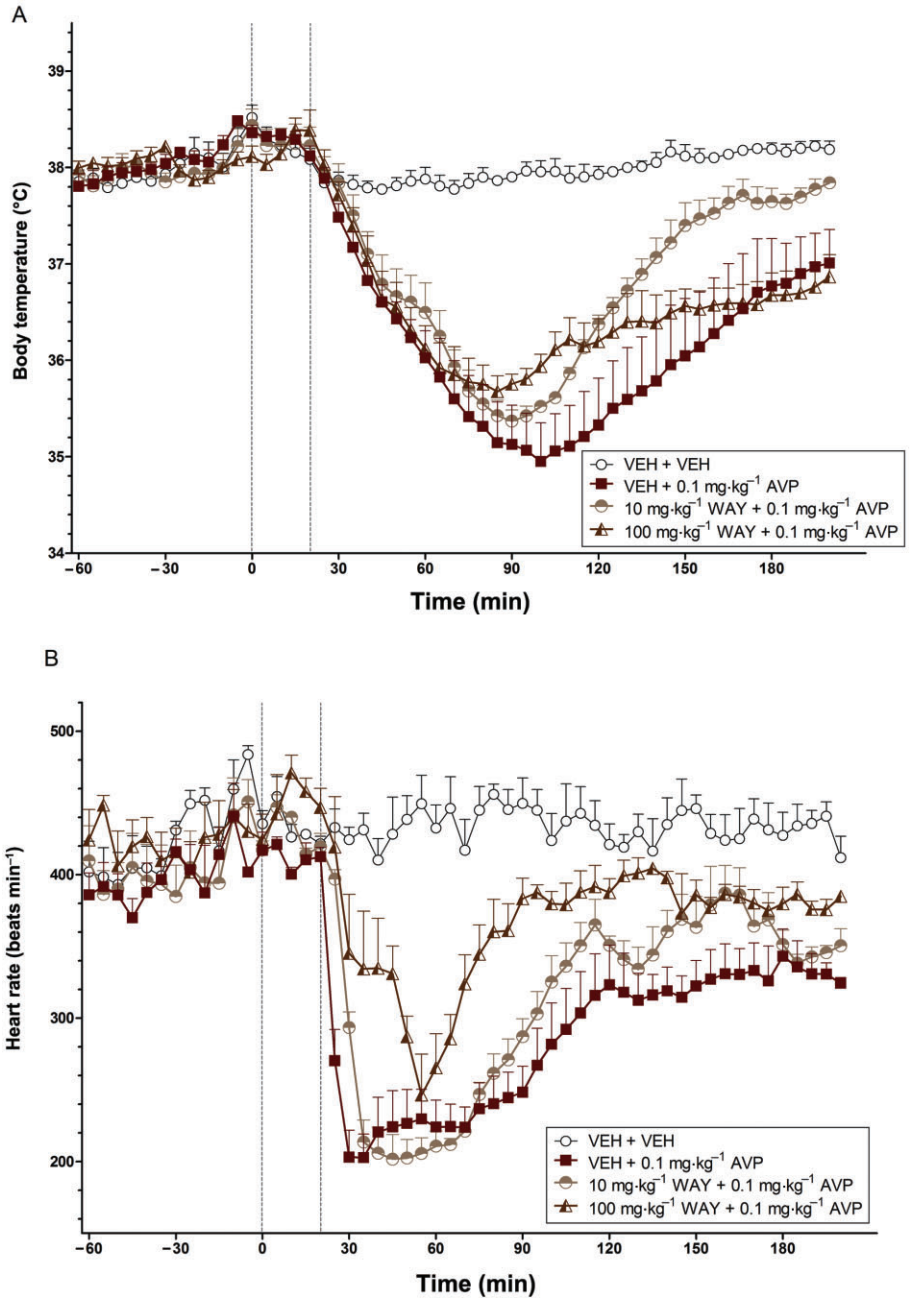

Figure 9

The effects of WAY 267,464 on the body temperature (°C) (A) and heart rate (beats min^{-1}) (B) changes induced by AVP over time (min). The vertical grey hashed line on the *X*-axis at 0 indicates the time of vehicle or WAY 267,464 administration, while the second line at *X* = 20 indicates the time of vehicle or AVP injection. Data are the means + SEM. VEH, vehicle; WAY, WAY 267,464.

et al., 2009), while systemically delivered AVP has a well-documented antipyretic action (Richmond, 2003). It is particularly striking then, that here we have documented potent thermoregulatory and cardiovascular effects of OT and AVP under basal conditions, thus demonstrating the capacity of OT, AVP and WAY 267,464 to influence tonic thermoregulatory and cardiac functions of rats at doses that are typically used in behavioural studies.

It is important to consider whether the effects of OT, AVP and WAY 267,464 on body temperature and heart rate might, to a certain extent, reflect locomotor hypoactivity observed after exogenous administration of these drugs. OT has well-established sedative effects in rodents following central (Uvnäs-Moberg *et al.*, 1992) or peripheral administration (Carson *et al.*, 2010; Hicks *et al.*, 2012), while AVP (Andrews *et al.*, 1983), and AVP-like analogues (Krejci *et al.*, 1979), also reduce locomotor activity in rats when given peripherally. Similarly, WAY 267,464 induces locomotor hypoactivity at the dose of 100 mg·kg^{-1} used in the present study (Hicks *et al.*, 2012). However, it seems unlikely that sedation accounts for

the hypothermia and bradycardia induced by OT, AVP and WAY 267,464. Notably, hypothermic and bradycardic effects were obtained in the current study with lower doses of OT and AVP that are unlikely to sedate (Andrews *et al.*, 1983; Hicks *et al.*, 2012). Moreover, observations suggest that sleep (i.e. complete immobility), without drug treatment, only reduces body temperature by 1.25 °C and heart rate by 75 beats min^{-1} (Sei *et al.*, 1997), which is less than the effects reported here. The hypothermia, bradycardia and locomotor hypoactivity seen with OT and AVP, has been suggested to reflect a common shift from sympathetic to parasympathetic autonomic control with an inhibition of the hypothalamic–pituitary–adrenal axis (Uvnäs-Moberg, 1998), to facilitate a passive and non-defensive physiological and behavioural state that permits effective social engagement (Porges, 2003).

An interesting question from the current study relates to the relative involvement of central and peripheral mechanisms in the observed effects. There is increasing evidence that circulating OT directly penetrates the blood–brain barrier in small, but physiologically significant amounts (Neumann *et al.*, 2013), and may also indirectly increase brain OT levels via stimulation of vagal afferents (McEwen, 2004). It appears possible then that a significant portion of the thermoregulatory and cardiovascular effects of OT, and AVP too, may be centrally mediated. Indeed, central infusion of high doses of OT and AVP induce body temperature and heart rate effects that mimic those produced after peripheral injection (Rogers and Hermann, 1985; Diamant and De Wied, 1993; Drago *et al.*, 1997). Moreover, peripheral administration of OT, AVP and WAY 267,464 at the doses used in the current study stimulates neuronal activity in key autonomic centres in the brain including the hypothalamus and the nucleus of the solitary tract (NTS) (Wu *et al.*, 1995; Hicks *et al.*, 2012). When infused directly into the NTS, OT can potentiate the bradycardic response to a pressor challenge (Higa *et al.*, 2002). Furthermore, the hypothermic effect of i.v. AVP was significantly reduced by i.c.v. infusion of an L-glutamate receptor antagonist, suggesting a central site of action of peripheral AVP (Paro *et al.*, 2003).

However, there is also evidence that OT and AVP act directly on peripheral tissues to regulate body temperature and heart rate. Okuno *et al.* (1965) showed that bilateral electrolesions of the anterior hypothalamus did not prevent the fall in body temperature after i.v. infusion of AVP, which the authors suggested was due to a reduction in brown adipose tissue (BAT)-mediated thermogenesis. Indeed, i.v. administration of AVP significantly reduces BAT temperature (Paro *et al.*, 2003), while OT receptor knockout mice show altered interscapular BAT morphology accompanied by impaired cold-induced thermogenesis (Takayanagi *et al.*, 2008). There is also evidence suggesting that OT and AVP can act directly on cardiac receptors to modulate heart rate, as both neuropeptides reduced heart rate and the force of atrial contractions in isolated atria from perfused rat hearts (Favaretto *et al.*, 1997; Kaygisiz *et al.*, 2001). Future studies might disentangle the sites at which OT, AVP and WAY 267,464 act to influence thermoregulation and heart rate using peripheral administration of these compounds in combination with direct intracranial administration of specific receptor antagonists.

The V_{1A} receptor antagonist SR49059 caused a striking cancellation of OT- and AVP-induced hypothermia and bradycardia at doses that had no significant intrinsic effects. In contrast, the body temperature and heart rate effects of OT were not prevented by pretreatment with the selective OT receptor antagonist C25. These results suggest a primary role for the V_{1A} receptor, rather than the OT receptor, in the hypothermic and bradycardic effects of peripherally administered OT (and AVP). The V_{1A} receptor is widely distributed in the brain and on peripheral organs including the heart, kidney and adrenal glands, and has a well-documented role in thermoregulation and cardiac function (Frank and Landgraf, 2008). Importantly, the present findings contribute to an emerging body of research showing that major functional effects of OT are mediated by AVP receptors (Gupta *et al.*, 2009; Schorscher-Petcu *et al.*, 2010; Loyens *et al.*, 2011; Sala *et al.*, 2011; Ramos *et al.*, 2013).

SR49059 is a potent V_{1A} receptor antagonist that shows >50-fold selectivity over the OT, V_{1B} and V_2 receptors in a number of species (Serradeil-Le Gal *et al.*, 1993). The effects of OT were completely abolished with a 1 $mg{\cdot}kg^{-1}$ dose of SR49059, although a higher dose (10 $mg{\cdot}kg^{-1}$) was necessary to obtain a full antagonism of AVP's effects. This may reflect a difference in the potency of the maximal doses of OT and AVP employed in the present study. Indeed, a prior study showed that a 100-fold higher dose of OT was required to induce cardiovascular effects comparable with AVP (Petty, 1987). At a dose of 10 $mg{\cdot}kg^{-1}$, SR49059 may also act on OT and V_{1B} receptors, and therefore the possibility that AVP may act on these receptors, in addition to the V_{1A} receptor, to alter body temperature and heart rate cannot be discounted. In a reciprocal manner to OT, AVP exhibits significant cross-talk with the OT receptor (Gimpl and Fahrenholz, 2001), and some reports have implicated the V_{1B} receptor in the blood pressure changes (Milutinovic *et al.*, 2006), and the OT receptor in the tachycardic effects (Roozendaal *et al.*, 1993), induced by central infusion of AVP.

Although blood pressure data were not collected in the present study, it is likely that the potent bradycardia induced by OT and AVP may be a consequence of increased arterial pressure leading to baroreflex-mediated reductions in heart rate. Peripheral administration of high doses of OT and AVP acutely increase blood pressure (Ludwig *et al.*, 2013), and enhance baroreceptor control of heart rate (Michelini, 2007). The hypertensive actions of OT and AVP are likely mediated by the V_{1A} receptor as they can be prevented by pretreatment with the V_{1A} receptor antagonist $(d(CH_2)_5^1,Tyr(Me)^2,Arg^8)$-AVP (Costa-e-Sousa *et al.*, 2005), or SR49059 (Serradeil-Le Gal *et al.*, 1993) respectively. It appears plausible then, that SR49059 prevented OT- and AVP-induced bradycardia in the present study by inhibiting the associated increase in arterial pressure induced by these neuropeptides through a V_{1A} receptor-dependent mechanism.

A particularly novel finding in the present study was the antagonist action of WAY 267,464 on OT-induced hypothermia and bradycardia. This provides further evidence of a V_{1A} receptor-mediated mechanism underlying the hypothermic and bradycardic effects of OT, and consolidate our recent observations that WAY 267,464 functions as a V_{1A} receptor antagonist *in vitro* (Hicks *et al.*, 2012). Interestingly, WAY

267,464 only tended to reduce the effects of AVP, which might again conceivably be attributable to a difference in potency between OT and AVP at the maximal doses used in the present study.

In contrast to the effects of SR49059, the OT receptor antagonist C25 failed to prevent the hypothermic and bradycardic effects of the maximal dose of OT, and WAY 267,464. Given that C25 is relatively uncharacterized *in vivo* it is possible that antagonist effects might have been achieved with a higher dose. However, C25 given alone caused a subtle alteration of heart rate, indicating functional effectiveness, as well as a role for endogenous OT in cardiovascular homeostasis. Such a role is supported by observations that direct infusion of an OT receptor antagonist into the NTS disrupts the baroreflex response on heart rate to exercise (Braga *et al.*, 2000) or a pressor challenge (Higa *et al.*, 2002), and that i.c.v. administration of an OT receptor antagonist significantly potentiates the tachycardia induced by air jet stress (Wsol *et al.*, 2008).

When interpreting the current findings, it is important to consider the apparent contradiction whereby WAY 267,464 produces an intrinsic hypothermic and bradycardic effect, while largely preventing these effects with peripheral OT and AVP. Although the mechanisms underlying this are not entirely clear, it likely relates to the subtly different pharmacological properties of WAY 267,464 compared with OT and AVP. So while OT and AVP exhibit agonist properties at both the OT and V_{1A} receptors, WAY 267,464 functions as a selective OT receptor agonist but also as a V_{1A} receptor antagonist. In addition, Grundschober *et al.* (2012) recently suggested that WAY 267,464 may also act as a potent V_2 receptor agonist *in vitro*. The V_2 receptor is also involved in thermoregulation and cardiovascular control: i.c.v. administration of a V_2 receptor antagonist blocks the antipyretic effect of AVP (Kovacs *et al.*, 1992), while i.v infusion of the V_2 receptor agonist 1-deamino-4-valin-8-D-arginine-AVP increases baroreflex-mediated bradycardia in normotensive rats (Budzikowski *et al.*, 1992). Therefore, WAY 267,464, at higher doses, may reduce body temperature and heart rate via a V_2 receptor-mediated agonist action while attenuating the effects of OT and AVP at the V_{1A} receptor. The enormous complexity of the peptide and non-peptide ligand-receptor interactions reported in the current study, highlights the need to develop more selective pharmacological research tools that demonstrate high binding and functional selectivity across several species both *in vitro* and *in vivo* (Manning *et al.*, 2012).

In conclusion, we show here that peripherally administered OT, AVP and WAY 267,464 have powerful autonomic effects that include hypothermia and bradycardia. These actions should be taken into account in studies involving the administration of high peripheral doses of these drugs to rodents, as they may impair performance on complex behavioural and cognitive tasks. Our study also shows that OT-induced effects on thermoregulation and heart rate after peripheral administration are most likely mediated by the V_{1A} receptor, rather than the OT receptor, contributing to an expanding body of research showing interplay between OT and AVP systems. Finally, our findings confirm our previous *in vitro* work suggesting important functional actions of WAY 267,464 at the V_{1A} receptor.

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Conflicts of interest

The authors declare no conflicts of interest.

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