Anti-Nociceptive Effects of Oxytocin Receptor Modulation in Healthy Volunteers – a Randomized, Double-Blinded, Placebo-Controlled Study

Running title

Anti-nociceptive effects of carbetocin in healthy volunteers

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Funding

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/EJP.1781

This study was funded by the Swiss National Science Foundation (SPUM 33CM30-124117) and the Scientific Funds of the University Department of Anesthesiology and Pain Therapy at the University of Bern.

Conflicts of interests

José A. Biurrun Manresa: No conflicts of interests
Jürg Schliessbach: No conflicts of interests
Pascal Vuilleumier: No conflicts of interests
Monika Müller: No conflicts of interests
Frank Musshoff: No conflicts of interests
Ulrike Stamer: No conflicts of interests
Frank Stüber: No conflicts of interests
Lars Arendt-Nielsen: No conflicts of interests
Michele Curatolo: No conflicts of interests

Keywords

Oxytocin / analogs & derivates, hyperalgesia, spinal cord dorsal horn, interneurons

Significance

This study provides evidence of the anti-nociceptive effect of intravenous administration of the oxytocin agonist carbetocin in healthy male volunteers.

ABSTRACT

Background

There is increasing evidence for oxytocin as a neurotransmitter in spinal nociceptive processes. Hypothalamic oxytocinergic neurons project to the spinal dorsal horn, where they activate GABA-ergic inhibitory interneurons. The present study tested whether the long-acting oxytocin-analogue carbetocin has anti-nociceptive effects in multi-modal experimental pain in humans.

Methods

Twenty-five male volunteers received carbetocin 100 mcg and placebo (0.9% NaCl) on two different sessions in a randomized, double-blinded, cross-over design. Multi-modal quantitative sensory testing (QST) including a model of capsaicin-induced hyperalgesia and allodynia were performed at baseline and at 10, 60 and 120 minutes after drug administration. QST data were analyzed using mixed linear and logistic regression models. Carbetocin plasma concentrations and oxytocin receptor genotypes were quantified and assessed in an exploratory fashion.

Results

An anti-nociceptive effect of carbetocin was observed on intramuscular electrical temporal summation (estimated difference: 1.26 mA, 95%-CI 1.01 to 1.56 mA, $p = 0.04$) and single-stimulus electrical pain thresholds (estimated difference: 1.21 mA, 95%-CI 1.0 to 1.47 mA, $p = 0.05$). Furthermore, the area of capsaicin-induced allodynia was reduced after carbetocin compared to placebo (estimated difference: -6.5 cm^2, 95%-CI -9.8 to -3.2 cm^2, $p < 0.001$).

Conclusions

This study provides evidence of an anti-nociceptive effect of carbetocin on experimental pain in humans.

INTRODUCTION

There is increasing evidence for the involvement of oxytocin in the modulation of nociceptive transmission in the peripheral and central nervous system (Hilfiger et al., 2020; Xin et al., 2017). Early animal studies demonstrated the presence of oxytocinergic synapses in laminae I-II of the spinal dorsal horn (Rousselot et al., 1990), and the accumulated evidence afterwards suggests that they mediate spinal anti-nociception by activating inhibitory GABA-ergic interneurons (Breton et al., 2008; Brussaard et al., 1996; Gimpl & Fahrenholz, 2001; Jo et al., 1998; Rojas-Piloni et al., 2007). Specifically, stimulation of the paraventricular nucleus, where oxytocin is produced, as well as direct administration of oxytocin have shown analgesic effects in rats, probably mediated by GABA-ergic neurotransmitter release and consecutive inhibition of A-δ and C-fibers in the spinal cord (Condés-Lara et al., 2006). More recently, animal experiments have shown that oxytocin can inhibit peripheral neuronal activity (Nersesyan et al., 2017) and that peripheral oxytocin receptors inhibit the nociceptive input signal to spinal dorsal horn (González-Hernández et al., 2017).

In humans, the first report of intrathecal administration of oxytocin indicated that it was able to relieve chronic low back pain in a large, double-blinded study, although very little details of this trial were published (Yang, 1994). More recently, a phase 1 safety trial of intrathecal administration of oxytocin did not reproduce anti-nociceptive effects to thermal stimuli previously observed in animals (Eisenach et al., 2015), although epidural oxytocin showed efficacy alleviating intense pain in severely-ill cancer patients (Condés-Lara et al., 2016). Furthermore, intranasal oxytocin administration revealed promising analgesic effects in double-blind, placebo-controlled studies (Ohlsson et al., 2005; Rash & Campbell, 2014; Singer et al., 2008) and one study found significant increases in pain thresholds for visceral perception in patients with irritable bowel syndrome, using intravenous administration of oxytocin (Louvel et al., 1996).

Carbetocin is an oxytocin analogue with similar biological action, but with stronger oxytocin-receptor coupling affinity and longer plasma half-life (Passoni et al., 2016). A study on post-partal bleeding incidentally found that women who had received carbetocin reported significantly lower pain scores after cesarean section than women who had received the shorter-acting oxytocin (De Bonis et al., 2012). However, a retrospective analysis of 76 patients after cesarean section failed to replicate this result (Gawecka & Rosseland, 2014).

The present study investigated whether administration of carbetocin to healthy male volunteers could confirm these suspected anti-nociceptive effects of oxytocinergic mechanisms. For this purpose, multiple experimental pain tests were used, including multi-modal quantitative sensory tests and a model of experimentally induced hyperalgesia and allodynia. In addition, plasma concentrations of carbetocin at different time points as well as three single nucleotide polymorphisms (SNP) of the oxytocin receptor were assessed and analyzed in an exploratory fashion.

METHODS

Ethical considerations

This randomized, double-blinded, placebo-controlled cross-over study was approved by the ethics committee of the Canton Bern, Switzerland (No. 140/12), and by the Swiss Medication Agency (Swissmedic, 2013DR1021). It was registered on clinicaltrials.gov (NCT01918475) prior to patient enrolment. All subjects gave written informed consent. The manuscript adheres to the applicable Equator guidelines.

Sample characteristics

The experiments were performed at the Department of Anesthesiology and Pain Medicine, University Hospital, Inselspital, Bern, Switzerland. Participants were recruited by advertisement at the University Hospital of Bern. Twenty-five healthy male volunteers aged 18-65 years were studied and received a compensation of 200 Swiss francs.

Inclusion and exclusion criteria

Women were not included in the study since the potential side effects of carbetocin (uterine contractions, belly pain, menstrual syndromes, and early pregnancy termination) outweigh the potential benefits in a study at this stage. For safety purposes, participants had to be in good physical shape, quantified as a MET score equal or above 4 (metabolic equivalent task, defined as being capable of walking up two flights of stairs without dyspnea). Exclusion criteria were any acute or chronic pain at the time of testing, known or suspected neurological dysfunction at the tested sites, ongoing medication having a potential effect on pain or nociception (e.g. antidepressants, opioids, benzodiazepines, anticonvulsants, and nonsteroidal anti-inflammatory drugs), intake of any analgesic drug within 48 h before testing, known allergy to carbetocin, any health condition affecting the cardiovascular system, any family history of coronary heart disease before the age of 55, any history of sudden death in first- or second-degree relatives, any abnormal electrocardiographic tracings (performed before the experiments), present or past epilepsy, history of kidney or liver disease, migraine, asthma, or hyperreactive airway disease.

Experimental intervention

At two different sessions, 100 µg of carbetocin (Pabal®, Ferring, Baar, Switzerland) or placebo (NaCl 0.9%) was injected intravenously in a randomized, double-blinded cross-over fashion. A minimal washout period of 2 weeks between sessions was ensured in order to avoid carry-over effects. The choice of dosage was based on a previous publication that found analgesic effects of carbetocin after cesarean section (De Bonis et al., 2012), as well as on the standard dosing recommendation of the Swiss Medical Compendium. Treatment allocation (i.e. placebo first or carbetocin first) was determined by a computer-generated random list and stored in sealed envelopes for every individual participant. Immediately prior to an experiment, a study nurse not otherwise involved in the project prepared the syringes containing the allocated study medication and labelled them with the subject

number, sequence number, date and time, thus leaving the investigator blinded as to the content of the syringe.

Quantitative sensory tests

General methodological aspects

The multi-modal quantitative sensory testing (QST) used in this study has been shown to have acceptable test-retest reliability (Biurrun Manresa et al., 2014; Vuilleumier et al., 2015). Before starting each session, training measures were performed until the volunteers were familiar with the testing procedures. Volunteers were positioned in a comfortable supine position with the upper body elevated by 30 degrees. All tests were performed on the dominant body side, except for the cold pressor test, which was applied on the non-dominant body side. All subjects were tested in the morning. QST were performed for training purposes, then at baseline before drug administration, and subsequently after 10, 60, and 120 minutes (a succinct overview is shown in Fig. 1). Three measurements were obtained and their arithmetical mean was used for data analysis, except for the cold pressor test and the assessment of conditioned pain modulation (CPM), for which only one measurement was taken.

Thresholds to cutaneous and intramuscular electrical stimulation

Cutaneous electrical stimulation was performed using Ag/AgCl-electrodes (Alpine Biomed Adhesive Disposable Surface Electrodes, Denmark), placed just distal to the lateral malleolus in the innervation area of the sural nerve. A 25-ms, train-of-five, 1-ms, square-wave impulse (perceived as a single stimulus) was delivered using a constant current stimulator (Digitimer DS7A Ltd, Welwyn Garden City, UK). The current intensity was increased from 1 mA in steps of 1 mA until a pain sensation was evoked (cutaneous single-stimulus electrical pain threshold; CSSEPT). Cutaneous temporal summation electrical pain threshold (CTSEPT) was measured using five identical bursts at a frequency of 2 Hz. The current intensity was increased from 1 mA in steps of 0.5 mA until the subjects reported pain during the last two to three of the five stimuli.

Intramuscular electrical stimulation was performed using two needle electrodes placed in the tibialis anterior muscle (Alpine Biomed Disposable Monopolar Needle Electrode, 37 mm × 0.33 mm, 28 G, Denmark), 14 cm distal from the caudal end of the patella and 2 cm in depth. Intramuscular single-stimulus electrical pain threshold (ISSEPT) was recorded analogous to CSSEPT. Intramuscular temporal summation electrical pain threshold (ITSEPT) was measured identical to the cutaneous temporal summation threshold (EPRS), repeating 5 identical bursts at 2 Hz. The current intensity was increased from 1 mA in steps of 0.5 mA until the subjects felt pain during the last two to three of the five bursts.

Pressure pain detection threshold

Pressure pain detection threshold (PPDT) was measured with an electronic pressure algometer (Somedic AB, Horby, Sweden) applied at the center of the pulp of the second toe. The probe had a surface area of 1 cm^2. The pressure was increased from 0 at a rate of 30 kPa/s to a maximum pressure

of 1000 kPa. The pain detection threshold was defined as the time point at which the pressure sensation turned to pain. The subjects were instructed to press a button when this point was reached. The algometer displayed the pressure intensity at which the button was pressed. If the subjects did not press the button at a pressure of 1000 kPa, this value was considered the threshold.

Thermal pain thresholds

Thermal stimulation was performed using a peltier thermode with a surface of 30 × 30 mm applied to the skin at the lateral aspect of the leg midway between the knee and the lateral malleolus (TSA-II, Medoc, Ramat Yishai, Israel). Temperature was increased from 30ºC to a maximum of 50.5ºC at a rate of 1.0ºC/s. Heat pain detection threshold (HPDT) was defined as the temperature at which the warm sensation turned to pain. Heat pain tolerance threshold (HPTT) was defined as the temperature at which the subjects could no longer tolerate the pain. At that point, the temperature was recorded and the thermode cooled back to 30ºC. A maximal temperature limit was set at 50.5°C. In case a subject did not reach the HPTT, this limit was recorded as the threshold. Cold stimulation was performed with the same thermode cooling down from 30ºC to a minimum of 0ºC at a rate of 1.0ºC/sec. The cold pain detection threshold (CPDT) was defined as the temperature at which the cold sensation turned to pain. Immediately after the threshold was reached, the thermode reheated to 30ºC. If the threshold was not reached at 0ºC, 0ºC was considered the threshold.

Cold pressor test and conditioned pain modulation

The hand of each subject was immersed in ice-saturated water (0.7 ± 1°C). The device consisted of a container separated by a mesh screen into an outer and an inner part. The mesh screen prevented direct contact between the ice (placed in the outer part) and the hand of the subject (placed in the inner part). The water was regularly stirred to maintain the temperature in the inner part near 0.7 ± 1°C as monitored by a thermometer with a digital display (± 0.1°C). The subjects placed their hand wide open and to the wrist into the container. They were asked to keep the hand in the water until the cold pain intensity was rated as 7/10 on a numeric rating scale (NRS with 0=no pain, 10=worst pain imaginable), or for a maximum time of 2 min. The elapsed cold pressor time (CPT) was recorded, followed immediately by one additional PPDT measurement at the toe (same site as before). An increase in PPDT at the toe as compared to the measurement before the cold pressor test was considered an indication of conditioned pain modulation (CPM).

Intradermal capsaicin

Once the baseline QST were obtained, capsaicin was injected intradermally on the volar side of the dominant forearm. The injection site was marked midway between the elbow and the wrist. Using a surgical skin marker, four lines were drawn through the injection site so that they intersected the corners of a regular octagon. These lines were marked every 0.5 cm from the center to the edges using a predefined grid. This resulted in the creation of triangles radiating from the intersection to the outward lines. The skin temperature was measured using a digital infra-red thermometer and was 32 ± 1°C in all subjects. Using a tuberculin syringe with a 27-gauge needle, 100 mcg of capsaicin (0.1 ml)

were injected at the point of intersection. The appearance of a white skin wheal confirmed correct injection. Capsaicin was produced specifically for this study by the Pharmacy of the University Hospital of Berne, Switzerland, following ICH guidelines and formal approval by the Swiss Medication Agency Swissmedic (EMA equivalent for Switzerland) regarding capsaicin production and administration to volunteers (Swissmedic 2013DR1021). The following substances were used for capsaicin production: Capsaicin USP, Fagron, Charge Fagron: 07H27-N09; Polysorbat 80 EP, Hänseler, Charge Hänseler: 2009.03.0770; Natrium chloratum "Bichsel" 0.9 % 100 ml.

Areas of capsaicin-induced hyperalgesia and allodynia

The area of hyperalgesia was assessed using a 512mN pinprick filament. The punctuated probe was applied along the eight radial lines defined above, from outside to inside at each mark in steps of 0.5 cm for 2 seconds at each point. The volunteers were asked to report at which point the pricking sensation changed to pain. At least 4 s elapsed between consecutive stimuli. This procedure was repeated for each vector, resulting in an octagon-shaped area of hyperalgesia. The area allodynia was determined by gently stroking a hand-held cotton wool tip along each of the lines at a rate of approximately 1 cm/s from the periphery to the center. Subjects were asked to report when the stroke changed from a non-painful touch to a painful sensation. The borders of allodynia were delineated similarly to the determination of hyperalgesia.

Carbetocin plasma concentration and genetic analysis

Methods for measurement of carbetocin plasma concentrations and oxytocin receptor genotyping

Carbetocin plasma concentrations were measured at the Forensic Toxicological Centre in Munich, Germany. The genetic analysis was performed at the Laboratory of the Department of Anesthesiology and Pain Medicine of the University Hospital, Bern, Switzerland. Three genetic variants (rs237902, rs2228485 and rs4686302) of the oxytocin receptor gene that previously revealed modest modulatory effects on pain perception were investigated (Landau et al., 2010; Vuilleumier, Ortner, et al., 2013). The chi-squared-goodness of fit test was used to assess the Hardy–Weinberg equilibrium for each single-nucleotide polymorphism (SNP). If the Hardy–Weinberg equilibrium was not met, the SNP was excluded.

Blood samples

EDTA blood samples were drawn at 10, 60, and 120 min after carbetocin administration and were stored on ice until processing within 1-2 hours. Blood cells and plasma were frozen separately at -20°C or -80°C, respectively, after centrifugation (3000 × *g*, 5 min at 4°C). All laboratory analyses were performed after enrolment of the last patient, and the laboratory staff was blinded to the volunteers' data.

Carbetocin plasma concentrations

Certified reference substance of benzoylecgonine-D3 (BZE-D3) was purchased from Sigma-Aldrich (Steinheim, Germany). Carbetocin secondary internal standard (QR 9351 and QRL 1457) was supplied by Ferring GmBH, Germany. Acetonitrile, ammonium formate solution 10 M in H_2O, formic acid, and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). Aliquots of 100 µl plasma were transferred into 2 ml Eppendorf vials and 5 ng of BZE-D3 were added as an internal standard. After addition of 1 ml of acetonitrile, each vial was immediately agitated for 1 min and centrifuged for 5 min at 14500 rpm. The supernatant organic layer was transferred to another 2 ml Eppendorf vial. The supernatant was added to 2 µl of formic acid 98% and evaporated to dryness under a stream of nitrogen at 37°C. The dry extract was reconstituted in 150 µl reconstitution buffer (5 mM ammonium formate in 85 % H_2O and 15% methanol) and transferred to HPLC vials. Analysis was performed with an Agilent 1290 Infinity LC system (Agilent Technologies, Waldbronn, Germany) coupled to an AB Sciex QTrap 6500 Linear Ion Trap Quadrupole LC/MS/MS mass spectrometer (AB Sciex Instruments, Darmstadt, Germany) equipped with a turbo spray IonDrive source. The column used was a Phenomenex Kinetex C18 (3.0 mm × 50 mm, 2.6 µm) reverse phase column heated at 30°C. Mobile phases consisted of eluent A (5 mmol/L ammonium formate in H_2O with 0.1 % formic acid) and eluent B (5 mmol/L ammonium formate in MeOH with 0.01% formic acid). Starting from 10% B after 0.5 minutes of equilibration at the same conditions, gradient elution was carried out by increasing B to 20% at 1.5 min, then increasing B to 100% at 3.5 min and running isocratically for 2.5 min after which B was decreased to 10% for 1 min. The flow rate was held at 300 µl/min over the whole run. The injection volume was 15 µl. Collision energies were 39 V for the transition 988.3 > 704.2 with collision cell exit potential of 44V, 49 V for the transition 988.3 > 659.2 with collision cell exit potential of 40 V, 43 V for the transition 988.3 > 676.2 with collision cell exit potential of 40 V, and 61 V for the transition 293 > 171 (IS BZE-D3) with collision cell exit potential 27 V. Data processing was done with Multiquant 3.0 software from AB Sciex. The limit of detection (LOD) with a signal-to-noise ratio of 3 and limit of quantification (LOQ) with signal-to-noise ratio of 9 were calculated with 0.025 ng/ml and 0.05 ng/ml, respectively.

Genetic analysis

Genetic data was assessed from the blood sample at admission on the first session, when the IV was inserted. DNA was extracted (ReliaPrep Blood gDNA Miniprep System, Promega, Madison, WI, USA) and genotyping was performed for three genetic variants within the oxytocin receptor. Rs4686302 was analyzed with a LightSNiP assay (TIB Molbiol, Berlin, Germany) using a LightCycler 2 (Roche Diagnostics, Rotkreuz, Switzerland). Genotyping of rs2228485 and rs237902 was performed by PCR amplification and restriction enzyme digestion as described previously (Wermter et al., 2010; Wu et al., 2005). PCR reagents were from Roche Diagnostics (Rotkreuz, Switzerland), primers from Microsynth (Balgach, Switzerland), and BsmI from New England Biolabs

(Ispwich, MA, USA). For rs237902, the isoschizomer MluCI from New England Biolabs (Ispwich, MA, USA) was used.

Data analysis

Intramuscular temporal summation electrical pain threshold (ITSEPT) was the primary outcome measure. All other sensory tests were secondary outcome measures. Intramuscular and cutaneous electrical pain thresholds, pressure pain detection thresholds and cold pressor times were log-normally distributed. These results are reported using medians and interquartile ranges. Heat pain detection thresholds, hyperalgesic and allodynic areas were normally distributed and are reported using means and standard deviations. Heat pain tolerance thresholds and cold pain detection thresholds were not normally distributed, neither on the original scale nor after log transformation, and were either left or right censored. Therefore, these variables were dichotomized using the maximally attainable stimulus as cut-off (i.e., 50.5°C or 0°C) and results are reported as number of patients and percentages with these thresholds at limit or not-at-limit. Similarly, conditioned pain modulation is presented as number of patients and percentage of patients with conditioned pain modulation effect larger than zero.

Statistics

Mixed linear regression models (for continuous QST) and mixed logistic regression models (for dichotomized QST) were performed to account for the multilevel data structure in this cross-over study with repeated measurements. We assumed the same treatment effect at all time-points and thus calculated overall treatment effects without modelling an interaction between treatment group and time point of assessment (main analyses). To evaluate this assumption, we also modelled an interaction between treatment group and time point of assessment (sensitivity analyses). We reported time specific treatment effects along with overall treatment effects in case of interaction. To correct for multiple testing at different time points, we used the Bonferroni-Holm correction and reported the corrected p-values. We adjusted all analyses considering baseline measurements and treatment order between sessions (carbetocin as first treatment vs. placebo as first treatment) including these variables as covariates in the models, according with current statistical recommendations (Senn, 2006; Vickers, 2001). A carry-over effect was excluded by design and not tested for. A random intercept was added for each subject to account for intra-subject clustering and for each subject in each treatment phase to account for repeated measures. For log-normally distributed QST, the treatment effect was presented on the raw scale as geometric mean ratio with values above one suggesting an anti-nociceptive effect of carbetocin. For normally distributed QST, the treatment effect was presented on the raw scale as mean difference with values above zero suggesting an anti-nociceptive effect of carbetocin (heat pain detection threshold) and values below zero suggesting an anti-hyperalgesic effect of carbetocin (areas of hyperalgesia and allodynia). For dichotomized QST the treatment effect was presented on the raw scale as odds ratio, with values above one suggesting an analgesic effect of carbetocin. Pearson product-moment correlations were used to explore the relationship between carbetocin plasma

concentrations and changes in QST measures from baseline. As this is an exploratory analysis, no correction for multiple comparisons was applied, so results should be interpreted with caution because the familywise error rate was not controlled. Possible effects of oxytocin receptor mutations on QST were assessed by graphical/visual inspection only, because the sample size was too small to detect genetic influences with sufficient statistical power. All statistical analyses were performed with STATA version 12 (StataCorp LP, College Station, TX, USA).

Sample size considerations

The sample size was estimated based on the primary outcome measure (intramuscular temporal summation electrical pain threshold, ITSEPT). In a previous study investigating the GABA-agonist clonazepam, the difference in ITSEPT between verum and placebo was 1.1 mA, with a SD of 1.3 mA (Vuilleumier, Besson, et al., 2013). According to our hypothesis, oxytocin receptor modulation activates GABA-ergic interneurons. We therefore expected a similar effect on ITSEPT. A paired t-test analysis resulted in a sample size of 14 subjects to detect this effect with 90% power at a two-tailed significance level of α=0.05. In order to account for a possible higher variability and expecting that some of the subjects might cancel the experiments, we included 25 subjects.

RESULTS

Twenty-six subjects were assessed for eligibility. One subject with an ongoing medical treatment for epilepsy was excluded. All other volunteers completed the study, and no adverse effects were observed. Median age was 26 years (IQR: 4 years), median body weight was 79 kg (IQR: 20 kg), and median body mass index was 24.4 kg/m^2 (IQR: 5.6 kg/m^2).

Effect of carbetocin vs. placebo on QST

Intramuscular temporal summation electrical pain thresholds (ITSEPT) showed an anti-nociceptive effect of carbetocin vs. placebo; the estimated difference was 1.26 mA (95%-CI 1.01 to 1.56, p = 0.04). Intramuscular single-stimulus electrical pain thresholds (ISSEPT) were also increased after carbetocin compared to placebo, with an estimated difference of 1.21 mA (95%-CI 1.0 to 1.47, p = 0.05). Furthermore, the area of allodynia was smaller after carbetocin administration (estimated difference: -6.53 cm^2, 95%-CI -9.84 to -3.21, p < 0.001). On the other hand, cold pressor times (CPT) were shorter after carbetocin compared to placebo (estimated difference: 0.88 s, 95%-CI 0.81 to 0.96, p = 0.005). None of the remaining QST measures showed differences between carbetocin and placebo. The detailed results are shown in Table 1, and estimated differences and their confidence intervals are displayed in Table 2. The estimated anti-nociceptive effects of carbetocin on ITSEPT and ISSEPT, as well as the changes in the hyperalgesic and allodynic areas are illustrated in Fig. 2. Individual values for all variables are presented in Supplementary Figs. 1, 2 and 3.

Plasma concentrations of carbetocin and receptor genotyping

Correlations between changes in QST and carbetocin plasma concentrations

The results of the Pearson correlation between changes in QST from baseline and carbetocin plasma concentrations are shown in Table 3. No significant correlation was found except for the electrical pain detection threshold to single intramuscular stimulation at 60 min (r = 0.507, p = 0.01), which most probably reflects a chance finding. Visual inspection of the results (figure 1 of main manuscript) does not suggest that the effects measured by QST behave in parallel to the decay of plasma concentration displayed in Fig. 3.

Genetic analysis

Allele frequencies of genetic variants were within the Hardy-Weinberg equilibrium except for rs4626302 (CC N = 24, TT N = 1, and CT N = 0). This SNP was thus excluded. Figs. 4 and 5 show a comparison of SNPs rs237902 and rs2228485 for intramuscular electrical pain thresholds and areas of hyperalgesia and allodynia (presented as mean values and standard deviations at 10, 60 and 120 minutes). For SNP rs237902, there were N = 8 wild-type, N=12 heterozygous and N = 5 homozygous mutants. There was a tendency to higher intramuscular electrical single and repeated pain thresholds for the mutant compared to wild type variants and a tendency to larger areas of hyperalgesia, although the statistical power was not sufficient to verify if this is an actual treatment effect or not (Fig. 4). For

SNP rs2228485, there were N = 18 wild-type and N = 7 heterozygous subjects. The heterozygous variant might be associated with slightly lower intramuscular electrical pain thresholds and areas of allodynia (Fig. 5), but again, the small sample size precludes conclusions about the effect of oxytocin receptor polymorphisms on QST measures.

DISCUSSION

The analgesic and anti-nociceptive effects of oxytocin have been under increasing scrutiny over the past years (González-Hernández et al., 2014; Rash et al., 2014). They can be explained by two principal mechanisms of action. A central nervous effect may be due to hypothalamo-spinal projections to the superficial and deep dorsal horn, where abundant oxytocin receptors are located and where the nociceptive A-delta and C-fibres synapse (Breton et al., 2008). On the other hand, peripheral effects may be present. Oxytocin receptors are found not only in the uterus and mammary glands, which are the most studied, but also in the cardiovascular and digestive systems (Gimpl & Fahrenholz, 2001) and the skin (González-Hernández et al., 2017). Crucially, oxytocin has a non-specific, short-lasting effect, which requires administration by continuous infusion. The long-acting oxytocin analogue carbetocin, on the other hand, can be administered as a single dose, which makes it a more attractive substance for clinical use. The present study provides evidence that carbetocin has anti-nociceptive effects. Whether they are central or peripheral in nature cannot be ascertained with certainty. Rather, the results are useful to generate hypotheses that justify further investigations using this compound both in basic science, elucidating the mechanisms of action, as well as in clinical research, finding a potential therapeutic application.

Effects of carbetocin on muscular and cutaneous electrical pain thresholds

Carbetocin caused an increase in intramuscular electrical pain thresholds, both for single stimulation and temporal summation. A similar effect had been detected in a previous study from our group using the GABA-agonists clonazepam and clobazam (Vuilleumier, Besson, et al., 2013). One possible anti-nociceptive effect of oxytocin relies on activation of GABA-ergic inhibitory dorsal horn neurons by descending oxytocinergic projections from the hypothalamus (Breton et al., 2008; Condés-Lara et al., 2009; Jiang et al., 2014; Jo et al., 1998; Juif et al., 2013; Paloyelis et al., 2016; Robinson et al., 2002; Rojas-Piloni et al., 2007). The similarity of the observed effects on the same assessment outcomes therefore lends support to this hypothesis.

The present study did not detect an effect of carbetocin on cutaneous electrical pain thresholds. This is consistent with another human study, where intranasal administration of 32 IU oxytocin did not affect electrical pain thresholds at the dorsum of the hand (Singer et al., 2008). The differential effect of oxytocin receptor modulation on cutaneous and muscular electrical pain thresholds might be explained by the fact that superficial and deep somatic afferents synapse with different second-order neurons (Schaible & Grubb, 1993) and thus carbetocin-mediated effects might predominantly influence the deep somatic nociceptive pathways. A peripheral modulatory effect of oxytocin might be conceivable as well, given that a recent study found oxytocin receptors on nociceptive terminals which were able to inhibit nociceptive input on activation (González-Hernández et al., 2017). The fact that cutaneous electrical pain thresholds were not modulated by carbetocin might then be explained by

direct activation of the sural nerve axons by transcutaneous electrical stimulation, thereby bypassing free nerve endings where oxytocin receptors are located.

Effects of carbetocin on capsaicin-induced allodynia and hyperalgesia

The present study showed a reduction of the capsaicin-induced allodynic skin area, although it could not detect a similar effect for hyperalgesia, assessed by pinprick filament. This is not necessarily surprising, since hyperalgesia and allodynia are mediated by different nociceptive mechanisms (Sandkühler, 2009). Thus, either specific mechanisms for allodynia are influenced by oxytocin receptor modulation, or the lack of observed effect on hyperalgesia represents a type II error due to insufficient statistical power. The fact that two previous human studies using GABA-agonists could demonstrate a modulation of both hyperalgesia and allodynia (Besson et al., 2015; Vuilleumier, Besson, et al., 2013) argues for the latter. It cannot be ruled out that the observed anti-allodynic effect might be due to a purely peripheral action of carbetocin. In-vitro studies have shown that oxytocin inhibits the TRPV1 receptor, at which capsaicin acts as an agonist (Hobo et al., 2012; Nersesyan et al., 2017). However, such peripheral TRPV1-effects of oxytocin have not yet been confirmed in human studies (Gonzalez-Hernandez & Charlet, 2018).

Effects of carbetocin on thermal pain and ice water tolerance

A puzzling finding of the present study is the reduced ice water tolerance after carbetocin, which could be interpreted as a cold hyperalgesia. The existing literature in this respect is controversial: Grewen *et al.* found that ice water tolerance time was not associated with plasma oxytocin concentrations, once the variance due to ethnicity was accounted for (Grewen et al., 2008). Rash *et al.*, on the other hand, performed a crossover study using hand immersion into ice water with 37 pain-free volunteers and found that 40 IU intranasal oxytocin was associated with higher cold pain threshold compared to placebo, as assessed by the time from immersion to reach pain (Rash & Campbell, 2014). As in many cases involving the cold pressor test, data is difficult to compare across studies due to methodological differences. Whereas tolerance threshold in baseline measurements in this study was reached within a median time of 18 s (IQR: 12 – 29 s), participants in Rash & Campbell (2014) reported a mean pain threshold time of 38.3 s (with a much larger variance, SD: 59.1 s), and the researchers reported that pain tolerance was not analyzed because most participants reached 300 s without withdrawing the hand from the ice-cold water (i.e., pain tolerance threshold). Thus, these contradictory effects are not easy to explain, and the possibility that any of these may reflect a false-positive must be borne in mind. Nonetheless, if this effect would prove to be consistent in follow-up studies, potential explanations include oxytocin-induced peripheral vasodilatation and the consequent increase in cutaneous microcirculation that might increase the pain perception during the cold pressor test. An increased cutaneous blood flow might also influence the number of activated nociceptors or result in a larger temperature difference of the immersed hand in the ice-water bath.

Oxytocin may also act via the release of nitric oxide or atrial natriuretic peptide, with possible direct effects on the adrenergic receptor (Gutkowska et al., 2000; Petersson, 2002; Xu et al., 2017).

We did not detect an influence of carbetocin in the remaining thermal QST measures. Eisenach et al. (2015) observed similar results after intrathecal administration of oxytocin, although this was a phase 1 safety trial with only 5 participants, so it was not sufficiently powered to reliably detect differences. Nevertheless, Zunhammer et al. (2015) did not find any effect on thermal thresholds after intranasal administration of oxytocin in a larger sample, which is consistent with the finding in this study.

Strengths, limitations, and future perspectives

This study assessed the effect of carbetocin in a multi-modal experimental pain setting in humans. It is important to highlight that this study was pre-registered and that the choice of primary outcome and statistical analysis was done prior to the study commencement, in order to avoid p-hacking, HARKing (making hypothesis after results are known) and the problem of the "garden of the forking paths" (Gelman & Loken, 2014; Munafò et al., 2017). A multi-modal testing procedure was used because there is currently no information on which experimental pain models would be most sensitive to the effect of carbetocin, which makes the study exploratory in nature. The complexity of the study design, including its cross-over nature, the intradermal or intravenous administration of two substances (capsaicin and carbetocin, respectively), and the repeated assessment of painful outcomes limit the duration of the experiment. Thus, even though the effect of carbetocin might extend for several hours (as observed in obstetric patients), the assessment of the outcomes was extended up to two hours after carbetocin administration, which should match the time to reach peak effects. The lack of a pharmacokinetic analysis prevents conclusions about the relationship between plasma concentrations of carbetocin and QST measures. With a potential perspective of use in clinical pain relief, future studies using oxytocinergic compounds should be carefully designed to individualize the nature and location of the mechanisms involved in the anti-nociceptive effects, considering larger and more diverse samples with particular emphasis on gender-related differences and specific clinical pain phenotypes, the dose-response relationship and potential safety concerns (Hilfiger et al., 2020). Finally, although both oxytocin and carbetocin are agonists at the same receptor, their pharmacodynamics profile may not be identical. One possibility may be a different pattern of association to inhibitory or excitatory G-Proteins (Passoni et al., 2016). Specifically, carbetocin is not a full agonist of the OTR, but a Gq-biased agonist of OTR. The OTR can be coupled to multiple G proteins, and thus the effects from carbetocin do not necessarily translate to oxytocin studies in a 1:1 manner.

Conclusion

This study demonstrated an anti-nociceptive effect of carbetocin on deep somatic experimental pain in humans, and an anti-allodynic effect in an experimental capsaicin model of sensitization. These results encourage research on oxytocin receptor modulation in clinical pain.

ACKNOWLEDGEMENTS

We thank Christoph Lippuner from the Lab of the Department of Anesthesiology and Pain Medicine, Bern University Hospital, for his work performed at the laboratory, as well as Christine Riggenbach and Fabienne Treichel from the Department of Anesthesia and Pain Medicine, Bern University Hosptial, for their support in data handling and patient recruitment. The Clinical Trial Unit of Bern Univesity provided a valuable support in the statistical analysis, the pharmacy of the Bern University hospital prepared and randomized the study medication. Shellie Boudreau from Aalborg University provided artwork for Fig. 1 from the "Feature extraction APP for Pain Profiles"

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FIGURE LEGENDS

Figure 1: Schematic overview of the testing sequence and location of the quantitative sensory tests.

Figure 2: Intramuscular electrical pain thresholds and areas of hyperalgesia and allodynia after capsaicin injection. Bars represent means with corresponding 95% confidence intervals as estimated by mixed linear regression models (continuous outcomes) or mixed logistic regression models (binary outcomes) adjusted for baseline test values and sessions. *: $p \leq 0.05$, **: $p < 0.001$, ns: $p > 0.05$.

Figure 3: Carbetocin plasma concentrations (in logarithmic scale) as function of time. Points represent individual measurements for each subject. Boxes delimit 25th and 75th percentiles, whiskers delimit 5th and 95th percentiles and the central line marks the median value.

Figure 4: Intramuscular electrical pain thresholds and areas of hyperalgesia and allodynia after capsaicin injection, according to single-nucleotide polymorphism (SNP) rs237902 variants. Bars represent means with corresponding standard deviations. Wild type: N = 8; mutant/heterozygous: N = 17.

Figure 5: Intramuscular electrical pain thresholds and areas of hyperalgesia and allodynia after capsaicin injection, according to single-nucleotide polymorphism (SNP) rs2228485 variants. Bars represent means with corresponding standard deviations. Wild type: N = 18; mutant/heterozygous: N = 7.

Table 1. Values of quantitative sensory tests at baseline as well as 10 min, 60 min and 120 min after carbetocin administration.

Test	Time	Placebo		Carbetocin	
		N	Median (IQR)	N	Median (IQR)
Intramuscular temporal summation electrical pain threshold ITSEPT (mA)	Baseline	24	1.4 (1.0 – 1.9)	24	1.9 (1.4 – 2.8)
	10min	24	1.2 (1.0 – 2.0)	23	2.0 (1.5 – 3.2)
	60min	24	1.3 (1.0 – 2.3)	22	2.2 (1.5 – 3.8)
	120min	24	1.7 (1.0 – 2.2)	21	2.0 (1.2 – 3.8)
Intramuscular single-stimulus electrical pain threshold ISSEPT (mA)	Baseline	24	2.2 (1.7 – 2.7)	24	3.1 (2.0 – 5.1)
	10min	24	1.9 (1.6 – 2.8)	23	3.0 (1.7 – 5.0)
	60min	24	1.9 (1.3 – 2.8)	23	3.7 (1.7 – 5.0)
	120min	24	2.1 (1.5 – 3.4)	21	3.8 (1.7 – 5.7)
Cutaneous temporal summation electrical pain threshold CTSEPT (mA)	Baseline	25	5.0 (3.8 – 7.0)	25	4.7 (3.7 – 6.0)
	10min	25	5.5 (4.0 – 6.7)	25	4.7 (4.0 – 6.0)
	60min	25	5.5 (4.5 – 6.7)	25	4.7 (3.8 – 5.7)
	120min	25	5.7 (4.3 – 7.3)	25	4.7 (4.0 – 6.7)
Cutaneous single-stimulus electrical pain threshold CSSEPT (mA)	Baseline	25	7.0 (4.7 – 8.5)	25	5.7 (4.7 – 7.7)
	10min	25	7.2 (5.0 – 9.0)	25	6.0 (4.7 – 9.5)
	60min	25	7.2 (5.0 – 8.2)	25	7.8 (6.3 – 7.8)
	120min	25	6.7 (5.5 – 8.5)	25	6.7 (5.0 – 7.7)
Pressure pain detection threshold PPDT (kPa)	Baseline	25	352 (318 – 413)	25	423 (292 – 468)
	10min	25	359 (330 – 440)	25	411 (337 – 474)
	60min	25	358 (302 - 403)	25	380 (324 – 438)
	120min	24	342 (317 - 431)	25	357 (327 – 462)
Cold pressor time CPT (s)	Baseline	25	18 (12 – 28)	25	18 (12 – 29)
	10min	25	18 (13 – 25)	25	17 (11 – 26)
	60min	25	18 (13 – 24)	25	13 (10 – 24)

	120min	25	16 (11 – 25)	25	14 (11 – 22)

		N	Mean (SD)	N	Mean (SD)
Heat pain detection threshold HPDT (°C)	Baseline	25	47.7 (1.7)	25	47.9 (2.0)
	10min	25	48.4 (1.6)	25	48.6 (1.7)
	60min	25	48.0 (1.4)	25	48.0 (1.9)
	120min	25	47.8 (1.4)	25	47.7 (1.7)
Hyperalgesia: area (cm^2)	Baseline	24	41.1 (13.1)	24	42.7 (16.3)
	10min	24	44.0 (16.7)	24	46.2 (16.6)
	60min	24	43.4 (15.6)	24	38.5 (16.0)
	120min	24	41.3 (18.8)	24	38.4 (16.3)
Allodynia: area (cm^2)	Baseline	24	21.9 (11.5)	24	24.9 (14.5)
	10min	24	21.2 (13.5)	24	15.8 (11.6)
	60min	24	16.4 (8.5)	24	11.8 (10.1)
	120min	24	13.9 (9.9)	24	8.8 (8.3)

		N	N (Proportion)	N	N (Proportion)
Heat pain tolerance threshold HPTT (cut-off ≥ 50.5°C)	Baseline	25	15 (60%)	25	15 (60%)
	10min	25	17 (68%)	25	16 (64%)
	60min	25	15 (60%)	25	16 (64%)
	120min	25	14 (56%)	25	14 (56%)
Cold pain detection threshold CPDT (cut-off ≤ 0.0°C)	Baseline	25	19 (76%)	25	21 (84%)
	10min	25	19 (76%)	25	22 (88%)
	60min	25	21 (84%)	25	21 (84%)
	120min	25	22 (88%)	25	21 (84%)
Conditioned pain modulation CPM	Baseline	24	20 (83%)	24	19 (79%)
	10min	24	18 (75%)	24	17 (71%)
	60min	24	21 (88%)	24	21 (88%)

Table 2. Main analysis of the effect of carbetocin vs. placebo on quantitative sensory tests. Treatment effect was adjusted for baseline values of quantitative sensory tests, time point and assessment session with corresponding 95% confidence intervals (95% CI) and p-values.

Test	**Treatment effect**	
	Geometric Mean Ratio[#] (95% CI)	**p-value**
ITSEPT (mA)	1.26 (1.01, 1.56)	**0.04**
ISSEPT (mA)	1.21 (1.00, 1.47)	**0.05**
CTSEPT (mA)	1.02 (0.95, 1.10)	0.58
CSSEPT (mA)	1.08 (0.99, 1.19)	0.09
PPDT (kPa)	1.00 (0.94, 1.07)	0.91
CPT (s)	0.88 (0.81, 0.96)	**0.005**
	Mean difference[*] (95% CI)	**p-value**
HPDT (°C)	0.00 (-0.48, 0.46)	0.99
Hyperalgesia: area (cm^2)	-2.85 (-6.05, 0.35)	0.08
Allodynia: area (cm^2)	-6.53 (-9.84, -3.21)	**<0.001**
	Odds Ratio° (95% CI)	**p-value**
HPTT (cut-off ≥ 50.5°C)	0.96 (0.30, 3.08)	0.95
CPDT (cut-off ≤ 0.0°C)	0.97 (0.30, 3.12)	0.95
CPM	0.98 (0.30, 3.20)	0.97

[#]Analysis on the log-scale using mixed linear regression models and results presented on the normal scale as geometric mean ratio with values > 1 suggesting an anti-nociceptive effect of carbetocin.

[*]Analysis on the normal scale using mixed linear regression models and results presented on the normal scale as mean difference with values > 0 suggesting an anti-nociceptive effect of carbetocin (HPDT) and values < 0 suggesting anti-hyperalgesic/anti-allodynic effect of carbetocin.

°Analysis on the normal scale using mixed logistic regression models and results presented as odds ratios with values > 1 suggesting an anti-nociceptive effect of carbetocin.

ITSEPT: intramuscular temporal summation electrical pain threshold (primary outcome measure); ISSEPT: intramuscular single-stimulus electrical pain threshold; CTSEPT: cutaneous temporal summation electrical pain

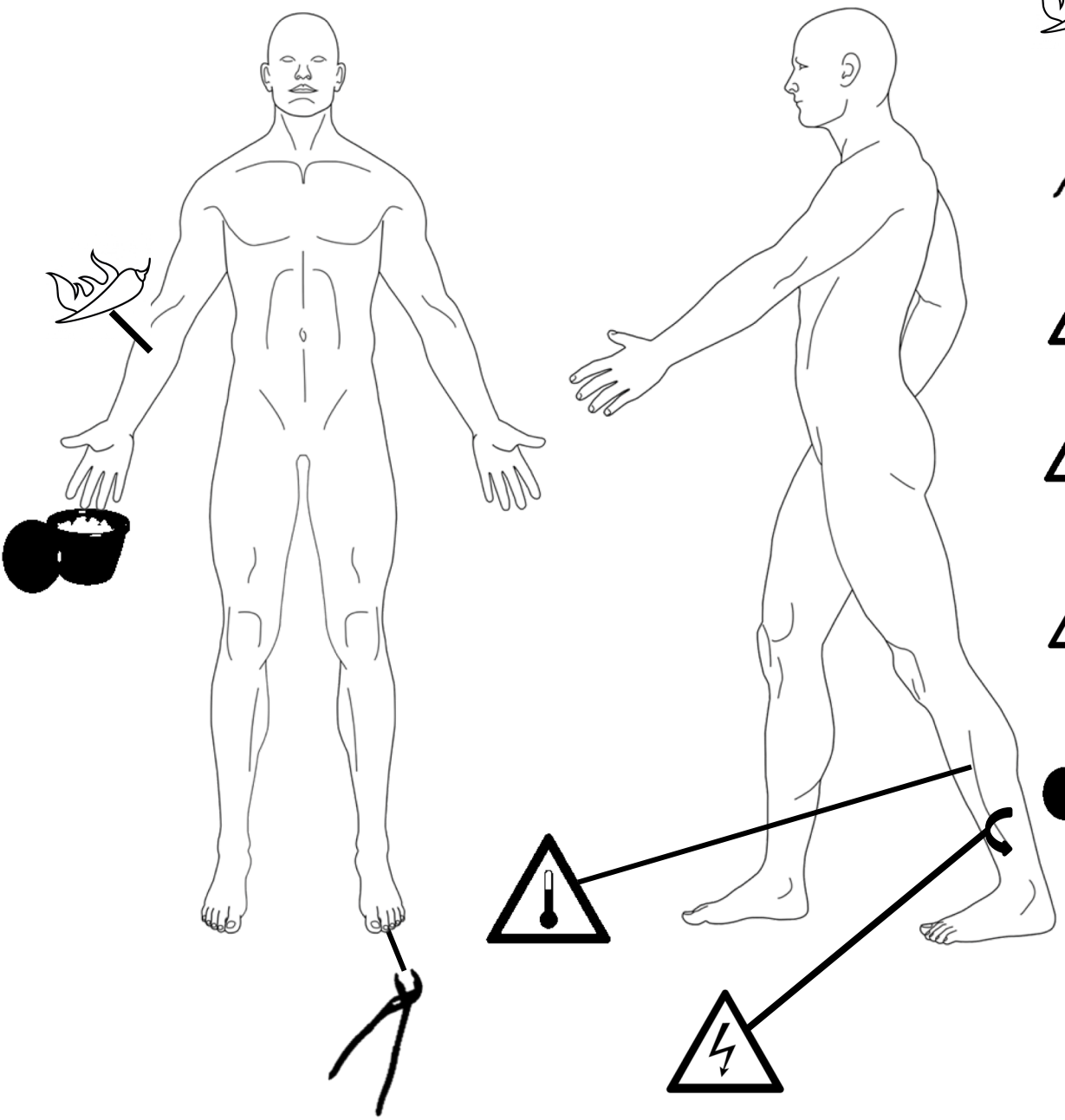
threshold; CSSEPT: cutaneous single-stimulus electrical pain threshold; PPDT: pressure pain detection threshold at the 2nd toe; CPT: cold pressor test; HPDT: heat pain detection threshold; HPTT: heat pain tolerance threshold; CPDT: cold pain detection threshold; CPM: conditioned pain modulation

Table 3: Results of the correlation analyses between carbetocin plasma levels and quantitative sensory tests.

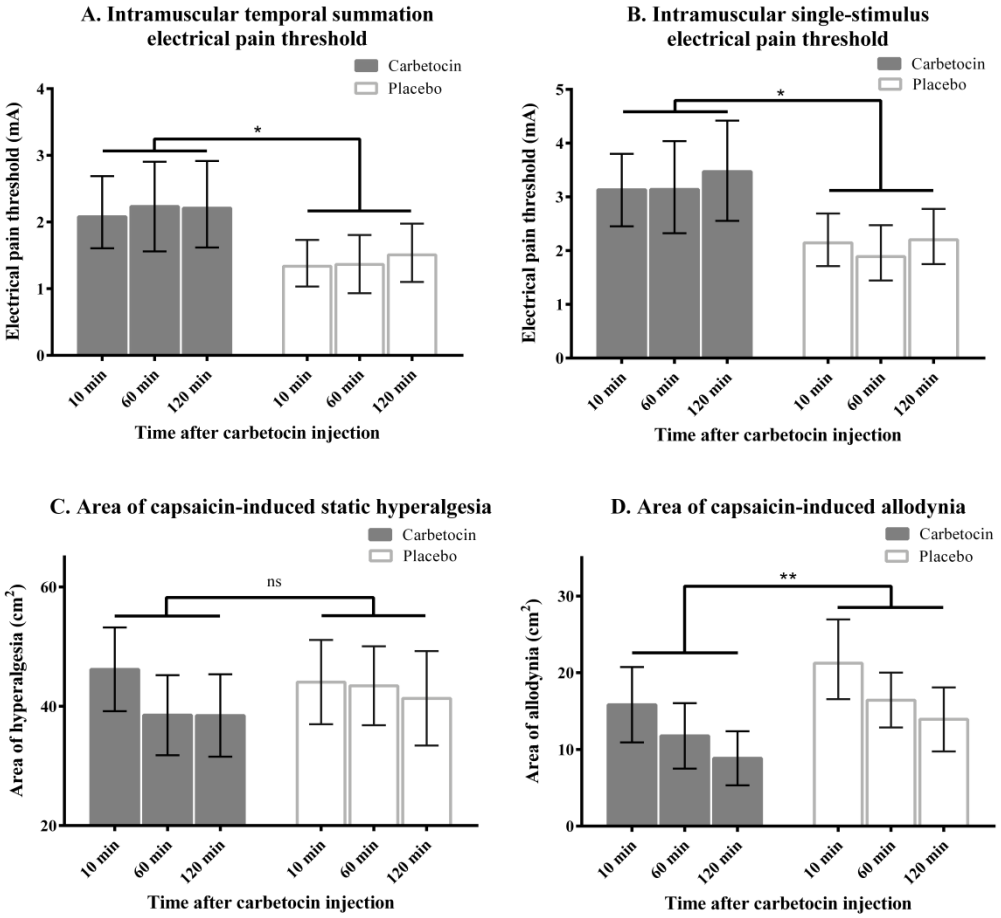
Time	Plasma carbetocin concentration Mean in ng/ml (SD)	Quantitative Sensory Tests (Difference to baseline)	N	Pearson correlation coefficient	p-value (2-tailed)
10 min	7.32 (2.11)	ITSEPT (mA)	23	0.047	0.83
		ISSEPT (mA)	23	-0.005	0.98
		CTSEPT (mA)	25	0.066	0.75
		CSSEPT (mA)	25	0.327	0.11
		PPDT(kPa)	25	-0.146	0.49
		CPT (sec)	25	0.030	0.89
		HPDT (°C)	25	-0.090	0.67
		Hyperalgesia: Static area (cm^2)	25	0.040	0.85
		Hyperalgesia: Dynamic area (cm^2)	24	0.129	0.55
		HPTT (cut-off $\geq$ 50.5°C)	25	-0.274	0.19
		CPDT (cut-off $\leq$ 0.0°C)	25	-0.042	0.84
		CPM (patients with increase in PPDT)	24	-0.044	0.83
60 min	0.65 (0.24)	ITSEPT (mA)	22	0.130	0.56
		ISSEPT (mA)	23	0.507	0.01
		CTSEPT (mA)	25	-0.171	0.42
		CSSEPT (mA)	25	0.190	0.36
		PPDT(kPa)	25	-0.193	0.36
		CPT (sec)	25	0.224	0.28
		HPDT (°C)	25	0.041	0.85
		Hyperalgesia: Static area (cm^2)	25	0.214	0.32
		Hyperalgesia: Dynamic area (cm^2)	24	0.255	0.23
		HPTT (cut-off $\geq$ 50.5°C)	25	-0.202	0.33
		CPDT (cut-off $\leq$ 0.0°C)	25	0.162	0.44
		CPM (patients with increase in PPDT)	24	-0.206	0.33
120 min	0.20 (0.08)	ITSEPT (mA)	21	-0.153	0.51
		ISSEPT (mA)	21	0.207	0.37
		CTSEPT (mA)	25	-0.114	0.59
		CSSEPT (mA)	25	0.126	0.55
		PPDT(kPa)	25	-0.064	0.76
		CPT (sec)	25	-0.132	0.53
		HPDT (°C)	25	-0.116	0.58
		Hyperalgesia: Static area (cm^2)	24	0.334	0.11
		Hyperalgesia: Dynamic area (cm^2)	24	0.012	0.96

HPTT (cut-off ≥ 50.5°C)	25	-0.064	0.76
CPDT (cut-off ≤ 0.0°C)	25	0.209	0.32
CPM (patients with increase in PPDT)	24	0.010	0.96

Quantitative sensory tests were calculated as difference to baseline and correlated with carbetocin plasma concentration at the respective time point. SD: standard deviation; min: minutes; ITSEPT: intramuscular temporal summation electrical pain threshold; ISSEPT: intramuscular single-stimulus electrical pain threshold; CTSEPT: cutaneous temporal summation electrical pain threshold; CSSEPT: cutaneous single-stimulus electrical pain threshold; PPDT: pressure pain detection threshold at the 2nd toe; CPT: cold pressor test; HPDT: heat pain detection threshold; HPTT: heat pain tolerance threshold; CPDT: cold pain detection threshold; CPM: conditioned pain modulation.



1. **Cutaneous allodynia and hyperalgesia after capsaicin**
2. **Pressure pain detection**
3. **Cold pain detection**
4. **Heat pain detection and heat pain tolerance**
5. **Electrical stimulation thresholds**
6. **Conditioned pain modulation and ice water tolerance**



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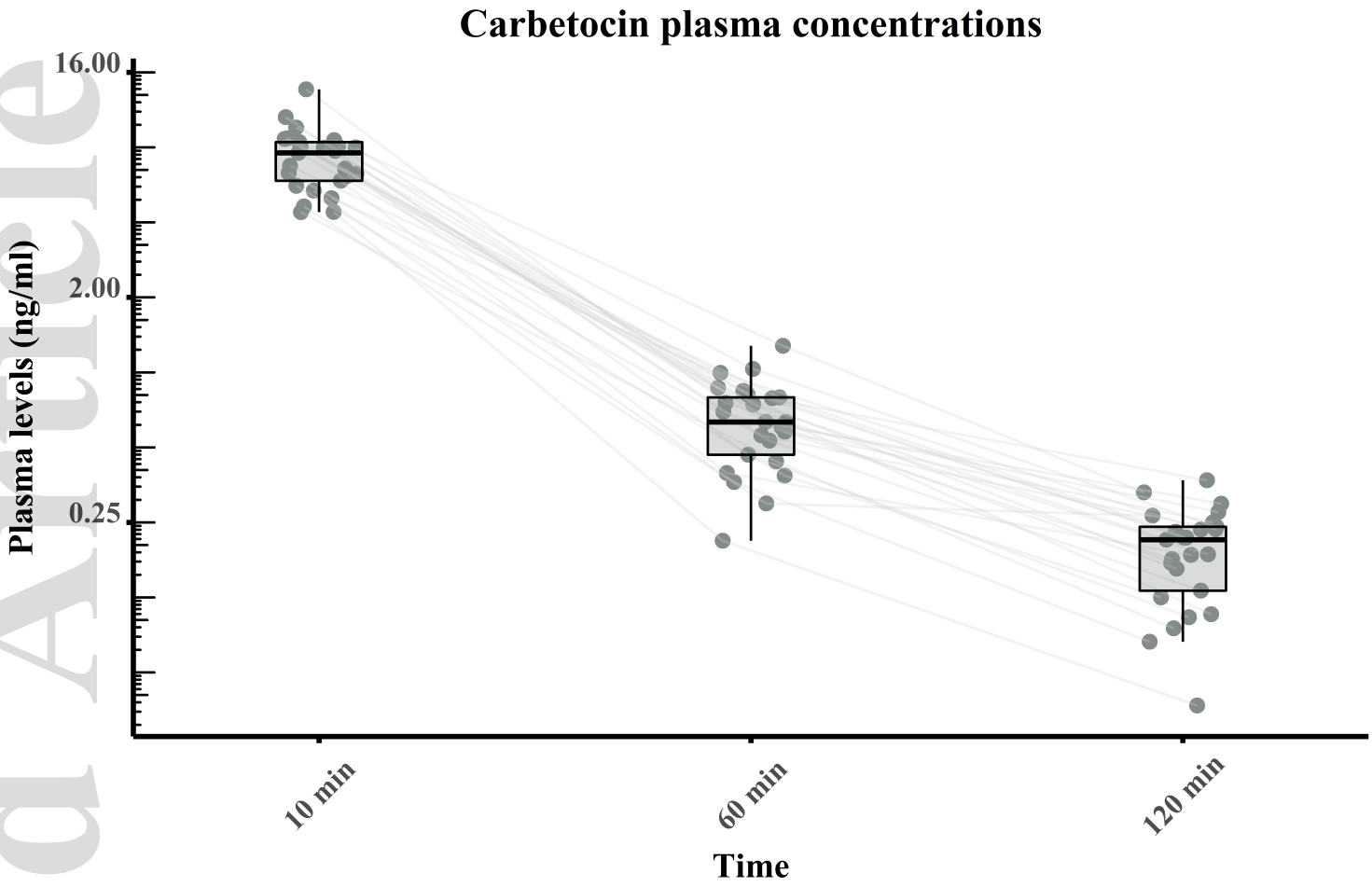

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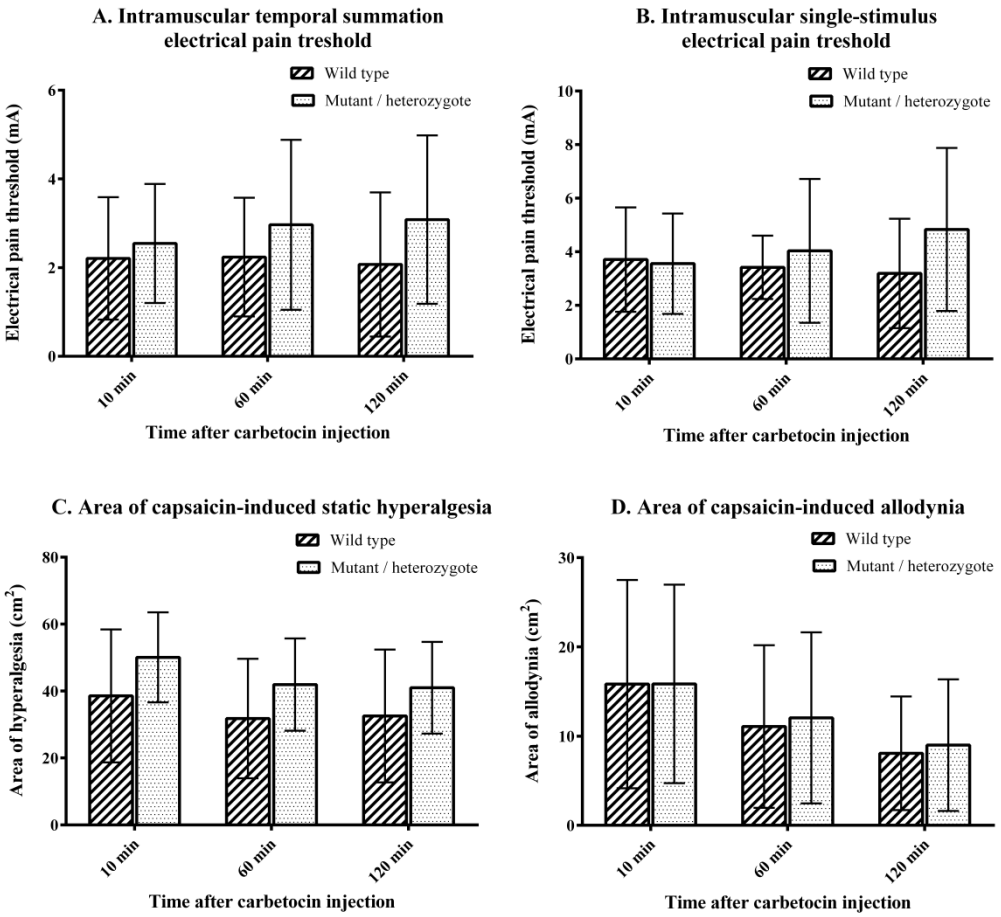

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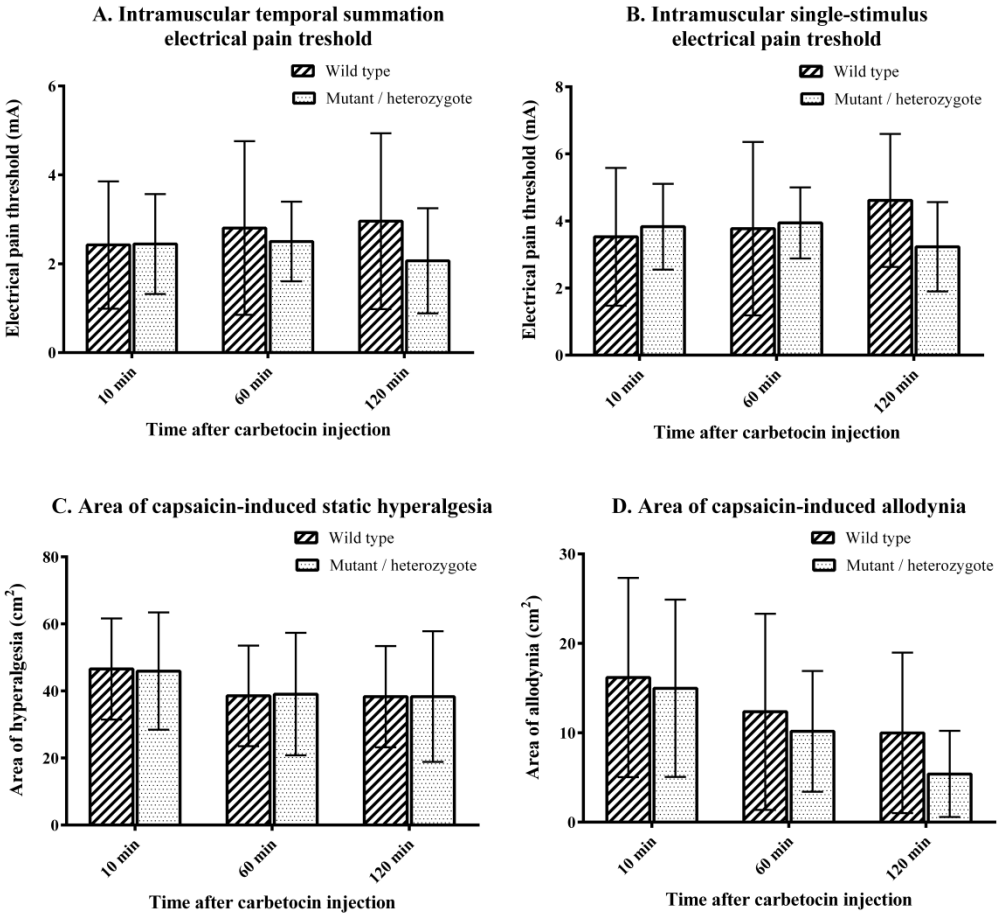

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