Low Dose Ketamine Reduces Pain Perception and Blood Pressure, but Not Muscle Sympathetic Nerve Activity, Responses During a Cold Pressor Test

**Running title:** Ketamine Reduces Pain and Pressor, but Not Sympathetic, Responses

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Key Points Summary

- Low dose ketamine is a leading medication used to provide analgesia in pre-hospital and hospital settings. Low dose ketamine is increasingly used off-label to treat conditions such as depression.

- In animals, ketamine stimulates the sympathetic nervous system and increases blood pressure, but these physiological consequences have not been studied in conscious humans.

- Our data suggest that low dose ketamine administration blunts pain perception and reduces blood pressure, but not muscle sympathetic nerve activity burst frequency, responses during a cold pressor test in healthy humans.

- These mechanistic, physiological results inform risk-benefit analysis for clinicians administering low dose ketamine in humans.
Abstract

Low dose ketamine is an effective analgesic medication. However, our knowledge of the effects of ketamine on autonomic cardiovascular regulation is primarily limited to animal experiments. Notably, it is unknown if low dose ketamine influences autonomic cardiovascular responses during painful stimuli in humans. We tested the hypothesis that low dose ketamine blunts perceived pain, and blunts subsequent sympathetic and cardiovascular responses during an experimental noxious stimulus.

Twenty-two adults (10F/12M; 27±6 y; 26±3 kg·m⁻², mean±SD) completed this randomized, crossover, placebo-controlled trial during two laboratory visits. During each visit, participants completed cold pressor tests (CPT; hand in ~0.4 °C ice bath for two minutes) pre- and five minutes post-drug administration (20 mg ketamine or saline). We compared pain perception (100 mm visual analog scale), muscle sympathetic nerve activity (MSNA; microneurography, 12 paired recordings) and beat-to-beat blood pressure (BP; photoplethysmography) during the pre- and post-drug CPT’s separately using paired, two-tailed t-tests. For the pre-drug CPT, perceived pain (p=0.4378), MSNA burst frequency responses (p=0.7375), and mean BP responses (p=0.6457) were not different between trials. For the post-drug CPT, ketamine compared to placebo administration attenuated perceived pain (p<0.0001) and mean BP responses (p=0.0047), but did not attenuate MSNA burst frequency responses (p=0.3662). Finally, during the post-drug CPT, there was a moderate relation between cardiac output and BP responses after placebo administration (r=0.53, p=0.0121), but this relation was effectively absent after ketamine administration (r=-0.12, p=0.5885). These data suggest that low dose ketamine administration attenuates perceived pain and pressor, but not MSNA burst frequency, responses during a CPT.

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Southwestern Medical Center. Mu is interested in examining the interaction between neural control of circulation and the manifestation of clinical pain in the hopes of enhancing pain management in order to improve function and prevent disability.

**Introduction**

A critical component of medical care is pain management (Butler *et al.*, 2014; Gausche-Hill *et al.*, 2014). When selecting a pain medication, clinicians must consider both the analgesic properties of a medication and any side effects that medication may have on vital physiological processes, such as autonomic nervous system control of arterial blood pressure (BP) (Wallin & Nerhed, 1982; Joyner *et al.*, 2010). BP support (i.e., the avoidance of severe hypotension) is crucial in the pre-hospital setting because advanced cardiovascular support may not be immediately available (Gausche-Hill *et al.*, 2014). One analgesic demonstrated to have little to no risk for eliciting hypotension is low dose ketamine, a N-methyl-D-aspartate receptor (NMDA) antagonist (Pfenninger *et al.*, 1994; Ahern *et al.*, 2015; Shackelford *et al.*, 2015; Liebe *et al.*, 2017; Riva-Posse *et al.*, 2018). For this reason, low dose ketamine has gained recent attention as a leading option for analgesia in the pre-hospital setting for civilians (Porter, 2004; Bredmose *et al.*, 2009; Jennings *et al.*, 2011) and military (Butler *et al.*, 2014) populations. However, to date, our knowledge about the effects of ketamine on autonomic cardiovascular regulation is primarily limited to studies in animals (Traber & Wilson, 1969; Slogoff & Allen, 1974; Lundy *et al.*, 1985; Ogawa *et al.*, 1993; Asmundsson *et al.*, 1998; Wang *et al.*, 2001; Camargo *et al.*, 2013) and one study in humans using a high (i.e., anesthetic) dose of ketamine (Kienbaum *et al.*, 2000). Determining how autonomic cardiovascular regulation is affected by low dose ketamine administration in conscious humans would inform clinical risk-benefit analysis and complement existing observational studies of analgesic use in pre-hospital settings (Losvik *et al.*, 2015; Petz *et al.*, 2015; Shackelford *et al.*, 2015; Schauer *et al.*, 2019). Therefore, the purpose of this study was to determine to what extent low dose ketamine affects autonomic cardiovascular regulation during a noxious stimulus.

During experimental noxious stimuli, autonomic cardiovascular responses are largely dependent on the intensity of perceived pain (Fagius *et al.*, 1989; Peckerman *et al.*, 1994; Stancak *et al.*, 1996; Butler *et al.*, 2014; Fazalbhoy *et al.*, 2014; Metzler-Wilson *et al.*, 2019). For example, during mechanostimulation (i.e., skin pinching), adults without hypertension had a significant correlation
between pain ratings and mean BP or muscle sympathetic nervous activity (MSNA) responses (Schobel et al., 1996). However, a more recent investigation using slow intramuscular infusion of hypertonic saline as a stimulus reported divergent BP responses, with mean BP increasing in some participants and decreasing in others, despite a consistent rating of the painful stimulus (5 to 6 out of 10) pain in all participants (Fazalbhoy et al., 2012). Consistent with other literature, Fazalbhoy et al., reported relatively homogenous increases in MSNA burst frequency during the moderate pain stimulus. Thus, when taken together, we still do not have a full understanding of the autonomic cardiovascular responses associated with experimental pain as they are likely dependent on the pain stimulus employed. The cold pressor test (CPT) is a commonly used stimulus that has been consistently reported to elicit pain-related cardiovascular responses. For example, during the CPT, perceived pain has been repeatedly associated with sympathetic nervous system mediated (Victor et al., 1987; Fagius et al., 1989; Kregel et al., 1992; Nakamura et al., 2008) increases in BP (Peckerman et al., 1991; Peckerman et al., 1994): i.e., greater perceived pain is associated with larger increases in sympathetic nerve activity and BP. We too recently have demonstrated that adults with greater pain scores had greater MSNA responses during the CPT (Huang et al., 2019). Thus, utilizing the CPT in the present study, we tested the hypothesis that low dose ketamine attenuates perceived pain, and the subsequent MSNA burst frequency and BP responses. Further, since there is evidence that the pain-mediated increases in BP are predominately associated with increases in cardiac output (Peckerman et al., 1994), we also sought to determine the contribution of cardiac output on increases in BP during the CPT. Thus, our secondary hypothesis was that the relation between increases in cardiac output and increases in mean BP during the CPT would be reduced following low dose ketamine administration – i.e., when pain sensations are attenuated.

The results from this work will inform guidelines for the use of low dose ketamine in both pre-hospital and hospital-based setting. For example, low dose ketamine provides cardiovascular support during anesthesia and improves peri- and post-operative analgesia (Mayer et al., 1990; Atashkhoyi et al., 2013; Singh et al., 2013; Basuni, 2016). Also, out-patient use of low dose ketamine has recently increased because of its efficacy as a non-opioid based analgesic alternative to treat chronic pain conditions in light of the opioid addiction crisis (Galinski et al., 2007; Azari et al., 2012; Volkow & Collins, 2017; Karlow et al., 2018; Li & Chen, 2019). Finally, low dose ketamine is increasingly being used for off-label uses such as for treatment of depression (Fava et al., 2018; Domany et al., 2019). Thus, our work will provide fundamental information regarding the effects of low dose ketamine on autonomic cardiovascular regulation.
Methods

Ethical Approval. The U.S. Army Medical Research & Material Command Human Research Protection Office, Institutional Review Board for Human Subjects Research at the University of Texas Southwestern Medical Center (IRB# 092017-068), and Texas Health Presbyterian Hospital Dallas approved this protocol. We recruited participants from the Dallas-Fort Worth metroplex. This study conformed with the standards set by the latest revision of the Declaration of Helsinki. Each of the 22 participants provided verbal and written consent prior to enrollment in the study. The data in this manuscript are associated with a registered clinical trial (ClinicalTrials.gov Identifier: NCT03621085).

Experimental overview. This experiment was a randomized, crossover, placebo-controlled design consisting of two experimental visits separated by at least 48 hours. The experimental visits included an initial pain assessment (Pre-Drug administration), a drug/placebo administration, a progressive lower-body negative pressure (LBNP) test, a second drug/placebo administration (30 minutes after the first drug administration), and a second pain assessment (Post-Drug administration; see timeline in Figure 1). The data from this protocol aimed to address two distinct research questions, i.e., the effect of low dose ketamine administration on 1) pain perception, and sympathetic and cardiovascular responses during the CPT, from which data are presented herein, and 2) tolerance to progressive LBNP, which are presented in a companion manuscript.

Participants. The inclusion criteria for this study included: age between 18-45 years, body mass ≥65 kg, and body mass index <32 kg/m², with each variable obtained during the initial screening visit. Study participants were also free of any known overt cardiovascular disease and had no evidence of cardiac, neurological, renal, metabolic, or pulmonary disease. Exclusion criteria included current or recent (within past three years) use of nicotine, prescription-based pain medications, and/or antihypertensive medications.

Experimental Protocol. Before the experimental visits, participants were instructed to refrain from large quantities of water for two hours; eating for at least six hours; caffeine for 12 hours; strenuous
exercise, alcohol, Naproxen, and NSAID’s for 24 hours; and over-the-counter cold or allergy medication and Aspirin for 36 hours prior to each trial. For female participants, both experimental visits were completed in the same phase of the menstrual cycle. Participants arrived to the laboratory and provided a urine sample to confirm 1) a negative urine drug screen, 2) euhydration with a urine specific gravity ≤1.025 (Cheuvront et al., 2010) (Atago Inc., Bellevue, WA, USA), and 3) a negative pregnancy screen for females. After an intravenous catheter was placed, participants were instrumented for the measurement of MSNA, brachial BP, beat-to-beat BP, and heart rate. Once these signals were acquired, participants relaxed quietly in the supine position in our temperature-controlled laboratory (22.9 ± 0.9 °C).

Following a five-minute baseline data collection period and blood sample collection, participants completed a pressure pain tolerance test and a CPT (Pre-Drug administration). Then, over five seconds, we intravenously administered either saline (0.9 % NaCl) or the US Army’s recommended dose (20 mg) of racemic ketamine (Ketalar - racemic ketamine hydrochloride, JHP Pharmaceuticals, Rochester, MI, USA) (Butler et al., 2014). Following this first drug/placebo administration, participants completed a progressive LBNP test (these data are presented in a companion manuscript). Because previous work suggests that the analgesic effects of intravenous low dose (0.25 mg/kg) ketamine last between 5 and 10 minutes (Clements & Nimmo, 1981), we administered a second drug/placebo dose thirty minutes after the first drug/placebo administration. Thirty minutes was chosen to minimize carryover effects from the first drug administration. Following this second drug/placebo administration, participants repeated the pain assessments, inclusive of another CPT (Post-Drug administration). For both pain assessments, participants relaxed for two minutes before completing the algometer testing (see description below). Next, they relaxed for two minutes (defined as post-Drug administration “Rest” data) prior to the CPT (defined as post-Drug administration “CPT” data), at which time another blood sample was collected. Thus, by design the second CPT started approximately five minutes following the drug administration (see timeline in Figure 1).

Participants, but not the researchers, were blind to experimental condition.

Pain assessments. In all 22 participants, the CPT – an experimental noxious stimulus (Victor et al., 1987; Peckerman et al., 1991; Kregel et al., 1992; Peckerman et al., 1994) – was completed by having a laboratory team member submerge the participants’ hand in a stirred ice bath (water temperature:
0.35 ± 0.09 °C) for two minutes. We instructed participants to avoid holding their breath or hyperventilating during the CPT. Immediately following removal of the hand from the ice bath, we asked participants to rate their pain during the CPT using a 100 mm visual analog scale. The left anchor, 0 mm, was “no pain or discomfort” and the right anchor, 100 mm, was “worst imaginable pain or discomfort.” The distance from the left anchor, 0 mm, is reported in the results as the pain rating for that CPT.

In 12 participants, we also assessed pressure pain tolerance by applying pressure with the tip of a handheld digital algometer (model FPX, Wagner Instruments, Greenwich, CT, USA) just proximal to the webbing of the second and third digit. We instructed participants to “report the first feeling of discomfort, not the most pain that can be tolerated.” We gradually applied force, and the peak force was recorded when the participant first reported a feeling of discomfort. We stopped applying pressure upon reaching 3 kg to limit any potential for injury and assigned that test result a value of 3 kg. We repeated this test three to five times, approximately 10 seconds apart, depending on when we recorded three readings within 10% of one another (Lacourt et al., 2012). We report the average of those three values.

Muscle sympathetic nerve activity. As previously described (Gagnon et al., 2017; Huang et al., 2019), we directly recorded MSNA using ultrasound-guided radial microneurography in 12 participants (6 males, 6 females), from which we could obtain adequate MSNA recordings for all assessments in both visits. Briefly, with real-time ultrasound imaging we inserted a tungsten recording microelectrode into a radial nerve of the upper arm and inserted a reference microelectrode ≤3 cm from the recording electrode. The electrical signal was amplified (80-90,000x), bandpass filtered (700-2,000 Hz), rectified, and integrated (time constant 0.1s) using a nerve traffic analyzer (Nerve Traffic Analyzer, model 662c-4; University of Iowa, Bioengineering, Iowa City, IA, USA).

Cardiovascular measures. We used single-lead ECG to continuously assess heart rate (GE Medical Systems, WI, USA). We used photoplethysmography to assess beat-to-beat BP, as well as Modelflow-derived cardiac output and total peripheral resistance (Finometer; Finapres Medical Systems, Netherlands) at rest and during the CPT (Guelen et al., 2003). Briefly, Modelflow is a three-element
model that uses the arterial input impedance, including continuous correction for estimated variations in the diameter, the compliance of the aorta, and total peripheral resistance, describing the relationship between aortic flow and pressure, and computing valid estimates of stroke volume (Wesseling et al., 1993). Thus, Modelflow allows for valid estimates of cardiac output (Jansen et al., 2001). We used auscultatory dimensional K-sound analysis, using a microphone placed over the brachial artery to detect Korotkoff sounds triggered from the ECG signal, to assess brachial BP (Tango M2 Stress Test Monitor, SunTech Medical, SC, USA). Finally, the brachial BP values obtained at rest were used to calibrate the beat-to-beat BP.

Data analysis. Data were collected at a sampling rate of 625 Hz using Biopac (MP150, Biopac, Santa Barbara, CA, USA). While blind to condition, an experienced (Watso et al., 2019a; Watso et al., 2019b) investigator (JCW) visually inspected the sympathetic neurogram on a beat-to-beat basis to determine the presence/absence of MSNA bursts using Ensemble (Elucimed, Wellington, New Zealand). MSNA analysis was conducted in accordance with recent guidelines (White et al., 2015) using the following criteria: (1) >3:1 signal-to-noise ratio, (2) burst morphology consistent with MSNA bursts, and (3) a pulse-synchronous signal. MSNA was quantified as burst frequency (bursts • minute⁻¹) and burst incidence (bursts • 100 heart beats⁻¹). MSNA burst amplitude and area were not assessed because we could not confirm that the position of the microelectrode was the same for all four CPT’s; i.e., MSNA burst amplitude and area varied within participants and between visits given the repeated measures design (MSNA recordings from opposing arms on separate occasions due to the proximity of experimental trials) as well as within visits (due to LBNP-related shifts in the neurogram, which occurred between the repeated CPT’s within each visit). Therefore, it would be inappropriate to estimate the MSNA burst amplitude or area for this particular protocol given that, even with burst amplitude and area normalization, alterations in baseline characteristics of the neurogram have a significant influence on the resultant CPT-related changes in burst amplitude and area (White et al., 2015; Macefield, 2020).

We obtained venous blood samples, before drug administration #1 and approximately four minutes after drug administration #2. These samples were collected in Lithium Heparin spray-coated tubes (BD Vacutainer®, Oakville, ON, USA). We stored these tubes on ice until centrifugation (2,000 g for 10 minutes) less than 60 minutes later. Lastly, we stored plasma samples at −80 °C until shipping on
dry-ice to Arup Laboratory (Salt Lake City, UT, USA) for high performance liquid chromatography assessment of plasma epinephrine and norepinephrine concentrations (n=20 for baseline, n=17 for post-Drug).

We compared pressure pain tolerance via the algometer, and pain perception during the CPT, between trial days prior to saline/drug administration (i.e., during the “Pre-Drug” time point). We also compared absolute cardiovascular and sympathetic measures at rest and during the CPT between trial days prior to saline/drug administration. We calculated cardiovascular and sympathetic responses (delta values) during the CPT by subtracting the values collected during the second minute of the CPT (“CPT”) from the values collected during the rest period prior to that CPT (“Rest”). Separately, and pertinent to the hypotheses of this manuscript, we compared the same data between trial days as described above within the “Post-Drug” time point.

Statistical analysis. The hypotheses in this manuscript address a secondary aim from a larger registered clinical trial (ClinicalTrials.gov Identifier: NCT03621085). Thus, we did not conduct an a priori power analysis for this particular dataset. We compared spot urine specific gravity values between trials using paired, two-tailed t-tests. We compared pain measurements between trials separately within each time point (i.e., Pre-Drug and Post-Drug) using paired, two-tailed t-tests. We compared plasma catecholamine concentrations between trials separately within each time point (i.e., Pre-Drug and Post-Drug) using paired, two-tailed t-tests. We compared absolute sympathetic and cardiovascular measures at rest and during the CPT between trials separately within each time point (i.e., Pre-Drug and Post-Drug) using repeated measures two-way ANOVAs (main effects of rest/CPT and trial). Tukey multiple comparison testing was employed for post-hoc analyses when appropriate. We compared the magnitude of the change in sympathetic and cardiovascular measures during the CPT between trials within each time point (i.e., Pre-Drug and Post-Drug) using paired, two-tailed t-tests. We examined the relation between reductions in pain and mean BP responses after low dose ketamine administration using a Pearson correlation. Finally, we used Pearson correlations to determine if changes in cardiac output and changes in total peripheral resistance were each related to the changes in mean BP during the Post-Drug CPT’s. We analyzed these data using GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA, USA). We set significance a priori at p < 0.05. Data are presented as means ± SD with individual responses.
Results

A schematic of the experimental visit timeline is included in Figure 1. Participant screening characteristics are provided in Table 1. No participants reported a past or current diagnosis of post-traumatic stress disorder during health history review. Upon arrival to the laboratory, spot urine specific gravity was not different between trials (pre-placebo 1.014 ± 0.010 vs. pre-ketamine 1.015 ± 0.010; p=0.9605).

Pre-Drug Administration Pain Assessments, Sympathetic, and Cardiovascular Measures. Pressure pain tolerance was not different between trials (pre-placebo 1.7 ± 0.5 vs. pre-ketamine 1.6 ± 0.6 kg, p=0.3056). Perceived pain during the initial (i.e., prior to drug/placebo administration) CPT was also not different between trials (pre-placebo 66 ± 14 vs. pre-ketamine 68 ± 17 mm, p=0.4378). While MSNA burst frequency was slightly higher pre-placebo compared to pre-ketamine (Rest: pre-placebo 23 ± 11 vs. pre-ketamine 19 ± 10; CPT: pre-placebo 40 ± 10 vs. pre-ketamine 35 ± 12 bursts • min⁻¹; perturbation: p<0.0001, trial: p=0.0225, interaction: p=0.7337), MSNA burst frequency was not different between trials during Rest (post hoc p=0.3036) or CPT (post hoc p=0.2970). Plasma epinephrine concentrations (pre-placebo 22 ± 12 vs. pre-ketamine 32 ± 32 pg/mL, p=0.1149) and norepinephrine concentrations (pre-placebo 253 ± 129 vs. pre-ketamine 280 ± 136 pg/mL, p=0.4942) were not different between trials. We did not observe a significant interaction effect for mean BP during the CPT (Rest: pre-placebo 91 ± 9 vs. pre-ketamine 93 ± 8; CPT: pre-placebo 108 ± 12 vs. pre-ketamine 109 ± 14 mmHg; perturbation: p<0.0001, trial: p=0.6248, interaction: p=0.6410). We did not observe a significant interaction effect for heart rate during the CPT (Rest: pre-placebo 61 ± 10 vs. pre-ketamine 62 ± 15; CPT: pre-placebo 73 ± 14 vs. pre-ketamine 74 ± 14 bpm; perturbation: p=0.0001, trial: p=0.5337, interaction: p=0.8291). Similarly, we did not observe a significant interaction effect for any other cardiovascular and sympathetic values during the CPT (p≥0.1914 for all Pre-Drug data, data not shown). We did not observe a significant effect of trial for the increase in MSNA burst frequency (pre-placebo Δ 17 ± 6 vs. pre-ketamine 16 ± 11 bursts • min⁻¹, p=0.7375), mean BP (pre-placebo Δ 17 ± 10 vs. pre-ketamine 16 ± 10 mmHg, p=0.6457), or heart rate (pre-placebo Δ 11 ± 12 vs. pre-ketamine 11 ± 10 bpm, p=0.8311) during the CPT. Similarly, no other sympathetic or cardiovascular responses during the CPT were different between trials (p≥0.2041 for all Pre-Drug data, data not shown).
Post-Drug Administration Pain Assessments. Pressure pain tolerance was significantly higher after low dose ketamine compared to placebo administration (post-ketamine 2.8 ± 0.4 vs. post-placebo 1.6 ± 0.6 kg; p<0.0001). Seven of 12 participants reached our pre-determined upper limit of 3 kg after low dose ketamine administration, whereas zero participants reached this upper limit after placebo administration. Pain perception during the CPT was significantly lower after low dose ketamine compared to placebo administration (Figure 2). Seven of 22 of participants reported “0” as their CPT pain rating after low dose ketamine administration, whereas zero participants reported “0” after placebo administration.

Post-Drug Administration Sympathetic Measures. Representative BP and MSNA tracings before and during a CPT for one participant after placebo and low dose ketamine administrations are displayed in Figure 3. MSNA burst frequency and burst incidence values increased from Rest (i.e., Post-Drug administration rest period but prior to the CPT) to CPT in both placebo/ketamine conditions (Figure 4A & 4C). There was a trend for higher absolute MSNA burst frequency values during the CPT after placebo compared to low dose ketamine administration (Figure 4A). That said, an important observation is that the increases in MSNA burst frequency and burst incidence during the CPT were not different between placebo and ketamine trials (Figure 4B & 4D). Plasma epinephrine concentrations (post-placebo 28 ± 14 vs. post-ketamine 55 ± 31 pg/mL, p=0.0003) and norepinephrine concentrations (post-placebo 237 ± 102 vs. post-ketamine 316 ± 106 pg/mL, p=0.0085 were higher after ketamine compared to placebo administration.

Post-Drug Administration Cardiovascular Measures. Systolic, mean, and diastolic BP values were higher during Rest after low dose ketamine compared to placebo administration, and increased from Rest to CPT in both ketamine/placebo conditions (Figure 5A, 5C, & 5E). Additionally, there was a trend for higher systolic BP during the CPT after low dose ketamine compared to placebo administration (Figure 5A). Despite a lack of ketamine-related changes in MSNA responses, the increases in systolic, mean, and diastolic BP during the CPT were attenuated after low dose ketamine compared to placebo administration (Figure 5B, 5D, & 5F). Interestingly, reductions in pain perception and mean BP responses after low dose ketamine administration were not significantly related (r=-0.27, p=0.2249).
Heart rate and cardiac output were higher, and total peripheral resistance was lower, during Rest after low dose ketamine compared to placebo administration (Figure 6A, 6C, & 6E). Additionally, heart rate, cardiac output, and total peripheral resistance values were higher during the CPT compared to Rest in both placebo/ketamine conditions (Figure 6A, 6C, & 6E). The increases in heart rate and cardiac output, but not total peripheral resistance, during the CPT were attenuated after low dose ketamine compared to placebo administration (Figure 6B, 6D, & 6F).

Post-Drug Administration Relations Between Cardiac Output & Total Peripheral Resistance and Mean BP Responses During the CPT. Cardiac output responses to the CPT were significantly related to mean BP responses after placebo, but not after low dose ketamine, administration (Figure 7A). Conversely, total peripheral resistance responses to the CPT were significantly related to mean BP responses after low dose ketamine, but not placebo, administration (Figure 7B).

Discussion

Our findings provide new information regarding the effects of low dose ketamine administration on autonomic cardiovascular regulation during the CPT in humans. The primary findings of this study are that during the CPT, low dose ketamine: 1) reduced pain perception, 2) did not alter increases in MSNA burst frequency, 3) attenuated increases in systolic, mean, and diastolic BP, and 4) blunted the contribution of cardiac output on mean BP responses. Additionally, low dose ketamine administration, compared to placebo, increased pressure pain tolerance. Together, these data suggest that low dose ketamine effectively reduces pain perception during two different acute experimental pain stimuli and attenuates BP, but not MSNA responses during the CPT.

In clinical pain conditions, pain severity is associated with greater alterations in autonomic cardiovascular regulation (Hallman et al., 2011; Zamunér et al., 2015; Adlan et al., 2017; Bruehl et al., 2018). In adults without chronic pain, perceived pain during noxious stimuli is associated with sympathetic nervous system mediated (Victor et al., 1987; Fagius et al., 1989; Kregel et al., 1992; Nakamura et al., 2008) increases in BP (Peckerman et al., 1991; Peckerman et al., 1994). The CPT, a
somatosensory-dependent reflex, stimulates pain- and temperature-sensitive free nerve endings in the skin and activates the spinal anterolateral system of ascending tracts (Nakamura et al., 2008). Most (Asmundsson et al., 1998; Camargo et al., 2013), but not all (Busnardo et al., 2009), prior work in animals demonstrated reduced BP responses during acute sympathoexcitatory stimuli following NMDA-antagonism. Thus, we hypothesized that low dose ketamine, a NMDA-antagonist, would reduce pain perception and the subsequent increases in MSNA and BP. Our finding of attenuated BP responses during the CPT after low dose ketamine administration is consistent with other data in humans reporting that low dose ketamine, compared to the nonsteroidal anti-inflammatory drug ketorolac, attenuated increases in BP elicited by tourniquet inflation in otherwise healthy individuals undergoing elective knee surgery (Zaidi & Ahmed, 2015). Therefore, our findings complement and support this previous work. Interestingly, the relation between reductions in pain and mean BP was not even modestly strong (r=-0.27, p=0.2249). Such an observation suggests a discordance between low dose ketamine-induced changes in pain perception and BP responses during the CPT that should be further explored.

One prior study reported increased BP and reduced MSNA burst frequency at rest after racemic ketamine administration (2 mg/kg body mass, i.e., anesthetic dose) (Kienbaum et al., 2000). But, given the high dose used, these data could not inform our hypothesis. Thus, the current study is the first to report MSNA following low dose ketamine administration. While we found low dose ketamine to attenuate BP responses during the CPT, MSNA responses to this noxious stimulus were not attenuated, suggesting that these attenuated BP responses may not be sympathetically mediated. This observation is inconsistent with both our hypothesis and one previous study in rodents demonstrating that rostral ventrolateral medulla injection of an NMDA-antagonist attenuated increases in plasma norepinephrine concentrations to exercise pressor reflex activation — another somatosensory reflex (Asmundsson et al., 1998). Though, it is difficult to compare results given species differences, differences in the route of delivery, and differences in the sympathoexcitatory stimulus used. Additionally, it is conceivable that ketamine-related reductions in anxiety (Sanacora et al., 2017; Banov et al., 2019) would have reduced sympathetic and cardiovascular responses during the CPT, given that anticipation and anxiety are known to influence pain perception (Burgmer et al., 2011). However, we did not observe ketamine to attenuate MSNA burst frequency responses during the CPT. Investigation into the psychological effects of ketamine are beyond the scope of the present manuscript. Therefore, future investigations should consider objectively assessing psychological
status and mental stress following low dose ketamine administration to better elucidate these potential interactions experimentally.

An investigation assessed MSNA responses to moderate (5 to 6 out of 10) pain induced by intramuscular infusion of hypertonic saline (7%) in a cohort of 12 participants (Fazalbhoy et al., 2012). The authors identified two divergent groups, those who exhibited increases (n=7) and decreases (n=5) in MSNA burst amplitude during the pain stimulus. Mean BP responses were in parallel with MSNA burst amplitude responses, such that the ‘increasing amplitude’ group experienced increases in mean BP and ‘decreasing amplitude’ group experienced decreases in mean BP. Conversely, the ‘increasing amplitude’ group had no significant changes in MSNA burst frequency whereas the ‘decreasing amplitude’ group had significant increases in burst frequency. Together, these prior results indicate that both MSNA burst occurrence (i.e., frequency) and intensity (i.e., amplitude) participate in the integrative autonomic responses during a pain stimulus. For the present study, the lack of parallel increases in BP, despite increases in MSNA burst frequency during the CPT after low dose ketamine administration raises the possibility that: a) ketamine reduced MSNA burst amplitude responses while increasing MSNA burst frequency, b) ketamine altered sympathetic outflow directed to other vascular beds, and/or c) ketamine reduced the effects of muscle sympathetic nerve bursts on BP (i.e., sympathetic vascular transduction (Fairfax et al., 2013)).

While the elevated plasma norepinephrine concentrations after low dose ketamine support the notion of greater MSNA burst amplitude at rest, it is important to note that prior work in animals suggests that ketamine impairs norepinephrine reuptake transporter function (Lundy et al., 1985) and reduces vasodilatory capacity (Miyawaki et al., 1995). Thus, the altered functional relation between circulating norepinephrine concentrations and vascular responses to a given amount of synaptic norepinephrine release limits one’s ability to conclude that elevated plasma catecholamine concentrations observed following low dose ketamine are indicative of increased MSNA burst amplitude. Unfortunately, due to several limitations addressed in the ‘data analysis’ section, we did not assess MSNA burst amplitude or area for this work. Therefore, we are unable to elucidate the potential influence of MSNA burst amplitude on our findings. Thus, future work on the effects of low dose ketamine on sympathetic vascular transduction, vascular function, and norepinephrine metabolism in humans is warranted.
Previous findings have reported that low dose ketamine raises resting heart rate and BP (Pfenninger et al., 1994; Ahern et al., 2015; Shackelford et al., 2015; Liebe et al., 2017; Riva-Posse et al., 2018). Consistent with these reports, we found that resting heart rate was 14 bpm higher (post-hoc p=0.0011) and resting mean BP was 14 mmHg higher (post-hoc p=0.0004) after low dose ketamine compared to placebo administration. However, we did not observe parallel increases in resting MSNA after low dose ketamine administration, as previous animal studies have suggested (Traber & Wilson, 1969; Wang et al., 2001). A combination of species differences and the use of higher doses of ketamine in these earlier animal studies likely explain this discrepancy. Given the prior work in animals identifying baroreflex dysfunction as a potential mediator of ketamine-induced increases in resting BP (Slogoff & Allen, 1974; Ogawa et al., 1993), future work in humans is warranted to examine these potential mechanisms. By design, our current work did not include time epochs (i.e., ≥10-minute evaluation rest period after drug administration) sufficient to assess arterial baroreflex function properly, thus, additional experiments are necessary to address this mechanism of low dose ketamine in humans.

We also demonstrated that low dose ketamine administration blunted the increases in cardiac output during the CPT. Consistent with our second hypothesis, cardiac output and mean BP responses were significantly related during the post-placebo CPT, but cardiac output and mean BP responses were not significantly related during the post-ketamine CPT. These observations inform future investigations that aim to determine the effects of low dose ketamine administration on cardiac function in humans.

In the past decade, low dose ketamine has been added to several prehospital pain management guidelines (Porter, 2004; Bredmose et al., 2009; Butler et al., 2014). For the United States military in particular, the use of low dose ketamine has appreciably increased in combat settings (Petz et al., 2015; Shackelford et al., 2015; Schauer et al., 2019). Limited to observational data, one of these previous reports mentioned that future research needs to address the cardiovascular side effects of low dose ketamine (Petz et al., 2015). As such, the present data support of previous observational findings (Jennings et al., 2011; Butler et al., 2014; Losvik et al., 2015) concluding that low dose ketamine has value as an analgesic medication. Finally, low dose ketamine has additional clinical uses that should be mentioned. Namely, low dose ketamine improves BP support and analgesia in peri- and post-operative settings (Mayer et al., 1990; Atashkhoi et al., 2013; Singh et al., 2013; Basuni, 2016), and

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reduces opioid requirements to maintain analgesia (Galinski et al., 2007; Li & Chen, 2019). Separately, the National Institutes of Health and National Institute on Drug Abuse seek to reduce the burden of the opioid addiction crisis (Azari et al., 2012; Volkow & Collins, 2017). Here, we present data for the analgesic and cardiovascular effects of low dose ketamine, a non-opioid based analgesic that may be considered to reduce opioid medication overuse and abuse. Lastly, the effects of low-dose ketamine on cardiovascular regulation demonstrated herein may also inform the off-label clinical use to treat other disorders, such as for treatment of major depressive disorders (Domany et al., 2019), anxiety disorders (Banov et al., 2019), and migraine pain (Lauritsen et al., 2016).

**Limitations.** While our study contributes new knowledge related to how low dose ketamine alters pain perception, sympathetic, and cardiovascular responses to an acute experimental pain stimulus, there are several limitations to mention. 1) This study contained a progressive LBNP test between the Pre- and Post-Drug administration pain assessments. However, we would not expect to have different conclusions had the LBNP test been omitted from this investigation for two reasons. First, the LBNP protocol was identical between experimental visits. Thus, the physiological conditions after the LBNP were likewise identical between trials (these LBNP data will be addressed in a separate manuscript). Second, a minimum of 10 minutes separated the end of LBNP testing from the onset of Post-Drug data collection (i.e., the first time point of interest to this manuscript after the LBNP test). We propose that this is sufficient time to restore physiological responses to a pre-LBNP state. 2) During the Pre-Drug administration CPT’s, we noted a significant main effect of trial for MSNA burst frequency, despite there being no differences in the conditions between these CPT trials. We contend that this is unlikely to have confounded the results or conclusions for several reasons. First, the Pre-Drug post hoc analysis demonstrated that MSNA burst frequency was not different between trials during either Rest or CPT. Second, the magnitude of the increase in MSNA burst frequency (i.e., delta values) was not different between trials prior to drug/placebo administration. Finally, Post-Drug sympathetic and cardiovascular responses during the CPT were the primary data of interest for this manuscript, not the Pre-Drug dataset. 3) While previous studies reported that increases in BP and MSNA during repeated CPT’s are reproducible within a visit, increases in HR have been reported to be attenuated during the second compared to the first CPT when these CPTs were separated by three to four minutes (Fagius et al., 1989; Stancak et al., 1996). Nevertheless, being that we directly compared low dose ketamine to placebo in the Post-Drug time point alone, with each trial performed on different days, any potential effect of repeated CPT on the observed physiological responses would be inconsequential with respect
to the obtained findings. Moreover, 30 minutes separated the two CPT’s in the present study whereas the two previous studies only allowed three to four minutes. 4) The present dataset does not allow us to draw conclusions on how low dose ketamine might affect autonomic cardiovascular function with pain associated with real-world severe trauma. Of note, there are well-founded ethical concerns rightfully preventing the use of extreme painful stimuli for investigative purposes. 5) Given the repeated measures design and LBNP-related baseline shifts in the neurogram (see ‘data analysis’ section for more specific details) between repeated CPT’s, we were unable to accurately assess MSNA burst amplitude or burst area for the present work. Future studies are warranted to determine the effects of low dose ketamine on MSNA burst amplitude and area at rest and during acute sympathoexcitation.

Summary. Consistent with previous literature, we observed increases in resting heart rate and BP following low dose ketamine administration. Our current data support observational findings indicating that low dose ketamine significantly reduces pain perception. Importantly, these data are the first to demonstrate that low dose ketamine administration attenuates increases in BP, but not MSNA burst frequency, during the CPT. Finally, we demonstrated that low dose ketamine blunted the contribution of cardiac output on increases in mean BP during the CPT. Together, these data suggest a discordance between MSNA and BP responses during an acute pain stimulus following low dose ketamine administration that should be further examined in future experiments.

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Author Contributions

Experiments were performed in the Thermal and Vascular Physiology Research Laboratory within the Institute of Exercise and Environmental Medicine of Texas Health Presbyterian Hospital Dallas. MH, GM, MNC, JMH, CH, and CGC contributed to the conception or design of the work; all authors contributed to the acquisition, analysis, or interpretation of the data for the work; all authors drafted the work or revised it critically for important intellectual content. All authors approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References


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Figure 1. Experimental Protocol Timeline. In random crossover fashion, participants completed two experimental trials, on different days, that were identical except during one visit they received two administrations of low dose ketamine and during the other visit they received two administrations of placebo (saline). Prior to drug/placebo administration, we collected data during a rest period before participants completed a pressure pain tolerance test and cold pressor test. Following the second drug/placebo administration, we repeated these assessments. The data from the lower-body negative pressure (LBNP) test are presented in a companion manuscript. The primary data of interest for this manuscript were the Post-Drug administration data between placebo and low dose ketamine administrations.
Figure 2. Pain Perception During the Cold Pressor Test (CPT) Post-Drug Administration.
Perceived pain during the CPT was significantly lower after low dose ketamine compared to placebo administration. Data are presented as means ± SD with individual responses (N=22) and were compared using a paired, two-tailed t-test.
Figure 3. Representative Blood Pressure and Sympathetic Tracings During the Cold Pressor Test Post-Drug Administration. Representative mean blood pressure (BP) and muscle sympathetic nerve activity (MSNA) tracings during the cold pressor test are presented from one participant after placebo (left) and low dose ketamine (right) administration. The dashed line indicates the onset of the two-minute cold pressor test.
Figure 4. Muscle Sympathetic Nerve Activity (MSNA) Responses During the Cold Pressor Test (CPT) Post-Drug Administration. MSNA burst frequency and burst incidence values increased from Rest to CPT in both conditions (panels A & C). There was a trend (P=0.0637) for higher absolute MSNA burst frequency values during the CPT after placebo compared to low dose ketamine administration (panel A). However, the increases in MSNA burst frequency and burst incidence during the CPT were not different between placebo and ketamine trials (panels B & D). Data are presented as means ± SD with individual responses (N=12). For panels A and C, data were compared using repeated measures two-way ANOVAs (main effects of rest/CPT and trial). For panels B and D, data were compared using paired, two-tailed t-tests.
Figure 5. Blood Pressure (BP) Responses During the Cold Pressor Test (CPT) Post-Drug Administration. Systolic, mean, and diastolic BP values were higher during Rest after low dose ketamine compared to placebo administration, and increased from Rest to CPT in both conditions (panels A, C, & E). Additionally, there was a trend for higher systolic BP during the CPT after low dose ketamine compared to placebo administration (panel A). The increases in systolic, mean, and diastolic BP during the CPT were attenuated after low dose ketamine compared to placebo administration (panels B, D, & F). Data are presented as means ± SD with individual responses (N=22). For panels A, C, and E, data were compared using repeated measures two-way ANOVAs (main effects of rest/CPT and trial). For panels B, D and F, data were compared using paired, two-tailed t-tests.
Figure 6. Hemodynamic Responses During the Cold Pressor Test (CPT) Post-Drug Administration. Heart rate and cardiac output were higher, and total peripheral resistance was lower, during Rest after low dose ketamine compared to placebo administration. Heart rate, cardiac output, and total peripheral resistance values were higher during the CPT compared to Rest in both conditions (panels A, C, & E). The increases in heart rate and cardiac output, but not total peripheral resistance, during the CPT were attenuated after low dose ketamine compared to placebo administration (panels B, D, & F). Data are presented as means ± SD with individual responses (N=22). For panels A, C, and E, data were compared using repeated measures two-way ANOVAs (main effects of rest/CPT and trial). For panels B, D, and F, data were compared using paired, two-tailed t-tests.
Figure 7. Contribution of Cardiac Output and Total Peripheral Resistance on Mean Blood Pressure (BP) Responses During the Cold Pressor Test Post-Drug Administration. The increase in cardiac output was significantly related to the increase in mean BP during the cold pressor test after placebo (dashed line and open circles), but not after low dose ketamine (solid line and closed circles) administration (panel A). Conversely, the increase in total peripheral resistance was significantly related to the increase in mean BP during the cold pressor test after low dose ketamine, but not placebo, administration (panel B). Individual data (N=22 for each variable and condition) are presented. Pearson’s correlations were conducted to determine the relation between cardiac output (panel A) and total peripheral resistance (panel B) responses on mean BP responses.
Table 1. Participant screening information

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD (range)</th>
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<tbody>
<tr>
<td>Number of participants</td>
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</tr>
<tr>
<td>Age, yrs</td>
<td>27 ± 6 (19-37)</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>80 ± 10 (65-101)</td>
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<tr>
<td>Body mass index, kg • m⁻²</td>
<td>26 ± 3 (22-32)</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>120 ± 10 (102-140)</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>73 ± 9 (60-94)</td>
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