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SHORT COMMUNICATION

Tachycardia in response to remote capsaicin injection as a model for nociception in the ball python (*Python regius*)

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Abstract

Objectives To quantify the effect of subcutaneous (SC) capsaicin injection on heart rate (HR) in ball pythons (*Python regius*) and to assess the efficacy of two opioids (morphine and butorphanol) in modifying this response.

Study design Prospective, randomized, unmatched study.

Animals Eleven mixed-sex, captive-bred ball pythons.

Methods Snakes were randomly assigned to three groups (n = 6) by intramuscular premedication: 1) control: saline (0.9 mL); 2) morphine (10 mg kg^{-1}); and 3) butorphanol (10 mg kg^{-1}). Three snakes were tested twice and another two were tested three times in different treatments administered 1 month apart. Under isoflurane anaesthesia, snakes were instrumented with SC electrocardiogram (ECG) electrodes and an SC catheter for remote stimulus delivery. After recovery from anaesthesia, all snakes, in visual and audial isolation from the experimenter, received a sham stimulus of saline (0.4 mL) via the SC catheter. A nociceptive stimulus of SC capsaicin (3 mg in 0.2 mL saline with 7% Tween 80) was then applied by catheter at 7 hours after premedication. In a subset (n = 3), two sham injections (saline 0.2 mL) preceded the capsaicin treatment. HR was recorded via ECG, and changes in HR (ΔHR) from baseline were calculated for all stimulations.

Results Capsaicin injection was associated with a significant increase in HR [peak ΔHR: saline group: 8.8 ± 7.1 beats minute^{-1}; capsaicin group: 21.1 ± 5.8 beats minute^{-1} (p = 0.0055)] and integrated ΔHR as a function of time. The administration of morphine or butorphanol 7 hours prior to nociception failed to significantly reduce the peak and integrated ΔHR. Butorphanol caused marked, long-lasting sedation as assessed by muscle tone.

Conclusions and clinical relevance The HR response to an SC capsaicin injection can serve as a nociceptive model in *P. regius*. Morphine and butorphanol administration did not reduce HR response to capsaicin stimulation but produced significantly different effects on pre-stimulation HR and sedation.

Keywords analgesia, butorphanol, capsaicin, morphine, reptile.

Introduction

The alleviation of pain is a goal in research and clinical practice and is aimed at both ensuring animal welfare and increasing the reliability of experimental results. The efficacy of analgesics in reptiles, however, remains poorly understood (Mosley 2011). The use of opioid μ-agonists (μ-receptor/MOP receptor agonists), which represents the reference standard method in mammals, appears to be effective in multiple classes of reptile, whereas while mixed agonists (e.g. butorphanol κ-receptor/KOP

receptor agonist and μ-receptor antagonist) are commonly used, their efficacy is questioned (Mosley 2011). There is particular controversy surrounding snakes. Despite the presence of endogenous opioids (Ng et al. 1990), recent studies show an insignificant response to a routine dose of butorphanol at surgery (Olesen et al. 2008), and thermal nociceptive responses that differ from those in other taxa (Sladky et al. 2008). There is, therefore, a current need for a reliable nociceptive test in snakes.

Here, we evaluate whether capsaicin might represent a reliable chemical nociceptive stimulus when administered subcutaneously (SC) in snakes. The hypothesis of this study was that SC capsaicin administration would elicit tachycardia, and that this model would elucidate the efficacy of opioid administration in *Python regius*.

Materials and methods

Animals

Eleven ball pythons with a mean ± standard deviation (SD) body mass of 916 ± 336 g were housed at 26–27 °C in the animal care facility at Aarhus University. All snakes were feeding regularly prior to the study and maintained appropriate body mass. Snakes were fasted for 5–7 days before experiments to reduce the influence of digestion on heart rate (HR) (Enok et al. 2012), and were not studied while shedding. Experiments were conducted under licence from the Danish Animal Experiments Inspectorate (licence no. 2013-15-2934-00847).

Experiment groups

Snakes were randomly assigned by lottery to one of three groups for premedication: 1) 0.9 mL isotonic saline (0.9% NaCl; Fresenius Kabi Norge AS, Norway); 2) 10 mg kg^{-1} morphine (Morfin SAD, 5 mg mL^{-1}; Amgros I/S, Denmark); and 3) 10 mg kg^{-1} butorphanol (Torbugesic Vet, 10 mg mL^{-1}; Scanvet Animal Health A/S, Denmark). All opioid injections were given into the epaxial muscles and were split over multiple sites (0.3 mL at each site). The experimenter was aware of the treatment. Group size was determined according to data from a pilot study using seven snakes. A sample of at least six snakes per group was required to detect a change in HR after administration of capsaicin with a power of 80% and a significance level of 5% (Power and Sample Size Calculator; MGH Biostatistics Center, MA, USA). Five individuals were reassigned to different groups for retesting; of these, three snakes were tested twice and two were tested three times. All tests were separated by rest periods of 1 month. The remaining six snakes were tested once only.

Instrumentation

Anaesthesia was induced by placing the snake in a ziplock bag containing a swab saturated with isoflurane (Isoflo Vet; Orion Pharma Animal Health AS, Finland) at 15 minutes after premedication. The snakes were endotracheally intubated with 0.5 cm diameter soft polyethylene (modified 3.5 mL transfer pipettes; Sarstedt AG & Co., Germany) and placed on a ventilator (Anaesthesia Workstation; Hallowell EMC, MA, USA) and mini-circle system (Fluotec Mark 3 vaporizer; Simonsen & Weel A/S, Denmark). An oxygen flow of 250 mL $minute^{-1}$ kg^{-1} was provided with tidal volume adjusted for weight, respiratory frequency of 4 breaths $minute^{-1}$ and maximum airway pressure of 10 cm H_2O. Snakes were placed on covered heating pads (Melissa Electric Heating Pad 631-015; Adexi A/S, Denmark) to maintain a mean ± SD cloacal temperature of 30 ± 1 °C. Anaesthesia was maintained with 3% isoflurane. Three electrocardiogram (ECG) electrodes were introduced SC and sutured ventrally (Polysorb 3–0; Covidien, Inc., MA, USA), at 2 cm cranial and caudal to the heart. A 50 cm, saline-filled catheter (0.58 mm internal diameter polythene; Smiths Medical Danmark ApS, Denmark) was placed dorsolaterally at a point two-thirds along the length of the body so that a 1 cm length lay SC. Catheter and ECG placement were performed aseptically. Isoflurane administration ended at the completion of surgery and snakes were ventilated with 100% oxygen to wash out the isoflurane, measured by end-tidal gas analysis (Cardell Veterinary Monitor MAX 12-HD; Midmark Corp., OH, USA). Snakes were extubated upon voluntary head retraction. After recovery, snakes were placed, unrestrained, within a 40 × 20 × 15 cm terrarium in a metabolic chamber maintained at 30 °C.

Sedation and capsaicin stimulation

Sedation was assessed according to muscle tone, extent of voluntary movement and righting reflex at extubation and at 30 minutes before stimulation. All stimulations were performed within the metabolic