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SHORT COMMUNICATION

Inhaled vasopressin increases sociability and reduces body temperature and heart rate in rats

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Summary The neuropeptides vasopressin (AVP) and oxytocin (OT) have therapeutic potential across a range of psychiatric disorders. However, there is uncertainty about the effectiveness of the intranasal route of administration that is often used to deliver these neuropeptides. Recent preclinical studies, typically involving anesthetized or restrained animals, have assessed intranasal AVP or OT effects, and have obtained somewhat inconsistent results. Here we obtained intranasal administration of AVP in rats by nebulizing the peptide (1 ml of 5 or 10 mg/ml solution) into a small enclosed chamber over a 2 min period in which well-habituated, unanesthetized, unrestrained, rats were placed. Rats were immediately removed from the chamber and tested in the social interaction test, or assessed for changes in heart rate and body temperature using biotelemetry. Results showed that rats exposed to nebulized AVP (5 or 10 mg/ml) showed increased social proximity (adjacent lying) and decreased anogenital sniffing in the social interaction test. Biotelemetry showed substantial and long lasting (>1 h) hypothermic and bradycardic effects of nebulized AVP. These behavioral and physiological effects of nebulized AVP mimic those observed in recent studies with peripherally injected AVP. Plasma AVP concentrations were substantially increased 10 min after nebulized AVP, producing levels above those seen with a behaviorally effective injected dose of AVP (0.005 mg/kg intraperitoneal). This study thus provides a novel and effective method for neuropeptide administration to rodents.

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1. Introduction

Arginine vasopressin (AVP) is a neuropeptide with well documented behavioral and physiological functions in humans and other animals. AVP acts centrally to modulate a range of psychological functions including memory, stress responses and aggression (Benarroch, 2013). Central administration of AVP improves social recognition and memory in rats (Veenema et al., 2011), and facilitates partner preference formation in male prairie voles (Winslow, 1993). Our group recently showed that peripherally injected AVP increases social proximity in rats meeting for the first time in a social interaction test (Ramos et al., 2013).

AVP and the closely related neuropeptide oxytocin (OT) are typically given to humans intranasally. This helps overcome their poor oral bioavailability and blood–brain barrier penetration. Despite good evidence that intranasal AVP raises levels of AVP in cerebrospinal fluid (Born et al., 2002), some doubts remain about the efficacy of the intranasal route. Several recent preclinical studies have sought to clarify the effectiveness of intranasal methods. This has typically involved direct application of peptides around the nostrils of anesthetized or restrained animals and the observation of various behavioral, physiological and/or neurochemical endpoints (Bales et al., 2013; Chang et al., 2012; Ebitz et al., 2013; Jarcho et al., 2011; Ludwig et al., 2013; Neumann et al., 2013).

For example, application of intranasal OT to anesthetized mice and rats increased brain levels of the peptide as detected by microdialysis (Neumann et al., 2013). Enhanced prosocial behaviors were seen in male prairie voles following intranasal OT (delivered to restrained animals), however, partner preference was impaired in male prairie voles after chronic intranasal OT (Bales et al., 2013). Nebulized, inhaled OT facilitated prosocial and altruistic behaviors in rhesus macaques and reduced vigilance toward social threats (Chang et al., 2012; Ebitz et al., 2013). In coppery titi monkeys, a high dose of intranasal AVP increased preference for a familiar partner over a stranger (Jarcho et al., 2011). In contrast, a recent study found that intranasal AVP (applied to the nostrils of rats during brief anesthesia) failed to affect social recognition, anxiety-like behavior or Fos expression (Ludwig et al., 2013). It is notable then, that intranasal administration of OT and AVP to rodents has largely relied on the passive mucosal absorption of the drug by anesthetized or restrained animals rather than active insufflation/inhalation used in human studies.

Here we explored the utility of a novel alternative method to deliver AVP to rats. We employed nebulizer delivery of small (2–5 μm in diameter) aerosol particles of AVP solution into a small enclosed environment in which a rat is located. Importantly, rats are obligate nose breathers meaning that these nebulized peptides will be actively and intranasally self-administered as a consequence of normal breathing (Haidarliu et al., 2012).

Recently we have used biotelemetry to demonstrate strong bradycardic and hypothermic effects of intraperitoneally (i.p.) injected AVP in rats (Hicks et al., 2014), in addition to the prosocial effects we have also recently described (Ramos et al., 2013). The aim of the current study was to determine whether nebulized AVP produces the same prosocial, bradycardic and hypothermic effects as peripherally injected AVP.

2. Methods

2.1. Animals and surgical procedures

Experiments were conducted on experimentally naïve adult male Long–Evans rats (total $n = 62$) purchased from Adelaide University (Adelaide, SA, Australia) and weighing between 250 and 300 g. Rats were housed in groups of 8 in large plastic tubs (640 mm × 400 mm × 220 mm) and maintained under a reverse 12:12 h light–dark cycle (lights off at 09:00 h) in a temperature (21 ± 1 °C) controlled colony room. Rats used in the biotelemetry experiment were single housed in translucent Plexiglas tubs (420 mm × 260 mm × 180 mm) after surgery in a separate test room, which was maintained under the same light and temperature conditions as the main colony room. Animals had *ad libitum* access to food and water, except during the 30 min social interaction test. All experiments were conducted in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (7th Edition, 2004) with approval of The University of Sydney Animal Ethics Committee.

Some rats ($n = 8$) were implanted with biotelemetry probes as previously described (Hicks et al., 2014). These rats were anesthetized using isoflurane gas. After testing of withdrawal reflexes to ensure adequate depth of anesthesia, a 2 cm abdominal incision was made, and the sterile radiotelemetry transmitter (Data Sciences International CTA-F40) was placed into the peritoneal cavity. The two biopotential leads were placed near the right foreleg and left hind leg, and were secured in place using non-absorbable sutures. The abdominal wall was closed using absorbable sutures according to the manufacturer's protocol (Data Sciences International, St. Paul, MN, USA). Rats were allowed to recover for 7 days prior to testing.

2.2. AVP preparation and delivery

AVP was purchased from AusPep Ltd (Parkville, VIC, Australia) and dissolved in physiological saline (0.9%) to a concentration of 5 mg/ml or 10 mg/ml. A total of 1 ml of these solutions was nebulized over a 2 min period. These doses were arrived at after extensive pilot studies, which were conducted to ensure the effectiveness of the treatment and the welfare of the animals involved. During these studies, rats received nebulized AVP (0.5–10 mg/ml) for 1–4 min and were closely monitored for any signs of distress (e.g. gasping for air, immobility, piloerection) during AVP exposure, and then when observed in the social interaction test and in their home cage afterwards. These pilot studies suggested the effectiveness of higher range AVP nebulized doses in increasing subsequent social interaction.

A Medix pocket nebulizer (sound frequency: 180 kHz; particle size: 2–5 μm; Asthma Foundation of South Australia, Hilton, SA) was connected via polyethylene tubing to an inlet in a small chamber made of clear acrylic (235 mm × 170 mm × 120 mm) located in a fume hood. The chamber contained several air holes (5–10 mm in diameter) to ensure that rats did not become hypoxic.

Rats were habituated to the chamber for 5 min a day for 7 days prior to testing. In these habituation sessions, saline was nebulized into the chamber for 3 min followed by a