European Journal of Pharmacology 809 (2017) 203–208

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

Gender differences in the effects of cathinone and the interaction with caffeine on temperature and locomotor activity in the rat

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ARTICLE INFO

Keywords:
Cathinone
Caffeine
Gender differences
Hyperthermia
Locomotor activity
Radiotelemetry
Stimulants

ABSTRACT

We have investigated gender differences in the effects of cathinone and the interaction with caffeine on temperature and movement activity in Wistar rats. Telemetry probes were implanted in rats under isoflurane anaesthesia, and 7 days later, temperature and activity were recorded in conscious unrestrained animals. Caffeine (10 mg/lkg) or vehicle, and 30 min later, cathinone (5 mg/kg) or vehicle, were injected subcutaneously. Cathinone produced significant and marked increases in activity, and the response to cathinone was significantly greater in female animals. The combination of caffeine and cathinone causes a short lived potentiation followed by a prolonged inhibition of the activity response to cathinone. Cathinone alone had minor effects on temperature. However, the combination of caffeine and cathinone produced a significant acute rise in temperature only in male rats in the 90 min after cathinone injection. Hence, cathinone caused greater increases in activity in female than in male rats. Secondly, caffeine produced an initial potentiation followed by a prolonged inhibition of the activity response to cathinone. Thirdly, cathinone in combination with caffeine significantly raised temperature acutely in male but not female rats. These differences highlight the need to carry out gender studies of the actions of stimulants.

1. Introduction

Leaves of Khat (Catha edulis Forsk) contain the β-keto-amphetamine cathinone that has amphetamine-like actions (Kalix, 1990). The Khat chewing habit is a problem that is growing world-wide (Weir, 1985; Randall, 1993). In the past, less women chewed Khat due to social stigma (Australia: Stevenson et al., 1996). In Yemen, It has been estimated that 82% of men have used Khat compared to 43% of women (NIDA, 2013), and in the Jazan region of Saudi Arabia 42.2% of men compared to 11.3% of women (Mahfouz et al., 2015), but approximately equal numbers of men and women in two Yemeni cities (Nakajima et al., 2014) and in the UK (ACMD, 2005). In addition, cathinones are widely used as drugs of abuse.

Actions of Khat include euphoria and erratic behaviour, with typically enlarged pupils (Brenneisen et al., 1990; Toennes and Kauert, 2004), and cardiovascular actions to increase blood pressure and heart rate (Widler et al., 1994; Toennes et al., 2003), and increased cardiac morbidity and mortality in both males and females (Ali et al., 2011). Cathinone is the major active amphetamine-like stimulant in Khat. Cathinone can be detected in the urine of infants during lactation among Khat chewing mothers (Graziani et al., 2008; Kuczkowski, 2005).

Cathinone probably produces its major actions at the noradrenaline (NET) and dopamine (DAT) transporters. Cathinone has similar potency to MDMA at the noradrenaline transporter (approximately 1 μM) (Horn, 1973; Cleary and Docherty, 2003), with some additional direct receptor mediated actions (see Docherty, 2008; Docherty and Green, 2010; Alsufyani and Docherty, 2015). Cathinone was equipotent at DAT and up to a 100 times less potent at SERT as compared to MDMA (Simmler et al., 2013). Cathinone has similar low potency at SERT to that of amphetamine (Rothman et al., 2003).

Major adverse effects of amphetamine-like stimulants include hyperthermia which is dependent on the environmental and social conditions such as occur in nightclubs (Cole and Sumnall, 2003), and increased motor activity. Increased motor activity in rats may be a useful indicator of stimulant actions in man and allow correlation between stimulant action and adverse temperature and cardiovascular actions.

In social environments including that of Khat chewing, coffee is often combined with the stimulant, and there may be additive effects In rats, caffeine increases the locomotor response produced by cocaine (Schenk et al., 1990) and mephedrone (Shortall et al., 2016) but not by

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http://dx.doi.org/10.1016/j.ejphar.2017.05.028
Received 30 November 2016; Received in revised form 1 May 2017; Accepted 12 May 2017
Available online 18 May 2017

MDMA (Vanattou-Saïfoudine et al., 2010b). Vanattou-Saïfoudine et al. (2010b) reported a small increase in locomotor activity to caffeine and Zancheta et al. (2012) found no effect or increased locomotor activity depending on habituation to environment. In mice, caffeine (10 mg/kg) caused a marked increase in locomotor activity (Camarasa et al., 2006), although in other studies, caffeine (5–50 mg/kg) did not increase locomotor activity (Szopa et al., 2016). Interaction with caffeine may influence the overall effect of cathinone.

Hence, in this study, we have investigated gender differences in the effects of cathinone and the interaction with caffeine on temperature and motor activity monitored by radiotelemetry in Wistar rats.

2. Materials and methods

2.1. General

Male (230–300 g) and female (190–230 g) Wistar rats were obtained from Harlan (UK). Animals were housed in pairs in home cages (plastic, environmentally controlled) and bedding (wood shavings). The Principles of laboratory animal care were followed, and all studies have been approved by the Health Products Regulatory Agency (HPRA), and by the RCSI Research Ethics Committee.

Animals were implanted with telemetry probes (TA11TA-F1; Data Sciences International, St Paul, MN, U.S.A.) under isoflurane anaesthesia, The implant was placed in the abdominal cavity, and the abdominal wall and skin incision were closed with silk suturing. Motion and temperature sensors built into the device measure motor activity (including locomotor and stereotypy) and core body temperature. Animals were given Vetergesic (buprenorphine hydrochloride 0.05 mg/kg, sub-cutaneously) post-operatively. Animals were housed singly from atleast at least 1 day before the experimental day.

On the experimental day, approximately 7 days later (range 6–9), animals were transferred from the home cage to an experimental cage together with bedding, food and water from the home cage. The home cage was too large for the telemetry receiver plate. A PhysioTel-Receiver (model RPC- 1: 33×22 cm plate) was placed under each individual animal experimental cage, enabling recording of locomotor and temperature parameters. Data signals were acquired from 25 min prior to and for 270 min after drug administration, and analysed using the Dataquest A.R.T., Version 4.3. All recordings were obtained at room temperature (22 °C). The initial 20 min of recording was used to confirm the baseline, but time zero was set after 20 min of recording, and drug injection was at 5 and 35 min after time zero.

2.2. Experimental protocol

Four treatment groups were employed in studies of both male and female rats: vehicle/vehicle; vehicle/cathinone (5 mg/kg); caffeine (10 mg/kg)/vehicle; caffeine (10 mg/kg)/cathinone (5 mg/kg). In each group, n=7 animals were employed. Animals were randomized for treatment using a random number generator. Animals were not habituated to vehicle injections prior to the experimental day. The experimenter who also carried out initial analysis was blind to the treatment. Each experimental day, a male and a female animal were investigated employing the same treatment regime. The cages were kept apart and a barrier was placed between the cages so that the animals could not see each other.

Animals were slowly injected over 30–60 s subcutaneously (into the thigh area) with vehicle (distilled water, 1 ml/kg) or test drugs. Vehicle or caffeine (10 mg/kg) was injected at 5 min, and vehicle or cathinone (5 mg/kg) was injected at 35 min, all in a volume of 1 ml/kg s.c., and recording continued for another 240 min. At the end of the recording period, animals were killed by the injection of an overdose of pentobarbitone (100 mg/kg, i.p.) and exsanguination.

2.3. Data analysis and statistics

Data was initially compiled using Microsoft Excel, and temperature data was converted to change in temperature (ΔT) from the zero min baseline. Activity and ΔT data were then transferred to GraphPad Prism for MacIntosh computers for statistical analysis and graphical presentation. Results are expressed as mean ± S.E.M. from 7 animals in each group. The minimum level for statistical significance was $P < 0.05$. Total responses were compared by one-way ANOVA, followed by Bonferroni test (comparing groups) or Dunnett test (comparing with control). Time course responses were compared by repeated measures two-way ANOVA, followed by Bonferroni test. Three-way ANOVA was used to compare interactions between pretreatment (vehicle or caffeine), treatment (vehicle or cathinone) and gender. Post-tests were carried out only when differences were significant ($P < 0.05$).

3. Results

3.1. Activity

One-way ANOVA showed a significant effect of treatment/gender ($F_{(7,385)}=92.6$, $P < 0.0001$). Post-hoc comparisons showed that all treatments significantly increased total activity (5–275 min) in both male and female rats as compared with the effects of vehicle (Dunnett's post-test $P < 0.05$). Total activity (5–275 min) was significantly increased in female rats as compared with male rats for all except vehicle groups (Bonferroni post-test $P < 0.05$).

Although caffeine increased total activity in male and female, the caffeine/vehicle group did not differ significantly from vehicle/vehicle at any 5 min time point in male rats, and differed significantly from vehicle/vehicle only towards the very end of recording in female rats (Fig. 1). However, taking 30 min intervals, the caffeine/vehicle group had significantly higher activity at 35–65 min in male and at 65–95, 185–215 and 245–275 min in female rats.

Two-way ANOVA showed significant effects of treatment ($F_{(3,24)}=6.77$, $P < 0.01$), time ($F_{(55,1320)}=4.35$, $P < 0.0001$) and the interaction of treatment and time ($F_{(165,1320)}=3.49$, $P < 0.0001$) in male animals. Post-hoc comparisons showed that the vehicle/cathinone group differed significantly from vehicle/vehicle in the 5 min periods from 65 to 95 min and 130, 180 and 195 min (compare Fig. 1 with Fig. 2), and in the 30 min intervals from 65 to 215 min in male animals (Bonferroni post-test, $P < 0.05$). Two-way ANOVA showed significant effect of treatment ($F_{(3,24)}=17.7$, $P < 0.0001$), time

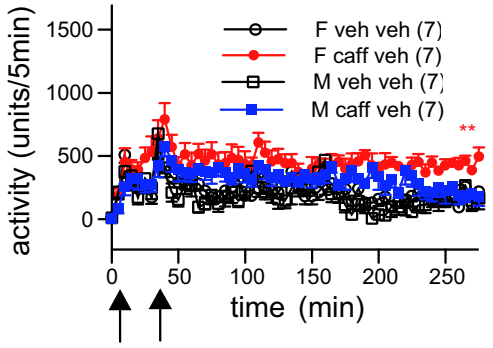

Fig. 1. Locomotor activity recordings in conscious male and female rats given vehicle or caffeine (10 mg/kg) at 5 min (indicated by first arrow), and vehicle at 35 min (indicated by second arrow), at room temperature of 22 °C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate S.E.M. from 7 rats. Two groups of animal are shown for both Male and Female: vehicle/vehicle (veh veh) and caffeine/vehicle (caff veh). Significant differences from vehicle/vehicle in the same gender are shown by colour-coded asterisks (blue for male, red for female): in this case there were significant differences only in female (Two-way ANOVA, and Bonferroni post test, $P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).