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Oxytocin injected into the hippocampal ventral subiculum induces penile erection in male rats by increasing glutamatergic neurotransmission in the ventral tegmental area

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ABSTRACT

Oxytocin (100 ng) injected unilaterally into the ventral subiculum of the hippocampus induces penile erection episodes, which started 30 min after treatment and were abolished by the prior injection of d$(CH_2)_5$Tyr(Me)^2-Orn^8-vasotocin (2 μg), an oxytocin receptor antagonist, into the ventral subiculum. Oxytocin-induced penile erection occurred 15 min after the increase of the concentration of extracellular dopamine in the dialysate obtained from either the nucleus accumbens or the prelimbic medial prefrontal cortex, which was also abolished by d$(CH_2)_5$Tyr(Me)^2-Orn^8-vasotocin. An increase in extracellular glutamic acid concentration was also observed in the same dialysate obtained from the ventral tegmental area, but not from the prelimbic medial prefrontal cortex or the nucleus accumbens in which dopamine concentration was measured, 15 min after the injection of oxytocin into the ventral subiculum. This effect was also abolished by the prior injection of d$(CH_2)_5$Tyr(Me)^2-Orn^8-vasotocin into the ventral subiculum. These results confirm previous findings showing that ventral subiculum oxytocin-induced penile erection is mediated by an increase of glutamic acid neurotransmission in the ventral tegmental area. This in turn increases mesolimbic and mesocortical dopaminergic activity, releasing dopamine in the nucleus accumbens and in the prelimbic medial prefrontal cortex. These results are in line with previous studies supporting the hypothesis that the ventral subiculum participates in a complex neural circuit controlling not only penile erection and copulation, but also sexual motivation, arousal and rewarding.

1. Introduction

Penile erection is the result of a complex interaction between central and peripheral autonomous nervous system and plays a key role in male copulatory behaviour and reproduction of mammals, including humans. Several brain areas involved in male erectile function and copulatory behaviour have been identified. These include the medial preoptic area, the hypothalamus and its nuclei, mainly the paraventricular nucleus (PVN), which contains the cell bodies of oxytocinergic neurons projecting to extra-hypothalamic brain areas, i.e. the hippocampus, the amygdala, the ventral tegmental area (VTA), the medulla oblongata and the spinal cord (see Hull et al., 1995, 2002; Giuliano and Rampin, 2000; McKenna, 2000; Andersson, 2001; Argiolas and Melis, 2004, 2005; Melis et al., 2007, 2009a,b, 2010; Succu et al., 2007, 2008). We have previously reported that oxytocinergic neurons projecting to the spinal cord facilitate erectile and copulatory performance, while those projecting to the VTA, the hippocampus and the amygdala control aspects of the anticipatory phase of sexual behaviour, from arousal to motivation and reward (Melis et al., 2007, 2009a,b, 2010; Succu et al., 2007, 2008; Melis and Argiolas, 2011).

In particular, oxytocin injected into the ventral subiculum of the hippocampus induces penile erection and increases the concentration of extracellular dopamine in the dialysate obtained from the nucleus accumbens shell. Apparently, these responses are secondary to an increased nitric oxide production and glutamic acid neurotransmission in the ventral subiculum (Melis et al., 2009a,b, 2010). Interestingly, penile erection episodes induced by oxytocin injected into the ventral subiculum were abolished by (+) MK-801, a non competitive antagonist of glutamic acid receptors of the N-methyl-D-aspartic acid (NMDA) subtype (Woodruff et al., 1987), given into the VTA but not into the nucleus accumbens,

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suggesting that glutamatergic afferents to the VTA, rather than to the nucleus accumbens, are involved in these oxytocin responses (Melis et al., 2009a,b, 2010). In line with this hypothesis, glutamatergic projections from the ventral subiculum, travelling through the prelimbic medial prefrontal cortex to reach the VTA (see Alquicer et al., 2004; Balfour et al., 2006; Witter, 2006) were reported to modulate dopamine release in the nucleus accumbens shell (Legault and Wise, 1999, 2001; Legault et al., 2000; Floresco et al., 2001), although a direct glutamatergic projection from the ventral subiculum to the VTA cannot be excluded (Witter, 2006). In order to provide evidence for an involvement of hippocampal glutamatergic efferents on the increase of mesolimbic dopaminergic neurotransmission in the nucleus accumbens and on penile erection induced by oxytocin given into the ventral subiculum, the effect of a pro-erectile dose of oxytocin given into the ventral subiculum on the concentration of extracellular glutamic acid and dopamine was studied in the same dialysate obtained from the prelimbic medial prefrontal cortex, the VTA and the nucleus accumbens. The effect of $d(CH_2)_5Tyr(Me)^2$-Orn^8-vasotocin, a potent and selective oxytocin receptor antagonist (Bankowski et al., 1980) on oxytocin-induced responses was also studied.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (250–300 g) (Charles River, Como, Italy) were used in all the experiments. The animals were caged in groups of 4–6 at 24 °C, humidity 60%, lights on from 07:00 to 19:00 h with water and standard laboratory food ad libitum. The experiments were performed between 09:00–13:00 h. All experiments were carried out in accordance with the guidelines of the European Communities Directive of 24 November 1986 (86/609/EEC) and the Italian Legislation (D.P.R. 116/92). In order to avoid any effect of handling-stress on extracellular dopamine and glutamic acid concentrations (Del Arco and Mora, 2008), rats were habituated to handling for at least one week before the experiments. No experiment was performed to ascertain whether these rats were sexually active or inactive when exposed to a receptive female.

2.2. Drugs and peptides

Oxytocin was purchased from Sigma (S. Louis, MO, USA); $d(CH_2)_5Tyr(Me)^2$-Orn^8-vasotocin from Bachem AG (CH). All other reagents were from available commercial sources.

2.3. Microinjections into the ventral subiculum and microdialysis in prelimbic medial prefrontal cortex, in the VTA and in the shell of the nucleus accumbens

For microinjections of oxytocin into the ventral subiculum and microdialysis either in the prelimbic medial prefrontal cortex or in the VTA or in the shell of the nucleus accumbens of the same male rat, a stainless-steel chronic guide cannula (22 gauge) aimed unilaterally at the ventral subiculum (coordinates: 6.3 mm posterior, 4.5 mm lateral and 2.0 mm ventral to dura), (Paxinos and Watson, 1987) and a microdialysis probe with a U-shaped dialysis membrane (approximately 2 mm of free surface for dialysis), prepared as previously described (Melis et al., 2003) and aimed unilaterally either at the ipsilateral prelimbic medial prefrontal cortex (coordinates: 2.7 mm anterior, 0.5 mm lateral and 4.0 mm ventral to dura), or at the ipsilateral VTA (coordinates: 6.04 mm posterior, 1.0 mm lateral and 8.4 mm ventral to dura), or at the ipsilateral nucleus accumbens shell (coordinates: 1.7 mm anterior, 0.7 mm lateral and 7.0 mm ventral to dura) (Paxinos and Watson, 1987), were implanted stereotaxically (Stoelting Co., Wood Dale, IL, USA) in the skull of male rats during the same stereotaxic surgery under chloral hydrate anaesthesia (400 mg/kg I.P.). Rats were given two days for recovery. When the experiments were performed, the microdialysis probe aimed either at the prelimbic medial prefrontal cortex or at the VTA or at the shell of the nucleus accumbens was perfused with Ringer's solution, containing 147 mM NaCl, 3 mM KCl and 1.2 mM $CaCl_2$, pH 6.5, at a constant flow rate of 2.5 μL/min by using a Stoelting microsyringe pump. After a 2-h equilibration period, the dialysate was collected every 15 min in aliquots of 37.5 μL in polyethylene loops and transferred in polyethylene tubes kept at 10–15 °C for the determination of dopamine and glutamic acid concentrations, as described below. After the collection of three dialysate aliquots, oxytocin dissolved in saline or vehicle alone was injected into ventral subiculum in a volume of 0.3 μL in 2 min via an internal cannula (28 gauge), which extended 5.3 mm below the tip of the guide cannula (Paxinos and Watson, 1987), and connected by polyethylene tubing to a 10-μL Hamilton syringe driven by a Stoelting microsyringe pump (Stoelting Co., Wood Dale, IL, USA). When $d(CH_2)_5Tyr(Me)^2$-Orn^8-vasotocin was used, the oxytocin antagonist was dissolved in saline and injected into the ventral subiculum in a volume of 0.3 μL in 2 min, 15 min before oxytocin. After injections, the tip of the cannula was left in the injection site for 30 s to allow the spreading of the injected solution. Rats were observed for 75 min, during which five additional dialysate fractions of 37.5 μL each were collected every 15 min and penile erection episodes were counted.

2.4. Behavioural studies

Rats implanted with a microinjection cannula aimed at the ventral subiculum and a microdialysis probe aimed at the VTA, or at the medial prefrontal cortex or at the nucleus accumbens, respectively, were placed individually in Plexiglas cages (30 × 30 × 30 cm). The microdialysis probe was connected via polyethylene tubing to 2500 μL Hamilton syringe driven by a Stoelting microsyringe pump on one end and to the polyethylene collecting loop on the other end. After the equilibration phase three dialysate aliquots of 37.5 μL were collected for the determination of basal levels of dopamine and of glutamic acid, then oxytocin or vehicle was given into the ventral subiculum and five additional dialysate aliquots collected. When $d(CH_2)_5Tyr(Me)^2$-Orn^8-vasotocin was used, this was given into the ventral subiculum 15 min before oxytocin. In all the above experimental conditions, after treatments rats were observed for the entire duration of the experiment in order to count penile erection episodes and to replace filled loops with empty ones every 15 min. Penile erections were scored when the penis emerged from the penile sheath, which was usually accompanied by penile grooming and hip flexions.

2.5. Determination of glutamic acid concentration in the dialysate from the prelimbic medial prefrontal cortex, the VTA or the shell of the nucleus accumbens

The concentration of glutamic acid in the dialysate obtained from the prelimbic medial prefrontal cortex, the VTA or the shell of the nucleus accumbens was measured in the same sample used for dopamine measurement. Briefly, glutamic acid concentration was measured in 5 μL aliquots of dialysate samples added to 5 μL of $HClO_4$ 30 mM after pre-column derivatization with orto-phtalaldialdehyde and 2-mercaptoethanol, by high pressure liquid chromatography (HPLC) (Melis et al., 2004). The chromatograph was equipped with a 15 × 0.4 cm Supelco C_{18} column, 5 μm particle size, and coupled to fluorescence detection (excitation wavelength: 318 nm; emission wavelength: 452 nm; SFM 25 spectrofluorimeter, Kontron, Milan, Italy), using an automatic injector. The mobile phase was phosphate buffer 0.1 M, pH 6.2 containing methanol 28% v/v at a flow rate of 1 mL/min. The column temperature was maintained at 25 °C. The sensitivity of the assay was 10 nM.

2.6. Determination of dopamine concentration in the dialysate from the prelimbic medial prefrontal cortex, the VTA or the shell of the nucleus accumbens

The concentration of dopamine in the dialysate from the prelimbic medial prefrontal cortex, the VTA or the shell of the nucleus accumbens was measured in 20 μL of the same dialysate used for glutamic acid by HPLC on a 7.5 cm × 3.0 mm i.d., Supelcosil C18, 3 μm particle size column, (Supelco, Supelchem, Milan, Italy) coupled to electrochemical detection (Coulochem II, ESA, Cambridge, MA, USA) using a 4011 dual cell (Melis et al., 2003). Detection was performed in reduction mode with potentials set to +350 and −180 mV. The mobile phase was 0.06 M citrate/acetate pH 4.2, containing methanol 20% v/v, 0.1 mM EDTA, 1 μM trimethylamine, 0.03 mM sodium dodecyl sulphate at a the flow rate of 0.6 mL/min. The sensitivity of the assay was 0.125 pg.

2.7. Histology

At the end of the experiments the animals were killed by decapitation, the brains were immediately removed and stored in 2% aqueous formaldehyde for 12–15 days. Fifty μm transverse brain sections were then prepared by means of a freezing microtome, stained with Neutral Red and inspected on a phase contrast microscope. The position of the tip of the microinjection cannula in the ventral subiculum or the position of the tip of the microdialysis probe in the prelimbic medial prefrontal cortex, the VTA or the shell of the nucleus accumbens was localised by following the tract of both the microinjection cannula and of the microdialysis probe through a series of brain sections. Only those animals found to have the tip of the microinjection cannula and the tip of the microdialysis probe positioned correctly into the brain areas under study (see Results section) were considered for the statistical evaluation of the results.

2.8. Statistics

The area under the curves (AUC) obtained by plotting penile erection, dopamine and glutamate values vs. time in each animal was first calculated with the classical trapezoidal rule. The AUCs were then statistically compared between groups with one way ANOVA followed by Tukey's multiple comparison test in order to show significant differences between groups. A $P < 0.025$ was considered significant, in