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SHORT COMMUNICATION

Ketamine modulates catecholamine transmission in the bed nucleus of stria terminalis: The possible role of this region in the antidepressant effects of ketamine

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Abstract
Since the therapeutic treatment of depression is far from being satisfactory, new therapeutic strategies ought to be pursued. In addition, further investigation on brain areas involved in the action mechanism of antidepressants can shed light on the aetiology of depression. We have previously reported that typical and atypical antidepressants strongly stimulate catecholamine transmission in the bed nucleus of stria terminalis (BNST). In this study, we have built on that work to examine the effect of ketamine, an unusual antidepressant that can produce a fast-acting and long-lasting antidepressant effect after administration of a single sub-anaesthetic dose. Ketamine is an antagonist of the ionotropic N-methyl-D-aspartate (NMDA) receptor but can also act through its metabolite (2R-6R)-hydroxynorketamine. Using the microdialysis technique in freely moving rats, we monitored the acute effect of ketamine on catecholamine release in the BNST to gain clues to its prompt antidepressant effect. Male Sprague-Dawley rats were implanted with a microdialysis probe in the BNST and 48 h later, were injected with ketamine (10, 20, and 40 mg/kg, i.p.). Ketamine increased norepinephrine (127%, 155%, 186%) and dopamine (114%, 156%, 176%) extracellular concentration above basal in a time and dose dependent manner, without significantly modifying motility. Since the effect of ketamine, although lower, was not substantially different from that produced by classical antidepressants, we suggest that catecholamine increase in BNST is not likely to be related to a rapid ketamine antidepressant effect, though it might be related to its performance in predictive tests of antidepressant properties.

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1. Introduction

Depression is a common psychiatric disorder characterised by personal suffering and elevated societal cost (Pincus and Pettit, 2001). The aetiology of depression is uncertain but it is widely accepted that it can be triggered by the interaction of genetic and epigenetic factors (Feder et al., 2009). Among epigenetic factors, stress has a pivotal role because it can alter neurotransmitter release and neuronal circuitry in several brain areas (Caspi et al., 2003). In particular, noradrenergic transmission plays a major role in the physiological response to environmental challenges and stress (Itoi and Sagimoto, 2010). Among brain areas involved in stress control, the bed nucleus of stria terminalis (BNST) is one of the brain areas most innervated by norepinephrine neurons and has a general role in stress-induced hypothalamic-pituitary-adrenal (HPA) activation (Choi et al., 2007). In particular, the relationship between norepinephrine and corticotrophin releasing factor (CRF) neurons in the BNST suggests that the BNST is involved in the adaptive response to stress (Morilak et al., 2005), and possibly in the aetiology of depression (Jennings et al., 2013).

Depression therapy is mostly based on drugs that target monoaminergic neurotransmissions, but the slow onset of the clinical response and the significant number of non-responders, suggest that drugs with alternative mechanism of action ought to be investigated. In particular, the modulation of the ionotropic N-methyl-D-aspartate (NMDA) receptor is considered to be a promising target for the treatment of depression (Gerhard et al., 2016). This view is in agreement with the consideration that glutamate neurotransmission dysfunction may be a feature of stress-related mental illnesses (Musazzi et al., 2013). In addition, an altered glutamate function has been found in animal models of depression (Sanacora et al., 2012). Among NMDA acting drugs, the intravenous infusion of a single sub-anaesthetic dose of ketamine evoked a fast-acting and long-lasting antidepressant effect (Berman et al., 2000). Depressed patients, even those resistant to monoaminergic-acting antidepressants, reported alleviation of core symptoms within 2 h of a single low-dose infusion, with effects lasting up to 2 weeks (Zarate et al., 2006). Interest in the ketamine effect on brain neurotransmission has been greatly enhanced by a recent report that proposes an NMDA independent antidepressant action of ketamine metabolites (Zanos et al., 2016). We have previously reported that the systemic acute administration of various antidepressants, namely desipramine, reboxetine, imipramine, fluoxetine, citalopram and bupropion increased norepinephrine and dopamine extracellular concentration (output) in the BNST, suggesting that catecholamine transmission in the BNST may be part of a common downstream pathway that is involved in the mechanism of action of antidepressants (Cadeddu et al., 2014). Interestingly, it has been reported that the BNST is involved in the behavioural affective effects of systemic ketamine (Louderback et al., 2013).

Hence, considering the potential role of BNST in stress generated depression, and in the mechanism of action of classical antidepressants, as well as of ketamine, we believed it important to investigate the effect of acute administration of ketamine on catecholamine release in the BNST of freely moving rats through the microdialysis technique.

2. Experimental procedures

2.1. Animals

All animal experimentation was conducted in accordance with the guidelines for care and use of experimental animals set by the European Communities Council Directive and approved by the "Ethics Committee" at the University of Cagliari.

Male Sprague-Dawley rats weighing 250–300 g (Harlan, S. Pietro al Natisone, Italy) were group-housed under standard conditions of humidity (60%), temperature (22 °C) and artificial light (light, 8 a.m. to 8 p.m.). Food and water were available *ad libitum*.

2.2. Probes and surgery

Probes were house constructed (as previously described in Cadeddu et al. (2014)) using AN 69 dialysis fibre, (0.310 and 0.220 μm, outer and inner diameter, respectively), cut-off 40.000 Da (Hospal-Dasco, Bologna, Italy), active-membrane length=2 mm. On the day of the surgery rats were anaesthetised with 400 mg/Kg i.p. chloral hydrate and placed in stereotaxic apparatus (Kopf, Germany). A small hole was drilled on the side of the exposed skull and a microdialysis probe was implanted in the BNST (AP −0.4; L ±1.2; V −8.0) from dura mater (coordinates are in mm from Bregma), according to the atlas by Paxinos and Watson (2007). Probes were then fixed to the skull with dental cement (Shofu Cx-Plus, GmbH, Ratingen, Germany) and the skin sutured. The rats were individually housed in transparent plexiglass hemispheric cages, covered with a top hemisphere, with food and water made available.

2.3. Dialysis experiments

Experiments were performed on freely-moving animals 48 h after the probe implantation. On the day of the experiment, artificial cerebrospinal fluid (Ringer's solution, NaCl 147 mM, $CaCl_2$ 2.2 mM, KCl 4 mM, pH 6.5) was pumped through the dialysis probe at a constant rate of 1 μL/min via a microinjection pump. Dialyzed samples (20 μL) were collected every 20 min and immediately injected with no purification into a HPLC system equipped with reversed-phase column (C-18, 15 cm × 4.6 mm, 3.5 μm Supelco, Milan, Italy) and a coulometric detector (ESA Coulochem II, Bedford, MA, USA; oxidation +125 mV, reduction −175 mV). The mobile phase composition was 0.1 M sodium acetate, 0.3 mM Na2EDTA, 1.8 mM octanesulfonic acid, 120 ml/L methanol, and pH 5.4. The sensitivity of the assay allowed for the detection of 5 fmoles of norepinephrine and dopamine. When the basal output of norepinephrine and dopamine reached stable values (mean of three consecutive samples differing less than 10% from the mean of the previous three samples), rats (n=6 per each dose, n=5 for saline) were given a single acute i.p. injection of ketamine (10, 20, and 40 mg/kg) or saline. Stable levels were usually obtained after the first 2-3 h of dialysis. Basal values (as a mean±SE) of norepinephrine and dopamine were 36.25 (±5.2) and 21.89 (±3.8,) fmol/20 μl sample respectively (n=23).

2.4. Motility

For each experimental group, four rats were in turn exposed in a new environment for three 10 min periods, for time intervals of 15–45 min after drug administration. Total and locomotion activity was evaluated through an "Open Field" metre (Columbus Instruments, Columbus OH, USA). Each 10 min period activity and global exposition period (30 min) activity were statistically analysed (ANOVA) for differences in total and locomotion activity between groups in each period and between periods in each experimental group.

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