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Strain differences in the neurochemical response to chronic restraint stress in the rat: Relevance to depression

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ABSTRACT

The neurochemical basis of depression focuses on alterations in the monoaminergic and amino acid neurotransmitter systems. Moreover, decreases in serum levels of the neurotrophin brain-derived neurotrophic factor (BDNF) have led to the more recent neurotrophic hypothesis of depression. Chronic stress is one of the major predisposing factors to developing the disorder and thus we investigated the impact of chronic restraint stress on the levels of several neurotransmitters and their metabolites in a genetic animal model of depression, the Wistar Kyoto (WKY) rat. Behavioural analysis of WKY rats indicated both a depressive and anxiety-like phenotype compared to their Sprague Dawley (SD) controls. WKY animals showed similar stress-induced decreases in hippocampal GABA, noradrenaline and dopamine as their SD counterparts while exhibiting a divergent decrease in 5-HT, 5-HIAA and DOPAC. WKY rats also showed a stress-dependant increase in GABA concentrations in the amygdala compared to the SD animals. Moreover, WKY but not SD rats had a chronic stress-induced decrease in serum BDNF levels. Together these data show that there are specific strain-dependent changes in neurotransmitter and neurotrophin levels in response to chronic stress which may predispose WKY animals to a depressive-like phenotype.

1. Introduction

Life stress and genetic predisposition are considered to be key factors in the development of psychiatric illnesses including major depression (Charney and Manji, 2004). Stress may be defined as a real or interpreted threat to the physiological or psychological integrity of an individual that results in physiological and/or behavioural responses (McEwen, 2007). However, excessive stress induces abnormal changes in brain function and physiology that impair its ability to appropriately regulate physiological and behavioural responses to subsequent stressors. The mechanisms underlying this stress × genetic interaction in vulnerable populations still remain elusive. Animal studies have shown that chronic stress induces dysfunction at a multisystem level including morphological changes in the hippocampus and the amygdala (McEwen, 2005; McLaughlin et al., 2007; Vyas et al., 2002), alterations in a variety of neurotransmitters (Adell et al., 1988; Sunanda et al., 2000; Torres et al., 2002), changes in the expression of both hippocampal and serum brain-derived neurotrophic factor (BDNF) (Bergstrom et al., 2008; Smith et al., 1995; Vollmayr et al., 2001) , alterations in behaviour and reductions in body weight (Bravo et al., 2009).

In parallel, ongoing efforts using animal models and clinical neuroimaging aim to illuminate which brain structures are involved in depression. Current data implicate key limbic structures (e.g. prefrontal cortex, and hippocampus), the hypothalamus and the anterior temporal cortex in depression (Davidson et al., 2002; Singewald, 2007). From a neurotransmitter standpoint the monoamine theory of depression has long purported a specific role of serotonin (5-HT), noradrenaline (NA) and dopamine (DA) in clinical depression (Coppen, 1967; Cryan and Leonard, 2000; Nestler and Carlezon, 2006; Nutt, 2002; O'Leary and Cryan, 2010). However, increasing evidence also suggests a role for γ-aminobutyric acid (GABA) in the disorder (Cryan et al., 2005a; Sanacora et al., 2004; Slattery and Cryan, 2006).

A variety of clinical studies have shown that serum levels of the neurotrophin BDNF are decreased in patients with major depressive disorder (Kim et al., 2007, Sen et al., 2008; Shimizu et al., 2003) giving rise to a neurotrophin hypothesis of depression (Duman and Monteggia, 2006) (but see Groves, 2007) . These findings are bolstered by data from animal studies, with most but not all, demonstrating that central BDNF is downregulated by chronic stress (Bravo et al., 2009; Nibuya et al., 1999; Smith et al., 1995) and elevated by chronic antidepressant treatment (Nibuya et al., 1995; Shirayama et al., 2002).

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Genetically inbred rodent strains are one of the most useful tools in dissecting the genetic basis of complex stress-related disorders (Jacobson and Cryan, 2007; Ramos et al., 1997) .The Wistar Kyoto (WKY) rat is an inbred strain that has been proposed to be a genetic model of depression (Lopez-Rubalcava and Lucki, 2000; Will et al., 2003) compared to the outbred Sprague Dawley (SD) rat strain, commonly used as control (Malkesman and Weller, 2009). WKY rats have dysregulation of the hypothalamic pituitary adrenal (HPA) axis, (Rittenhouse et al., 2002; Solberg et al., 2001) and show marked differences in their behaviour in response to acute stress such as the open field (Drolet et al., 2002; Pardon et al., 2002) and the forced swim test (Lahmame et al., 1997b; Tejani-Butt et al., 2003; Will et al., 2003). The WKY also has an increased susceptibility to chronic stress-induced gastric ulceration (Paré and Redei, 1993).

In this study we sought to investigate if WKY rats respond differently to chronic restraint stress in terms of central neurotransmitter levels, plasma corticosterone and serum BDNF concentrations. We hypothesise that WKY animals will have an aberrant neurochemical response to stress that may predispose them to elevations in depression and anxiety-like behaviour.

2. Experimental procedures

2.1. Animals

Male Sprague Dawley (SD) and Wistar Kyoto (WKY) were acquired from Harlan Laboratories (Oxon, UK). All animals were given at least 1 week to habituate in the animal facility with food and water *ad libitum,* on a 12/12 h reversed dark–light cycle with temperature 22 ± 1 °C (lights on 7 am). All animals were group housed (5/6 per cage). Three separate cohorts of animals were used in this study. (I) Forced swim test (weight of group; 330–430 g), (ii) Open field group (weight of group; 283–401 g), (iii) neurotransmitters, body weight and serum BDNF in response to restraint stress (weight of group; 285–345 g). Animals from both strains were of similar weight at the time sacrifice in each cohort and daily body weight was noted. All experiments were conducted following institutional ethics guidelines and were in full accordance with the European Community Directive (86/609/EEC).

2.2. Restraint stress

Restraint is a well validated method to induce stress (Buynitsky and Mostofsky, 2009).The duration of stressor used is based on the work of Chattarji and colleagues (Mitra et al., 2005). Rats were exposed to 2 h restraint stress for 10 days, using a transparent plastic tube with air holes to increase ventilation. Rats were then immediately returned to their home cage. Control animals were left undisturbed in their home cage. The restraint procedure was performed between 8.00 am and 12.30 pm.

2.3. Corticosterone ELISA

Plasma corticosterone levels were measured using a Corticosterone Immunoassay Kit (Assay Designs, Ann Arbor, MI, USA). Briefly, plasma samples were diluted in assay buffer and incubated with a sheep polyclonal corticosterone antibody on a 96-well plate. Following washes, bound antigen was incubated with *p*-nitrophenyl phosphate substrate and alkaline phosphatase. Following colour development, the reaction was stopped using trisodium phosphate. Wells in the plate were read at 405 nm and the optical density was calculated. The concentration of each sample was extrapolated from a standard curve. Sensitivity of this assay is 26.99 pg/ml.

2.4. Detection of serum BDNF

Trunk blood was collected 2 h after last restraint stress into serum tubes, allowed to clot for 30 min and centrifuged at 1000 × *g* for 15 min. The serum was then removed and stored at −80 °C until analysis was performed. Serum BDNF concentrations was measured using a quantitative enzyme immunoassay technique (Quantikine, R&D Systems, Inc., USA). The assay was performed in duplicate according to the manufacturer's instructions. Briefly, standards and samples were pipetted into the wells of a microplate that was pre-coated with a monoclonal antibody specific for BDNF. Any BDNF present is bound by the immobilized antibody. An enzyme-linked monoclonal antibody (specific for BDNF) was then added to the wells. Any unbound antibody-enzyme reagent was removed by washing the 96-well plate and a substrate solution was then pipetted into wells, that allowing colour development to occur in proportion to the concentrations of BDNF which was bound in the initial step. Colour development was stopped after 30 min with the addition of the stop solution and the intensity of the colour was immediately measured by a microplate reader (Bio-tek instruments-Synergy HT), set for 450 nm with a correction at 540 nm. Four parameter logistic curve-fit was used to analysis the data using Gen 5 software.

2.5. Forced swim test

The modified forced swim test was conducted as previously described (Bissiere et al., 2006; Cryan et al., 2002, 2005a,b). The rats were placed individually in 21 cm × 46 cm Pyrex cylinders filled to a 30 cm depth with 21–25 °C water. Two swimming sessions were conducted: a 15 min pre-test followed by a 5 min test 24 h later. After 15 min on day 1, the rats were removed, towel dried and placed back in their home cage. The water in each cylinder was changed between animals. Twenty-four hours after their first exposure, rats were placed back in the swim apparatus for 5 min and a video camera positioned above the cylinders was used to monitor and record the animals for subsequent analysis. The total duration of predominant behaviour in each 5 s period of the 300 s test was scored on day 2. Swimming, immobility and active climbing were the main behaviours scored with swimming described as horizontal movements throughout all 4 quadrants of the cylinder, climbing was defined as vigorous fore-paw movements directed toward the walls of the apparatus and immobility defined as floating, with only enough movement necessary for the rat to keep its head above water (see Cryan et al., 2002 for pictorial representations). The experiments were carried out between 9.00 am and 12.30 pm and analysed by two observers blind to the experimental conditions.

2.6. Open field

The open field test was performed as previously described (O'Mahony et al., 2009). Briefly, the open field consisted of a circular white arena, 90 cm in diameter, 40 cm in height, 900 lux light. Testing was conducted between 9:00 a.m. and 1:00 p.m. At the beginning of each trial the rat was placed gently into the centre of the arena and allowed to explore the arena for 10 min. The behaviour of the animals was recorded by an overhanging camera that was attached to a personal computer. Ethovision 3.0 (Noldus, The Netherlands) was used to track the movement of the animal. The total distance moved in the arena, time spent and distance moved in the inner zone of the arena were recorded. The number of fecal pellets present in the arena at the end of the 10 min trial was also recorded. When rats are anxious they usually display freezing behaviour and stay close to the sides of the arena, which will result in a reduction in the amount of time spent in the brightly lit inner zone and distance travelled. An increased number of fecal pellets can also be indicative of the anxious state of the animal.