Increased 5-HT$_{2c}$ receptor responsiveness occurs on rearing rats in social isolation

Abstract

To investigate whether isolation rearing alters 5-hydroxytryptamine$_{2c}$ (5-HT$_{2c}$) receptors, the effect of the serotonin agonist m-chlorophenylpiperazine (mCPP) was examined on elevated plus-maze behaviour, plasma corticosterone and brain 5-HT$_{2c}$ receptor protein levels in rats. There was no distinction between behaviour or corticosterone levels in drug-free isolates or socially housed rats exposed to the elevated plus-maze. The anxiogenic response to mCPP (decrease in open arm entry and time and an increase in stretch attend postures) on the elevated plus-maze was greater in isolation than in socially reared controls without any concomitant difference in the hypolocomotor effect of mCPP in the two groups. mCPP produced a greater elevation in plasma corticosterone in isolates than in group-housed controls. Hippocampal 5-HT$_{2c}$ receptor protein-like immunoreactive levels were significantly lower following mCPP than saline only in rats reared in isolation. These results indicate that increased 5-HT$_{2c}$ receptor responsiveness accompanies isolation-rearing and may contribute to the enhanced response to stress and the increased neophobia seen in this animal model of trait anxiety/depression. In isolation reared rats, rapid down-regulation of supersensitive 5-HT$_{2c}$ receptors may occur in the hippocampus following 5-HT agonist challenge.

Key words
5-Hydroxytryptamine$_{2c}$ receptors • Social isolation • Elevated plus-maze • Anxiety • Corticosterone • Serotonin

Introduction

Rats reared in isolation from early post-weaning weeks show marked behavioural alteration from socially reared litter mates. These include hyperphagia (Einon et al. 1978), impaired spatial learning (Greenough et al. 1972; Juraska et al. 1984) and an increased response to reward (Jones et al. 1990). Isolation-reared rats also exhibit locomotor hyperactivity (Sahakian et al. 1975; Wright et al. 1991a) and a blunted corticosterone response (Gentsch et al. 1981) when placed in a novel environment, together with an anxiogenic profile on the elevated plus-maze (Parker and Morinan 1986). These behavioural changes are not mimicked by isolation of adult rats and, furthermore, the anxiogenic response is not reversed by resocialisation (Wright et al. 1991b), suggesting that they result from permanent developmental CNS alterations. Indeed, these characteristic changes have been proposed to be analogous to some of those occurring in several human mental disorders including schizophrenia, depression and anxiety (Parker and Morinan 1986; Jones et al. 1990), although it does not model any single specific disorder. Considerable effort has therefore been made to investigate the underlying neurochemical basis of the ‘isolation syndrome’.

Rats reared in isolation have altered catecholaminergic neurotransmission, including enhanced dopamine release (Jones et al. 1990) and presynaptic $\alpha_2$-adrenoceptor supersensitivity (Fulford et al. 1994). In addition, isolates have reduced presynaptic neuronal release of 5-HT in the frontal cortex (Bickerdike et al. 1993) and a compensatory post-synaptic supersensitivity to 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor agonists (Wright et al. 1991a). Such changes in serotonergic neuronal function could contribute to the anxiogenic profile of isolation-reared rats and may be pertinent to the aetiology of human trait anxiety. The current study further investigates alterations in serotonergic neurotransmission in isolation-reared
rats. Increasing evidence suggests that blockade of 5-HT$_{2C}$ receptors results in anxiolysis (Kennett 1992; Kennett et al. 1995); therefore, supersensitivity of this post-synaptic receptor subtype may contribute to the heightened response to stress observed in isolation-reared rats. To test this hypothesis, in the current study, changes in 5-HT$_{2C}$ receptor sensitivity in isolation-reared rats were examined behaviourally, in the ethologically based animal model of anxiety (the elevated plus-maze) and neurochemically by correlating with changes in brain 5-HT$_{2C}$ receptor protein levels using a receptor radioimmunoassay.

Materials and methods

Animals

Male Lister hooded rats were separated into two groups (n = 20 each) of equal weight at weaning (21 days postnatal) to be reared either socially (in groups of five in cages 54 x 38 x 20 cm) or in isolation (in an opaque plastic cage 34 x 28 x 20 cm high with a sawdust lining). Both groups were given food and water ad libitum, maintained on a 12-h light-dark cycle (lights on 0800 hours) and kept in the same room, so isolates had auditory, visual and olfactory contact with other rats. Behavioural studies commenced 5 weeks post-weaning when rats weighed 260 ± 54 g and were performed with approval of the UK Home Office under the Animal (Scientific Procedures) Act 1986.

Open field arena

The open field arena comprised a uniformly lit (200 LUX) cylindrical chamber (75 cm diameter with 45 cm matt black walls) from which behaviour was monitored in the inner (55 cm diameter) and outer zones using a video-camera and a computerised analysis system (VideoTrak, CPL Systems Ltd). Five weeks after weaning, rats were placed in the centre of the arena (0930–1130 hours) facing away from the experimenter and allowed to explore for 5 min. The arena was cleaned between trials with 5% (v/v) ethanol to remove odour cues.

Elevated plus maze

Six weeks post-weaning (between 0930 and 1230 hours) exploratory behaviour was monitored 30 min after injection of either l-(3-chlorophenyl) piperazine (mCPP, 1 or 2.5 mg/kg IP, n = 10 each) or saline (0.154 M, 1 ml/kg) on an elevated plus-maze (made of matt black plastic) comprising two opposite open (45 x 15 cm long) arms arranged perpendicularly to two enclosed (10 cm high walls) arms (raised to 65 cm at 200 LUX in the central square). Thirty minutes after drug injection, rats were placed in the centre of the maze facing a closed arm. The numbers of open and closed arm entries (all four feet out of the central square), head-dips, stretch attend postures and rears and the locomotor speed were determined using a computerised video-tracking system (VideoTrak CPL Systems) over 5 min (Beckett and Marsden 1995). The apparatus was cleaned between trials with 5% (v/v) ethanol to remove odour cues and a blind protocol with random drug allocation was used for all behavioural studies.

Biochemical analyses

Within 1 min of the end of the elevated plus-maze test, rats were decapitated and mixed arterio-venous blood collected in heparinised tubes which were centrifuged (1200 g, for 5 min at room temperature) and plasma corticosterone levels determined in duplicate using a commercial double antibody radioimmunoassay kit (Gammma-B-125I, Immunodiagnostic Systems Ltd).

Rat brains were rapidly isolated and divided into 2 mm coronal slices by a hand-held vibratome after brief immersion in liquid nitrogen, to enable dissection of frontal cortex, nucleus accumbens, striatum, septum, hypothalamus, hippocampus, amygdala and a 1 mm central cube from the midline of the midbrain-pons-medulla containing the brainstem raphe nuclei. Brain regions were frozen separately in liquid nitrogen and stored at −80 °C prior to determining 5-HT$_{2C}$ receptor protein-like immunoreactivity by radioimmunoassay.

To determine 5-HT$_{2C}$ receptor protein-like immunoreactivity, brain regions were homogenised (in 1 ml 50 mM TRIS-HCl containing 5% (v/v) aprotonin pH 7.4) separately and membrane preparations (following centrifugation 30 000 g for 30 min) assayed in triplicate from the linear portion of a radioimmunoassay curve (Sharma et al. 1994), as follows. Serial dilutions of the synthetic rat 5-HT$_{2C}$ receptor peptide (MVNLGNAVRSYC, amino acids 1–10 + YC, 10–4000 pg tube$^{-1}$) or tissue homogenates (100 µl) were incubated (4°C for 24 h) with a polyclonal sheep antiserum (30 µl, 1:5000) and [125I]$^{-}$5-HT$_{2C}$ peptide (50 µl). Free label was precipitated (2000 g for 30 min, 4°C Mistral 6000, Fisons) after incubation (room temperature, 30 min) with human plasma (50 µl) and charcoal (300 µl, 5 g l$^{-1}$ in 0.012 M phosphate buffer containing 0.154 M NaCl and 2.5 x 10$^{-5}$ M dextran) and counted for 180 s on an LKB gamma counter (Model 1272 Clinigamma). In order to determine absolute 5-HT$_{2C}$-like immunoreactive levels, the assumption is made that the extracted endogenous 5-HT$_{2C}$ receptor protein and the synthetic 5-HT$_{2C}$ receptor peptide bind to the polyclonal antiserum in a ratio of 1:1. Recovery of 5-HT$_{2C}$-LI from ventral spinal cord homogenates was 84 ± 6% (mean ± SE, n = 10). The intra-assay coefficient of variation measured in cortical homogenates (n = 13) was 7.5% and the inter-assay coefficient of variation as determined from ED$_{50}$ values (n = 8) was 14.9% (data not shown).

Drug sources and preparation

The only drug used was 1-((3-chlorophenyl) piperazine (mCPP), which was dissolved in 0.154 M saline. All injections were given IP at a volume of 1 ml/kg.

Statistical analysis

All data are represented as mean (± SE). Open field data not involving drug administration was analysed by Student’s unpaired t-test. Behavioural results from the elevated plus-maze were analysed by two-way analysis of variance (ANOVA) with post-hoc Duncan’s new multiple range test. Rears, head-dips and stretch attend postures, which have a skew (Poisson) distribution as a consequence of their low incidence were square-root normalised before parametric analysis was performed (Shepherd et al. 1994). Plasma corticosterone was analysed by one-way ANOVA followed by Fisher post-hoc test. In all statistical tests, P < 0.05 was considered as significant.

Results

Open field arena

Rats reared in isolation showed the expected marked alteration in behavioural response to a novel open field,