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AN INTACT DOPAMINERGIC SYSTEM IS REQUIRED FOR CONTEXT-CONDITIONED RELEASE OF 5-HT IN THE NUCLEUS ACCUMBENS OF POSTWEANING ISOLATION-REARED RATS

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Abstract—We investigated the effect of the tyrosine hydroxylase inhibitor, alpha-methyl-para-tyrosine (AMPT) on extracellular dopamine and 5-HT levels in the nucleus accumbens of group- and isolation-reared rats. Microdialysis with high-performance liquid chromatography–electrochemical detection was used to quantify dopamine and 5-HT efflux in the nucleus accumbens following foot shock and in association with a conditioned emotional response (CER). Isolation- and group-reared rats received i.p. injections of either saline (0.9%) or AMPT (200 mg/kg) 15 h and 2 h prior to sampling. There was no significant difference between saline-treated isolation- or group-reared rats for basal efflux of dopamine or 5-HT, however as expected, AMPT-treatment significantly reduced dopamine efflux in both groups to an equivalent level (50–55% saline-treated controls). Exposure to mild foot shock stimulated basal dopamine efflux in saline-treated groups only, although the effect was significantly greater in isolation-reared rats. In AMPT-treated rats, foot shock did not affect basal dopamine efflux in either rearing group. Foot shock evoked a prolonged increase in 5-HT efflux in both isolation- and group-reared saline-treated rats but had no effect on 5-HT efflux in AMPT-treated rats. In response to CER, isolation-rearing was associated with significantly greater efflux of both dopamine and 5-HT in saline-treated rats, compared to saline-treated, group-reared controls. However in AMPT-treated rats, efflux of dopamine or 5-HT did not change in response to CER. These data suggest that unconditioned or conditioned stress-induced changes in 5-HT release of the nucleus accumbens are dependent upon intact catecholaminergic neurotransmission. Furthermore, as the contribution of noradrenaline to catecholamine efflux in the nucleus accumbens is relatively minor compared to dopamine, our findings suggest that dopamine efflux in the nucleus accumbens is important for the local regulation of 5-HT release in this region. Finally, these findings implicate the isolation-enhanced presynaptic dopamine function in the accumbens with the augmented ventral striatal 5-HT neurotransmission characterized by isolation-reared rats.

Key words: isolation-rearing, AMPT, dopamine, 5-HT, conditioned fear, microdialysis.

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Abbreviations: AMPT, α-methyl-*p*-tyrosine; ANOVA, analysis of variance; CER, conditioned emotional response; HPLC, high performance liquid chromatography; Nacc, nucleus accumbens; TH, tyrosine hydroxylase; 6-OHDA, 6-hydroxydopamine.

Ongoing characterization of the neurochemical phenotype of isolation-reared rats has informed understanding of the isolation behavioral syndrome and the remarkable effect that the early social environment has on development of cortico-limbic function. Previous research has established that isolation-rearing in rats leads to substantial dysregulation in forebrain catecholaminergic systems. Autoregulation of hippocampal noradrenaline release is increased in isolation-reared rats reflecting presynaptic terminal α_2-autoreceptor supersensitivity (Fulford et al., 1994; Fulford and Marsden, 1997a,b). Postweaning isolation also alters presynaptic dopamine function in the mesolimbic system. Dopamine agonists, psychostimulant administration and acute stress exposure are all associated with enhanced extracellular dopamine efflux in the nucleus accumbens (NAcc) of isolation-reared rats *in vivo* (Jones et al., 1992; Fulford and Marsden, 1998b; Howes et al., 2000). Correlates between novelty-induced locomotor hyperactivity and amphetamine-induced hyperactivity in isolates and enhanced mesolimbic presynaptic dopamine release have been drawn (Jones et al., 1992). Isolation-rearing also disrupts firing characteristics of prefrontal cortical pyramidal neurons in response to stimulation of the ventral tegmental area *in vivo* (Peters and O'Donnell, 2005) and reduces dopamine turnover in the frontal cortex (Heidbreder et al., 2000; Jones et al., 1992). Both predicate aberrant mesocortical dopamine function following isolation that may be instrumental to disordered mechanisms of sensorimotor gating and the temporal organization of behaviors (Geyer et al., 1993; Weiss and Feldon, 2001; Cilia et al., 2005).

The neurobiological organization of behavior in rats is highly complex therefore it is not surprising that alterations in neurotransmitter release following isolation-rearing in rats encompass presynaptic 5-HT terminal release in limbic-corticostriatal circuits. Isolation-induced changes in presynaptic 5-HT neuronal function are regionally-specific, characterized by attenuated presynaptic function in the hippocampus (Bickerdike et al., 1993; Whitaker-Azmitia et al., 2000; Muchimapura et al., 2002) that is related to loss of 5-HT neuronal terminals and dendrites in Ammon's horn of hippocampus (Whitaker-Azmitia et al., 2000). Presynaptic 5-HT efflux is also reduced in frontal cortex (Bickerdike et al., 1993; Dalley et al., 2002) but is apparently upregulated in the NAcc (Fulford and Marsden, 1998a). Long-term adaptations to early social isolation from weaning on presynaptic 5-HT terminal function in limbic–corticostriatal pathways in the context of integration of monoaminergic inputs remains poorly understood. Both dopa-

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mine and 5-HT in the NAcc are associated with impulsive choice behavior (Winstanley et al., 2005) and imbalance between these monoamine neurotransmitters in NAcc and prefrontal cortex has been implicated in isolation-induced deficits in impulsivity (Dalley et al., 2002).

The NAcc represents a major integrating center receiving descending prefrontal cortical and limbic inputs (see Levita et al., 2002). Several studies have described the tight interrelationship between dopamine and 5-HT systems within mesolimbic and cortico-striatal circuits (Benloucif and Galloway, 1991; Parsons and Justice, 1993; Yadid et al., 1994; Mendlin et al., 1998; Balcioglu and Wurtman, 1998) and dopamine function in the dorsal striatum appears to be necessary for acute stress-induced changes in presynaptic 5-HT function in this region (Mendlin et al., 1999). Close contact between 5-HT terminals and dopamine neurons in the NAcc has also been reported (Van Bockstaele and Pickel, 1993). Our previous reports of isolation-induced increases in both extracellular dopamine and 5-HT levels in the NAcc following foot shock and conditioned emotional response (CER) (Fulford and Marsden, 1998a,b) highlight parallel stress-induced changes in presynaptic dopamine and 5-HT function following early isolation. Compensatory changes in 5-HT nerve terminal innervation and regulation of extracellular 5-HT levels in the striatum follow 6-hydroxydopamine (6-OHDA) lesioning of the adult central dopamine system in rats (Balcioglu et al., 2003) raise the possibility that serotonergic output is closely coupled to dopaminergic activity.

The aim of this study was to elucidate the functional association between presynaptic dopaminergic and serotonergic systems in the context of postweaning social isolation and response to fear conditioning using an *in vivo* pharmacological approach. We induced acute suppression of central dopaminergic content to assess the impact on basal and stress-induced changes in serotonergic neurotransmission in isolation-reared rats. To preserve integrity of dopaminergic nerve terminals we employed tyrosine hydroxylase (TH) inhibition, rather than neuronal lesioning, to assess functional regulation of extracellular 5-HT levels in the NAcc. Dopamine release from presynaptic dopaminergic terminals in the brain is sensitive to α-methyl-*p*-tyrosine (AMPT), an inhibitor of TH, the rate-limiting enzyme in catecholamine synthesis. AMPT-induced inhibition of TH, induces a significant reduction in total catecholamine levels when administered systemically (Uehara et al., 2004) and of presynaptic dopamine release when perfused locally into the dorsal striatum or NAcc of rats (Watanabe et al., 2005b). In the present study we pretreated isolation- and group-reared rats with AMPT prior to exposure to foot shock and a CER in order to assess the role of presynaptic dopamine function in the release of 5-HT in the NAcc.

EXPERIMENTAL PROCEDURES

Animals

Male, Lister hooded rats (Biomedical Services Unit, Nottingham University Medical School, Nottingham, UK) were obtained at weaning age (21–25 days postnatal) and divided into two rearing groups counterbalanced by weight. Rats were housed in plastic-bottomed, sawdust-lined cages either singly or in groups of five per cage for 6 weeks postweaning. All rats were housed in the same holding room on a standard 12-h light/dark photoperiod (lights on 07:00 h) in a temperature- (22±0.5 °C) and humidity- (40–60%) regulated room. Isolation-reared rats were able to see, hear and smell other rats in the holding room. Food and water were available *ad libitum*. Experiments were performed in accordance with the UK Home Office and Animal (Scientific Procedures) Act, 1986. Every effort was made to minimize the number of animals used and their suffering.

Microdialysis probe implantation

At 6 weeks postweaning rats were prepared for implantation of microdialysis probes. Concentric-style probes were prepared by hand using semi-permeable renal dialysis tubing (reconstituted cellulose) with a molecular weight cutoff point of 20,000 (220 μm o.d., 180 μm i.d.). Prior to surgery dialysis probes (1 mm active membrane length) were connected to slow infusion pumps via a liquid swivel system and continuously perfused with artificial cerebrospinal fluid (composition in mM: NaCl 125.0, $NaCO_3$ 27.0, KCl 2.5, $CaCl_2$ 1.0, $MgCl_2$ 1.0, Na_2HPO_4 1.2, NaH_2PO_4 0.5, Na_2SO_4 0.5, pH 7.4) at a flow rate of 1 μl/min.

Anesthesia was induced using halothane (4%) in a N_2O:O_2 mixture (1:2) and rats were positioned in a stereotaxic frame using atraumatic ear bars with the incisor bar set at −3.3 mm below the intra-aural line. Anesthesia was maintained during surgery with 2% halothane. The skull was exposed and a small bore hole drilled to allow vertical probe implantation targeted at the ventromedial portion of the anterior NAcc (coordinates: AP +1.6 mm, ML −0.8 mm relative to Bregma, DV −8.5 mm relative to dura according to the atlas of Paxinos and Watson (1986)). Microdialysis sampling was therefore largely confined to the region of the ventromedial NAcc shell with minor contribution of the medial NAcc core (see Fig. 1). Microdialysis probes were affixed to the skull using two watchmaker screws and dental cement. The open incision was sutured and sprayed with antibacterial powder and plastic wound dressing. Following surgery rats were returned to holding cages and allowed 16–18 h recovery prior to experimentation.

Dialysis samples were collected at 20 min intervals into ice-cold Eppendorf vials (0.5 ml) containing 5 μl perchloric acid (0.4%) and were either injected directly into the high performance liquid chromatography (HPLC) system or were snap frozen and retained at −70 °C until assay. Monoamine content was unaffected by freezing and frozen samples remained stable for up to1 month. At the completion of the experiment, rats were killed by anesthetic overdose and the dialysis probes perfused with Pontamine Sky Blue solution. The brains were then removed and fixed in 4% paraformaldehyde in 0.9% saline. Probe position was subject to verification by histological examination (see Fig. 1).

HPLC–electrochemical detection analysis of dialysates

Microdialysis samples were analyzed using HPLC–electrochemical detection systems dedicated to measurement of dopamine and 5-HT. Equipment involved a solvent delivery pump (2400 p.s.i. at 0.3 ml/min) used to circulate mobile phase (0.15 M Na_2HPO_4, 1.0 mM EDTA, 0.5 mM sodium octanylsulphonate, 8% methanol, pH 4.0). Samples were injected onto a column (100×2 mm i.d., Phenomenex) packed with 3 μm ODS C18 material (Hichrom), via a Rheodyne 7125 sample injector. Dopamine and 5-HT were measured electrochemically using a glassy carbon working electrode set at +0.65 V maintained by a Ag/AgCl reference electrode (Antec VT-03 cell). Changes in potential were