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BRAIN RESEARCH

Research Report

Normal [^{3}H]flunitrazepam binding to GABA$_A$ receptors in the locus coeruleus in major depression and suicide

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ABSTRACT

Major depression and suicide are associated with altered concentrations of specific noradrenergic proteins in the human locus coeruleus (LC). Based on experimental studies that can reproduce these LC abnormalities in laboratory animals, we hypothesized that noradrenergic pathobiology in depression is a result of overactivity of the LC. LC activity is under the control of both excitatory and inhibitory inputs. A major inhibitory input to the LC is GABAergic, arising from the nucleus prepositus hypoglossi. Numerous studies demonstrating low levels of GABA in the CSF and plasma of subjects with major depressive disorder (MDD) raise the possibility that LC overactivity in depression may be secondary to reduced GABAergic input to the LC. Here, GABAergic input to the LC in depression was evaluated by studying the binding of [^{3}H]flunitrazepam to GABA$_A$ receptors at three anatomically defined levels of the human postmortem LC. LC tissues were collected from subjects with MDD, subjects with depressive disorders including MDD that died as a result of suicide, and psychiatrically normal control subjects. A modest rostral–caudal gradient of GABA$_A$ receptor binding density was observed among all subjects. No significant differences in the amount of binding to GABA$_A$ receptors were observed between control subjects (n=21) and MDD subjects (n=9) or depressed suicide victims (n=17). These results demonstrate that GABA$_A$ receptor binding in the LC measured with [^{3}H]flunitrazepam is not altered in subjects with depressive illnesses.

1. Introduction

The locus coeruleus (LC) is the largest noradrenergic nucleus in the brain projecting extensively to a number of cortical and subcortical regions (Simpson and Lin, in press). Norepinephrine (NE) released from LC neurons plays a crucial role in the regulation of emotional activation, vigilance, sleep–wake cycle, and mood. Disruptions of noradrenergic transmission have long been believed to underlie certain psychiatric disorders, particularly depression. Converging evidence from studies of the LC from postmortem depressed subjects and from catecholamine depletion studies in living depressed subjects has provided compelling evidence that depression is associated with a deficit in noradrenergic

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transmission (Delgado and Coconcea, in press; Ordway, in press; Ordway et al., 2002).

Previous observations from postmortem studies have revealed that major depressive disorder (MDD) and suicide are associated with altered concentrations of several noradrenergic proteins in the LC. For example, elevated levels of tyrosine hydroxylase (Ordway et al., 1994a; Zhu et al., 1999), increased agonist binding to α_2-adrenergic receptors (Ordway et al., 2003, 1994b), and reduced levels of norepinephrine transporters (Klimek et al., 1997) have been reported in the LC from MDD subjects and/or from suicide victims. Studies utilizing laboratory animals provide interesting insights into the possible basis for these postmortem findings. For example, depletion of NE or repeated stress in rats increases tyrosine hydroxylase expression, increases binding to α_2-adrenergic receptors, and decreases binding to the norepinephrine transporter (Cubells et al., 1995; Lee et al., 1983; Melia et al., 1992; Torda et al., 1985; U'Prichard et al., 1979; Wang et al., 1998; Zafar et al., 1997). Together, these findings are highly suggestive of dysfunctional noradrenergic neurotransmission in depression, possibly through sustained stress-induced LC activation and the ultimate depletion of NE.

The possibility that there is elevated LC activity in depression brings attention to the possibility of depression-associated deficits in inputs to the LC from neurons that regulate LC activity. Recently, abnormalities in excitatory inputs (corticotropin releasing factor, glutamate) to the LC in depression and/or suicide have been reported (Austin et al., 2003; Bissette et al., 2003; Karolewicz et al., 2005; Merali et al., 2006). It is reasonable to speculate that elevated LC activity in depression may be simultaneously associated with diminished inhibitory input to the LC. GABAergic fibers, originating in the nucleus prepositus hypoglossi, project to the LC and inhibit LC firing (Aston-Jones et al., 1991). Receptors responding to GABA in the LC include $GABA_A$ receptors (Palacios et al., 1981; Zezula et al., 1988), which are ligand-gated chloride channels [for a review, see Barnard et al., 1998; Olsen et al., 1991]. Interestingly, there is a wealth of information suggesting that there is a deficit of inhibitory neurotransmitter GABA in depression (Brambilla et al., 2003; Lloyd et al., 1989; Merali et al., 2004; Sanacora et al., 1999, 2002, 2004; Tunnicliff and Malatynska, 2003).

Given the putative role of GABA in depressive disorders and GABA-modulation of the noradrenergic LC, the present study investigated the distribution of the $GABA_A$ receptors along the rostral–caudal axis of the human LC and potential abnormalities in the concentrations of $GABA_A$ binding sites in the LC that might occur in MDD and suicide. MDD and suicide subjects were matched with control subjects for age, sex, cigarette smoking history, and postmortem interval as closely as possible. Brain tissue was collected from carefully screened subjects (postmortem) who were diagnosed retrospectively with depressive disorders at the time of death and from control subjects who lacked major (Axis I) psychiatric disorders, except as indicated for nicotine dependence.

2. Results

A representative autoradiogram of the binding of [^{3}H]flunitrazepam to $GABA_A$ receptors in the middle LC from a control subject is shown in Fig. 1. The amount of specific binding of [^{3}H]flunitrazepam to $GABA_A$ receptors was measured in the immediate region of the compact neuromelanin-containing cell body region of the LC, shown by Nissl stains of same section. The specific binding of [^{3}H]flunitrazepam to $GABA_A$ receptors in the entire region of the LC was low relative to the density of binding in the cerebellum, as observed in the upper left corner of Fig. 1. Both the compact cellular region of the LC and the area of the dendritic expanse of the LC demonstrated relatively low [^{3}H]flunitrazepam binding levels. There was an uneven distribution of [^{3}H]flunitrazepam binding along the

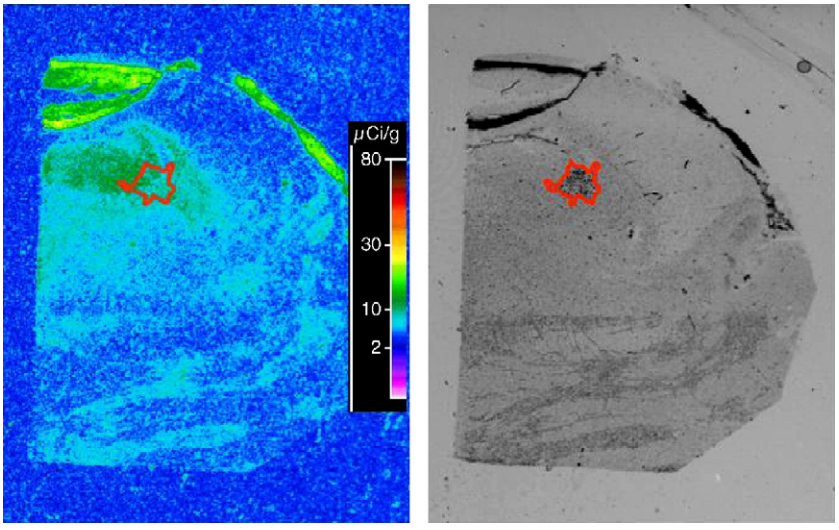

Fig. 1 – Digital images of an autoradiogram depicting the specific binding of [^{3}H]flunitrazepam to $GABA_A$ receptors (left panel) and the Nissl staining of same section (right panel) at the middle level of a representative control subject. The image of specific binding was generated by digitally subtracting the image of non-specific binding from that of total binding using autoradiograms generated from adjacent tissue sections. The drawing on image of the Nissl-stained section, overlaid onto the autoradiogram, depicts the region of the compact LC cell group that was measured.