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Immunohistochemical localisation of the NK_1 receptor in the human amygdala: Preliminary investigation in schizophrenia

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Abstract

The amygdala has a role in the modulation of moods and emotion, processes that are known to be affected in people with psychiatric disorders such as schizophrenia and depression. The tachykinin NK_1 receptor is known to be expressed in the amygdala. However to date, there is limited knowledge of the distribution of the NK_1 receptor in this region. This study used immunohistochemistry to analyse the distribution of the NK_1 receptor in fixed human amygdala tissue in control subjects with no history of psychiatric illness and matched subjects with a diagnosis of schizophrenia ($n=4$ pairs). The NK_1 receptor was observed sparsely distributed in cell bodies in all amygdaloid nuclei with the basolateral and lateral having a greater relative density of NK_1 receptor-immunoreactive cell bodies than the other nuclei. Double labelling with antibodies to microtubule associated protein and the NK_1 receptor revealed that the NK_1 receptor is expressed by large pyramidal, small stellate and large bipolar neurons. Interestingly, the basal nucleus of Meynert, which is just dorsal to the amygdala, was observed to have a significantly higher relative density of NK_1 receptor-immunoreactive cell bodies compared to any of the amygdaloid nuclei. Preliminary analysis of the density of NK_1 receptor-immunoreactive cell bodies in the major amygdaloid nuclei and the basal nucleus of Meynert revealed no significant differences between schizophrenia and control subjects. Real-time PCR showed that the mRNA for both the short and long isoforms of the NK_1 receptor was expressed at low levels in fresh frozen human amygdala tissue from control subjects and that this was not different in matched subjects with schizophrenia ($n=11$ pairs). In conclusion, this study has demonstrated that the NK_1 receptor is widely distributed in the amygdala, and has shown for the first time a high relative density of NK_1 receptor-immunoreactive cell bodies in the basal nucleus of Meynert.

Keywords: Basal nucleus of Meynert; Human amygdala; NK_1 receptor; Schizophrenia; Tachykinins

1. Introduction

The tachykinins are a family of neuropeptides. The three main tachykinins, substance P, neurokinin A and neurokinin B exert their effects through binding to the tachykinin receptors NK_1, NK_2 and NK_3 respectively but not exclusively (Wijkhuisen et al., 1999). These neuropeptides function in nociception, immunomodulation, inflammation, exocrine secretion, control of smooth muscle activity (Wijkhuisen et al., 1999) and synaptic transmission (Jobling et al., 2001). A fourth family of tachykinins, hemokinin-1 and the endokinins (Page, 2004), is produced in haematopoietic cells and is important for B cell development (Zhang et al., 2000).

Abbreviations: DAB, 3,3′-diaminobenzidine; MAP2, microtubule associated protein 2; NK_1-IR, NK_1 receptor immunoreactivity.

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Evidence from the literature suggests the tachykinins may play a role in the modulation of emotions such as anxiety and fear. Animal studies have shown modulation of behaviours equated to emotional states in guinea pigs by an NK_1 receptor antagonist (Kramer et al., 1998; Rupniak, 2002; Rupniak and Kramer, 1999). For example, an NK_1 receptor antagonist is efficacious in attenuating foot tapping, induced by foot shock, in gerbils (Ballard et al., 2001), whilst Teixeira et al. (1996) identified effects on anxiety of both NK_1 receptor agonists and antagonists in mice. Since the amygdala is involved in emotion and memory processing, in particular fear memory, this suggests that many of the effects of the NK_1 receptor in controlling emotion, anxiety and fear may be mediated by receptors localised to the amygdala. Interestingly, Rupniak et al. (2000) showed that injection of the NK_1 antagonist, GR73632, directly into the amygdala of guinea pigs attenuated the vocalisations of neonates on separation from their mother. Furthermore, double-blind placebo-controlled clinical trials of the tachykinin antagonist, MK-869, showed that this compound was efficacious in treating depression (Kramer et al., 1998). Although this finding has since been replicated (Rupniak, 2002) and an additional NK_1 receptor antagonist, L-759274, was also shown to be efficacious in treating major depression in a separate double blind placebo controlled study (Kramer et al., 2004), to date there are no NK_1 receptor antagonists in clinical use for depression. This is likely to be due to the lack of efficacy of the NK_1 receptor antagonist, aprepitant, in phase III clinical trials (Kramer et al., 2004; Keller et al., 2005). Animal studies have shown the amygdala (Kramer et al., 1998; Maubach et al., 2001), in particular the basolateral nucleus (Boyce et al., 2001), may be the site of action of the NK_1 receptor antagonist in treating depression. Depression has some signs and symptoms in common with schizophrenia including social withdrawal and flat affect. Indeed, dysfunction of emotion processing is also a major symptom of schizophrenia (Aleman and Kahn, 2005; Shayegan and Stahl, 2005) and alterations in the neural circuits of emotion, particularly in the amygdala have been identified in schizophrenia (Takahashi et al., 2004). Furthermore, two recent reviews have highlighted studies which implicate the amygdala in the production of these symptoms (Aleman and Kahn, 2005; Shayegan and Stahl, 2005).

Functional magnetic resonance imaging has identified an increased response to facial expressions from the amygdala of patients with schizophrenia (Kosaka et al., 2002), and structural magnetic resonance imaging studies have identified reductions in amygdala grey matter volume in schizophrenia (Hulshoff Pol et al., 2001; Wright et al., 1999). Immunohistochemical analysis of G protein isoforms in the amygdala has shown a correlation between decreases in specific isoforms in paranoid type schizophrenia compared to disorganised type, although there was no difference in the expression of G protein isoforms between control and schizophrenia groups (Yang et al., 1998).

There is evidence for the existence of a C-terminally truncated form of the NK_1 receptor (Fong et al., 1992). The level of mRNA of these two NK_1 isoforms was assessed in different human brain regions by Caberlotto et al. (2003) using *in situ* hybridisation and real-time quantitative PCR analysis. The long isoform of the NK_1 receptor was found to be more abundant than the short isoform in most brain regions, whereas the short isoform was more prevalent in peripheral tissues. Moderate levels of NK_1 mRNA expression were detected in the various amygdaloid nuclei, with similar levels of expression observed in the lateral, basal and accessory basal nuclei (Caberlotto et al., 2003). Immunohistochemical analysis of the human basolateral amygdala by Maubach et al. (2001) using an antibody to the C-terminus, which would thus detect the long isoform of the NK_1 receptor, identified expression of the NK_1 receptor on pyramidal cells and also smaller neurones with characteristics similar to GABAergic interneurons. However the distribution of the NK_1 receptor in other amygdala nuclei was not reported.

The distribution of the NK_1 receptor was previously studied in the human prefrontal cortex (Tooney et al., 2000) and a follow-up study observed increased relative density of NK_1 receptor immunoreactivity in subjects with schizophrenia (Tooney et al., 2001). In this study, the distribution of the NK_1 receptor in the human amygdala was determined using immunohistochemistry with an NK_1 receptor selective polyclonal antibody directed against the N-terminus that would detect the long and short forms of the NK_1 receptor. Both the distribution and relative density of these receptors have been assessed in post-mortem amygdala tissue from non-psychiatric controls and subjects with schizophrenia. We predict that given our previous studies in the prefrontal cortex observed an increased relative density of NK_1 receptor immunoreactivity (Tooney et al., 2001) and that NK_1 receptor antagonists appear to act in the amygdala in depressed states, there would be increased density of NK_1 receptor cell bodies in the amygdala from subjects with schizophrenia.

2. Methods

2.1. Case characteristics

Post-mortem amygdala tissue was obtained from the NSW Tissue Resource Centre (The University of Sydney). Consent was obtained from the next of kin and a diagnosis of schizophrenia according to the DSM-IV criteria was confirmed by medical file review using the Item Group Checklist of the Schedules for Clinical Assessment in Neuropsychiatry. All subjects were male Caucasians, cases were excluded if they had a significant history of drug or alcohol abuse, a diagnosis of another neurological disorder, a medical illness that may influence agonal state, any abnormality on neuropathological examination, head injury, or where the post-mortem interval (PMI) exceeded 48 h. Only non-psychiatric controls who had not committed suicide were included.

Immunohistochemical analysis was performed on formalin fixed tissue from four subjects with schizophrenia that were matched for age, gender and PMI to four non-psychiatric control subjects (Table 1). Real-time PCR was performed on fresh frozen amygdala tissue from 11 pairs of schizophrenia and control subjects (Table 2) which were matched for PMI, tissue storage time at −80 °C, pH, age and gender. This latter cohort of cases were used in a recent study using DNA microarray analysis to assess global gene expression changes in the amygdala in schizophrenia (Weidenhofer et al., 2006).