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Effects of olanzapine and quetiapine on corticotropin-releasing hormone release in the rat brain

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ABSTRACT

An altered regulation of the corticotropin-releasing hormone (CRH) system in the CNS is consistently associated with anxiety and depression; several drugs used to treat CNS disorders modulate – usually in a negative manner – CRH turnover in the brain, and it can be postulated that their effectiveness may be at least in part related to their effects on CRH. This study was aimed to investigate the effects of two atypical antipsychotics also employed in the treatment of bipolar disorders, i.e. quetiapine (QTP) and olanzapine (OLZ), on CRH release from isolated rat brain regions. Acute rat hypothalamic and hippocampal explants were exposed for 1 h to plain medium or medium containing the test drugs, either under baseline conditions or after stimulation of CRH release by veratridine or 56 mM KCl. CRH immunoreactivity present in the incubation medium and in the tissues was assessed by radioimmunoassay. QTP 10 μM but not OLZ inhibited baseline CRH secretion from the hypothalamus; neither drug affected basal CRH release from the hippocampus. Both QTP and OLZ, 1 and 10 μM, inhibited veratridine- or K^+-stimulated CRH release from the hypothalamus, whereas OLZ only, when given at 10 μM, was able to inhibit stimulated CRH release from the hippocampus. In conclusion, two widely used atypical antipsychotics, QTP and OLZ are able to acutely reduce the release of CRH from isolated rat hypothalami and hippocampi.

1. Introduction

The corticotropin-releasing hormone (CRH) system currently includes two receptor sub-families, CRH-R1 and R2, four ligands, CRH and the CRH related peptides urocortin I, II and III, as well as the CRH-binding protein. A large body of evidence has accumulated in the last years indicating that an altered functioning of CRH system may be critical in the development of affective disorders, including anxiety, depression and stress-related pathologies (Müller and Wurst, 2004; Belmaker and Agam, 2008). As a corollary to the main theory, it was suggested that the therapeutic effects of antidepressive agents might be due to reduction of central CRH production and release (Holsboer and Barden, 1996); our research group contributed to develop this concept by showing that different drugs used in mood disorders, including mirtazapine, valproic acid and lamotrigine, share the ability to reduce – although via different mechanisms – the production and release of CRH from rat hypothalamic explants (Tringali et al., 2004; Fabricio et al., 2005; Tringali et al., 2006). A concrete clinical outcome of these research efforts is represented by the fact that two phase II/III studies to test the efficacy of pexacerfont, a selective non-peptidic CRH-R1 receptor antagonist, in the treatment of major depressive disorders and generalized anxiety disorders, respectively, have now been completed; other compounds of the same class are in earlier phases on clinical development.

Most of the evidence obtained in animal models of anxiety and depression indicate that a derangement of central CRH system may be common to both disorders, raising the question as to whether anxiety and depression are different presentations of the same pathology (Müller and Wurst, 2004), which would be at variance with currently accepted nosography. While such dichotomy made necessary to test the clinical efficacy of CRH antagonists in both disorders, pre-clinical data with the CRH-R1 selective agonist cortagine showed that the stimulation of central CRH-R1 receptors is anxiogenic in the rat, as expected, but it also mimics the effects of antidepressive drugs in animal models of depression (Tezval et al., 2004).

The above picture suggests that a need still exists to broaden our knowledge on the links between the biology of CRH and the pathophysiology of human affective disorders. Within this framework, in the present study we investigated the effects of olanzapine (OLZ) and quetiapine (QTP) on the release of CRH from two rat brain areas, namely hypothalamus and hippocampus. Originally developed and

Abbreviations: CRH, Corticotrophin-releasing hormone; OLZ, Olanzapine; QTP, Quetiapine; RIA, Radioimmunoassay; Vera, Veratridine.

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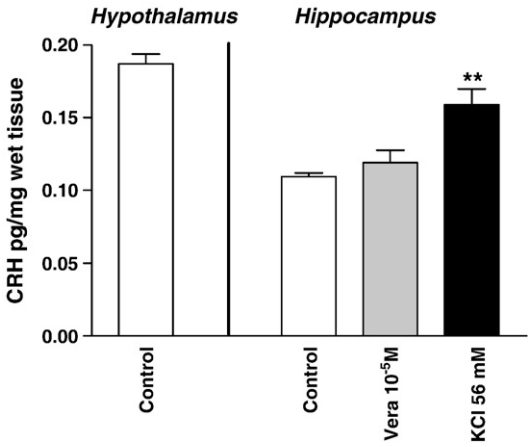

Fig. 1. Basal and stimulated CRH release from the rat hippocampus. Left panel: representative experiments showing the average amount of CRH released from isolated hypothalami in 1-h static incubations. Right panel: levels of CRH released after 1-h incubations with plain medium (control) or medium containing 10 μM veratridine or 56 mM KCl. Data are expressed as pg CRH/mg of wet tissue, the means ± SEM of 12 replicates per group. **$p<0.01$ vs control hippocampi.

marketed as atypical antipsychotics, OLZ and QTP were recently shown to be effective in the treatment of depressive episodes occurring in bipolar patients (Philip et al., 2008). Thus, OLZ and QTP share with previously-studied valproic acid and lamotrigine (Tringali et al., 2004, 2006) the same rationale for investigation in the above mentioned experimental paradigm.

While the hypothalamus is a well-established model to study the effects of drugs and other agents on CRH release, in this work for the first time we carried out static incubations of hippocampi, i.e. a rat brain area with the highest concentrations of CRH-binding sites and immunoreactive nerve terminals (De Souza, 1995); the experiments were conducted in parallel on hypothalami and hippocampi taken from the same animals. To this purpose, a preliminary characterization of CRH release from the rat hippocampus was also carried out.

2. Methods

2.1. Drugs

Olanzapine and quetiapine were kindly provided to J-M Aubry by Eli Lilly and Astra Zeneca, respectively; both drugs were dissolved in DMSO and further diluted to working concentrations in incubation medium or 56 mM KCl medium when appropriate. Veratridine, which elicits neurotransmitter release by opening both Na^+ and Ca^{2+} channels (Lingamaneni and Hemmings, 1999), was purchased from Sigma Chemicals Co. (St. Louis, MO); the drug was dissolved in absolute ethanol and further diluted to working concentrations in incubation medium or 56 mM KCl medium when appropriate. DMSO and ethanol (diluted 1/1000 at least) did not interfere with CRH release in the experimental setting, nor with CRH radioimmunoassay.

2.2. Experimental procedure

The entire preparative procedure has been previously described in detail (Tringali et al., 2004, 2006; Fabricio et al., 2005). Male Wistar rats (200–250 g) were decapitated between 09.00 and 10.00 a.m. and the brains rapidly removed. Hypothalami were dissected with the following limits: the posterior border of the optic chiasm, the anterior border of the mamillary bodies and the lateral hypothalamic sulci, with a depth of approximately 2 mm (Navarra et al., 1991). Hippocampi were dissected after removal of the posterior and temporal neocortex, with the following limits: dorsal portion (lying just behind the septum), posterior portion (where the hippocampus begins to bend ventrally and laterally) and ventral portion (lying in the temporal part of the brain). The tissues were then divided longitudinally in two halves to aid diffusion of medium. The total dissection time was less than 3 min from decapitation.

The hypothalami and hippocampi were incubated in a 24-well plate (one hypothalamus or one hippocampus per well), at 37 °C in a humidified atmosphere consisting of 5% CO_2 and 95% O_2 in a 300-μl incubation medium, Minimum Essential Medium (MEM) with Earle's salts, supplemented with 0.2% bovine serum albumin, 60 μg/ml ascorbic acid and 20 IU/ml aprotinin, pH 7.4. In this experimental model, explanted brain tissues remain viable and functional during the timeframe of the experiments, as assessed by the LDH assay for

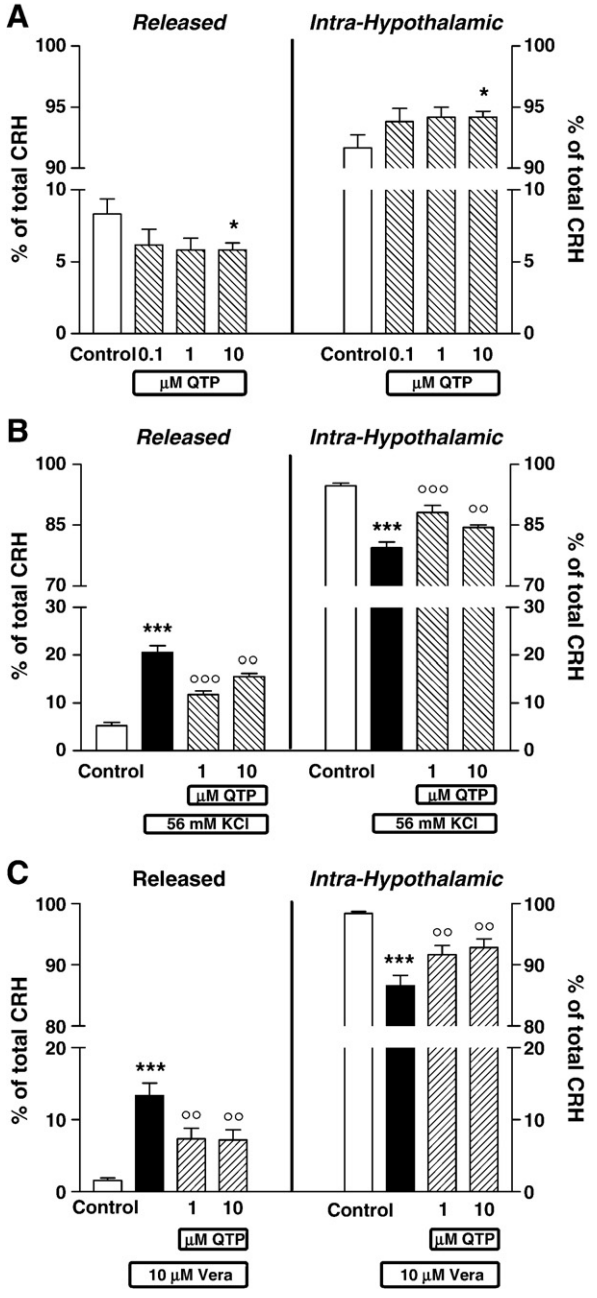

Fig. 2. Effects of QTP on CRH release from the rat hypothalamus under basal conditions (A) or after stimulation by 56 mM KCl (B) or veratridine (C). Results are from 2 (A) or 3 (A and B) independent experiments, according to a randomized block design. Total CRH content for each hypothalamus was calculated as the sum of the intra-hypothalamic peptide and the released fraction; subsequently, the released and intra-hypothalamic fractions were expressed as percentage of total CRH content. Data are the means ± SEM of 8 (A) or 12 (A and B) replicates per group. * and ***: $p<0.05$ and $p<0.001$ vs control; °° and °°°: $p<0.01$ and $p<0.001$ vs the secretagogue given alone.