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.

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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 (Holboer 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 pexacertone, 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.
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 Ca²⁺ 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% CO₂ and 95% O₂ 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. 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.](image1)

![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.](image2)
cellular toxicity (Pozzoli et al., 2001). Thus, variations in CRH release did not appear to be correlated with toxic damage of the tissues.

After a 60-min preincubation period (during which the medium was changed every 20 min), the medium was aspirated and replaced with fresh medium alone (control), or medium containing graded concentrations of test drugs. In experiments with KCl, the tissues were treated with medium alone (control), 56 mM KCl alone or 56 mM KCl in the presence of graded concentrations of test drugs; whenever KCl was used, MEM was replaced by a medium consisting of 56 mM KCl and 67 mM NaCl, with the same concentration of the other ions as found in MEM. Tissues were exposed to plain medium or 56 mM KCl, either alone or added with test substances, for 1 h.

At the end of experiments, incubation media were collected and stored at −35 °C until assay for CRH immunoreactivity. In order to measure intra-hypothalamic or intra-hippocampal CRH, the tissues were snap-frozen and kept at −80 °C until homogenization. The latter was performed as follows: the wet tissues were weighted immediately after thawing and were placed in ice-cold Tris–HCl 50 mM, pH 7.4, containing 40 IU/ml aprotinin and 0.2% bovine serum albumin, at a fixed ratio of 100 mg wet tissue/1 ml Tris. Tissues were homogenized using a Teflon glass homogenizer for 30–45 s at 4 °C; the tubes were then centrifuged for 45 min at 20,000 rpm and 4 °C. Supernatants were collected and 100-µl aliquots were assayed by radioimmunoassay as described below.

### 2.3. CRH radioimmunoassay

CRH was measured by radioimmunoassay (RIA) as previously described (Navarra et al., 1991), with the following modifications: a CRH antiserum which specifically recognizes the mature CRH form (kindly donated by Prof. R. Bernardini) and (2-[125I]-iodohystidyl32) CRF were used. The detection limit of the assay was 1 pg/tube (100-µl sample volume for incubation media), with intra- and inter-assay coefficients of variation of 5% and 10% respectively.

### 2.4. Statistical analysis

Each experiment was repeated two or three times, according to a randomized block design. Data were analyzed by two-way ANOVA for the factors time and treatment; since no significant difference was found between different experiments conducted with the same study design, integrated pooled analysis of the data was justified, and all results were presented as the mean ± SEM of n replicates per experimental group. Subsequent to ANOVA, post-hoc test Newman–Keul’s for comparison between group means, or Student’s t-test was carried out when appropriate. All descriptive statistics and statistical testing were performed with a Prism™ computer program (GraphPad, San Diego, CA, USA); differences were considered significant if \( p < 0.05 \).

### 3. Results

In preliminary experiments, we measured CRH release from isolated hippocampi and hypothalamai taken from the same animals. On a wet weight basis, hippocampal release about 50% of the amount of CRH released from the relative hypothalamai under the same experimental conditions (Fig. 1). After incubation in the presence of two well-established CRH secretagogues, 10 µM veratridine and media containing 56 mM KCl, CRH secretion was significantly increased after KCl stimulation only (Fig. 1); such secretory profile is partially at

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

**Fig. 4.** Effects of OLA on CRH release from the rat hypothalamus (A) and hippocampus (B) under basal conditions. Results are from 3 independent experiments, according to a randomized block design. Total CRH content for each brain area was calculated as the sum of the intra-tissue peptide and the released fraction; subsequently, the released and intra-tissue fractions were expressed as percentage of total CRH content. Data are the means ± SEM of 15 replicates per group.
In 1-h experiments under basal conditions, QTP reduced CRH release from hypothalamic explants, with statistical significance reached at 10 µM (Fig. 2A, left panel); in parallel, the drug increased CRH contents within the tissue (Fig. 2A, right panel). In experiments with KCl, hypothalami were treated with medium alone (control), 56 mM KCl alone or 56 mM KCl in the presence of 1–10 µM QTP. One-hour exposure to KCl significantly increased CRH release, and in parallel significantly reduced CRH intra-hypothalamic content (Fig. 2B). In this paradigm, 1 and 10 µM QTP significantly inhibited the stimulatory action of KCl on CRH release (Fig. 2B, left panel), as well as KCl-induced reduction of intra-hypothalamic peptide levels (Fig. 2B, right panel). Another series of experiments was conducted using the Na+–channel opener veratridine, and very similar results were obtained (Fig. 2C). In parallel experiments conducted on hippocampi, QTP failed to modify CRH release, both under basal conditions (Fig. 3A) and after stimulation with the only secretagogue effective in this paradigm, i.e. 56 mM KCl (Fig. 3B).

Olanzapine elicited no significant effect on CRH release under basal conditions, as assessed through simultaneous evaluations of released and intra-tissue CRH levels, both in the hypothalamus (Fig. 4A) and the hippocampus (Fig. 4B). However, 1 and 10 µM OLZ significantly inhibited the stimulatory action of K+ on CRH release (Fig. 5A, left panel), as well as KCl-induced reduction of intra-hypothalamic peptide levels (Fig. 5A, right panel), and the same results were obtained with veratridine stimulation (Fig. 5B). At variance with QTP, OLZ was also able to significantly counteract the stimulatory action of KCl on the release of CRH from the hippocampus (Fig. 5C, left panel), while at the same time antagonizing KCl-induced reduction of intra-hippocampal peptide levels (Fig. 5C, right panel). However, in this paradigm OLZ proved to be less potent compared to the hypothalamus, since it attained significant effects at the higher concentration (10 µM) only (Fig. 5C).

4. Discussion

In this study we found that isolated rat hippocampi release sizable amounts of immunoreactive CRH. On average, after 1 h of incubation each hippocampus releases about one half of the CRH released by the hypothalamus from the same animal. However, if one looks at the fraction of CRH released over the total CRH content (i.e. released + intra-tissue), the fraction of CRH released from the hippocampus is on average 2- to 3-fold higher compared to the fraction of total CRH released from the hypothalamus. At variance with the hypothalamus, the hippocampus is not sensitive to stimulation of CRH release by veratridine, which activates both Na+ and Ca2+ channel to elicit neurotransmitter release (Lingamani and Hemmings, 1999). Early studies showed that the hippocampus is among the rat brain areas with the highest concentration of CRH-binding sites, which corresponds well with the high density of CRH-immunoreactive nerve terminals in that region (De Souza, 1995). More recently, Chen et al. (2004) showed that releasable pools of CRH in the pyramidal cell layer of the hippocampus are associated to axon terminals of short GABAergic interneurons, and that stress conditions activate such neuronal population. The iontophoretic application of CRH in the cortex and hippocampus increases the spontaneous firing of CRH-ergic neurons, whereas the spontaneous activity of these neurons in the paraventricular nucleus of the hypothalamus is depressed after superfusion with CRH in vitro (De Souza, 1995). Thus, differences exist between hypothalamus and hippocampus in the sensitivity of the CRH releasable pool to the iontophoretic application of CRH; we also found a clear-cut difference between hypothalamus and hippocampus in response to veratridine stimulation, suggesting that the releasable pool of CRH in the hippocampus is only sensitive to a mechanism requiring Ca2+, but not Na+, channel activation to induce peptide release.

Olanzapine and QTP display a broadly similar spectrum of drug-receptor interactions; both drugs bind primarily to D2 and 5-HT2A receptors, with OLZ being about 1–2 orders of magnitude more potent than QTP on both binding sites (Arnt and Skarsfeldt, 1998; Baldessarini and Tarazi, 2006). Such similarity justifies the fact that the indications of the two drugs in clinical practice are largely overlapping. OLZ and QTP also showed a similar profile of actions in the present experimental paradigm. Under baseline secretory conditions, QTP at the highest concentration – but not OLZ – inhibited CRH release from the hypothalamus, whereas both drugs failed to modify CRH release.
from the hippocampus. On this regard, it should be pointed out that the effects of test drugs on baseline CRH release are less relevant than those observed on stimulated CRH release, since the latter represents a closer in vitro correlate to any condition of the CRH-system hyperactivation occurring in vivo in association to anxiety and/or depression. Under stimulated CRH release, both QTP and OLZ share the ability to counteract the increase in CRH secretion elicited at hypothalamic level by both secretagogues, high K+ and veratridine. Such pattern of activity differs from those of other CNS drugs previously tested by us in this paradigm: lamotrigine (which inhibits veratridine- but not K+-induced CRH release) and mirtazapine (which, on the contrary, inhibits K+- but not veratridine-induced CRH release) (Tringali et al., 2006; Fabricio et al., 2005). The inhibitory effects of QTP and OLZ on both Na+- and Ca2+-driven secretory mechanisms are seemingly attributed to the known antagonism of dopamine and 5-HT receptors. While the role of 5-HT in the control of hypothalamic CRH release has been extensively studied in basic research, the specific contribution of dopamine receptors has been scarcely investigated (Costa et al., 2001; Kasckow et al., 2003); however, extensive clinical experience with antipsychotic agents – including selective D2 antagonists – demonstrated that these drugs reduce stress-induced activation of CRH secretion (Baldessarini and Tarazi, 2006). The present findings also nicely fit with the observation by Park et al. (2007) that chronic treatments with QTP abolish stress-induced increase in CRH mRNA expression in the rat hypothalamus in vivo.

Based on the above considerations, a similar inhibitory effect of QTP and OLZ on K+-stimulated CRH release from the hippocampus was expected. It was therefore surprising to see that QTP had no effect in this paradigm whereas OLZ had inhibitory activity, although it was less potent by about 1 order of magnitude compared to the hypothalamus. A possible explanation for these diverging effects lays on the above discussed differences in CRH secretory patterns between hypothalamus and hippocampus. Moreover, the density of the 2 main target receptors, D2 and 5-HT2A, might be lower in the hippocampus compared to the hypothalamus, and an effect may emerge for OLZ – but not QTP – because of higher affinity of the former for these receptors.

5. Conclusions

In conclusion, here we found that QTP and OLZ significantly inhibit stimulated CRH release from the rat hypothalamus in vitro, and that OLZ shows such property on the isolated rat hippocampus as well. It remains to be established whether such activity is also present in humans and contributes to the clinical efficacy of these drugs.

References