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Olanzapine-induced Fos expression in the rat forebrain; cross-tolerance with haloperidol and clozapine

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Abstract

Acute administration of the atypical antipsychotic drug olanzapine (5 mg kg^{-1} i.p.) increased the number of Fos-positive cells moderately in the prefrontal cortex and the striatum; more pronounced were the effects in the nucleus accumbens, the lateral septum, the hypothalamic paraventricular nucleus and the amygdala. The acutely-induced Fos responses of olanzapine were significantly reduced in all brain areas investigated after a 3-week treatment period, indicating the development of tolerance. Through evaluation of cross-tolerance we investigated whether the effects of olanzapine, haloperidol and clozapine on Fos expression and on plasma corticosterone are mediated by the same or by different mechanisms. Cross-tolerance between olanzapine and either haloperidol or clozapine was assessed by the administration of a challenge dose of olanzapine to rats, that were pretreated for 3 weeks with either the same drug, with saline (1 ml kg^{-1} day^{-1}), haloperidol (1 mg kg^{-1} day^{-1}) or clozapine (20 mg kg^{-1} day^{-1}). A competitive dose of olanzapine in long-term haloperidol-treated rats showed cross-tolerance in the rostral part of the cingulate cortex, the dorsomedial and the dorsolateral striatum, the nucleus accumbens and the lateral septum. Cross-tolerance between olanzapine and clozapine, however, was limited to limbic nuclei, including the prefrontal cortex, the lateral septum, the hypothalamic paraventricular nucleus and the amygdala, with minor effects in the mid- and caudal parts of the cingulate cortex. Interesting are the common effects in the lateral septum, possibly an important target for antipsychotic efficacy. Olanzapine administration induced elevated levels of plasma corticosterone and cross-tolerance was seen in haloperidol- and clozapine-pretreated rats.

Keywords: Olanzapine; Haloperidol; Clozapine; Fos protein; Tolerance; Cross-tolerance

1. Introduction

Olanzapine is considered as an atypical antipsychotic, which pharmacologic profile resembles that of clozapine (Bymaster et al., 1996, 1997). Antipsychotic drugs have been shown to induce regionally-specific patterns of the protein product of the immediate early gene c-*fos* in the rat forebrain that can differentiate between typical and atypical compounds (Robertson et al., 1994). Acute administration of the typical antipsychotic drug haloperidol, that produces extrapyramidal side-effects, increases the number of Fos-positive cells in the dorsolateral striatum, where both clozapine and olanzapine had little, if any, effect (Casey, 1997; Dragunow et al., 1990; Deutch et al., 1992; Nguyen et al., 1992; Fink-Jensen and Kristensen, 1994; MacGibbon et al., 1994; Merchant et al., 1994; Robertson et al., 1994; Robertson and Fibiger, 1996; Sebens et al., 1995). Olanzapine and clozapine differ from typical antipsychotics as they target both, positive and negative symptoms of schizophrenia (Beasley et al., 1997). These drugs produce elevated levels of Fos-protein in the prefrontal cortex (Deutch and Duman, 1996; Robertson et al., 1994; Robertson and Fibiger, 1996), that may correlate with their effect on negative symptoms (Robertson and Fibiger, 1992). All antipsychotics known to date can increase Fos in limbic structures, such as the nucleus accumbens and the lateral septum, possibly but not necessarily mediated by common mechanisms of action (Dragunow et al., 1990; Fink-Jensen and Kristensen, 1994; MacGibbon et al., 1994; Robertson et al., 1994; Robertson and Fibiger, 1992, 1996; Sebens et al., 1995). After long-term haloperidol or clozapine treatment the acutely-induced Fos responses were attenuated in most brain areas, thus indicating the development of tolerance (Sebens et al., 1995, 1996).

The observation of attenuated Fos expression allows to assess whether antipsychotics of various classes act through

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the same or different mechanisms by cross-tolerance designs. In rats that develop tolerance after long-term olanzapine treatment, cross-tolerance with either haloperidol or clozapine will occur when the observed Fos response of an olanzapine challenge in long-term haloperidol- or clozapine-pretreated animals is different from the response to olanzapine in saline treated rats and equal to the effect of a single dose of olanzapine after long-term olanzapine pretreatment.

The main issue of the present study was to investigate in vivo whether the actions of olanzapine, haloperidol and clozapine were mediated by common or by different systems, probably receptors, in the rat forebrain. Rats were treated with either saline, olanzapine, haloperidol or clozapine for 3 weeks and finally received a challenge dose of olanzapine, 24 h after the last injection of the long-term treatment period. The numbers of Fos-positive cells were quantified in the following rat forebrain regions: the medial prefrontal cortex; the rostral, the mid- and the caudal part of the cingulate cortex; the dorsomedial, the dorsolateral and the ventrolateral striatum; the mid- and caudal regions of the nucleus accumbens; the lateral septum; the paraventricular nucleus of the hypothalamus and the central amygdala.

In this study, we determined also whether evidence for cross-tolerance between antipsychotics exists in enhancements of corticosterone release.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 200–220 g at the start of the experiment were housed individually in a 12-h light/dark cycle environment with free access to food and water during the treatment period. Experiments were performed during the light period.

2.2. Design of the study

Four groups of animals were treated once daily for 21 days with either saline (1 ml kg^{-1}), olanzapine (5 mg kg^{-1}), haloperidol (1 mg kg^{-1}) or clozapine (20 mg kg^{-1}) by intraperitoneal injection.

To study the effects of acute treatment, one group of animals received saline for 21 days, followed by a single dose of olanzapine (Sal + Olz). The 21-day saline-pretreatment was given to avoid stress-induced Fos responses of handling and injections. For the long-term experiment olanzapine was administered for 22 days (Olz + Olz). Cross-tolerance was determined by injecting two groups of rats with either haloperidol or clozapine for 21 days, followed by a single dose of the competitive drug olanzapine, (Hal + Olz) and (Cloz + Olz), respectively. All groups consisted of six to seven animals. Two hours after the last injection the animals were perfused transcardially under pentobarbital anesthesia. Before the perfusion fixation was started a blood sample was taken from the left ventricle to determine plasma corticosterone levels. The blood samples were transferred to a centrifuge tube containing 10 μl heparin solution (500 U ml^{-1}) and centrifuged for 15 min at $500 \times g$. The plasma was separated from the blood cells and stored at −20°C for the corticosterone assay. All experimental procedures were approved by the Committee on Animal Bio-ethics of the University of Groningen.

2.3. Drugs

Olanzapine, generously supplied by Eli Lilly, Indianapolis, USA, and clozapine, a gift from Sandoz Basel (Switzerland), were dissolved in slightly acidified saline. Haloperidol (Janssen Pharmaceutica, Beerse, Belgium) for intravenous use was obtained commercially and diluted with saline. The solutions were adjusted to pH 5.5 with NaOH. Neither saline nor clozapine solutions were

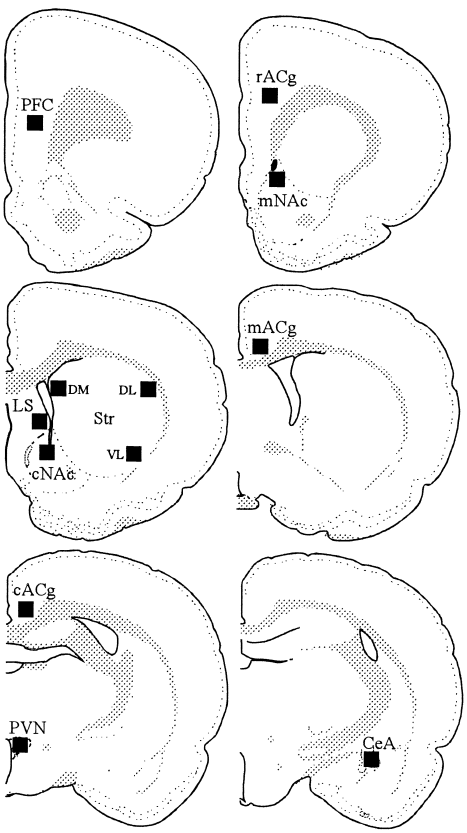

Fig. 1. Schematic representations of the different levels used for the counting of Fos-positive cells. Black squares indicate the counted regions. PFC, medial prefrontal cortex; rACg, rostral cingulate cortex; mACg, mid cingulate cortex; cACg, caudal cingulate cortex; DM, dorsomedial striatum; DL, dorsolateral striatum; VL, ventrolateral striatum; mNAc, mid nucleus accumbens; cNAc, caudal nucleus accumbens; LS, lateral septum; PVN, paraventricular nucleus of the hypothalamus; CeA, central amygdala.

buffered; the injection of the solutions did not produce apparent discomfort.

2.4. Corticosterone assay

Corticosterone was extracted from 75 μl plasma using a liquid extraction method. Quantification of corticosterone was performed with high-performance liquid chromatography (HPLC) in combination with ultraviolet detection (Dawson et al., 1984). The absolute detection threshold for corticosterone in plasma was 8 ng ml^{-1}.

2.5. Immunohistochemistry

Animals were perfused under deep anesthesia (pentobarbital 100 mg kg^{-1} i.p.) with saline for 1 min followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer pH 7.4 for 15 min. Brains were removed and postfixed overnight at 4°C in 4% paraformaldehyde solution before being stored in 50 mM Tris-buffered saline pH 7.4 containing 0.1% Na-azide. Brains were cryoprotected by overnight storage in 30% sucrose in 50 mM Tris/HCl buffer pH 7.4 at room temperature and sliced to 30 μm coronal sections using a cryostat microtome. The immunohistochemical procedure was performed on free floating sections, according to the previously described procedure (Sebens et al., 1995, 1996). Briefly, sections were pretreated with 0.3% H_2O_2 to block endogenous peroxidase activity, preincubated for 4 h at room temperature in 4% normal rabbit serum (Sigma, St. Louis, MO, USA) to decrease background staining. Subsequently, the Fos primary antiserum (Cambridge Research Biochemicals, CRB, OA-11-824, UK) was added, diluted (1:2000) in Tris-buffered saline, containing 2% bovine serum albumin (BDH Laboratory Supplies, Poole, UK), 2% normal rabbit serum and 0.5% Triton X-100 (Baker Grade). A biotinylated anti-sheep secondary antibody, diluted (1:800, Pierce Chemical, Rockford, IL, USA) was used, followed by an avidin–biotinylated horseradish peroxidase complex (1:125, Vector Laboratories, Burlingame, CA, USA). The peroxidase reaction was developed with DAB-nickel/H_2O_2. To control for the specificity of immunoreactivity, some of the sections were incubated with omission of the primary or the secondary antibody.

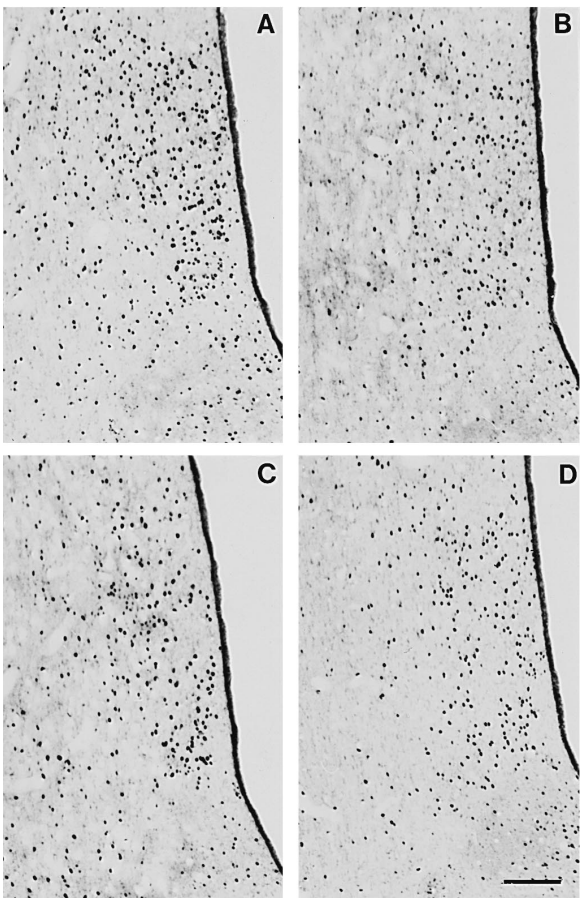

Fig. 2. Photomicrographs illustrating the distribution of Fos-positive nuclei in response to olanzapine treatment, at the level of the lateral septum. (A) acute response (Sal + Olz); (B) after long-term treatment (Olz + Olz); (C and D) after an olanzapine challenge in, respectively, long-term haloperidol (Hal + Olz) or clozapine (Cloz + Olz) treated rats. Scale bar: 100 μm.

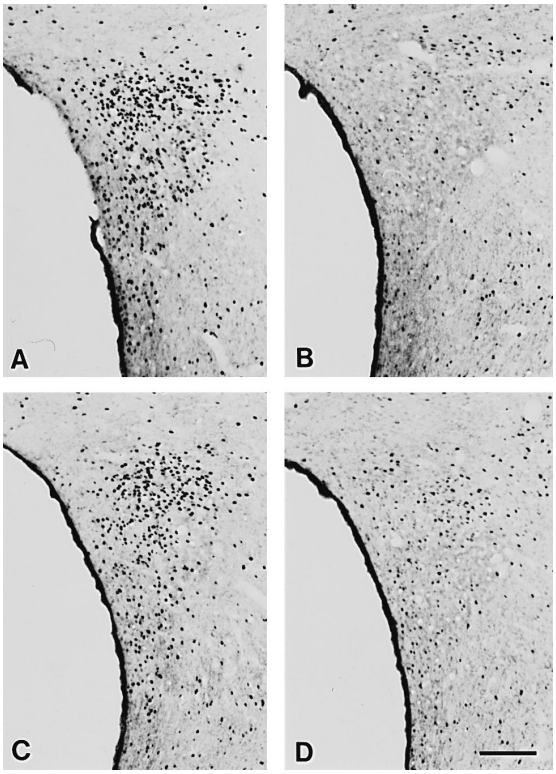

Fig. 3. Photomicrographs illustrating the Fos response to olanzapine administration in the paraventricular nucleus of the hypothalamus. (A) after a single dose of olanzapine (Sal + Olz); (B) after long-term treatment (Olz + Olz); (C and D) after a challenge dose of olanzapine following a 3-week pretreatment with haloperidol or clozapine, respectively. Scale bar: 100 μm.

2.6. Quantification and statistical analysis

Schematic drawings of the representative sections used for counting Fos-positive cells are shown in Fig. 1. The counted areas are indicated by black squares. Fos-immunoreactive cells were counted within a 400×400 μm grid at a magnification of $125\times$ in the medial prefrontal cortex, the rostral, the mid- and the caudal part of the cingulate cortex, the dorsomedial, the dorsolateral and the ventrolateral striatum, the mid- and caudal nucleus accumbens, the lateral septum, the hypothalamic paraventricular nucleus and the central nucleus of the amygdala. Fos-positive cells were counted bilaterally and averaged per animal. The mean number (±S.E.M.) of Fos-positive cells per experimental group was determined. The data of the differently treated groups were compared using a one-way analysis of variance (ANOVA), followed by the Dunnett's test for multiple comparison procedures. The similarities and differences of the regional effects on Fos expression between olanzapine, haloperidol and clozapine were deter-

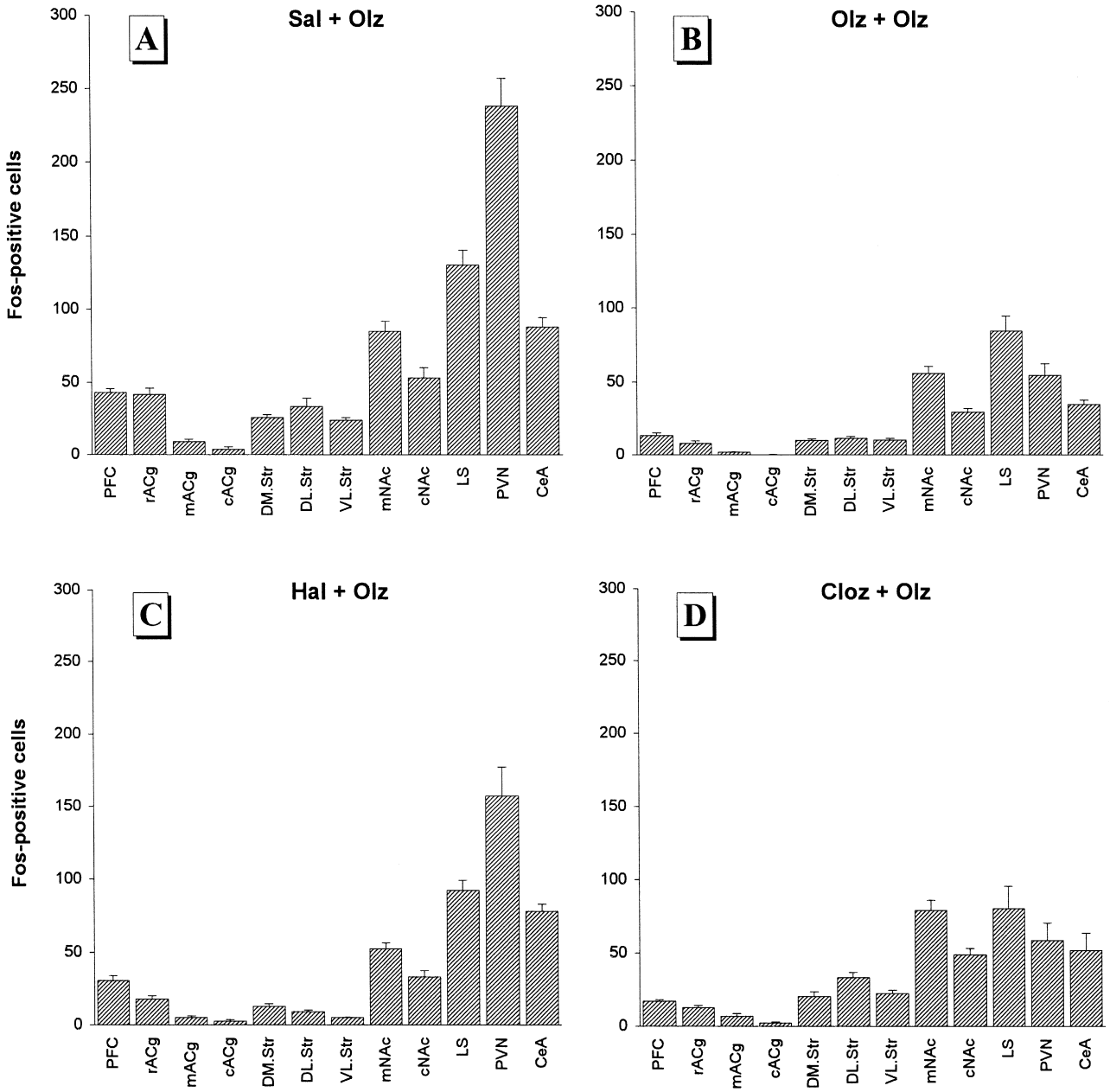

Fig. 4. Histogram showing the mean number (±S.E.M.) of Fos-positive cells after (A) acute (Sal + Olz) treatment and after a challenge dose of olanzapine in long-term (B) olanzapine- (Olz + Olz), (C) haloperidol- (Hal + Olz) or (D) clozapine- (Cloz + Olz) pretreated rats. Brain areas include: PFC, medial prefrontal cortex; rACg, rostral cingulate cortex; mACg, mid cingulate cortex; cACg, caudal cingulate cortex; DM.Str, dorsomedial striatum; DL.Str, dorsolateral striatum; VL.Str, ventrolateral striatum; mNAc, mid nucleus accumbens; cNAc, caudal nucleus accumbens; LS, lateral septum; PVN, paraventricular nucleus of the hypothalamus; CeA, central amygdala. For statistical significance of the differences, see Table 1.

mined. Accordingly, Pearson correlation and regression analyses were assessed of the relationship between the data, obtained from the present and from our previous study. The differences in number of Fos-positive cells and the correlation between differently treated groups were considered significant if $P < 0.05$.

3. Results

3.1. Acutely olanzapine-induced Fos-positive cells

A single dose of olanzapine induced a pattern of Fos expression that partially overlaps the Fos distribution observed after a single dose of haloperidol and clozapine. The medial part of the prefrontal cortex and the rostral cingulate cortex exhibited moderate increases in the number of Fos-positive cells, but along the rostro-caudal extent of the cingulate cortex an almost complete decline in Fos expression was observed (Fig. 4A). Olanzapine also affected the dorsomedial, the dorsolateral and the ventrolateral striatum, areas where haloperidol, but not clozapine, is active too (Fig. 4A and Fig. 5A,B). Notable were the olanzapine-induced increases in Fos-protein in limbic areas, like the nucleus accumbens, the lateral septum and the central amygdala, whereas the effect in the paraventricular nucleus of the hypothalamus was conspicuous (Fig. 2A, Fig. 3A and Fig. 4A). Particularly in limbic areas the similarity of the Fos responses of olanzapine and clozapine became obvious as there was a significant correlation in the various regions (Fig. 5A). Such a significant correlation was not seen when the numbers of Fos-positive nuclei induced by either olanzapine and haloperidol or clozapine and haloperidol were compared (Fig. 5B,C).

3.2. Long-term treatment

After a 3-week treatment with olanzapine, tolerance was developed in all brain areas investigated. Repeated olanzapine-treatment decreased the acutely-induced number of Fos-positive cells by 69% in the medial prefrontal cortex and by 81% in the cingulate cortex. Large reductions were also found in the striatal regions (61%), in the paraventricular nucleus of the hypothalamus (77%) and in the amygdala (60%). Less pronounced was the decreasing effect of long-term olanzapine-treatment in the nucleus accumbens where the decrease was 39% and in the lateral septum with a 35% reduction of Fos immunoreactivity (Fig. 2B, Fig. 3B and Fig. 4B). In Table 1, the significant differences are given. Striking was the resemblance between the percentages of decline in limbic regions, such as the nucleus accumbens, the lateral septum, the hypothalamic paraventricular nucleus and the amygdala of rats that received a single dose of olanzapine or clozapine, respectively, and long-term treated animals; for clozapine data see our previous report (Sebens et al., 1996). These data support the idea of overlapping working profiles of the two antipsychotics in limbic nuclei, in contrast to haloperidol. Chronic haloperidol administration did not develop tolerance towards Fos in these limbic nuclei, apart from the nucleus accumbens, where the decrease in Fos expression after a 3-week haloperidol administration was even more pronounced (Sebens et al., 1996).

3.3. Cross-tolerance

The number of Fos-positive cells produced by a challenge dose of olanzapine in haloperidol-pretreated rats and that induced by a single injection of olanzapine were different in the prefrontal and the rostral part of the

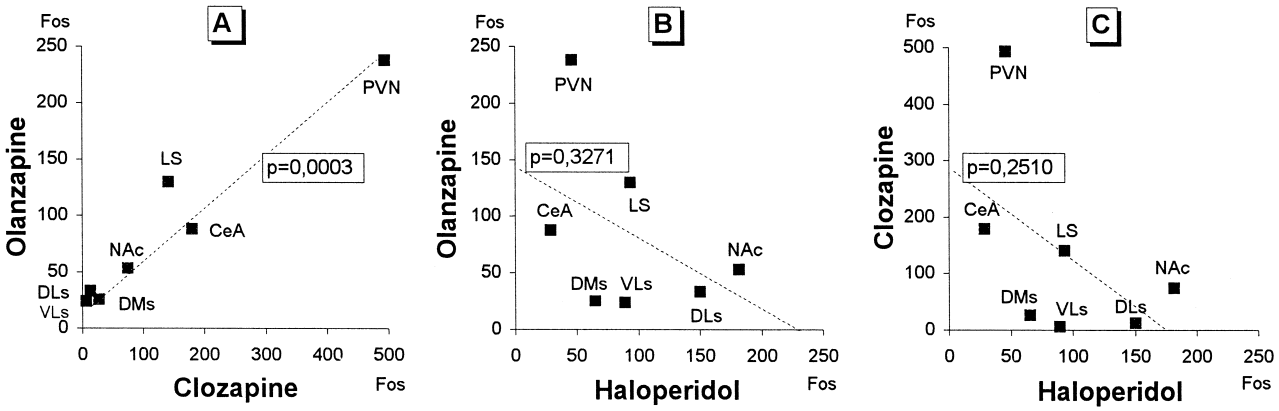

Fig. 5. Correlation between the number of Fos-positive cells (indicated as Fos) after acute: (A) olanzapine vs. clozapine administration, (B) olanzapine vs. haloperidol treatment and (C) clozapine vs. haloperidol injection. Data of olanzapine were applied from this study, whereas the data of haloperidol and clozapine originated from our previous study (Sebens et al., 1996). Linear regression analyses illustrate the correlation between the data and were considered significant if $P < 0.05$. Brain regions include: DMs, dorsomedial striatum; DLs, dorsolateral striatum; VLs, ventrolateral striatum; NAc, caudal nucleus accumbens; LS, lateral septum; PVN, paraventricular nucleus of the hypothalamus; CeA, central amygdala.

Table 1
Statistical significance of the differences in number of Fos-positive cells between different experimental groups

	PFC	rACg	mACg	cACg	DM.S	DL.S	VL.S	mNAc	cNAc	LS	PVN	CeA
Olz+Olz / Sal+Olz	<	<	<	<	<	<	<	<	<	<	<	<
Hal+Olz / Sal+Olz	<	<	NS	NS	<	<	<	<	<	<	<	NS
Hal+Olz / Olz+Olz	>	NS	NS	NS	NS	NS	<	NS	NS	NS	>	>
Cloz+Olz / Sal+Olz	<	<	<	<	NS	NS	NS	NS	NS	<	<	<
Cloz+Olz / Olz+Olz	NS	>	NS	NS	>	>	>	>	>	NS	NS	NS
Hal+Olz / Cloz+Olz	>	NS	NS	NS	NS	<	<	<	<	NS	>	>

Groups are defined as: (Sal + Olz) acute and (Olz + Olz) long-term olanzapine-treated, (Hal + Olz) olanzapine challenged long-term haloperidol-treated and (Cloz + Olz) olanzapine-challenged long-term clozapine-treated rats.
Brain regions include: PFC, medial prefrontal cortex; rACg, cingulate cortex, rostral part; mACg, mid cingulate cortex; cACg, cingulate cortex, caudal part; DM.S, dorsomedial striatum; DL.S, dorsolateral striatum; VL.S, ventrolateral striatum; mNAc, mid nucleus accumbens; cNAc, nucleus accumbens, caudal part; LS, lateral septum; PVN, paraventricular nucleus of the hypothalamus; CeA, central amygdala.
First group/second group: ⟨or⟩ indicates the first group is significantly smaller or larger than the second group at $P < 0.05$.
NS, not significant.
□ indicates cross-tolerance between the mentioned drug treatments.

cingulate cortex, the dorsomedial, dorsolateral and ventrolateral striatum, the nucleus accumbens, the lateral septum and the hypothalamic paraventricular nucleus. The Fos distributions observed after an olanzapine challenge in long-term haloperidol- and olanzapine-treated animals were not significantly different in the cingulate cortex, the dorsomedial and the dorsolateral striatum, the nucleus accumbens and the lateral septum (Fig. 2A,B,C, Fig. 3A,B,C, Fig. 4A,B,C, and Table 1). Apparently, cross-tolerance between olanzapine and haloperidol was limited to the rostral cingulate cortex, the dorsomedial and dorsolateral striatum, the nucleus accumbens and the lateral septum. Both agents seem to have their actions mediated by common (receptor) sites, in striatal as well as in some limbic regions of the rat forebrain.

A challenge of olanzapine after either long-term clozapine- or olanzapine-pretreatment induced virtually the same Fos response in the prefrontal cortex, the lateral septum, the hypothalamic paraventricular nucleus and the amygdala, while the olanzapine-induced number of Fos-positive cells in clozapine- and saline-pretreated rats differed in the prefrontal and the cingulate cortex, the lateral septum, the hypothalamic paraventricular nucleus and the amygdala (Fig. 2A,B,D, Fig. 3A,B,D, Fig. 4A,B,D and Table 1). Cross-tolerance between clozapine and olanzapine towards Fos expression appeared only in limbic areas, such as the prefrontal cortex, the lateral septum, the hypothalamic paraventricular nucleus and the amygdala; in the mid- and caudal part of the cingulate cortex the effects were small but significant.

Taken together, pretreatment with either haloperidol or clozapine leads to significantly differential patterns of Fos expression after a final dose of olanzapine in most brain areas, except in the lateral septum, where cross-tolerance between olanzapine, haloperidol and clozapine was seen. These observations may emphasize the importance of the lateral septum for the therapeutic effects of antipsychotics.

3.4. Plasma corticosterone levels

In rats that received a single dose of olanzapine the levels of corticosterone became more than six times higher, when compared to controls. Long-term treatment with olanzapine reduced the acutely elevated levels of cortico-

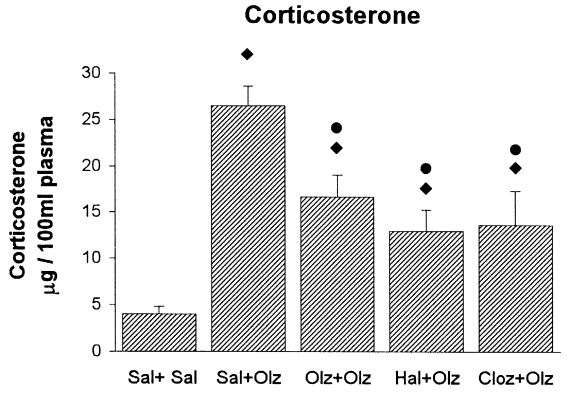

Fig. 6. Plasma corticosterone levels (mean ± S.E.M. of six to seven rats) of controls (Sal + Sal) and rats treated acutely with olanzapine (Sal + Olz) and after a challenge dose of olanzapine in long-term olanzapine- (Olz + Olz), haloperidol- (Hal + Olz) or clozapine- (Cloz + Olz) pretreated rats. All drug treatments resulted in significant elevations of plasma corticosterone levels (◆); (●) indicates statistically significant different from acute olanzapine treatment ($P < 0.05$).

sterone by 43%, which confirms the development of tolerance towards corticosterone release. No significant differences could be observed between the olanzapine-, haloperidol- and clozapine-pretreated groups after a last injection with olanzapine (Fig. 6).

These results suggest that common mechanisms are responsible for the antipsychotic-induced increment of plasma corticosterone levels.

4. Discussion

The pattern of Fos-protein distribution in the rat forebrain seen after acute administration of olanzapine resembles that of clozapine, apart from the striatal regions, where the small Fos-responses have more conformity with the effect of haloperidol. The Fos results of acute olanzapine-treatment in the striatum, the nucleus accumbens and the prefrontal cortex are in agreement with the report of Robertson and Fibiger (1996); in the present study we enlarged the number of investigated brain regions. Long-term olanzapine-treatment attenuated the acutely-induced Fos effect in all brain areas examined, a condition necessary for the assessment of cross-tolerance. Cross-tolerance between olanzapine and clozapine was limited to limbic areas, while olanzapine and haloperidol shared common sites of action in both striatal and limbic nuclei. Interesting are the Fos effects of either antipsychotic agent in the lateral septum, indicating a significant contribution of this nucleus in, at least, some of the antipsychotic actions. Olanzapine elevated the levels of plasma corticosterone and showed also mutual cross-tolerance.

The acute Fos effects of olanzapine in the striatum and the nucleus accumbens confirm the atypical profile of this drug. The striatal Fos response to olanzapine is presumably a result of a direct action on dopamine D_2 receptors. Haloperidol-induced Fos expression matches the dopamine D_2 receptor distribution in this area and both have their highest density in the dorsolateral part of the striatum (Boyson et al., 1986), a region involved in the regulation of movement (McGeorge and Faull, 1989). Moreover, there was cross-tolerance between olanzapine and haloperidol. Antipsychotic-induced Fos expression in this region may therefore predict the risk of extrapyramidal side-effects (Deutch et al., 1992; Robertson et al., 1994; Robertson and Fibiger, 1996). Recent observations indicate that at clinical doses olanzapine, like clozapine, produces hardly any of the motor side-effects (Coward et al., 1989; Fulton and Goa, 1997; Moore et al., 1997). Even though both agents elicit low levels of striatal Fos, olanzapine and clozapine block a substantial number of dopamine D_2 receptors in vivo (Farde et al., 1992; Pilowsky et al., 1996; Nyberg et al., 1997). The discrepancy in Fos response and dopamine receptor occupancy may be attributed to their, though different, potential to block muscarinic acetylcholine receptors in vivo, whereas the affinity of haloperidol for this receptor type is considerably lower (Bolden et al., 1991; Bymaster et al., 1997).

The ability of all antipsychotics examined to date, including olanzapine, to increase the number of Fos containing cells in the nucleus accumbens and the lateral septum, emphasizes the importance of these limbic nuclei for the antipsychotic actions (Deutch et al., 1992; Robertson et al., 1994).

In the nucleus accumbens olanzapine and haloperidol seem to have their actions mediated by identical mechanisms, but cross-tolerance did not occur between clozapine and haloperidol (Sebens et al., 1996) nor between clozapine and olanzapine (this study) in this area. These findings suppose a particular mechanism of action of clozapine in the nucleus accumbens and it is tempting to suggest that this nucleus has a crucial role in the clinical effects of clozapine in therapy-resistant patients. Olanzapine and haloperidol also have common targets in the dorsomedial part of the striatum. The coincidence of similar working mechanisms in both the nucleus accumbens and the dorsomedial striatum is not surprising, since these regions have many of their neuronal connections in common, such as the afferent projections from the amygdala, the association cortices and the dopaminergic neurons of the ventral tegmental (A10) area (Beckstead et al., 1979; Carter and Fibiger, 1977; McGeorge and Faull, 1989). In view of the high concentrations of dopamine D_3 receptors in the nucleus accumbens and the dorsomedial striatum (Sokoloff et al., 1992), this dopaminergic receptor subtype may be the main target for olanzapine and haloperidol in these limbic nuclei. Although the affinities of the two antipsychotics for the dopamine D_3 receptor are different (Bymaster et al., 1997), when the differences in dose were taken into account the affinities became almost equal. The lateral septum, too, is rich in dopamine D_3 receptors. Of all nuclei investigated, the lateral septum was the only region, where the interactions between olanzapine, clozapine and haloperidol became apparent, considering the Fos responses. As such, this septal nucleus seems to be a common locus in the actions of both typical and atypical antipsychotics. The fact, that the lateral septum has interconnections with a variety of other limbic nuclei, including the amygdala and the hypothalamus, regions, in which both olanzapine and clozapine elicit substantial Fos responses, may underline the significance of neuronal circuitry for the actions of antipsychotics.

Whereas all clinically effective antipsychotics induce Fos-protein in the nucleus accumbens and the lateral septum, only olanzapine and clozapine increased the number of Fos-positive cells in the prefrontal cortex. The patterns of Fos distribution in this region were consistent with activity against negative symptoms of schizophrenia, regarding the therapeutic profile of both antipsychotics (Beasley et al., 1996, 1997; Kane et al., 1988; Moore et al., 1997). Likewise, the clinical effects of clozapine have been attributed to its influence in the prefrontal cortex

(Friedman et al., 1991), presumably by intervention of several receptor types simultaneously, as no particular involved receptor could be delineated (Deutch and Duman, 1996). Considering the existence of cross-tolerance between olanzapine and clozapine towards Fos in this region, identical receptor systems are most probably affected.

Acutely administered olanzapine increased the levels of plasma corticosterone. This acute corticosterone response was significantly reduced after an olanzapine challenge in long-term olanzapine, haloperidol and clozapine pretreated animals. Furthermore, the magnitude of attenuation was the same. Thus, cross-tolerance between olanzapine, haloperidol and clozapine towards corticosterone release occurs, presumably in the paraventricular nucleus of the hypothalamus. In the parvocellular part of this nucleus the release of corticosterone is controlled by corticosterone-releasing factor-containing cells, while in the same area the most pronounced Fos effects were observed after a single dose of olanzapine. Several receptor-types that can be affected by atypical antipsychotics have been identified in this hypothalamic region (Aoki et al., 1994; Bealer, 1993; Charnay et al., 1997; Rivkees and Lachowicz, 1997). Interactions of olanzapine-like antipsychotics with such receptors (Eaton et al., 1996) most likely underlie the development of endocrine adverse-effects (Beasley et al., 1996; Casey, 1997).

5. Conclusion

The present study has demonstrated through Fos-protein induction that olanzapine and clozapine have similar actions in limbic regions of the rat forebrain, with the exception of the nucleus accumbens, where the interaction of olanzapine and haloperidol became obvious. Cross-tolerance between olanzapine and clozapine also occurred on levels of plasma corticosterone.

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