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Neuropeptide Y (NPY) and its mRNA in discrete brain areas after subchronic administration of neuroleptics

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Abstract. Classical (chlorpromazine, haloperidol) and atypical (sulpiride and clozapine) neuroleptics applied for a period of 2 weeks diminished neuropeptide Y (NPY) levels in nucleus accumbens, but not in striatum. These changes were correlated with NPY mRNA level. However, the effect of these neuroleptics on the level of NPY in hypothalamus was different. It is suggested that changes in NPY levels caused by neuroleptics are mediated by dopaminergic D1/D2 receptors.

Key words: neuroleptics, neuropeptide Y, nucleus accumbens, striatum, hypothalamus, rat

INTRODUCTION

It is very well known that pharmacological effects of neuroleptics are elicited by antagonism of dopaminergic receptors. After the discovery of the first neuropeptides, namely endogenous opioid peptides, data appeared that these new discovered neuropeptides very often colocalized in the same neurones with classical neurotransmitters, such as dopamine, and may interact with them and modulate their function in the central nervous system. Shortly after the discovery of enkephalins we studied the influence of long term treatment (1-12 months) with classical and atypical neuroleptics on the level of enkephalins in the rat striatum (Herman et al. 1984, 1991, 1993). We have shown that chronic administration of classical or atypical neuroleptic drugs increased the level of enkephalins. This finding supports the hypothesis that activation of dopaminergic neurones tonically inhibits the synthesis of enkephalins in the striatum.

After showing an abundant concentration and uneven localization of neuropeptide Y (NPY) in the brain we focused our attention on the distribution of this peptide in the brain. The aim of this paper was to examine the levels of NPY in discrete brain areas after acute as well as after subchronic administration of classical or atypical neuroleptics.

METHODS

Experiments were carried out on white male Wistar rats (initial body weight 240-260 g) from Central Animal Farm, Silesian University School of Medicine, housed five per cage (55 x 32 x 18 cm) under constant light conditions (artificial light from 7 a.m. to 7 p.m.) with free access to granular standard diet (containing 24% crude protein) and tap water. All experiments were performed between 9 a.m. and 3 p.m. Chloropromazine (CPZ), haloperidol (HAL), sulpiride (SULP) (Sigma), or clozapine (CLOZ) (Polfa) were used. Chlorpromazine hydrochloride was dissolved in a minimum quantity of 1% acetic acid. Sulpiride and clozapine were initially dissolved in deionized water, acidified with glacial acetic acid to pH 5.7 or 4.7 respectively. After dilution to the appropriate volume with deionized water the pH of the solutions was adjusted with 1 NaOH to 6.2 in the case of haloperidol and sulpiride. Clozapine solution was adjusted to pH 5.3.

Neuroleptics were administered i.p. in a volume of 2 ml/kg in doses given as a free base in mg/kg: CPZ 2 or 10, HAL 0.5 or 2.0, SULP 50 or 100, CLOZ 10 or 25, as a single injection or for 14 or 28 days. Control groups were treated with 0.9% sodium chloride. Each group of animals consisted of seven to eight rats.

Animals were killed 24 h after the last dose of neuroleptic or 8 days after drug withdrawal, and nucleus accumbens, striatum and hypothalamus were immediately separated, weighed and stored at -20°C before estimation of NPY. In some groups 3 h before sacrifice animals received an i.p. injection of the D1 receptor agonist SKF38393 (25 mg/kg) or D2 receptor agonist quinpirole (3 mg/kg). SCH23390 (1 mg/kg), a D1 receptor antagonist, was administered for 14 days and rats were killed 24 h after the last dose.

NPY was estimated radioimmunologically using NPY ^{125}I-labelled with Bolton and Hunter reagent (Amersham) and NPY antiserum for RIA from rabbit (Peninsula). NPY mRNA was estimated by the hybridization method described by Larhammar et al. (1987).

RESULTS

The most evident changes of NPY levels were observed 2 weeks after neuroleptic administration in discrete brain areas. In nucleus accumbens both classical (chlorpromazine, haloperidol) and atypical (sulpiride, clozapine) neuroleptics decreased NPY levels in a dose-dependent manner (Fig. 1). Haloperidol after a single administration, and clozapine after 2 weeks of administration, diminished the level of NPY mRNA in this area (Fig. 2).

Withdrawal of haloperidol, chlorpromazine and sulpiride caused an evident increase in NPY concentration, namely the inverse effect in comparison

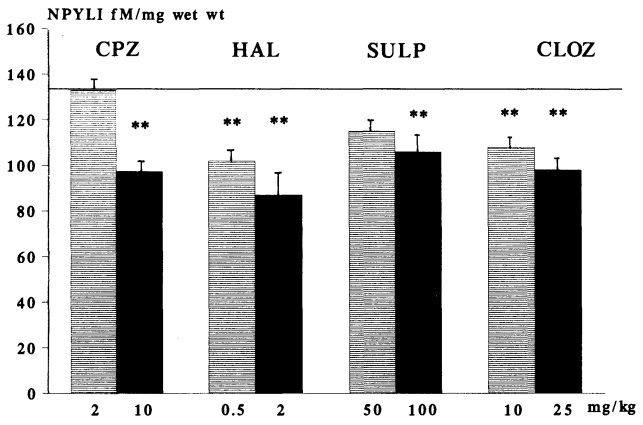

Fig. 1. The effect of 14-day treatment with neuroleptics: chlorpromazine (CPZ); haloperidol (HAL); sulpiride (SULP) or clozapine (CLOZ) on NPY-LI content in the rat nucleus accumbens. Doses of drugs (mg/kg) administred intraperitoneally are given under abcissa of graph. The horizontal line means the average value for both control groups (n=14). Bars represent the mean ±SEM (n=7). $^{**}P<0.01$ vs. respective control (Student's t-test).

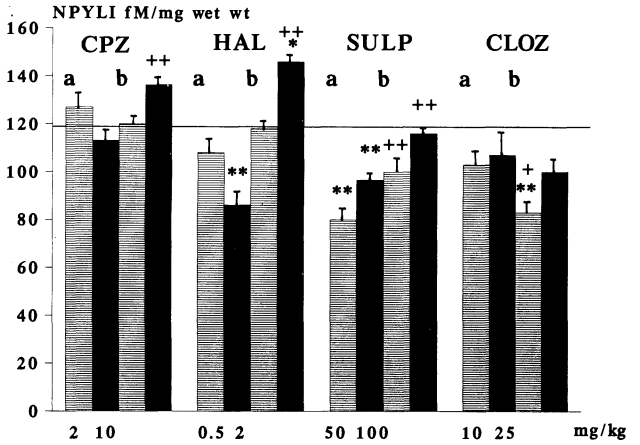

Fig. 3. The effect of 8-day withdrawal after chronic treatment with neuroleptics: chlorpromazine (CPZ); haloperidol (HAL); sulpiride (SULP) or clozapine (CLOZ) on NPY-LI content in the rat nucleus accumbens. a, 28-day tretment; b, withdrawal. Doses of drugs (mg/kg) administered intraperitoneally are given under abcissa of graph. The horizontal line mens the average value for control and control of withdrawal groups (n=24) $^{*}P<0.05$, $^{**}P<0.01$ vs. respective control; $^{+}P<0.05$, $^{++}P<0.01$ drug vs. withdrawal (Student's t-test).

with that observed after 28 days of administration of the drugs. The effect of haloperidol was mostly expressed (Fig. 3).

The administration of neuroleptics for 2 weeks did not affect the level of NPY in the striatum, with one exception. After treatment with the lower dose of sulpiride, NPY concentration was diminished.

No changes were observed after 2 week administration of haloperidol and clozapine in striatal concentration of NPY mRNA (results not shown).

In hypothalamus neuroleptics elicited different effects on the concentration of NPY in comparison with the changes observed in nucleus accumbens.

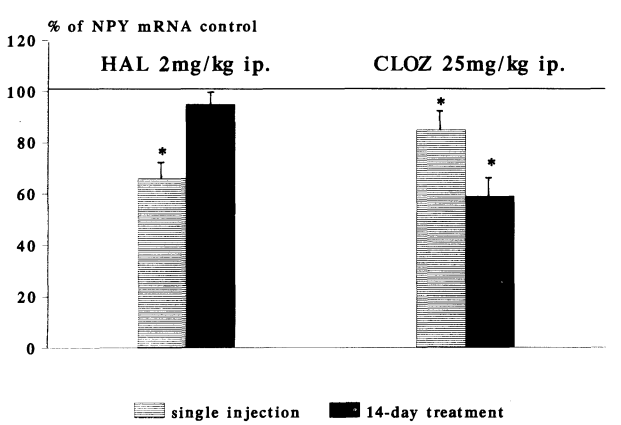

Fig. 2. The effect of treatment with haloperidol (HAL) or clozapine (CLOZ) on NPY mRNA level in the rat nucleus accumbens. Results are experessed as percentage of control SEM (n=3). $^{*}P<0.05$ vs. control (Duncan's test).

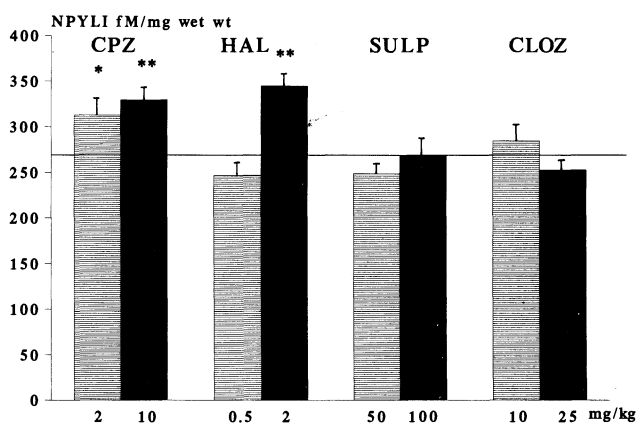

Fig. 4. Theeffect of 14 -day treatment with neuroleptics: chlorpromazine (CPZ); haloperidol (HAL); surpride (SULP), clozapine (CLOZ) on NPY-LI content in the rat hypothalamus. Details as in Fig. 1. $^{*}P<0.05$, $^{**}P<0.01$ vs. respective control (Student's t-test).

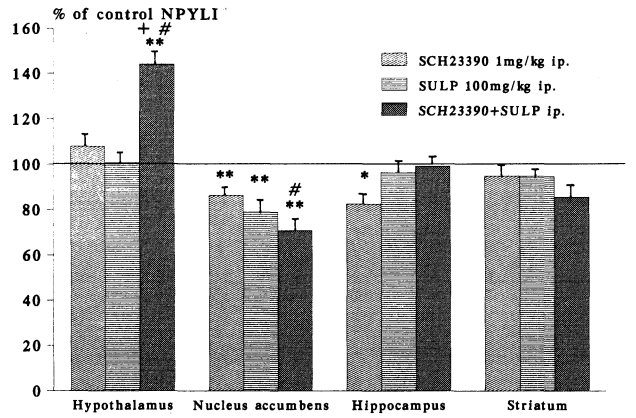

Fig. 5. The effects of 14-day intraperitoneally treatment with SCH23390 (1 mg/kg) and sulpiride (SULP) (100 mg/kg) on rat regional brain NPY-LI content. Results are expressed as percentage of control ±SEM (n=7-8). *P<0.05, **P<0.01 vs. control (Student's t-test). ^{+}P<0.05 vs. SULP, $^{\#}P$<0.05 vs. SCH23390 (Duncan's test).

Chlorpromazine and haloperidol increased and sulpiride and clozapine caused no changes of this concentration (Fig. 4).

To explain which dopaminergic receptor subtypes are involved in effects of these neuroleptics on NPY levels in discrete brain areas, a specific dopaminergic antagonist and two specific agonists were

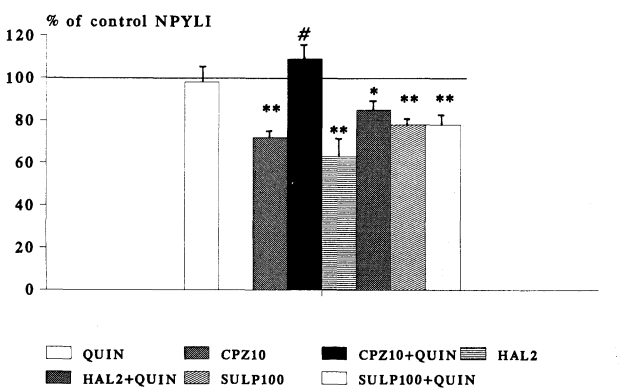

Fig. 6. The effect of acute quinpirole (QUIN) administration after 14-day treatment with neuroleptics: chlorpromazine (CPZ) (10 mg/kg); haloperidol (HAL) (2mg/kg); sulpiride (SULP) (100 mg/kg) in the rat nucleus accumbens. QUIN (3 mg/kg ip.) was injected 3 h prior to killing. Results are expressed as percentage of control ±SEM (n=7). *P<0.05, **P<0.01 vs. control (Student's test); $^{\#}P$<0.05 drug+QUIN vs. drug (Duncan's test).

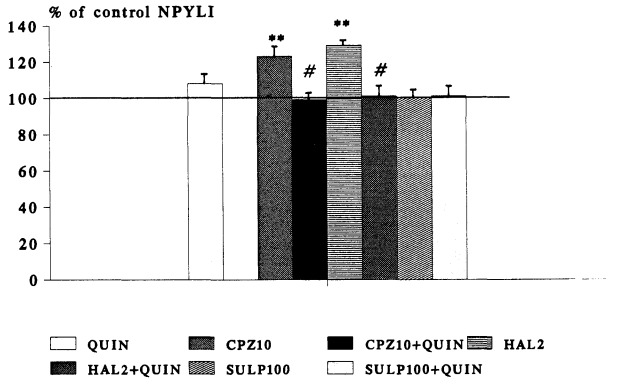

Fig. 7. The effect of acute quinpirole (QUIN) administration after 14-day treatment with neuroleptics: chlorpromazine (CPZ) (10 mg/kg), haloperidol (HAL) (2 mg/kg). sulpiride (SULP) (100 mg/kg) in the rat hypothalamus. Details as in Fig. 6.

used as pharmacological tools. SCH23390 (D1 receptor antagonist) evidently increased NPY levels after concomitant treatment with sulpiride for 14 days in hypothalamus. Sulpiride alone caused no changes. In nucleus accumbens this antagonist acted synergically with sulpiride on NPY levels, and exerted no influence on the effect of sulpiride in striatum as well as in hippocampus (Fig. 5). SKF38393, a D1 receptor agonist, had no influence on the subchronic administration of clozapine in examined brain areas (results not shown).

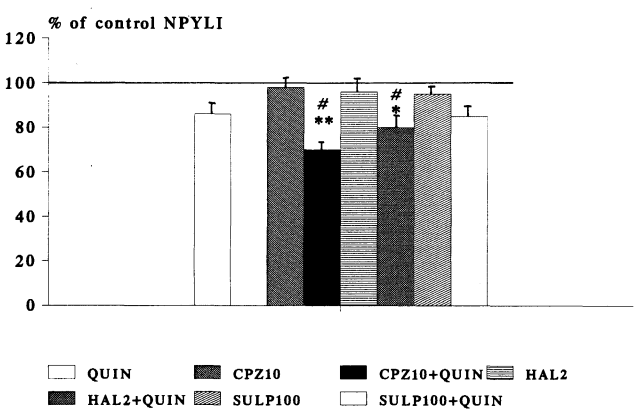

Fig. 8. The effect of acute quinpirole (QUIN) administration after 14-day treatment with neuroleptics: chlorpromazine (CPZ) (10 mg/kg); haloperidol (HAL) (2 mg/kg); sulpiride (SULP) (100 mg/kg) in the rat striatum. Details as in Fig. 6.

Quinpirole, a D2 receptor agonist, antagonized the effect of 2 week administration of chlorpromazine on NPY concentration in nucleus accumbens (Fig. 6). In the rat hypothalamus a similar antagonism was seen between quinpirole and haloperidol (Fig. 7). In striatum quinpirole diminished the NPY level in comparison with that elicited by chlorpromazine and haloperidol (Fig. 8).

DISCUSSION

We have examined the NPY concentration in three discrete brain areas after 2 or 4 weeks of administration of two classical or two atypical neuroleptics. These particular brain areas were chosen because the drugs used elicit their pharmacological effects in these places and because NPY is located here in high concentrations (Chronwall et al. 1985, De Quidt and Emson 1986a,b, Martel et al. 1988).

In nucleus accumbens all applied neuroleptics, particularly in the higher dose, diminished NPY levels only in n.accumbens. The effects evoked by the higher dose of haloperidol or clozapine could be explained by the diminution of NPY mRNA levels. The other finding was that 8 days of withdrawal of haloperidol after its 4 week administration elicited the opposite effect on NPY level. These data indicate that neuroleptics in this structure inhibit the function of neurones synthesizing NPY, and suggest their regulatory role in dopaminergic system. In fact, Salin et al. (1990) provided morphological evidence for a functional role of dopamine in controlling the metabolic activity of NPY neurones in nucleus accumbens.

In contrast, subchronic administration of all examined neuroleptics did not affect the striatal level of NPY and NPY mRNA. Śmiałowska and Legutko (1992) provided evidence that haloperidol administered four times but in doses of 2.5 and 5.0 mg/kg, higher than that used in our study, did not produce changes in the NPY level in the striatum. However, other data suggest a link between dopaminergic and NPY neuronal systems in this structure. It was suggested that nigro-striatal dopaminergic neurones may monosynaptically influence striatal NPY neurones (Kubota et al. 1988), and that dopamine has an inhibitory effect on NPY release (Tatsuoka et al. 1987). However, recently Kerkerian et al. (1992) using voltametric methods provided evidence that NPY facilitates striatal DA turnover by activation of dopamine release.

Concomitant administration of the specific D1 receptor antagonist SCH23390 and sulpiride for 2 weeks enhanced the NPY level only in the hypothalamus but not in the other examined brain areas.

Using different experimental models it was shown that NPY immunoreactivity increases in response to a D1 receptor antagonist and decreases in response to a D2 receptor antagonist, and it was suggested that dopaminergic D1 and D2 receptor subtypes play opposite roles in the dopaminergic control of the striatal NPY neuronal system (Kerkerian et al. 1988).

The D1 receptor agonist SKF38393 in our experimental conditions did not change NPY content in studied brain areas elicited by clozapine. The D2 receptor agonist quinpirole antagonized the influence on NPY concentration of two-week administration of chlorpromazine in examined brain areas (except striatum). The same type of antagonism was observed in hypothalamus after haloperidol treatment. Surprisingly, quinpirole did not alter sulpiride's effect. Our results suggest that changes of NPY levels induced by these neuroleptics are mediated by D2/D1 receptors.

Midgley et al. (1994) examined NPY-like immunoreactivity histochemically after administration of the same selective dopaminergic antagonist or agonists used in our experiments. However, our results are only partly consistent with their findings, which can be explained by different times of drug application and different methods of NPY estimation. These authors indicate that when histochemical methods are used, regions that remain unchanged in response to drug treatments mask the change of NPY-like immunoreactivity in responsive areas. They suggest that the neuropeptide system may be regulated by dopamine receptors. However, these authors have not studied the influence of these selective dopaminergic substances on NPY levels

elicited by neuroleptics. They also suggest that regional differences in response to these drugs might reflect differences in basal dopaminergic tone, which dictates the response to antagonists. Schwartz et al. (1992) have observed that quinpirole has considerably higher activity for D3 than for other types of dopaminergic receptors. Some data indicate that NPY systems in limbic structures of the rat are partly mediated by GABA-ergic (Midgley et al. 1993), serotonergic (Kakigi and Maeda 1992) or adrenergic mechanisms (Martire and Pistritto 1992). It may be hypothesized that subchronic administration of neuroleptics which act with different intensity on D1/D2 receptors and influence neurones producing the other neurotransmitter could extinguish the action of the substances used by us.

This study allows us to conclude that: (1) Classical and atypical neuroleptics elicited most evident and similar changes of NPY level in nucleus accumbens. (2) As shown by thr example of haloperidol and clozapine, the above-mentioned changes were caused by diminished peptide synthesis. (3) No evident changes in striatal NPY or NPY mRNA levels were observed after treatment with neuroleptics. (4) Classical neuroleptics influenced NPY levels in hypothalamus and in nucleus accumbens in opposite ways. (5) It is suggested that changes in NPY levels caused by neuroleptics are mediated by D2/D1 receptors.

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