Polish Journal of Pharmacology
Pol. J. Pharmacol., 2003, 55, 1153–1158
ISSN 1230-6002

PRELIMINARY COMMUNICATION

OPPOSITE EFFECTS OF CLOZAPINE AND SULPIRIDE ON THE LIPOPOLYSACCHARIDE-INDUCED INHIBITION OF THE GR-MEDIATED GENE TRANSCRIPTION IN FIBROBLAST CELLS

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Effects of clozapine and sulpiride on the lipopolysaccharide-induced inhibition of the GR-mediated gene transcription in fibroblast cells. A. BASTA-KAIM, B. BUDZISZEWSKA, L. JAWORSKA-FEIL, M. LEŚKIEWICZ, M. TETICH, M. KUBERA, S. SCHARPE, W. LASOŃ. Pol. J. Pharmacol., 2003, 55, 1153–1158.

Previously, we have found that some antipsychotic drugs are able to inhibit glucocorticoid receptor (GR)-mediated gene transcription. Since these drugs are known not only to inhibit hypothalamic-pituitary-adrenal (HPA) axis activity, but also to modulate the immunological system, the aim of the present study was to compare the effect of sulpiride and clozapine on GR function under basal culture conditions and during activation by lipopolysaccharide (LPS). The effect of clozapine and sulpiride alone and with LPS, the immune system activator, on glucocorticoid-mediated gene transcription was investigated in fibroblast cells, stably transfected with a mouse mammary tumor virus – chloramphenicol acetyltransferase plasmid (LMCAT cells).

Treatment of the cells with clozapine (3–10 μM) for 2 days significantly and in concentration-dependent manner decreased the chloramphenicol acetyltransferase (CAT) activity, while sulpiride (1, 3, 5 and 10 μM) was without any effect. LPS (1 μg/ml) given alone inhibited the corticosterone-induced gene transcription by ca. 35%. Clozapine (3, 5 and 10 μM) inhibited the effect of LPS (1 μM), while sulpiride, which alone had no effect on GR function, enhanced LPS (1 μM) action. The obtained results indicate that inhibition of GR-mediated gene transcription by LPS and clozapine can be a mechanism by which these compounds blocked some effects induced by glucocorticoids. Opposite effect of clozapine and sulpiride on LPS action may result from their distinct effect on activity of some kinases involved in regulation of GR transcriptional function and may determine their utility in the treatment of schizophrenia with or without immune system activation.

Key words: *lipopolysaccharide, clozapine, sulpiride, GR-mediated gene transcription*

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INTRODUCTION

A number of studies suggest that, apart from functional changes in monoamine systems, particulary hyperfunction of postsynaptic dopamine D_2 receptors, dysregulation of the neuroendocrinological and immune systems may be responsible for the development of schizophrenia, and that some antipsychotic drugs can attenuate this process [1, 4, 15, 30]. Indeed, hyperactivity of hypothalamic-pituarity-adrenal (HPA) axis, most probably evoked by impairment of the inhibitory feedback mechanism, has been observed in some schizophrenic patients [21, 29]. It has been well documented that the interaction between HPA axis and immune system is bidirectional and complex. It involves the effect of glucocorticoids on cytokine synthesis and action, and, on the other hand, the cytokine-induced activation of HPA axis and inhibition of glucocorticoid receptor (GR) function [23, 28]. Antipsychotic drugs are known to modulate the immunological systems and correct HPA activity, but the mechanisms of their action are poorly understood yet [4, 19].

Recently, it has been indicated that antipsychotic drugs can act not only on a function of membrane receptor, but also they can directly regulate gene transcription [5, 18]. Our previous studies have shown that some antipsychotic drugs are able to inhibit GR-mediated gene transcription under *in vitro* conditions [3]. Since the pro-inflammatory IL-1α also inhibits GR function in the same cell line [23], it is not unlikely that the effects of antipsychotics on GR-mediated gene transcription depend on immune system activity.

In order to shed more light on the effect of clozapine and sulpiride under basal culture conditions and during activation by lipopolysaccharide (LPS), we investigated the effect of those drugs alone or in combination with LPS on the GR-mediated chloramphenicol acetyltransferase (CAT) gene expression in fibroblast (LMCAT) cells. This cell line contains a CAT reporter gene, controlled by a mouse mammary tumor virus (MMTV) promoter.

Furthermore, the effect of LPS, clozapine and sulpiride on LMCAT cell viability was investigated in order to exclude the possibility that the action of these substances on the GR-mediated gene transcription was due to their non-specific, toxic effect.

MATERIALS and METHODS

Cell culture conditions

Effects of LPS, clozapine and sulpiride on the GR-mediated gene expression were determined in mouse fibroblast cells (L929), stably transfected with a mouse mammary tumor virus-chloramphenicol acetyltransferase (MMTV-CAT) – reporter plasmid (LMCAT cells). The LMCAT cell line was generously provided by Dr. E.R. Sanchez (Department of Pharmacology, Medical College of Ohio, Toledo, OH, USA). The cells were grown in DMEM (Life Technologies, Paisley, UK) supplemented with a 10% heat-inactivated fetal bovine serum (Life Technologies, New Zealand) and a 0.02% geneticin (Life Technologies, Paisley, UK) at 37°C in a 5% CO_2 /95% air atmosphere.

Drug treatments

LMCAT cells (final confluence: 90%) were treated with LPS (*E.coli*. 026:B6; Sigma, St. Louis, MO, USA, 1 μg/ml) or the drugs: clozapine (Polfa, Warszawa, Poland, 1–10 μM) or sulpiride (RBI, Natick, MA, USA, 1–10 μM) for 2 days. The control cells were cultured with the appropriate vehicle. In the next part of study, the cells were treated with LPS (1 μg/ml) and clozapine or sulpiride (1, 3, 5 and 10 μM) jointly for 2 days. CAT activity was stimulated by addition of 1 μM corticosterone (Sigma, St. Louis, MO, USA) for 2 h before harvesting the cells. LPS was dissolved in water, while clozapine, sulpiride and corticosterone were dissolved in a small amount of ethanol and diluted with water (final concentration of ethanol was below 0.5%).

CAT activity

Cell lysates were prepared by a freezing/thawing procedure. CAT activity was determined by the method of Pariante et al. [23]. Aliquots of the lysate (after heating for 10 min at 60°C) were incubated in 0.25 M Tris-HCl buffer (pH = 7.8) supplemented with 0.25 μCi D-threo-[dichloroacetyl-1-^{14}C]-chloramphenicol (Amersham Pharmacia Biotech, Buckinghamshire, UK) and 0.2 mM n-butyryl coenzyme A (Sigma, USA) at 37°C for 1 h. The butyrylated forms of chloramphenicol (in direct proportion to the CAT gene expression) were extracted twice with xylene, washed with 0.25 M Tris-HCl buffer, and the radioactivity was measured in a β-counter (Beckmann LS 335 liquid scintillation counter).

The results are presented as dpm of the butyrylated fraction of chloramphenicol per 10 μg of protein per one hour of incubation. Protein concentration in cell lysates was determined by a method of Lowry et al. [22].

Effect of LPS, clozapine and sulpiride on cell viability

LMCAT cells were treated with the vehicle, LPS (1 μg/ml), clozapine (1–10 μM) or sulpiride (1–10 μM) alone or jointly for 2 days. The effect of these compounds on cell viability was determined by counting viable and non-viable (blue) cells in a hemocytometer. The cell suspensions were mixed (at the 1:1 ratio) with a 0.4% trypan blue, and the number of non-viable cells per a total of 100 cells was counted.

Statistical analysis

The data are presented as the means ± SEM from 3–4 independent experiments (in duplicate wells). The significance of differences between the means was evaluated by the Duncan's test following a two-way analysis of variance (ANOVA).

RESULTS and DISCUSSION

Addition of corticosterone at a concentration of 1 μM for 2 h increased CAT activity about 30–35-fold. As shown previously, the CAT activity induced by corticosterone was completely blocked by RU 38486, a specific antagonist of the type II GR, which confirms involvement of the GR in that response [7]. Treatment of cells with antipsychotic drugs or LPS did not change the low, basal CAT activity (data not shown).

In the present study, LPS at concentration of 1 μM added for 2 days inhibited the stimulatory effect of corticosterone on the GR-mediated gene transcription (Figs. 1, 2). Treatment of cells with clozapine at concentrations of 3, 5 and 10 μM for 2 days significantly and in concentration-dependent manner decreased the CAT activity, but at the lowest concentration (1 μM) this drug was without ef-

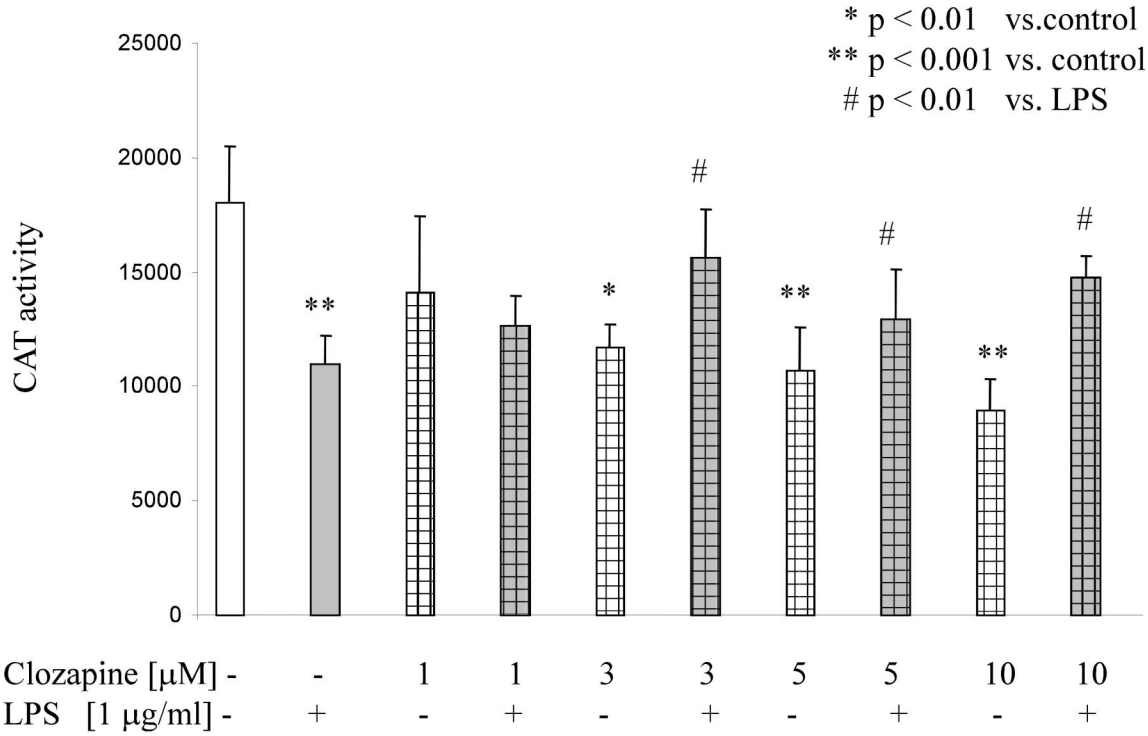

Fig. 1. The effect of clozapine or/and LPS on the CAT gene transcription induced by corticosterone in LMCAT cells. The test compounds at the indicated concentrations were applied for 2 days. Corticosterone (1 μM) was added 2 h before harvesting the cells for an assay of CAT enzyme activity. The data are presented as the dpm of the butyrylated fraction of chloramphenicol per 10 μg protein per hour of incubation; n = 6–8*;* the significance of differences between the means was evaluated by the Duncan's test following a two-way analysis of variance (* $p < 0.01$; ** $p < 0.001$ vs. control group; # $p < 0.01$ vs. LPS)

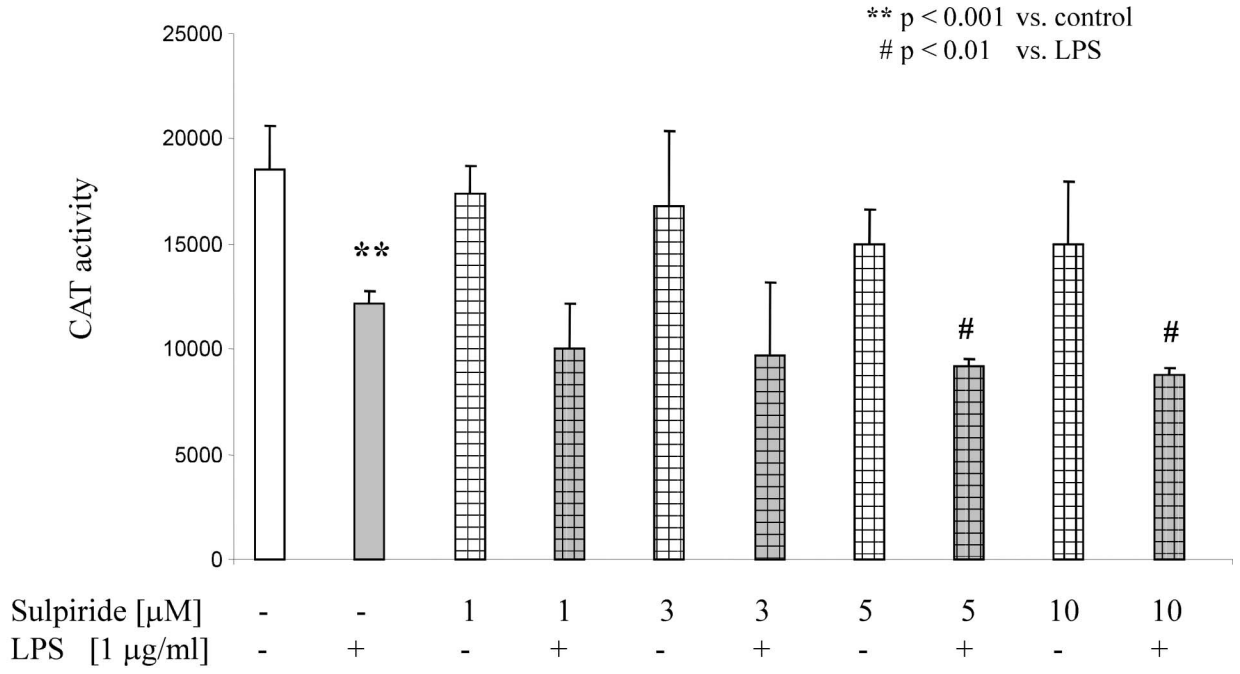

Fig. 2. The effect of sulpiride or/and LPS on the CAT gene transcription induced by corticosterone in LMCAT cells. The test compounds at the indicated concentrations were applied for 2 days. Corticosterone (1 μM) was added 2 h before harvesting the cells for an assay of CAT enzyme activity. The data are presented as the dpm of the butyrylated fraction of chloramphenicol per 10 μg protein per hour of incubation; n = 6–8*;* the significance of differences between the means was evaluated by the Duncan's test following a two-way analysis of variance (* $p < 0.01$; ** $p < 0.001$ vs. control group; # $p < 0.01$ vs. LPS)

fect (Fig. 1). Concomitant treatment of the cells with LPS (1 μM) and clozapine at 3, 5 and 10 μM but not at 1 μM increased the CAT activity in comparison with cultures treated with LPS alone (Fig. 1).

Two-day incubation with sulpiride (1, 3, 5 and 10 μM) was without any effect on the GR-mediated gene transcription in LMCAT cells (Fig. 2), however, combined treatment with LPS (1 μM) and sulpiride (5 and 10 μM) produced stronger inhibition of CAT activity than LPS given alone (Fig. 2).

The exposure of LMCAT cells to clozapine (1–10 μM), sulpiride (1–10 μM) and LPS (1 μM) alone or jointly for 2 days produced no toxic effect, as estimated by counting non-viable cells (data not shown).

The present data showed that treatment of LMCAT cells with LPS inhibited the corticosterone-induced gene transcription. LPS, a major amphiphilic molecule located at the outer membrane of Gram-negative bacteria, is a potent antigen known to induce specific humoral immune response in infected animals [12]. It has been described as a polyclonal activator of immune system, especially B lymphocytes. Furthermore, it has been reported that LPS injection, induces synthesis of various cytokines, especially interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF) by many cells [14]. Acting through incompletely understood mechanisms, cytokines can activate the HPA axis and modulate glucocorticoid-regulated gene transcription. Our finding concerning inhibitory effect of LPS on corticosterone-induced gene transcription is in line with the results of Pariante et al. [23], who observed that exogenous pro-inflammatory cytokine IL-1α reduced GR translocation from cytoplasm to nucleus and weakened GR function. Because LPS is known to induce synthesis of some pro-inflammatory cytokines also in fibroblast cells [25, 27, 31], it is possible that it affects GR function *via* influencing cytokine production. Inhibitory effects of LPS on GR function may involve several mitogen-activated protein kinase (MAPK) pathways: extracellular signal-regulated kinases (ERK) 1 and 2, c-Jun N-terminal kinase (JNK) and p38 kinase. Activation of MAP kinases is known to phosphorylate and decrease transcriptional activity of GR in some cell types [2, 16, 20, 26].

A question arises why clozapine and sulpiride act in opposite way on LPS-inhibitory effect on GR function. The ability of clozapine to decrease the

GR-mediated gene transcription, confirms results of our previous study, where longer incubation with this drug (5 days instead of 2 days) was used [3]. Clozapine, an atypical drug, shows higher affinity for serotonin 5-HT_{2A} than for dopamine D_2 receptors. However, regarding a possible mechanism for clozapine effects on the GR function, neither dopaminergic nor serotoninergic receptors are likely to be involved since fibroblast cells contain a very low, if any, number of these receptors [6, 11]. Inhibitory effect of clozapine on GR function may result rather from inhibition of phospholipase C/protein kinase C (PLC/PKC) pathway. Indeed, an *in vivo* study has shown that chronic treatment with clozapine and some other antipsychotics (chlorpromazine or haloperidol) decreased PKC activity in discrete rat brain regions [10, 17, 24]. Similarly, our previous results showed that inhibition of PLCβ and PKCα are connected with inhibitory effect of some antipsychotics on GR function [3]. Differences in the effects of antipsychotics on particular PKC or PLC isozymes [9] may explain dissimilarity in some of their effects, including action on the GR function. In fact, contrary to inhibitory effect of clozapine on PKC, sulpiride has been shown to enhance activity of this kinase [13]. Noteworthy, these two drugs affected also MAP kinase pathway in opposite way, e.g. clozapine inhibited ERK-MAPK, whereas sulpiride enhanced p38-MAPK activity [8, 24], both of these enzymes are reported to inhibit GR function. This may be important for their differential effects on LPS-induced inhibition of GR function, since LPS action in this model depends on activity of MAP kinases (our unpublished data).

It can be concluded that LPS and clozapine are capable of inhibiting GR-mediated gene transcription, which may account for a mechanism by which these substances block some effects induced by endogenous glucocorticoids. Moreover, opposite effects of clozapine and sulpiride on LPS inhibitory effect on GR function may result from their distinct effect on activity of some kinases involved in regulation of GR transcriptional function and may determine their utility in the treatment of schizophrenia with or without immune system activation.

Acknowledgments. The authors are grateful to Dr. E.R. Sanchez for generously providing an LMCAT cell line. They are also indebted to Ms. B. Korzeniak for her skillful technical assistance. This study was supported by the statutory funds of the Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland.

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Received: November 5, 2003; in revised form: November 24, 2003.