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Short communication

Agonist-stimulated GTPγ[^{35}S] binding to 5-HT$_{1A}$ receptors in human post-mortem brain

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Abstract

In this study, we have demonstrated that the technique of agonist-stimulated guanosine-5′-*O*-(3-[^{35}S]thio)-triphosphate (GTPγ[^{35}S]) binding can be successfully used to study the functional activity of the human 5-HT$_{1A}$ receptor in post-mortem tissue. Full agonist and antagonist actions of ligands specific for this site have been shown. Utilising 4-(2′-methoxy-phenyl)-1-[2′-(*n*-2″-pyridinyl)-*p*-fluorobenzamido]-ethyl-piperazine ([^{3}H]MPPF), the affinity of several antipsychotics for the 5-HT$_{1A}$ receptor was determined; clozapine and quetiapine were found to have K_i values at this receptor that, relative to their dopamine D$_2$ receptor affinities, indicated at least partial receptor occupancy at clinical doses. The agonist/antagonist activity of these two antipsychotics was studied using GTPγ[^{35}S] binding. Both compounds show partial agonism, and in addition, clozapine exhibited a larger degree of antagonism against 5-HT-stimulated binding than did quetiapine.

Keywords: 5-HT$_{1A}$ receptor; GTPγ[^{35}S]; Brain, human; Antipsychotic drug; Clozapine; Quetiapine

1. Introduction

The treatment of schizophrenia needs to address a variety of symptoms in addition to psychosis, which include disturbances of affect and cognition. The 5-HT$_{1A}$ receptor is attracting increasing interest as a site of action of psychoactive drugs. In addition to its potential importance in anxiolytic and antidepressant action (De Vry, 1995), it may be involved in cognitive function (Ridley et al., 1996). Furthermore, this receptor may contribute to the clinical efficacy of some of the atypical antipsychotic drugs. For example, clozapine has an affinity for this receptor which may be of importance at normal clinical doses (Mason and Reynolds, 1992). Thus, although the role of 5-HT$_{1A}$ receptors in antipsychotic efficacy remains speculative, this relationship deserves further study to enhance our understanding of the mechanisms underlying antipsychotic drug action.

Stimulation of guanosine-5′-*O*-(3-[^{35}S]thio)-triphosphate (GTPγ[^{35}S]) binding to neuronal membrane preparations has proved useful as a measure of agonist activity at receptors. In particular, 5-HT$_{1A}$ receptors have been studied in this way in both cell lines and rat hippocampal membranes (Alper and Nelson, 1998; Newman-Tancredi et al., 1998). As part of a study to determine the functional activity of several atypical antipsychotic drugs at the 5-HT$_{1A}$ site, we have applied the technique of agonist-stimulated GTPγ[^{35}S] binding to the naturally occurring receptor in human brain tissue taken post-mortem. We have also studied the receptor by conventional radioligand binding techniques employing the selective antagonist 4-(2′-methoxy-phenyl)-1-[2′-(*n*-2″-pyridinyl)-*p*-fluorobenzamido]-ethyl-piperazine ([^{3}H]MPPF) (Elliott and Reynolds, 1999).

2. Materials and methods

Briefly, for ligand binding assays with [^{3}H]MPPF (NEN Life Sciences), frozen human post-mortem hippocampal tissue was prepared in 50 mM Tris–HCl buffer as described by Kung et al. (1996). The tissue samples used were from six males and four females free of known neurological or psychiatric disease or treatment, with a mean age of 67 ± 3 years and a post-mortem interval of

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30 ± 7 h. Experiments were conducted at 37°C for 20 min, in triplicate, in a volume of 0.5 ml and a tissue concentration of 1.8 mg/ml. The incubation was terminated by rapid filtration and washing with ice-cold buffer. For saturation binding assays, concentrations of [^{3}H]MPPF ranged from 0.05 to 2.5 nM. Non-specific binding was determined in the presence of 10 μM 5-HT.

GTPγ[^{35}S] (Amersham) agonist-stimulated binding assays were conducted in a similar manner as described above. Tissue was homogenised in 50 mM Tris–HCl buffer containing 125 mM NaCl, 3 mM $MgCl_2$, 0.2 mM EGTA, pH 7.4, followed by centrifugation at 49 000 × *g* for 15 min and re-suspension in the buffer. All experiments were performed at a tissue concentration of 1 mg/ml, and in the presence of 30 μM GDP and 0.1 nM GTPγ[^{35}S], unless otherwise stated. Results were analysed by non-linear regression methods (Prism, Graphpad), and all errors quoted are S.E.M.s (expressed as confidence intervals or error bars where appropriate).

The following antipsychotics were gifts provided by various companies: quetiapine (Zeneca), clozapine (Sandoz), fluphenazine (Squibb), olanzapine (Lilly) and loxapine (Cyanamid). 8-Hydroxy-2-dipropylaminotetralin (8-OH-DPAT) and 4-(2′-methoxy-phenyl)-1-[2′-(*n*-2″-pyridinyl)-*p*-iodobenzamido]-ethylpiperazine (MPPI) were purchased from RBI, whilst chlorpromazine, haloperidol, 5-HT creatinine sulfate complex and GDP were from Sigma.

3. Results

[^{3}H]MPPF saturation binding yielded a B_{max} of 16.9 ± 1.7 fmol/mg tissue and a K_D of 0.63 ± 0.07 nM ($n = 4$). The affinity of the agonists 5-HT and 8-OH-DPAT, the selective antagonist MPPI (Kung et al., 1995), and the atypical antipsychotics clozapine and quetiapine, were determined and gave K_i values of 3.4 ± 1.0, 0.40 ± 0.05, 1.75 ± 0.09, 158 ± 11 and 383 ± 21 nM, respectively (n = 3–4). Other antipsychotics that were tested, that is, chlorpromazine, fluphenazine, olanzapine, haloperidol and loxapine, gave less than 50% inhibition of binding at 1 μM (i.e., K_i values > 560 nM).

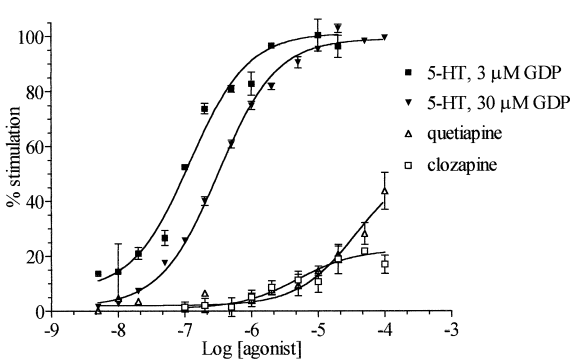

Fig. 1. 5-HT$_{1A}$ receptor-mediated agonist action on GTPγ[^{35}S] binding in human hippocampus.

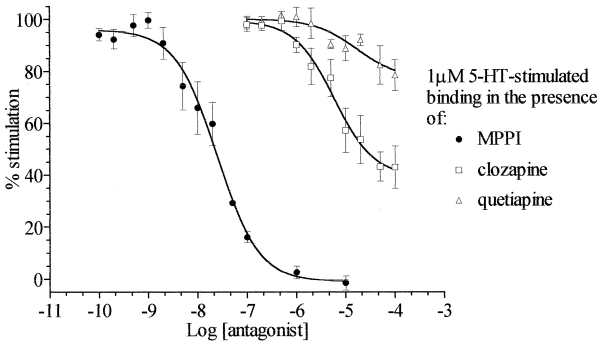

Fig. 2. Antagonism of 5-HT-stimulated GTPγ[^{35}S] binding in human hippocampus.

5-HT stimulated GTPγ[^{35}S] binding by 100–250% above basal binding, with an EC_{50} of 323 nM (302–347). 8-OH-DPAT stimulated GTPγ[^{35}S] binding as a full agonist with an EC_{50} of 62 nM (56–68). Fig. 1 demonstrates the agonist action of clozapine and quetiapine; each compound exhibits partial agonist actions relative to the effect of 5-HT, with quetiapine demonstrating greater efficacy at the highest concentrations studied. These effects were confirmed to be 5-HT$_{1A}$-mediated as the selective antagonist MPPI, at a concentration of 1 μM, inhibited stimulated binding (data not shown).

It proved possible to determine the affinity of antagonist effects by examining the inhibition of binding stimulated by 1 μM 5-HT. Apparent K_i values of MPPI and clozapine could be generated using the Cheng–Prusoff equation and a K_D value of 323 nM for 5-HT. MPPI yielded an apparent K_i of 4.9 nM (3.2–7.6). Clozapine too demonstrated considerable antagonist effects, producing 63 ± 6% maximal inhibition, with an apparent K_i of 1.2 μM (0.7–2.0), while quetiapine, at the concentrations studied, showed a smaller effect of approximately 20% inhibition (Fig. 2).

Decreasing the concentration of GDP in the GTPγ[^{35}S] assay buffer is found to increase the amount of basal binding, but decreases the proportion of agonist-stimulated binding. Lowering the concentration of GDP to 3 μM also significantly decreased the EC_{50} of 5-HT to a mean of 116 nM (95–145) ($P < 0.05$).

4. Discussion

Of the antipsychotic drugs studied, only clozapine and quetiapine showed significant affinity for the 5-HT$_{1A}$ receptor. The pK_i values for clozapine and quetiapine at human striatal dopamine D_2 receptors are 6.54 and 6.31, respectively (Blake et al., 1998). These affinities are similar to those for the 5-HT$_{1A}$ receptor, that is, 6.81 for clozapine and 6.42 for quetiapine, thus indicating at least

partial occupancy of the 5-HT_{1A} receptor at normal clinical doses. The other antipsychotics studied show more than 20-fold selectivity for the dopamine D_2 site over that for the 5-HT_{1A} receptor.

In this assay, both clozapine and quetiapine were found to have partial agonist effects. Under different conditions, Newman-Tancredi et al. (1998) obtained an efficacy for clozapine of 50–60% in recombinant human 5-HT_{1A} receptors expressed in Chinese hamster ovary cells. However, antagonistic activities of these two antipsychotics were not investigated by these authors.

There is a notable discrepancy between 5-HT's inhibition of [^{3}H]MPPF binding (K_i of 3.5 nM) and that observed for its effect on GTPγ[^{35}S] binding, which is approximately 100-fold lower in affinity. This effect is not observed for a full antagonist such as MPPI, which has a K_i vs. [^{3}H]MPPF of 1.8 nM, close to its value of 4.9 nM in inhibiting 5-HT-stimulated GTPγ[^{35}S] binding. While apparent K_i values of MPPI and clozapine could be obtained using a K_D value for 5-HT, this value is dependent on GDP concentration in the assay (Fig. 1). As described previously, GDP concentration is chosen to optimise the proportion of stimulated binding relative to the levels of basal binding in the GTPγ[^{35}S] assay (Alper and Nelson, 1998), but the concentration used routinely has the effect of decreasing this measure of agonist affinity. The variability of agonist K_D value depending on GDP concentration presumably accounts for the discrepancy in agonist K_i values between this assay and the [^{3}H]MPPF binding assay; the difference would then provide a measure of relative agonist activity. In this respect, it is notable that the 10-fold difference seen with clozapine is intermediate between that of 5-HT and MPPI, indicative of partial agonist/antagonist properties.

Quetiapine's results are less conclusive, perhaps because its maximal effects have not been achieved at the concentrations studied. However, the present data suggest this drug to be an agonist at the 5-HT_{1A} receptor with a lower affinity and higher efficacy than clozapine.

Comparison of EC_{50} values with those from other studies in different systems is not straightforward. Other authors used different concentrations of GDP, that is, 300 μM (Alper and Nelson, 1998) and 3 μM (Newman-Tancredi et al., 1998), and the latter authors also incubated the membranes at 22°C and not 37°C. In addition, the use of post-mortem tissue would indicate that the ratio of G-proteins to receptor is optimal for the assessment of human receptor function.

It is possible that actions at the 5-HT_{1A} receptor could contribute to the clinical effects of clozapine and quetiapine. Our results indicate differences in their 5-HT_{1A} effects which may relate to differences in the profile of symptom relief, and perhaps also in side effects. The two drugs are notable in being essentially devoid of extrapyramidal side effects, although the known function of 5-HT_{1A} receptors would suggest actions on cognition and/or affect, as mentioned above. Studies with some of the antipsychotics currently in development may serve to shed further light on the role of the 5-HT_{1A} receptor in antipsychotic drug action.

In conclusion, we have applied radioligand binding techniques that permit the identification of both agonist and antagonist effects at the natural human 5-HT_{1A} receptor. Of the currently available antipsychotic drugs, only quetiapine and clozapine show receptor affinities at the human 5-HT_{1A} receptor that are likely to indicate (partial) receptor occupancy at normal clinical doses. Thus, GTPγ[^{35}S] binding is a valuable tool that can be used in post-mortem human tissue, as well as in animal tissue and cell lines, with the advantages it brings to an understanding of human in vivo actions, to investigate the functional activity of drugs binding to the 5-HT_{1A} receptor.

Acknowledgements

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