Abstract Real-time analysis of dopamine:antagonist interactions at the recombinant D_{2short} receptor was performed by measuring time-dependent Ca^{2+} responses following activation of a chimeric \( G_{\alpha q/o} \) protein in CHO-K1 cells. Terguride (57%), (+)-UH 232 (20%) and buspirone (16%) demonstrated dopamine-like intrinsic activity at the presumably unoccupied, dopamine-free receptor; remoxipride, pipamperone and L 741626 being silent at 1 \( \mu M \). Each of the putative antagonists (1 \( \mu M \)) displayed a transient reversal capacity of the low-magnitude Ca^{2+} phase in the dopamine-bound receptor state (\( E_{\text{rev}} \): 68%–92% vs. 1 \( \mu M \) tropapride) with a \( t_{1/2} \) between 8.8 s and 13.9 s upon antagonist addition; this capacity was either almost fully [remoxipride, pipamperone and (+)-UH 232] or partially [buspirone (31%), terguride (45%) and L 741626 (70%)] lost upon further incubation. The biphasic reversal Ca^{2+} profile of these dopamine antagonists is different from previously characterised dopamine antagonists which display either full reversal of the high-magnitude Ca^{2+} response with a fast or slow onset of action, or partial reversal stably present over the entire incubation period. The dynamic Ca^{2+} data strongly suggest that the dopamine D_{2short} receptor can be blocked via multiple molecular mechanisms.

Keywords Recombinant human dopamine D_{2short} receptor · Ca^{2+} response · Dopamine antagonist · CHO-K1 cells

Introduction

One of the most important classes of D_{2} receptor ligands are the antipsychotic drugs. These comprise a wide range of structural chemical classes with the common ability to act as clinically effective antipsychotic agents, and are considered to be antagonists at D_{2-like} (D_{2}, D_{3} and D_{4}) receptors. A limitation of available accounts of the interactions of dopamine antagonists with D_{2} receptors concerns the resolution with which receptor activity has been measured and analysed. Investigating whether a high-resolution analysis of the different behaviours that central nervous system stimulants induce, may offer a more powerful account of the antipsychotic potential of neuroleptic compounds, Koek and Colpaert (1993) demonstrated antipsychotics to differ in terms of the relative doses at which they antagonise the stereotyped behaviors induced by methylphenidate in rats. It was hypothesized that this variation among antipsychotics may be based on differences in the extent to which they exert agonist activity at dopamine receptors. Also, some of the effects of antipsychotic drugs have been shown to occur with only a moderate occupancy (50%–75%) of D_{2-like} receptors and it is difficult to see how these could be achieved simply by antagonism of the effects of endogenous dopamine (Strange 1999).

Kinetic analyses of dopamine antagonist properties by monitoring dynamic interactions between antagonists and dopamine at the recombinant human dopamine D_{2short} receptor indicated that antagonists differed in terms of their abilities to prevent the high-magnitude Ca^{2+} phase in the antagonist-bound receptor state, and to reverse the low-magnitude Ca^{2+} phase in the dopamine-bound state (Pauwels et al. 2001). Few compounds (i.e., tropapride and nemonapride) fully antagonised both the high- and low-magnitude Ca^{2+} response. Most compounds displayed partial antagonism, in particular for reversing the low-magnitude Ca^{2+} response, though most of them did not display intrinsic activity at the unoccupied, dopamine-free D_{2short} receptor. This observation suggests that a weak antagonism cannot be solely explained by the putative presence of the ligand’s intrinsic activity. Two major subgroups were identified within these dopaminergic ligands: compounds with either a fast (i.e., olanzapine) or a slow [i.e., (+)-butaclamol] onset of action. In the present study,
we report on the atypical kinetics for a series of weak putative dopamine antagonists to reverse the low-magnitude Ca\(^{2+}\) phase in the dopamine-bound D\(_{2}\)short receptor state.

**Materials and methods**

Construction of human dopamine D\(_{2}\)short receptor and chimeric G\(_{\alpha}\)q/o protein genes. The short splice variant of the human dopamine D\(_{2}\) receptor cDNA (RC: 2.1.DA.02) was cloned by PCR (Pauwels et al. 2001) using oligonucleotide primers designed according to the sequence deposited in the Genbank database (accession no. S69899). The chimeric G\(_{\alpha}\)q/o protein was constructed by exchanging the last five amino acids (Glu355-Tyr-Asn-Leu-Val) of a mouse G\(_{\alpha}\)q/o protein by those corresponding to rat G\(_{\alpha}\) (Gly-Cys-Gly-Leu-Pro) (Pauwels et al. 2001). Receptor and chimeric G\(_{\alpha}\) protein constructions were inserted into a pCR3.1 mammalian expression vector.

Measurement of intracellular Ca\(^{2+}\) responses. Subconfluent CHO-K1 cells were transiently transfected with a human D\(_{2}\)short receptor and G\(_{\alpha}\)q/o protein plasmid in an equimolecular amount (10 \(\mu\)M) by electroporation. Cells were assayed between 24 h and 48 h upon transfection for intracellular Ca\(^{2+}\) responses upon 1-h pulse with 2 \(\mu\)M Fluo-3 fluorescent calcium indicator dye as described (Pauwels et al. 2001). Ligand-mediated intrinsic activity was defined as the ligand’s maximal high-magnitude Ca\(^{2+}\) response (\(E_{\text{max}}\)) in percentage vs. that obtained with 1 \(\mu\)M dopamine. Antagonists were either pre-incubated for 10 min before dopamine (1 \(\mu\)M) to prevent the high-magnitude Ca\(^{2+}\) phase in the antagonist-bound receptor state, or added 3.5 min upon the stimulation by dopamine (1 \(\mu\)M) to reverse the low-magnitude Ca\(^{2+}\) phase in the dopamine-bound state. Antagonist capacity (%) of dopamine-induced high-magnitude Ca\(^{2+}\) response was calculated as the surface area between the dopamine and ligand condition for a period of 4 min upon addition of dopamine. The antagonist-induced reversal of the dopamine-mediated low-magnitude Ca\(^{2+}\) response in general, a biphasic appearance (see Results); the Ca\(^{2+}\) concentration decreased to a minimum during a first phase, and increased to an apparent asymptote during a second phase. These biphasic time-response data could be described adequately by an equation consisting of the sum of four exponentials [i.e., response = \(\sum_{i=1}^{4} A_i e^{-k_i t} + C\), where \(A_i=\text{asymptote}\) and \(k_i=\text{time constant}\), which was fitted to the data by the solver function of Microsoft Excel. From this equation, the following measures were calculated: maximum reversal response (\(E_{\text{rev}}\)), time (in s) at which half-maximum response was reached (t\(_{1/2,E_{\text{rev}}}\)), asymtote (\(E_{\text{asym}}\)), and time (in s) at which half-asymptote was reached (t\(_{1/2,E_{\text{asym}}}\), calculated from the time at which \(E_{\text{rev}}\) occurred).

**Results**

A typical dopamine-mediated Ca\(^{2+}\) response displayed two phases in CHO-K1 cells transfected with a human D\(_{2}\)short receptor and chimeric G\(_{\alpha}\)q/o protein (Pauwels et al. 2001): a high-magnitude phase which was transient and a low-magnitude phase which continued throughout the recorded time period (10 min). Terguride (57±5), (+)-UH 232 (20±2) and busiprine (16±4) demonstrated various levels of intrinsic activity \(E_{\text{max}}\) at 1 \(\mu\)M vs. 1 \(\mu\)M dopamine at the presumably unoccupied, dopamine-free receptor; the other compounds being silent at 1 \(\mu\)M. Pre-incubation of transfected CHO-K1 cells for 10 min with the putative antagonist (1 \(\mu\)M) prior to 1 \(\mu\)M dopamine exposure to prevent the high-magnitude Ca\(^{2+}\) phase in the antagonist-bound receptor state indicated nearly full antagonism for terguride (94±2%) as compared to the efficacious antagonist tropapride. The other ligands being investigated were less effective as antagonists of the dopamine-mediated high-magnitude Ca\(^{2+}\) response; they displayed the following rank order of partial antagonism [antagonist capacity (%) vs. tropapride]: L 741626 (76±5) >> busiprine (49±3) > pipamperone (27±2) > remoxipride (24±0). Putative antagonists (1 \(\mu\)M) were also added 3.5 min upon the stimulation by dopamine to reverse the low-magnitude Ca\(^{2+}\) phase in the dopamine-bound receptor state. Figure 1 illustrates the kinetics of the dopamine-mediated low-magnitude Ca\(^{2+}\) phase for a series of putative antagonists. Tropapride (1 \(\mu\)M) reversed with a t\(_{1/2}\) of 13.1±1.0 s the low-magni-