Haloperidol increases prolactin release and cyclic AMP formation in vitro: inverse agonism at dopamine D2 receptors?

Rapid Communication

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Summary. Haloperidol (30 nM, 3 μM) was found to increase prolactin release from GH4C1 cells transfected with the D2 receptor cDNA (GH4ZR 7) and from wild-type (untransfected) GH3 cells, but not from wild-type GH4C1 cells. In addition, haloperidol (3 μM) stimulated cAMP formation in GH3 cells. It is suggested that haloperidol may act as an inverse agonist rather than as a neutral antagonist at dopaminergic D2 receptors.

Keywords: Haloperidol, dopamine, D2 receptors, prolactin, cyclic AMP, inverse agonist, reverse agonist, GH4ZR 7 cells, GH4C1 cells, GH 3 cells, cell culture, in vitro.

Introduction

GH3 and GH4C1 are prolactin-producing cell strains derived from the transplantable rat tumour MtTW 5 (Gourdji et al., 1982). Both cell lines respond to various prolactin secretagogues, such as VIP and TRH, in a physiological manner. In contrast to normal rat lactotrophs, however, neither GH3 nor GH4C1 cells respond to dopamine or dopamine D2 receptor agonists with a marked decrease in prolactin release (Albert et al., 1990; Johnston et al., 1991; for ref see Gourdji et al., 1982). Hence, for studies of D2 receptor mechanisms, the GH3 and GH4C1 strains have been no useful tools. In order to establish a prolactin-producing cell line that does respond to dopamine, Albert and coworkers (1990) transfected GH4C1 cells with D2 receptor cDNA, hence producing a stable transfectant (GH4ZR 7) expressing D2 receptors (short isoform) that are functionally coupled to adenylyl cyclase. Indeed, exposure of GH4ZR 7 to dopamine or D2 receptor agonists leads to a dose-dependent inhibition of VIP-induced prolactin secretion (Albert et al., 1990; Nilsson and Eriksson, 1992).
Haloperidol is a classical neuroleptic drug which antagonizes brain as well as pituitary D2 receptors. In man and rat, haloperidol has been shown to elevate baseline serum levels of prolactin as well as to antagonize the prolactin-suppressive effect of D2 receptor agonists (see, e.g., Rubin et al., 1976). Also, haloperidol has been demonstrated to antagonize D2 receptor agonist-induced suppression of prolactin release from normal anterior pituitary cells (Quijada et al., 1973) as well as from cells of the GH4ZR 7 line in vitro (Nilsson and Eriksson, 1992). In the present study, the effects of haloperidol per se on prolactin release from cells of the GH4ZR 7, GH4C 1 and GH 3 strains were investigated. In addition, cyclic AMP (cAMP) formation in GH 3 cells exposed to haloperidol was measured.

Materials and methods

Cell cultures

GH4ZR 7 and GH4C 1 cells were kindly donated from Dr. O. Civelli, The Vollum Institute for Advanced Biomedical Research, Oregon Health Science University, Portland, Oregon, U.S.A., and Dr. K. Törnquist, The Minerva Foundation, University of Helsinki, Finland, respectively. GH 3 cells were obtained from the American Type Culture Collection, Rockville, Maryland, U.S.A.. All cell strains were kept frozen in liquid N2 before being thawed, propagated and passaged by means of trypsin-EDTA dissociation. Stock cultures (monolayers) of the three cell strains were maintained in Nunclon T-25 flasks (Nunc A/S, Roskilde, Denmark) with approximately 10ml of Ham’s F-10 medium (Biochrom KG, Berlin, Germany) supplemented with 3.7 g/l sodium bicarbonate (Sigma, St Louis, MO, U.S.A.), 15% horse serum (Biochrom), 2.5% fetal calf serum (Biochrom), 100 U/ml penicillin G + 100 μg/ml streptomycin (=PeSt), 0.6 μg/ml amphotericin B, and 1 mM l-glutamine (Sigma) and incubated at 37 °C in a water-saturated atmosphere of 5% CO2 in air. Total medium was changed every 4th to 5th day.

Prolactin experiments

Approximately one week before an experiment, cells were seeded in Nunclon 16 mm 24-multiwell plates in an approximate amount of 100,000 cells in 500 μl of the same medium as in stock growth. Immediately before the experiment, the cells were washed twice (15 min each time) with warmed, CO2-equilibrated Earle’s Balanced Salt Solution (Sigma) supplemented with 0.2% bovine serum albumine (Sigma) (EBSS + BSA). Haloperidol (Leo, Sweden) was solubilized and dissolved in tartaric acid and ethanol and added to the wells diluted in EBSS + BSA (total volume: 500 μl); the final concentrations of tartaric acid and ethanol were < 0.5 nM and < 0.5%, respectively. On one 24-multiwell plate, 12 wells were given one dose of haloperidol (30 nM/3 μM) while 12 wells were given identical vehicle. In order to avoid systematic errors due to factors related to the position of the well on the plate (such as the ‘edge effect’), every second well was given haloperidol and vehicle, respectively. After 30 min of incubation with haloperidol or vehicle, the supernatants were gently pipetted from the wells, centrifuged (300 g; 5 min), in order to remove any remaining, floating cells, and frozen for subsequent rat prolactin RIA analysis.

In order to evaluate the capacity of the three cell strains to secrete increased amounts of prolactin in response to a secretagogue, in one experiment VIP (vasointestinal peptide, culture grade, Sigma) (300 nM) was administered instead of haloperidol. The design of this experiment was identical to that of the haloperidol experiments described above, the only exception being that VIP was dissolved directly into EBSS + BSA.