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European Journal of Pharmacology 310 (1996) 273–276

ejp

Short communication

Imidazoli(di)ne compounds interact with the phencyclidine site of NMDA receptors in the rat brain

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Received 19 June 1996; accepted 2 July 1996

Abstract

The effects of several imidazoli(di)ne compounds on the binding of the non-competitive NMDA receptor antagonist [^{3}H](+)-MK-801 (dizocilpine) to rat brain membranes were studied. These compounds fully inhibit radioligand binding with potencies in the micromolar range. The obtained profile of drug affinity correlated well with the potency of the same compounds promoting insulin release by blocking ATP-sensitive K^+ channels in the rat insulinoma cell line RIN-5AH. It is suggested that imidazoli(di)ne compounds interact with cation channels sharing a common phencyclidine binding site (e.g. NMDA receptors, K^+ channels and nicotinic acetylcholine receptors) and that this could be the basis of some biological effects of imidazoli(di)nes.

Keywords: NMDA receptor; Phencyclidine site; [^{3}H](+)-MK-801; Imidazoline drug; K^+ channel, ATP-sensitive

1. Introduction

Imidazoline derivatives and structurally related compounds bind not only to α-adrenoceptors but also to distinct non-adrenergic sites. Among these, the existence of two classes of imidazoline receptors denoted as I_1 and I_2 is well established which differ in their pharmacological profiles and also in their tissue and subcellular distributions (Michel and Ernsberger, 1992).

Recent studies have shown that certain imidazoli(di)ne/guanidine compounds, independently of their affinity on imidazoline I_1 and I_2 receptors, also interact (in the micromolar range) with cation channels, thus producing several biological effects. These compounds block ATP-sensitive K^+ channels in pancreatic β-cells and rat insulinoma (RIN) cells and this leads to the stimulation of insulin release (Dunne, 1991; Jonas et al., 1992; Olmos et al., 1994). Imidazoli(di)ne drugs inhibit the acetylcholine-induced secretion of catecholamines in adrenal chromaffin cells (Ohara Imaizumi and Kumakura, 1992) by blocking nicotinic acetylcholine receptors (Musgrave et al., 1995). Finally, these compounds also interact with 5-HT_3 receptors in N1E-115 cells inhibiting the veratridine-induced influx of guanidinium to those cells (Molderings et al., 1995). Imidazoli(di)ne drugs have been shown to interact with the phencyclidine binding site of the nicotinic acetylcholine receptor (Musgrave et al., 1995). Phencyclidine is an arylcyclohexylamine that also binds to other receptors and ion channels, including K^+ channels (Bartschat and Blaustein, 1988) and NMDA receptors (Lodge et al., 1983). The present study was therefore designed (1) to assess whether imidazoli(di)ne drugs can bind to the phencyclidine site of the NMDA receptor labelled with [^{3}H](+)-MK-801 in the rat brain and (2) to examine whether a common phencyclidine site located on distinct cation channels might underlie the effects mediated by high concentrations of imidazoli(di)ne drugs.

2. Materials and methods

2.1. Membrane preparation

Male Sprague-Dawley rats (250–300 g) were used. The animals were decapitated and membranes (P_2 fractions) were prepared by established methods from the parieto-occipital cortex. Briefly, the tissue samples were ho-

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PII S0014-2999(96)00519-5

mogenised in 5 ml of ice-cold Tris-sucrose buffer (5 mM Tris-HCl, 250 mM sucrose, 1 mM $MgCl_2$, pH 7.4). Homogenates were centrifuged at $1000 \times g$ for 10 min at 4°C. The supernatant was centrifuged at $40000 \times g$ for 10 min and the resulting pellet was washed twice with 2 ml of fresh incubation buffer (5 mM Tris-HCl, pH 7.4). The final pellet was resuspended in an appropriate volume of fresh incubation buffer to a final protein content of 0.6–0.8 mg/ml.

2.2. [^{3}H](+)-MK-801 binding assays and analyses of binding data

Radioligand binding assays with [^{3}H](+)-MK-801 were performed as previously described by Foster and Wong (1987). Drug competition studies were performed in a total volume of 500 μl, containing 400 μl of membrane suspension and [^{3}H](+)-MK-801 (4×10^{-9} M), in the absence or presence of various concentrations of the competing drugs (10^{-10} M or 10^{-7} to 10^{-2} M; 11–15 concentrations). Non-specific binding was determined in the presence of 10^{-4} M ketamine. The mixture was incubated for 45 min at 23°C and then subjected to rapid filtration through Whatman GF/C filters using a Brandel 48 R cell harvester (Biomedical Research and Development Laboratories, USA). The filters were then rinsed twice with 5 ml of ice-cold incubation buffer and counted for radioactivity by liquid scintillation spectrometry at 50% efficiency. Analyses of competition experiments as well as the fitting of data to the appropriate binding models were performed by computer-assisted non-linear regression using the EBDA-LIGAND programs as previously described (Olmos et al., 1994).

2.3. Drugs

[^{3}H](+)-MK-801 (20.3 Ci/mmol) was supplied by New England Nuclear/Du Pont (USA). Other drugs (and their sources) included: antazoline HCl (Sigma Chemical Co., St. Louis, MO, USA); cirazoline HCl (Synthélabo Recherche, Paris, France); clonidine HCl (Boehringer Sohn Ingelheim, Ingelheim am Rhein, Germany); efaroxan HCl (Sigma); idazoxan HCl (synthesized by Dr. F. Geijo, Lasa Laboratorios, Barcelona, Spain); ketamine HCl (Sigma); (+)-MK-801 (dizocilpine) maleate (RBI, Natick, MA, USA); naphazoline HCl (Sigma); phentolamine HCl (Ciba-Geigy, Barcelona, Spain); RX821002 (2-methoxy idazoxan) HCl (synthesized by Dr. F. Geijo, Lasa Laboratorios); and tolazoline HCl (Sigma). Other reagents were obtained from Sigma.

3. Results

In competition experiments, (+)-MK-801 completely inhibited the specific binding of [^{3}H](+)-MK-801 to rat brain membranes from a single site and with high potency (Table 1). Ketamine, another non-competitive NMDA receptor antagonist, also displaced the binding of the radioligand although with a lesser potency (Table 1, Fig. 1A).

When several compounds with the imidazoli(di)ne structure were tested, complete inhibition was obtained and computer-assisted non-linear analysis indicated that these drugs competitively inhibited [^{3}H](+)-MK-801 binding from a single site (slope factors near unity) and with potencies in the micromolar range (Table 1, Fig. 1A). Among the drugs tested, the chemically closely related compounds antazoline and phentolamine displayed the highest potency. The obtained profile of drug affinity for the [^{3}H](+)-MK-801 binding sites on NMDA receptors was very similar to that reported for the effects of the same

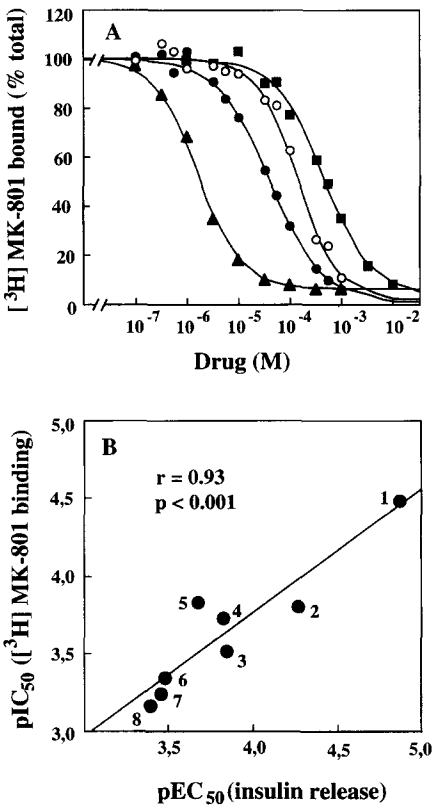

Fig. 1. (A) Inhibition of binding of [^{3}H](+)-MK-801 to NMDA receptors by ketamine (▲), antazoline (●), cirazoline (○) and clonidine (■) in the rat cerebral cortex. Membranes were incubated at 23°C for 45 min with [^{3}H](+)-MK-801 (4×10^{-9} M) in the absence or presence of selected concentrations of the competing drugs. Data shown are mean of 2–3 experiments and expressed as a percentage of total control binding (about 12000 dpm). See Table 1 for binding parameters and others details. (B) Correlation between the potency (expressed as $pEC_{50} = -\log EC_{50}$) of several imidazoli(di)ne drugs as promoters of insulin release from rat insulinoma (RIN-5AH) cells (data taken from Olmos et al., 1994) and the potency (expressed as $pIC_{50} = -\log IC_{50}$) inhibiting the binding of [^{3}H](+)-MK-801 to NMDA receptors in the rat brain (data taken from Table 1). The data were best described by the equation $y = 0.80x + 0.57$ ($r = 0.93$; $P < 0.001$). The identification of drugs is as follows: (1) antazoline, (2) cirazoline, (3) idazoxan, (4) efaroxan, (5) naphazoline, (6) clonidine, (7) RX821002 and (8) tolazoline.

Table 1
IC_{50} values for the inhibition of binding of [^{3}H](+)-MK-801 to rat brain membranes

Drug	[^{3}H](+)-MK-801 binding (IC_{50}, μM)
(+)-MK-801	0.008
Ketamine	1.6
Antazoline	31.5
Phentolamine	122
Naphazoline	150
Cirazoline	155
Efaroxan	190
Idazoxan	299
Clonidine	482
RX821002	562
Tolazoline	654

IC_{50} values were determined directly by simultaneous non-linear regression analysis (EBDA-LIGAND programs) of data from 2–3 independent experiments for each drug. IC_{50} standard errors were less than 5% of the reported mean values. Computer-assisted curve fitting demonstrated that, for all the drugs tested, the best fit was to a single-site binding model ($P < 0.0001$).

imidazoli(di)ne drugs as promoters of insulin release from rat insulinoma (RIN-5AH) cells (Olmos et al., 1994). In fact, there was a very good correlation ($r = 0.93$) between the IC_{50} values for the inhibition of [^{3}H](+)-MK-801 binding to rat brain membranes and the EC_{50} values of the same compounds stimulating insulin release (Fig. 1B). This close correlation suggests that these imidazoli(di)ne drugs interact with a similar phencyclidine binding site linked to both cation channels.

4. Discussion

The present data demonstrate the novel finding that several imidazoli(di)ne drugs fully inhibit [^{3}H](+)-MK-801 binding to NMDA receptors in rat brain membranes. Electrophysiological studies have demonstrated that a number of compounds, including phencyclidine, ketamine and MK-801, are functional antagonists of the effects of glutamate at the NMDA receptor by interacting with a site distinct from the agonist binding site that is within or closely associated with the ion channel of the receptor complex (Huettner and Bean, 1988). This phencyclidine site is also present in the nicotinic acetylcholine receptor (Oswald et al., 1983) and in the delayed rectifier K^+ channel (Bartschat and Blaustein, 1988).

These findings suggest the hypothesis that the imidazoli(di)ne drugs interact with a common phencyclidine binding site located in the NMDA receptor, nicotinic acetylcholine receptor and ATP-sensitive K^+ channel. If this was true, a similar pharmacological profile would be expected for the interaction of the imidazoli(di)ne drugs with the different cation channels. Patch-clamp experiments have demonstrated that stimulation of insulin release by imidazoli(di)ne compounds in pancreatic β-cells and RIN cells results from the blockade of ATP-sensitive K^+ channels (Dunne, 1991; Jonas et al., 1992). In support of the above hypothesis, a very good correlation was found between the potency of eight imidazoli(di)ne drugs inhibiting [^{3}H](+)-MK-801 binding and the potency of the same drugs stimulating insulin release (i.e. blocking ATP-sensitive K^+ channels) from RIN-5AH cells (Fig. 1B).

NMDA receptors have been implicated in the pathophysiology of neuronal death and clear protective effects of the NMDA receptor blocker MK-801 have been reported in animal models of ischaemia (Ozyurt et al., 1988). The present results demonstrating an interaction of imidazoli(di)ne compounds with NMDA receptors could be the basis of the reported neuroprotective actions of the imidazoline idazoxan and the structurally related drug, rilmenidine, in the ischaemic brain (Gustafson et al., 1989; Maiese et al., 1992).

Acknowledgements

This study was supported by DGICYT Grant PB94-0002-Mod C from the Ministerio de Educación y Ciencia (Spain). G.O. was also supported by the DRAC program of mobility (Institut Joan LLuís Vives). J.A.G.-S. is a member of the Institut d'Estudis Catalans.

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