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Research Report

Moclobemide attenuates anoxia and glutamate-induced neuronal damage in vitro independently of interaction with glutamate receptor subtypes

Marc Verleye[a,*], *Remy Steinschneider*[b], *François Xavier Bernard*[b], *Jean-Marie Gillardin*[a]

[a]*Biocodex, Département de Pharmacologie, Zac de Mercières-Chemin d'Armancourt, 60200 Compiègne, France*
[b]*BIOalternatives, 86160 Gençay, France*

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ABSTRACT

Recent data suggested the existence of a bidirectional relation between depression and neurodegenerative diseases resulting from cerebral ischemia injury. Glutamate, a major excitatory neurotransmitter, has long been recognised to play a key role in the pathophysiology of anoxia or ischemia, due to its excessive accumulation in the extracellular space and the subsequent activation of its receptors. A characteristic response to glutamate is the increase in cytosolic Na^+ and Ca^{2+} levels which is due mainly to influx from the extracellular space, with a consequent cell swelling and oxidative metabolism dysfunction. The present study examined the in vitro effects of the antidepressant and type-A monoamine oxidase inhibitor, moclobemide, in neuronal–astroglial cultures from rat cerebral cortex exposed to anoxia (for 5 and 7 h) or to glutamate (2 mM for 6 h), two in vitro models of brain ischemia. In addition, the affinity of moclobemide for the different glutamate receptor subtypes and an interaction with the cell influx of Na^+ and of Ca^{2+} enhanced by veratridine and K^+ excess, respectively, were evaluated. Moclobemide (10–100 μM) included in the culture medium during anoxia or with glutamate significantly increased in a concentration-dependent manner the amount of surviving neurons compared to controls. Moclobemide displayed no binding affinity for the different glutamate receptor subtypes ($IC_{50} > 100$ μM) and did not block up to 300 μM the entry of Na^+ and of Ca^{2+} activated by veratridine and K^+, respectively. These results suggest that the neuroprotective properties of moclobemide imply neither the glutamate neurotransmission nor the Na^+ and Ca^{2+} channels.

1. Introduction

Depressive symptoms are observed in many organic brain diseases in the elderly, particularly in stroke, degenerative or vascular dementias and Parkinson's disease. Emotional disturbances, such as depressed mood, apathy, anxiety, and aggression, are among the most common and problematic symptoms in neurodegenerative disorders (Reisberg et al.,

* *Corresponding author*. Fax: +33 3 44 86 82 34.
E-mail address: m.verleye@biocodex.fr (M. Verleye).

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; bFGF, basic fibroblast growth factor; KA, kainic acid; MAO, monoamine oxidase; MCB, moclobemide; n_H, Hill coefficient; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; QA, quisqualic acid; VSCCs, voltage-sensitive calcium channels; VSSCs, voltage-sensitive sodium channels

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1987). In the past 15 years, several studies in the elderly population have suggested the existence of a bidirectional relation between depression and various neurological disorders including dementia, epilepsy, stroke and cerebrovascular diseases. These latter diseases might predispose, precipitate, or perpetuate some late-life depressive syndromes (Alexopoulos et al., 1997). Conversely, depression may lead to cognitive impairment and cardiovascular diseases, which in turn may lead to subtle brain impairment, thereby causing more depression and cognitive impairments, and so on (Gallarda, 2004; Krishnan et al., 2005; Naarding et al., 2005). Oxygen delivery to brain tissues is closely regulated and essential for brain function. Failure of oxidative metabolism is the core destructive mechanism in stroke, and pure hypoxia–ischemia also produces irreversible brain damage selective for neurons (Gorgias et al., 1996). Single neuronal lesions or an accumulation of lesions exceeding a threshold are hypothesized to be central mechanisms in vascular depression in elderly people (Alexopoulos et al., 1997). In addition, it is well known that a massive increase of extracellular glutamate levels, resulting from an increased release and/or from a decreased uptake (Jabaudon et al., 2000) with a subsequent activation of its receptors, occurs during hypoxic–ischemic events with consequent neuronal death by excitotoxicity (Benveniste et al., 1984). Glutamate activates three major families of ionophore-linked receptors identified by their preferred agonists: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) or quisqualic acid (QA) and kainic acid (KA) (Watkins et al., 1990). Their prolonged stimulation triggers excessive cell Ca^{2+} influx, but it has been shown that these different glutamate receptor subtypes do not participate equally in excitotoxicity (see review Choi, 1992). However, the step implying cell Ca^{2+} entry is preceded by an early and short step marked by an excessive Na^{+} influx (with passive influx of Cl^{-} and water) resulting in cell volume expansion with damage (Choi, 1987, 1992; Lipton, 1999). Although our knowledge of glutamate-induced excito- or neurotoxicity is far from complete, the increased intracellular accumulation of Na^{+} and mainly of Ca^{2+} following an excessive entry elicited by activation of glutamate receptors (mainly the *N*-methyl-D-aspartate type) is largely recognized as an essential event and is suspected to contribute to cell death (Choi, 1992; Doble, 1999; Kahlert et al., 2005; Monaghan et al., 1989; Olney, 2002; Nicholls, 2004). In some cell types such as neurons, oxidative glutamate toxicity requires monoamine metabolism as a source of free radicals (Maher and Davis, 1996), which are important mediators of neurodegeneration resulting from cerebral ischemia (Ames and Shigenaga, 1992). The present studies focused on moclobemide because it is an antidepressant (Bonnet, 2003; Da Prada et al., 1989a) and type-A monoamine oxidase inhibitor with in vivo neuroprotective properties in rodent models (Ulugol et al., 1995). However, the mechanism(s) of these neuroprotective effects are not understood (Boland et al., 2002, 2003; Li et al., 2003). Although monoamine oxidase A inhibition could explain at least in part the neuroprotective effects of moclobemide (Haefely et al., 1993), there are fragmentary and incomplete data about possible interactions between moclobemide and the different glutamate receptor subtypes (Burkard et al., 1989). Since it has been shown that both NMDA and non-NMDA antagonists display neuroprotective properties both in vitro and in vivo (Bonde et al., 2005; Choi et al., 1988; Glass et al., 2004; Volbracht et al., 2006), a series of studies were designed to elucidate the mechanism(s) of the neuroprotective actions of moclobemide. A first study examined moclobemide effects in two in vitro models of cerebral ischemia: anoxia followed by re-oxygenation and glutamate-induced neuronal toxicity in rat cerebrocortical mixed neuron–astrocytes cultures. In a second set of experiments, a possible interaction between moclobemide and the subtypes of ionotropic glutamate receptors was examined by using radioligand binding assays in membrane preparations of rat cerebral cortex. In a third set of experiments, considering the complexity associated with time course of cell ionic influxes elicited by intense exposure to glutamate, the effects of moclobemide on sodium and calcium cell entries were investigated in suitable simple cell models: sodium cell entry was measured following veratridine-induced voltage-sensitive Na^{+} channels (VSSCs) coupled to glutamate receptors activation in neuroblastoma human cells. Calcium cell entry was evaluated following Ca^{2+}-permeable NMDA-sensitive glutamate receptors and/or voltage-sensitive calcium channels (VSCCs) activation by K^{+} excess in rat cultured A7r5 aortic smooth muscle cells. Veratridine, a VSSCs activator (Alkadhi and Tian, 1996) and K^{+} at a high concentration were cell membrane depolarizing agents mimicking glutamate exposure.

2. Results

2.1. *Neuroprotective effect against anoxia or glutamate-induced toxicity*

The cultures exposed for 5 and 7 h to anoxia showed a significant decrease in neuronal labelling of 76% and 95%, respectively, compared with control cell cultures (Figs. 1–3). The presence of moclobemide during 5 and 7 h anoxia challenge significantly inhibited in a concentration-dependent manner the anoxia-elicited decrease in neuronal labelling with a significant effect from the 10 μM dose (Fig. 1) and from the 30 μM dose (Figs. 2 and 3), respectively. The positive reference, bFGF at 10 ng/ml reversed the reduction of neuronal labelling induced by the exposure of 5 and 7 h of anoxia. When applied alone, the two compounds, moclobemide at the highest tested dose (Fig. 3) and bFGF at 10 ng/ml, had no intrinsic effects (neurotrophic or neurotoxic) in control cultures. Neuronal labelling revealed that the cell viability was decreased by 85% to 97% after 6 h treatment with 2 mM glutamate compared to the neuronal labelling of control cell culture (Figs. 4 and 5). The concomitant addition of moclobemide at the three concentrations tested or 10 ng/ml bFGF significantly reduced the glutamate-associated decrease in neuronal labelling. The magnitude of the protective effect of moclobemide against glutamate neurotoxicity increased in a concentration-dependent manner. As before, when applied alone, moclobemide at the highest tested dose (Fig. 5) and bFGF were devoid of any effects in control cell cultures.