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Signal transduction underlying carbachol-induced PGE_2 generation and cox-1 mRNA expression of rat brain

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Abstract

In this paper we have determined the different signal pathways involved in M_1 and M_3 muscarinic acetylcholine receptor (mAChR) dependent stimulation of cyclo-oxygenase 1 (cox-1) mRNA gene expression and PGE_2 production on rat cerebral frontal cortex. Carbachol stimulation of M_1 and M_3 mAChR exerts an increase in cox-1 mRNA gene expression without affecting cox-2 mRNA expression and increased PGE_2 generation. Besides, increased phosphoinositide (PI) turnover and stimulation of nitric oxide synthase (NOS) and cyclic GMP (cGMP) production. Inhibitors of phospholipase A_2 (PLA_2), COX and phospholipase C (PLC), calcium/calmodulin (CaM), NOS and soluble guanylate cyclase prevent the carbachol effect. These results suggest that carbachol-activation of M_1 and M_3 mAChR increased PGE_2 release associated with an increased expression of cox-1 and NO–cGMP production. The mechanism appears to occur directly to PLC stimulation and indirectly to PLA_2 activation. These results may contribute to understand the effects and side effect of non-steroidal anti-inflammatory drugs in patients with cerebral degenerative diseases.
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1. Introduction

Cyclo-oxygenase (COX) is the enzyme that catalyzes the first steps in the biosynthesis of prostaglandins (PGs) from the substrate arachidonic acid (AA).

COX activity has long been studied in preparations from sheep seminal vesicles and this enzyme was cloned by three separate groups (Merlie et al., 1988; Yokoyama et al., 1988). The discovery of a second form of COX in the early 1990s was a landmark event in prostanoid biology (Herschman, 1996; Bakhle and Bottin, 1996; Jouzeau et al., 1997).

The inducible enzyme COX-2 is very similar in structure and catalytic activity to the constitutive COX-1. The biological activity of both isoforms can be inhibited by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) (Vane, 1971).

The main reason for labeling COX-1 and COX-2 as physiological and pathological, respectively, is that most of the stimuli known to induce COX-2 are those associated with inflammation, i.e. bacterial lipopolysaccharide (LPS) and cytokines such as interleukin (IL)-1, IL-2 and tumor necrosis factor (TNF)-α. Corticosteroids decrease induction of COX-2 as well as the

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anti-inflammatory cytokines (IL-4, IL-10, IL-13) (Bakhle and Bottin, 1996; Onoe et al., 1996).

COX-1, as a constitutive form, is distributed in neurons throughout the brain, but is most prevalent in forebrain, where prostaglandin (PG) of E series (PGE_2) may be involved in complex integrative functions, such as modulation of the autonomic nervous system (Yamagata et al., 1993; Breder et al., 1995). COX-2 is expressed constitutively in only a few tissues including the brain and this expression is restricted to certain parts of the central nervous system (CNS), mainly the cortex, hippocampus, hypothalamus and spinal cord (Breder and Saper, 1996). It is the predominant isoform in the brains of neonate pigs (Jones et al., 1993) and in the spinal cord of the rat (Beiche et al., 1996), while human brain tissues contain equal amounts of cox-1 and cox-2 mRNAs (O'Neil and Fort-Hutchinson, 1993).

The correlation between COX isoforms, PGE_2 and CNS diseases, such as Alzheimer's disease, has been analyzed but the true significance and the normal risk for these diseases taking NSAIDs as anti-inflammatory therapy are still controversial (Cochran and Vitek, 1996; McGeer and McGeer, 1995). The powerful techniques of molecular biology have provided an extensive description of cox-1 and cox-2 gene expression and their mRNA, supporting the involvement of one or both isoforms in physiological or/and pathological processes. All such evidence, nowadays, is subject to debate and further studies are essential to reach definitive conclusions (Beiche et al., 1996; O'Neil and Fort-Hutchinson, 1993).

Release of AA in response to muscarinic acetylcholine receptor (mAChR) agonists has been reported in astrocytoma cells that possess M_3 mAChR subtypes (Wall et al., 1991) and in cells transfected with cDNAs that code for human M_1, M_3 and M_5 mAChRs (Felder et al., 1989). Although responses to agonists that engage G-protein-coupled receptors show some overlap, there are several distinct responses for each agonist, attributable to efficient coupling of G-protein α subunits to receptors (Aragay et al., 1995; Post et al., 1996) or to triggering of additional signaling pathways (Grabham and Cunningham, 1995; Collins et al., 1997).

Muscarinic AChR-triggered phospholipase A_2 (PLA_2)-catalyzed AA release with subsequent increase in COX activity with augmented PGE_2 generation, has been shown to be coupled to the activation of calcium entry from the extracellular medium and intracellular calcium signals (Shuttleworth and Thompson, 1998), playing a central role in the release of AA triggered by neurotransmitters (Bayon et al., 1997). This AA can be used by COX enzyme and exert physiological functions on its own. Like angiotensin, carbachol has been shown to stimulate PGE_2 release through PLA_2 activation (Schlondorff et al., 1997). Indeed, carbachol is also able to activate selected populations of mAChRs, thus increasing PGE_2 production in cat brain (Navarro et al., 1998).

Previously we have demonstrated that carbachol acts on rat frontal cortex as an early positive regulator of M_1 mAChR-mRNA expression, closely correlating with NOS-mRNA using common enzymatic pathways (Sterin-Borda et al., 2003). The aim of this work was to determine whether the mAChR agonist carbachol is able to induce early cox mRNA gene expression and whether such induction correlates with PGE_2 production. The implication of PLC and PLA_2 signal transduction underlying carbachol-induced PGE_2 generation was also studied.

In the present study, we show that the activation of rat cerebral frontal cortex M_1 and M_3 mAChR preparations leads to increased generation of PGE_2 preceded by an activation of PLA_2 and PLC; and increased induction of cox-1 without affecting cox-2 mRNA levels. These events are associated with selective activation of several mAChR subtypes, which was prevented by mAChR antagonist agents. In addition, these findings suggest the participation of cerebral mAChR subtypes in a cross-talk mechanism that involves carbachol-mediated coupling of PLA_2 and PLC and cox-1 gene expression.

2. Materials and methods

2.1. Rat cerebral frontal cortex membrane preparations

Male Wistar rats (obtained from the Pharmacology Unit, School of Dentistry, University of Buenos Aires) were housed in our colony in small groups and kept in automatically controlled lighting (lights on 08:00–19:00) and uniform temperature (25 °C) conditions. All animals were used at 3–4 months of age and were cared for in accordance with the principles and guidelines of the National Institutes of Health (NIH N° 8023, revised 1978). Efforts were made to minimize animal suffering such as: killing under anesthesia and reducing the number of animals, as well as using the same animal for all enzymatic assays. Membranes from cerebral frontal cortex were prepared as previously described (Borda et al., 1998). Briefly, tissues were homogenized in an Ultraturrax at 4 °C in 5 volumes of 10 mM potassium phosphate buffer, 1 mM $MgCl_2$, 0.25 M sucrose pH 7.5 (buffer A), supplemented with 0.1 mM phenyl methyl sulfonyl fluoride (PMSF), 2 μg ml^{-1} leupeptin and 1 μM pepstatin A. The homogenate was centrifuged twice for 10 min at 3000 g, then at 10,000 g and 40,000 g at 4 °C, for 15 and 90 min, respectively. Resulting pellets were resuspended in 50 mM phosphate buffer with the same protease inhibitor pH 7.5 (buffer B).

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