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Promiscuous coupling and involvement of protein kinase C and extracellular signal-regulated kinase 1/2 in the adenosine A_1 receptor signalling in mammalian spermatozoa

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ABSTRACT

Mammalian spermatozoa require a maturational event after ejaculation that allows them to acquire the capacity for fertilisation. This process occurs spontaneously during the transit through the female reproductive tract where spermatozoa are in contact with micromolar concentrations of adenosine that might act as a capacitative effector. This study shows that the adenosine A_1 receptor agonist, 2-chloro-N^6-cyclopentyladenosine, can induce capacitation, i.e., the ability to undergo the acrosome reaction and to become fertile. This receptor, already known to be bound to $G\alpha_{i2}$, is also bound to $G_{q/11}$. These G proteins are functional in the signalling pathway elicited by the A_1 receptor and correlate with the multiple intracellular events that follow its activation. The use of protein kinase C isoform inhibitors and MEK inhibitors, resulting in the abolition of the biological response to the selective agonist, indicates the involvement of protein kinase C and MEK in its signalling. In agonist-treated spermatozoa an extracellular calcium influx, involvement of α and γ PKC isoforms and transient phosphorylation of ERK1/2 have been observed. Our results, besides showing that adenosine A_1 receptor prompts mammalian spermatozoa to undergo the acrosome reaction hence supporting a role for adenosine as agent for fertilisation, show that 2-chloro-N^6-cyclopentyladenosine triggers signalling mechanisms that involve both $G\alpha_{i2}$ and $G_{q/11}$, extracellular calcium influx, modulation of classical Ca^{2+}-dependent PCK isoforms and up-regulation of the ERK1/2 phosphorylation.

1. Introduction

Adenosine is an important signalling molecule that elicits a large number of biological effects via four G protein-coupled proteins denoted A_1, A_{2A}, A_{2B}, A_3 receptors (ARs) [1]. The four adenosine receptors couple via G proteins to an intricate network of signalling pathways that enables the endogenous modulator adenosine to induce a variety of responses in cells [2].

A multiplicity of G proteins coupling has been documented for a variety of GPCRs and the simultaneous functional coupling with unrelated G proteins provides a mechanism for the activation of multiple intracellular effectors by a single receptor [3]. It is known that adenosine A_1Rs couple to

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pertussis toxin (PTX) sensitive G proteins ($G_{\alpha i1}$, $G_{\alpha i2}$, $G_{\alpha i3}$, and G_o) and stimulates numerous intracellular signalling events [4–6]. Experimental results obtained with cultured smooth muscle cells [4] and astrocytes [7], chick embryo ventricular myocytes [6], cardiac myocytes [8,9], transfected CHO [10] and COS-7 cells [11] link the adenosine A_1 receptors (A_1R) to phospholipase C (PLC), protein kinase C(PKC), and extracellular-regulated kinase (ERK)1/2 activation and suggest that $G_{i/o}$ βγ-subunit might be involved either in the direct coupling of A_1R to PLC or in the augmentation of $G_{q/11}$-coupled receptor response [12]. Changes in second messengers suggest that this receptor is able to adopt agonist-specific conformations that lead to the differential activation of G_i, G_s and G_q [13].

Adenosine A_1 receptors, present in mammalian spermatozoa and mainly localised at the acrosomal domain, the equatorial segment, and the middle piece [14], are coupled to a PTX sensitive $G_{\alpha i2}$ protein and cyclopentyladenosine (CPA), a specific adenosine A_1R agonist, activates PLC with subsequent IP_3 generation and release of intracellular calcium [15]. Studies with adenosine A_1R knockout mice show that the receptor must be functional for optimal sperm activation and in vivo fertility [16]. Mammalian ejaculated spermatozoa are infertile and two sequential activation processes, namely capacitation and acrosome reaction, are needed to successfully transit through the female reproductive tract and fertilize an egg. Spermatozoa, undergoing a series of biochemical and ultrastructural changes as responses to changes in the surrounding environment [17,18], presumably possess receptors that sense environmental signals and translate them into cellular responses that are necessary to acquire the ability to fertilize an egg. During the transit in the female genital tract, spermatozoa are in contact with adenosine at micromolar concentrations [19,20] hence, the proposal that the nucleosides might represent one of the multiple capacitative effectors. Capacitation is mainly regulated by increase in intracellular cAMP and increase in PKA activity that mediate protein tyrosine phosphorylation, an event regarded as a capacitation marker [15,21–23].

The capacitative effect of adenosine A_1R selective agonist is not related to receptor-mediated cAMP increase since this receptor is coupled with the inhibitory subtype $G_{\alpha i2}$ that leads to the inhibition of adenylyl cyclase. However, this receptor stimulates phospholipase C (PLC) and can increase IP_3 levels [15] that, in turn, acting on specific IP_3 receptors, stimulates intracellular calcium release, an event strongly related to capacitation [24]. IP_3 receptors are present in mammalian spermatozoa [25–27]. Components of the ERK pathway are also present in spermatozoa and involved in capacitation [28,29] and acrosome reaction [30] but sperm receptors responsible for activation of the ERK pathway during capacitation and acrosome reaction have not yet been identified.

It is suggested that GPCRs can be functionally coupled to multiple G proteins of different classes and that such promiscuous coupling is probably critical in modulating the efficacy and the potency of cell signalling [31]. This study investigates whether a promiscuous G protein coupling to adenosine A_1R could explain the multiple intracellular signalling events elicited by its activation. Assuming sperm capacitation as the biological response to the agonist, we were able of clarifying some aspects, i.e., cellular calcium influx, involvement of PKC and ERK1/2, of the signalling triggered by the selective A_1 agonist chloro-N^6-cyclopentyladenosine (CCPA) in mammalian spermatozoa.

2. Materials and methods

2.1. Materials

All reagents used in the study were from Sigma Chemical Co. (St. Louis, MO), unless otherwise stated. Bisindolylmaleimide V and U0124 were from Calbiochem (La Jolla, CA).

2.2. Medium

BWW medium consisted of 95 mM NaCl, 44 μM sodium lactate, 25 mM $NaHCO_3$, 20 mM Hepes, 5.6 mM D-glucose, 4.6 mM KCl, 1.7 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 0.27 mM sodium pyruvate, 5 U/ml penicillin, 5 μg/ml streptomycin, pH 7.4 and 0.3% BSA, unless otherwise indicated.

2.3. Mice

Adenosine A_1R knockout and wild type C57BL/6 mice were a gift of Dr. Johansson (Karolinska Institutet, Stockholm, Se). All the experiments were conducted using protocols following EUR directives, approved by the Institutional Animal Care and Use Committee of Perugia University.

2.4. Sperm collection and assessment of capacitation

Murine epididymal spermatozoa were extracted from adult mice (8–14 weeks) and Zonae Pellucida (ZP) were prepared from homogenised ovaries of 22-day-old virgin female as described [16]. The ability of the spermatozoa to respond to ZP and undergo the acrosome reaction was utilised as an assay for capacitation since the ZP-induced acrosome reaction occurs only in capacitated spermatozoa. 10×10^6 sperm, incubated for the indicated times in BWW at 37 °C in 5% CO_2/95% humidified air, were assessed for the capacitated state following incubation of the sperm with 2 ZP/μL for 30 min.

Human semen with normal sperm characteristics according to World Health Organisation criteria (vol. ~2 ml, concentration $>20 \times 10^6$ cells/ml; motility >50%, normal morphology >15%) was collected by masturbation from healthy donors. Approval for the donation of human semen samples was obtained from the University of Perugia human ethics committee. After liquefaction at 37 °C, motile spermatozoa were selected and suspended in BWW medium as described [15]. Sperm were assayed for capacitation by induction of AR with L-α-lysophosphatidylcholine (LPC) at 100 μg/ml. This concentration of LPC was previously shown to induce the AR in capacitated sperm while having no effects on uncapacitated sperm. Prior to drying and staining, randomly selected slides containing ~10^5 cells were examined to verify sperm motility and viability. The acrosomal status of the sperm was determined on air-dried sperm smears by Coomassie blue staining. At least 200 cells were scored with an Axioplan Zeiss microscope (Gottingen, Germany). The