HVCN1 Channels Related Sperm Motility

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In mammals, sperm capacitation is characterized by a set of physiological changes preparing the male gamete for fertilization, being the intracellular alkalinization a key event. Changes in intracellular pH (pHi) during capacitation are induced by different channels, including HCO3- membrane transporters, Na+-H+ exchangers (NHEs), monocarboxylate transporters (MCTs) and voltage-gated proton channels (HVCN1). HVCN1 channels belong to the superfamily of voltage-gated cation channels; they drive protons more quickly and efficiently than transporters or exchangers do and lead them unidirectionally to the extracellular medium. HVCN1 channels have been identified in human, bull and boar sperm, forming dimers of a molecular weight of 70-73 kDa; however, their action and regulation mechanisms are poorly understood.

A recent study has focused on the physiological role of HVCN1 channels during in vitro capacitation using the pig sperm as a model. This functional approach was carried out pharmacologically through using 2-guanidino benzimidazole (2-GBI), a specific blocker of HVCN1 channels. Sperm samples were incubated in in vitro capacitating medium for 300 min; after 240 min of incubation, progesterone was added to induce sperm hyperactivation and acrosomal exocytosis. To address the physiological role of HVCN1 channels during in vitro capacitation of pig sperm, some samples were incubated in the presence of 2-GBI blocker added at time 0 (Experiment 1). Moreover, and in order to understand the functional relationship between progesterone and HVCN1 channels, 2-GBI blocker was added together with progesterone at 240 min of incubation, in a second group of samples (Experiment 2). Sperm viability, sperm motility and kinematics, acrosomal exocytosis, membrane lipid disorder, intracellular calcium levels of the sperm head and tail, and mitochondrial membrane potential were evaluated after 0, 60, 120, 180, 240, 250, 270 and 300 min of incubation.

The results obtained showed that HVCN1 channels are essential for the maintenance of viability, motility and kinematics of pig sperm during in vitro capacitation and progesterone-induced acrosomal exocytosis. While a close relationship between HVCN1 activation and mitochondrial membrane potential was observed, HVCN1 channels were not found to be involved in the regulation of Ca2+ influx to the sperm tail. Despite further research being necessary, HVCN1 activation could also modulate Ca2+ entrance to the sperm head and prevent premature acrosomal exocytosis during in vitro capacitation of pig sperm.

Keywords: In vitro capacitation ; mammal sperm ; HVCN1 channels ; sperm motility ; 2-GBI ; progesterone

1. Introduction

Intracellular alkalinization during capacitation is induced by different channels, including HCO_3^- membrane transporters, Na^+-H^+ exchangers (NHEs), monocarboxylate transporters (MCTs) and voltage-gated proton channels (HVCN1) [1][2], despite their specific role in pHi regulation being unclear [1][2][3][4]. Taking into account the relevance of sperm pHi alkalinization for oocyte fertilization, the knowledge of the regulation mechanisms of sperm pHi would provide new insights on the etiology and treatment of male infertility.

2. Development

HVCN1 channels belong to the superfamily of voltage-gated cation channels [16,17]; they are composed of two subunits, each containing a proton-permeable voltage-sensing domain (VSD), joined by a coiled-coil domain in the C-terminus [4][5][6]. HVCN1 channels differ from other voltage-gated ion channels because they do not have a pore-forming domain; the proton pathway locates within the VSD, so each subunit has its own pore and can function independently [4][7][8][9]. HVCN1 channels drive protons more quickly and efficiently than transporters or exchangers do, and lead them unidirectionally to the extracellular medium [10].

Interestingly, not only does HVCN1 gating rely on membrane voltage, but also on the pH difference (DpH) across the plasma membrane [11]. Therefore, when changes in intracellular (pHi) and extracellular pH (pHo) are not associated to changes in DpH (pHo – pHi = 0), the voltage dependence of HVCN1-activation is low; conversely, when changes result in

DpH>0 or DpH<0 its voltage activation is high [12]. Therefore, the voltage activation threshold of HVCN1 channels is dependent on the DpH across the plasma membrane [13].

HVCN1 channels have an essential regulating role in several cell types [14]. They have been identified in human, bull and boar sperm, forming dimers of a molecular weight of 70-73 kDa; interestingly, these channels are not present in mouse sperm [11][13]. HVCN1 channels are implicated in the activation of sperm motility after ejaculation and showed enhanced activity in capacitated spermatozoa [11][13], despite their action and regulation mechanisms being poorly understood.

3. Data, Model, Applications and Influences

In a recent study, we investigated the physiological role of HVCN1 channels during in vitro capacitation using the pig sperm as a model [15]. This functional approach was carried out pharmacologically through using 2-guanidino benzimidazole (2-GBI), a specific blocker of HVCN1channels that inhibits their proton conductance via binding the intracellular VSD domain in the opened channel [10][6]. Sperm samples were incubated in in vitro capacitating medium for 300 min; after 240 min of incubation, progesterone was added to induce sperm hyperactivation and acrosomal exocytosis. To address the physiological role of HVCN1 channels during in vitro capacitation of pig sperm, some samples were incubated in the presence of 2-GBI blocker at 1, 5 or 10 mM added at time 0 (Experiment 1). Moreover, and in order to understand the functional relationship between progesterone and HVCN1 channels, 2-GBI blocker was added together with progesterone at 240 min of incubation, in a second group of samples (Experiment 2) [15]. Sperm viability, sperm motility and kinematics, acrosomal exocytosis, membrane lipid disorder, intracellular calcium levels of the sperm head and tail, and mitochondrial membrane potential were evaluated after 0, 60, 120, 180, 240, 250, 270 and 300 min of incubation [15].

Our approach demonstrated that HVCN1 channels are essential for the maintenance of sperm viability and motility during in vitro capacitation. In both experiments, HVCN1 blockage lead to a decreased progressive sperm motility, which manifested associated with an impaired sperm kinematics, mainly due to alterations in sperm velocity and linearity, and a reduction in mitochondrial membrane potential [15]. In bull [1] and human sperm [16], HVCN1 channels are considered to be essential for the activation of progressive motility after ejaculation and in the regulation of hypermotility during capacitation. In both non-capacitated and capacitated human sperm, the intracellular alkalinization is critical for sperm motility activation, since it triggers the sperm-specific soluble adenylyl cyclase/protein kinase A pathway (sAC/cAMP/PKA), which stimulates cell metabolism and propels the axoneme [16]. In humans, HVCN1 activity is higher in capacitated than in non-capacitated sperm due the phosphorylation of the channel during capacitation [16][13]; the phosphorylation of this channel during sperm capacitation has not been demonstrated in other mammal species. The reduction in mitochondrial membrane potential after blockage suggest that HVCN1 channels are essential for pig sperm to increase their oxidative phosphorylation during sperm capacitation and acrosomal exocytosis induced by progesterone [15]. In agreement with our results, Musset et al. [17] reported that HVCN1 channels are involved in the generation of superoxide radicals in human sperm, which are reactive oxygen species resulting from the activity of the mitochondrial membrane chain. In humans, alterations in mitochondrial membrane potential observed after HVCN1 inhibition are also associated to the decrease of sperm viability [18].

During capacitation, HVCN1 channels have been suggested to induce hypermotility through the cAMP/PKA pathway in bull sperm [1], and via activating CatSper channels in human sperm [16][13][18][19]. In capacitated pig sperm, a clear functional relationship between HVCN1 activation and Ca^{2+} entrance to the sperm tail was not observed, thus suggesting that while HVCN1 channels are essential for pig sperm motility during in vitro capacitation, they do not appear to be crucial in the regulation of Ca^{2+} influx [15]. Despite further research being required, we hypothesize that Ca^{2+} entrance to the flagellum in capacitated pig sperm does not rely upon the activity of HVCN1 channels, but rather depend a direct and non-direct effect of progesterone on Ca^{2+} channels [15].

In rodents [20], cattle [21] and humans [16], intracellular alkalinization has been correlated with the activation CatSper channels and the rise in intracellular Ca^{2+} levels during sperm capacitation. Moreover, in human sperm, HVCN1 and CatSper channels are localized in the same plasma membrane domains thus evidencing their strong functional relationship. In our study, however, the absence of a strong relationship between HVCN1 activity and Ca^{2+} influx to the sperm tail suggests that not only is intracellular alkalinization regulated by HVCN1 channels in pig sperm, but also by other H^+ transporters [15]. Nevertheless, further research in necessary in order to identify and localize the channels implicated in pHi regulation and their functional relationship with calcium channels.

Little data exist about the effects of HVCN1 blockage on acrosomal exocytosis in mammalian species. In pig sperm, HVCN1 inhibition had a different effect on acrosomal exocytosis depending on whether the 2-GBI blocker was added at the beginning of the experiment (i.e. 0 min) or after 240 min of incubation [15]. In sperm incubated with 2-GBI from time 0

(Experiment 1), a premature acrosomal exocytosis was observed, which was associated to high plasma membrane lipid disorder and to increased Ca^{2+} levels in the sperm head. In these samples, the addition of progesterone acted as a potent acrosomal exocytosis inducer, neither a new rise in the Ca^{2+} levels of the sperm head nor a further increase in membrane lipid disorder were observed [15]. In agreement with our results, a close relationship between pHi and cholesterol content of the plasma membrane has been reported in human sperm [22]. Moreover, recent studies in rodents have demonstrated that the sterol efflux induces a local depolarizing effect on the plasma membrane that may activate different types of transient voltage-gated cation channels, leading to Ca^{2+} rises and triggering acrosomal exocytosis [23][24]. In contrast, when added at 240 min together with progesterone (Experiment 2), 2-GBI blocker had little effect on acrosomal exocytosis and plasma membrane lipid disorder, but it led to reduced Ca^{2+} levels of the sperm head [15]. Besides, zinc blocking of HVCN1 channels in capacitated human sperm reduces progesterone-induced acrosomal exocytosis due to the inhibition of CatSper channels, which again underpins the close functional relationship between these two kinds of channels in this species [18]. In capacitated pig spermatozoa, SLO1 blockage also inhibits progesterone-induced acrosomal exocytosis by reducing Ca^{2+} entrance to the sperm head [25], whereas the presence of zinc prevents acrosomal membrane modifications associated to acrosomal exocytosis [26].

The alterations observed after total blockage in Experiment 1 suggest that HVCN1 channels are essential to maintain pig sperm homeostasis during in vitro capacitation and for preventing premature sperm activation [15]. From Experiment 2, one can conclude that while HVCN1 channels are relevant for the rise in Ca^{2+} levels after progesterone addition, they are not specifically involved in the mechanism that triggers acrosomal exocytosis [15]. On the other hand, another interesting finding of our study was that HVCN1 blockage affected differently the intracellular Ca^{2+} flux to the sperm head and the sperm tail, thereby indicating that the involvement of HVCN1 channels in regulating these two stores is distinct [15]. These results are in line with previous studies performed in human [27], mouse [28] and pig sperm [25].

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