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Bicarbonate-induced membrane processing in sperm capacitation

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Abstract

During capacitation, major changes take place in the sperm plasma membrane so as to render it fusogenic and responsive to zona pellucida glycoproteins. However, the mechanisms involved have not been defined. As bicarbonate is known to be the key component that induces capacitation, we have investigated the bicarbonate-dependent changes in the boar sperm's plasma membrane architecture. We have discovered that bicarbonate induces a rapid collapse of phospholipid transverse asymmetry, exposing phosphatidylethanolamine and phosphatidylserine at the outer surface of the lipid bilayer. The collapse, which is reversible, is brought about as a result of activation of the phospholipid scramblase that exchanges phospholipids in a non-specific fashion between the two leaflets of the lipid bilayer. The activation takes place via a cyclic AMP–protein kinase A-dependent pathway and is initiated via stimulation of the so-called 'soluble' adenylyl cyclase in the sperm cell by bicarbonate. As a result of the collapse and the concurrent increase in phospholipid exchange, removal of cholesterol by albumin is facilitated (perhaps due to increased lipid packing disorder). This finding is in conflict with earlier surmises that cholesterol loss precedes activation of the cyclic AMP–protein kinase A axis. We have noted that not all cells in a given sperm population show rapid changes in response to bicarbonate stimulation; samples from individual boars also differ in their response. Maturation differences between cells have been found to play an important role in such functional heterogeneity.

Keywords: Sperm capacitation; Membrane processing; Bicarbonate; Phospholipid scrambling; Lipid packing disorder

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1. Introduction

At fertilization, the mammalian spermatozoon binds in a specific manner to the zona pellucida (outer coating of the egg) and, in response to this contact, exocytoses its acrosome. It then penetrates the zona pellucida by means of a combination of flexured ('hyperactive') motility and the activity of released or unmasked acrosomal hydrolases; once through the zona, it fuses with the egg cell beneath. As taken from the epididymis, the mature spermatozoon is unable to perform these functions. It needs to undergo a prior priming sequence known as capacitation. Capacitation is a lengthy process, taking some two hours on average to complete in the case of boar spermatozoa, and it clearly involves a number of molecular changes. However, although many biochemical and cell biological indicators of capacitation have been described, the sequence of steps to achieve full capacitation (i.e., the ability to interact immediately with the egg) has yet to be defined. Nevertheless, one aspect of capacitation is clear. The zona binding, and the two fusion events described above, all involve sperm plasma membrane function. Since the mature epididymal spermatozoon has no innate fusibility, nor does it show specific egg recognition, the capacitation process must involve considerable remodelling of the sperm plasma membrane. (For recent reviews on capacitation and capacitative processes, see [1–6].)

An important concept in connection with capacitation is that the process is a positive one. While some have implied that capacitation is a continuation of the maturation changes that take place in the epididymis, spermatozoa do not become capacitated during prolonged storage, either within this organ or after extrusion into a simple medium. Indeed, one of the major difficulties in achieving in vitro fertilization was to develop specific media for successful sperm–egg interaction in which capacitation necessarily had to take place. Some years ago, inspection indicated to us that the key components of IVF media were Ca^{2+}, serum albumin, and bicarbonate/CO_2. Studies by ourselves and others indicated that bicarbonate was the crucial in vitro capacitating agent for several animal species, including the pig. Bicarbonate is generally considered an ubiquitous ion. However, in the region of the epididymis where mature spermatozoa are stored, its levels are maintained considerably below those in the circulation; thus, the spermatozoa encounter a sudden rise in bicarbonate/CO_2 concentration on deposition in the female tract. Such a situation supports the concept that bicarbonate may also play an important capacitating role in vivo (see [2]). Over the last 10 years, therefore, we have sought to elucidate the action of bicarbonate.

As indicated above, the slowness with which capacitation takes place implies strongly that it consists of a series of sequential events. At the start of our studies, it was clear to us that a major problem in trying to understand capacitation was the fact that no serious attempts had been made to place the very many changes associated with capacitation in any temporal order. Given that bicarbonate had been identified as the essential capacitating agent, we sought to identify and understand the very earliest changes induced by the ion, hypothesizing that it was responsible for initiating (and sustaining) the process. Our investigations soon showed that physiological levels of bicarbonate induce rapid and major changes in plasma membrane architecture, which, we believe, have profound implications for the cell's fertilizing capability.