Lectins binding on human sperm surface increase membrane permeability and stimulate acrosomal exocytosis

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Cross-linked complexes formed between certain lectins and their specific multivalent carbohydrates and glycoconjugates on the sperm surface were studied for their ability to modify sperm membrane permeability and to induce the acrosome reaction. Wheat germ agglutinin (WGA), concanavalin A (Con A) and peanut agglutinin (PNA) increased the proportions of human spermatozoa permeable to the impermeable propidium iodide (31.9 compared with 13.8%, 38.4 compared with 18.4% and 72.7 compared with 18.9% respectively). Removal of sperm surface sialic acid by neuraminidase treatment was a prerequisite for Con A and PNA binding to the sperm surface. The percentage of permeable and acrosome-reacted spermatozoa was not affected by sperm treatment with 500 mU/ml Arthrobacter ureafaciens neuraminidase. WGA did not induce the acrosome reaction, whereas PNA induced the acrosome reaction regardless of the sperm capacitation status, allowing the proportion of acrosome-reacted spermatozoa to reach 27.7% of capacitated spermatozoa. However, the ability of Con A to induce the acrosome reaction was limited to uncapacitated spermatozoa. To test the physiological relevance of this study, uncapacitated human spermatozoa were incubated with the human zona pellucidae and the permeability of spermatozoa bound to the zona surface was analysed according to the time post-insemination. Two-thirds of spermatozoa bound to zona pellucida became permeable to propidium iodide in the first 30 min post-insemination and almost all bound spermatozoa became permeable to the impermeable dye after 60 min. Our results show that molecular interactions between human zona pellucida and sperm surface increase the permeability of sperm membranes; the cross-linked complexes formed by PNA lectin and its specific multivalent carbohydrates and glycoconjugates on the sperm surface were also able to increase sperm membrane permeability and to induce the acrosome reaction. These results suggest a role for the saccharide moieties of sperm surface glycoconjugates in the induction of the acrosome reaction.

Key words: acrosome reaction/capacitation/lectins/peanut agglutinin/receptor aggregation

Introduction

The capacitation event is considered to involve a series of cellular and molecular changes that prepare the spermatozoon for an exocytotic process, the acrosome reaction (reviewed by Langlais and Roberts, 1985; Sidhu and Guraya, 1989; Yanagimachi, 1994). Sperm capacitation has been viewed as a reversible phenomenon that results in a net decrease in negative surface charge, an efflux of membrane cholesterol, and an influx of calcium ions between the plasma and the outer acrosomal membranes (reviewed by Langlais and Roberts, 1985). Alteration of the cholesterol content of the erythrocyte membrane leads to changes in membrane properties including ion permeability and membrane fragility (reviewed by Langlais and Roberts, 1985). The acrosome reaction is characterized by sperm membrane vesiculation, fusion of the plasma membrane with the outer acrosomal membrane and release of the acrosomal content (reviewed by Sidhu and Guraya, 1989; Yanagimachi, 1994).

Human spermatozoa, especially those with normal morphology and an intact acrosome, bind to the zona pellucida (Liu and Baker, 1992, 1994) where the acrosome reaction occurs (Cross et al., 1988; Tesarik, 1989; Lee et al., 1992), probably induced by the human zona pellucida glycoprotein ZP3 (Barratt et al., 1993; Van Duin et al., 1994). In the mouse, acrosomal exocytosis seems to be initiated by the aggregation of sperm molecules recognized by ZP3 glycoproteins (Leyton and Saling, 1989). Whereas intact and solubilized human zonae pellucidae (Cross et al., 1988; Bielfeld et al., 1994) have acrosome reaction-inducing activity, neither ZP3 glycopeptides derived from pronase-digested ZP3 nor isolated oligosaccharides are able to induce the acrosome reaction (Florman et al., 1984; Leyton and Saling, 1989; Bunch et al., 1992). However, when ZP3 glycopeptides previously bound to the sperm surface were cross-linked by anti-ZP3 antibodies, induction of the acrosome reaction was elicited to the same extent as that occurring following exposure to whole zona pellucida (Leyton and Saling, 1989). Macek et al. (1991) hypothesized that ZP3 induces the acrosome reaction by virtue of multiple sperm-binding oligosaccharides bound to a protein core, which are able to cross-link sperm surface receptors. Moreover, aggregation of sperm surface receptors in the absence of zona pellucida components may induce the acrosome reaction, as
has been reported for aggregation of progesterone receptors in human (Tesarik and Mendoza, 1993) sperm surface galactosyltransferase (Macek et al., 1991) and proteinase inhibitor in mice (Aarons et al., 1992), or sperm plasma membrane antigen in ram (McKinnon et al., 1991). Receptor aggregation in response to biological stimuli has been described in other systems as an important mechanism for initiating signal transduction across the membrane (Schechter et al., 1979; Detmers et al., 1987; Ratcliffe et al., 1992; Gold et al., 1994).

The aim of our work was to study changes in membrane integrity and the occurrence of the acrosome reaction in human spermatozoa consequent to the binding of specific lectins to their respective receptors at the sperm surface. Propidium iodide, a cellular DNA dye impermeable to the cell plasma membrane, has been previously used to evaluate sperm membrane integrity (Harrison and Vickers, 1990; Garner and Johnson, 1995). Lectins are multimeric protein or glycoprotein molecules bearing more than one binding site for their characteristic sugar ligands (reviewed by Koehler, 1981). They are able to induce the aggregation of cell surface carbohydrate receptors and to form distinct cross-linked complexes with specific multivalent carbohydrates and glycoconjugates (Bhattacharyya et al., 1989; Gupta et al., 1994). Certain lectins have been reported to mimic typical signal transduction mechanisms induced by epidermal growth factor (EGF) (Zeng et al., 1995). Moreover, the role of carbohydrates in glycoprotein–hormone signal transduction has been established (Sairam 1989). In this study, we used the three following lectins: wheat germ agglutinin (Triticum vulgaris, WGA) which recognizes both sialic acid (NeuNaC) and N-acetylgalactosamine (GlcNAc) (Monsigny et al., 1980); peanut agglutinin (PNA) which had a strong specificity for disaccharides with terminal galactose (Gal) especially the D Gal α (1,3) D GalNAc disaccharide (Pereira et al., 1976); and concanavalin A (Con A) which recognizes mannose (Man) and glucose (Glc) and exhibits a particularly high affinity for complex and high mannose type glycopeptides on cell membrane (Brewer et al., 1985). WGA binds to terminal NeuNAc α (2,6) Gal/GalNAc glycoconjugates on the plasma membrane of human motile spermatozoa (Lassalle and Testart, 1994). The removal of sialic acid from the human sperm surface increases sperm–zona pellucida recognition, probably by unmasking certain sperm surface antigens implicated in the recognition mechanism (Lassalle and Testart, 1994). Binding sites for PNA and Con A on the sperm surface are also unmasked by removal of sperm surface sialic acid. The ability of these lectins to alter sperm membrane integrity and to induce the acrosome reaction was assessed taking into account the carbohydrate specificity and biochemical characteristics of each lectin. To test the physiological relevance of this study, uncapacitated human spermatozoa were incubated with human zonae pellucidae and the permeability to impermeable propidium iodide of spermatozoa bound to the zona pellucida surface was analysed according to the time post-insemination.

Materials and methods

**Media and chemicals**

BM1 medium was obtained from Ellios Bio Media (91430 Igny, France) and Percoll from Pharmacia Biotech S.A. (78051 Saint Quentin en Yvelines, France). Arthrobacter ureafaciens neuraminidase (EC 3.2.1.18), calcium ionophore A23187 and all lectins used in this study were purchased from Sigma (38297 La Verpillère, France).

**Sperm preparation and labelling with lectins**

Ejaculated fresh semen samples were obtained with consent from normal donors and liquefied for 30 min at 37°C and ~0.6 ml of samples was separated on a Percoll gradient (90/45%) as described by Lassalle and Testart (1994). After centrifuging for 25 min at 400 g, the 90% Percoll fractions were carefully separated and washed by dilution with 5 ml phosphate-buffered saline (PBS) and then centrifuged at 600 g for 7 min. The pellets were resuspended in 1 ml B2 medium. Before treatment, motile and non-motile spermatozoa were counted on a Malassez cell to verify the high (>80%) percentage of motile spermatozoa recovered in the 90% Percoll gradient. Sperm populations recovered in the 90% Percoll fraction after centrifugation on a discontinuous Percoll gradient have a high proportion of normal motile spermatozoa (Le Lannou and Blanchard, 1988) with intact plasma membrane (Lassalle and Testart, 1994) and high fertilizing ability (Hyne et al., 1983; Berger et al., 1985; Sapienza et al., 1993).

Preliminary studies (Lassalle and Testart, 1994) showed that <20% of spermatozoa recovered in the 90% Percoll fraction bound PNA and Con A lectins, whereas WGA binding occurred in at least 80% of this sperm population. However, the proportion of spermatozoa with binding sites for PNA and Con A reached >70% of the sperm population after removal of sperm surface sialic acid with 500 mIU/ml neuraminidase. Consequently, pretreatment of spermatozoa with 500 mIU/ml neuraminidase was performed before Con A or PNA treatment to optimize the binding of these two lectins to the sperm plasma membrane. Thus, sperm suspensions were either incubated for 30 min at 37°C with 20 μg/ml WGA or preincubated for 30 min at 37°C with 500 mIU neuraminidase/ml and then incubated for 30 min at 37°C with 20 μg/ml PNA or Con A.

When sperm neuraminidase or lectin treatments were performed immediately after separation on the discontinuous Percoll gradient and the washing procedure (<60 min after the semen liquefaction step), spermatozoa were considered to be uncapacitated. Sperm capacitation was obtained by a 24 h incubation of spermatozoa in B2 medium at room temperature (Lassalle and Testart, 1994).

**Assessment of sperm acrosome reaction**

Pisum sativum agglutinin (PSA) was shown to stain the acrosome contents of permeabilized acrosome-intact spermatozoa (Centola et al., 1990). Acrosome reaction was scored by using fluorescein isothiocyanate-conjugated PSA (FITC-PSA) (Cross et al., 1986; Centola et al., 1990). Lectin-treated sperm suspensions were washed twice in PBS to remove free lectin. Spermatozoa were permeabilized after dropwise addition of 0.5 ml 95% ethanol directly onto the sperm pellet followed by a 30 min incubation at 4°C. Ethanol was removed by a minimum of two centrifugation cycles; 40 μl of FITC-PSA stock solution (100 μg/ml) was then added and mixed with the resultant pellet. Sperm suspension (10 μl) was placed between slide and coverslip for observation under a Leitz microscope equipped with epifluorescence. Acrosome-reacted and acrosome-intact spermatozoa were scored according to Cross et al. (1986). The percentages of acrosome-reacted spermatozoa were estimated from at least 150 spermatozoa per preparation (Centola et al., 1990). A positive control was carried out by inducing the acrosome reaction by incubating (30 min at 37°C) a sperm suspension with calcium ionophore A23187 [stock solution in dimethyl sulphoxide (DMSO)] and frozen at −20°C at a final concentration of 20 μM.
**Assessment of sperm plasma membrane permeabilization changes**

Propidium iodide (PI), which binds to and stains cellular DNA, is impermeable to the plasma membrane and therefore can only enter and stain cells with permeable plasma membrane (Harrison and Vickers, 1990; Garner and Johnson, 1995). PI has previously been used to assess membrane integrity in mammalian spermatozoa and has been shown not to stain intact spermatozoa (Harrison and Vickers, 1990). The number of spermatozoa permeable to PI among highly motile sperm populations was scored. Stock solution (10 μl) of PI (0.5 mg/ml in PBS, stored in the dark at −20°C) was added to 1 ml of sperm preparation. Then 10 μl of the resulting suspension was placed between slide and coverslip for observation under both phase contrast and epi-fluorescence microscopy. This simultaneous observation was obtained by maintaining the light intensity at a sufficiently high level to score both non-fluorescent and fluorescent spermatozoa under phase contrast microscopy and sufficiently low to visualize the red fluorescence of PI-permeant spermatozoa. Percentages of spermatozoa permeant to PI were estimated from at least 200 spermatozoa per preparation.

**Effect of zona pellucida on the permeability of the sperm plasma membrane to propidium iodide**

Human oocytes remaining unfertilized 40–48 h after in-vitro insemination were stored at 4°C in ammonium sulphate solution for optimal zona pellucida conservation (Yoshimatsu et al., 1988). At 2 h before insemination, oocytes were thoroughly rinsed over a 90 min period in four successive baths of PBS-bovine serum albumin (BSA) then placed in B2 medium at 37°C before insemination.

To distinguish spermatozoa newly-bound to the zona pellucida surface from those previously bound during the in-vitro fertilization (IVF) procedure, spermatozoa recovered from the 90% Percoll fraction were stained (15 min, 37°C) with 0.3 ml FITC solution (0.25 mg/ml in PBS-glucose) according to the method of Liu et al. (1988). The number of motile spermatozoa was assessed before and after FITC staining. The sperm suspension was then washed twice with PBS and the final pellet was resuspended in B2 medium before dilution to obtain a concentration of 5×10⁶ motile spermatozoa/ml. Human zona pellucidae were placed in the sperm medium for 10–120 min before washing in PBS supplemented with 2% propidium iodide stock solution (0.5 mg/ml PBS). Zonae pellucidae were placed between slide and coverslip for observations performed under a Leitz microscope equipped with epifluorescence. The numbers of green (FITC) and red (PI) spermatozoa on each zona pellucida were determined using extinction and emission filters appropriate to each fluorescent probe. Spermatozoa previously bound during the IVF procedure showed red fluorescence on the head with an absence of green fluorescence on the tail. Spermatozoa bound to the zona pellucida during the experimental period were those with green fluorescence on the tail; among this latter population those spermatozoa which also showed red fluorescence on the head were considered to be permeable spermatozoa (Figure 1). The percentage of spermatozoa permeable to PI after binding to the zona pellucida was estimated for each zona pellucida. The mean number of bound spermatozoa, the mean number of bound permeable spermatozoa and the mean percentage of permeable spermatozoa per zona pellucida were calculated according to the time post-insemination.

**Inhibition of the ability of PNA to induce the acrosome reaction**

Two inhibitors of protein kinase (PK), calphostin C (PKC inhibitor) and KT 5720 (PKA inhibitor) (Calbiochem 92039, La Jolla, CA, USA) have previously been used to inhibit the acrosome reaction induced by PKA or PKC activators in human spermatozoa. The optimal concentration of each inhibitor was found to be 100 nM (Doherty et al., 1995). Stock solutions of calphostin C and KT 5720 were prepared by diluting 50 μg in 0.5 ml DMSO and aliquots were stored at 4°C. Each inhibitor was added at the final concentration of 100 nM) 15 min before addition of PNA and was present during the time of sperm incubation with PNA. Since the inhibitory properties of calphostin C are most effective in the presence of light (Bruns et al., 1991), an incandescent light was directed at all test tubes during the time of incubation (Doherty et al., 1995).

**Results**

**Permeabilization of the sperm membrane to propidium iodide induced by zona pellucida**

Two-thirds of zona pellucida-bound spermatozoa had become permeable to propidium iodide 15–30 min post-insemination. This proportion of permeable cells reached >89% after 45 min in culture (Table I) whereas only 13.1% (± 7.3%) of unbound spermatozoa present in the medium surrounding the zonae pellucidae were permeable to propidium iodide.

**Permeabilization of the sperm membrane to propidium iodide induced by lectins**

Treatment of spermatozoa with 500 IU/ml neuraminidase had no effect on their permeability to propidium iodide (22.3 compared with 18.6%, not significant; Table II). The percentage of spermatozoa with membranes permeable to propidium iodide showed a two-fold increase after treatment with either Ca²⁺ ionophore (27.7 compared with 14.5%, P <0.03), 20 μg/ml WGA (31.9 compared with 13.8%, P <0.004) or neuraminidase (20 μg/ml) Con A (38.4 compared with 18.4%, P <0.002; Table II). The percentage of spermatozoa with permeable membranes reached 72.7% after treatment with neuraminidase (20 μg/ml) PNA compared with only 18.9% of spermatozoa (P <0.0001) in the control samples (Table II).

**Acrosome reaction induced by lectins**

The percentages of acrosome-reacted spermatozoa were not affected by sperm treatment with 500 μIU/ml A. ureafaciens neuraminidase (uncapacitated, 12.7 compared with 10.7%; capacitated spermatozoa, 14.4 compared with 15.0%; Tables III and IV). Percentages of uncapacitated spermatozoa with reacted acrosome were significantly increased (as compared with their own control) after sperm treatment with 25 μM calcium ionophore A23187 (14.3 compared with 7.3%, P <0.0008) and with 500 μIU/ml neuraminidase followed by 20 μg/ml PNA (15.1 compared with 7.9%, P <0.0002) or 20 μg/ml Con A (17.2 compared with 10.7%, P <0.006; Table III). After capacitation (24 h at room temperature), the acrosome reaction was ~2.5-fold increased for neuraminidase-PNA (27.7 compared with 10.3%, P <0.0003) and Ca²⁺ ionophore-treated spermatozoa (33.2 compared with 12.8%, P <0.0001) whereas...
Figure 1. Effect of zona pellucida on the permeability of human sperm plasma membrane to propidium iodide. Spermatozoa previously bound during the in-vitro fertilization (IVF) procedure show red fluorescence on the head with an absence of green fluorescence on the tail. Spermatozoa bound to the zona pellucida during the experimental period are those with green fluorescence on the tail; among this sperm population those with red fluorescence on the head are considered to be permeable spermatozoa. This photograph was a superimposition of two epifluorescence images obtained after a dual recording procedure performed under a Biorad MRC-600 confocal laser scanning microscope equipped with extinction and emission filters appropriate to each fluorescent probe. Bar = 20 μm.

Table I. The ability of zona pellucida to increase the permeability of human spermatozoa to propidium iodide. The proportion of permeable spermatozoa is documented according to the time post-insemination. Figures in parentheses are the numbers of zonae pellucidae used per experiment.

<table>
<thead>
<tr>
<th>Time post-insemination</th>
<th>15–30 min</th>
<th>31–45 min</th>
<th>46–60 min</th>
<th>&gt;60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean sperm number bound per zona pellucida</td>
<td>15.6 ± 5.1 (5)*</td>
<td>27.6 ± 6.1 (8)</td>
<td>27.4 ± 4.3 (12)*</td>
<td>35.0 ± 3.3 (15)*</td>
</tr>
<tr>
<td>Mean number of permeable spermatozoa bound per zona pellucida</td>
<td>10.4 ± 3.3 (5)*</td>
<td>23.5 ± 5.6 (8)</td>
<td>24.5 ± 4.1 (12)</td>
<td>34.5 ± 4.1 (15)*</td>
</tr>
<tr>
<td>Percentage of permeable bound spermatozoa</td>
<td>65.9 ± 7.4 (5)*</td>
<td>82.4 ± 4.4 (8)*</td>
<td>88.4 ± 3.7 (12)*</td>
<td>98.9 ± 0.4 (15)*</td>
</tr>
</tbody>
</table>

Means (± SEM) were compared using Fisher’s PLSD test.

*P < 0.03; **P < 0.005; ***P < 0.01; ****P = 0.0001; *****P < 0.001; ******P < 0.008.

the acrosome reaction was not affected by sperm treatment with neuraminidase-Con A (15.0 compared with 14.4%; Table IV). Treatment of spermatozoa with 20 μg/ml WGA had no effect on acrosome reaction regardless of sperm capacitation status when compared with untreated spermatozoa (7.5 versus 8.2% and 17.0 versus 13.1% respectively; Tables III and IV). The proportion of acrosome-reacted spermatozoa observed after PNA sperm treatment was similar (27.7%) to that obtained with acrosome reaction-inducing agents previously used in the literature (Table V).

**Effect of protein kinase A and C inhibitors on the acrosome reaction induced by PNA lectin**

The acrosome reaction induced by PNA lectin was not inhibited by sperm pretreatment with 100 nM Calphostin C (23.9% of acrosome-reacted spermatozoa), with 100 nM KT 5720 (27.1%)
Human sperm acrosome reaction induced by lectins

Table II. Ability of various treatments to increase the permeability of human spermatozoa to propidium iodide (values are means ± SEM). Figures in parentheses are the numbers of ejaculates tested

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ca²⁺ ionophore</th>
<th>Neuraminidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuraminidase (PNA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrosome-reacted spermatozoa in control samples (%)</td>
<td>7.3 ± 1.0 (4)</td>
<td>7.9 ± 1.0 (5)</td>
</tr>
<tr>
<td>Acrosome-reacted spermatozoa in treated samples (%)</td>
<td>14.5 ± 2.4 (4)</td>
<td>15.1 ± 1.4 (5)</td>
</tr>
<tr>
<td><strong>P</strong> (Fisher’s PLSD test)</td>
<td>P = 0.0008</td>
<td>P = 0.0008</td>
</tr>
</tbody>
</table>

WGA = wheat germ agglutinin; PNA = peanut agglutinin; Con A = concanavalin A.

Table III. Ability of various treatments to induce the acrosome reaction of uncapacitated human spermatozoa (values are means ± SEM). Figures in parentheses are the numbers of ejaculates tested

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ca²⁺ ionophore</th>
<th>Neuraminidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuraminidase (PNA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrosome-reacted spermatozoa in control samples (%)</td>
<td>12.8 ± 2.4 (4)</td>
<td>10.3 ± 1.8 (4)</td>
</tr>
<tr>
<td>Acrosome-reacted spermatozoa in treated samples (%)</td>
<td>23.2 ± 5.8 (4)</td>
<td>27.7 ± 2.6 (4)</td>
</tr>
<tr>
<td><strong>P</strong> (Fisher’s PLSD test)</td>
<td>P = 0.0001</td>
<td>P = 0.0003</td>
</tr>
</tbody>
</table>

WGA = wheat germ agglutinin; PNA = peanut agglutinin; Con A = concanavalin A.

Table IV. Ability of various treatments to induce the acrosome reaction of capacitated human spermatozoa (values are means ± SEM). Figures in parentheses are the numbers of ejaculates tested

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ca²⁺ ionophore</th>
<th>Neuraminidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuraminidase (PNA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrosome-reacted spermatozoa in control samples (%)</td>
<td>14.4 ± 2.2 (4)</td>
<td>14.4 ± 2.2 (4)</td>
</tr>
<tr>
<td>Acrosome-reacted spermatozoa in treated samples (%)</td>
<td>17.2 ± 1.3 (4)</td>
<td>17.2 ± 0.6 (3)</td>
</tr>
<tr>
<td><strong>P</strong> (Fisher’s PLSD test)</td>
<td>P = 0.0001</td>
<td>P = 0.89</td>
</tr>
</tbody>
</table>

WGA = wheat germ agglutinin; PNA = peanut agglutinin; Con A = concanavalin A.

Table V. The ability of specific agents to induce the acrosome reaction in capacitated human spermatozoa. All spermatozoa used in these studies were previously capacitated in different culture conditions

<table>
<thead>
<tr>
<th>Acrosome reaction induc ing agents</th>
<th>Acrosome-reacted spermatozoa (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact zona pellucida</td>
<td>46</td>
<td>Cross et al., 1988; Hoshi et al., 1993</td>
</tr>
<tr>
<td>Solubilized zona pellucida</td>
<td>24–56</td>
<td>Cross et al., 1988; Bielfeld et al., 1994</td>
</tr>
<tr>
<td>Human follicular fluid</td>
<td>25–40</td>
<td>Saragietta et al., 1994; Thomas and Neizel, 1988; Yudin et al., 1988</td>
</tr>
<tr>
<td>Monoclonal antibody CRC-10</td>
<td>29</td>
<td>Garcia-Framis et al., 1994</td>
</tr>
<tr>
<td>Progestins</td>
<td>8–25</td>
<td>Tesarik and Mendoza, 1993; Tomiyama et al., 1995; Benoff et al., 1995</td>
</tr>
<tr>
<td>Protein kinase A activators (forskolin, dibutyryl cyclic AMP)</td>
<td>22–33</td>
<td>Bielfeld et al., 1994; Doherty et al., 1995</td>
</tr>
<tr>
<td>Protein kinase C activators (diacylglycerols, phorbol ester)</td>
<td>29–35</td>
<td>Bielfeld et al., 1994; Doherty et al., 1995; Byrd et al., 1989; White et al., 1990; Liu and Baker, 1990</td>
</tr>
<tr>
<td>Ca²⁺ ionophore A23187</td>
<td>25–40</td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different from their own controls.

Discussion

In this study, we have shown that molecular interactions between human zona pellucida and sperm surface molecules increase the sperm membrane permeability to impermeable propidium iodide. Changes in sperm membrane permeability induced by the zona pellucida were previously observed in mouse (Lee and Storey, 1989) and were described as an intermediary stage leading to acrosome reaction (Lee and Storey, 1989; Kligman et al., 1991). The endpoint of the first stage (S stage) in the zona pellucida-induced acrosome reaction in mouse spermatozoa was characterized by an increase in acrosomal H⁺ and Ca²⁺ permeability (Lee and Storey, 1989). We observed that two-thirds of zona pellucida-bound spermatozoa were permeable to propidium iodide in the first 30 min post-insemination and that by 60 min after sperm insemination, almost all spermatozoa bound to the zona pellucida surface had become permeable to propidium iodide. Our results suggested a rapid alteration in the permeability of the sperm membrane following binding to the zona pellucida.

In mouse, the interaction of ZP3 with the sperm surface or 0.8% DMSO (25.1%) when compared with control non-pretreated spermatozoa (28.1%).
may occur in a multivalent process, and multiple interactions are followed by receptor aggregation that may ultimately lead to signal transduction and acrosomal exocytosis (reviewed in Leyton and Saling, 1989 and in Kligman et al., 1991). Moreover, aggregation of sperm surface receptors in the absence of zona pellucida components were found to induce the acrosome reaction. In our study, we have shown that cross-linked complexes formed between certain lectins and their specific multivalent carbohydrates and glycoconjugates on the sperm surface were able to modify membrane permeability and to induce the acrosome reaction. We observed that although the lectins WGA, Con A and PNA were able to increase the proportion of permeabilized spermatozoa, the consequences for the induction of the acrosome reaction varied from one lectin to another. The binding of WGA to sperm surface receptors containing terminal sialic acid did not induce the acrosome reaction regardless of the sperm capacitation status.

Using capacitated spermatozoa, we observed that PNA induced the acrosome reaction in the same proportions (27%) as calcium ionophore A23187 (Tables I and II), human follicular fluid, or solubilized human zonae pellucidae (see Table V for references) whereas Con A was unable to induce the acrosome reaction. Whereas the capacitation of spermatozoa seems to be a prerequisite process which leads to physiological acrosomal exocytosis, we observed that PNA and Con A were able to induce the acrosome reaction in uncapsacitated human spermatozoa. However, removal of sperm surface sialic acid by neuraminidase seems to be a prerequisite for Con A and PNA binding to the sperm surface. Sialic acid removal may unmask Con A and PNA binding sites by exposing subterminal sugar residues of sperm glycoconjugates. Sialic acid has been shown to protect the spermatozoon and sperm surface antigens from the immune system (Holt, 1980; Schauer, 1985; Toshimori et al., 1991) and its removal from the sperm plasma membrane could be considered to be one of the capacitation events necessary to unmask certain sperm surface antigens implicated in zona pellucida recognition (Lassalle and Testart, 1994). If certain capacitation events were artificially induced by sperm neuraminidase treatment, the effects of PNA and Con A on the sperm acrosome reaction may comprise partially-capacitated spermatozoa.

The biochemical characteristics of lectins (including sugar specificity, amino acid composition, molecular size and valency) as well as the saccharide structure recognized by each lectin or its biological effects on the cell (as mitogenicity and cytotoxicity) or even its ability to induce signal transduction (Pandolfino et al., 1983; Gupta et al., 1994, Rini, 1995) may be responsible for its ability to induce sperm acrosome reaction. PNA and Con A become mitogenic for human and rat lymphocytes only after neuraminidase treatment, and binding of these lectins on the cell surface increases the free cytoplasmic calcium concentration (Novogrodsky et al., 1975; Pereira et al., 1976; Hesketh et al., 1983; Bijsterbosch and Klaus, 1986; Dixon et al., 1987). Con A has been reported to mediate cell cytotoxicity and apoptosis (Novogrodsky, 1975; Kulkarni and McCulloch, 1995). However WGA, a non-mitogenic lectin, also causes an increase in free cytoplasmic calcium concentration and early stimulation of lymphocytes, although this stimulation is self-aborted before DNA synthesis occurs (Hesketh et al., 1983). Pandolfino et al. (1983) suggested that mitogenic lectins are able to bind and cross-link more membrane receptors than non-mitogenic lectins.

In our results the proportions of uncapacitated spermatozoa permeable to propidium iodide were higher than the proportions of acrosome-reacted spermatozoa. Moreover, human zona pellucida induced greater differences in the proportions of either permeabilized or acrosome-reacted spermatozoa than occurred with PNA. Changes in the sperm membrane permeability induced by zona pellucida in mouse were described as an intermediary stage leading to the acrosome reaction (Lee and Storey, 1989; Kligman et al., 1991). Mouse spermatozoa previously incubated with zona pellucida to induce acrosome reaction were blocked in a transition step (S stage) by addition of a PKC inhibitor and possessed intact acrosomes; however, the plasma and outer acrosomal membranes had started to lift away from the sperm head (Kligman et al., 1991). According to Lee and Storey (1989) permeabilization of the membranes marking a transition step are probably due to pore formation induced by punctate fusion of the plasma and outer acrosomal membranes. The penetration of impermeable propidium iodide across sperm membranes may suggest a loss of sperm membrane integrity. Changes in sperm membrane permeability consequent to lectin binding to the sperm surface or to attachment of spermatozoa to the zona pellucida surface could be considered to be a preliminary step that may or may not lead to acrosomal exocytosis.

Receptor aggregation in response to biological stimuli has been described in other systems to initiate signal transduction across the membrane (Detmers et al., 1987; Ratcliffe et al., 1992; Gold et al., 1994). The mechanisms by which aggregated receptors transmit signal into the cell are not fully understood. However, lymphocyte activation was induced after chemical transformation of terminal sugar residues of glycoconjugates on the cell surface or by lectin-inducing aggregation of cell membrane glycoconjugates (Novogrodsky, 1975). It has been reported that Con A–carbohydrate interaction can mimic typical signal transduction mechanisms (Hesketh et al., 1983) and that biosignalling via lectins may involve modulation of protein kinase activities (Zeng et al., 1995). The human sperm acrosome reaction seems to occur via the activation of at least two signal transduction pathways, involving PKA and PKC (Rotem et al., 1992; Doherty et al., 1995). We observed here that two potent inhibitors of PKA (KT 5720) and PKC (calphostin C) did not inhibit the acrosome reaction induced by PNA lectin. From our results, we conclude that signalling events which lead to acrosome reaction induced by PNA binding (i.e. cross-linking complexes formed between the lectin and the specific multivalent glycoconjugates) on the sperm surface do not involve the activation of protein kinase pathways.

The mechanism generally accepted as responsible for sperm–oocyte recognition involves saccharide moieties of zona pellucida glycoconjugates and complementary zona pellucida-binding proteins on the spermatozoa (Macek and Shur, 1988; Miller and Ax, 1990). It has been suggested that the active binding component is the glycan portion of ZP3 glycoprotein and that the protein backbone functions, in part, to crosslink the glycan
(review by Miller and Ax, 1990). Our results and those of others (Macek et al., 1991; Aarons et al., 1992; Tesarik and Mendoza, 1993) confirm that aggregation of sperm surface receptors per se (in the absence of zona pellucida components) is able to induce the acrosome reaction. The use of lectins as a tool for evaluating glycoconjugates in spermatozoa membranes and acrosomal status may influence and distort results if applied to live, unfixed spermatozoa. Therefore, histochemical techniques using lectins should be limited to previously fixed spermatozoa rather than live spermatozoa, although we accept that fixation procedures may also modify membrane integrity. Moreover, we have demonstrated that aggregation of certain sperm surface glycoconjugates mediated via their glycan component is able to induce the acrosome reaction.

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