3,3',4,4',5-Pentachlorobiphenyl (PCB 126) Induces Ca^{2+} Influx and Membrane Depolarization in Human Spermatozoa

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Introduction

Polychlorinated biphenyls (PCBs) have been widely shown to be present in human body fluids among which reproductive tract fluids and secretions (1), including cervical mucus. Recently, non-ortho, "dioxin"-like congeners 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and 3,3',4,4'-tetrachlorobiphenyl (PCB 77) have been reported to effect acrosome reactions in human spermatozoa (2). This evidence, together with the findings that sperm motility and viability are not affected by PCB concentrations far higher than those measured in cervical mucus (3), prompted us to get more insight into the mechanism of PCB-sperm interaction. In this report, for the first time we show that human spermatozoa exposed to PCB 126 develop ionic and electric responses similar to those evoked by progesterone, a physiological initiator of sperm acrosome reaction at the site of fertilization.

Materials and methods

Semen specimens were obtained by healthy, fertile volunteers and analyzed following the WHO criteria (4). Spermatozoa were isolated from samples found to be normal according to the aforesaid criteria and loaded with either the Ca^{2+} fluorescent chelator fura2-AM or the potential-sensitive fluorescent probe bis-oxonol according to a previously reported procedure (5). Aliquots (50-µl) of the sperm suspension (2.5×10^6 cells) were mixed with 1.5-ml buffer amounts (5) in the reading cuvette of a spectrofluorometer. After recording the basal fluorescence emission for approximately two minutes, PCB 126 was added from stock solutions in DMSO to obtain the selected final concentrations (5 to 150 nM; DMSO concentration never exceeding 0.1 %). Fluorescence readings, fura2-AM fluorescence calibration, and conversion into intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) were carried out as described elsewhere (6).

Results and discussion

Figure 1a shows a typical response of sperm [Ca^{2+}]i to PCB 126, consisting of a rapid increase of calcium concentration to the maximum value, observed after 20 seconds from the addition, followed by a slow decline towards the basal level. In the nominal absence of extracellular Ca^{2+} (no calcium added, 2-mM EGTA), the above response was abolished (not shown), indicating that the PCB 126-stimulated increase of [Ca^{2+}]i is totally dependent on an influx from the extracellular medium. Figure 1b shows that PCB 126 also induces membrane depolarization. This response is transient, and the maximal depolarization, observed within 15 seconds from PCB 126 addition, is followed by a decline to a new plateau slightly above the prestimulus mean value. Both effects are dose-dependent, with maximal and half-maximal PCB 126 concentrations of 100 and 25 nM, respectively. Interestingly, membrane depolarization was reproduced upon subsequent additions of PCB 126, this likely meaning that sperm response to this pollutant is not receptor-mediated. These results are similar to those we obtained by exposing human spermatozoa to the organochlorine insecticide lindane (5). In that case we demonstrated that, by intercalating among membrane phospholipids and thereby altering intramembrane intrinsic potential, the insecticide induced membrane depolarization and Ca^{2+} influx. The present results strongly suggest that a similar mechanism of action can be triggered by PCB 126. By analogy, the partition of this highly lipophylic molecule into the membrane bilayer and the subsequent intramembrane dipole reorienting caused by its high dipole moment (7) would result in the dissipation of the intrinsic membrane potential. The latter event activates low-threshold voltage-operated Ca^{2+} channels and Ca^{2+} influx, followed by further membrane depolarization and opening of additional Ca^{2+} channels. In comparison with lindane, however, PCB 126 was about three orders of magnitude more effective in inducing the same effects.

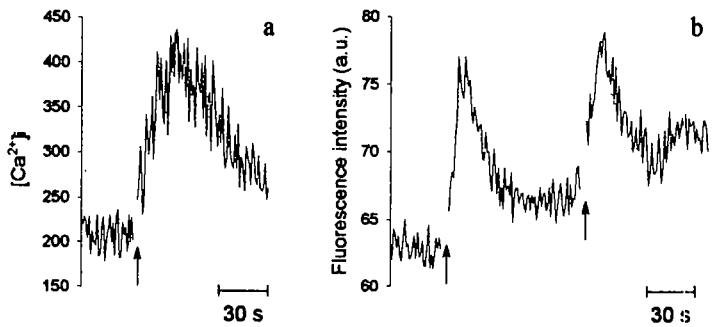

Figure 1

Effect of PCB 126 on human sperm [Ca2+]i (a) and membrane potential (b) (the arrow indicates addition of 100-nM PCB 126).

From the biological point of view, it is worth to mention that the effects of PCB 126 on sperm $[Ca^{2+}]i$ and membrane potential are quite similar to those elicited by progesterone, a physiological initiator of the acrosome reaction in capacitated spermatozoa. The PCB 126 concentrations used in this study are higher than those found in human cervical mucus (3). However, since many different organochlorine chemicals accumulate in this matrix, it cannot be ruled out that – due to synergism – even low doses of single toxic chemicals may be detrimental to sperm functions in the female tract.

References

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