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Intercellular communication in phytohemagglutinin – induced lymphocyte agglutinates

Intercellular communication, as indicated by ion flow between cells, was measured with micro-glass-electrodes in lymphocytes immediately after addition of phytohemagglutinin.

1. Introduction

Small lymphocytes can be induced to form blast cells by addition of phytohemagglutinin (PHA) as well as other stimulants. Blast transformation is associated with the synthesis of new RNA, DNA replication and cell division. One of the early reactions to the stimulant is the cell agglutination which occurs immediately upon addition of PHA. The present investigation was performed in order to test whether a criterion for cellular interconnection, namely the measurement of electrical "communication" between cells, can be detected in PHA-induced lymphocyte agglutinates.

The flow of ions and other substances of lower molecular weight from one cell to another can be induced and measured by the use of microelectrodes. Since the early experiments of Loewenstein and Kanno [1] this coupling of closely associated cells is known as "intercellular communication", which occurs in many non-excitabile tissues [2]. Some observations favor the idea that this electrical communication may be correlated with a functional cooperation of cells [3].

2. Materials and methods

Bovine lymphocytes were purified, cultivated, and stimulated with PHA-P according to methods described elsewhere [4]. For the electrical measurements, the cells were sedimented and resuspended in a solution of Eagle's medium, containing 10 % calf serum, 0.6 % gelatine (Merck) and 0.8 % agar (Agar noble special, Difco). This suspension was transferred to a plastic petri dish. The resulting 20 μm thin agar film immobilized the agglutinates after gelatinization. Eagle's medium (2.5 ml) plus 10 % calf serum was poured on the agar layer.

Intercellular communication was measured with two Ling-Gerard-glass-microelectrodes (resistances $\geq 40 \text{ M}\Omega$, tip

potential $\leq 5 \text{ mV}$, tip diameter $< 0.5 \mu\text{m}$). After successful impalement of a cell the membrane sealed around the electrode, therefore no leakage occurred. The "current-electrode" was supplied with 0.1 Hz rectangular pulses of current and indicated the successful impalement of a cell by the decreased current-flow, which was due to the high ohmic resistance of the lymphocytes. The "recording-electrode" registered the potential difference of another impaled lymphocyte and – in the case of communication – periodical voltage changes corresponding to the pulse of the "current-electrode". Besides this, the "recording-electrode" was used for continuous resistance measurements of the electrode and the lymphocyte-membranes by 1 Hz pulses. For details see [5].

3. Results

In control experiments with non-stimulated cells, a maximal ion flux between the two electrodes was observed when both electrodes were inserted into the same lymphocyte. By way of contrast, when each electrode was inserted into a different non-stimulated lymphocyte, there was a minimal flow of ions between the electrode, even when both cells were close together. In experiments with PHA agglutinated cells, electrical communication could be measured not only between adjacent cells, but even over a distance of more than 10 lymphocytes.

Under certain conditions agglutination of lymphocytes can be effected without causing blast transformation [6]. It was found that horse anti-pig thymocyte serum exhibited this effect on bovine lymphocytes. In order to test whether a 24 h treatment with this horse serum caused lymphocyte stimulation, cells were assayed for [^{14}C]uridine incorporation into cellular RNA. The rate of incorporation did not differ from that of calf serum-treated control cultures. Cell viability after a 24 h treatment was tested by cell counts and trypan blue exclusion, as well as by testing the capacity of cells to react to PHA. No cytotoxic or stimulation-inhibiting effect of this horse serum was found. As shown in the left part of Fig. 1, agglutination with this serum does not cause electrical communication between the agglutinated lymphocytes.

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Abbreviation: PHA: Phytohemagglutinin

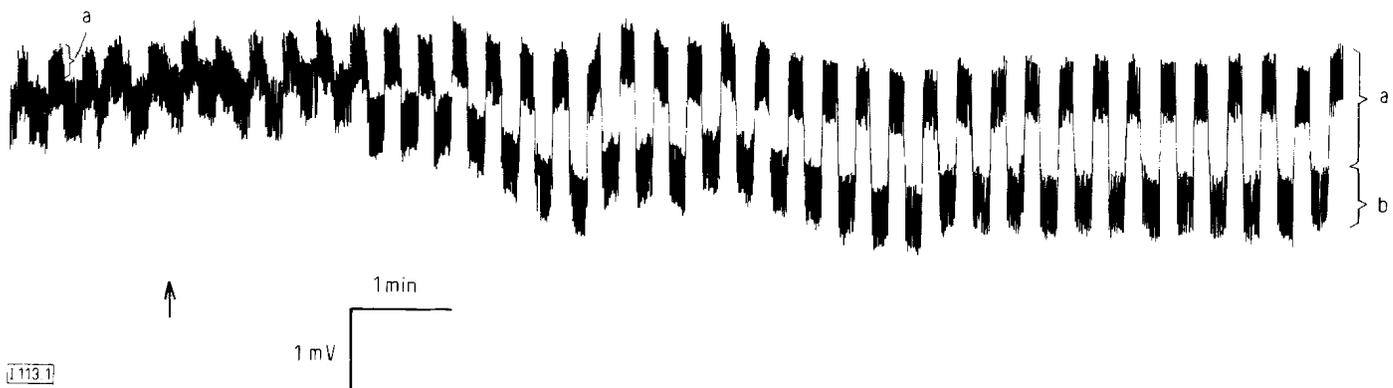


Figure 1. Influence of PHA on the establishment of intercellular communication between agglutinated lymphocytes. Lymphocytes were agglutinated with a non-stimulating horse serum. Intercellular communication was measured with two Ling-Gerard glass-microelectrodes by sending current pulses from one cell interior to another. The left side of the graph shows the unspecific ion flux in this system. After the addition of PHA to the medium (arrow) the onset of intercellular communication can be seen two min later, as indicated by increasing pulse a (frequency: 3/min). Pulse b (frequency: 60/min) indicates the resistance of the system membrane plus electrode, showing successful impalement of the intact cell.

Furthermore, the time course of the establishment of communication can be demonstrated in this system, when PHA is added to the culture during the measurement of electrical coupling in this horse serum-induced lymphocyte agglutinate. Two minutes after the addition of PHA, communication can be measured between the agglutinated cells (Fig. 1). Since it can be estimated that this time interval is required for the diffusion of the stimulant through the agar film, it is concluded that intercellular communication begins immediately after attachment of PHA to the lymphocyte surface. Communication remains detectable after at least 36 h stimulation.

4. Discussion

When lymphocytes react to PHA, the earliest detectable effects are connected with the cell membrane. At the early phase of PHA action processes of active and passive transport are stimulated [7–9]. As another property of the cell membrane we have now demonstrated that intercellular communication is established within minutes of the addition of the stimulant. This means that the normally high ohmic resistance of the cell membrane is reduced at the sites of cell contact, which allows the ion flux between the cells without increased leakage into the medium. Lymphocyte agglutination is not the only prerequisite for the establishment of intercellular communication, as has been shown by a non-stimulating, but agglutinating serum. Therefore we conclude,

that intercellular communication is closely connected with the process of stimulation.

The results presented here suggest that information exchange may be possible between agglutinated lymphocytes during PHA stimulation. Recent experiments (J.H. Peters, unpublished results) have shown that in lymphocyte stimulation the rate of uridine incorporation is in fact a function of the degree of agglutination. This favors the concept of a “contact-cooperation” between stimulated lymphocytes.

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5. References

- 1 Loewenstein, W.R. and Kanno, Y., *J. Cell Biol.* 1964. 22: 565.
- 2 Borek, C., Higashino, S. and Loewenstein, W.R., *J. Membrane Biol.* 1969. 1: 274.
- 3 Potter, D.D., Furshpan, E.J. and Lennox, E.S., *Proc. Nat. Acad. Sci. U.S.* 1966. 55: 328.
- 4 Hausen, P., Stein, H. and Peters, H., *Eur. J. Biochem.* 1969. 9: 542.
- 5 Hülser, D.F., *Pflügers Arch. Eur. J. Physiol.* 1971. 325: 174.
- 6 Pogo, B.G.T., *J. Cell Biol.* 1969. 40: 571.
- 7 Quastel, M.R. and Kaplan, J.G., *Exp. Cell Res.* 1970. 63: 230.
- 8 Peters, J.H. and Hausen, P., *Eur. J. Biochem.* 1971. 19: 502.
- 9 Peters, J.H. and Hausen, P., *Eur. J. Biochem.* 1971. 19: 509.