コンカナバリンA刺激によるリンパ球の膜流動性

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AgriKnowledge

Membrane Fluidity of Equine, Bovine and Canine Lymphocytes during Stimulation with Concanavalin A

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Several studies in regard of membrane fluidization have been reported by the use of various methods including fluorescence depolarization [1], electron spin resonance (ESR) [8], nuclear magnetic resonance (NMR) [14] and fluorescence photobreaching recovery [9]. It seems that there are some disagreements about the temporal fluidization of lymphocyte membranes stimulated with mitogen [3, 6, 7, 11, 16], partly because of the difference in the analytical methods. There was no report to compare the different methods for investigating the change of membrane fluidity through lymphocyte activation pathway. In this study, the membrane fluidity of equine, bovine and canine peripheral blood lymphocytes (PBLs) during the stimulation with concanavalin A (ConA, Pharmacia, Milwaukee) was investigated by means of fluorescence depolarization and ESR measurements, which were the most widely used methods for the study of membrane fluidity.

A mixed breed horse (19 years old), a Holstein-Friesian cow (9 years old) and a mongrel dog (5 years old) were used in this study. They were clinically healthy by physical examination. PBLs were separated from venous blood by Ficoll-Conray density centrifugation as previously described [15]. The separated PBLs were washed three times with Hank's balanced salt solution (HBSS, pH 7.4). The purity and viability of collected lymphocytes were more than 95%.

1, 6-diphenyl-1, 3, 5-hexatriene (DPH, Sigma, St. Louis) was used for fluorescent probe. Two mM DPH solution in tetrahydrofuran was added to 1,000-fold volume of HBSS during vigorously stirrig. It was stirred for 15 minutes at room temperature to make DPH dispersion. PBLs were suspended in the DPH dispersion at a concentration of 1.6×10^6 per m*l* and incubated for 30 minutes at 37°C. After incubation, the cells were washed twice with HBSS and used for the experiment.

The values of steady-state anisotropy (r_s) of DPH fluorescence in the membrane of equine, bovine and canine PBLs were observed at every 5 or 10 minutes for 60 minutes after stimulation with ConA. No significant changes in r_s values were observed after the stimulation with ConA for each case. The maximum range of the changes observed was within that observed in unstimulated PBLs (Fig. 1).

2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxy (5-doxyl stearic acid, Aldrich, Milwaukee) was used as a spin probe. One μl of 1 mM 5-doxyl stearic acid solution in ethanol was evaporated in a glass tube, then added with 50 μl of PBL suspension containing 1×10^7 cells, and incubated for 30 minutes at 37°C with gentle shaking.

The ESR observation was continued for 40 minutes at every 5 minutes. The values of order parameter were not changed by ConA stimulation as shown in Fig.2. Slight decrease in the value observed in bovine and canine PBLs at 20 to 30 minutes after the stimulation and increases in equine PBLs at 30 to 35 minutes were not statistically significant.

Both probes used in this study were intercalated into phospholipid array, and the movement of probes reflects the molecular motions of phospholipids in the membrane. In ESR measurement, carboxyl group of spin labeling reagent was trapped on the outer layer of membrane and could not be intercalated deeply in lipid bilayers. The time range in the movement of the spin probe is almost the same as that of DPH is fluorescence method. Thus order parameter obtained from ESR spectra might reflect the movement of outer half of membrane than the fluorescence anisotropy. In the case of using ConA as a stimulator, the fluidities of three animals' PBLs measured by both methods were not changed so much.

There were ESR studies demonstrating that

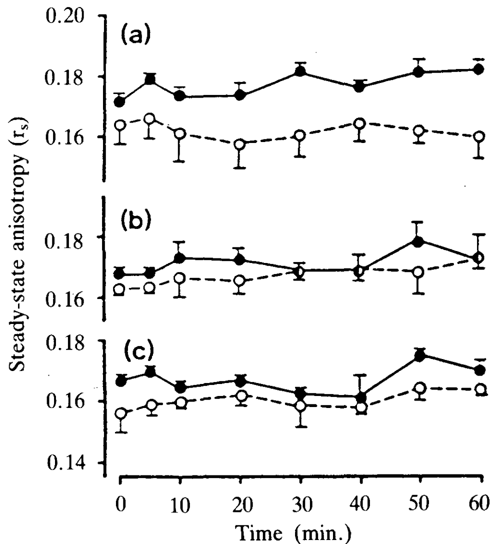

Fig. 1. The changes of the fluorescence anisotropy of DPH-labeled equine (a), bovine (b) and canine (c) peripheral blood lymphocytes (PBLs) after stimulation (straight line) and non-stimulation (broken line) with ConA. Each value represents average and standard error of three experiments. The DPH-labeled PBLs were resuspended in HBSS (1.6×10^6 per m*l*), and introduced into two different optical cuvettes. To one of the cuvettes, ConA was added at a concentration of 50, 5 or 25 μg/m*l* for stimulating equine, bovine or canine PBLs as previously described [10, 12, 15]. The steady-state anisotropy of the fluorescent light, $r_s=(I_{||}-I_{\perp})/(I_{||}+2I_{\perp})$, where values of $I_{||}$ and $I_{\perp}$ were modified according to Araiso and Koyama [2], was calculated as an indicator of the membrane fluidity [1]. All measurements were performed at 37°C.

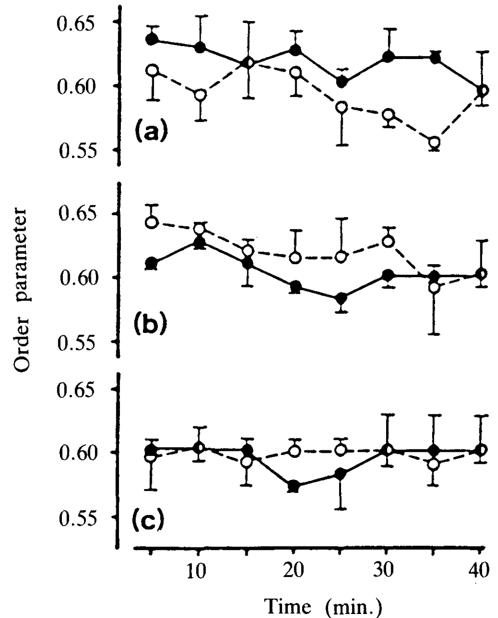

Fig. 2. The changes of the order parameter of 5-doxyl stearic acid-labeled equine (a), bovine (b) and canine (c) peripheral blood lymphocytes (PBLs) after stimulation (straight line) and non-stimulation (broken line) with ConA. Values represent as shown in Fig. 1. Immediately after the spin labeled PBLs were stimulated with ConA as shown in Fig. 1, the stimulated and unstimulated PBLs suspension each 50 μl containing 10^7 cells were placed in a hematocrit capillary. Order parameter was calculated from ESR spectrum as an indicator of the membrane fluidity according to Hubbel *et al.* [8].

the movement of spin-labeled phospholipids is changed in human PBLs by PHA or ConA stimulation [4, 5]. From above method, it was concluded that the disarrangement of lipid bilayer was brought about by the sequestration of the more rigid glycosphingolipids and glycoproteins into the patches and caps formed by receptor-ligand complexes. The fluidity change in PBLs from three animal species is much smaller than of human. It may be attributed to the different lipid composition and/or different unsaturated fatty acid content in these membranes as observed in these species erythrocytes [13].

From the data described above, no membrane fluidization was recognized in equine, bovine and canine PBLs when the PBLs used in this study were stimulated with the mitogenic concentration of ConA.

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要　約

コンカナバリンＡ刺激によるリンパ球の膜流動性（短報）：田島誉士・荒磯恒久[1]・小山富康[1]・藤永　徹・大友勘十郎・小池寿男（北海道大学獣医学部家畜外科学講座，[1]北海道大学応用電気研究所生理部門）――ウマ，ウシおよびイヌの末梢血リンパ球（PBL）のコンカナバリンＡ（ConA）刺激による膜流動性の変化を，蛍光偏光解消法および電子スピン共鳴法により観察した．ConA 刺激により，３種の動物の PBL のわずかな膜流動性の変化が観察されたが，それらの変化は未刺激 PBL においても観察される程度の変化であった．したがって，少なくともウマ，ウシおよびイヌ PBL の ConA 刺激後の反応は，膜（リン脂質二重層）流動性の変化を伴っていないことが示唆された．