Guanine nucleotides inhibit poly-L-arginine-induced membrane damage in polymorphonuclear leukocytes

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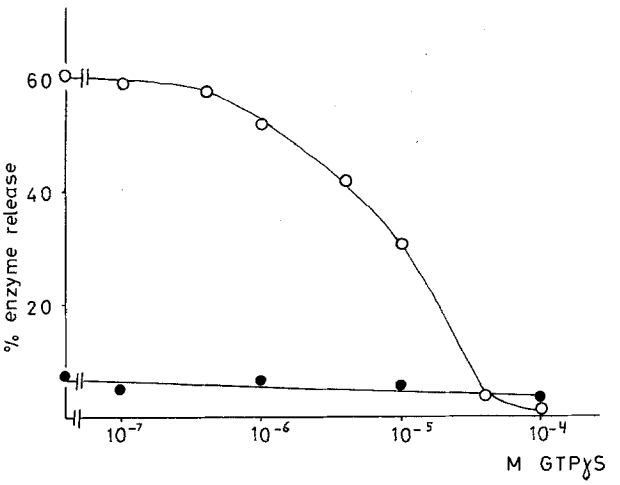
Summary. Poly-L-arginine induces a strong release of the cytoplasmic enzyme lactate dehydrogenase from rabbit polymorphonuclear leukocytes, indicating that plasma membrane damage occurs. $GTP_\gamma S$, a stable guanine nucleotide, completely inhibits poly-L-arginine-induced LDH release whereas pretreatment of the cells with pertussis toxin gives a moderate inhibition. The results suggest that poly-L-arginine-induced plasma membrane damage is mediated by guanine nucleotide binding structures.
Key words. Poly-L-arginine; polymorphonuclear leukocytes; plasma membrane damage; guanine nucleotides.

Cationic macromolecules have been shown to act as bactericidal, antiviral and antifungal agents, to agglutinate and lyse several types of mammalian cells, and to cause inflammation[1-3]. Because the charge of cationic proteins is due to a high proportion of the amino acids, arginine and lysine, polymers of these amino acids may serve as model systems to study the effects of these proteins. Polyarginine induces histamine release from basophil leukocytes, and modulates chemotaxis and superoxide generation by polymorphonuclear leukocytes (PMNs)[4-6]. Polycations such as polylysine and polyarginine induce plasma membrane damage in PMNs which is evident from the release of cytoplasmic constituents, such as lactate dehydrogenase (LDH)[7]. Recently it was found that a GTP-binding regulatory protein (G-protein) is involved in a number of PMN functions such as exocytosis and production of superoxide[8-10]. We studied the possible involvement of this protein in polyarginine-induced membrane damage and considered the effect of a stable GTP analogue on poly-L-arginine-induced LDH release from PMNs.

Materials and methods. Polymorphonuclear leukocytes were obtained from the peritoneal cavity of rabbits, as described earlier[11]. The cells were suspended in a medium consisting of 140 mM NaCl, 5 mM KCl, 10 mM glucose and 20 mM Hepes, pH 7.3. During the experiments the cells, in a final concentration of 3×10^6 PMNs per ml, were incubated with the polycation for 20 min at 37 °C. Then the cells were centrifuged, and the supernatant was analyzed. The release of the cytoplasmic enzyme lactate dehydrogenase (LDH) was considered as a measure for plasma membrane damage. LDH was determined by measuring the conversion of NADH into NAD^+ during the conversion of pyruvate into lactate. The release of the granule enzyme lysozyme was measured to determine the involvement of granules in the release process. Lysozyme was determined by measuring the rate of lysis of *Micrococcus lysodeikticus*. Enzyme release was expressed as a percentage of a maximum value, obtained by treating the cells with 0.05% Triton X-100. Poly-L-arginine (M = 40 000), poly-L-lysine (M = 90 000), and pertussis toxin were obtained from Sigma Chemical Co. Guanosine-5'-0-[3-thio]tri-phosphate ($GTP_\gamma S$), guanosine-5'-0-[2-thio]diphosphate ($GDP\beta S$), and other nucleotides were obtained from Boehringer Mannheim.

Results and discussion. A concentration of 100 nM poly-L-arginine induces extensive LDH release from PMNs (fig.), indicating that the polycation causes damage to the plasma membrane. Under the conditions of our experiments, where no extracellular Ca^{2+} was present, poly-L-arginine induced only little lysozyme release. This implies that granule membranes are little involved under these conditions. In the presence of extracellular Ca^{2+} the situation becomes more complex because then activation of exocytosis occurs, a process which is superimposed on the cytotoxic effect. Because we were primarily interested in the membrane-damaging effect of poly-L-arginine, the experiments described in this investigation were carried out in the absence of extracellular Ca^{2+}. In the presence of increasing concentrations of $GTP_\gamma S$ a decrease of LDH release occurs, and at a concentration of 50 μM $GTP_\gamma S$ the poly-L-arginine-induced enzyme release, and thus the membrane-damaging effect is completely inhibited. Pretreatment of PMNs with pertussis toxin, which is considered as a selective inhibitor of some G-proteins, gives a moderate inhibition of poly-L-arginine-induced LDH release (table 1). A central role of G-protein in PMN function was first demonstrated by using pertussis toxin in activation of PMNs by chemotactic peptide[8,12]. The inhibition of this activation by pertussis toxin led to the suggestion that a G-protein coupled chemotactic receptor interaction to phospholipase C activation[13]. In the meantime, it has been shown that GTP analogues have several roles in PMN func-



Enzyme release from poly-L-arginine-treated PMNs in the presence of increasing concentrations of $GTP_\gamma S$. PMNs (3×10^6 per ml) were exposed to 100 nM poly-L-arginine and the indicated concentration of $GTP_\gamma S$ for 20 min at 37 °C —○—: LDH release; —●—: lysozyme release.

Table 1. Comparison between poly-L-arginine- and poly-L-lysine-induced LDH release: effect of $GTP_\gamma S$ and pertussis toxin. PMNs (3×10^6 per ml) were preincubated without or with 500 ng pertussis toxin per ml, for 1 h at 37 °C. Subsequently $GTP_\gamma S$ was added as indicated, and 100 nM poly-L-arginine (pArg) or 100 nM poly-L-lysine (pLys) was added, followed by incubation at 37 °C for 20 min. Values given are the means of three experiments ± SD.

	% LDH release induced by	
	pArg	pLys
–	50 ± 2	55 ± 2
50 μM $GTP_\gamma S$	7 ± 2	39 ± 1
Pertussis toxin	38 ± 3	38 ± 1
Pertussis toxin, 50 μM $GTP_\gamma S$	4 ± 3	33 ± 2

Table 2. Effect of some nucleotides on poly-L-arginine-induced LDH release from PMNs. PMNs (3×10^6 per ml) were exposed to 100 nM poly-L-arginine and the indicated concentration of nucleotide for 20 min at 37 °C. Values given are the means of three experiments ± SD.

	% LDH release
–	55 ± 3
50 μM $GTP_\gamma S$	5 ± 1
50 μM GDPβS	40 ± 3
50 μM GTP	15 ± 2
50 μM ATP	43 ± 2

tions, and it has been suggested that different G-proteins might be involved [14–16].

There is a remarkable difference between the effect of $GTP_\gamma S$ on poly-L-arginine-induced LDH release, and on LDH release induced by poly-L-lysine. Whereas inhibition by pertussis toxin is about the same for both polycation, the inhibition by $GTP_\gamma S$ is much stronger for poly-L-arginine than for poly-L-lysine. It might therefore be that a G-protein has a modulating effect on the membrane-damaging effect of polycations. There remain some uncertainties, however, because the protective effect of pertussis toxin is modest, and because some other nucleotides, such as GTPβS and ATP also give a (relatively small) inhibition of poly-L-arginine-induced LDH release (table 2). GTP itself is furthermore a good inhibitor which could suggest that the guanine nucleotide-binding structure has no high GTPase activity. Shielding of positive charges on poly-L-arginine might affect its ability to interact with the PMN but it seems unlikely that inhibition by ATP and GDPβS is due to a charge effect because these nucleotides have no effect on poly-L-lysine-induced LDH release.

Though the molecular mechanism of guanine nucleotide inhibition of poly-L-arginine-induced membrane damage is far from clear, the results presented in this paper demonstrate that the damaging effect of the polycation on the plasma membrane is not direct. One or more guanine nucleotide-dependent structures are involved, which either induce membrane damage after activation by poly-L-arginine, or modify the membrane structure and thus enable poly-L-arginine to damage the membrane.

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Intact teratogenic immunoglobulins may reach the rat embryo

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Summary. It is known that heterologous antiserum against rat kidney homogenate may induce congenital malformations when injected into pregnant rats during the period of organogenesis. Teratogenic rabbit antibodies against a purified rat renal tubular glycoprotein were isolated, labelled with ^{125}I and injected into pregnant rats on the 10th day of gestation. Extracts of visceral yolk-sacs (VYS) and embryos were obtained 16 h later and chromatographed separately on a Sephacryl S-300 gel filtration column. The resultant chromatograms showed several radioactive peaks, one of which coincided with the eluate of intact rabbit immunoglobulins G (IgG). We interpret the result as an indication that some undigested intact teratogenic IgG were present in VYS and the embryo.
Key words. Teratogenesis; visceral yolk-sac; antibodies; embryos.

The discovery that rabbit antiserum against rat kidney homogenate induced abnormal embryonic development when injected into pregnant rats during the critical organogenetic period was reported more than a quarter of century ago [1]. Many investigators have since confirmed and extended these findings [2–8]. The biologic effects of the kidney antiserum appeared to be dependent on the dosage of antiserum administered but not on complement or other non-specific immunologic mediators [3]. Since many reported fluorescent antibody localization studies [4–7] demonstrated that the teratogenic antibodies localized in vivo within the visceral yolk-sac (VYS) endodermal cells of the developing embryo, it was hypothesized that the teratogenic antibodies might induce birth defects by causing VYS placental dysfunction. Both biochemical [9] and morphological [10] evidence has been presented to support such a hypothesis. However, it has not been determined if the injected teratogenic antibodies ever reach the developing embryo. Although immunofluorescent studies seemed to fail to demonstrate the presence of such antibodies in the embryo itself [7], it is possible that a small amount of antibodies might escape from detection by the immunofluorescent technique.

A high molecular weight glycoprotein (gp340) has been isolated from rat renal proximal tubules and antibodies against