Developmental changes in ANP-stimulated guanylyl cyclase activity enhanced by ATP in rat lung membrane fractions

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1 ANP (atrial natriuretic peptides)- or ANP/ATP-stimulated guanylyl cyclase activities were compared in adult (2 month old) and neonatal (5–7 day old) rat lung membrane fractions.

2 The enzyme activities of both membranes depended on the incubation time and ATP concentration: although the activities of both membranes were similar after a short incubation time (4 min), those in adult membranes were lower than those of neonatal membranes after longer incubation times (10 and 30 min) or at lower concentrations of ATP.

3 ANP/ATP γ S-stimulated guanylyl cyclase activities, which were much higher than ANP/ATP-stimulated activities, were similar in both membranes.

4 ATPase activity of adult membranes was higher than that of neonatal membranes, suggesting that hydrolysis of ATP leads to a decrease of ANP/ATP-guanylyl cyclase activity in adult membranes. Triton X-100 enhanced and diminished ANP/ATP-stimulated guanylyl cyclase activities of adult and neonatal membranes, respectively, and thereby abolished the adult/neonatal difference in the membrane response to ATP.

5 ANP-stimulated activities of both membranes were much more activated by pre-incubation with ATP γ S than those induced by simultaneous addition of ATP γ S. The former activities were decreased to levels of the latter by Triton X-100. The latter activities were not affected by Triton X-100.

6 The present results suggested that conformation of lung plasma membranes is related to activation of the ANP receptor/guanylyl cyclase system.

Keywords: Atrial natriuretic peptide; ATP; guanylyl cyclase; rat lung membranes; developmental change

Introduction

Atrial natriuretic peptide (ANP) synthesized in atrial cardiocytes and released into the circulation evokes a variety of physiological responses. Some of the physiological actions of ANP are mediated via production of guanosine 3':5'-cyclic monophosphate (cyclic GMP) by the activation of membrane-bound guanylyl cyclase (Atlas & Laragh, 1986; Chinkers *et al.*, 1989; Inagami, 1989; Pandey & Singh, 1990). Recently, enhancement of guanylyl cyclase by ATP was demonstrated (Kurose *et al.*, 1987; Chang *et al.*, 1990; Gazzano *et al.*, 1991; Marala *et al.*, 1991; Chamienia & Johns, 1992; Lebowitz *et al.*, 1992), and coexistence of ANP receptors and guanylyl cyclase was identified in membranes prepared from various mammalian tissues and cells (Kuno *et al.*, 1986; Pual *et al.*, 1987; Takayanagi *et al.*, 1987; Meloche *et al.*, 1988). However, little is known about the definitive biochemical mechanism of the ATP action.

ANP receptors coupled to guanylyl cyclase are in abundance in lung plasma membranes of mammals (Lad & White, 1979; Inomata *et al.*, 1987; Imamura *et al.*, 1988). The function of lung is greatly changed after birth, and hence it is interesting to investigate how effects of ATP on ANP-stimulated guanylyl cyclase activity are changed during development. In the present study, we examined changes of ANP-stimulated guanylyl cyclase activity during the course of development by using lung plasma membranes from adult and neonatal rats. The results obtained show that there are large differences in incubation time-dependency and ATP effect on ANP-stimulated guanylyl cyclase activities between adult and neonatal rat lung membranes.

Methods

Preparation of rat lung plasma membranes

Male rats of a Wistar strain, adult (2 month old) and neonate (5–7 day old) were used. Each lung was minced in 0.25 M sucrose-1 mM EDTA-20 mM HEPES buffer (pH 7.5) containing aprotinin (50 μ l ml⁻¹) and homogenized with polytron and subsequently Teflon homogenizer. Homogenates were centrifuged for 5 min at 600 g and the supernatant was centrifuged for 30 min at 25000 g. Pellets were washed, resuspended with 0.25 M sucrose: 1 mM EDTA: 20 mM HEPES buffer (pH 7.5), and stored at –80°C until use as crude plasma membrane fractions.

Measurement of guanylyl cyclase activity

Crude plasma membrane fractions (40–50 μ g protein) were suspended in the assay medium of a final volume of 0.3 ml containing 20 mM HEPES buffer (pH 7.5), 0.25 mM GTP, 0.4 mM IBMX, 3 mM MgCl₂, 0.5 mM EDTA, 5 mM creatine phosphate, 30 μ l ml⁻¹ creatine phosphokinase, and 0.1% bovine serum albumin (BSA) and incubated with ANP at 30°C. ANP was added at 500 nM unless otherwise noted. Reaction was terminated by addition of 0.03 ml of 1 N HCl. The amount of cyclic GMP produced by guanylyl cyclase was measured by radioimmunoassay as described previously (Honma *et al.*, 1977) and assessed as guanylyl cyclase activity.

Measurement of ATPase activity

Crude plasma membrane fractions (40–50 μ g protein) were suspended in 20 mM HEPES buffer (pH 7.5) containing 3 mM MgCl₂, 0.5 mM EDTA, 0.1% BSA, and various doses of ATP in a final volume of 0.3 ml and incubated at 30°C. Reaction was terminated by addition of 0.03 ml of 3 N HClO₄. To 1 ml of the deproteinized supernatant were added

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2 ml of 1.25% ammonium molybdate in 0.15 N H₂SO₄, and a further 3 ml of 0.08% *p*-aminonaphthol sulphonic acid reagent described by Fiske & Subbarow (1925). Samples were allowed to stand for 30 min at 30°C and measured at 750 nm. The amount of inorganic phosphate released by hydrolysis of ATP was assessed as ATPase activity.

[³⁵S]-adenosine 5'-(3-thio) triphosphate binding to crude plasma membrane fractions

Crude plasma membrane fractions (40–50 µg protein) were suspended with 20 mM HEPES buffer (pH 7.5) containing 100 mM NaCl, 3 mM MgCl₂, 0.5 mM EDTA, and various doses of [³⁵S]-adenosine 5'-(3-thio)-triphosphate ([³⁵S]-ATPγS) in a final volume of 0.3 ml and incubated at 30°C for 30 min. The measurement of non-specific binding was carried out by the addition of unlabelled ATPγS at a concentration of 250 µM under similar conditions.

Treatment of crude plasma membrane fractions with Triton X-100

Crude plasma membrane fractions (40–50 µg protein) were incubated with or without ATP or ATPγS in the presence or absence of 0.1% Triton X-100 in the medium for assay of guanylyl cyclase. In the case of pretreatment of membranes with ATPγS, membrane fractions (40–50 µg protein) were pre-incubated with 200 µM ATPγS at 37°C for 5 min in 20 mM HEPES buffer (pH 7.4) containing 0.25 M sucrose, 3 mM MgCl₂, 0.5 mM EDTA, and 0.1% BSA in a final volume of 0.2 ml. After pre-incubation, the medium for assay of guanylyl cyclase was added into the pre-incubated membrane suspensions and guanylyl cyclase activities were measured in the presence or absence of 0.1% Triton X-100 under similar conditions to those described above.

Measurement of plasma membrane fluidity

The degree of fluidity of the nonpolar regions of biological membranes has been estimated by the introduction of a highly efficient hydrocarbon fluorophore, 1,6-diphenyl 1,3,5-hexatriene (DPH) which is localized in the hydrophobic regions of biological membranes. The fluorescence polarization of the DPH in the rat lung membranes was measured as follows. Two µl of 1 mM DPH solution in tetrahydrofuran were added to the lung membrane suspension (100 µg protein) in 2 ml of 25 mM HEPES buffer (pH 7.5) containing 145 mM NaCl, and 4 mM KCl in a cuvette. After the membrane suspension containing DPH was stirred for 15 min at 25°C, the polarization of the DPH fluorescence was measured as described previously (Nomura & Kurihara, 1987). The following relationship was employed to obtain the polarization (P) of the DPH fluorescence:

$$P = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + I_{\perp})$$

Here *I*_∥ and *I*_⊥ represent the fluorescence intensity parallel to, and perpendicular to the plane of polarization of the excitation beam, respectively. The emission of DPH was excited at 360 nm and monitored at 430 nm through a 390-nm cut-off filter. The molar ratio of DPH was used for measurement of the fluorescence polarization.

Plasma membrane proteins

Proteins were measured by the method of Lowry *et al.* (1951).

Materials

Cyclic GMP assay kits and aprotinin were provided by Yamasa Shoyu (Japan) and Hoechst Japan, respectively. ANP, ATP, ATPγS, creatine phosphate, creatine phosphokinase, 3-isobutyl-1-methylxanthine (IBMX), and bovine

serum albumin (BSA) were purchased from Sigma (USA). 1,6-Diphenyl 1,3,5-hexatriene (DPH) was from Tokyo Chemical Ind. (Japan).

Analysis of data

Scatchard plots of equilibrium binding of [³⁵S]-ATPγS were analyzed by a nonlinear least-squares curve-fitting procedure with a one-site or two-site model using the modified LIGAND programme (Munson & Rodbard, 1980). Statistical differences were analyzed by Student's *t* test.

Results

Effects of ATP on ANP-stimulated guanylyl cyclase activities and its incubation time-dependency

ANP slightly activated guanylyl cyclase of adult and neonatal rat lung membranes in a concentration-dependent manner, and its action was markedly enhanced in both membranes by simultaneous addition of 100 µM ATP (Figure 1). There was no significant difference in the enzyme activities between adult and neonatal membranes under the conditions incubated at 30°C for 3 min. ATP itself had no effect on guanylyl cyclase activity.

Figure 2 shows the time course for ANP-stimulated guanylyl cyclase activities of adult and neonatal lung membranes with or without 100 µM ATP. Stimulation of guanylyl cyclase activity by ANP in adult membranes reached a maximal level at 4 min. In contrast, the enzyme activity of neonatal membranes continued to increase up to incubation time 10 min, resulting in 2 fold that of adult membranes at incubation times 10 min and 30 min (Figure 2a). The enzyme activities enhanced by addition of 100 µM ATP were increased time-dependently in both membranes. However, as in the absence of ATP at the incubation times 10 min and 30 min, enzyme activity of adult membranes was one-half that of neonatal membranes (Figure 2b).

The dose-dependent effects of ATP on ANP-stimulated guanylyl cyclase activities were examined at various incubation times in adult and neonatal lung membranes (Figure 3). ATP enhanced ANP-stimulated guanylyl cyclase in a concentration-dependent manner in both membranes. ANP-stimulated guanylyl cyclase activities of adult and neonatal

Figure 1 Effects of ATP on ANP-stimulated guanylyl cyclase activities in plasma membrane fractions of adult and neonatal rat lungs. Crude plasma membranes were incubated with indicated concentrations of ANP for 3 min in the presence (dashed lines) and absence (solid lines) of ATP (100 µM). Measurement of guanylyl cyclase activity was carried out as described in the Methods. Adult (●); neonate (○). Values are means ± s.e. mean of five separate experiments. For abbreviations in this and other legends, see text.

Figure 2 Effects of incubation times and ATP addition on ANP-stimulated guanylyl cyclase in plasma membrane fractions of adult and neonatal rat lungs. Crude plasma membranes were incubated for indicated incubation times in the presence (b) and absence (a) of ATP (100 μ M). Measurement of guanylyl cyclase activity was carried out as described in the Methods. Adult (●); neonate (○). Values are means $\pm$ s.e. mean of five separate experiments. * P < 0.05; ** P < 0.01.

Figure 3 Relationship between incubation time and ATP concentration in ANP-stimulated guanylyl cyclase activities of plasma membrane fractions of adult and neonatal rat lungs. Crude plasma membranes were incubated with indicated concentrations of ATP for 4 min (a), 10 min (b), and 30 min (c). Measurement of guanylyl cyclase activity was carried out as described in the Methods. Adult (●); neonate (○). Values are means $\pm$ s.e. mean of four separate experiments.

membranes at incubation time of 4 min were similar at all ATP concentrations tested (Figure 3a). In contrast, at incubation time 10 min, the enzyme activities of neonatal membranes were significantly higher than those of adult membranes at concentrations of ATP below 300 μ M (see Figure 2). Concentrations of ATP above 500 μ M enhanced the enzyme activities of both membranes to a similar extent, and hence there was no significant difference in the enzyme activities between the two membranes (Figure 3b). After an incubation time of 30 min, the enzyme activities of neonatal membranes were always significantly higher than those of adult membranes at all ATP concentrations tested (Figure 3c).

Relationship between ATP effect on ANP-stimulated guanylyl cyclase activity and ATPase activity

The amount of ATP-binding to adult and neonatal lung membranes was measured using various concentrations of ATPyS. Scatchard plots of the data are shown in Figure 4. The analysis of the binding data in both membranes exhibited curvilinear plots, indicating that two classes of ATP-binding sites (high affinity with a small number and low affinity with a large number) exist in rat lung membranes. The values of K_d and B_{max} for ATPyS binding are shown in

Figure 4 Scatchard plot analysis of [³⁵S]-ATPyS binding to plasma membrane fractions in adult and neonatal rat lungs. Crude plasma membranes were incubated with various concentrations of [³⁵S]-ATPyS and [³⁵S]-ATPyS binding activity to each membrane was measured as described in Methods. Adult (●); neonate (○).

Figure 5 Comparison of effects of ATP and ATPyS on ANP-stimulated guanylyl cyclase activities in plasma membrane fractions of adult and neonatal rat lungs. Crude plasma membranes were incubated for indicated incubation times with (a) ATP or ATPyS at 50 μ M and (b) ATP or ATPyS at 200 μ M. Measurement of guanylyl cyclase activity was carried out as described in the Methods. Adult (●); neonate (○); ATP (solid lines); ATPyS (dashed lines). Values are means $\pm$ s.e. mean of four separate experiments.

Table 1 The values of K_d and B_{max} of [35 S]-adenosine 5'-(3-thio)triphosphate ([35 S]-ATP γ S) binding to lung membrane fractions

	High affinity binding		Low affinity binding	
	K_d (μ M)	B_{max} (pmol mg $^{-1}$ protein)	K_d (μ M)	B_{max} (nmol mg $^{-1}$ protein)
Adult	2.25 $\pm$ 0.30	664.5 $\pm$ 45.7	373.4 $\pm$ 28.5	8.10 $\pm$ 0.20
Neonate	2.54 $\pm$ 0.15	789.7 $\pm$ 100.1	448.5 $\pm$ 54.2	8.71 $\pm$ 0.15

The activities of [35 S]-ATP γ S binding to lung membranes of adult and neonate were measured as described in Methods. Values are means $\pm$ s.e. mean of five separate experiments.

Table 1. There were no significant differences in K_d values or B_{max} values between adult and neonatal membranes.

ANP-stimulated guanylyl cyclase activities enhanced by ATP were compared with those enhanced by non-hydrolyzable ATP γ S in adult and neonatal membranes (Figure 5). The effects of 50 μ M and 200 μ M ATP γ S on the enzyme activities of both membranes were larger than those of 50 μ M and 200 μ M ATP, respectively. In addition, ANP/ATP γ S activated guanylyl cyclase of both membranes to a similar extent. These results raised the possibility that the difference in the ATP effect on ANP-stimulated guanylyl cyclase activities between adult and neonatal membranes is explained by the difference in the ATP hydrolytic activity of the two membrane preparations. To test this possibility, ATPase activities of adult and neonatal lung membranes were compared (Figure 6). ATPase activation depended on ATP concentrations. The order of the efficacy of ATPase activities was adult > neonate at incubation time 4 min at all ATP concentrations tested. At incubation times 10 min and 30 min, the efficacies in both membranes were similar at low concentrations of ATP, but higher in adult than neonate at high concentrations of ATP. These results suggest that the hydrolysis of ATP in part contributes to the smaller ATP effect on guanylyl cyclase in adult membranes. The degree of the ATP effects on ANP-stimulated guanylyl cyclase activities was, however, similar in both membranes at incubation time of 4 min. In addition, at incubation times at 10 min, ANP/ATP-stimulated guanylyl cyclase activity of adult membranes was lower than that of neonatal membranes. Hence, there is a possibility that other factor(s) besides ATPase affects the ATP effect on guanylyl cyclase activity.

Effects of Triton X-100 on guanylyl cyclase activities

There is evidence that some detergents perturb the interaction between ATP and guanylyl cyclase (Kurose *et al.*, 1987; Chang *et al.*, 1990). Hence, we examined effects of 0.1% Triton X-100 on ANP/ATP-stimulated guanylyl cyclase

activities at various incubation times in adult and neonatal lung membranes (Figure 7). Addition of 0.1% Triton X-100 diminished the enzyme activity in neonatal membranes and enhanced the activity in adult membranes. As a result of the opposite actions of Triton X-100, the magnitude of the stimulatory effect of ANP/ATP on the enzyme activities became similar in both membranes. Effects of pre-incubation with ATP γ S on ANP-stimulated guanylyl cyclase activities of both membranes were compared with those of simultaneous addition of ATP γ S in the presence or absence of Triton X-100 (Figure 8). Pre-incubation with 200 μ M ATP γ S caused much more elevation of ANP-stimulated guanylyl cyclase activities compared to those with simultaneous addition of ANP and 200 μ M ATP γ S (see Figure 5b). Addition of Triton X-100, however, almost completely abolished the enhanced effects of pre-incubation with ATP γ S. The resulting activities were similar to those induced by simultaneous addition of ATP γ S in both membranes. On the other hand, guanylyl cyclase activation by simultaneous addition of ANP and ATP γ S was not affected by Triton X-100 in either membrane (see Figure 4b).

It is known that plasma membrane fluidity affects the membrane-related reactions, regulation of intracellular metabolism, and membrane-bound enzymes (Singer & Nicholson, 1972; Shinitzky & Inbar, 1976; Freedman, 1981). It is possible that membrane fluidity of lung membranes affects the ANP/ATP-stimulated guanylyl cyclase activity. Accordingly, the membrane fluidity of adult and neonatal lung membranes was observed by measuring fluorescence polariza-

Figure 6 ATPase activities in plasma membrane fractions of adult and neonatal rat lungs. Crude plasma membranes were incubated with indicated concentrations of ATP for 4 min (a), 10 min (b), and 30 min (c). Inorganic phosphates released by ATPase were measured as described in the Methods. Adult (●); neonate (○). Values are means $\pm$ s.e. mean of four separate experiments.

Figure 7 Effects of Triton X-100 treatment on ANP/ATP-stimulated guanylyl cyclase activities in plasma membrane fractions of adult and neonatal rat lungs. Crude plasma membranes were incubated with simultaneous addition of ANP (0.5 μ M) and ATP (100 μ M) in the presence (dashed lines) and absence (solid lines) of 0.1% Triton X-100 for indicated incubation times. Measurement of guanylyl cyclase activity was carried out as described in the Methods. Adult (●); neonate (○).

Figure 8 Effects of Triton X-100 on ANP/ATP γ S-stimulated guanylyl cyclase activity of plasma membrane fractions of adult (closed symbols) and neonatal (open symbols) rat lungs. Crude plasma membranes were pre-incubated for 5 min with (circles and triangles) or without (squares) ATP γ S (200 μ M). Thereafter ATP γ S (200 μ M) was added to the membrane suspensions without ATP γ S (squares). All the membrane suspensions containing ATP γ S and ANP were used for the measurement of guanylyl cyclase activity according to the Methods in the presence (triangles and squares) and absence (circles) of Triton X-100 for indicated incubation times.

Table 2 Fluorescence polarization of lung membrane fractions

	Fluorescence polarization
Adult	0.2443 $\pm$ 0.0018
Neonate	0.2176 $\pm$ 0.0009

The groups are significantly different from each other at $P < 0.001$. Measurement of fluorescence polarization in lung membranes of adult and neonate is described in Methods. Values are means $\pm$ s.e.mean of six separate experiments.

tion of DPH-membrane suspension. As shown in Table 2, the order of the fluorescence polarization was adult > neonate, indicating that the order of magnitude of membrane fluidity was neonate > adult.

Discussion

Developmental changes of ANP-receptor/guanylyl cyclase system were studied using plasma membrane fractions prepared from adult (2 month old) and neonatal (5–7 day old) rat lungs. ANP slightly activated guanylyl cyclase and its activity was markedly enhanced by addition of ATP in both membranes. ATP γ S caused much greater enhancement of ANP-stimulated guanylyl cyclase than ATP. ATP and ATP γ S had no effect on basal guanylyl cyclase activity of rat lung membranes, in agreement with observations by Marala *et al.* (1991), and in disagreement with observations by other investigators (Kurose *et al.*, 1987; Chang *et al.*, 1990; Gazzano *et al.*, 1991). The stimulatory effects of ATP analogues on basal guanylyl cyclase activities were dependent on Mg^{2+} concentration in incubation medium (Gazzano *et al.*, 1991). The Mg^{2+} concentration and membrane preparation used in

our experiments may not be suitable for activation of basal guanylyl cyclase by ATP.

ANP- or ANP/ATP-stimulated guanylyl cyclase activity of adult membranes was approximately one-half that of neonatal membranes when the incubation time was prolonged (10–30 min). The lower enzyme activity of adult membranes was restored to the level of neonatal membranes by addition of increasing ATP concentrations, but fell again with prolongation of incubation time. In contrast, addition of ATP γ S exerted a similar degree of activation of ANP-stimulated guanylyl cyclase in both membranes. There was no significant difference in the ability of ATP γ S binding between the membranes. ATPase activity of adult membranes was higher than that of neonatal membranes. These results suggest that an increase in ATP hydrolysis decreases the effect of ATP on guanylyl cyclase activity. There was, however, not always an inverse correlation between the incubation time- and ATP concentration-dependent ATPase activities and guanylyl cyclase activities in both membranes. For example, the response of guanylyl cyclase to ANP was similar in both membranes regardless of the presence of ATP, while ATPase activity of adult membranes was already higher than that of neonatal membranes at the early incubation time of 3–4 min. In addition, Kurose *et al.* (1987) reported that ADP and ADP β S also enhanced ANP-stimulated guanylyl cyclase, but their efficacies were only about half that of ATP. These results raised the possibility that other factor(s) besides ATPase affect(s) the ATP effect on guanylyl cyclase.

More recent studies (Leitman *et al.*, 1986; Fuller *et al.*, 1988; Pandey *et al.*, 1988; Chang *et al.*, 1989; Chinkers *et al.*, 1989; Lowe *et al.*, 1989; Schulz *et al.*, 1989; Uchida *et al.*, 1989) using the co-purification of ANP receptor/guanylyl cyclase and molecular cloning, demonstrated that there are three distinct ANP receptor molecules with different molecular weight in various mammalian tissues: types A- and B-receptors are preferentially activated by ANP and BNP (brain natriuretic peptide), and by CNP (clearance natriuretic peptide), respectively. Both receptors are coupled to guanylyl cyclase. Type C-receptors without guanylyl cyclase are related to clearance of excess of ANP, BNP and CNP which are coupled to the adenylyl cyclase/cyclic AMP signal transduction system through inhibitory GTP-binding protein (Anand-Srivastava *et al.*, 1990). Co-purified ANP receptor/guanylyl cyclase which retained ANP-binding characteristics, however, could not be stimulated by ANP, suggesting that some components essential for guanylyl cyclase activation were lost during the purification procedure or that the regulatory domain of the enzyme was labile (Pual *et al.*, 1987; Takayanagi *et al.*, 1987; Meloche *et al.*, 1988).

It was demonstrated that allosteric nucleotide-binding sites for ATP participate in the activation of ANP-stimulated guanylyl cyclase (Kurose *et al.*, 1987; Singh *et al.*, 1988; Chinkers & Garbers, 1989; Marala *et al.*, 1991). In addition, Kurose *et al.* (1987) demonstrated that adenosine analogues, ADP, ADP β S, or AppNHp but not UTP, CTP, and AMP also enhanced ANP-stimulated guanylyl cyclase, although they produced smaller effects than ATP itself. These results suggested that ATP or ATP γ S activated guanylyl cyclase via best occupation of the allosteric site(s). The present study showed that there are two classes of ATP-binding sites on plasma membranes of rat lung from Scatchard analysis of ATP γ S binding. Therefore, the protein kinase-like domain appears to contain ATP-binding sites.

Particulate guanylyl cyclase activities were enhanced by some detergents due to the unmasking of receptor binding domains (Lad & White, 1979; Garbers & Radnay, 1981; Meloche *et al.*, 1988). In addition, Chang *et al.* (1990) and Gazzano *et al.* (1991) suggested that the effects of ATP γ S on stimulating ANP-dependent guanylyl cyclase activity are exerted via the action of accessory proteins or non-protein cofactor which is susceptible to membrane washing and detergents, and is stabilized by ATP γ S. The present study showed that Triton X-100 brought about stimulation in adult

membranes and suppression in neonatal membranes of ANP/ATP-stimulated guanylyl cyclase activities, thereby resulting in the abolition of the difference in the responsiveness between the different membranes. In contrast, ATPyS pre-incubation caused much more elevation of ANP-stimulated guanylyl cyclase activities compared with that produced by simultaneous addition of ATPyS. The markedly elevated activities were, however, decreased by Triton X-100 to levels induced by simultaneous addition of ATPyS in both membranes.

The above results, together with results observed by previous investigators suggested the following two possibilities. There exist modulatory factor(s) regulated by ATP. The factor(s) is more abundant in adult membranes than in neonatal membranes, and exhibits time-dependent inhibitory effects on ANP- or ANP/ATP-stimulated guanylyl cyclase activity. The inhibitory effect is counteracted by non-hydrolyzable ATPyS, and the ATPyS effect is not affected by Triton X-100. Another possibility is that conformation of plasma membranes is related to activation of the ANP receptor/guanylyl cyclase system. The guanylyl cyclase activities of membranes were only slightly activated by ANP alone, but markedly activated by ANP/ATP or ANP/ATPyS. The active conformation of the membranes is stabilized by ATPyS pre-incubation and this effect is abolished by Triton

X-100. No direct evidence is yet available to support our hypotheses, and the mechanism of actions of Triton X-100 on ANP/ATP-dependent guanylyl cyclase activity cannot be clearly explained. In any case, it seems premature to conclude that Triton X-100 diminishes ANP/ATP-stimulated guanylyl cyclase activity simply by denaturation of the enzyme.

The degree of fluorescence polarization of neonatal membranes was lower than that of adult membranes, indicating that membrane fluidity of neonatal membranes was higher than that of adult membranes. This fact may imply that an essential component for the activation of guanylyl cyclase embedded in the membranes becomes more exposed, and thereby contributes to the enhanced response to ANP (Freedman, 1981).

Weil *et al.* (1986) reported that plasma concentrations of ANP are elevated during the first 2–4 days of human life and decline rapidly, suggesting that the enormous changes which were related to elevated pulmonary arterial pressure occurred in the circulation after birth. The phenomenon that plasma ANP concentration is high in neonates may contribute to an increase in ANP/ATP-dependent cyclic GMP production of neonatal rat lung membranes. Therefore, the characteristics of guanylyl cyclase in adult and neonatal lung membranes should be examined from different aspects.

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