*Biochemical Pharmacology*, Vol. 38, No. 8, pp. 1359–1361, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00


# SHORT COMMUNICATIONS

## Interaction of gentamicin with atrial natriuretic polypeptide receptors in renal cells

(*Received* 16 *February* 1988; *accepted* 3 *August* 1988)

Atrial natriuretic polypeptide (ANP), which is produced by and secreted from the heart, was found to possess potent natriuretic and diuretic activities, and the kidney was demonstrated to be one of the main target tissues of ANP [1–3]. We reported that specific receptors for human (h) ANP were localized in the cultured renal epithelial LLC-$PK_1$ cells, and that cyclic GMP production in these cells was stimulated by α-hANP [4]. In addition, specific binding sites for α-hANP were found on basolateral membranes isolated from rat renal cortex [5, 6], comparable to binding studies by Napier *et al.* [7].

Aminoglycoside antibiotics appear to cause nephrotoxicity, in which the initial event occurs at the plasma membranes of the proximal tubular cells [8–10]. It was also suggested that plasma membranes isolated from renal cortex possess binding sites for aminoglycosides [11–13]. Although anionic phospholipids were implicated as binding sites for the drugs [14, 15], the target molecules for aminoglycosides on the plasma membranes have not been fully identified [16]. During the course of the characterization of ANP receptors in the kidney, we found that gentamicin interacts with ANP receptors in the LLC-$PK_1$ cells and in basolateral membranes isolated from rat renal cortex.

### *Materials and Methods*

The following reagents were used: [$^{125}$I]α-hANP (2000 Ci/mmol, Amersham International, Buckinghamshire, U.K.), α-hANP (Protein Institute Inc., Osaka, Japan), bacitracin and gentamicin sulfate (Sigma Chemical Co., St. Louis, MO, U.S.A.), bovine serum albumin (BSA, Poviet Producten B.V., Amsterdam, Holland), Tris, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), 3-isobutyl-1-methylxanthine, spermine and tetraethylammonium (TEA, Nakarai Chemicals, Kyoto, Japan). A cyclic GMP assay kit was purchased from the Yamasa Shoyu Co., Ltd. (Chiba, Japan). According to previous reports, binding studies were performed for basolateral membranes [5, 6] and for the LLC-$PK_1$ cells [4], and the determination of cyclic GMP production in the LLC-$PK_1$ cells was carried out using the monolayer cells grown on 35 mm diameter plastic dishes [4].

### *Results and Discussion*

The effects of gentamicin, spermine and tetraethylammonium as well as unlabeled α-hANP on [$^{125}$I]α-hANP binding to the LLC-$PK_1$ cells and to renal basolateral membranes were examined. As reported previously [4–6], the binding of [$^{125}$I]α-hANP to both preparations was inhibited competitively by increasing concentrations of unlabeled α-hANP. In the LLC-$PK_1$ cells (Fig. 1), gentamicin and spermine inhibited the specific binding of [$^{125}$I]α-hANP in a dose-dependent manner. The concentrations of both drugs required for half-maximal displacement of [$^{125}$I]α-hANP binding ($IC_{50}$) were approximately 1 mM. On the other hand, tetraethylammonium caused no significant inhibition. In basolateral membranes (Fig. 2), gentamicin

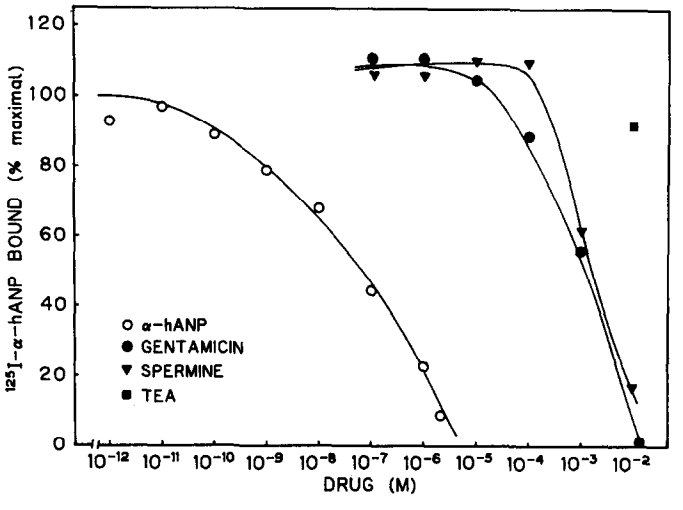


Fig. 1. Effects of gentamicin and cationic drugs on [$^{125}$I]α-hANP binding to LLC-$PK_1$ cells. The LLC-$PK_1$ cells (100 μl, $6 \times 10^5$ cells/tube) were incubated at 0° for 60 min with [$^{125}$I]α-hANP (100 μl, 32,000 cpm) in phosphate-buffered saline (PBS) buffer containing 0.2% BSA, in the presence and absence of unlabeled α-hANP or several drugs at concentrations shown on the abscissa. The bindings of [$^{125}$I]α-hANP in the presence and absence of 3 μM unlabeled α-hANP were 1.3 and 3.5% of the total radioactivity added respectively. Each point represents the mean of three determinations. TEA = tetraethylammonium.

and spermine inhibited [$^{125}$I]α-hANP binding, whereas tetraethylammonium did not.

We previously demonstrated that the LLC-PK$_1$ cells respond to α-hANP by a dose-dependent increase in cyclic GMP production [4]. Therefore, the effect of gentamicin on cyclic GMP production stimulated by α-hANP in the LLC-PK$_1$ cells was examined. As shown in Table 1, cyclic GMP production stimulated by α-hANP at 10 nM, which is the concentration of the peptide required for half-maximal activity ($EC_{50}$) [4], was unaffected in the presence of gentamicin at concentrations of 30 mM or below. However, cyclic GMP production induced by 1 nM α-hANP was further stimulated in the presence of gentamicin at concentrations above 1 mM. As shown in Fig. 3, furthermore, gentamicin stimulated cyclic GMP production in a dose-dependent manner, whereas spermine and tetraethylammonium had no effect on cyclic GMP production.

The findings that α-hANP binding to both the LLC-PK$_1$ cells and basolateral membranes was inhibited by gentamicin and spermine but not by tetraethylammonium suggest that the polycationic charge of the drugs is responsible for the binding to the ANP receptor. These observations raise the possibility that the specific binding of ANP to its receptors is, in part, due to the cationic property of the peptide. In fact, α-hANP is a cationic polypeptide composed of 22 neutral, 1 acidic and 5 basic amino acid residues. Among the cationic drugs tested, gentamicin stimulated cyclic GMP production dose-dependently in the LLC-PK$_1$ cells, whereas spermine and tetraethylammonium did not. However, gentamicin caused no significant further increase in cyclic GMP production, when the cells were stimulated by 10 nM α-hANP. If cyclic GMP production is induced by gentamicin through a pathway different from that of α-hANP, it should be induced even in the presence of α-hANP. Therefore, the stimulation of cyclic GMP production by gentamicin may be related to activation of α-hANP receptor-coupled guanylate cyclase.

Since the identification of the binding sites for aminoglycosides in the plasma membranes is important for understanding their toxicity to renal cells, several groups have examined the target molecules for aminoglycoside antibiotics. Although anionic phospholipids were identified as the binding sites for aminoglycoside in the renal plasma membranes [13–15], the binding capacity of the membranes cannot be fully explained only by the phospholipids [16]. A similar class of binding sites was observed in both brush border and basolateral membranes, while the binding capacity was greater in basolateral membranes than brush border membranes [13, 17].

Based on the present results, specific receptors for ANP in both the LLC-PK$_1$ cells and renal basolateral membranes may contribute to the binding of gentamicin to renal plasma membranes.

Table 1. Effect of gentamicin on stimulation of cyclic GMP production by α-hANP in LLC-PK$_1$ cells

| Gentamicin (mM) | Cyclic GMP formed (pmol/10$^6$ cells) 1 nM α-hANP | 10 nM α-hANP |
|---|---|---|
| 0 | 3.15 ± 0.09 | 5.84 ± 0.11 |
| 1 | 3.57 ± 0.07* | 5.78 ± 0.64 |
| 3 | 3.50 ± 0.06 | 5.53 ± 0.31 |
| 10 | 4.05 ± 0.09† | 6.10 ± 0.03 |
| 30 | 4.49 ± 0.14† | 5.61 ± 0.11 |

The cells (3 × 10$^6$ cells) grown on 35 mm plastic dishes were incubated at 37° for 15 min with α-hANP in the presence and absence of gentamicin. Each value represents the mean ± SE of three determinations.

*† Significant differences from no addition of gentamicin using one-way analysis of variance followed by Dunnett's *t*-test: *$P < 0.05$, and †$P < 0.01$.

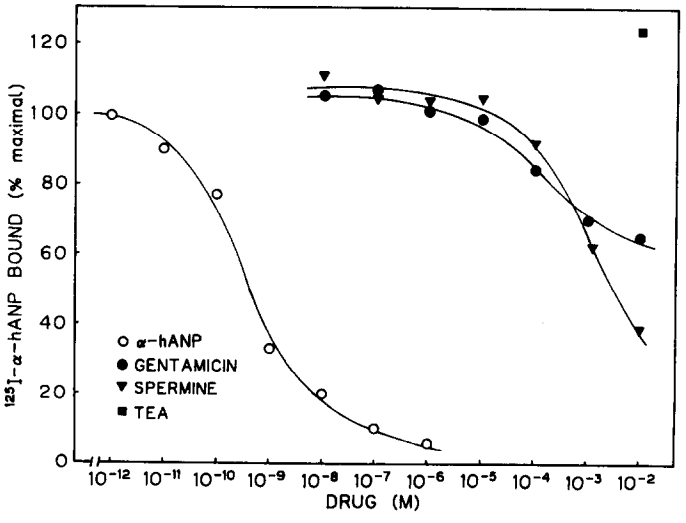


Fig. 2. Effects of gentamicin and cationic drugs on [$^{125}$I]α-hANP binding to renal basolateral membranes. Basolateral membranes (100 μl, 52 μg protein/tube) were incubated at 0° for 4 hr with [$^{125}$I]α-hANP (100 μl, 45,000 cpm) in 50 mM Hepes–Tris, pH 7.5, 5 mM $MgCl_2$, 0.2% BSA and 0.1% bacitracin, in the presence and absence of unlabeled α-hANP or several drugs at concentrations shown on the abscissa. The bindings of [$^{125}$I]α-hANP in the presence and absence of 3 μM unlabeled α-hANP were 5 and 13% of the total radioactivity added respectively. Each point represents the mean of three determinations. TEA = tetraethylammonium.

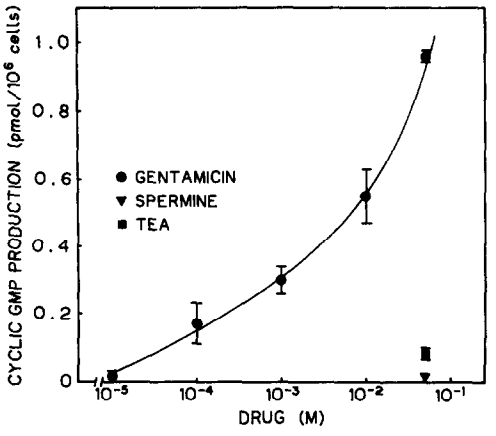


Fig. 3. Effects of gentamicin and cationic drugs on cyclic GMP production in the LLC-PK$_1$ cells. The LLC-PK$_1$ cells ($3 \times 10^6$ cells) grown on 35 mm plastic dishes were incubated at 37° for 15 min in buffer B containing 0.5 mM 3-isobutyl-1-methylxanthine in the presence of the drugs [gentamicin, spermine, and tetraethylammonium (TEA)] at concentrations shown on the abscissa. Each point represents the mean ± SE of three determinations.

*Acknowledgement*—This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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