Biochemical Pharmacology, Vol. 32, No. 23, pp. 3601–3604, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

THE EFFECT OF GUANINE NUCLEOTIDES ON [^{125}I]-ANGIOTENSIN BINDING IN RAT KIDNEY CORTEX EPITHELIAL MEMBRANES

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(*Received* 18 *May* 1983; *accepted* 11 *July* 1983)

Abstract—Specific [^{125}I]-angiotensin binding to crude basolateral/brush border membranes of rat kidney cortex was influenced by guanine nucleotides. The order of potency of nucleotides in their ability to decrease specific binding, was Gpp(NH)p > GTP ≥ ITP > GDP > ATP > GMP > IDP. The kinetic alterations induced by a maximally effective concentration of Gpp(NH)p were: (a) a reduced steady state level of binding and (b) a markedly slower rate of ligand dissociation. The presence of Gpp(NH)p was found to increase the affinity of [^{125}I]-angiotensin binding sites in rat renal cortex membranes. This contrasts with the decreases in affinity of [^{125}I]-angiotensin binding reported in adrenal and mesenteric artery membranes.

It is well established that guanine nucleotides may influence many hormone and neutrotransmitter-receptor interactions. Guanosine triphosphate (GTP) has been proposed to bind to different nucleotide regulatory proteins within the target cell membranes. These proteins in turn mediate stimulation or inhibition of adenylate cyclase [1].

Specific binding of the hormone angiotensin II (AII) to various target tissues has been reported to be modulated by guanine nucleotides. Glossmann and co-workers [2] reported that these substances induced decreases in AII binding in rat adrenal glomerulosa cell membranes. Similar results have since been obtained in rat hepatic plasma membranes [3] and in mesenteric artery membranes [4]. As in other guanine nucleotide-sensitive binding systems it is the agonist rather than the antagonist binding which is modified since [^{125}I]-[Sar^{1}Ala^8] angiotensin binding was unaffected [3].

Recently, we have identified high affinity [^{125}I]-AII binding sites in partially purified basolateral/brush border membranes from rat renal cortex [5]. These sites exhibited an equilibrium constant and a specificity for angiotensin agonists and antagonists which resembled AII receptors in other target tissues. Furthermore, this binding of AII to renal sites required the presence of sodium ions for maximal binding activity. This requirement has been found in studies of AII binding to other target tissues [6, 7]. This study has investigated the ability of guanine nucleotides to influence the binding of [^{125}I]-AII to rat renal cortex epithelial membranes.

MATERIALS AND METHODS

Male Wistar rats (250 g) were killed by cervical dislocation and the renal cortex rapidly dissected on ice. Tissue was homogenised in a loose fitting glass homogeniser fitted with a Teflon pestle, in ice-cold 10% (w/v) isolation medium containing 250 mM sucrose, 10 mM triethanolamine HCl and 0.1 mM phenylmethylsulphonylfluoride (PMSF) pH 7.6. A crude basolateral/brush border membrane fraction of renal cortex was prepared by differential centrifugation as described previously [8]. Membranes (10–20 μg protein) were incubated with 1 nM [^{125}I]-AII for 5 min at 22° in 20 mM Tris–HCl buffer, pH 7.4, containing 120 mM NaCl, 5 mM Na_2EDTA, 0.1 mM PMSF and 0.2% BSA. The incubation volume was 250 μl. In studies performed in the absence of sodium, NaCl was omitted and 5 mM EDTA was used in the incubation buffer. Specific [^{125}I]-AII binding was defined by the addition of 1 μM unlabelled AII. Free and bound hormone were separated by rapid filtration through GF/B filters (Whatman) under vacuum. Bound radioactivity was determined in a Beckman γ Counter with a minimum counting efficiency of 76%. Filter blanks were determined routinely by incubation in the absence of renal cortex membranes [5]. Protein was determined with Coomassie Brilliant Blue Dye as described by Bradford [9].

[^{125}I]-AII was obtained from N.E.N. (Boston, MA) with a specific activity of 1880 Ci per mmole and quoted radiochemical purity of greater than 97%. The labelled hormone was dissolved in deionised water and aliquots were flash frozen, stored at −20°C and used once after thawing. Unlabelled AII and nucleotides were purchased from Sigma (Poole, U.K.). Solutions of nucleotides were made immediately prior to use in binding assays and kept on ice at all times.

RESULTS

A decrease in specific [^{125}I]-AII binding was obtained with a range of nucleotides, as shown in

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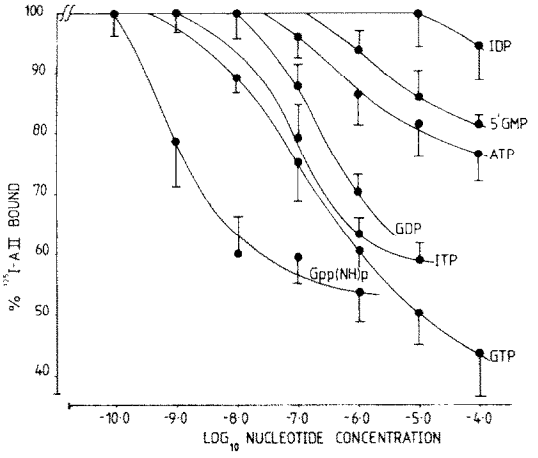

Fig. 1. Comparison of the effects of different nucleotides on [^{125}I]-AII binding in rat renal cortex epithelial membranes. Renal membranes were incubated for 5 min at 22° with 0.9 nM [^{125}I]-AII and at least 3 different concentrations of each nucleotide. Each point represents the mean ± S.E.M. from triplicate incubations.

Fig. 1. The most potent of these was the non-hydrolysable GTP analogue, 5'guanylylimidodiphosphate (Gpp(NH)p) which produced a maximal decrease in binding of 50% at a concentration of 1 μM. Therefore the potency of each nucleotide was compared by determining the concentration required to inhibit 25% of the binding. Gpp(NH)p was the most potent of the guanine nucleotides (IC_{25} 1.5 nM) being 67 times more potent than GTP. ITP was of a similar potency as GTP (IC_{25} 100 nM) while GDP was 333-fold less active than Gpp(NH)p. ATP, 5'GMP and IDP were virtually inactive at reducing specific [^{125}I]-AII binding. At high concentrations of GTP there is a suggestion (Fig. 1) that further reductions in specific binding might occur although this was not statistically significant.

The alterations in binding kinetics induced by the most potent nucleotide, Gpp(NH)p were investigated. The presence of 0.1 μM Gpp(NH)p did not alter the observed rate at which specific [^{125}I]-AII binding reached equilibrium (Fig. 2). The observed association rate constants (k_{obs}) calculated from these data, using a pseudo first order rate equation, were not significantly different from controls as shown in Table 1. In contrast, the rate of dissociation (k_{-1}) was markedly altered by the presence of Gpp(NH)p. Addition of Gpp(NH)p alone at equilibrium resulted in a 5.6-fold slower k_{-1} than the control dissociation rate initiated by 1 μM AII (Fig. 3a). Simultaneous addition of the nucleotide and AII at equilibrium, resulted in a k_{-1} that was still slower than control.

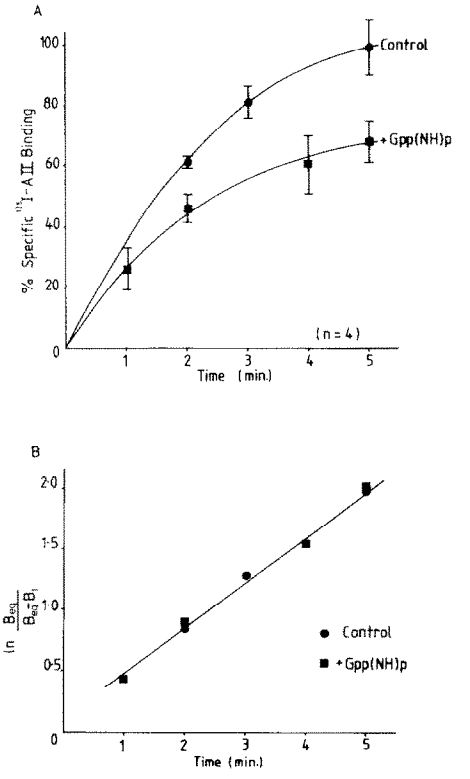

Fig. 2. (a) Association rate data under control conditions and in the presence of 0.1 μM Gpp(NH)p. Renal membranes were incubated for increasing time periods at 22° with 0.9 nM [^{125}I]-AII in the presence and absence of 0.1 μM Gpp(NH)p. Unlabelled AII was added at 1 μM to half of the tubes to define specific binding. (b) Data from (a) was transformed using a pseudo-first order rate equation. The slopes of the resulting best straight lines were obtained by linear regression to give k_{obs} which was used to calculate the association rate constant k_1 using the equation:

$$k_1 = \frac{k_{obs} - k_{-1}}{[L]}$$

where [L] is the ligand concentration.

Table 1. The effect of $G_{pp}(NH)_p$ on the rate constants of ^{125}I-AII specific binding.

Conditions	k_{obs} (sec^{-1})	k_{-1} (sec^{-1})	k_1 (M^{-1}·sec^{-1})	K_{eq} (nM)
Control	$6.39 \pm 0.33 \times 10^{-3}$	$6.49 \pm 0.03 \times 10^{-3}$	$11.36 \pm 0.53 \times 10^{6}$	0.57 ± 0.03
Gpp(NH)p, 0.1 μM at equilibrium	6.94×10^{-3}	$1.17 \pm 0.08 \times 10^{-3}$	11.59×10^{6}	0.10
Gpp(NH)p, 0.1 μM + AII, 1 μM at equilibrium	6.94×10^{-3}	4.18×10^{3}	11.12×10^{6}	0.38

Association (k_1) and dissociation (k_{-1}) rate constants were obtained using pseudo first order and first order rate equations respectively.

Individual rate constants were calculated from association and dissociation data of which Figs. 2b and 3b are representative. The ratio of k_{-1}/k_1 was used to calculate the equilibrium constant (K_{eq}) under the conditions described above.

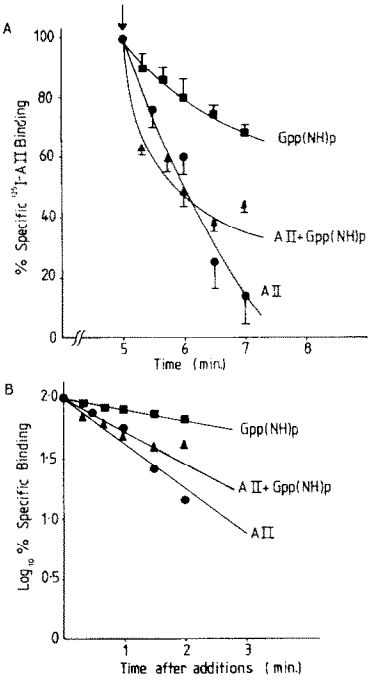

Fig. 3. (a) Effect of AII (1 μM) and Gpp(NH)p (0.1 μM) on the dissociation rate of [125I]-AII binding. Time dependent decreases in [125I]-AII binding (0.7–0.9 nM) were measured following the addition of AII, Gpp(NH)p separately or together after 5 min preincubation of membranes with ligand at 22°. (b) Dissociation rate data from (a) were fitted to a first order rate equation and the resulting best straight lines obtained by linear regression. The dissociation rate constants (k_{-1}) were obtained directly from the slopes of these lines.

but more rapid than with the nucleotide alone (Fig. 3a). Fitting the data to a first order rate equation resulted in a single component straight line (Fig. 3b), suggesting ligand dissociation from a single population of sites under different conditions. Since the rate of association of [125I]-AII binding was not significantly altered by Gpp(NH)p (see Table 1 and Figs. 2a and b) the slower rates of dissociation resulted in increases in the affinity constant (*K*eq) compared with that of controls (Table 1).

An investigation of the cation sensitivity of these guanine nucleotide-induced alterations appear to indicate a dependence on NaCl for the reduction of specific [125I]-AII binding. Absence of NaCl from the incubations reduced the steady-state level of binding as described previously [5]. The presence of Gpp(NH)p (1 nM–1 μM) under these conditions failed to significantly reduce specific binding further (Table 2). A maximal 20% inhibition of specific [125I]-AII binding was achieved with 10 nM Gpp(NH)p in the absence of sodium ions.

DISCUSSION

The results demonstrate that specific [125I]-AII binding to rat renal brush border/basolateral membranes was reduced in a dose-dependent manner by the addition of guanine nucleotides. The order of potency was Gpp(NH)p> GTP≥ ITP> GDP; whilst ATP, GMP and IDP were ineffective. This result is in agreement with observations reported for [125I]-AII binding in other target tissues [2, 3]. However, a close examination of the effects of guanine nucleotides upon the kinetics of binding revealed differences in the present study from those previously reported in other tissues. Thus, in the presence of Gpp(NH)p the rate of dissociation of the ligand from its binding site was markedly reduced, while the association constant was unaffected. This resulted in a significantly higher equilibrium dissociation constant (0.1 nM) in the presence of Gpp(NH)p compared with control values (0.57 nM). This is in direct contrast to the nucleotide-induced reductions in AII receptor affinity described in the adrenal cortex [10] and mesenteric artery [14], and to the absence of changes in the affinity in hepatic membrane preparations. It is unclear whether or not these differences reflect altered tissue sensitivity arising possibly from a different membrane environment, or are a reflection of a different mechanism of action of the hormone in these tissues.

Although guanine nucleotide-induced changes in senstivity of binding sites often suggests that the site is linked to either an inhibitory or stimulatory adenylate cyclase there are exceptions to this rule [1]. Thus the binding of some ligands which are affected by

Table 2. The effect of Gpp(NH)p on specific [125I]-AII binding in the presence and absence of NaCl

Condition	Specific [125I]-AII binding (fmol/mg protein)
Control + 120 mM NaCl	119.6 ± 12.6
Control − 120 mM NaCl + 1 nM Gpp(NH)p	80.6 ± 2.8*
Control − 120 mM NaCl	26.8 ± 6.8**
Control − 120 mM NaCl + 1 nM Gpp(NH)p	24.7 ± 10.4
Control − 120 mM NaCl + 10 nM Gpp(NH)p	21.7 ± 2.1
Control − 120 mM NaCl + 100 nM Gpp(NH)p	19.6 ± 6.5
Control − 120 mM NaCl + 1 μM Gpp(NH)p	21.8 ± 4.8

Incubations were performed for 5 min at 22° with 0.9 nM [125I]-AII in 20 mM Tris incubation buffer, pH 7.4 in the presence and absence of NaCl. A range of Gpp(NH)p concentrations were tested for their ability to affect specific binding in the absence of NaCl. The values are the mean ± S.E.M. from a single experiment performed in triplicate. Significance was determined using an unpaired Student's *t*-test with *$P < 0.05$ and **$P < 0.01$ (difference from control values in the presence of NaCl).

guanine nucleotides are non-cyclase linked. Angiotensin has been proposed to fall into this classification [1]. Certainly there is evidence to suggest that AII-induced stimulation of sodium and water transport in rat kidney cortex occurs without changes in renal cAMP or adenylate cyclase activity [11]. On the other hand, more recently, a small but significant inhibition of basal and PTH-stimulated renal adenylate cyclase has been reported [12]. This has been interpreted as evidence for AII being linked to an inhibitory adenylate cyclase. The data obtained from studies of angiotensin effects upon adenylate cyclase activity in other tissues is also controversial. Changes in adenylate cyclase activity appear to play no part in the stimulation of aldosterone biosynthesis [13, 14]. However, small (40%) but consistent inhibition of glucagon-stimulated adenylate cyclase activity by AII was reported in isolated hepatocytes [15]. At present it seems likely that if angiotensin is inducing changes in adenylate cyclase these are of an inhibitory nature.

Thus the present observations could be interpreted to suggest that there is a guanine nucleotide-regulatory protein near to the renal cortex angiotensin binding site; binding of GTP or its analogues, altering specific [^{125}I]-AII binding. Whether or not, binding of GTP to this protein leads to an inhibition of adenylate cyclase or to some other, as yet undescribed membrane event is open to speculation.

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