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Guanine Nucleotides and Monovalent Cations Increase Agonist Affinity of Prostaglandin E_2 Receptors in Hamster Adipocytes

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SUMMARY

In hamster adipocyte membranes, prostaglandin E_1 (PGE_1) and PGE_2 inhibit adenylate cyclase by a GTP-dependent process which is amplified by sodium ions. The binding of [^{3}H]PGE_2 and its regulation by guanine nucleotides and monovalent cations were studied in hamster adipocyte ghosts. Binding of [^{3}H]PGE_2 reached equilibrium within 30–60 min at 37° and was slowly reversible upon addition of unlabeled PGE_2. Saturation analysis of [^{3}H]PGE_2 binding suggested a simple, noncooperative bimolecular interaction between the ligand and a single receptor population. The equilibrium dissociation constant (K_D) and the receptor density (B_{max}) were about 10 nM and 4 pmoles/mg of protein, respectively, under the conditions used. Prostaglandins competed with the binding of [^{3}H]PGE_2 in the order of potency $PGE_1 \geqq PGE_2 > PGF_{2\alpha} > PGD_2$, which order paralleled their reported ability to inhibit adipocyte adenylate cyclase. Other agents inhibiting or stimulating adipocyte adenylate cyclase did not affect [^{3}H]PGE_2 binding. At low [^{3}H]PGE_2 concentrations (~1 nM), GTP had a biphasic effect on [^{3}H]PGE_2 binding; at concentrations up to 10 μM GTP, the binding increased by up to about 3-fold and again reached control binding at 1 mM GTP. Saturation analysis showed that GTP (10 μM) decreased the K_D by about 3-fold, without major change in the B_{max} and the slopes of the Hill plots ($n_H = 1.0$). Guanylyl 5′-imidodiphosphate exhibited the same potency as GTP but was less efficient in increasing [^{3}H]PGE_2 binding. Other nucleotides (GDP > ITP > ATP > GMP) were far less potent than GTP. Monovalent cations, in the potency order sodium > lithium > potassium > choline, caused a similar increase in [^{3}H]PGE_2 binding as observed with GTP. Studies on the time kinetics of [^{3}H]PGE_2 binding suggested that the increase in binding affinity induced by guanine nucleotides and monovalent cations is largely due to an increased association rate constant for the agonist. The data suggest that [^{3}H]PGE_2 labels physiologically relevant PGE_2 receptors in hamster adipocyte ghosts and that the observed increase in receptor affinity for the agonist induced by guanine nucleotides and monovalent cations is somehow related to the adenylate cyclase inhibition mediated by these receptors.

INTRODUCTION

In binding studies on hormone receptors that are involved in regulation of adenylate cyclase activity, it has been shown that guanine nucleotides such as GTP and Gpp(NH)p[1] decrease the receptor affinity for hormone agonists but not antagonists. Such an effect of guanine nucleotides has been reported for receptors mediating adenylate cyclase stimulation, e.g., *beta*-adrenoceptors (1–3) or glucagon receptors (4, 5), and also for receptors involved in adenylate cyclase inhibition, e.g., *alpha*$_2$-adrenoceptors (6–8) and opiate receptors (9). The observed decrease in agonist affinity appeared to be due to a guanine nucleotide-induced increased dissociation rate constant for the hormone. In studies on receptors mediating adenylate cyclase inhibition, e.g., *alpha*$_2$-adrenoceptors and opiate receptors (7–10), sodium ions have been shown to cause a similar but not an identical decrease in receptor affinity toward agonists. Whereas guanine nucleotides appear to affect agonist receptor binding and adenylate cyclase stimulation or inhibition by a regulatory coupling component, the target system of sodium ions is not yet known.

In hamster adipocytes, PGE_1, PGE_2, *alpha*-adrenergic agonists, adenosine, and nicotinic acid decrease intracellular cyclic AMP levels, apparently by a receptor-mediated inhibition of adenylate cyclase. We have recently shown that inhibition of adenylate cyclase in adipocyte membrane preparations induced by these antilipolytic agents is mediated by a GTP-dependent process which is amplified by sodium ions (11–14). To determine whether the binding of prostaglandins to their receptor

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[1] The abbreviations used are: Gpp(NH)p, guanylyl 5′-imidodiphosphate; PGE_1, PGE_2, $PGF_{2\alpha}$, PGD_2, prostaglandins E_1, E_2, $F_{2\alpha}$, and D_2; App(NH)p, adenylyl 5′-imidodiphosphate.

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sites in adipocyte membranes is regulated by guanine nucleotides and monovalent cations, we studied the binding of [^{3}H]PGE_2 to hamster adipocyte ghosts. Here we report that guanine nucleotides and monovalent cations do not decrease but increase the apparent affinity of the [^{3}H]PGE_2 binding sites for the agonist ligand.

MATERIALS AND METHODS

GTP, Gpp(NH)p, ATP, ITP, GMP, guanosine, and App(NH)p were obtained from Boehringer Mannheim (Mannheim, Federal Republic of Germany), and GDP was obtained from Sigma Chemical Company (St. Louis, Mo.). [^{3}H]PGE_2 (165 Ci/mmole) and [^{3}H]PGE_1 (89.5 Ci/mmole) were purchased from New England Nuclear Corporation (Boston, Mass.). PGE_1, PGE_2, $PGF_{2\alpha}$, and PGD_2 were kindly donated by Dr. J. Pike, The Upjohn Company (Kalamazoo, Mich.). All other reagents were obtained as described before (11–14) and were of the highest purity available. Hamster adipoycte ghosts were prepared as described (11) and stored at −85° until use. Protein was determined by the technique of Lowry *et al.* (15), with human serum albumin as standard.

The binding of [^{3}H]PGE_2 to hamster adipocyte ghosts was performed in a reaction mixture containing, if not otherwise stated, 2 mM $MgCl_2$, 30 μM App(NH)p, 50 mM Tris-HCl (pH 7.4), and [^{3}H]PGE_2 at the indicated concentrations in a total volume of 100 μl. Binding was initiated by the addition of hamster adipocyte ghosts (4–10 μg of protein) to the prewarmed reaction mixtures and conducted for 60 min or as indicated at 37°. Incubations were terminated, and bound and free [^{3}H]PGE_2 were separated by the addition of 1 ml of ice-cold incubation buffer to the reaction mixture and subsequent rapid filtration over Whatman GF/C glass-fiber filters. The filters were washed four times with 5 ml of ice-cold incubation buffer. The separation was completed within about 10 sec. Radioactivity on the filters was determined in Triton X-100-toluene-based scintillation fluid with a counting efficiency of about 40%. Assays were performed in duplicate or triplicate with variation of less than 5% of the means and were repeated at least twice. Nonspecific binding of [^{3}H]PGE_2 to hamster adipocyte ghosts was defined as the amount of [^{3}H]PGE_2 bound in the additional presence of 10 μM unlabeled PGE_2 or PGE_1 and was about 2% of total [^{3}H]PGE_2 bound. Specific binding of [^{3}H]PGE_2 is given except where indicated. The identity of [^{3}H]PGE_2 in both the free and the membrane-bound form, at the end of a 2-hr incubation period with adipocyte ghosts (50 μg of protein), was verified by thin-layer chromatography as described (16). More than 95% of [^{3}H]PGE_2 co-migrated with the original labeled compound. The binding of [^{3}H]PGE_1 was performed as described above for [^{3}H]PGE_2 binding.

RESULTS

Influence of App(NH)p on [^{3}H]PGE_2 binding. It has been reported that the stable ATP analogue, App(NH)p, decreases GTP degradation by nonspecific membrane phosphohydrolases (17). Therefore, we studied the influence of App(NH)p on the binding of [^{3}H]PGE_2 to hamster adipocyte membranes in the absence and presence of GTP. App(NH)p had no effect on control binding of [^{3}H]PGE_2 at concentrations up to 1 mM and caused a small increase at 3 mM (Fig. 1). However, in the presence of GTP (10 μM), which increased the binding by about 70%, App(NH)P caused a large increase in the efficacy of GTP to enhance [^{3}H]PGE_2 binding. At 30 μM App(NH)p, GTP increased the binding by about 3.5-fold; App(NH)p at higher concentrations caused a further small increase in the effect of GTP. By using different concentrations of GTP, we found that App(NH)p (30 μM) shifted the concentration response of GTP to the left by about one order of magnitude without causing a change in the maximal effect of GTP (data not shown). Therefore, App(NH)p (30 μM) was routinely included in the binding assay mixture.

Influence of guanine nucleotides on [^{3}H]PGE_2 binding. In the absence of GTP, binding of [^{3}H]PGE_2 to hamster adipocyte ghosts reached equilibrium within about 30 min at 37° (Fig. 2). GTP had a biphasic effect on the binding equilibrium. A maximal increase in binding was observed at 10 μM GTP; at higher GTP concentrations, the binding equilibrium was decreased and almost reached control values at 1 mM GTP. The rapidity with which binding equilibrium was reached was increased by GTP in a concentration-dependent manner. The calculated pseudo-first order association rate constants (k_{ob}) at 1.35 nM [^{3}H]PGE_2 were 0.08, 0.13, 0.23, and 0.32 min^{-1} in the presence of 0, 0.1 μM, 10 μM, and 1 mM GTP, respectively.

The addition of unlabeled PGE_2 (10 μM) after the binding equilibrium was reached (60 min) caused a slow dissociation of the receptor-ligand complex (Fig. 3). The dissociation reaction appeared to be biphasic, consisting of a first rapid phase followed by a second slow phase. This was particularly evident when GTP was added together with the unlabeled PGE_2. Whereas the slow phase with a k_{-1} of about 0.01 min^{-1} was apparently not

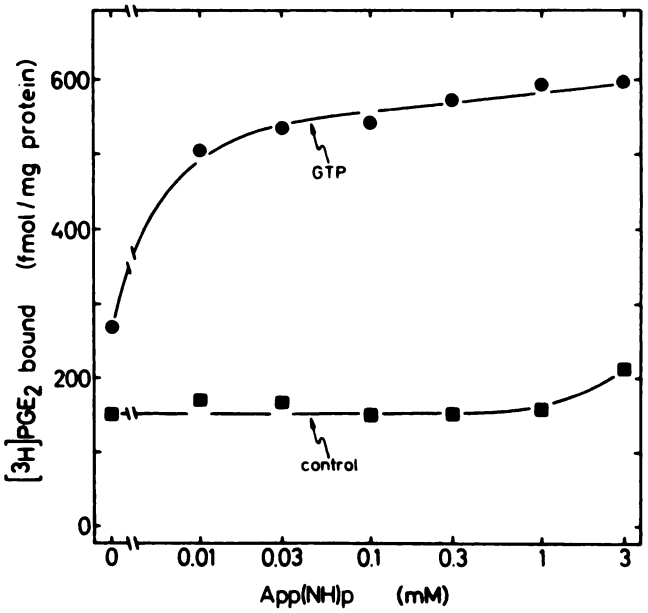

FIG. 1. *Influence of App(NH)p on [^{3}H]PGE_2 binding in hamster adipocyte ghosts*

In the absence (■) and presence (●) of GTP (10 μM), binding of [^{3}H]PGE_2 (0.88 nM) was measured at increasing concentrations of App(NH)p for 60 min.

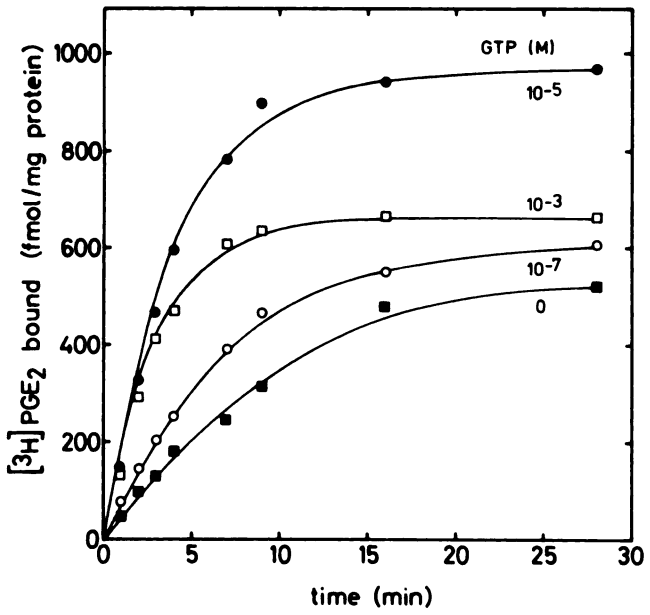

FIG. 2. *Influence of GTP on the time course of [^{3}H]PGE$_2$ binding*
Binding of [^{3}H]PGE$_2$ (1.35 nM) was measured in the absence (■) and presence of 0.1 μM (○), 10 μM (●), or 1 mM (□) GTP for the indicated periods of time.

changed by GTP, GTP increased the rapid rate of dissociation. The approximate values of the rates of this first phase were 0.07, 0.11, 0.17, and 0.29 min^{-1} in the presence 0, 0.1 μM, 10 μM, and 1 mM GTP, respectively.

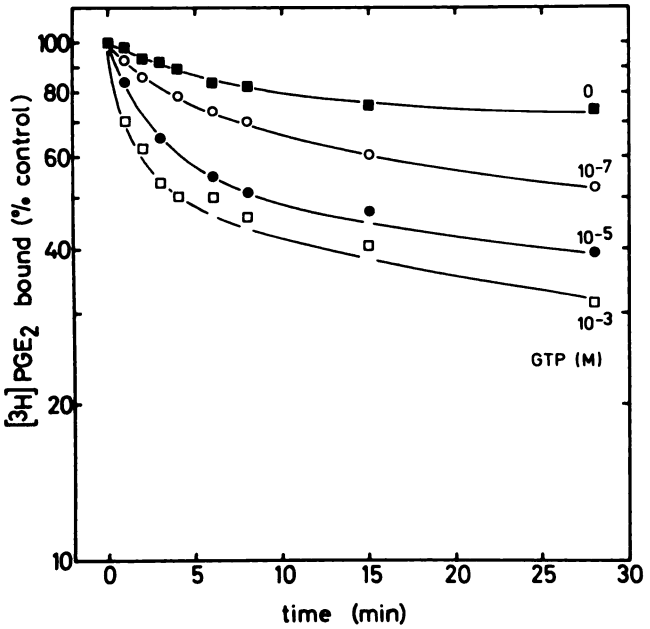

FIG. 3. *Influence of GTP on the dissociation of [^{3}H]PGE$_2$ from its binding sites*
After a 60-min preincubation of adipocyte ghosts with [^{3}H]PGE$_2$ (1.18 nM) but without GTP, unlabeled PGE$_2$ (10 μM final concentration) was added without (■) and with 0.1 μM (○), 10 μM (●), or 1 mM (□) GTP, and specific binding of [^{3}H]PGE$_2$ was then assayed at subsequent intervals. The time of PGE$_2$ addition is defined as time 0. Initial binding (100%) before the addition of unlabeled PGE$_2$ and GTP was 725 fmoles/mg of protein. The ordinate has a log scale.

However, when GTP (1 or 10 μM) was added without unlabeled PGE$_2$ to the reaction mixture after equilibrium was reached, the binding of [^{3}H]PGE$_2$ was not decreased, but GTP increased the binding as shown before in the association experiments (data not shown).

To determine the specificity of the effect of GTP, we examined the effects of various related nucleotides on [^{3}H]PGE$_2$ binding. The binding experiments were performed for 60 min, and the nucleotides studied were present throughout the complete reaction time. None of the nucleotides examined at concentrations up to 1 mM decreased [^{3}H]PGE$_2$ binding as compared with control binding (Fig. 4). GTP had a biphasic effect on the binding of [^{3}H]PGE$_2$. Binding increased at GTP concentrations up to 10 μM, followed by a decrease at higher GTP concentrations; at 1 mM GTP, control binding was observed. The stable GTP analogue, Gpp(NH)p, had a similar biphasic influence on [^{3}H]PGE$_2$ binding. A maximal increase in binding was also observed at 10 μM, but this increase was less than that seen with 10 μM GTP. GDP was almost two orders of magnitude less potent than GTP in affecting [^{3}H]PGE$_2$ binding. The increase in [^{3}H]PGE$_2$ binding observed in the presence of ITP occurred at 500- to 1000-fold higher concentrations of ITP than of GTP. Again about one order of magnitude less potent than ITP, ATP increased [^{3}H]PGE$_2$ binding. GMP had only a small effect at 1 mM, and guanosine was completely ineffective.

Since GTP increased the binding of [^{3}H]PGE$_2$ at low concentrations, we studied in saturation experiments whether this increase was due to a change in the apparent receptor number or in the binding affinity. Shown in Fig. 5 is the influence of GTP (10 μM) on the binding of [^{3}H]

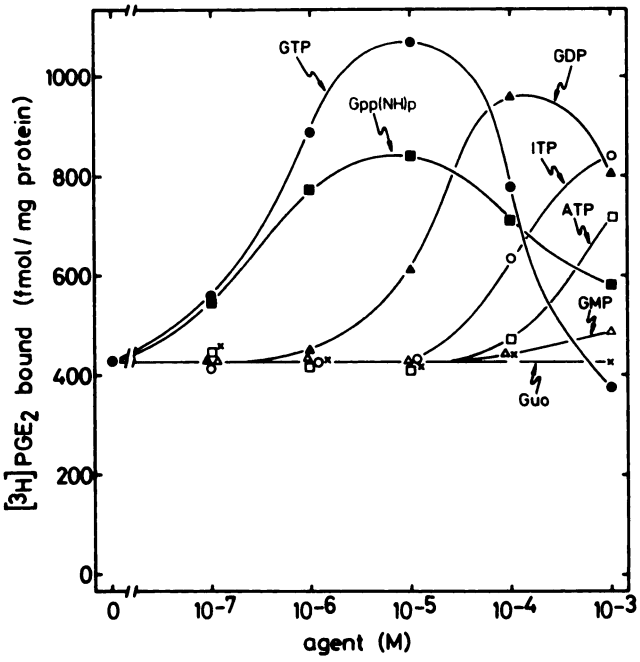

FIG. 4. *Influence of various nucleotides and guanosine on [^{3}H] PGE$_2$ binding*
Binding of [^{3}H]PGE$_2$ (0.75 nM) was measured in the absence and presence of increasing concentrations of GTP (●), Gpp(NH)p (■), GDP (▲), ITP (○), ATP (□), GMP (△), and guanosine (*Guo*, ×). The agents were present throughout the complete reaction time of 60 min.

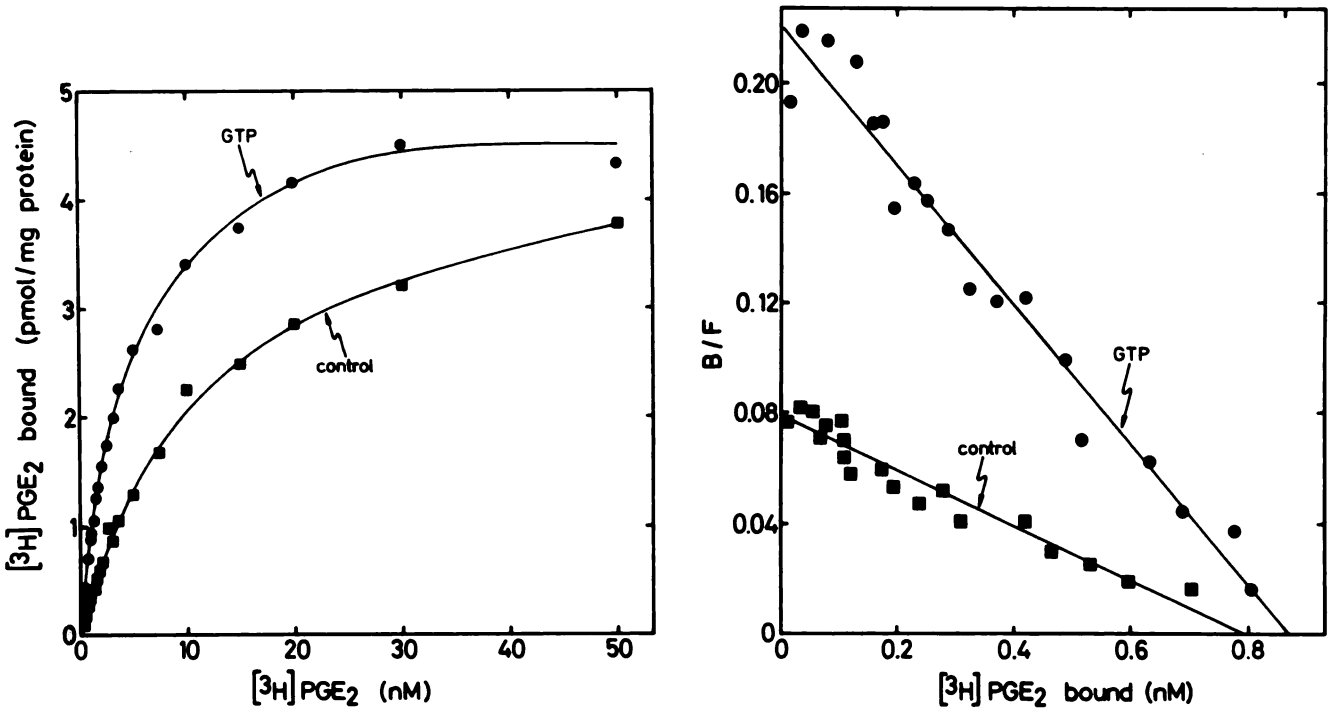

FIG. 5. *Saturation analysis of [^{3}H]PGE$_2$ binding in hamster adipocyte ghosts*
In the absence (■) and presence (●) of GTP (10 μM), binding of [^{3}H]PGE$_2$ (0.2–50 nM) was determined for a 60-min reaction period. Left, Plot of relationship of total [^{3}H]PGE$_2$ added to specific binding of [^{3}H]PGE$_2$. Right, Scatchard plot of these data, with [^{3}H]PGE$_2$ bound given on the abscissa and bound over free (B/F) [^{3}H]PGE$_2$ given on the ordinate. The lines shown were obtained by linear regression analysis ($r \geq 0.98$).

PGE$_2$ to hamster adipocyte membranes measured at concentrations of 0.2–50 nM [^{3}H]PGE$_2$. In the absence of GTP, binding of [^{3}H]PGE$_2$ was a saturable process and was half-maximal at about 10 nM [^{3}H]PGE$_2$. GTP increased the binding of [^{3}H]PGE$_2$ at any [^{3}H]PGE$_2$ concentration studied, but mainly at low concentrations. After transformation of these data according to Scatchard (18), straight lines were obtained for both control binding and binding measured in the presence of GTP. From the intercept with the abscissa and the slope of the line, control B_{max} and K_D values of 4.04 ± 0.18 pmoles/mg of protein and 10.4 ± 0.71 nM, respectively, were obtained (mean ± standard error of the mean of five separate experiments). In the presence of GTP, the equilibrium dissociation constant was decreased to 4.0 ± 0.17 nM, whereas the maximal number of binding sites was slightly but not significantly increased, to 4.53 ± 0.16 pmoles/mg of protein. The approximate K_D values derived from the association and dissociation (first phase) rate constants were 3 and 9 nM in the absence and presence of 10 μM GTP, respectively. Hill plots of the saturation experiment data revealed straight lines with Hill coefficients (n_H) of unity in both the absence and presence of 10 μM GTP (data not shown).

Influence of monovalent cations on [^{3}H]PGE$_2$ binding. We have recently shown (14) that monovalent cations, in the potency order sodium > lithium > potassium > choline, amplify GTP-dependent, PGE$_1$-induced inhibition of adenylate cyclase in hamster adipocyte membrane preparations. Therefore, the influence of these ions as their respective chloride salts was studied on [^{3}H]PGE$_2$ binding (Fig. 6). NaCl increased the binding of [^{3}H]PGE$_2$. A half-maximal increase was observed at about 100 mM NaCl, and a maximal increase (almost 3-fold) occurred at about 300 mM NaCl. LiCl was somewhat less potent than NaCl and exhibited a pronounced biphasic

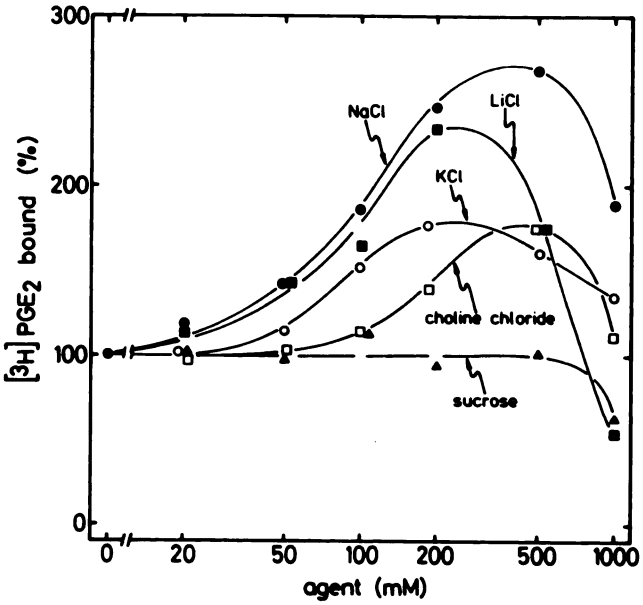

FIG. 6. *Influence of various salts and sucrose on [^{3}H]PGE$_2$ binding*
Binding of [^{3}H]PGE$_2$ (1.33 nM) was measured in the absence and presence of increasing concentrations of NaCl (●), LiCl (■), KCl (○), choline chloride (□), and sucrose (▲). The agents were present throughout the complete reaction time of 60 min; their concentrations are given on the abscissa in a log scale. Control binding (100%) was 681 fmoles/mg of protein.

response. KCl and choline chloride were about 2- and 5-fold, respectively, less potent than NaCl in increasing [^{3}H]PGE_2 binding. Besides this difference in the potency, these salts also did not reach the same maximal level of [^{3}H]PGE_2 binding as observed with NaCl. Sucrose had no effect on the binding at concentrations up to 500 mM and caused a 40% decrease at 1 M concentration.

As shown in a time course of the association reaction (Fig. 7), NaCl (250 mM) increased the binding of [^{3}H]PGE_2 both when added together with the labeled ligand to the membranes and when added 15 min after the reaction had been started, i.e., when two-thirds of the binding equilibrium was reached. Similar to GTP, NaCl increased the dissociation of the labeled ligand-receptor complex when added together with unlabeled PGE_2 (10 μM) after binding equilibrium was reached. In saturation experiments, NaCl decreased the equilibrium dissociation constant of [^{3}H]PGE_2 binding without significant change in the apparent receptor number. When added together with 10 μM GTP, NaCl (100 mM) did not cause a further increase in [^{3}H]PGE_2 binding, whereas at lower GTP concentrations the effects of both agents were additive. Specific binding of [^{3}H]PGE_2 was linear with regard to the amount of membrane preparation added, at least up to the 10-fold protein concentration usually used. Similarly, the increases in [^{3}H]PGE_2 binding induced by GTP and NaCl were independent of the membrane concentration (data not shown).

Pharmacological characterization of the [^{3}H]PGE_2 binding. To ensure that [^{3}H]PGE_2 labels physiologically relevant receptor sites in hamster adipocyte ghosts, we studied the interaction of various prostaglandins with the binding of [^{3}H]PGE_2 (Fig. 8). PGE_1 was at least as potent as PGE_2 in competing with [^{3}H]PGE_2 for the binding sites. $PGF_{2\alpha}$ was about 20-fold less potent than PGE_2, and about two orders of magnitude higher concentrations of PGD_2 than of PGE_2 were required to compete with [^{3}H]PGE_2. The K_I values, calculated from the IC_{50} values by the method of Cheng and Prusoff (19), were 6.3, 8.1, 193, and 768 nM for PGE_1, PGE_2, $PGF_{2\alpha}$ and PGD_2, respectively. Thus, the K_I value of PGE_2 observed in competition experiments was in good agreement with the K_D value (10 nM) obtained in saturation experiments. Other agents known to affect hamster adipocyte adenylate cyclase activity, such as isoproterenol (300 μM) and adrenocorticotropic hormone (10 μM), both of which stimulate adenylate cyclase, and nicotinic acid (30 μM), N^6-phenylisopropyladenosine (10 μM), and epinephrine (300 μM, added in combination with 30 μM propranolol), all of which cause adipocyte adenylate cyclase inhibition, had no effect on control [^{3}H]PGE_2 binding. The concentrations of these agents studied were those required for maximal stimulation or inhibition of the hamster adipocyte adenylate cyclase (11–14). Similar to the data shown above for [^{3}H]PGE_2 binding, GTP increased the binding of [^{3}H]PGE_1 at concentrations up to 10 μM, and at higher GTP concentrations binding was again decreased almost to control values at 1 mM GTP (data not shown).

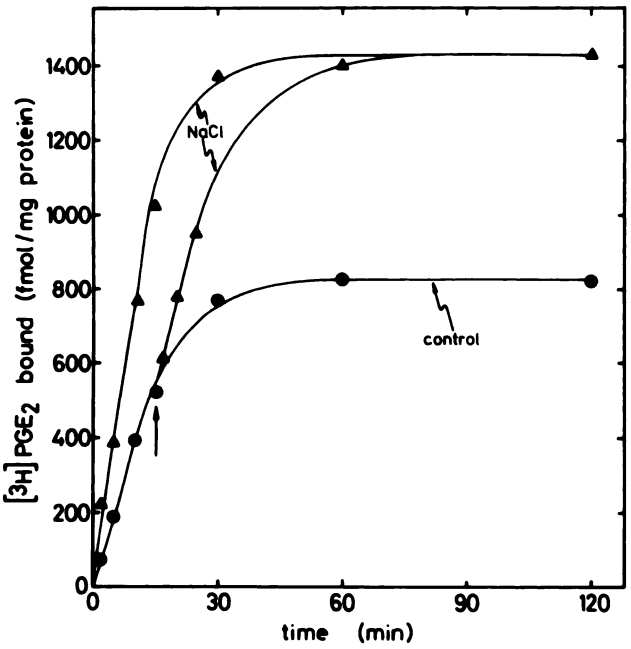

FIG. 7. *Influence of NaCl on the time course of [^{3}H]PGE_2 binding*
In the absence (●) and presence of 250 mM NaCl (▲), binding of [^{3}H]PGE_2 (1.31 nM) was measured for the indicated periods of time. NaCl was added at time = 0 or 15 min after the binding reaction had been started, as indicated by the *arrow*.

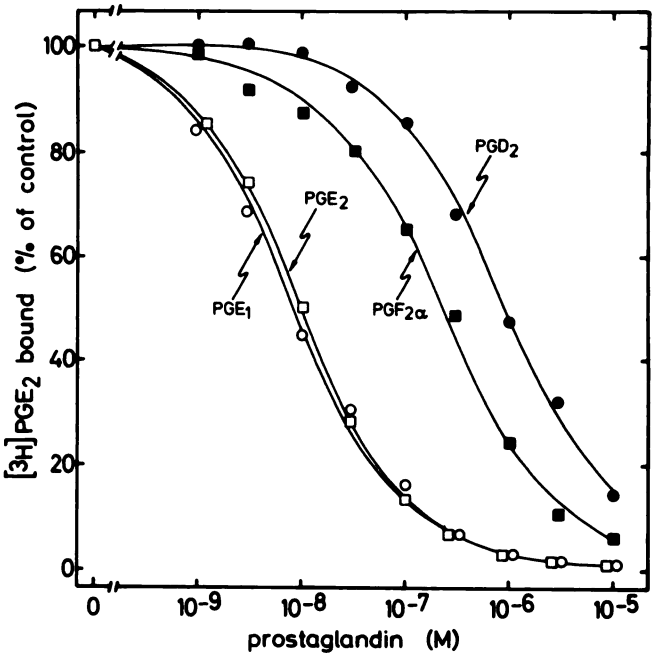

FIG. 8. *Competition of various prostaglandins with [^{3}H]PGE_2 binding in hamster adipocyte ghosts*
Binding of [^{3}H]PGE_2 (2.22 nM) was measured in the absence and presence of increasing concentrations of PGE_1 (○), PGE_2 (□), $PGF_{2\alpha}$ (■), and PGD_2 (●). Total control binding (100%) was 1230 fmoles/mg of protein.

DISCUSSION

In binding studies on various hormone receptors that are coupled to adenylate cyclase, it has been observed that guanine nucleotides decrease the receptor affinities for hormone agonists but not antagonists (when available). This guanine nucleotide effect has been described for receptors mediating adenylate cyclase stimulation (1–5) and also for receptors involved in adenylate cyclase

inhibition (6–9). The affinities of PGE_1 receptors mediating adenylate cyclase stimulation appear to be regulated by guanine nucleotides in a similar manner (20, 21). Additional modulators of agonist receptor binding, at least of those receptors that are involved in adenylate cyclase inhibition, are sodium and other monovalent cations (7–10). In hamster adipocytes, PGE_1 and PGE_2, with almost identical potencies, cause inhibition of adenylate cyclase by a GTP-dependent and sodium-amplified process (11, 12, 14). Therefore, we were interested in whether the affinity of the adipocyte PGE_1/PGE_2 receptor is also regulated by guanine nucleotides and monovalent cations as described for various other hormone receptors. Since no antagonist ligand is available for this receptor, the binding studies were performed with a labeled receptor agonist.

The data presented in this report suggest that [^{3}H]PGE_2 labels physiologically relevant PGE_1/PGE_2 receptors in hamster adipocyte ghosts and, most interestingly, they demonstrate that guanine nucleotides and monovalent cations, instead of decreasing receptor affinity, cause an increase in affinity for the agonist ligand. In adenylate cyclase studies (11), we observed that prostaglandins inhibited adenylate cyclase in the potency order $PGE_1 \geqq PGE_2 > PGF_{2\alpha} > PGD_2$. In the binding studies shown here, an identical potency order of the prostaglandins in competing with [^{3}H]PGE_2 binding was obtained. Furthermore, the PGE_2 concentration required for half-maximal inhibition of adenylate cyclase (about 10 nM) (11) was essentially like the K_D and K_I values for PGE_2 reported herein. These findings suggest that [^{3}H]PGE_2 labels the receptors that mediate adenylate cyclase inhibition by these prostaglandins. Other hormone agonists stimulating or inhibiting adipocyte adenylate cyclase had no effect on [^{3}H]PGE_2 binding. In the absence of guanine nucleotides, the binding of [^{3}H]PGE_2 to its receptor sites was relatively slow and was not readily reversible by the addition of excess unlabeled PGE_2. These kinetic characteristics of PGE_2 binding in hamster adipocyte membranes resemble those seen in PGE_1 binding studies in other systems (20–23) and also in agonist binding to different receptors, e.g., *beta*-adrenoceptors in frog erythrocytes (3).

Guanine nucleotides had several effects on the binding of [^{3}H]PGE_2. Under equilibrium conditions, these agents affected [^{3}H]PGE_2 binding in a biphasic manner, stimulating at low concentrations followed by a decrease to control binding at higher concentrations. Similar data were observed with [^{3}H]PGE_1 as receptor ligand. The potency order of the nucleotides studied was GTP $\cong$ Gpp(NH)p $\gg$ GDP > ITP > ATP > GMP, which suggests that the observed purine nucleotide effect is specific for guanine nucleotides with high selectivity for the triphosphate derivatives. In the absence of GTP, binding saturation analysis resulted in a single linear line in the Scatchard plot. GTP at a maximally stimulatory concentration (10 μM) decreased the equilibrium dissociation constant by about 3-fold. The affinity increase induced by GTP was not accompanied by an alteration of the apparent noncooperative behavior of the binding sites.

Since the decrease in receptor affinity for agonists induced by guanine nucleotides is thought to be due to an increased dissociation rate constant for the hormone (3, 4), it may be asked how guanine nucleotides increase the affinity of the adipocyte PGE_1/PGE_2 binding sites for the agonists. In association and dissociation experiments, GTP and Gpp(NH)p (data not shown) caused several effects on the binding of [^{3}H]PGE_2. First, GTP accelerated the association reaction in a concentration-dependent manner. On the other hand, GTP also increased the dissociation of the labeled ligand when added together with an excess of unlabeled ligand. These effects of guanine nucleotides are similar to those observed in agonist binding studies on other hormone receptors, e.g., *beta*-adrenoceptors (3). However, when added without unlabeled PGE_2, GTP did not induce dissociation of the receptor-ligand complex after equilibrium was reached, but increased the binding of [^{3}H]PGE_2 as observed in the association experiments. Thus, the increase in the apparent affinity induced by GTP is not due to a decreased dissociation rate constant but apparently is due to an increased association rate constant. The apparent increases in both rate constants, furthermore, suggest that GTP (at any concentration) leads to an increased rate of formation of receptor-ligand complex.

Similar to guanine nucleotides, monovalent cations increased binding of [^{3}H]PGE_2. The characteristics of the cation effects on the PGE_2 binding were not different from those observed with GTP. As mainly studied with NaCl, sodium accelerated the association reaction and also increased dissociation of the labeled ligand-receptor complex after equilibrium was reached, but only when added together with an excess of unlabeled PGE_2 and not when added alone. Therefore, the observed increase in [^{3}H]PGE_2 binding also appears to be due to an increased association rate constant for the ligand. The observed potency order of the monovalent cations studied, sodium > lithium > potassium > choline, is identical with that observed in adenylate cyclase studies with regard to inhibition of the enzyme by PGE_1 (14). This finding suggests that the cations act via the same (unknown) component on the agonist receptor binding as on the adenylate cyclase inhibition mediated by these receptors. From the available data it cannot be determined whether these actions are independent of each other or whether, by one action on its target system, monovalent cations increase agonist-receptor binding and amplify hormone-induced adenylate cyclase inhibition. The same is true for the action of guanine nucleotides, since the guanine nucleotide-sensitive system mediating hormone-induced adenylate cyclase inhibition has not yet been identified.

The observed increase in agonist receptor affinity induced by guanine nucleotides appears not to be unique to the adipocyte PGE_2 binding sites studied. It has been shown (8) that GTP can increase the binding of the agonists clonidine and epinephrine to brain *alpha*$_2$-noradrenergic receptors. The stimulatory effect of GTP was seen only in the presence of divalent cations such as magnesium (as we used), manganese, or calcium, whereas in the absence of divalent cations GTP (>1 μM) decreased the binding.[2] Since no saturation experiments were performed under these stimulatory conditions, it is not clear from these studies whether the GTP-induced increase in

[2] Similar data were recently observed on adipocyte PGE_2 and adenosine receptors (24) (K. H. Jakobs, manuscript in preparation).

agonist binding is due to an increased affinity of the brain *alpha*$_2$-adrenoceptor for the agonists studied, as shown here for the adipocyte PGE_1/PGE_2 binding sites. In studying binding on hamster adipocyte adenosine receptors, we found that GTP can increase the binding of the labeled agonist ligand N^6-phenylisopropyladenosine; this enlargement was accompanied by a GTP-induced increase in adenosine receptor affinity for the agonist (24). Thus, it appears that the observed increase in receptor affinity for agonists is not unique to the PGE_1/PGE_2 binding sites and also not to the adipocyte system studied. Further studies on different receptor systems may reveal how general this type of agonist receptor binding regulation is and by which molecular mechanisms guanine nucleotides exert this regulation.

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