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INVOLVEMENT OF GTP-REGULATORY PROTEIN IN BRAIN PROSTAGLANDIN E_2 RECEPTOR AND SEPARATION OF THE TWO COMPONENTS

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The specific binding protein for prostaglandin (PG) E_2 solubilized from porcine brain was sensitive to guanine nucleotides. GTP inhibited the association and enhanced the dissociation of the specific [^{3}H]PGE_2 binding. Scatchard analyses showed that GTP (10 μM) decreased the binding affinity more than 3-fold without major change in the number of binding site. Gel filtration separated the binding site from GTP-regulatory component (N). The separated binding protein had a reduced affinity to PGE_2 and lost its sensitivity to GTP. The addition of the separated N restored its responsiveness to GTP, and also increased the binding affinity to the original level. These results provide direct evidence for the molecular interaction between the PGE_2 binding protein and N in the brain.

Prostaglandin (PG) E series stimulate the synthesis of cyclic AMP in brain slices (1), neuroblastoma cells (2,3) and primary culture cells from rat fetal brains (4). This fact suggests that PGE series exert various neurophysiological actions (5) such as regulation of body temperature and food intake via the adenylate cyclase system. Hormone-sensitive adenylate cyclase system is composed of at least three types of proteins in the membrane as revealed by recent studies (6,7): the hormone receptor (R), the guanine nucleotide-regulatory protein (N), and

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Abbreviations used: PG, prostaglandin; N, guanine nucleotide-regulatory protein; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate.

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the catalytic unit of adenylate cyclase (C). The binding protein, presumably a receptor, of PGE_2 was found in membrane preparations from bovine pineal gland (8) and from rat (9) and human (10) brains. The localization of the PGE_2 binding protein was investigated in our laboratory using in vitro autoradiography and computerized image analyses (11). In monkey hypothalamus, PGE_2 binding was predominant in the lateral and median preoptic area (the center of body temperature regulation), anterior hypothalamic nucleus (the autonomic center), and lateral hypothalamic area (the center of food intake), suggesting the close relationship between the localization of the PGE_2 binding and its action (12). However, the evidence for the molecular interaction between the PGE_2 receptor and N in the brain was not available. Even in peripheral tissues, where the PGE binding to the receptor is suggested to be regulated via N by GTP (13-15), the separation and reconstitution of the two components (R and N) have not yet been demonstrated. Recently, we solubilized the PGE_2 binding protein in an active form from porcine brain by 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS) (16). In this study, we report that the PGE_2 binding to the solubilized fraction is sensitive to GTP and that R and N can be separated by gel filtration using HPLC.

EXPERIMENTAL PROCEDURES

Materials——[5,6,8,11,12,14,15-^{3}H(N)]PGE_2 (165 Ci/mol) was purchased from New England Nuclear. Unlabeled PGE_2 was a generous gift from Ono Pharmaceutical Company. Nucleotides and guanyl-5'-yl imidodiphosphate were obtained from Boehringer Mannheim. CHAPS was from DOJINDO Laboratories. TSKgel G5000PW and G3000SW columns were from Toyo Soda.

Solubilization of PGE_2 binding protein——The PGE_2 binding protein was solubilized from porcine cerebral cortex with 10 mM CHAPS according to the procedure described previously (16).

Binding assay——The PGE_2 binding to the solubilized fraction was determined by the polyethylene glycol method described previously (16) with a slight modification. The standard reaction mixture

(0.2 ml) contained the solubilized fraction (0.2-1.4 mg of protein), 50 mM Tris-HCl (pH 7.4), 0.1 M NaCl, 10 nM [^{3}H]PGE_2, 10% glycerol, 2 mM 2-mercaptoethanol, and 1 mM EDTA. The latter three compounds activated the specific binding about twofold as compared with that using the previous reaction mixture. The final concentration of CHAPS in the reaction mixture was adjusted to 5 mM. Nonspecific binding was determined by carrying out incubations with [^{3}H]PGE_2 in the presence of 100 μM unlabeled PGE_2. The specific binding was calculated by subtracting the nonspecific binding from the total binding. All values were expressed as the means of triplicate determinations.

Protein determination——Proteins were assayed by the method of Lowry et al. (17) with bovine serum albumin as the standard.

RESULTS

Effects of guanine nucleotides on association and dissociation of [^{3}H]PGE_2 binding to the solubilized fraction——The addition of GTP to the reaction mixture inhibited the specific [^{3}H]PGE_2 binding to the solubilized fraction as shown in Fig. 1. Half-maximal inhibition was obtained at around 10 μM GTP.

By diluting the reaction mixture, the binding protein-[^{3}H]PGE_2 complex formed after 60-min incubation does not dissociate to a significant extent even 60 min after the dilution as

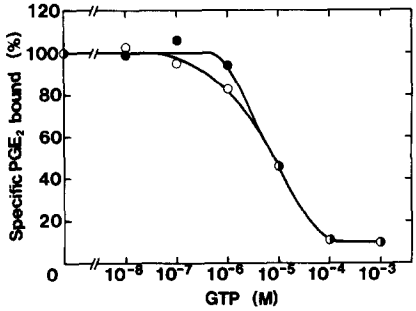

Fig. 1. Effects of GTP on association (o) and dissociation (•) of PGE_2 binding. The specific [^{3}H]PGE_2 (10 nM) binding to the solubilized fraction (0.68 mg of protein) was determined in the presence of increasing concentrations of GTP. The effect of GTP on the dissociation reaction was determined as follows. After the incubation of the solubilized fraction (0.31 mg of protein) with 5 nM [^{3}H]PGE_2 under standard assay conditions, the reaction mixture was diluted 10 times with the same buffer containing GTP and kept at 37°C with shaking. The amount of specific [^{3}H]PGE_2 bound was determined after 60 min. Results are expressed as percent of control. Control experiments were carried out in the absence of GTP. Control values for association and dissociation experiment are 14000 and 2750 dpm, respectively.

described previously (16). As shown in Fig. 1, the addition of GTP to the diluting buffer enhanced the dissociation of the complex in a dose-dependent manner similar to the inhibition of the association reaction.

GDP had an effect similar to that of GTP on the association and dissociation of PGE_2 binding. Guanyl-5'-yl imidodiphosphate (GppNHp) was more than one order of magnitude less potent than GTP in affecting PGE_2 binding. ATP (up to 1 mM) had no significant effect. The nonspecific binding was not affected by these nucleotides.

Scatchard plot analyses——To elucidate whether the effect of GTP on the PGE_2 binding was due to a change in the number of binding site (B_{max}) or in the binding affinity (K_D), we carried out saturation experiments. Figure 2 shows the scatchard plot analyses of the specific [^{3}H]PGE_2 binding in the presence or absence of 10 μM GTP which causes a half-maximal inhibition of the binding as described above. In the absence of GTP, high affinity site (K_D = 2.1 nM, B_{max} = 29 fmol/mg of protein) and low affinity site (K_D = 17 nM, B_{max} = 170 fmol/mg of protein) were found. In the presence of GTP, a single binding component was found and the K_D value increased to 44 nM, whereas the B_{max} value

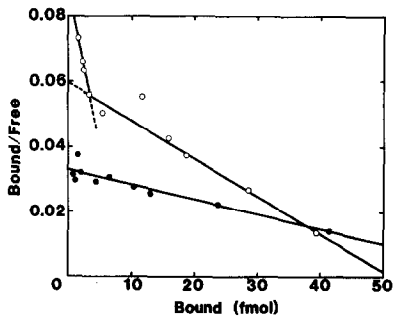

Fig. 2. Scatchard plot analyses of [^{3}H]PGE_2 binding in the presence or absence of GTP. In the absence (o) or presence (•) of 10 μM GTP, the specific binding of [^{3}H]PGE_2 (0.4 - 61 nM) to the solubilized fraction (0.30 mg of protein) was determined as described under "Experimental Procedures".

did not decrease (240 fmol/mg of protein). This result suggests that the inhibition of the binding by GTP is due principally to reduced affinity of the binding site to the ligand.

Separation of the binding protein from the GTP-regulatory component——To elucidate whether the binding protein and the GTP-regulatory component can be separated, we carried out an HPLC analysis. Figure 3 shows the gel filtration pattern of the solubilized fraction on TSKgel G5000PW and G3000SW tandem-linked columns. When the fractions were assayed for the PGE_2 binding (closed circles in Fig. 3), the specific binding was very low and the recovery was less than 30% of the original value. By the addition of GTP (0.5 mM), the PGE_2 binding to the solubilized fraction before HPLC was inhibited more than 80%, whereas that to the combined fractions 25-29 was not. This result suggests that

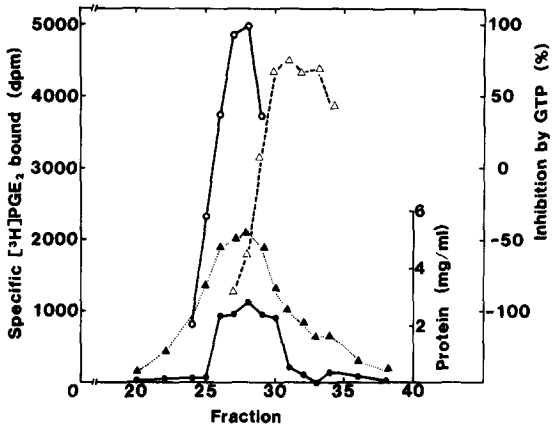

Fig. 3. HPLC analysis of solubilized fraction. The solubilized fraction (50 mg of protein) was chromatographed on TSKgel G5000PW (7.5 x 600 mm) and G3000SW (7.5 x 600 mm) tandem-linked columns, which were equilibrated and eluted with 20 mM Tris-HCl (pH 7.4) containing 10 mM CHAPS. Fractions of 1.0 ml were collected and protein concentrations (▲) were determined. Aliquots (25 µl) of the fractions were assayed for the specific [^{3}H]PGE_2 binding in the presence (○) or absence (●) of the GTP-regulatory component fraction (fraction 30, 25 µl). GTP-sensitivity of the binding protein fraction (fraction 27) was determined in the presence of fractions 28-34 as follows. The reaction mixture contained 25 µl of each fraction and 25 µl of fraction 27, and inhibition by GTP (0.5 mM) was determined (△).

the fractions contain the PGE_2 binding protein separated from the GTP-regulatory component.

To determine where the GTP-regulatory component was eluted, we added the pooled fractions 19-24, 30-35, and 36-42 to the separated binding protein. Only the addition of the fractions 30 - 35 was effective for restoring the GTP-sensitivity to the binding protein, suggesting that it contains the GTP-regulatory component. The separation of the two components was demonstrated more clearly, when the GTP-sensitivity of the binding protein (fraction 27) was determined in the presence of each aliquot of fractions 28-34 (open triangles in Fig. 3). The addition of fractions 30-33 to the fraction 27 restored the GTP-sensitivity similar to that of the solubilized fraction before HPLC.

The reconstitution of the functional coupling of the two components also enhanced the binding. The maximal activation was observed by the addition of the fraction 30 to the binding protein fraction (open circles in Fig. 3). Scatchard analyses revealed that this activation reflected the increase of the binding affinity. The K_D value of the separated binding protein (> 50 nM) was nearly identical to that of the solubilized fraction determined in the presence of GTP, and was decreased to the original level ($\sim$ 10 nM) by the reconstitution. In the reconstitution experiment, the recovery of the binding activity was calculated to be almost 100%.

DISCUSSION

Concerning the sensitivity to GTP, three types of PGE receptors are reported in peripheral tissues: (i) GTP decreases the binding affinity [PGE receptors of bovine thyroid (having a pH optimum of 7.0) (13) and frog erythrocytes (14)]; (ii) GTP increases the binding affinity [hamster adipocyte PGE receptor

(15)]; (iii) GTP does not affect the binding affinity [bovine thyroid PGE receptor having a pH optimum of 3.6 (13)]. The present results show that the PGE receptor in the brain belongs to the first type. Although the solubilization of the PGE receptor was reported in a few peripheral tissues (18,19), the interaction between the solubilized receptor and GTP-regulatory protein (N) was not so far observed. A number of hormone receptors which are thought to be coupled to the adenylate cyclase system have been solubilized in their active forms. However, in most cases, the sensitivity to GTP is lost during the solubilization. Several receptors solubilized in GTP-sensitive forms (20-23) are not separated from N, so that R and N seem to be solubilized in a complex form. In the case of β-adrenergic receptor (24-26) and muscarinic acetylcholine receptor (27,28), reconstitution of functional interactions between R and N is reported. However, the addition of phospholipids and removal of detergents are necessary for the functional coupling. Moreover, the sources and/or the procedures for solubilization of the two components are different. In the present study, we showed that the solubilized PGE_2 binding protein is sensitive to GTP and can be separated from N by gel filtration. These results suggest that R and N are obtained simultaneously in their active forms from the same tissue and the functional reconstitution can be easily attained by mixing the two components in the presence of the detergent without added phospholipids. Therefore, the present work will provide the basis for the precise elucidation of the molecular interaction between R and N.

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