ORIGINAL

Guanosine triphosphate can directly regulate cortisol production by activating Ca^{2+} -messenger systems in bovine adrenal fasciculata cells

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Abstract. Adenosine triphosphate (ATP) is known to stimulate cortisol production *in vitro*, however, the effect of guanosine triphosphate (GTP) on cortisol production is not known. We studied the effect of GTP on cortisol production and investigated the regulation of intracellular signal transduction systems, including the cyclic AMP-dependent and Ca^{2+} -messenger systems, in bovine adrenal fasciculata cells. GTP clearly induced cortisol biosynthesis but only to a level less than half the adrenocorticotrophic hormone (ACTH)-induced maximum. The binding site for $[\gamma\text{-}^{35}\text{S}]\text{-GTP}\gamma\text{S}$ was shown to differ completely from that for ATP and also from those for G_s and G_i , as indicated by the fact that binding was not influenced by pretreatment with cholera toxin and pertussis toxin. GTP significantly increased cytosolic calcium ($[\text{Ca}^{2+}]_i$) and inositol 1, 4, 5-triphosphate without affecting cyclic AMP formation. GTP-induced cortisol production was suppressed by H-9 and Calphostin C (specific protein kinase C inhibitors) but not by H-8 and KT5720 (specific inhibitors of cyclic AMP-dependent protein kinase), suggesting that GTP activates cortisol biosynthesis possibly *via* a protein kinase C-dependent pathway. Extracellular calcium may be essential for GTP activity since GTP-induced cortisol production was almost completely suppressed in its absence. In conclusion, it can be postulated that GTP-induced steroid secretion in bovine adrenal fasciculata cells is under paracrine or autocrine control.

Key words: Steroidogenesis, Cyclic AMP, Protein kinase C, Cytosolic calcium, Inositol 1, 4, 5-triphosphate

ADRENAL gland produces steroid hormones and plays a central role in situations with disrupted homeostasis; however, it is still challenging to clarify the exact mechanisms involved in the regulation of adrenal steroidogenesis and growth [1]. Paracrine control mechanisms for adrenal steroidogenesis exist, although ACTH and angiotensin II are possibly the main regulators that promote steroid production [2, 3].

GTP is known to be involved in regulating the secretion of various substances, both at the signal transduction level [4, 5] and at a later stage in the form of a distinct GTP-binding protein designated as G_e [6, 7]. In general, the regulation of cortisol production in adrenocortical fasciculate cells by ACTH has been considered to be mediated *via* the cyclic AMP (cAMP)-dependent systems [8, 9]. However, ACTH has been considered to promote adrenal steroidogenesis *via* other mechanisms

as well. ATP stimulates cortisol production by activating the membrane phosphoinositide turnover after binding to its specific receptor in bovine adrenal fasciculata cells [10]. This suggests the existence of a paracrine and neuroendocrine control of adrenocortical steroidogenesis *via* a purine receptor. In addition, extracellular ATP stimulates steroidogenesis in bovine adrenocortical fasciculata cells *via* P2 purinoceptors [11]. Thus, synthesis of cortisol may be regulated not only by ACTH but also by purine nucleosides and nucleotides, including ATP. However, there have been no reports describing the effect of GTP on cortisol production. Thus, we attempted to investigate the role of extracellular GTP on adrenal steroidogenesis in bovine fasciculata cells.

Experimental Procedures

Chemicals

KT5720 and calphostin C were purchased from Kyowa Medex Co., Ltd. (Tokyo, Japan). H-8 and H-9 were obtained from Seikagaku-Kogyo (Tokyo). Bovine serum albumin (BSA, fraction V), collagenase type I, deoxyribonuclease type I, $\text{N}^6, 2'$ -O-dibutyryladenine

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3': 5' -cyclic monophosphate (Bt₂cAMP), N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid] (HEPES), GTP, GTP γ S, GDP, GMP, cyclic GMP and trilostane, which is a specific inhibitor of 3 β -hydroxysteroid dehydrogenase, were obtained from Sigma Chemical Co. (St. Louis, MO). 3-Isobutyl-1-methylxanthin (IBMX), which is a non-specific inhibitor against phosphodiesterase for cyclic AMP or cyclic GMP, was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Fura-2 AM was purchased from Wako Chemical Co. (Osaka, Japan). ACTH was purchased from Daiichi Pharmaceutical Co., Ltd. (Tokyo) and Eagle's minimum essential medium (MEM) was obtained from GIBCO. Cyclic AMP and cyclic GMP assay kits were purchased from YAMASA Co. (Tokyo). The inositol-1,4,5-triphosphate (IP₃) [³H] assay system was purchased from Amersham International Plc (Buckinghamshire, UK). [γ -³⁵S]-GTP γ S (Du Pont-NEN Research Products) was obtained from Daiichi Kagaku Yakuhin Co. (Tokyo).

Preparation of bovine adrenal fasciculata cells

Isolated bovine adrenal fasciculata cells were prepared as described previously by Nishikawa *et al.* [12]. Bovine adrenal glands were obtained from a local slaughterhouse. After removing the adherent fat and medulla, 0.5 mm-thick slices containing fasciculate tissue were cut using a Staddie-Riggs microtome (Natsume Seisakusho, Tokyo). The first two slices from the inner-most portion were discarded to avoid including part of the medulla and the next two slices were pooled and placed in MEM containing 0.1% BSA. The pooled slices were minced and incubated in MEM containing 0.1% BSA, 0.25% collagenase type I, and 0.05 mg/mL deoxyribonuclease type II at 37°C for 10 min. After each incubation, the cells were dispersed mechanically by pipetting them 20 times using a 5-mL pipette. The dissociated cells were filtered through cotton gauze and washed three times with Krebs-Ringer bicarbonate buffer, pH 7.4, containing 20 mM HEPES, 1.3 mM CaCl₂, 0.2% glucose, and 0.1% BSA (KRBGA-HEPES).

Steroidogenesis

Adrenal fasciculata cells were resuspended to a final cell density of 20×10^4 cells/mL in KRBGA-HEPES with or without 1.3 mM CaCl₂ and incubated in the presence of various additives for 60 min at a constant temperature of 37°C after a 15-min preincubation with or without various inhibitors. The cortisol content in the media was measured directly by a specific radio-

immunoassay [12]. The cells were also incubated in the presence of 40 μ M trilostane for 60 min in order to study the effect of GTP and ACTH on pregnenolone formation. The amount of pregnenolone in the media was estimated as previously described [13].

cAMP formation

Bovine adrenal fasciculata cells were preincubated with or without various inhibitors for 15 min in KRBGA-HEPES containing 0.5 mM IBMX. After the preincubation, various concentrations of GTP, ATP, and ACTH were added and the mixture was incubated for 15 min. Incubation was terminated by adding 200 μ L of 30% trichloroacetic acid, followed by sonication. The samples were centrifuged and the supernatants were treated four times with cold diethylether. cAMP was estimated by a specific radioimmunoassay [14].

[Ca²⁺]_i Measurement

Isolated bovine adrenal fasciculata cells were incubated with 2 μ M Fura-2 AM in KRBGA-HEPES buffer at 37°C for 30 min. The cells loaded with Fura-2 AM were washed twice in the same buffer without Fura-2 AM. Shimadzu RF-500 fluorescence spectrophotometer (Shimadzu Seisakusho, Kyoto, Japan) equipped with a thermostated cuvette holder (maintained at 37°C) and magnetic stirrer was used for the spectrofluorometric analysis [14]. Loading with Fura-2 AM did not affect cell viability as assessed by trypan blue exclusion staining nor did it affect the ACTH-induced cortisol production (data not shown).

Estimation of IP₃

The amount of IP₃ formed in adrenal fasciculata cells was estimated using an IP₃ assay system. The adrenal fasciculata cells were stimulated by GTP. The reactions were terminated by adding 0.2 volume ice-cold 20% perchloric acid and the mixture was centrifuged at $2000 \times g$ for 15 min at 4°C. The supernatants were then titrated to a pH of 7.5 using 10 N KOH and precipitated with KClO₄, and the sediments were then removed by centrifugation at $2000 \times g$ for 15 min at 4°C. Next, 100- μ L aliquots of the supernatants were incubated with 100 μ L of D-myo-[³H] inositol 1, 4, 5-triphosphate (0.0075 μ Ci) and 100 μ L of IP₃-binding protein solution for 15 min on ice and the reaction mixtures were centrifuged at $2000 \times g$ for 10 min at 4°C. The supernatants were discarded, and the pellets were resuspended in 200 μ L of distilled water. Each sample

was mixed with 2 mL of scintillant and the radioactivity was measured in a β -scintillation counter.

Binding assay of [γ - 35 S]-GTP γ S

The cells isolated from the bovine adrenal fasciculata tissues (2×10^5 cells/tube) were incubated in KRBGA-HEPES buffer (total volume: 500 μ L) containing [γ - 35 S]-GTP γ S (0.1 μ Ci/ 0.083 pmol/ tube) in the presence or absence of an appropriate amounts of GTP γ S and various additives. The incubations were performed at 37°C for 8 min and the temperature was then lowered to 4°C to stop the reaction. The tubes were centrifuged, and the pelleted cells were washed twice using fresh KRBGA-HEPES buffer. The cells were lysed using organic solvents and the radioactivity was measured. The binding was analyzed using Scatchard plots [15]. The cells were pretreated with cholera toxin and pertussis toxin for 15 min, and were then incubated with [γ - 35 S]-GTP γ S as described above in order to study the effect of the cholera toxin and pertussis toxin on [γ - 35 S]-GTP γ S binding.

Statistical analysis

Statistical analysis was performed using Student's *t* test and the Stat View software. Results were expressed as mean $\pm$ standard error of the mean (S.E.) of three experiments performed in duplicate.

Results

Effects of GTP on cortisol production and pregnenolone formation

As shown in Fig. 1A, both GTP and ACTH induced a dose-dependent increase in cortisol production at concentrations between 0.01 mM and 1.0 mM in case of GTP and between 10^{-13} M and 10^{-7} M in case of ACTH. The maximum cortisol production with GTP was almost half of that observed with ACTH. Pregnenolone formation was also enhanced in a dose-dependent manner by GTP (Fig. 1B). GTP-induced cortisol production was markedly reduced in the absence of Ca^{2+} in the medium (Fig. 1C), and there was a significant difference in cortisol production between cells cultured in the absence and

Fig. 1 Effects of GTP on the production of cortisol and pregnenolone by bovine adrenal fasciculata cells (A) Effects of GTP (0.01 mM – 1.0 mM) and ACTH (10^{-13} – 10^{-7} M) on cortisol production. (B) Effects of GTP (0.01 mM – 1.0 mM) and ACTH (10^{-7} M) on the production of pregnenolone by cells incubated in the presence of trilostane. (C) Effects of GTP on cortisol production by bovine adrenal fasciculata cells incubated with and without calcium. Experiments were performed using three different preparations of isolated cells from 6 different bovine adrenal glands. Each point represents mean $\pm$ S. E.

presence of Ca^{2+} in the medium when incubated with 1 mM GTP ($p < 0.001$). We also examined the effect of GTP with ACTH on cortisol production (Fig. 2). As shown in Fig. 2, 10^{-13} M ACTH or 1.0 mM GTP could significantly increase cortisol production, and cortisol production was additively enhanced when the cells were simultaneously incubated with GTP and ACTH (Fig. 2).

Effects of GTP, GDP, GMP, cyclic GMP, and GTP γ S on cortisol production

Neither GDP nor cyclic GMP enhanced cortisol production, while GMP at a high concentration of 1.0 mM induced a slight increase in the production (not significant, as compared to the control) (Fig. 3). Both GTP γ S and GTP induced a significant increase in cortisol production ($p < 0.001$, in comparison to the control).

Effects of GTP on cyclic AMP formation and cytosolic calcium movement

Neither GTP nor GTP γ S increased cyclic AMP forma-

tion (Fig. 4A), while ACTH induced a marked increase (Fig. 4B). It is well known that IBMX is not a specific inhibitor of phosphodiesterase for cyclic AMP but a non-specific inhibitor of phosphodiesterase for cyclic nucleotides including cGMP. Thus, we also measured cyclic GMP content by using the same sample as used for determining cyclic AMP content. 1 mM GTP demonstrated a significant increase in cyclic GMP content ($p < 0.001$) (control; 0.08 ± 0.01 pmol/ 10^4 cells/15 min, 1 mM GTP; 5.25 ± 0.23 pmol/ 10^4 cells/15 min).

The basal level of $[\text{Ca}^{2+}]_i$ was 258.0 ± 1.0 nmol/ 10^5 cells, and each value when incubating with GTP, GDP and cyclic GMP was expressed as subtracting the amount of the basal $[\text{Ca}^{2+}]_i$. GTP induced an increase in $[\text{Ca}^{2+}]_i$ mobilization in a dose-dependent manner (Fig. 4C), while neither GDP nor cyclic GMP had a significant effect on $[\text{Ca}^{2+}]_i$ mobilization ($[\text{Ca}^{2+}]_i$ was 0.3 ± 0.2 nmol/ 10^5 cells and 0.8 ± 0.4 nmol/ 10^5 cells when incubated with 1 mM GDP and 1 mM cyclic GMP, respectively).

Fig. 2 Effects of simultaneous addition of GTP and ACTH to the incubation mixture on cortisol production. The cells isolated from bovine adrenal fasciculata tissues were incubated with GTP (1.0 mM) and ACTH (10^{-13} M) for 60 min. Experiments were performed by using three different preparations of isolated cells from 6 different bovine adrenal glands. Each bar represents mean $\pm$ S. E. Basal cortisol production that was described as control (-) was 0.247 ± 0.021 ng/60 min/ 10^4 cells. a, $p < 0.001$, vs control; b, $p < 0.01$, vs control; c, $p < 0.01$, vs + GTP; d, $p < 0.01$, vs + ACTH.

Fig. 3 Effects of GTP, its analogue, and metabolites on cortisol production. The cells isolated from bovine adrenal fasciculata tissues were incubated with either GTP, GDP, GMP, cyclic GMP (cGMP), or GTP γ S for 60 min at various concentrations. Experiments were performed by using three different preparations of isolated cells from 6 different bovine adrenal glands. Each point represents mean $\pm$ S. E.

Effects of various inhibitors of cAMP-dependent protein kinase and protein kinase C on GTP-induced cortisol production

The effect of pretreatment with H-8 and KT5720, which inhibit cAMP-dependent protein kinase, and H-9 and calphostin C, which are antagonists of protein kinase C, on GTP-induced cortisol production are shown in Fig. 5. GTP-stimulated cortisol production was significantly inhibited by H-9 and calphostin C, but not by H-8 or KT5720.

Effect of GTP on IP₃ formation

IP₃ formation was significantly increased by GTP (Fig. 6A), and GTP γ S could also significantly enhance IP₃ formation, although the effect of GTP γ S was significantly lesser than that of GTP ($p < 0.05$) (Fig. 6B).

Binding of [γ -³⁵S]-GTP γ S

The specific binding of GTP γ S increased and almost reached its maximum value at 8 min (Fig. 7A). The Scatchard plot of the specific binding of GTP γ S showed only one type of specific binding site for GTP γ S in the bovine adrenal fasciculata cells (Fig. 7B). The binding specificity was analyzed as shown in Fig. 7C. The bound GTP γ S was not dissociated by the addition of ACTH, angiotensin II, CDP, or UDP. There was almost 15% dissociation when the cells were incubated with CTP, UTP, and ATP, and around 40% dissociation was observed in the presence of GDP. The bound GTP γ S was completely dissociated when the cells were incubated with non-radioactive GTP γ S. The effects of pretreatment with cholera toxin and pertussis toxin, which are known to act on Gs and Gi, respectively, on the binding of GTP γ S were as shown in Table 1. There was no significant difference in K_d values or maximum binding sites after treatment with cholera toxin and pertussis toxin.

Effects of ATP on cortisol and cyclic AMP formation

Cortisol production was dose-dependently increased (almost 4-fold increase with 1 mM ATP) by ATP, which could also significantly enhance cyclic AMP formation (Table 2).

Discussion

This study clearly demonstrated that there is a specific binding site for GTP in bovine adrenal fasciculata cells. Previously it was reported that bovine adrenal fas-

ciculata cells possess a purinergic P2 receptor and that ATP can induce cortisol production [10, 11]. Moreover, it was reported that NCI-H295R (a human adrenal cortex-derived cell line) expressed multiple purinergic receptors and P2Y1 was related to glucocorticoid secretion [16]. The results of the present study demonstrated that the binding site of GTP seems to be completely different from that of ATP (Fig. 7C), although ATP shows almost 20% cross reactivity for GTP γ S-binding. Our data revealed that the signal transduction systems activated by GTP is completely different from those activated by ATP. While ATP could enhance cortisol production due to increasing cyclic AMP formation (Table 2), and the action of GTP on enhancing cortisol production was not induced by cyclic AMP-dependent systems. It is very interesting to know which purinergic receptor is regulated by GTP for cortisol production, although future studies are required to determine this fact.

The present study also provides new insights into the role of Ca²⁺-messenger systems in GTP-induced cortisol production by bovine adrenal fasciculata cells. These findings indicated that different concentrations of GTP induced cortisol production mainly *via* Ca²⁺-messenger systems, because the increase in [Ca²⁺]_i and IP₃ formation was apparently not associated with the activation of cAMP formation. It was reported that GTP could not enhance cAMP formation in rat adrenocortical cells [17] and our results are consistent with those findings, although cyclic GMP, which could not increase cortisol production (Fig. 3), was enhanced by GTP in our data. It is, therefore, suggested that cyclic GMP-dependent intracellular signaling systems activated by GTP may not directly relate to cortisol synthesis pathway. The present data revealed that the increase in cortisol production induced by GTP was apparently suppressed in the absence of calcium. We also demonstrated that GTP induced IP₃ formation, which is known to be induced by the activation of phospholipase C in the plasma membrane. Therefore, this indicates that the action of GTP on the regulation of cortisol production is related to the activation of phospholipase C. ACTH does not influence IP₃ formation in bovine adrenal fasciculata cells [18], suggesting that ACTH does not enhance cortisol production *via* the activation of phospholipase C. On the other hand, we had already reported that ACTH stimulates cytosolic calcium movement, which was biphasically enhanced by its concentrations, and also induces cortisol production mainly *via* phospholipase A₂-dependent processes

Fig. 4 Effects of GTP on cyclic AMP formation and cytosolic calcium movement
(A) Effects of GTP and GTP γ S on cyclic AMP formation.
(B) Effects of ACTH and GTP on cAMP formation.
(C) Effects of GTP (0.01 mM – 1.0 mM) on cytosolic calcium movement in cells loaded with Fura-2. The basal level of [Ca²⁺]_i was 258.0 $\pm$ 1.0 nmol/10⁵ cells, and each value with incubating GTP in this figure was expressed as subtracting the basal value of [Ca²⁺]_i. Experiments were performed by using three different preparations of isolated cells from 6 different bovine adrenal glands. Each point represents mean $\pm$ S. E.

Fig. 5 Effects of pretreatment with each inhibitor for 15min
The cells were pretreated with various inhibitors for 15min and then 1mM GTP was added to the incubation mixture. The tubes were incubated for 60min after adding GTP, subsequently the incubation media were obtained to estimate cortisol production. Experiments were performed by using three different preparations of isolated cells from 6 different bovine adrenal glands. Each point represents mean $\pm$ S. E. ^a P < 0.05 vs no pretreatment

Fig. 6 Effects of GTP on inositol 1, 4, 5 – triphosphate formation (IP₃)
(A) Time course of IP₃ formation in cells incubated with 1.0mM GTP. (B) Effects of 1.0mM GTP and 1.0mM GTP γ S on IP₃ after incubation of cells for 15sec..
Experiments were performed by using three different preparations of isolated cells from 6 different bovine adrenal glands. Each point represents mean $\pm$ S. E. ^a P < 0.001 vs control

Fig. 7 Binding assay of $[\gamma\text{-}^{35}\text{S}]$ -GTP γ S in cells isolated from bovine adrenal fasciculata tissues

(A) Time course of total, specific, and non-specific binding of $[\gamma\text{-}^{35}\text{S}]$ -GTP γ S. The cells were incubated with $[\gamma\text{-}^{35}\text{S}]$ -GTP γ S in the presence of 1000-fold quantity of non-radioactive GTP γ S to determine non-specific binding. (B) Analysis of specific binding by Scatchard plot. (C) Dissociation curves for the binding of $[\gamma\text{-}^{35}\text{S}]$ -GTP γ S in the presence of various concentrations of ACTH, angiotensin II, CTP, CDP, UTP, UDP, ATP, GDP, and GTP γ S. These experiments were done using several preparations of isolated cells; typical results are shown.

Table 1 Effects of cholera toxin and pertussis toxin on $[\gamma\text{-}^{35}\text{S}]$ -GTP γ S binding

Pretreatment	Kd ($\times 10^{-6}$ M)	Maximum binding sites (pmol/ 10^5 cells)
None	32 ± 5.2	1.6 ± 0.2
Cholera toxin, 20 $\mu\text{g/mL}$	48.3 ± 7.7	2.3 ± 0.3
Pertussis toxin, 1 $\mu\text{g/mL}$	34.7 ± 5.1	1.7 ± 0.2

The cells were pretreated with 20 $\mu\text{g/mL}$ cholera toxin and 1 $\mu\text{g/mL}$ pertussis toxin, which were activated just before use, at 37°C for 15 min and then the binding assay was performed as described in Methods. Values of Kd and maximum binding sites were calculated from the Scatchard analysis [15]. Each value was obtained from the results by using three different preparations of the isolated cells. Results are expressed as mean $\pm$ S. E.

Table 2 Effect of ATP on cortisol production and cyclic AMP formation

	+ATP			
	0	0.01mM	0.1mM	1.0mM
Cortisol (% of control)	100 ± 2.2	$222.5 \pm 19.2^{**}$	$304.9 \pm 36.0^{**}$	$389.4 \pm 98.3^{***}$
Cyclic AMP (pmol/ 10^4 cells/15min)	0.105 ± 0	$0.190 \pm 0.008^*$	$0.309 \pm 0.016^*$	$0.388 \pm 0.014^*$

The cells isolated from bovine adrenal fasciculata tissues were incubated with various concentrations of ATP. All experiments were performed and determination of cortisol and cyclic AMP was done as described under Methods. Experiments were performed by using three different preparations of isolated cells from 6 different bovine adrenal glands. Each value represents mean $\pm$ S. E. Basal cortisol production as described as control was 0.176 ± 0.007 ng / 60 min / 10^4 cells. * $p < 0.001$ vs control, ** $p < 0.01$ vs control, *** $p < 0.05$ vs control.

[19]. Therefore, cortisol production induced by ACTH at low concentrations seems to be regulated mainly by the Ca^{2+} messenger systems, and the cortisol production induced by ACTH at high concentrations is possibly regulated by the cAMP-dependent protein kinase system as well as Ca^{2+} messenger systems [19]. The present experiments also demonstrated additive effect of GTP on ACTH-induced cortisol production (Fig. 2). Thus, it is suggested that the cyclic AMP-dependent systems activated by ACTH and simultaneous activation of Ca^{2+} messenger systems by GTP could co-operatively enhance cortisol production.

Our results suggest that activation of protein kinase C systems that are activated by both $[\text{Ca}^{2+}]_i$ and IP_3 , may be partially essential for GTP-induced cortisol production, because H-9 and calphostin C (specific antagonists of protein kinase C) inhibited the activation of cortisol production by GTP, while H-8 and KT5029 (cAMP-dependent protein kinase inhibitor) did not. Purified bovine adrenocortical cytochrome P-450_{11 β} is phosphorylated by protein kinase C whose activity is tightly associated with the inner mitochondrial membrane, suggesting that protein kinase C possibly plays a crucial role in the regulation of mitochondrial functions by modifying mitochondrial components such as phospholipids and/or calcium [20]. We have previously demonstrated that the expression of the steroidogenic acute regulatory (StAR) protein, which induces the movement of cholesterol from the outer to the inner mitochondrial membrane resulting in activation of pregnenolone synthesis, was significantly enhanced by protein kinase C-dependent systems [12]. Therefore, it is suggested that GTP can activate phospholipase C and subsequently induce the activation of protein kinase C systems, resulting in increased cortisol production.

The binding site for $[\gamma\text{-}^{35}\text{S}]\text{-GTP}\gamma\text{S}$ seems to be different from that of classical GTP-binding proteins such as Gs and Gi because pretreatment with cholera and pertussis toxin did not affect the K_d values and maxi-

mum binding sites of GTP γS . However, we need to further investigate whether the binding site is different from those of other types of G-proteins related to the regulation of phospholipase C.

It is well known that GTP as well as ATP are essential to maintain fundamental cellular functions and there is a possibility of GTP leakage to blood stream from various cells, such as macrophages and neutrophils during inflammatory processes. We can detect blood GTP concentrations using gold nanoparticles modified indium tin oxide electrode [21]. Thus, GTP may physiologically play an important role in the regulation of cortisol synthesis when some inflammatory event occurs even after the stress-induced activation of ACTH secretion. On the other hand, we used un-physiologically high dose of GTP for these experiments since plasma concentration of GTP was reported to be 6 to 7×10^{-7} M [21]. Thus, further experiments are required to clarify the physiological action of GTP on adrenal steroidogenesis.

In conclusion, we have demonstrated the presence of a specific binding site for GTP in bovine adrenal fasciculata cells and that GTP induces cortisol production. GTP-induced cortisol production may be regulated partly by the protein kinase C-dependent system without activating the cAMP formation. The presence of a paracrine or autocrine regulatory mechanism for steroid secretion by GTP as well as by ATP has been suggested. Further studies are needed to elucidate the physiological significance of the effect of GTP on cortisol production.

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Conflict of Interest

The authors declare no conflicts of interest.

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