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Pertussis Toxin Blocks Activin A-induced Production of Inositol Phosphates in Rat Hepatocytes

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The present study was conducted to examine an involvement of G protein in the action of activin A in rat parenchymal liver cells. Activin A induced a dose-dependent increase in inositol phosphates in cells prelabelled with [^{3}H]inositol. The effect of activin A was completely blocked by pretreatment of the cells with pertussis toxin. In contrast, pertussis toxin had little effect on angiotensin II-induced production of inositol phosphates. Both activin A and angiotensin II inhibited glucagon-mediated production of cAMP. Pretreatment of the cells with pertussis toxin blocked the inhibition induced by both activin A and angiotensin II. In permeabilized cells, activin A augmented production of inositol phosphates. Activin-mediated production of inositol trisphosphate was enhanced by GTP-γS and was attenuated by GDP-βS. These results suggest that a pertussis toxin-sensitive G protein(s) may be involved in the action of activin A in hepatocytes.

Activin A is a dimeric protein, which resembles transforming growth factor- β in its structure (1). Besides its action on pituitary FSH secretion, activin A elicits diverse effects in various types of cells (2). Thus, activin A modulates growth and differentiation of many types of cells (3-6), regulates secretion of hormones (1,7-9), and affects carbohydrate metabolism (10). We have shown that activin A stimulates glycogenolysis in isolated rat hepatocytes (10). In this cell system, activin A increases cytoplasmic free calcium concentration, $[Ca^{2+}]_C$, by causing hydrolysis of polyphosphoinositide. In this regard, the action of activin A resembles those of vasoactive hormones including angiotensin II, vasopressin and norepinephrine.

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Receptors for these vasoactive agents couple to a G rotein of Gq family, which is insensitive to pertussis toxin (PTX) (11-13), and phospholipase C is activated via the G protein. Recently, Vale and his colleague have determined the cDNA for the activin A receptor (14). Of particular interest is that primary structure of the receptor indicates an existence of serine threonine kinase in its cytoplasmic domain. This raises a question as to the mechanism by which activin A activates phospholipase C in hepatocytes. In the present study, we examined an involvement of G protein in the action of activin A in rat hepatocytes. Results suggested that a PTX-sensitive G protein(s) may be involved in the action of activin A in hepatocytes.

Materials and Methods

Preparation of Hepatocytes

Isolated hepatocytes were prepared by collagenase digestion method described by Berry and Friend (15). Cells were incubated in modified Hanks' solution containing 137 mM NaCl, 3.5 mM KCl, 0.44 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 0.33 mM Na_2HPO_4, 1.0 mM $CaCl_2$ and 20 mM HEPES/NaOH (pH 7.4) equilibrated with 0_2 gas. For preparation of PTX-treated cells, rats were injected with PTX (25 μg/100g body weight) intraperitoneally, and hepatocytes were prepared 24 hrs later. Under this condition, α-subunit of G_i is ADP-ribosylated completely (11).

Measurement of Inositol Phosphates

Production of inositol trisphosphate was assessed in cells prelabelled with [^{3}H]inositol (10). Cells were incubated for 2 hrs 10 μCi/ml [^{3}H]inositol. After the labelling period, cells were washed and were then incubated in modified Hanks' solution containing 10 mM LiCl. Cells were then stimulated with activin A or angiotensin II. The reaction was terminated by adding perchloric acid (final concentration of 10 %) and cells were homogenized by repetitive aspiration through a 23-gauge needle. The homogenate was centrifuged at 800 x g for 5 min, the supernatant was neutralized with 5 M KOH and was applied to an anion exchange column. Inositol phosphates were separated as described by Berridge et al.(16). Isomers of inositol trisphosphate were not determined in this study.

Measurement of cAMP Production

Aliquots of cell suspension were incubated with various agents for 2 min in the presence of 0.5 mM 3-isobutyl-1-methylxanthine. The reaction was terminated by adding trichloroacetic acid. After the removal of trichloroacetic acid by washing with diethylether, cAMP was measured after succinylation by using radioimmunoassay kit (Yamasa, Tokyo,Japan).

Permeabilization of Hepatocytes

Hepatocytes were incubated with cytosol-like medium containing 20 mM NaCl, 100 mM KCl, 5 mM $MgSO_4$, 0.96 mM NaH_2PO_4, 25 mM HEPES/KOH (pH 7.05), 1 mM EGTA and 25 μg/ml saponin for 10 min. Permeabilization was confirmed by the fact that none of the cells extruded trypan blue. The calcium concentration of the medium was then adjusted to 200 nM using Ca-EGTA buffer (17).

Results

Effects of Activin A and Angiotensin II on Production of Inositol Phosphates

As reported previously (10), activin A increased inositol phosphates in a concentration dependent manner in naive hepatocytes. The effect was detected at 100 pM and was saturated at 10 nM (Fig.1). In PTX-treated hepatocytes, however, activin A did not increase inositol phosphates. As shown in Fig. 1, activin A was without effect at doses up to 100 nM in PTX-treated cell. In sharp contrast, the action of angiotensin II was not sensitive to PTX. As shown in Fig. 2, angiotensin II-induced increase in inositol phosphates in PTX-treated cells was identical to that observed in naive hepatocytes.

Effects of Activin A and Angiotensin II on Glucagon-induced Increase in cAMP

It is reported that angiotensin II inhibits glucagon-stimulated cAMP production via G_i (12). We tested whether activin A had a similar action. As demonstrated in Table 1, both angiotensin II and activin A attenuated glucagon-stimulated cAMP production in naive hepatocytes. In contrast, inhibitory action of either angiotensin II or activin A was observed in PTX-treated cells.

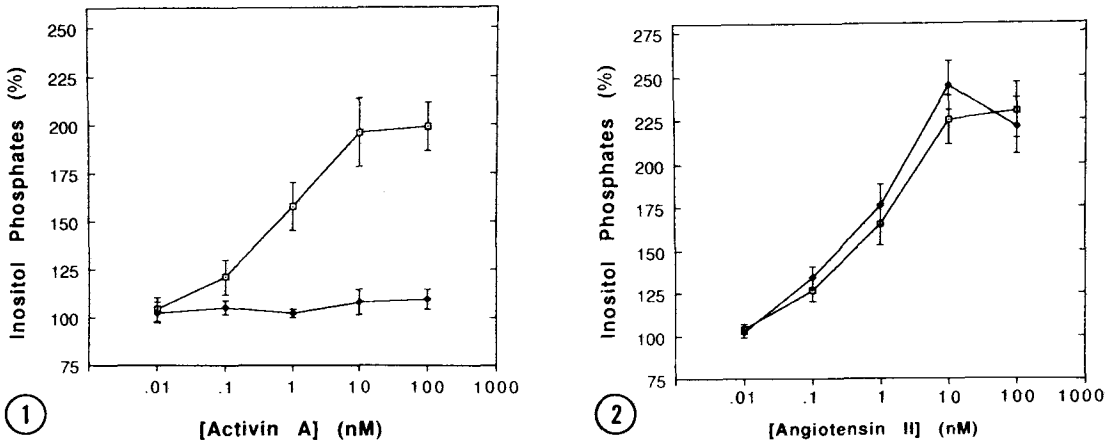

Figure 1. Effect of Pertussis Toxin on Activin A-induced Production of Inositol Phosphates in Hepatocytes
PTX-treated (•) or untreated (□) hepatocytes were obtained as described in Methods. Cells labelled with [^{3}H]inositol were incubated for 20 sec with varying concentrations of activin A and inositol phosphates were measured. Values are the mean ± for six determinations.

Figure 2. Effect of Pertussis Toxin on Angiotensin II-induced Production of Inositol Phosphates
PTX-treated (•) and untreated (□) hepatocytes were labelled with [^{3}H]inositol. Cells were incubated for 20 sec with varying concentrations of angiotensin II and inositol phosphates were determined. Values are the mean ± S.E. for six determinations.

Table 1. Effect of Pertussis Toxin on Inhibition of Glucagon-induced cAMP Production by Activin A and Angiotensin II

Addition	cAMP (pmol/mg/protein)	
	PTX-untreated	PTX-treated
none	5.2 + 1.1	5.6 + 0.8
glucagon	32.1 ± 2.8	43.5 ± 5.3
glucagon + activin A	26.5 ± 3.3*	40.4 ± 4.7
glucagon + angiotensin II	22.1 ± 4.5*	42.2 ± 5.3

Hepatocytes obtained from PTX-treated or untreated rats were incubated with 10^{-8} M glucagon in the presence or absence of either 10^{-8}M angiotensin II or 10^{-8} M activin A. cAMP was determined as described in Methods. Values are the mean ± S.E. for five determinations and statistical analysis is done by using Student t-test. * $P<0.05$ vs glucagon.

Effects of Guanine Nucleotides on Activin A-induced Production of Inositol Phosphates in Permeabilized Hepatocytes

In permeabilized hepatocytes, 10 nM activin A induced a 155 % increase in inositol phosphates. When 100 μM GTP- γS was included together with activin A, the action of activin A was significantly enhanced (Fig.3). Thus, in the presence of GTP- γS, activin A induced a 210 % increase in inositol phosphates. Conversely, when GDP- βS was included together with activin A, the effect of activin A was reduced. When 500μM GDP- βS was added, stimulatory action of activin A was completely blocked.

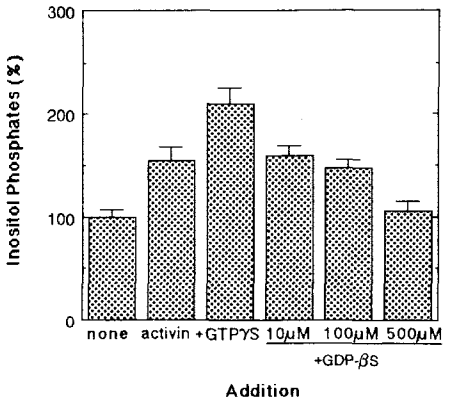

Figure 3. Effects of Guanine Nucleotides on Activin A-induced Production of Inositol Phosphates in Permeabilized Cells

Hepatocytes labelled with [^{3}H]inositol were permeabilized as described in Methods. Cells were then incubated with 10 μM activin A for 20 sec in the presence and absence of GTP- γS or GDP- βS. Values are the mean ± S.E. for four experiments.

Discussion

The present results indicate that activin A increases inositol phosphates by a mechanism sensitive to PTX. In addition, activin A inhibits glucagon-mediated production of cAMP and this reaction is sensitive to PTX. The latter observation suggests that activin A presumably activates G_i. Moreover, activin-mediated production of inositol phosphates is enhanced by GTP- γS and is blocked by GDP- βS. All these results are consistent with an idea that PTX-sensitive G protein(s) is involved in the action of activin A. Noteworthy is a fact that the action of activin A but not angiotensin II on inositol phosphates is blocked by PTX. It is now widely accepted that vasoactive hormones stimulate phospholipase C via a PTX-insensitive G protein of G_q family. Therefore, it appears that activin A receptor is coupled to a PTX-sensitive G protein which is distinct from G_q. It is shown that, in addition to Gq, angiotensin II receptor is coupled to G_i (12). Since activin A inhibits glucagon-mediated production of cAMP in a PTX sensitive manner, both angiotensin II receptor and activin A receptor may be coupled to G_i. Yet, only activin A-mediated production of inositol phosphates is sensitive to PTX. Hence, activin A may activate phospholipase C by involving a PTX-sensitive G protein other than G_i. Alternately, activin A modifies G_i and renders it interactable with phospholipase C. In any case, activin A activates phospholipase C by a mechanism slightly different from those for vasoactive hormones. Such a mode of action of activin A in hepatocytes is intriguing but is not totally unique to activin A. It is reported that production of inositol phosphates induced by epidermal growth factor (EGF) is also blocked by PTX (18). Furthermore, EGF-mediated calcium entry is sensitive to PTX (19). Although, involvement of tyrosine phosphorylation of phospholipase C is considered to be the mechanism for EGF-mediated activation of phospholipase C in other cell systems (20), above reports suggest that EGF receptor is either directly or indirectly coupled to a PTX-sensitive G protein at least in hepatocytes (21). Given the facts that both the EGF receptor and the activin A receptor have a single transmembrane domain and that both receptors have protein kinase activity in their cytoplasmic domain, it is possible that EGF and activin A may stimulate phospholipase C by a similar mechanism.

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