Gonadotropin releasing hormone stimulates the formation of inositol phosphates in rat anterior pituitary tissue

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The production of inositol phosphates in response to gonadotropin releasing hormone (GnRH) was studied in rat anterior pituitary tissue preincubated with [^3H]inositol. Prelabelled paired hemipituitaries from prepubertal female rats were incubated in the presence or absence of GnRH in medium containing 10 mM-Li $^+$. Li $^+$, which inhibits *myo*-inositol-1-phosphatase, greatly amplified the stimulation of inositol phosphate production by GnRH (10^{-7} M) to 159, 198 and 313% of paired control values for inositol 1-phosphate, inositol bisphosphate and inositol trisphosphate respectively after 20 min. The percentage distribution of [^3H]inositol within the phosphoinositides was 91.3, 6.3 and 2.4 for phosphatidylinositol, phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate respectively and was unaffected by GnRH. The stimulation of inositol trisphosphate production by GnRH was evident after 5 min incubation, was dose-dependent with a half-maximal effect around 11 nM, and was not inhibited by removal of extracellular Ca $^{2+}$. Elevation of cytosolic Ca $^{2+}$ by membrane depolarization with 50 mM-K $^+$ had no significant effect on inositol phosphate production. These findings are consistent with the hypothesis that GnRH action in the anterior pituitary involves the hydrolysis of phosphatidylinositol 4,5-bisphosphate. The resulting elevation of inositol trisphosphate may in turn lead to intracellular Ca $^{2+}$ mobilization and subsequent stimulation of gonadotropin secretion.

There is now considerable evidence to suggest that GnRH triggers a rise in cytosolic Ca $^{2+}$ levels within the gonadotroph cell which in turn leads to gonadotropin secretion (for review see Conn *et al.*, 1980). The mechanism whereby receptor activation is coupled to intracellular Ca $^{2+}$ mobilization is, however, unclear. The original proposal that PI turnover provided a mechanistic link in receptor-mediated generation of the Ca $^{2+}$ signal (Michell, 1975) has been recently modified to include a primary role for the polyphosphoinositides, PIP and PIP $_2$ (Michell *et al.*, 1981; Downes & Michell, 1982). Indeed, an agonist-induced hydrolysis of the polyphosphoinositides by phospholipase C has recently been reported in a number of systems employing Ca $^{2+}$ -mobilizing receptors (for review

see Downes & Michell, 1982; Hawthorne, 1983; Berridge, 1984). A number of studies have also reported an accompanying agonist-induced increase in the inositol phosphate products of phospholipase C action in various cell types including anterior pituitary cells (Rebecchi & Gershengorn, 1983; Martin, 1983; Baird *et al.*, 1983; Drummond *et al.*, 1984), the exocrine pancreas (Rubin *et al.*, 1984), the hepatocyte (Thomas *et al.*, 1984), insect salivary gland, mammalian parotid and brain (Berridge, 1983). The physiological relevance of these phenomena to intracellular Ca $^{2+}$ mobilization has been recently highlighted by the finding that inositol 1,4,5-trisphosphate in micromolar concentrations releases Ca $^{2+}$ from permeabilized pancreatic cells (Streb *et al.*, 1983) and hepatocytes (Joseph *et al.*, 1984). Inositol 1,4,5-trisphosphate may thus provide the long-sought-for link between agonist-stimulated phosphoinositide metabolism and the elevation of cytosolic Ca $^{2+}$.

The stimulation of PI turnover by GnRH has been previously reported in dispersed anterior

Abbreviations used: GnRH, gonadotropin releasing hormone (gonadoliberin); PI, phosphatidylinositol; PIP, phosphatidylinositol 4-monophosphate; PIP $_2$, phosphatidylinositol 4,5-bisphosphate; IP, inositol 1-phosphate; IP $_2$, inositol bisphosphate; IP $_3$, inositol trisphosphate; TRH, thyrotropin releasing hormone (thyroliberin).

pituitary cells (Snyder & Bleasdale, 1982) and an enriched population of gonadotrophs (Raymond *et al.*, 1984) and also in luteal (Leung *et al.*, 1983) and granulosa (Naor & Yavin, 1982; Davis *et al.*, 1983) cells. However, it is now generally thought that PI radiolabelling of the polar head group is a secondary consequence of phospholipase C-mediated phosphoinositide hydrolysis, which itself can be directly monitored by measuring the production of inositol phosphates. These inositol phosphates in turn are rapidly hydrolysed to free inositol which can then be reincorporated into the phosphoinositides. The fact that Li^+ blocks *myo*-inositol-1-phosphatase, thereby interrupting this cycle, has been recently exploited by Berridge *et al.* (1982) to amplify agonist-induced accumulation of inositol phosphate in brain and parotid slices. I have adopted this strategy to measure the effect of GnRH on anterior pituitary phosphoinositide metabolism in tissue obtained from prepubertal female rats, which contain a high proportion of gonadotrophs with enhanced responsiveness to GnRH (Naor & Catt, 1981). The present study demonstrates that GnRH stimulates an early specific increase in IP_3 in the presence of Li^+ , presumably resulting from an initial hydrolysis of PIP_2 by phospholipase C.

Experimental methods

Materials

GnRH was purchased from Ayerst, Andover, Hants, U.K. *myo*-[2- ^3H]inositol (15.8 Ci/mmol) was obtained from New England Nuclear, Boston, MA, U.S.A. Dowex 1 X8 (200–400 mesh), Hepes, phosphoinositide standards and bovine serum albumin were obtained from Sigma Chemical Co. Pre-coated silica gel G t.l.c. plates were purchased from Merck, Darmstadt, Germany. Solvents for t.l.c. and scintillation cocktails Fiso fluor 2 and 3 were obtained from Fisons, Loughborough, Leics., U.K. Anterior pituitary tissue was obtained from prepubertal female Lewis strain rats.

Incubation conditions

Female rats (4-weeks-old) were killed, decapitated and their anterior pituitary glands removed. Paired hemipituitaries were quartered and incubated in a control versus experimental protocol in 0.2 ml of Krebs–Ringer medium at 37°C containing 0.5% bovine serum albumin, 0.1% glucose and 20 mM-Hepes at pH 7.4. The tissue was preincubated for 1 h during which time the medium was replaced every 10 min. The tissue was then incubated for 1 h in 0.06 ml of medium containing [^3H]inositol (200 Ci/ml) under O_2 . After removal of the medium the tissue was washed in 1 ml of radioisotope-free medium and then incubated in

0.2 ml of medium for a further 1–20 min in the presence or absence of GnRH and in the presence of 10 mM- Li^+ . In experiments conducted in the absence of added Ca^{2+} , prelabelled hemipituitaries were washed in Ca^{2+} -free medium containing 0.5 mM-EGTA and then incubated in the same medium with or without GnRH in the presence of Li^+ . Incubations were terminated by snap freezing the tissue, after removal of the medium, in 0.6 ml of acidified (0.1 M-HCl) methanol at -70°C .

Extraction and isolation of phosphoinositides and inositol phosphates

Hemipituitary tissue was homogenized in 0.6 ml of acidified methanol. Lipid was then extracted for 30 min at room temperature after addition of 0.6 ml of chloroform. On addition of 0.6 ml of 5 mM-EDTA the mixture was vortex mixed and phase separation was achieved by centrifugation at 1000 g for 5 min. The aqueous and organic phases were then each washed once with 0.6 ml of chloroform and 1.2 ml of acidified methanol/5 mM-EDTA (1:1, v/v) respectively. A 20 μl aliquot of the washed organic phase was then dried and counted in Fiso fluor 3 to measure the total lipid-bound [^3H]inositol. The remainder was dried under N_2 and subjected to t.l.c. in chloroform/methanol/ NH_3 /water (90:90:7:20, by vol.) (Weiss *et al.*, 1982), to separate the different phosphoinositides. PI, PIP and PIP_2 were each resolved in this system with respective R_f values of 0.5, 0.3 and 0.04, and were detected with a Panax Betagraph imaging system and by iodine staining of authentic standards. Radioactivity corresponding to the different phosphoinositides was eluted from the gel with 0.4 ml of methanol/water/6 M-HCl (80:20:1, by vol.) and measured by scintillation counting in Fiso fluor 2.

The washed aqueous phase extracts were freeze-dried to remove methanol and HCl and the residue was resuspended in 2 ml of distilled water. Water-soluble [^3H]inositol metabolites were then separated by anion-exchange chromatography on Dowex 1 (formate form) resin as described previously (Berridge *et al.*, 1982). The identity of radioactive peaks corresponding to IP, IP_2 and IP_3 was confirmed by virtue of the fact that they were eluted with the same characteristics as ^3H -labelled IP, IP_2 and IP_3 prepared by acid hydrolysis by the method of Dawson & Dittmer (1961) of PI, PIP and PIP_2 extracted from rat anterior pituitary tissue prelabelled with [^3H]inositol. Aliquots (0.5 ml) from the eluate fractions corresponding to free inositol, glycerophosphoinositol, IP, IP_2 and IP_3 were counted in Fiso fluor 2 scintillation cocktail.

There was some variability in the amount of [^3H]inositol incorporated into total phospholipid

from different anterior pituitary glands, possibly reflecting small differences in gland size or the physiological state of individual animals at killing. However, the amount of lipid-bound [^3H]inositol associated with each hemipituitary pair was very similar. Furthermore, the relative distribution of the [^3H]inositol phosphates expressed as a percentage of the total lipid-bound [^3H]inositol was also very similar from one gland to another, the mean values during a 20 min incubation in the presence of 10 mM- Li^+ alone being $5.8 \pm 0.3\%$, $3.0 \pm 0.3\%$ and $0.9 \pm 0.1\%$ for IP , IP_2 and IP_3 respectively ($n = 13$). All values are presented as means $\pm$ S.E.M. Statistical evaluation of the data was by paired Student's t test.

Results

Effect of GnRH and Li^+ on inositol phosphate production

The data illustrated in Fig. 1 indicate the effect of Li^+ (10 mM) and GnRH (10^{-7} M), either alone or in combination, on the production of [^3H]inositol phosphates in anterior pituitary tissue from pre-pubertal female rats which have been pre-labelled with [^3H]inositol. Preliminary experiments demonstrated that 10 mM- Li^+ alone (versus a 10 mM elevation of Na^+ concentration) produced 3.6-, 3.9- and 2.9-fold increases in IP , IP_2 and IP_3 respectively (Fig. 1a; $n = 2$) during a

20 min incubation, suggesting that phosphoinositide breakdown is occurring in some cells in the absence of exogenously added agonist. A similar enhanced production of all three inositol phosphates with Li^+ alone has been observed in the exocrine pancreas (Rubin *et al.*, 1984). GnRH alone also produced a $221 \pm 11\%$ ($n = 3$) increase in IP_2 production after 20 min (Fig. 1b); however, the smaller increases in IP and IP_3 were not statistically significant. In the presence of Li^+ , GnRH stimulated the production of IP , IP_2 and IP_3 to $159 \pm 17\%$, $198 \pm 25\%$ and $313 \pm 59\%$ of paired control values respectively (Fig. 1c). A similar amplification by Li^+ of agonist-induced IP_2 and IP_3 production as well as IP has been previously observed in vasopressin-stimulated hepatocytes (Thomas *et al.*, 1984) and by carbachol in the exocrine pancreas (Rubin *et al.*, 1984).

The present findings in the anterior pituitary are consistent with a GnRH-stimulated hydrolysis of polyphosphoinositides by phospholipase C in the gonadotroph cell. In order to maximize changes in inositol phosphate production in response to GnRH, all subsequent experiments were performed in the presence of 10 mM- Li^+ . There was no effect of GnRH on the levels of [^3H]inositol associated with the peak of radioactivity corresponding to glycerophosphoinositol, the mean percent of control values being $100 \pm 5\%$ ($n = 11$). GnRH (10^{-7} M) also stimulated [^3H]inositol phos-

Fig. 1. Effect of Li^+ and GnRH on [^3H]inositol phosphate production

Paired hemipituitaries were incubated with [^3H]inositol ($200 \mu\text{Ci}/\text{ml}$) for 1 h, then washed with radioisotope-free medium. Tissue was then incubated for a further 20 min: (a) in Krebs-Ringer medium (open bars) or medium containing 10 mM- Li^+ (hatched bars) ($n = 2$); (b) in Krebs-Ringer medium (open bars) or medium containing GnRH (10^{-7} M) ($n = 3$); or (c) in medium containing 10 mM- Li^+ (open bars) or 10 mM- Li^+ plus GnRH (10^{-7} M) (hatched bars) ($n = 4$). The incubations were then stopped and the water-soluble [^3H]inositol products were extracted and separated by anion-exchange chromatography as described in the Experimental methods section. Values represent means $\pm$ S.E.M.; * $P < 0.05$ by paired Student's t test.

phate production in hemipituitaries from young (4-week-old) male rats during a 20 min incubation. The levels of IP, IP₂ and IP₃ were increased to $185 \pm 4\%$ ($P < 0.01$), $191 \pm 20\%$ ($P < 0.05$) and $289 \pm 28\%$ ($P < 0.05$) of paired control values respectively ($n = 4$).

Effect of GnRH on anterior pituitary phosphoinositides

The percentage distribution of radioactivity between the different phosphoinositides in rat anterior pituitary tissue preincubated with [³H]-inositol (200 Ci/ml) for 1 h was $91.3 \pm 1.3\%$, $6.3 \pm 1.2\%$ and $2.4 \pm 0.1\%$ for PI, PIP and PIP₂ respectively ($n = 4$). This relative distribution is similar to that previously reported for GH₃ pituitary cells prelabelled with [³H]inositol (Martin, 1983; MacPhee & Drummond, 1984). GnRH (10^{-7} M) had no effect on [³H]phosphoinositide levels after 5 or 20 min incubation in the presence of Li⁺ (results not shown). Although this observation is not consistent with a role for phosphoinositide breakdown in the GnRH-stimulated increases in inositol phosphates reported above, the heterogeneous nature of the cell population in the anterior pituitary and the rapid turnover of [³H]inositol lipid may well mask any accompany-

ing changes in phosphoinositide levels within the gonadotroph cell.

Time course of inositol phosphate production

The temporal changes in the production of [³H]-inositol phosphates in the presence and absence of GnRH is illustrated in Fig. 2. There were no detectable changes in any of the inositol phosphates up to 2 min. However, after 5 min GnRH caused a significant increase in IP₃ production (Fig. 2c) ($157 \pm 8\%$ of paired control values, $P < 0.001$, $n = 4$), without any change in IP levels (Fig. 2a). Significant increases in all inositol phosphates were demonstrable after 20 min incubation. This temporal relationship of phosphoinositide hydrolysis products would be consistent with an initial GnRH-mediated hydrolysis of PIP₂, and possibly PIP, as the principal mechanism operating during receptor activation rather than a breakdown of PI.

The temporal increases in inositol phosphates in the presence of Li⁺ alone are indicative of a basal turnover of phosphoinositides, possibly associated with cells such as the mammothroph which is actively secreting prolactin on removal from an inhibitory dopaminergic influence *in vivo*. In this respect it is interesting to note that dopamine

Fig. 2. Time course of inositol phosphate production

Paired hemipituitaries were prelabelled with [³H]inositol (200 μ Ci/ml) for 1 h, washed in radioisotope-free medium and then incubated for a further 1, 2, 5 or 20 min in the absence (●) and presence (○) of GnRH (10^{-7} M) in medium containing 10 mM-Li⁺. Incubations were stopped by snap freezing the tissue in acidified methanol and inositol phosphates were extracted, separated and measured as described in the Experimental methods section. Values represent means $\pm$ S.E.M., * $P < 0.05$, ** $P < 0.001$. Values for 1 and 2 min are the means for two experiments and for 5 and 20 min are means for four experiments. (a), IP; (b), IP₂; (c), IP₃.

inhibited PI turnover in rat anterior pituitary glands *in vitro* (Canonica *et al.*, 1982). Previous reports in a number of cell types have observed a much more rapid onset of inositol polyphosphate production in response to various agonists compared with the effect of GnRH in the present study. This may be partly due to the heterogeneous nature of the anterior pituitary tissue employed in the present study as well as the diffusion barrier of quartered hemipituitaries compared with isolated cells.

Dose dependency of GnRH-stimulated inositol polyphosphate production

The dose-response relationship of GnRH to IP_2 and IP_3 production is shown in Fig. 3. A maximum stimulation was observed at 10^{-7} M with a half-maximal effect around 11 nM, which closely approximates to the half-maximal effect of 10^{-8} M-GnRH on luteinizing hormone secretion from female rat anterior pituitaries *in vitro* (Williams, 1976). A significant increase in IP_3 production was readily detectable at 10^{-8} M-GnRH to $223 \pm 32\%$ of paired control values (Fig. 3) ($P < 0.02$, $n = 4$).

Fig. 3. Dose-dependence of GnRH-stimulated inositol polyphosphate production

Paired hemipituitaries were prelabelled with [3 H]-inositol and then incubated for a further 20 min (see the Experimental methods section) with varying doses of GnRH (10^{-9} – 10^{-6} M). After this time the water-soluble [3 H]inositol products were extracted, separated and quantified as described in the Experimental methods section. ○, IP_2 ; ●, IP_3 . GnRH-stimulated values are expressed as a mean ($\pm$ S.E.M.) percentage of paired control values and were obtained from three or more experiments.

Effect of Ca^{2+} on inositol phosphate production

It has been previously shown that the removal of extracellular Ca^{2+} has no effect on the ability of TRH to stimulate inositol phosphate production in GH $_3$ pituitary cells (Rebecchi & Gershengorn, 1983; Baird *et al.*, 1983). Although such observations do not resolve the issue of whether agonist-induced increases in cellular Ca^{2+} are a cause or effect of phosphoinositide hydrolysis, they are consistent with the idea that inositol lipid breakdown could lead to Ca^{2+} mobilization. In the present study, incubation of [3 H]inositol-labelled anterior pituitary tissue in the absence of exogenously added Ca^{2+} and in the presence of 0.5 mM-EGTA did not prevent the GnRH-stimulated increase in inositol phosphate production (Fig. 4b). The respective changes expressed as a percentage of paired control values in the presence (2.5 mM) and absence of Ca^{2+} were: for IP , 165 ± 15 and 170 ± 21 ; IP_2 , 229 ± 47 and 224 ± 24 ; IP_3 , 382 ± 82 and 298 ± 43 ($n = 6$). Under these relatively stringent Ca^{2+} -free conditions gonadotropin secretion is completely suppressed (Borges *et al.*, 1983), presumably due to a prevention of any GnRH-induced rise in free cytosolic Ca^{2+} . It is apparent, however, that these Ca^{2+} -free conditions

Fig. 4. Effect of Ca^{2+} removal on inositol phosphate production

Paired hemipituitaries were prelabelled with [3 H]-inositol (200μ Ci/ml) for 1 h. Tissue was then washed in radioisotope-free Krebs-Ringer medium (containing 2.5 mM- Ca^{2+}) or medium containing 0.5 mM-EGTA without Ca^{2+} . The medium was then replaced with either (a) medium containing 2.5 mM- Ca^{2+} , without (open bars) or with (hatched bars) GnRH (10^{-7} M) or (b) medium without added Ca^{2+} plus 0.5 mM-EGTA without (open bars) or with (hatched bars) GnRH (10^{-7} M). Values represent means $\pm$ S.E.M. ($n = 6$); * $P < 0.05$, ** $P < 0.02$.

did reduce the absolute basal and stimulated levels of all three inositol phosphates (Figs. 4a and 4b) which may reflect in part the known Ca^{2+} -dependent nature of phosphoinositide-specific phospholipase C (Irvine *et al.*, 1984).

Depolarizing concentrations of K^+ have been previously shown to cause $^{45}\text{Ca}^{2+}$ influx in the anterior pituitary and stimulate a Ca^{2+} -dependent release of gonadotropin (Kraicer, 1975). When paired hemipituitaries prelabelled with [^3H]-inositol were incubated in 10mM- Li^+ in the presence of 50mM- K^+ for 20min, relatively small non-significant increases in inositol phosphates were observed, the respective changes for IP, IP_2 and IP_3 being $145 \pm 27\%$, $151 \pm 46\%$ and $130 \pm 12\%$ of paired control values ($n = 3$). Similar findings were reported with 50mM- K^+ in GH_3 cells, though a small significant increase in IP was observed (Rebecchi & Gershengorn, 1983). These observations would not support a model advocating an elevation of cytosolic free Ca^{2+} as the primary trigger for polyphosphoinositide breakdown in the anterior pituitary.

Discussion

The observations in this study demonstrate that GnRH causes an increased accumulation of inositol phosphates in the anterior pituitary in the presence of Li^+ . Since the only known means available for IP_3 production in mammalian cells is a phospholipase C-mediated hydrolysis of PIP_2 , it seems reasonable to attribute GnRH-stimulated IP_3 production to such a mechanism. These findings are consistent with previous reports on GnRH-stimulated PI turnover in anterior pituitary cells (Snyder & Bleasdale, 1982; Raymond *et al.*, 1984), since the various phosphoinositides are in dynamic equilibrium with one another and re-synthesis is thought to accompany breakdown. The fact that GnRH had no apparent effect on phosphoinositide levels in the present study could possibly reflect the heterogeneous nature of the anterior pituitary tissue and the rapid restoration of [^3H]inositol lipid levels during stimulation. The relatively rapid increase in IP_3 compared with IP (Fig. 2) suggests that GnRH stimulates an initial hydrolysis of PIP_2 to produce IP_3 , which would then be further hydrolysed by inositol tris- and bisphosphatases to IP_2 and IP. A similar temporal relationship for agonist-stimulated inositol phosphate production was observed in TRH-stimulated GH_3 cells (Martin, 1983), carbachol-treated pancreatic acini (Rubin *et al.*, 1984) and vasopressin-stimulated hepatocytes (Thomas *et al.*, 1984). An increased phospholipase C-mediated hydrolysis of PIP due to GnRH may also occur and indeed GnRH, in the absence of Li^+ , caused a specific

increase in IP_2 (Fig. 1b). The employment of Li^+ , which inhibits inositol-1-phosphate phosphatase and enhances agonist-induced accumulation of IP (Berridge *et al.*, 1982) has in the present study greatly amplified the responses of both IP and IP_3 to GnRH stimulation. Whether the effect of Li^+ on IP_3 accumulation is due to feedback inhibition by IP or by direct inhibition of inositol 1,4,5-trisphosphate hydrolysis is not known.

An area that remains controversial with regard to phosphoinositide hydrolysis is the nature of the role played by Ca^{2+} in this process (Hawthorne, 1983). Since the elevation of cytosolic Ca^{2+} by membrane depolarization had no effect on IP_3 formation in the present study, and the removal of free Ca^{2+} by chelation with EGTA did not prevent GnRH-stimulated inositol phosphate formation, the hydrolysis of PIP_2 in the anterior pituitary does not appear to be a consequence of Ca^{2+} mobilization. These findings are consistent with observations on intact cells from a wide variety of tissues where the agonist-dependent breakdown of phosphoinositides occurs independently of changes in cytosolic Ca^{2+} (see Berridge, 1984). It is also of interest to note that the stimulation of PI turnover in isolated pituitary cells by GnRH was unaffected by incubation in Ca^{2+} -free medium containing 1mM-EGTA (Naor *et al.*, 1984). Although the mechanism for receptor-activated hydrolysis of PIP_2 by phospholipase C is unknown, a recent study by Irvine *et al.* (1984) indicates that the enzyme is not active below 0.1mM- Ca^{2+} when assayed under ionic and substrate conditions approximating to those of the intracellular environment. Manipulation of substrate presentation in that study led to the conclusion that changes in substrate availability would be a sufficient and sensitive regulator of enzyme activity (Irvine *et al.*, 1984).

The physiological relevance of agonist-stimulated PIP_2 hydrolysis within the gonadotroph cell has yet to be determined. This phenomenon has been linked to intracellular Ca^{2+} mobilization in a variety of cell types (for review see Downes & Michell, 1982; Berridge, 1984). Indeed, a number of recent reports have demonstrated the ability of inositol 1,4,5-trisphosphate to release Ca^{2+} from the endoplasmic reticulum of various cell types (see Berridge, 1984). Although a GnRH-induced rise in cytosolic Ca^{2+} levels appears to play a major role in the stimulation of gonadotropin secretion, the source of this Ca^{2+} is unknown. Nonetheless, the production of IP_3 in response to GnRH is a possible mechanism that the gonadotroph could employ to achieve a release of Ca^{2+} from intracellular stores such as the endoplasmic reticulum.

Another characteristic feature of phospholipase C-mediated phosphoinositide hydrolysis is the

production of diacylglycerol which, like IP_3 , may also function as a second messenger since it is a potent activator of protein kinase C (Nishizuka, 1983). In this respect it is interesting to note that a mediatory role for protein kinase C has recently been implicated in GnRH-stimulated gonadotropin secretion (Hirota *et al.*, 1984).

In summary, this study describes a GnRH-stimulated production of [3H]inositol phosphates, in particular IP_3 , in the anterior pituitary. This is probably the result of a receptor-activated hydrolysis of PIP_2 by phospholipase C within the gonadotroph cell. This GnRH-stimulated PIP_2 breakdown provides the gonadotroph cell with a mechanism for the simultaneous coupling of receptor activation to Ca^{2+} mobilization and protein kinase C activation (Berridge, 1984), both of which are implicated in GnRH-stimulated gonadotropin secretion.

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