

Identification of gonadotropin releasing hormone receptor in different tissues of pig

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The effect of gonadotropin releasing hormone (GnRH) is mediated by a membrane bound receptor called GnRH-receptor. GnRH-receptor was also characterized in placenta (Eidne *et al.* 1985, Butzow *et al.* 1987). The GnRH-receptor is a member of G-protein coupled receptor super family. Like other members of this family of receptors the GnRH-R has 7 transmembrane domains. Although GnRH-R is primarily found in anterior pituitary, it has been also found in several other tissues e.g. ovary, testis, endometrium liver, spleen etc. (Kakar *et al.* 1993). Presence of GnRH-receptor in these tissues indicates its role in paracrine or autocrine regulation of reproductive processes. Since GnRH was first isolated from the pig brain more than 3 decades ago, an additional 16 novel GnRH structural variants of decapeptide hormone were identified in different species of animals (Mammalian GnRH-I (pGlu-His-Trp-Gly-Leu-Arg-Pro-Gly-NH₂) and GnRH-II (pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH₂) (Sealfon *et al.* 1997). Presence of GnRH-R in cancer cells of various reproductive tissues particularly ovary epithelium, uterine endometrium and prostate has opened another area of intriguing research, generating potential of newer strategy for control of reproductive cancers. There are reports showing increased expression of GnRH-R gene in cancer cells than that in normal cells. There are several reports of regression of cancers of reproductive tissues with application of GnRH analogs.

Different tissues (liver, heart, lung and brain) of pig were collected immediately after slaughter under cold condition from a local abattoir and transported to laboratory. Particular care was taken to avoid contact of pituitary with hands.

The total cellular protein was extracted from lung, brain, heart and liver tissues by TRIZOL method (Chomczynski

and Sacchi 1987). The total cellular protein concentration was estimated by adding Bradford reagent to all tissue samples with a control (Bradford method) and kept it at room temperature for 2 min. OD of these samples was determined by using spectrophotometer at 595 nm. A standard graph had been plotted on the basis of absorbance against protein concentration. Western blot (dot-blot) analysis had been done for further processing of isolation of required protein. The total GnRH-R protein content was observed by the presence of antibody conjugation method. Two kinds of antibodies (primary and secondary) were used for the present study. A monoclonal antibody (mouse anti human GnRH-R) was taken as a primary antibody conjugant to bind with specific GnRH-R protein on PVDF membrane (2 cm×5 cm). The membrane was wetted in 100% methanol for few second and then thoroughly rinsed with distilled water followed by washing treatment and blocking by BSA. A secondary antibody (anti mouse-Ig-AP conjugate: raised in goat) was added to conjugate with primary antibody. At the final step, we developed the specific protein (GnRH-R) by treating the membrane with BCIP/NBT substrate solution for a colour reaction.

Protein estimation: Total protein content of different tissues for GnRH-R, proteins was estimated by spectrophotometer (Table 1).

By comparing the OD of unknown protein with OD of known concentration protein, the concentration of protein

Table 1. OD of known and unknown concentrated protein at 595 nm

Known sample	OD at 595	unknown sample	OD at 595
Blank	0.000 A°		
1	0.047 A°	Lung	0.140 A°
2	0.094 A°	Brain	0.110 A°
3	0.138 A°	Heart	0.162 A°
4	0.175 A°	Liver	0.130 A°

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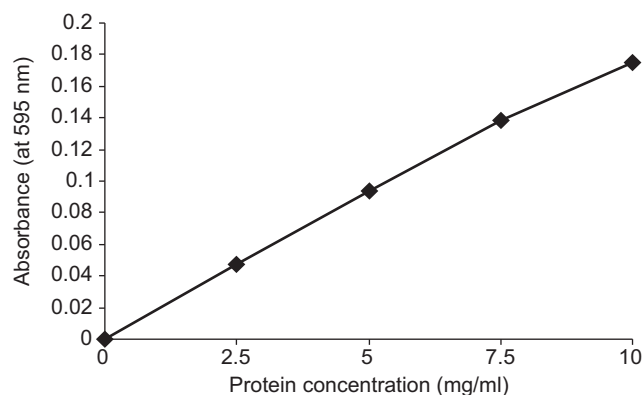


Fig 1. Plotted graph between OD on Y-axis and protein concentration on X-axis

sample of these tissues was measured as follows

Sample	Conc. of protein (mg/ml)
Brain	5.5
Heart	8.5
Lung	6.5
Liver	6.0

In our study, all the 4 tissues (heart, lung, liver and brain) showed blue signal i.e. all were positive for GnRH-receptor probing. Liver and brain were positive for GnRH-R in this study. These results are in accordance of several earlier reports (as presence of GnRH- receptor is already known in these 2 tissues in other mammals) thus these results are confirmative for the presence of this receptor in liver and brain in pig.

The colour reaction was developed and the results for the all the tissues under study were positive as all the blots gave blue colour. However intensity of colour in different blots was different (Fig. 2).



Fig 2. Result of western dot blot analysis.

Unlike the study done in buffalo (Ramakrishna and Srivastava 2006), western dot blot analysis of total protein from pig heart showed positive signal for GnRH-R. As no other report is there, showing the presence of GnRH-receptor

in heart and keeping in view the limitations of the technique used in this study, this result needs further investigation by running the PAGE with protein marker (to confirm the molecular size of protein) transferring the proteins on PVDF membrane and probing the membrane with primary and secondary antibody to develop specific band.

The present study gives strong evidences for presence of gonadotropin releasing hormone receptor (GnRH-R) in different extrahypophysial tissues of pig. Our results give strong reason to investigate the GnRH-R in pig at molecular level with advanced biotechnology tools, which will sure be of immense importance and us to understand the role and regulation of this receptor.

SUMMARY

Gonadotropin releasing hormone is hypothalamic decapeptide that is the prime controller of reproductive function. The aim of the present study is to verify the presence of the GnRH-receptor in physiologically important extrahypophysial tissues of pig. The total protein was extracted from tissues (liver, heart, lung and brain) of pig sample using standard protocol of protein extraction and analysis by western dot blot. The results for all tissues under study were positive as all the blots gave blue colour. However intensity of colour in different blots was different. Our results gave strong reason to investigate the GnRH-R in pig at molecular level with advanced biotechnology tools, which will sure be of immense importance and will help us to understand the role and regulation of this receptor.

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