Journal of Animal and Veterinary Advances 7 (6): 712-716, 2008
ISSN: 1680-5593

Peptidergic Innervation of the Mammary Gland in the Pregnant, Lactating and Non-lactating Periods of Guinea Pig

Mine Yaman, Berrin Gençer Tarakçi and Ali Bayrakdar
Department of Histology and Embryology, Faculty of Veterinary Medicine, Firat University, 23119, Elazig, Turkey

Abstract: The presence and distribution of some biologically active substances in nerve fibres supplying the mammary gland in the pregnant, lactating and non-lactating guinea pig were studied using immunohistochemical methods. The substances studied included: Calcitonon Gene Related Peptide (CGRP), substance P (SP), Vasoactive Intestinal Polypeptide (VIP), Galanin (GAL), Cholecystokinin (CCK) and neurotensin. Immunoreactive nerve fbres were associated with blood vessel, lactiferous ducts and alveoli. CGRP, SP, VIP, GAL, CCK and neurotensin immunoreactive fibres appeared to be much more numerous in the mammary gland of pregnant and lactating guinea pigs when compared to non-lactating guinea pigs. The results demonstrate a rich distribution of different types of nerve fibres in structures of mammary gland related to milk ejection. These nerve fibres may be involved in the local control of milk ejection.

Key words: Nerve fibres, mammary gland, guinea pig, immunohistochemistry

INTRODUCTION

The secretory tissue of the mammary gland has a tubuloalveolar arrangement. It is composed of numerous alveoli each consisting of a ball of secretory cells, surrounded by a network of contractile myoepithelium and blood vessels (Cowie, 1974; Mepham, 1987; Wakerly *et al*., 1988).

In early years, the knowledge of the innervation of the mammary gland was very limited and fragmentary. There was only few papers daling with the innervation of the gland and most of these studies were performed using histological (Cowie, 1974; Linzell, 1959; Michel, 1993) and histochemical methods (Balakina and Skopichev, 1986; Cowie, 1974; Hebb and Linzell, 1966; Franke-Radowiecka and Wasowicz, 2002). However, during the last decades, a lot of data have been gained dealing with the biologically active substances, mainly neuropeptides, found within neurons supplying the mammary gland of different mammalian. Several neuropeptides have been described within nerves supplying the mammary gland in woman (Eriksson *et al*., 1996), rat (Eriksson *et al*., 1996; Skakkebaek *et al*., 1999; Tasker *et al*., 1988; Thulesen *et al*., 1994; Traurig *et al*., 1984), pig (Franke-Radowiecka *et al*., 2002), marsupial and red kangaroo (Griffiths and Slater, 1988). Retrograde tracing in combination with immunohistochemistry has shown that sensory fibres of the rat mammary gland not only originate from dorsal root ganglia, but also in the nodose ganglia and that neurons in dorsal root ganglia contain calcitonin gene related peptide (CGRP), whereas those in the nodose ganglia contain vasoactive intestinal polypeptide (VIP) (Eriksson *et al*., 1996). The same study has also shown that sympathetic neuropeptide Y (NPY) containing fibres innervating the mammary gland originate in the stellate ganglion.

Since, no information on peptidergic innervation of mammary gland in guinea pig exist, we decided to investigate by immunohistochemistry, the localization and distribution of some neuropeptides such as CGRP, substance P, VIP, neurotensin, galanin and cholecystokinin (CCK) immunoreactivity in the mammary gland of pregnant, lactating and non-lactating guinea pig.

MATERIALS AND METHODS

Animals and tissue samples: Eighteen female guinea pig (pregnant, lactating and non-lactating guinea pig, each : 6 guinea pig) were used. Guinea pigs were housed under controlled conditions of temperature and light. Pellet food and water were supplied *ad libitum*. After euthanasia with ether, mammary glands were removed and fixed in 4% formaldehyde in sodium cacodylate

Corresponding Author: Mine Yaman, Department of Histology and Embryology, Faculty of Veterinary Medicine, Firat University, 23119, Elazig, Turkey

buffer (0.1 M, pH 7.4) for 24 h. They were placed in 0.1 M sodium cacodylate, pH: 7.4, containing 20% sucrose for at least 24 h at 4°C before snap freezing and sectioning at 10-15 µm in cryostat.

Immunohistochemistry: PAP (Peroxidase-Anti-Peroxidase) method: Immunohistochemical staining was carried out by using the Peroxidase-Antiperoxidase (PAP) method. Blocking of endogenous peroxidase was carried out with 0.008% hydrogen peroxidase (H_2O_2) in methanol for 5 min (Sternberger, 1986). In order to block unspecific binding, an incubation with normal goat serum in 0.1 M phosphate buffered saline (PBS), pH 7.2 (Dilution 1:10) was performed. Sections were incubated for 16-20 h at 4°C with rabbit IgG antibodies against calcitonin gene-related peptide (Chemicon, AB5920), substance P (Chemicon, AB1566), galanin (Chemicon, AB5909), neurotensin (Chemicon, AB5496), cholecystokinin (Chemicon, AB1973) and vasoactive intestinal polypeptide (Chemicon, AB982). Antibodies were diluted to 1:200, 1:200, 1:500, 1:1000, 1:500 and 1:500 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin. Sections were then incubated in goat anti-rabbit IgG (Dako, Z0421, Denmark) followed by rabbit peroxidase anti-peroxidase complex (Zymed Lab., 61.2003, San Francisco), both at dilution of 1:50 in PBS, for 1 hour at room temperature. Sections were washed in PBS for 30 min after each incubation and finally immersed in glucose oxidise-DAB-nickel ammonium sulphate substrate (Shu *et al*., 1988) for 10 min. After washing in distilled water and counterstaining with eosin, sections were dehydrated and coverslips mounted with aqueous permanent mounting medium.

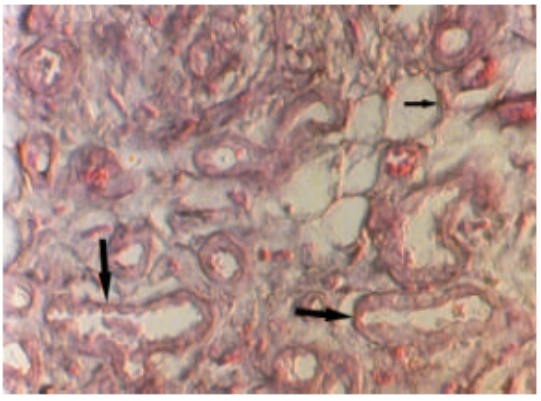

Fig. 1: CCK immunoreactive nerve fibres were observed numerous around of lactiferous ducts (big arrows) and alveoli (small arrow) in mammary gland of pregnant guinea pig. X200

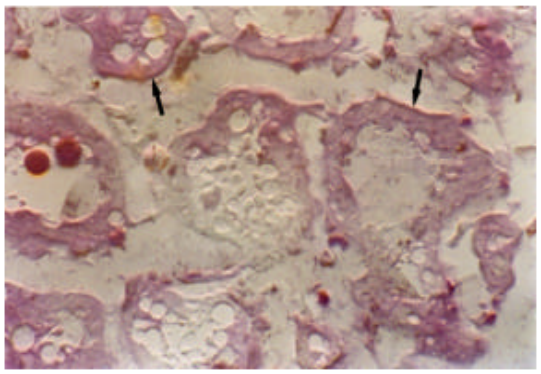

Fig. 2: CCK immunoreactive nerve fibres around of lactiferous ducts (big arrows) in mammary gland of lactating guinea pig. X200

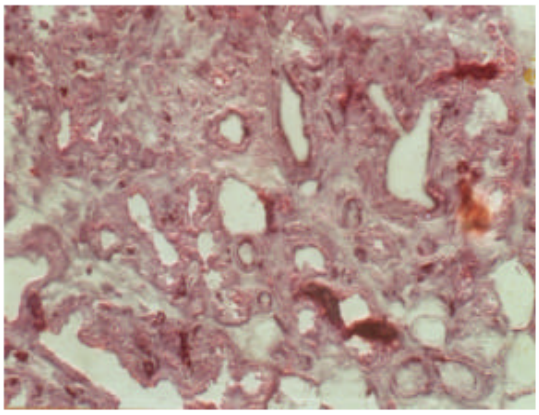

Fig. 3: CCK immunoreactive nerve fibres were found rare in the mammary gland of non-lactating guinea pig. X200

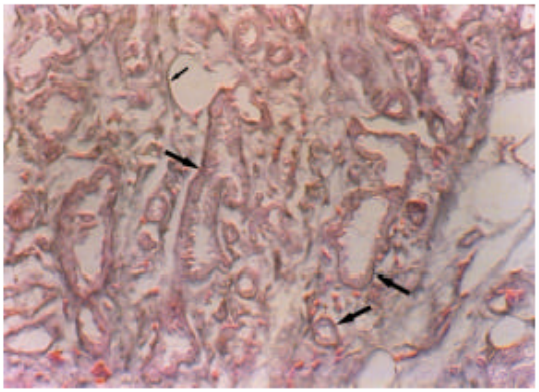

Fig. 4: VIP immunoreactive nerve fibres were found numerous around of lactiferous ducts (big arrows) and alveoli (small arrow) in mammary gland of pregnant guinea pig. X200

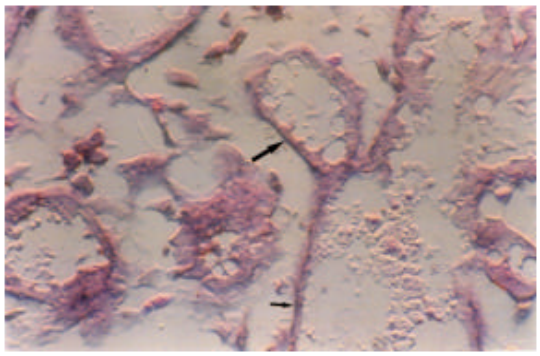

Fig. 5: VIP immunoreactive nerve fibres around of lactiferous ducts (big arrow) and alveoli (small arrow) in mammary gland of lactating guinea pig. X200

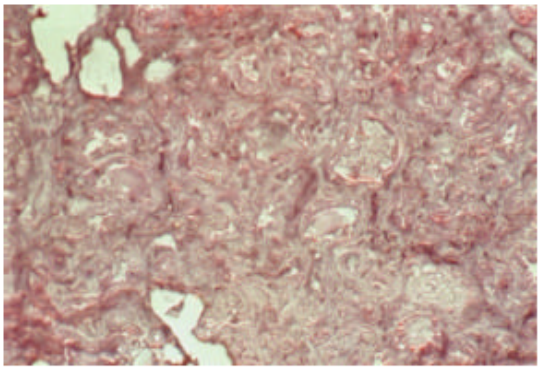

Fig. 6: VIP immunoreactive nerve fibres were found rare in the mammary gland of non-lactating guinea pig. X200

Table 1: Distribution and relative frequency of CGRP, SP, VIP, Galanin, CCK and Neurotensin immunoreactive nevre fibres in the guinea pig mammary gland (n:5)

Mammary gland	Lactiferus ductuli	Alveolus	Blood vessel
Pregnant			
CGRP	++	++	++
SP	+++	+++	+++
VIP	+++	+++	++
Galanin	++	++	+
CCK	++	++	++
Neurotensin	++	++	++
Lactating			
CGRP	+++	+++	+++
SP	+++	+++	+++
VIP	+++	+++	+++
Galanin	++	++	+
CCK	++	++	++
Neurotensin	++	++	++
Non- lactating			
CGRP	+	+	+
SP	++	++	++
VIP	+	+	+
Galanin	+	+	+
CCK	+ / -	+ / -	+
Neurotensin	+ / -	+ / -	+

Relative frequencies: +++: Numerous, ++: Moderate, +: Rare, -: Not detected

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger (1979) by using (including the replacement of) specific antiserum preincubated with its corresponding antigen. Sections were examined with light microscope and photographs were taken.

RESULTS

CGRP, SP, VIP, CCK, neurotensin and galanin immunoreactive nerve fibres were found in the mammary gland of the pregnant, lactating and non-lactating guinea pigs. No immunoreactive neuronal cell bodies were observed. In the pregnant and lactating guinea pigs, CGRP, SP, VIP, CCK, neurotensin and galanin immunoreactive fibres appeared to be much more numerous than in the non-lactating guinea pigs (Fig. 1-6). Positive nevre fibres were seen close to blood vessels, to alveoli and lactiferous ducts (Fig. 1, 4 and 5).

The regional distribution and relative frequencies of CGRP, SP, VIP, CCK, neurotensin and galanin positive fibres in guine pig mammary gland is shown in Table 1.

DISCUSSION

The present study has revealed a rich peptidergic innervation of the mammary gland in pregnant and lactating guinea pigs compared to non-lactating conditions. Nerve endings were present around blood vessels and in the tissue surrounding alveoli and ducts in the mammary gland. These nerve endings contained CGRP, SP, VIP, CCK, neurotensin and galanin. The present results compared to the findings reported by other authors (Eriksson *et al*., 1996; Skakkebaek *et al*., 1999; Tasker *et al*., 1988; Thulesen *et al*., 1994; Traurig *et al*., 1984; Franke-Radowiecka *et al*., 2002; Griffiths and Slater, 1988) suggest the existence of relatively small inter-species differences in the innervations of this gland.

CGRP influences vascular smooth muscle tone and induces a prolonged vasodilatation of resistance and capacitance vessels (Cathart *et al*., 1948) and CGRP and SP have been shown to increase blood flow in the skin (Sann *et al*., 1988) and they are both known to affect smooth muscle cells (Brain and Williams, 1988; Brain *et al*., 1985). The CGRP positive nerve fibres around blood vessels in the mammary gland may thus act as vasodilatators. In fact local injections of CGRP into the mammary gland increase mammary blood flow (Eriksson *et al*., 1996). The close relation of CGRP immunoreactive fibres to lactiferous ducts may indicate that this peptide also influences the resistance in the ductuli.

VIP immunoreactive fibres were also present around blood vessel and lactiferous ducts within the mammary gland. VIP is known to relax smooth muscle in many organs (Roussellot *et al*., 1994). Their presence in the mammary gland suggests that they might be involved in relaxing smooth muscle and thereby increasing blood flow and decreasing ductal tone in the mammary gland.

Galanin immunoreactive nerve fibres have been mentioned to be present around blood vessels and in connection with lactiferous duct and alveoli of the mammary gland in woman and rat (Eriksson *et al*., 1996) but in the pig these fibres were found only to surround blood vessels (Franke-Radowiecka *et al*., 2002). In the present study, galanin positive fibres were widely distributed around lactiferous ducts. It has been found that GAL has a contractile effect on smooth muscle cells in human and rat and thus, it is possible that this peptide plays similar role in the guinea pig mammary gland.

CCK and neurotensin immunoreactive nerve fibres were also found in the mammary gland of guinea pig in pregnant, lactating and non-lactating conditions which have not been previously described. The occurrence of CCK and neurotensin immunoreactive nerve fibres around of lactiferous ducts suggests that they can play role in ductal tone.

CONCLUSION

The present study has revealed that many biologically active substances exist in nerve fibres supplying the mammary gland in guinea pig. The distribution of these nerves strongly suggests that they can play a role in the process of milk ejection during the lactation periods and control of blood flow through the gland.

REFERENCES

Balakina, G.B. and V.G. Skopichev, 1986. Localization of choline acetyltransferase in the alveolar portion of the mammary gland of the white mouse. Arkh. Anat. Gistol. Embriol., 90 (4): 73-77.

Brain, S.D. and T.J. Williams, 1988. Substance P regulates the vasodilator activity of calcitonin gene related peptide. Nature, 335: 73-75.

Brain, S.D., T.J. Williams, J.R. Tippins, H.R. Morris and I. MacIntyre, 1985. Calcitonin gene- related peptide is a potent vasodilator. Nature, 313: 54-56.

Cathart, E.P., F.W. Gairns and H.S.D. Garven, 1948. The innervation of the human quiescent nipple with notes on pigmentation, erection and hyperneury. Trans. R. Soc. Edinburgh, 61: 699-717.

Cowie, A.T., 1974. Overview of the mammary gland. J. Invest. Dermat., 63: 2-9.

Eriksson, M., B. Lindh, K. Uvnas-Moberg and T. Hökfelt, 1996. Distribution and origin of peptide-containing nerve fibres in the rat and human mammary gland. Neuroscience, 70: 227-245.

Franke-Radowiecka, A. and K. Wasowich, 2002. Adrenergic and cholinergic innervation of the mammary gland in the pig. Anat. Histol. Embryol., 31 (1): 3-7.

Franke-Radowiecka, A., J. Kaleczyc, M. Klimczuk and M. Lakomy, 2002. Noradrenergic and peptidergic innervation of the mammary gland in the immature pig. Folia Histochem. Cytobiol., 40 (1): 17-25.

Griffiths, M. and E. Slater, 1988. The significance of striated muscle in the mammary gland of marsupials. J. Anat., 156: 141-156.

Hebb, C.O. and J. L. Linzell, 1966. A histochemical study of the innervation of the mammary gland. Physiol. Soc., 186: 82-83.

Linzell, J.L., 1959. Physiology of the mammary gland. Physiol. Rev., 39: 534-576.

Mepham T.B., 1987. The structure of mammary glands. In Physiology of Lactation. Ed. Mepham T.B. Butler and Tanner Ltd, London, pp: 15-21.

Michel, G., 1993. Histological and histochemical examination of the innervation of the teat and udder skin in the bovine. Schweiz Arch. Tierheilk, 135: 305-309.

Roussellot, P., D.A. Poulain and T. Thedosis, 1994. Ultrastructural visualization and neurochemical characterization of spinal projections of primary sensory afferents from the nipple: Combined use of transganglionic transport of HRP-WGA and glutamate immunocytochemistry. J. Histochem. Cytochem., 42: 115-123.

Sann, H., E. Pinter, J. Szolcsanyi and F.K. Pierau, 1988. Peptidergic afferents might contribute to the regulation of skin blood-flow. Agents Actions, 23: 14-15.

Shu, S., G. Ju and L.Fan, 1988. The glucose oxidase -DAB-nickel method in peroxidase histochemistry of the nervous system. Neurosci. Lett., 85: 169-171.

Skakkebaek, M., J. Hannibal and J. Fahrenkrug, 1999. Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) in the rat mammary gland. Cell. Tiss. Res., 298: 153-159.

Sternberger, L.A., 1979. The Unlabelled Antibody Peroxidase-Antiperoxidase (PAP) Method. In: Sternberger L.A. (Ed.). Immunocytochemistry. J. Wiley, Sons, New York, pp: 104-169.

Sternberger, L.A., 1986. Immunocytochemistry, 3rd Edn., John Wiley, New York.

Tasker, J.G., D.T. Theodosis and D.A. Poulain, 1988. The effects of neonatal capsaicin treatment on the sensory innervation of the nipple ond on the milk ejection reflex in the rat. Expl. Brain Res., 73: 32-38.

Thulesen, J., T.N. Rasmussen, P. Schmidt, J.J Holst and S.S. Poulsen, 1994. Calcitonin gene-related peptide (CGRP) in the nipple of the mammary gland. Histochem. Cell. Biol., 102: 437-444.

Traurig, H., R.F. Papka, A. Saria and F. Lembeck, 1984. Subtance P immunoreactivity in the rat mammary nipple and the effects of capsaicin treatment on lactation. Arch. Pharmacol. 328: 1-8.

Wakerly, J.B., G. Clarke and A.J.S. Summerlee, 1988. Milk ejection and its control. In the Physiology and Lactation. Ed. Knobil, E. and J. Neill. Raven Press Ltd, New York, pp: 2284-2316.