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# Localisation of the neuropeptide PACAP and its receptors in the rat parathyroid and thyroid glands

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ABSTRACT

PACAP (pituitary adenylate cyclase activating polypeptide) is widely distributed neuropeptide acting via three subtypes of receptors, $PAC_1$, $VPAC_1$ and $VPAC_2$. Here we examined the localisation and nature of PACAP-immunoreactive nerves in the rat thyroid and parathyroid glands and defined the distribution of $PAC_1$, $VPAC_1$ and $VPAC_2$ receptor mRNA's. In the parathyroid gland a large number of nerve fibres displaying PACAP-immunoreactivity were distributed beneath the capsule, around blood vessels and close to glandular cells. Most of the PACAP-nerves were sensory, since they co-stored CGRP (calcitonin-gene-related peptide) and were sensitive to capsaicin-treatment. mRNA's for $PAC_1$ and $VPAC_2$ receptors occurred in the parathyroid gland, mainly located in the glandular cells. In the thyroid gland PACAP-immunoreactive nerve fibres were associated with blood vessels, thyroid follicles and parafollicular C-cells. A high degree of co-existence between PACAP and VIP (vasoactive intestinal polypeptide) was observed in the intrathyroid nerve fibres and cell bodies of the thyroid ganglion indicating a common origin for the two peptides. A minor population of PACAP-immunoreactive nerve fibres with relation to blood vessels co-stored NPY (neuropeptide Y), whereas only a few fibres co-stored CGRP. $PAC_1$ and $VPAC_1$ receptor mRNA's occurred in follicular cells and blood vessels, whereas the expression of the $VPAC_2$ receptor was low. The findings suggest that PACAP plays a role in the regulation of parathyroid and thyroid blood flow and hormone secretion.



## 1. Introduction

The occurrence and possible functions of many neuropeptides in the thyroid and parathyroid glands have over the years been described [1,8–10,21,25,26,33,34] but so far no information on PACAP (pituitary adenylate cyclase activating polypeptide) in the thyroid gland exists. Two reports have described the presence of PACAP-containing nerve fibres in the parathyroid gland of a number of species, including man [25,26]. PACAP is a member of the secretin/glucagon/VIP (vasoactive intestinal polypeptide) family of peptides and occurs in two biologically active forms, PACAP 38 and the C-terminally truncated PACAP 27 [32]. In all tissues examined so far, PACAP 38 has been shown to be the dominating molecular form. PACAP can interact with three subtypes of receptors ($PAC_1$, $VPAC_1$ and $VPAC_2$) of which the latter two are shared with VIP [18]. PACAP is widely distributed in the brain and in nerve fibres of peripheral organs, and since its discovery in 1989 numerous studies have provided evidence that PACAP has a broad spectrum of biological functions [32].

The rat thyroid gland receives a rich supply of sympathetic, parasympathetic and sensory nerve fibres [11]. The sympathetic nerve fibres originate in the superior cervical ganglion. The parasympathetic nerve fibres emanate from vagal branches and one of these, the thyroid nerve, harbours a ganglionic formation at the dorso-medial aspects of the thyroid gland. This ganglion, designated the thyroid ganglion, gives rise to nerve fibres entering the thyroid. Most VIP-containing nerve fibres in the gland seem to originate in this ganglion, which also harbours a number of NPY-(neuropeptide Y) containing cell bodies. The sensory innervation of the rat thyroid gland seems to have three different sources: the jugular ganglion, the cervical dorsal root ganglia and the trigeminal ganglion [11].

The number of parathyroid glands varies among different species, and the rat has two glands. The parathyroid gland harbours, like the thyroid, peptide-containing nerve fibres of presumed sympathetic, parasympathetic and sensory nature [26]. Certain peptides can be used as markers for divisions of the peripheral nervous system. Thus, sympathetic nerves contain NPY, VIP occurs in parasympathetic nerves, while primary sensory nerve fibres harbour substance P and/or CGRP [24].

In the present study we have by immunohistochemistry examined the detailed distribution and localisation of PACAP in the rat thyroid and parathyroid glands. The nature and possible origin of the PACAP-immunoreactive nerve fibres were elucidated by co-localisation studies using NPY, VIP and CGRP as markers for

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sympathetic, parasympathetic and sensory nerves, respectively. To further clarify the possible origin of the PACAP-immunoreactive nerve fibres animals were treated neonatally with the sensory neurotoxin capsaicin. Finally we defined the distribution of $PAC_1$, $VPAC_1$ and $VPAC_2$ receptor mRNA's in the two glands by *in situ* hybridisation histochemistry.

## 2. Materials and methods

### 2.1. Animals and tissue

The thyroid and the parathyroid glands from adult Wistar rats (about 60 days old and weighing 180–200 g) of both sexes housed in 12 h light: 12 h dark were examined. No sex differences with respect to PACAP-immunoreactive nerves were found. For immunohistochemistry eight animals were anaesthetised with pentobarbital (Mebumal, SAD, Denmark) (15 mg i.p.) and perfused via the ascending aorta with a room temperature solution of saline (0.9%) to which heparin (15,000 IU/l) was added (75–100 ml) over 3–5 min. This perfusion was followed by 2% paraformaldehyde, 0.2% picric acid in 0.1 M sodium phosphate buffer, pH 7.2 (400 ml over 15 min). After perfusion fixation, the thyroid gland including the parathyroids and trachea was removed and postfixed in the same fixative for 24 h. The specimens were then rinsed repeatedly in tyrode solution, containing 30% sucrose, and frozen on dry ice for cryostat sectioning. For *in situ* hybridisation histochemistry, frozen sections from three animals were used.

To determine if PACAP immunoreactivity is present in sensory fibres in the rat parathyroid and thyroid glands, animals were treated neonatally with the sensory neurotoxin, capsaicin (Sigma, St. Louis, MO, USA). Five rats were injected intraperitoneally with capsaicin 50 mg/kg body weight at day two after birth as described by Nagy et al. [30]. Five litter-matched rats were injected with vehicle solution (Tween 80, saline, absolute alcohol, 1:8:1 by volume) at the same time as the experimental animals and were used as controls. The rats were killed after eight to 12 weeks.

### 2.2. Immunohistochemistry

Sections of 12 μm thickness were processed for immunohistochemistry using a well characterised mouse monoclonal anti-PACAP antibody (code No. Mab JHHI) [16] alone or in combination with rabbit anti-VIP antiserum (code No. 291E) [5], rabbit anti-NPY (code No. 8183), donated by P.J. Larsen [28], rabbit anti-calcitonin antiserum (code No. 6014, Peninsula Laboratories, St. Helens, UK) or anti-CGRP-antiserum (code No. B47-1, Euro diagnostica, Malmö, Sweden) following a slight modification of the previously described procedure [17].

### 2.3. Single antigen immunohistochemistry

After incubation for 12–18 h with anti-PACAP antibody (supernatant diluted 1:5) at 4 °C the sections were washed in phosphate-buffered saline 3 × 10 min in 0.25% bovine serum albumin +0.1% Triton X-100 (PBS-BT) followed by incubation for 60 min at room temperature in biotinylated rabbit anti-mouse antiserum (E464, Dako, Glostrup, Denmark, diluted 1:1600). The signals from the biotinylated antiserum were further amplified using the principles described by Berghorn et al. [3]. The sections were washed 3 × 5 min in PBS-BT and incubated for 30 min at room temperature in avidin–biotin complex (ABC)-streptavidin horseradish peroxidase complex (Vector, Burlingame, USA, diluted 1:150). After washing 3 × 5 min in PBS-BT, sections were incubated with biotinylated tyramide (Tyramide System amplification, DuPont NEN®, Boston, MA, diluted 1:100 in application buffer; Perkin Elmer, Waltham, MA, USA), then washed 3 × 5 min in PBS-BT and incubated in ABC-streptavidin horseradish peroxidase complex (diluted 1:150) for another 30 min. After washing in 2 × 5 min in PBS-BT

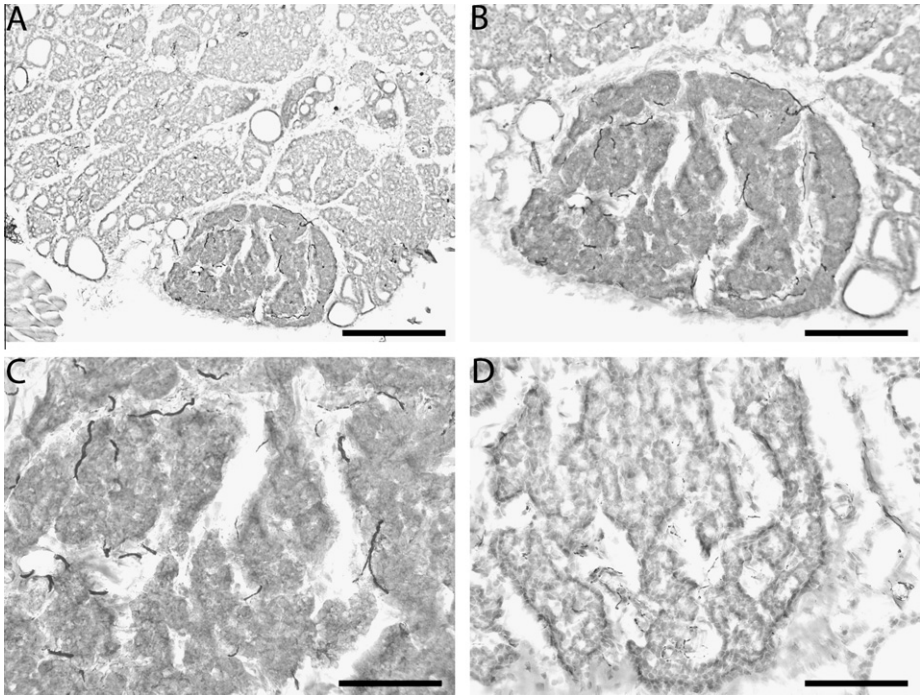


**Fig. 1.** PACAP-immunoreactive nerve fibres in the rat parathyroid gland and in capsaicin-treated rat visualised by DAB-staining. (A) Low magnification showing the abundance and distribution of PACAP-immunoreactive nerve fibres in the parathyroid gland as compared to the thyroid. (B and C) Higher magnifications demonstrating PACAP-containing nerve fibres in or beneath the capsule, around blood vessels and in the parenchyma sometimes forming bundles of fibres. (D) Comparable section from a capsaicin-treated rat demonstrating that the number of PACAP-immunostained fibres was markedly reduced, but some delicate, beaded fibres associated with parenchymal cells and blood vessels remained. Scale bars: A = 400 μm; B = 200 μm; C and D = 100 μm.