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Short Communication

**A MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL
INVESTIGATION OF GUINEA PIG SKIN AFTER THE
INTRODUCTION OF SUBSTANCE P AND VIP**

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Abstract: The aim of this study was to examine a morphological picture of guinea pig skin that had been injected with neuropeptides (NPS)² – substance P (SP) and guinea pig vasoactive intestinal peptide (VIP) – to elucidate their local influence. Routine histological stainings were performed, together with immunohistochemical reactions for T cells and for macrophages. In the deeper layers of the skin, T cell and macrophagic infiltrations were observed. The intensity of these changes was greater 24 hours after injections than that observed at the third hour of the experiment.

Key Words: Substance P, VIP, Guinea Pig, Skin, Histology, Immunohistochemistry

INTRODUCTION

The skin is innervated by primary afferent sensory nerves, postganglionic cholinergic parasympathetic nerves and postganglionic adrenergic and cholinergic sympathetic nerves [1]. NPS are present and released in all these nerve endings; however, their meaning for the physiology and pathology of the skin has not yet been explained. VIP and SP are sensory NPS, and additionally VIP is present in post-ganglionic cholinergic neurones [1]. Sensory NPS are collocated in the secretory vesicles of primary afferent nerve fibres and are released during the stimulation of these nerves (neurogenic inflammation). In addition, they have been identified as potent mediators of inflammatory and immunologic reactions [2, 3]. NPS affect the functions of smooth muscles, blood

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Abbreviations used: NPS - neuropeptides; SP - substance P.; VIP - vasoactive intestinal peptide

vessels and leukocytes directly, and indirectly through the actions of mediators released from mast cells stimulated by these peptides [3].

NPS have been extensively studied in several *in vitro* systems. The presence of SP receptors on T cells [4] and on macrophages [5] has been established. SP enhances the proliferation and chemotaxis of T cells [6] and increases the chemotaxis of neutrophils and monocytes [2,5]. Receptors for VIP on T cells have also been described [7]. Conflicting results were presented on T-cell chemotaxis upon VIP inhibition of T-cell proliferation and migration [8] and the induction of T-cell chemotaxis [6]. *In vivo* studies were much less numerous. SP applied intradermally in humans induced local vasodilation (10^{-9} M), protein extravasation (10^{-8} M - 10^{-6} M) and significant histamine release (10^{-5} M) [9]. Histological excisions were not investigated.

NPS have been postulated to be involved in the mechanisms of some dermatoses – psoriasis [10, 2], prurigo [11], vitiligo [11], eczema and atopic dermatitis [2] and various inflammatory skin disorders [2, 12]. NPS are/may be potentially effective medicines in various diseases including septic shock and rheumatoid arthritis [13], therefore, more detailed knowledge is needed on the local effects of NPS in the skin. The experiments reported on here were histological and immunohistochemical studies of the *in vivo* effects of SP and guinea pig VIP applied exogenously in a single dose into guinea pig skin.

MATERIALS AND METHODS

Twelve male guinea pigs (*Cavia porcellus*) at the age of 5-6 months, weighing 350-400 G each, were used. 0.3 ml samples of 3.3×10^{-6} M SP (Sigma) or 10^{-6} M guinea pig VIP (Tocris, GB) solutions were injected intradermally into the shaved dorsal area of the skin of 6 animals in each case. Material was taken from equal numbers of animals at 3 and 24 hours after application. As a control, skin from the opposite side of the body of the same animals injected with 0.3 ml of 0.9% NaCl was used. The excisions were fixed in Bouin's or Carnoy's solutions and paraffin embedded, and the sections were used for histology and immunohistochemistry analyses.

Stainings of guinea pig pan T-cell antigen and of macrophages/monocytes were performed using the/a two-step indirect immunohistochemical method.

Macrophages were detected using the monoclonal IgG1 mouse primary antibody, clone MAC387 (Serotec, GB), in dilution 1:100. For T cells, we used the monoclonal IgG1 mouse primary antibody, clone CT5, in dilution 1:120. The secondary antibody used was the peroxidase-labeled rabbit F(ab')₂ anti-mouse IgG (Serotec, GB), in dilution 1:50. The negative control was obtained by omitting the primary antibody. Visualization of the reactions was performed using a 3,3'-diaminobenzidine substrate solution (Sigma). Additionally, Astra blue staining for mast cells was performed. We established the infiltration intensity rates in 10 fields of vision per slide at magnification 240x.

RESULTS AND DISCUSSION

Histological changes were not found in any of the control specimens at 3 h and 24 h. After the NPS injections, characteristic vascular reactions – extension and overfilling of the blood vessels in the deep dermis – were noted. Infiltrations mainly appeared in the deep dermis and subcutaneous tissue, indicating an increased chemotaxis of lymphocytes and macrophages after 24 h compared to the 3 h post-injection changes (Tab. 1). The immunostainings used by us for T cells and macrophages were specific; no cells were visualized in the negative controls (without the primary antibodies).

Tab. 1. Changes in the distribution of T cells, macrophages and mast cells in guinea pig skin after VIP or SP intradermal injections.

	Time	Location	T cells	Macrophages	Mast cells
Control skin		upper dermis	++	+	++
		deep dermis	+	+	+
VIP	3h	upper dermis	++	++	++
		deep dermis	+++	+++	+
	24h	upper dermis	++	++	++
		deep dermis	+++	++++	+
SP	3h	upper dermis	++	++	+
		deep dermis	+++	++	+
	24h	upper dermis	+	++	++
		deep dermis	++++	++	++

After SP application, we observed the formation of subepidermal clefts and the widening of cutaneous lymphatic vessels (Fig. 1A). Subcutaneous lymph nodes were augmented, presumably showing T-cell presence (Fig. 1B); it is evident that exogenous SP stimulated local lymph nodes to T-cell production.

In the deep dermis, T cells predominated in infiltrations (Fig. 1C). Intradermal, exogenous SP injection produced a transient (visible after 3 h) decrease in the number of mast cells stainable with Astra blue, normalized after 24 h (7-12 in one field of vision in the upper dermis) (Tab. 1).

VIP evoked the extension and overfilling of the blood vessels, and, sometimes, erythrorrhages to the tissues. Cellular infiltrations in the deep dermis, mainly located around the nerves and blood vessels, were primarily composed of macrophages (above 50%) (Fig. 1D) and T cells (up to 25%). Neutrophils and other cells were rare. The intensity of the changes was greater after 24 hours.

Even though both SP and VIP application produced T-cell and macrophagic infiltration, T-cell ratio was higher after SP injection, suggesting a stronger influence of SP on T lymphocytes to that of VIP at the doses used. Furthermore, the VIP and SP doses used by us were sufficient to induce vascular and chemotactic effects, but too small to cause massive degranulation of the mast

cells [9]. NPS action is generally considered to be short-lasting [11, 12]. Thus, the observed late phase of SP and VIP reactions probably results from a sequence of events only initiated by the NPS injection, but followed by immunological cell recruitment and local lymphokine production. We conclude that possible therapeutic applications of NPS [13] must be handled with caution and preceded with detailed studies, with particular focus on the late effects.

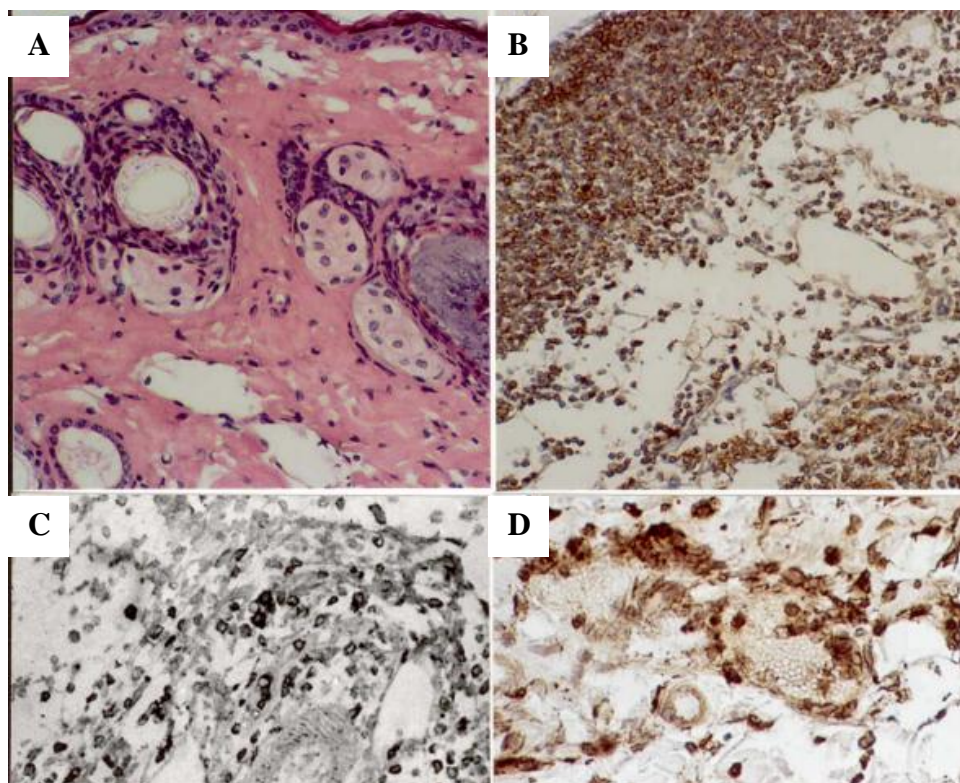


Fig. 1. Histological and immunohistochemical pictures of guinea pig skin injected with NPS. A. Guinea pig skin 24 h after an intradermal SP injection. Subepidermal clefts and widening of lymphatic vessels are visible. Haematoxylin and eosin. x 240. B. Guinea pig skin 24 h after an intradermal SP injection. Immunoreactivity to T cells. Subcutaneous lymph node showing signs of stimulation with T-cell predominance is visible. Haematoxylin counterstain. x 240. C. Guinea pig skin 24 h after an intradermal SP injection. Immunoreactivity to T cells. Above 50% of the infiltrating cells in the deep dermis are T cells. x 240. D. Guinea pig skin 3 h after an intradermal VIP injection. Immunoreactivity to macrophages. The majority of the infiltrating cells in the deep dermis are macrophages. x 600.

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