SZENZOROS NEUROTOXINOK ÁLTAL KIVÁLTOTT DEGENERATÍV ÉS NEUROPLASZTIKUS JELENSÉGEK PATKÁNY URETERÉBEN

SENSORY NEUROTOXIN-INDUCED DEGENERATIVE AND NEUROPLASTIC PHENOMENA IN THE RAT URETER

Andrea Ambrus

PhD Thesis

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Albert Szent-Györgyi Medical School,

Department of Physiology

and

Department of Anatomy, Embryology and Histology

Szeged

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Publications related to the subject of this thesis

I Jancsó G. and **Ambrus A.**: Capsaicin sensitivity of primary sensory neurones and its regulation. Pharmacological aspects of peripheral neurons involved in nociception, Peripheral neurons in nociception: Physio-pharmacological aspects. **Frontiers in pain research, Edited by Besson J.M.: Guilbaud G. and Oleat J.H., Libbey Eurotext 1994, Paris 71-88.**

II **Ambrus A.**, Jancsó G. and Fischer J.: Tomato lectin a new label for capsaicin sensitive neurons. **Lectins: Biology, Biochemistry, Clinical Biochemistry, Edited by E. Van Driessche, J. Fischer, S. Beeckmans, T.C. Bog-Hansen. (1994) 10: 3-6.**

III Sann H., Jancsó G., **Ambrus A.** and Pireau Fr-K.: Capsaicin treatment induces selective sensory degeneration and increased sympathetic innervation in the rat ureter. **Neuroscience (1995) 67 (4): 953-966.**

IV **Ambrus A.**, Kraftsik R. and Barakat-Walter I.: Ontogeny of calretinin expression in rat dorsal root ganglia. **Developmental Brain Research 106 (1-2): 101-108 (1998).**

Abstracts:

I Jancsó G., **Ambrus A.** and Török T.: Neurotoxic effect of resiniferatoxin on rodent and avian primary sensory neurones. **Neuropeptides (1992) 22(1) :34.**

II Sann H., Jancsó G., **Ambrus A.** and Pireau Fr-K.: Capsaicin pretreatment induces selective regional degeneration and neuroplastic changes in rat ureter. **Neuropeptides Supplement (1994) 26 (1): 65.**

III **Ambrus A.** and Barakat-Walter I.: Changes in calretinin expression by neurons in rat dorsal root ganglia. **Experientia (1996) 52: A74.**

IV **Ambrus A.** and Barakat-Walter I.: Ontogeny of calretinin expression in rat dorsal root ganglia. **European Journal of Neuroscience (1996) Suppl. 9 98: 54-01.**

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1. AIMS OF THE STUDY

The aims of the present study were

1. to study the neurotoxic effects of resiniferatoxin, an ultrapotent analogue of capsaicin on primary sensory neurons,

2. to reveal the morphology and anatomical organization of resiniferatoxin-sensitive neurons with particular emphasis on the innervation of the ureter,

3. to examine the general and specific innervation patterns of the rat ureter,

4. to investigate possible neuroplastic changes in the innervation pattern of the rat ureter induced by systemic capsaicin administration.

2. INTRODUCTION

2.1 Capsaicin Heritage

As early as 1878, A. Högyes[31] reported experimental results demonstrating that an oily extract of paprika caused a fall in body temperature when introduced in the stomach of dogs. He also tested its effect on different preparations such as frog heart and nerve-muscle and concluded that the site of action of the oily extract was primarily on the sensory nerves.

In 1912, W. Scoville[79] studied capsicum's ability to evoke the sensation of burning pain and calibrated the potency of pepper by extracting capsaicin in alcohol and diluting it until pungency was just detected after placing a drop on his tongue.

Further studies on capsaicin revealed a wide profile of biological activities, such as a hypothermic response[81], a pronounced hypotensive effect followed by variable actions on respiration[61] and a clear stimulating effect on gastrointestinal motility[61]. The first breakthrough in capsaicin research was N. Jancsó's discovery of local capsaicin desensitization. N. Jancsó and A. Jancsó-Gábor[47] observed that after repeated application of the pungent agents to the eye, the pain and inflammation produced by the chemical agents were abolished for days while responses to mechanical stimuli, e.g. the corneal reflex, remained unimpaired. Later, the Jancsós have also reported[48] that a similar desensitizing effect could also be produced by parenteral capsaicin pre-treatment on mice, and called it general capsaicin desensitization. A blockade of almost all types of chemical irritation led to the conclusion that capsaicin induced a very selective loss of responsiveness to heat and chemogenic stimuli[46]. Following these discoveries numerous experiments were undertaken to examine the neuroanatomical and ultrastructural effects of local and systemic capsaicin administrations on spinal ganglia and the spinal cord. F. Joó et al.[51] and J. Szolcsányi et al.[91] described abnormal mitochondrial morphology in adult small B-type neurons without alteration in the ultrastructure of any other neuronal or glial cell types, while G. Jancsó et al.[38, 39] presented evidence that neonatal capsaicin administration produced a selective and permanent loss of unmyelinated C-fibers and an irreversible degeneration of the entire population of small dark neurons in spinal ganglia. The same observation was provided by morphometric

studies demonstrating that neonatally administered capsaicin destroyed almost the entire small B cell population in spinal ganglia and unmyelinated C-fibers of the dorsal root[45, 58].

It is perhaps of interest to recall here, that on the basis of their cytological and ultrastructural features, the peripheral primary sensory neurons are divided into two main subpopulations: the so called large light or A-type cells with myelinated axons and the small dark or B-type neurons with mainly unmyelinated axons[22, 95]. The proportion of the large A-type neurons and small B-type neurons are similar in the mature lumbar ganglia. The two populations have different physiological properties. Thus, A- type neurons with thick myelinated axons (Aβ-fibers; velocity > 12 m/s) respond to very low-threshold stimuli such as hair movement in the skin, whereas the thin myelinated Aδ-fibers (velocity 2-12 m/s) form several subpopulations: the high-threshold mechanoreceptors (Aδ-HTMR) which respond exclusively to noxious stimuli, the low-threshold Aδ D-hair afferents, the afferents innervating subcutaneous structures, and the deep afferents[60]. The small B neurons project thin unmyelinated C-fibers (velocity < 1.3 m/s) which form the primary afferent pathways for a variety of painful stimuli[60].

In the late 70's and early 80's, a large number of neuropeptides were localized in capsaicin-sensitive primary sensory neurons and in the substantia gelatinosa of the spinal dorsal horn, such as substance P[36, 50] (SP), somatostatin[13, 29, 36] (SRIF), cholecystokinin[36] (CCK), vasoactive intestinal peptide[36] (VIP), calcitonin gene-related peptide[15, 19] (CGRP), bombesin[8] and opioid peptides[16, 29, 30]. Several of these reports have also shown that neonatal capsaicin treatment depleted substance P[36, 50], but also affected the SRIF system[13], CCK[36, 68] and CGRP[19]. It is now well established that in response to capsaicin, peptide mediators are released from central and peripheral nerve endings of capsaicin-sensitive neurons. A survey of them is beyond the scope of this introduction. (For more information see Holzer's review on capsaicin[24]). In addition to neuropeptides, neurohormones[8, 52] and several enzymes, such as the extralysosomal enzyme fluoride resistant acid phosphatase (FRAP)[8, 52] and the adenosine deaminase[69], were also found in many B-type neurons. FRAP, also called thiamine monophosphatase, was shown to be depleted following systemic capsaicin treatment[33, 44].

The findings showing the percentage of calcium-binding proteins in DRG neurons are of particular interest with respect to the mechanism(s) of the neurotoxic action of capsaicin. An increased influx of calcium ions has been shown to be a major event in capsaicin induced

neuronal degeneration[37, 94]. Therefore it has been assumed that buffering of intracellular free calcium level by calcium-binding proteins may have a protective effect against capsaicin-induced neurotoxicity[53]. However, immunohistochemical studies failed to support this suggestion; indeed, the proportion of calbindin-positive small sensory neurons decreased by 80-90% after neonatal capsaicin treatment[53].

Further studies on the localization of calretinin also indicated that the presence of calcium-binding proteins is unlikely to be related to neuronal capsaicin sensitivity. Thus, populations of both small and large DRG neurons displayed calretinin immunoeactivity[2].

Interestingly, capsaicin sensitivity displayed dose- and age-dependent differences. Indeed, the destruction of B neurons and the reduction of the substance P content by capsaicin revealed to be irreversible (despite the relative low doses of 50 mg/kg administered) if capsaicin was given within a week after birth, but reversible if animals were treated later in life[42]. When administered to the adult rat, capsaicin induced (even in doses as high as 100 mg/kg), a short or long-lasting desensitization depending on the type of sensory modality. This effect was shown to result from the depletion of neuropeptides and enzymes from the axon terminals of affected neurons and from an irreversible degeneration of a small proportion of B neurons[42].

From all these studies, a current view was inferred assuming that capsaicin-sensitive neurons belong to three classes: 1. nociceptive and thermal afferents, 2. reflex afferents, 3. regulatory afferents[34]. The first class consists of Aδ mechanonociceptors, C polymodal nociceptors and warm-receptor afferents. The polymodal nociceptors are involved in the mechanism of antidromic vasodilatation and neurogenic inflammation[88]. The second class includes various visceral afferents involved in the mediation of cardiovascular and respiratory reflexes[62], whereas the third class consists of visceral afferents involved in the local regulation or modulation of vascular, inflammatory[40] and immune[72] responses, via the release of neuropeptides from their nerve endings.

2.2 Natural Pungent Agents

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the pungent ingredient of fruits of various species of the plant genus Capsicum, including Mexican chilli pepper and Hungarian red pepper (Capsicum annuum also called paprika). On threshold concentration capsaicin elicits a warm sensation, whereas the perception of a hot feeling and pain develops when higher concentrations are tested. The capsaicin content of pepper pods varies in the range of 0.21 % - 1.43 %, and the threshold concentration of capsaicin on the tongue in producing lukewarm and definite painful burning sensations are 0.2 μg/ml and more than 1μg/ml, respectively[89]. Besides capsaicin other naturally occurring pungent agents exist, for example piperine, responsible for the pungency of black pepper (Piper nigrum), zingerone and shogaol, the pungent principles of ginger and resiniferatoxin which is found in some plants of the genus Euphorbia[21]. They all have structural similarities with capsaicin (Fig.1).

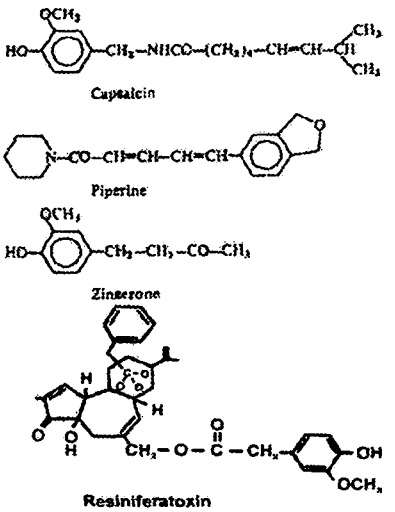

Fig.1. Chemical structure of natural pungent agents.

Ground pepper contains 4.6 % - 9.8% piperine; its threshold concentration on the human tongue is 3 μg/ml[90], whereas zingerone has only a moderate pungent effect, with a threshold concentration of 1mg/ml[90]. In addition to these natural agents, a large amount of congeners have

been synthesized and tested[71]. Correlation between chemical structure and pungency was established by systematic alteration in different groups of capsaicin molecules and revealed that the acylamid linkage was essential[70].

2.3 Molecular Actions of Capsaicin

Until recently, little was known about the precise molecular mechanism involved in capsaicin actions. Proposed models included direct perturbation of membrane lipids by hydrophobic capsaicin, activation of a non-selective cationic channel[23, 66], inhibition of voltage activated ion channels such as Ca^{++} channels[9] and activation of a specific receptor[85-87]. Several lines of evidence indicated that acute peripheral application of capsaicin causes a depolarization and excitation of the capsaicin-sensitive neurons which are accompanied by a large increase in membrane conductance due to activation of cation-specific ion channels[4, 23, 66]. These channels were supposed to provide a main route for the uptake of Ca^{++} observed during exposure to capsaicin[37, 94]. Calcium thus was thought to trigger the acute release of sensory neurotransmitters and neuropeptides and to contribute to capsaicin-induced neurotoxicity[66]. Further investigations presenting electrophysiological data found that the increase in intracellular calcium concentration produced a long-lasting inhibition of voltage-activated calcium currents and a subsequent reduction of transmitter release[9], thus providing a convenient explanation for the analgesic and anti-inflammatory actions of capsaicin.

These findings, together with the strict structural requirements needed to obtain capsaicin-like effects and the dose-dependent actions of capsaicin, strongly indicated the presence of a specific receptor linked to the capsaicin sensitive ion channel. Although capsaicin and its related compounds revealed to be inadequate to study the receptor because of their high lipophilicity, the discovery of resiniferatoxin (RTX), a highly potent analogue of capsaicin has made a direct demonstration of capsaicin binding sites possible[11, 83]. Indeed, Szállási and Blumberg[84, 85, 86] were able to show specific binding sites for 3[H] RTX on membranes isolated from spinal cord and dorsal root ganglia of rats and pigs. Because the vanilloid moity constitutes an essential chemical component of capsaicin and resiniferatoxin structures, the proposed

binding site was called the vanilloid receptor. RTX acts on the same cells via the same cellular mechanism as capsaicin but is at least 100 times more potent in most tissues[64]. Our own investigation[35] on rodent and avian primary sensory neurons, described below, was initiated to further characterize the actions of this analogue.

Very recently, the molecular nature of the capsaicin-binding site was obtained by means of the cloning of a gene encoding a capsaicin receptor[5]. To achieve this, the authors used a functional screening assay to isolate a cDNA clone that reconstitutes capsaicin responsiveness in non-neuronal cells. The cloned receptor, now called vanilloid receptor subtype 1 (VR1), revealed to be a moderately-selective ion channel that passes about ten calcium ions for every sodium ion. It is activated both by RTX and capsaicin. It is also a thermal sensor that is strongly activated by temperature known to elicit pain in humans. Moreover, it is expressed exclusively by small diameter B-type neurons in DRG, thus providing a definitive explanation for both the high selectivity of capsaicin action and the burning sensation elicited by capsaicin. One of the current hypothesis is that the real purpose of the receptor-channel is to sense noxious temperature, but the nature of its intrinsic agonist is still unclear.

2.4 Study of the Innervation of the Rat Ureter

In addition to the investigations focusing on the mechanisms implicated in capsaicin-induced effects, capsaicin was used as a neurotoxin to explore sensory functions. The easy accessibility and availability of capsaicin allowed the development of new experimental techniques, which proved to be an important pharmacological tool in exploring the structural and functional characteristics of primary sensory neurons and their receptive part in the peripheral and central tissues as well.

Early ultrastructural and immunohistochemical investigations of the sensory innervation of mammalian visceral organs, especially the ureter[28, 80, 92] were completed by the use of capsaicin. The rat ureter which was shown by Su et al.[82] to be supplied by primary afferents originating in thoracic and lumbar DRGs (T_{11}-L_3 and L_6-S_1), revealed to be particularly vulnerable to capsaicin. Capsaicin treatment resulted in a marked depletion of substance P and

CGRP-immunoreactive fibers of the rat and guinea-pig ureter[20, 26, 27, 65]. Chung et al.[6] reported a massive loss of unmyelinated fibers in all layers of the wall of the adult rat ureter following systemic capsaicin administration and suggested that autonomic efferent fibers were also affected. This observation was in contradiction with a previous report indicating that in the rat iris, capsaicin-induced loss of sensory fibers led to an increase in noradrenaline levels[63]. Our investigation, described below, was aimed to re-examine the effects of neonatal and adult capsaicin treatments on sensory and non-sensory innervation of the rat ureter in order to define whether the selective elimination of sensory afferents may result in a reorganization of the autonomic innervation. The results presented in this Ph.D. thesis show that the almost complete loss of capsaicin-sensitive fibers is accompanied by a sprouting of sympathetic (neuropeptide Y-positive) and paraszmpathetic (vasoactive intestinal peptide-positive) fibers[76].

3. MATERIALS AND METHODS

Experiments focused on the following aspects: general neurotoxic effects of RTX, determination of RTX-sensitive neuron population in DRGs, local neurotoxic effects of RTX applied directly on nerve fibers, comparison of neurotoxic effects in different species and neurotoxic effects of capsaicin on the ureter of neonatal and adult rats.

The description of methods emphasizes just the main points of each protocol which differ from the original methods.

3.1 Experimental Protocols for Resiniferatoxin and Capsaicin Administrations

In our studies neonatal and adult Wistar rats and 3-day-old chickens were used. For each experiment 3 to 5 animals were sacrificed. The doses and the manners of the administrations of neurotoxins were different in each experiment.

For the study of the effects of RTX on DRGs, the adult rats and the 3-day-old chickens received subcutaneously (s.c.) a single dose of RTX at concentrations of 300-500 μg/kg or capsaicin at a concentration of 100 mg/kg under anesthesia. The animals used as control were injected with vehicle containing 10% alcohol and 10% Tween 80.

By means of double administration of RTX followed by capsaicin to the same animal (adult rat), the identity of RTX-sensitive and capsaicin-sensitive neuronal populations in rat DRG were investigated. Animals first received s.c. RTX at a concentration of 500 μg/kg and received 48 hours later a dose of 100 mg/kg capsaicin.

The perineural effects of RTX on intraaxonal transports of both the endogenous thiamine monophosphatase and the intracutaneously given horseradish peroxidase were studied by the application of RTX directly onto a peripheral nerve of adult rats in amounts ranging from 0.3 μg to 5 μg.

In the experiments examining the effects of capsaicin on the ureter, the newborn rats were given a single s.c. capsaicin injection at a dose of 50 mg/kg and the adults were treated s.c. with

capsaicin at doses of 100 mg/kg or cumulatively 25, 50 and 75 mg/kg on consecutive three days under anesthesia.

The rats in the above experiments were sacrificed 30 minutes to 2 months after the administration of neurotoxins. According to the histological procedures, animals were perfused transcardially by 4 % formaldehyde in 0.1 M phosphate buffer (PB) or by a mixture of 2 % formaldehyde and 1 % glutaraldehyde in 0.1 M PB or by Zamboni's fixative.

3.2 Methods for the Observations of Neurotoxic Effects of Resiniferatoxin

The neurotoxic effects of resiniferatoxin were determined by various histochemical procedures. The free intracellular calcium ion level was detected in 30 μm thin frozen sections by a sensitive calcium staining[78]. The severe morphological changes in DRG neurons were studied at light microscopical level in semithin sections stained with toulidine blue. Fink-Heimer[12] silver impregnation gave information about the presence of the degenerated axon terminals in the Rexed's laminae I and II of the dorsal horn. Electronmicroscopic studies completed these observations obtained by light microscopy in order to detect neuronal degeneration in DRGs and axonal degeneration in the dorsal root and ureter.

The perineural effects of RTX on intraaxonal transport were studied with enzymhistochemistry and mustard oil-induced Evans blue plasma extravasation as a functional test[46]. The saphenous nerve innervates the medial part of the skin of the rat paw. Injections of a 1 % solution of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) in distilled water were given under the skin innervated by the saphenous nerve at 3 different sites in a total volume of 7.5 μl[74]. After a survival time of 72 hours, the presence of the enzyme was detected by the technique of Graham and Karnovsky[18] as modified by Adams[1] using 3,3'-diamino-benzidine (DAB) as chromogen. The anterograde intraaxonal transport of the endogenous enzyme, thiamin monophosphatase (TMP), was demonstrated according to a modified Gömöri method[56]. The enzyme activity of TMP in the central terminals of the treated nerve was also investigated in the related somatotpic areas of the spinal horn. After perineural RTX treatment, we also studied the remaining activity of the corresponding sensory nerves by means of provoking neurogenic inflammation.

Neurogenic inflammation was brought about by painting the skin of one of the hind paws with 5 % mustard oil in liquid paraffin after intravenous injection of 50 mg/kg Evans Blue dye.

3.3 Cell Counting and Morphometric Analysis in DRG

The cell counting was carried out in sections of rat L_4 and L_5 DRGs. The total number of neurons and the number of degenerating neurons were counted. Only cell profiles with at least one visible nucleolus were considered. At least 500 neurons were counted in a DRG at each experiment. The ratio of degenerating neurons to the total number of neurons in DRG is given in percentage.

Morphometric analysis was undertaken to estimate the distribution of cell cross-sectional areas: every 20^{th} section of one DRG was analysed using an Olympus microscope equipped with a camera lucida. The outlines of cell profiles were digitized and these data were exported and converted to a frequency distribution histogram using a computer program. Only cell profiles with at least one visible nucleolus were taken into account.

3.4 Immunohistochemistry of the Rat Ureter

Both sensory and autonomic innervation of the rat ureter were studied by light microscopic immunohistochemistry. To avoid problems arising from regional differences in the innervation and variations in immunohistochemistry, the control and capsaicin-pretreated tissues of about 1 cm length of a corresponding region in the middle of the ureter were processed under the same conditions on the same day. The general innervation pattern of the ureter in both control and capsaicin treated rats was determined by staining with antibodies against protein gene-product 9.5 (PGP) (polyclonal, 1:400) and neuron specific enolase (NSE) (polyclonal 1:1000). Antisera against neuropeptide Y (NPY) (polyclonal 1:400) or dopamine-β-hydroxilase (DBH) (polyclonal 1:200) and VIP (polyclonal 1:400) were used as markers for sympathetic and parasympathetic innervation. In addition, antisera to CGRP (polyclonal 1:400) and SP

(polyclonal 1:400) were used to investigate the presence of capsaicin-sensitive peptidergic sensory fibers. All antisera were raised in rabbits. The secondary antibodies were labelled with a fluorophore such as dichlorotriazynil aminofluorescein or carboxymethylindocyanine, and were used at a dilution of 1:100 or 1:200, respectively. The preparations were examined by means of an Olympus fluorescence photomicroscope (IMT-2).

Tissue from the upper (including the renal pelvis) and the lower end (close to the ureterovesical junction) of the ureter, regions particularly rich in parasympathetic innervation, were used for VIP and additonal PGP or NSE immunohistochemistry.

3.5 Quantification of Immunopositive Fibers in the Ureter

In order to quantify the effects of capsaicin treatment on immunoreactive fibers, the absolute number of fibers was determined in different layers of the ureter, namely, in the subepithelial plexus (SUB-EPI), in the submucosa (SUBM) and in smooth muscle layer and adventitia (SM/AD). At least three measurements in one piece of tissue were performed. Thin varicose, presumably single axons were counted separately from the nerve bundles. The ratios between the number of bundles and single fibers for each count were calculated, as an additional indicator of innervation density. For sake of clarity, only the total number of bundles and single axons will be presented below. The mean data of each animal were used for statistical comparisons using Student's t-test.

4. RESULTS

4.1 Neurotoxic Effects of Resiniferatoxin on DRG Cells

The neurotoxic effects of RTX were studied in adult rats and 3-day-old chicken DRGs after the systemic administration of RTX. The possible morphological changes were studied at different times after systemic injections to identify the consequences of the treatment.

The first important visible change was a marked accumulation of calcium ions in the perikarya of primary sensory neurons already 30 minutes after the injection. The detectable intracellular calcium accumulation persisted still another 30 minutes. Further light microscopic studies in semithin sections revealed serious structural changes in DRG neurons as early as 2 hours after the treatment. Many DRG neurons, namely 18-22 % of the total population became pyknotic and basophilic, and displayed severe cytoplasmic vacuolization (Fig. 2).

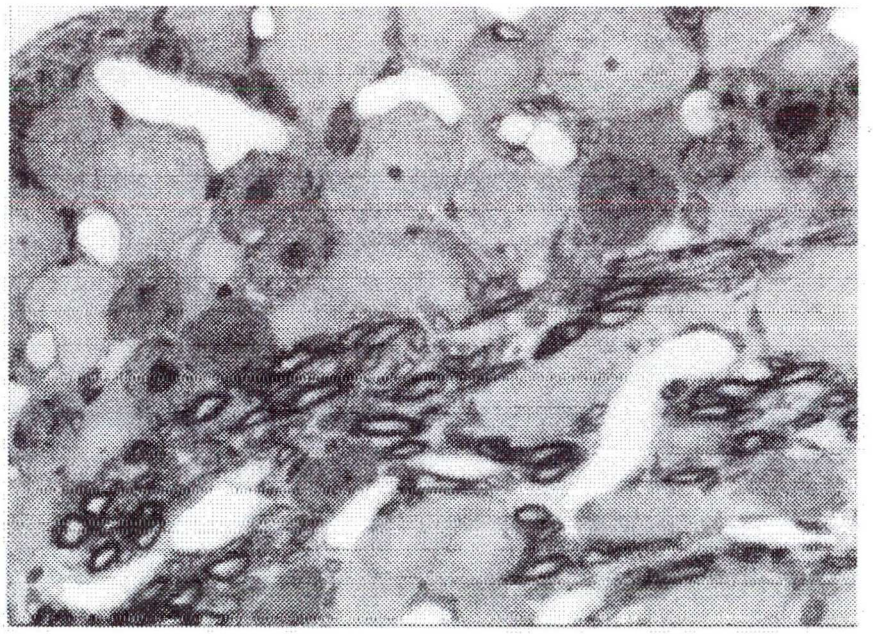

Fig. 2. Structural changes produced by a single injection of resiniferatoxin (500 μg/kg) given to an adult rat. Light microscopic photograph taken from the 4th lumbar spinal ganglion 2 hours

after treatment. Several small neurons exhibit irregular, pyknotic nucleus, increased nuclear and cytoplasmatic basophilia, and severe cytoplasmic vacuolization. The structure of large sized neurons is apparently intact.

Examination of the central terminals of DRG neurons with silver impregnation revealed several argyrophil particles in Rexed's laminae I and II of the spinal cord, indicating the presence of degenerated axon terminals. The electron microscopic observation reinforced this finding, showing that B cells underwent a rapid degeneration. The affected cells showed severe structural damages: their cytoplasmic organelles could hardly be recognized, mitochondrial swelling, dilatation of the cisternae of the rough endoplasmic reticulum and of the Golgi apparatus, and enhanced electron density in both nucleus and cytoplasm were observed at a 2 hour survival time. In addition, central and peripheral processes also displayed signs of degeneration. Several swollen and osmiophil unmyelinated fibers were observed in the dorsal root and in the ureter. Large myelinated afferent fibers, large light neurons, Schwann and satellite cells were unaffected by RTX.

A typical example of morphometric analysis performed on semithin sections of a rat lumbar 4th (L_4) ganglion is shown Fig 3., demonstrating that these RTX-sensitive cells were confined to the small dark neuronal subpopulation (Fig. 3).

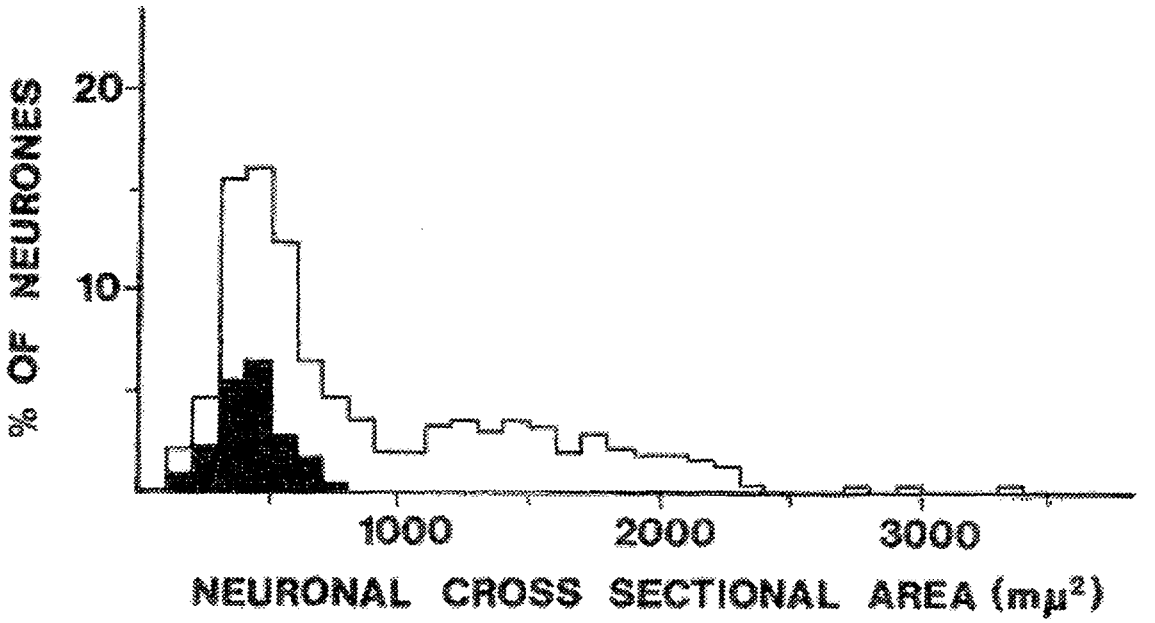

Fig. 3. Relative frequency of areas of degenerating neurons (shaded histogram field) and total neuronal profiles (open histogram). RTX was injected at a concentration of 500 μg/kg and the analysis was done 2 hours after treatment. Note that the cross-sectional areas of degenerating neurons are smaller than 800 μm^2.

The fact that capsaicin and RTX have the same neuronal target was further evidenced by the double administration protocol, where pretreatment of adult rats with RTX hindered capsaicin to induce additional changes in primary afferent neurons.

In other experiments we studied the effects of perineural application of RTX. The advantage of the perineural administration of a neurotoxin is that the effects of the neurotoxin on anterograde and retrograde intraaxonal transports can be selectively studied. Similarly to what was observed with capsaicin[14], the topical administration of RTX led to the blockade of both

anterograde and retrograde intraaxonal transport as demonstrated by the accumulation of endogenous thiamin monophosphatase proximally and that of horseradish peroxidase distally to the site of administration. In accordance with the blockade of axonal tarnsport, the perineural application of RTX led to the depletion of the thiamin monophosphatase from the central axon terminals in the substantia gelatinosa of the spinal cord. The perineural application of RTX also inhibited the neurogenic inflammatory response since the application of mustard oil onto the skin of the rat's paw did not evoke plasma extravasation, further confirming the inhibition of axonplasmic transport.

No degenerative histological changes were found in chicken DRG neurons after subcutaneous administration of RTX at any time observed. This finding demonstrated again the strong similarity between the actions of two neurotoxins.

4.2 Innervation of Rat Ureter

PGP 9.5 and NSE, as neuronal markers, were used to determine the general pattern of innervation of the rat ureter. Strong PGP 9.5 immunoreactive bundles and single varicose fibers were detected in all layers of the ureter except the epithelium. The general pattern and quantitative of PGP 9.5 immunoreactive fibers were studied in the various layers of the ureter (Fig. 4). A dense plexus of fibers, parallel with the longitudinal axis of the ureter, was found in the subepithelium (SUB-EPI) while in the adjacent submucosa (SUBM) a loose net-like organization of PGP 9.5-positive fibers was observed. In the adventitia, PGP 9.5 immunoreaction was located mainly in large nerve bundles and around blood vessels. Quantitatively, the most immunopositive fibers were found in the subepithelium and in the layers of the smooth muscle and adventitia (SM/AD).

The localization and number of NSE positive bundles and fibers were similar to those observed with PGP 9.5, though NSE displayed a weak labeling.

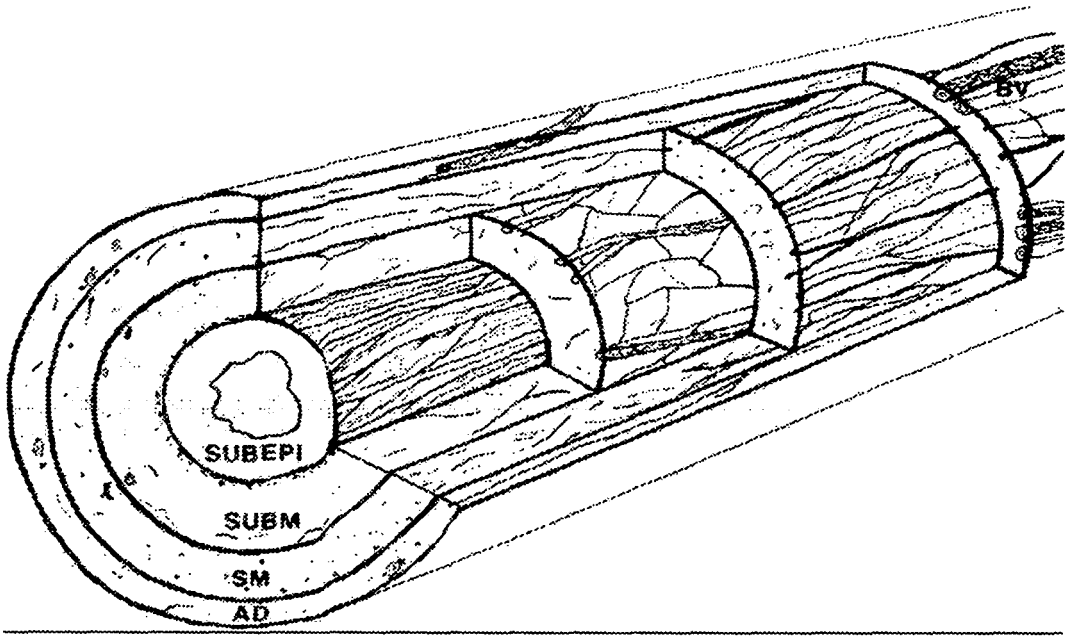

Fig. 4. Schematic drawing of the general innervation pattern of the rat ureter as deduced from preparations stained with an antibody against PGP 9.5. In our study, nerve fibers were counted (from left to right) in the subepithelial plexus just beneath the epithelium (SUB-EPI), in the outer submucosa (SUBM), in the smooth muscle layer (SM) and in the adventitia (AD). BV means blood vessels.

In addition to the general pattern of the innervation, the specific distribution and ratio of sensory and autonomic fibers were also determined. The most frequent transmitters in the peripheral sensory afferents are CGRP and substance P while VIP and NPY occur in the autonomic efferents. In control adult animals (see Table 2) similar number of CGRP and SP immunopositive nerve bundles was found in the SUB-EPI layer (87.6 and 70.5, respectively) and in SM/AD layers (70.6 and 64.6, respectively). However much less bundles were counted in the SUBM layer (18.9 and 16.1, respectively). The distribution of autonomic efferents was slightly different from that of primary afferents. The most NPY and VIP immunopositive fibers were found in SM/AD layers (62.9 and 30.4, respectively), less in the SUB-EPI layer (18.2 and 13.2) and the least in the SUBM layer (11.1 and 5.9). DBH-positive fibers showed a similar

distribution to that of NPY-positive fibers in all layers of the ureter. Mainly, single varicose fibers displayed VIP, NPY and DBH immunoreaction.

4.3 Capsaicin-Sensitive Afferents in the Rat Ureter

4.3.1 Neonatal Treatment

The neonatally treated rats were used as 2 months old adults for the immunohistochemical investigations. The quantitative data on the numbers of immunoreactive fibers (bundles + single axons) in the different layers are summarized in Table 1. PGP 9.5 immunoreactivity was almost completely abolished (99% reduction) in the subepithelial layer of the ureter. On the other hand, no significant change was observed in the number of PGP-positive fibers in the SUBM and SM/AD layers.

In the SUB-EPI layer the number of SP- and CGRP immunoreactive fibers was markedly reduced (to 0.4 and 5 per cent of the control, respectively), whereas VIP- and NPY-immunoreactive fibers remained apparently unaffected. In contrast, in the SUBM layer the number of NPY-positive fibers was increased (from 21.2 to 33.4) and significative reduction of CGRP (from 18.9 to 2.1) and SP-positive fibers (from 16.1 to 0) was found. Interestingly, there was a striking increase in number of VIP-positive fibers in the layer SUBM (from 1.8 to 11.4), whereas no significative changes were found in the other two layers.

Table 1. Mean numbers of immunoreactive fibers (bundles + single fibers) in the adult rat ureter after neonatal capsaicin pretreatment.

	SUB-EPI	SUBM	SM/AD
PGP control (n=3-5)	94.8 ± 13.1	25.1 ± 7.9	92.2 ± 7.7
Treated (n=3-5)	0.5 ± 0.3***	19.0 ± 6.5	74.4 ± 7.1
CGRP control (n=3-4)	105.2 ± 16.2	27.1 ± 6.4	112.0 ± 10.0
Treated (n=4)	4.8 ± 1.7***	13.9 ± 2.9*	39.3 ± 7.1***
SP control (n=3)	84.9 ± 15.9	19.3 ± 2.6	59.4 ± 8.6
Treated (n=3)	0.3 ± 0.3***	6.3 ± 1.0**	34.7 ± 9.8
NPY control (n=3-4)	19.1 ± 10.0	21.2 ± 2.7	50.3 ± 4.9
Treated (n=4-5)	8.9 ± 3.7	33.4 ± 7.7*	53.7 ± 5.6
VIP control (n=2-3)	7.3 ± 4.1	1.8 ± 1.5	15.8 ± 3.8
Treated (n=2-3)	6.5 ± 2.5	11.4 ± 3.1***	17.0 ± 12.7

Values are mean ± S.E.M.

Significant differences between control and capsaicin treatment are indicated: *P< 0.05, **P< 0.01, ***P < 0.001.

4.3. 2 Adult Treatment

The capsaicin or vehicle treated adults were sacrified 10-22 days after the s.c. injection. Since no significant difference was found between the number of immunoreactive ureteric nerve fibers of animals treated with either 100 or 150 mg/kg capsaicin, the results of both groups were analyzed together. As seen in Table 2, adult treatment affected particularly the SP containing cells indepedently of their locations: a total disappearance of SP immunoreactivity was found in the three layers. Capsaicin treatment also resulted in an almost complete depletion of CGRP immunoreactivities from all layers of the ureter. As far as autonomic fibers are concerned a significant increase (from 18.2 to 31.7) in NPY-positive fibers was found in the SUB-EPI layer, whereas no change was detected in the other layers. Opposite to what was seen after neonatal capsaicin treatment the number of VIP-positive fibers remained unaffected after adult treatment.

Table 2. Mean numbers of immunoreactive fibers (bundles + single fibers) in adult rat ureter after adult capsaicin treatment.

	SUB-EPI	SUBM	SM/AD
PGP control (n=4-5)	70.2 ± 5.0	14.7 ± 3.6	78.3 ± 13.7
Treated (n=5)	7.8 ± 2.0***	11.8 ± 0.6	85.4 ± 10.8
CGRP control (n=3-4)	87.6 ± 5.2	18.9 ± 1.3	70.6 ± 3.1
Treated (n=5)	0.1 ± 0.1***	2.1 ± 1.0***	7.6 ± 1.2***
SP control (n=5)	70.5 ± 6.8	16.1 ± 3.8	64.6 ± 6.9
Treated (n=4)	0***	0***	0***
NPY control (n=3-4)	18.2 ± 3.7	11.1 ± 1.7	62.9 ± 4.9
Treated (n=3-4)	31.7 ± 3.1*	14.4 ± 2.0	69.7 ± 5.5
VIP control (n=3)	13.2 ± 7.3	5.9 ± 2.1	30.4 ± 9.8
Treated (n=3)	16.7 ± 9.1	8.6 ± 5.2	17.8 ± 3.5

Values are mean ± S.E.M.

Significant differences between control and capsaicin treatment are indicated: *P< 0.05, **P< 0.01, ***P < 0.001.

Whereas in the SUB-EPI layer the absolute number of PGP-immunoreactive fibers was significantly reduced from 70.2 to 7.8, no significant change in the number of PGP-immunoreactive fibers was found in the SUBM and the SM/AD layers.

5. DISCUSSION

5.1 Neurotoxic Effects of Resiniferatoxin

In previous biochemical and biological studies[83-87], resiniferatoxin was shown to be an ultrapotent analogue of capsaicin. We now provide evidence demonstrating that the histological alterations caused by RTX are also similar to those of capsaicin, and that these alterations are induced at doses much lower than needed for capsaicin to produce similar effects (300-500 μg/kg versus 50-100 mg/kg, respectively).

Our investigations at light microscopic and ultrastructural levels reveal cellular lesions following RTX treatment similar to those seen in rat DRG neurons after capsaicin injection[42]. The affected neurons are exclusively confined to the subpopulation of B-type sensory neurons. Our morphometric analysis further provides quantitative evidence demonstrating that the severely damaged cells belong to the subpopulation of small dark neurons. Cross-desensitization between capsaicin and RTX reinforces these results. Indeed, when systemic RTX administration preceded the systemic injection of capsaicin, no additional histological changes in DRG neurons can be revealed, suggesting again that RTX-sensitive neuronal population is identical to the capsaicin-sensitive subpopulation in rat DRGs.

An other approach used in our study to demonstrate similarity of actions between RTX and capsaicin was the topical application of RTX on saphenous nerve. Perineural RTX treatment causes the accumulation of both retrogradely transported WGA-HRP and anterogradely transported TMP at the application site. RTX also induces profound histological changes in the substantia gelatinosa of the spinal cord where TMP activity is strongly reduced in the corresponding central projection areas of the treated nerve, indicating that RTX is able to modify markedly the function of the affected neurons via their peripheral fibers. Our finding that neurogenic inflammation could not be evoked after topical RTX treatment of the saphenous nerve, also support this conclusion. It is tempting to suppose that the functional impairment of sensory fibers induced by RTX results from the bidirectional blockade of axonal fluxes and / or

the depletion of enzymes and / or transmitters. Such a blockade was already observed in earlier studies evidencing that capsaicin inhibited the axonal transport of macromolecules[14, 41].

The biological and histological effects of capsaicin have been known to depend on the species used[24]. In our morphological investigations, RTX has produced species-related differences similar to that of capsaicin, since the systemic RTX treatment did not provoke any detectable histological changes in the neurons of 3 days-old chicken DRGs. We have to emphasize that the biological[83], biochemical[84-87] and histological[83] effects of RTX have been determined so far exclusively in mammals such as pigs, guinea-pigs, rats and in human spinal cord[84-87]. In avian species the effects of RTX have not been studied so far. In addition, all the previous studies were performed using systemic injections, thus ignoring the important consequences of the perineural application of RTX.

Among the cellular mechanisms underlying the actions of RTX, an increase in cytoplasmic free calcium ion concentration seems to be crucial. In vitro RTX treatment was described to increase the intracellular calcium concentration in primary cultures obtained from rat DRGs[93], and our present study is the first to demonstrate that this is also the case in vivo. This calcium accumulation may induce the dramatic histological changes observed in our studies. The irreversible damages due to calcium overload leading to cell death are probably also responsible for the RTX-induced cross-desensitization. Like capsaicin[42, 43], RTX is able to deplete neurotransmitters from the perikarya and central terminals of the affected neurons and to kill 18-22% of primary sensory neurons. Capsaicin administration was also reported to produce a detectable intracellular calcium accumulation in the affected small dark neurons of rat DRGs[37, 94].

Opposite to our results, Szállási et al.[83] found neither detectable calcium staining nor histological changes in adult DRG neurons after systemic RTX administration. The possible explanation of the discrepancy between ours and Szállási's results may reside in the different survival time allowed before sacrifying the animals, which was 30 min in ours and minimum 4 hours in their experiments. As we have shown, intracellular calcium accumulation is detectable as early as 30 min after injection and persists through another 30 min, whereas the severe histological changes are detected between 1 and 2 hours after the RTX injection. The long

survival time used in Szállási's experiments might have hindered the authors to detect these early events.

Very recently the capsaicin receptor, called as the vanilloid receptor subtype 1 (VR1) has been cloned[5]. It was found to be a moderately-selective calcium channel which opens when it is activated, allowing the influx of calcium and sodium ions into the cell. These findings provide a definitive explanation for the dramatic effects of capsaicin and RTX at the molecular level. Our double administration approach demonstrating a striking cross-desensitization between capsaicin and RTX supports the view that these two neurotoxins bind to the same receptor. But the fact that RTX is at least 100 times more potent than capsaicin suggests that RTX has a higher affinity to the receptor than capsaicin.

5.2 Sensory and Autonomic Innervation of the Rat Ureter

The aims of this study were to determine the proportion of capsaicin-sensitive sensory afferents in the layers of the rat ureter wall and to reveal any possible neuronal plasticity after both neonatal and adult capsaicin treatments. Although capsaicin and RTX stimulate the same subset of primary afferents and induce similar neurotoxic effects acting via a common membrane receptor site, referred to as the vanilloid receptor[5], we preferred to use capsaicin in this study, because of its well characterized effects following systemic pretreatment in both neonates and adults.

Our experiments provide additional data to previous studies[6, 82] about the innervation of the rat ureter. The various types of fibers such as autonomic and sensory axons innervate the different layers of the ureter in distinct manner. The autonomic fibers (NPY- and VIP-immunopositive fibers) preferentially innervate the SM/AD layers while similar number of sensory fibers (CGRP- and SP-immunopositive fibers) are equally distributed in both the SUB-EPI and the SM/AD layers. Much less VIP-containing processes are detected in SM/AD layers compared to that of NPY-containing fibers. The NPY-containing processes are observed mainly around the blood vessels. The VIP-immunostained fibers were not described in the rat ureter before. The SUBM layer, the thinnest layer of the ureter is sparsely supplied by neuronal fibers.

Previous studies pointed out that pronounced species differences in the innervation of the ureter exist. Whereas CGRP/SP processes predominate in rats[76] and guinea-pigs[80, 92], much less CGRP/SP-containing fibers were found in cat[92], pigs, ferrets, calves (H. Sann, personal communication), chickens[77] and human ureter[10, 32]. Our investigation reveals that most of the sensory processes in the rat ureter are capsaicin-sensitive fibers. Similar data have been published about the ureter of guinea-pigs[55] in which 70 % of the neuronal fibers were found to be capsaicin sensitive. In rat ureter, most capsaicin-sensitive processes (almost 90 %) are found in the SUB-EPI, a layer that receives a weak innervation by autonomic processes (10 % only). This ratio is higher in SM/AD layers where more autonomic fibers are detected. Most likely, the main transmitters of the capsaicin-sensitive fibers in the rat ureter are CGRP and SP as both neonatal and adult capsaicin treatments markedly decrease the number of CGRP- and SP-containing fibers. The similar distribution and capsaicin sensitivity of SP- and CGRP-immunopositive fibers suggest that most CGRP-positive fibers may contain also SP. Electrophysiological data from guinea-pigs suggest that the majority of sensory fibers in the ureter are not activated by physiological stimuli and might signal noxious events[7]. These noxious fibers are also activated and desensitized by capsaicin[75]. Afferent fibers with similar response characteristics have been described in the rat kidney and renal pelvis[73]. Capsaicin-sensitive CGRP- and SP-containing sensory fibers may be involved in local regulatory functions, including vascular and smooth muscle responses[24].

Since administration of capsaicin to adult rats leads to an almost complete loss of CGRP- and SP immunopositive fibers in the ureter, the disappearance of these immunostained fibers may result either from depletion of transmitters or from degeneration of sensory fibers. As the 10-22 days survival time should be enough for the neurons to recover from depletion and to resynthetize their transmitters, the lack of immunostained fibers after such a long survival time prompts us to rule out a simple depletion of neurotransmitters and to admit a degeneration of the fibers. In contrast to SP- and CGRP-immunoreactive fibers, the number of NPY-containing fibers increases in the SUB-EPI layer after the adult capsaicin treatment. The sympathetic and sensory fibers are known to depend on various neurotrophic factors such as nerve growth factor to survive in the postnatal life[3, 17]. The complete destruction of afferent fibers by capsaicin may

result in a relative excess of NGF which may promote the sprouting of sympathetic processes into the denervated areas[54, 57, 59].

The neonatal capsaicin treatment is known to cause the degeneration of almost all the small dark neurons in rat DRGs[39]. In accordance with this, CGRP- and SP-containing fibers practically disappeared from the SUB-EPI layer (4.8 and 0.3, respectively) while in SM/AD layers many immunostained fibers remained after the neonatal treatment (39.3 and 34.7, respectively). Holzers[25] has reported that a subpopulation of DRG neurons containing SP and CGRP is spared after the neonatal capsaicin administration and may account for this innervation. Following neonatal capsaicin treatment there is a striking increase in the number of VIP-positive fibers (SUBM) whereas the treatment seems not to affect the sympathetic innervation. This may indicate that early destruction of sensory fibers by capsaicin promotes the development of VIP-positive fibers. Interestingly, in the cornea of rats' eyes substantial increase in the number of tyrosine hydroxylase-immunopositive sympathetic fibers has been observed[67] after capsaicin application indicating an interaction between sensory and autonomic systems. Future studies should gain more insight into the mechanisms implicated capsaicin-induced neural plasticity.

6. CONCLUSION

Our results provide evidence that the neurotoxic effects of resiniferatoxin are similar to those of capsaicin in the adult rat and the RTX-sensitive and capsaicin-sensitive neuronal populations in rat DRGs are identical. Although the dominant effects of these neurotoxins are degenerative, our present work on the ureter highlights compensatory processes which follow the elimination of capsaicin-sensitive primary afferents. The reinnervation of the sensorily denervated areas by the sympathetic and the parasympathetic fibers may suggest a regulatory interaction between the sensory and autonomic system. This interaction may depend on certain factors such as the age of the animal, capsaicin sensitivity of the species and may involve molecular mechanisms including a possible competition for the availability of neurotrophic factors by sensory and autonomic nerves, and / or a reciprocal regulation of axonal growth between the sensory and autonomic terminals.

7. REFERENCES

1. Adams J.C.: Heavy metal intensification of DAB-based HRP reaction product. J Histochem Cytochem 29: 775 (1981)

2. Ambrus A., Kraftsik R. and Barakat-Walter I.: Ontogeny of calretinin expression in rat dorsal root ganglia. Dev Brain Res 106 (1-2): 101-108 (1998).

3. Barde Y.-A., Edgar D. and Thoenen H.: Purification of a new neurotrophic factor from mammalian brain. EMBO J 1: 549-553 (1982).

4. Bevan S.J. and Forbes C.A.: Membrane effects of capsaicin on rat dorsal root ganglion neurones in cell culture. J Physiol 398: 28 P (1988).

5. Caterina M.J., Schumacher M.A., Tomnage M., Rosen T.A., Levine J.D. and Julius D.: The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 816-824 (1997).

6. Chung K., Schwen R.J. and Coggeshall R.E.: Ureteral axon damage following subcutaneous administration of capsaicin in adult rats. Neurosci Lett 53: 221-226 (1985).

7. Cervero F. and Sann H.: Mechanically evoked responses of afferent fibres innervating the guinea-pig's ureter: an in vitro study. J Physiol 412: 245-266 (1989).

8. Decker M.W., Towle A.C., Bisette G., Mueller R.A., Lauder J.M. and Nemeroff C.B.: Bombesin-like immunoreactivity in the central nervous system of capsaicin-treated rats: a radioimmunassay and immunohistochemical study. Brain Res 342:1-8 (1985).

9. Docherty, R.J., Robertson B. and Bevan S.: Capsaicin causes a prolonged inhibition of voltage-activated calcium currents in adult dorsal root ganglion neurons in culture. Neuroscience 40: 513-521 (1991).

10. Edyvane K.A., Smet P.J., Trussel D.C., Jonavicius J. and Marshall V.R.: Patterns of neuronal colocalisation of tyrosine hydroxylase, neuropeptide Y, vasoactive intestinal polypeptide, calcitonin gene-related peptide and substance P in human ureter. J Auton Nerv Syst 48: 241-255 (1994).

11. Evans F.J. and Schmidt R.J.: An assay procedure for the comparative irritancy testing of esters in the tigliane and daphnane series. Inflammation 3: 215-223 (1979).

12. Fink R.P. and Heimer L.: Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. Brain Res 4:369-374 (1967).

13. Gamse R., Leeman S.E., Holzer P. and Lembeck F.: Differential effects of capsaicin on the content of somatostatin, substance P, and neurotensin in the nervous system of the rat. Naunyn Schmiedebergs Arch Pharmacol 317: 140-148 (1981).

14. Gamse R., Petsche U., Lembeck F. and Jancsó G.: Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin. Brain Res 239: 447-462 (1982).

15. Gibbins I.L., Furness J.B. and Costa M.: Pathway-specific patterns of the co-existence of substance P, calcitonin-gene related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. Neurosci Lett 57: 125-130 (1985).

16. Gibbins I.L., Furness J.B. and Costa M.:Pathway-specific patterns of the co-existence of substance P, calcitonin gene-related peptide. Cholecystokinin and dynorphin in neurons of the dorsal root ganglia of the guinea-pig. Cell Tiss Res 248: 417-437 (1987).

17. Goedert M., Stoeckel K. and Otten U.: Biological importance of the retrograde axonal transport of nerve growth factor in sensory neurons. Proc Natl Acad Sci USA 78 (9): 5895-5898 (1981).

18. Graham R.C. and Karnovsky M.J.: The early stages of absorption of injected horseradish peroxidase in the proximal tubule of mouse kidney: Ultrastructural cytochemistry by a new technique. J Histochem Cytochem 14: 291-302 (1966).

19. Gray J.L., Bunnet N.N., Muvihil S.J. and Debas H.T.: Capsaicin-stimulated release of CGRP from the isolated perfused rat stomach. Gastroenterology 96: A181 (1989).

20. Harti G., Sharkey K.A. and Pierau Fr.-K.: Effects of capsaicin in rat and pigeon on peripheral nerves containing substance P and calcitonin gene-related peptide. Cell Tiss Res 256: 465-474 (1989).

21. Hergenhahn M., Adolph W., and Hecker E.: Resiniferatoxin and other esters of novel polyfunctional diterpenes from Euphorbia resinifera and unispina. Tet Lett 19: 1595-1598 (1975).

22. Hess A.: The fine structure of young and old spinal ganglia, Anat Rec 123: 399-424 (1955).

23. Heyman I. and Rang HP.: Depolarizing responses to capsaicin in a subpopulation of rat dorsal root ganglion cells. Neurosci Lett 56: 69-75 (1985).

24. Holzer P.: Capsaicin: cellular targets, mechanism of action, and selectivity for thin sensory neurons. Pharmac Rev 43: 143-201 (1991).

25. Holzer P., Bucsics A. and Lembeck F.: Distribution of capsaicin-sensitive nerve fibres containing immunoreactive substance P in cutaneous and visceral tissue of the rat . Neurosci Lett 31: 253-257(1982).

26. Hoyes A. D.: Fine structure and response to capsaicin of primary afferent nociceptive axons in the rat and guinea pig ureter. In Sensory Receptor Mecahnisms (Eds: Hamann W. and Iggo A.) pp. 25-34 World Scientific, Singapore (1984).

27. Hoyes A. D. and Barber P.: Degeneration of axons in the ureteric and duodenal nerve plexuses of the adult rat following in vivo treatment with capsaicin. Neurosci Lett 25: 19-24 (1981).

28. Hoyes A.D., Bourne R. and Martin B.G.: Ultrastructure of the submucous nerves of the rat ureter. J Anat 119: 123-132 (1975).

29. Hökfelt T., Elde R., Johansson O., Luft R., Nilsson G., Arimura A.: Immunohistochemical evidence for separate population of somatostatin-containing and substance P-containing primary afferent neurons in the rat. Neurosci 1: 131-136 (1976).

30. Hökfelt, T., Johansson,O., Ljungdahl,A. Lundberg,J.,M., and Schultzberg, M.: Peptidergic neurons. Nature (London) 284: 515-521 (1980).

31. Högyes A.: Beträge zur physiologischen Wirkung der Bestandteile des Capsicum annuum. Arch Exp Pathol Pharmacol 9: 117-130 (1878).

32. Hua X.-Y.: Tachykinins and calcitonin gene-related peptide in relation to peripheral functions of capsaicin-sensitive neurons. Acta Phys Scan Suppl 551: 1-45 (1986).

33. Inomata K. and Nasu F.: Effects of neonatal capsaicin treatment on thiamine monophosphatase (TMPase) activity in substantia gelatinosa of spinal cord. Int J Dev Neurosci 2: 307-311 (1984).

34. Jancsó G.: B-afferents: A system of capsaicin-sensitive primary sensory neurons? Behav Brain Sci 13 (2): 306-7 (1990).

35. Jancsó G., Ambrus A. and Török T.: Neurotoxic effects of resiniferatoxin on rodent and avian primary sensory neurones. Neuropeptides 22 (1): 34 (1992).

36. Jancsó G., Hökfelt T., Lundberg J.M., Király E., Halász N., Nilsson G., Terenius L., Rehfeld J., Steinbusch H., Verhofstad A.E.R., Said S. and Brown M.: Immunohistochemical studies on the effect of capsaicin on spinal and medullary peptide and monoamine neurones using antisera to substance P, gastrin/CCK, somatostatin, VIP, enkephalin, neurotensin and 5-hydroxytryptamine. J Neurocytology 10: 963-980 (1981).

37. Jancsó G., Karcsú S., Király E., Szebeni A., Tóth L., Bácsy E., Joó F. and Párducz Á.: Neurotoxin induced nerve cell degeneration: possible involvement of calcium. Brain Res 295: 211-216 (1984).

38. Jancsó G. and Király E.: Sensory neurotoxins: Chemically induced selective destruction of primary sensory neurones. Brain Res 210:83-89 (1981).

39. Jancsó G., Király E. and Jancsó-Gábor A.: Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature (London) 270: 741-743 (1977).

40. Jancsó G., Király E. and Jancsó-Gábor A.: Chemosensitive pain fibers and inflammation. International J of Tiss Res 2:57-66 (1980).

41. Jancsó G., Király E. and Jancsó-Gábor A.: Direct evidence for an axonal site of action of capsaicin. Naunyn-Schmiedeberg's Arch Pharmacol 313: 91-94 (1982).

42. Jancsó G., Király E., Such G., Joó F. and Nagy A.: Selective degeneration by capsaicin of a subpopulation of primary sensory neurons in the adult rat. Neurosci Lett 59: 209-214 (1985).

43. Jancsó G., Király E., Such G., Joó F. and Nagy A.: Neurotoxic effect of capsaicin in mammals. Acta Physiol Hung 69 (3-4): 295-313 (1987).

44. Jancsó G. and Knyihár E.: Functional linkage between nociception and fluoride-resistant acid phosphatase activity in the Rolando substance. Neurobiol 5: 42-43 (1975).

45. Jancsó G. and Lawson S.N.: Ganglionic changes associated with transganglionic degeneration of capsaicin-sensitive primary afferents: a quantitative morphometric and imunohistochemical study. Reg Peptides 22: 97 (1988).

46. Jancsó N.: Desensitization with capsaicin and related acylamides as a tool for studying the function of pain receptors. In: Pharmacology of Pain. Proc. 3rd International Congress of Pharmacology of Pain Pergamon, Oxford, New York, 9: 33-55 (1968).

47. Jancsó N. and Jancsó-Gábor A.: Desensitization of sensory nerve endings. Kísérl Orvostud Suppl 2: 15 (1949).

48. Jancsó, N. and Jancsó-Gábor, A.: Daueraussschaltung der chemischen Schmerzempfindlichkeit durch Capsaicin. Naunyn-Schmiedeberg Arch Exp Pathol Pharmac 236: 142-145 (1959).

49. Jancsó N., Jancsó-Gábor A. and Szolcsányi J.: Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Br J Pharmac 31: 138-151 (1967).

50. Jessel T.M., Iversen L.L. and Cuello A.C.: Capsaicin-induced depletion of substance P from primary sensory neurons. Brain Res 152: 183-188 (1978).

51. Joó F., Szolcsányi J. and Jancsó-Gábor A.: Mitochondrial alterations in the spinal ganglion cells of the rat accompanying the long-lasting sensory disturbance induced by capsaicin. Life Sci 8: 621-626 (1969).

52. Kai-Kai M.A., Anderton B.H. and Keen P.: A quantitative analysis of the interrelationships between subpopulations of rat sensory neurons containing arginine vasopressin or oxytocin and those containing substance P, fluoride-resistant acid phosphatase or neurofilament protein. Neurosci 18 (2): 475-486 (1986).

53. Kashiba H., Senba E., Ueda Z and Tohzama M.: Calbindin D28k-containing splanchnic and cutaneous dorsal root ganglion neurons of the rat. Brain Res 528(2): 311-316 (1990).

54. Kessler J.A., Bell W.O. and Black I.B.: Interactions between the sympathetic and sensory innervation of the iris. J Neurosci 3: 1301-1307 (1983).

55. Király E., Jancsó G. and Hajós M.: Possible morphological correlates of capsaicin desensitization. Brain Res 540: 279-282 (1991).

56. Knyihár E., László I. and Tornyos S.: Fine structure and fluoride resistant acid phosphatase activity of electron dense sinusoid terminals in the substantia gelatinosa Rolandi of the rat after dorsal root transection. Exp Brain Res 19: 529-544 (1974).

57. Korsching S. and Thoenen H.: Nerve growth factor supply for sensory neurons: site of origin and competition with the sympathetic nervous system. Neurosci Lett 54: 201-205 (1985).

58. Lawson S.N. and Nickels S.M.: The use of morphometric techniques to analyse the effect of neonatal capsaicin on dorsal root ganglia and dorsal roots. J Physiol 300: 19P (1980).

59. Levi-Monthalchini R. and Angeletti P.U.: Nerve growth factor. Physiol Rev 48: 534-569 (1968).

60. Lewin G.R. and Mendel M.: Nerve growth factor and the nociception. TINS 16: 353-359 (1993).

61. Lille L. and. Ramirez E: Chem Abstr 29: 4836 (1935).

62. Lundberg J.M., Franco-Cereda A., Hua X.-Y., Hökfelt T. and Fischer J.: Co-existence of substance P and calcitonin gene-related peptide immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin. Eur J Pharmacol 108: 315-319 (1985).

63. Luthman J., Stromberg E., Brodin E. and Jonsson G.: Capsaicin treatment to developing rats induces increase of noradrenaline levels in the iris without affecting the adrenergic terminal density. Int J Dev Neurosci 7: 613-622 (1989).

64. Maggi C.A., Patacchini R., Tramontana M., Amann R., Giuliani S. and Santicioli S.: Similiraties and differences in the action of resiniferatoxin and capsaicin on central and peripheral endings of primary sensory neurons. Neurosci 37: 531-539 (1990).

65. Maggi C.A., Santicioli P., Giuliani S., Abelli L. and Meli A.: The motor effect of the capsacicin-sensitive inhibitory innervation of the rat ureter. Eur J Pharmac 126: 333-336 (1986).

66. March S.J., Stansfield C.E., Brown D.A., Davy R. and McCarthy D.: The mechanism of action of capsaicin on sensory C-type neurons and their axons in vitro. Neurosci 23: 275-289 (1987).

67. Marfurt C.F., Ellis L.C. and Jones M.A.: Sensory and sympathetic-nerve sprouting in the rat cornea following neonatal administration of capsaicin. Somatosensory Motor Res 10: 377-398 (1993).

68. Micevych P.E., Yaksh T.L. and Szolcsányi J.: Effect of intrathecal capsaicin analogs on the immunofluorescence of peptides and serotonin in the dorsal horn in rats. Neurosci 8: 123-131 (1983).

69. Nagy J.I., Buss L.A., LaBella and Dadonna P.E.: Immunhostpchemical localisation of adenosine deaminase in primary afferent neurones of the rat. Neurosci Lett 48: 133-138 (1984).

70. Nelson E.K.: Vanillyl-acyl amides. J Amer Chem Soc 41: 1115 (1919).

71. Newman A.A.: Natural and synthetic pepper-flavoured substances. (4) Synthetic substitutes. Chem Prod (London) 16: 467-471 (1953).

72. Payan D.G., Levine J.D. and Goetzl E.J.: Dual roles of substance P: Modulator of immune and neuroendocrine functions. Annals of New York Academy of Sciences 512: 465-75 (1984).

73. Recordati G.: Chemoreceptors in the kidney. News Physiol. Sci 1: 165-166 (1986).

74. Robertson B. and Ardvisson J.: Transganglionic transport of wheat germ agglutinin-HRP and choleragenoid-HRP in rat trigeminal primmary sensory neurons. Brain Res 348: 44-51 (1985).

75. Sann H., Hammer K., Rössler W. and Pierau Fr.-K.: Functions of peptidergic afferents from mammalian and avian ureter. Neuropeptides 22: 59 (1992).

76. Sann H., Jancsó G., Ambrus A. and Pierau Fr.-K: Capsaicin treatment induces selective sensory degeneration and increased sympathetic innervation in the rat ureter. Neurosci 67: 953-966 (1995).

77. Sann H., Rössler W., Hammer K. and Pierau Fr.-K.: Substance P and calcitonin gene-related peptide in the ureter of chicken and guinea pig: distribution, binding sites and possible functions. Neurosci 49: 699-326 (1992).

78. Sávay G. and Csillik B.: Acetylcholine-induced calcium release in the post-junctional sarcoplasm. Symp Biol Hung 5: 149-157 (1965).

79. Scoville, W. J. Am. Pharm. Assoc. 1: 453-454 (1912).

80. Sikri K.L., Hoyes A.D., Barber P. and Jagessar H.: Substance P-like immunoreactivity in the intramural nerve plexuses of the guinea-pig ureter: a light and electronmicroscopical study. J Anat 133: 425-442 (1981).

81. Stary, Z.: Über Erregung der Wörmenerven durch Pharmaca. Arch Exp Pathol Pharmacol 105: 76-87 (1925).

82. Su H.C., Wharton J., Polak J., Mulderry P.K., Ghatei M.A., Gibson S.J., Terenghi G., Morisson J.F.B., Ballesta J. and Bloom S.R.: Calcitonin gene-related peptide immunoreactivity in afferent neurons supplying the urinary tract: combined retrograde tracing and immunohistochemistry. Neurosci 18 (3): 727-747 (1986).

83. Szállási Á. and Blumberg P.M.: Resiniferatoxin, a phorbol-related diterpene, acts as an ultrapotent analog of capsaicin, the irritant constituent in red pepper. Neurosci 30: 515-520 (1989).

84. Szállási Á. and Blumberg P.M.: Specific binding of resiniferatoxin, an ultrapotent capsaicin analog, by dorsal root ganglion membranes. Brain Res 524: 106-111 (1990).

85. Szállási Á. and Blumberg P.M: Characterization of vanilloid receptors in the dorsal horn of pig spinal cord. Brain Res 547: 335-338 (1991).

86. Szállási Á . and Blumberg P.M.: Molecular target size of the vanilloid (capsaicin) receptor in pig dorsal root ganglia. Life Sci 48: 1863-18969 (1991).

87. Szállási Á. and Goso C.: Characterization by [^{3}H] resiniferatoxin binding of a human vanilloid (capsaicin) receptor in post-mortem spinal cord. Neurosci Lett 165: 101-104 (1994).

88. Szolcsányi J.: A pharmacological approach to elucidation of the different nerve fibers and receptor endings mediation of pain. J Physiol Paris 73: 251-259 (1977).

89. Szolcsányi J.: Capsaicin type pungent agents producing pyrexia. In: Handbook of Experimental Pharmacology 60: 437-478 Springer-Verlag, (1982).

90. Szolcsányi J. and Jancsó-Gábor A.: Sensory effects of capsaicin congeners I. Relationship between chemical structure and pain producing potency of pungent agents. Arzneim Forsch 25: 1877-1881 (1975).

91. Szolcsányi J., Jancsó-Gábor A., and Joó F.: Functional and fine structural characteristics of the sensory neuron blocking effect of capsaicin. Naunyn-Schmiedebergs Arch Pharmacol 12 (287): 157-169 (1975).

92. Wharton J., Polak J., Probert L., DeMey J., McGregor G.P., Bryant M.G. and Bloom S.R.: Peptide containing nerves in the ureter of the guinea-pig and cat. Neurosci 6: 969-982 (1981).

93. Winter J., Walpole C.S.J., Bevan S. and James I.F.: Characterization of resiniferatoxin binding sites on sensory neurons: co-regulation of resiniferatoxin binding and capsaicin sensitivity in adult rat dorsal root ganglia. Neurosci 57 (3): 747-757 (1993).

94. Wood J.N., Winter J., James I.F., Rang H.P., Yeats J. and Bevan S.: Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. J Neurosci 8: 3208-3220 (1988).

95. Yamadori T.: A light and electron microscopic study on the postnatal development of spinal ganglia. Acta Anat Nippon 45: 191-205 (1970).