Vas deferens sympathetic activity homeostasis involving capsaicin-sensitive neurons

Homeostasis de la actividad simpática del vaso deferente que involucra neuronas sensibles a capsaicina

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in memoriam[1]

Abstract

The evidence indicates the presence of crossed histamine-containing neuronal pathways at the level of the sympathetic ganglionic clusters of the vas deferens, which are involved in a peripheral short-loop reflex, in which noradrenergic neurons interact with contralateral sympathetic ganglionic histamine-containing neurons. The latter cause a contralateral reciprocal inhibitory modulation of vas deferens sympathetic activity. This short loop reflex requires the presence of interneurons which convey the message of the sympathetic discharge from one vas deferens to the contralateral ganglionic histaminergic neurons. In this work, we are presenting evidence concerning the possible peptidergic nature, distribution, and functioning of these interneurons. *Sprague-Dawley* rats were subjected to axotomy induced by capsaicin applied at the prostatic end of the vas deferens. For selective decentralization, capsaicin was locally applied to the preganglionic hypogastric nerve. After the surgery, animals received reserpine intraperitoneally to assess changes of sympathetic activity in the vas deferens. Our findings demonstrate that capsaicin-induced selective postganglionic axotomy does not affect noradrenaline or histamine levels in the vas deferens. The local application of capsaicin to the ipsilateral preganglionic sympathetic nerve did not alter sympathetic activity in the vas deferens. Capsaicin applied locally to only one sympathetic ganglion, caused the facilitation of sympathetic activity in both vasa deferentia. Capsaicin-induced postganglionic selective axotomy caused similar local facilitation of the sympathetic activity in the presence of normal histamine levels. Facilitation of sympathetic activity induced by surgical ganglionectomy was similar in magnitude as the one induced by capsaicin selective postganglionic axotomy. Furthermore, the combination of capsaicin-induced postganglionic selective axotomy plus surgical contralateral ganglionectomy, caused similar facilitation on the sympathetic activity as either one alone, suggesting that both inhibitory components of the reflex are sharing the same mechanism to damp down sympathetic activity in the vas deferens. Our results indicate that peripheral neuropeptidergic, as well as histaminergic neurons, are important components of the peripheral compensatory reflex of sympathetic activity, thus contributing to maintaining homeostasis of the sympathetic activity.

Key words: histamine, substance P, capsaicin, axotomy, ganglionectomy

Resumen

La evidencia indica la presencia de vías neuronales cruzadas que contienen histamina a nivel de los ganglios simpáticos de los conductos deferentes, que están involucrados en un reflejo periférico de asa corta, en el que las neuronas noradrenérgicas interactúan con neuronas ganglionares simpáticas contralaterales que contienen histamina. Estas últimos provocan una modulación inhibitoria recíproca contralateral de la actividad simpática del conducto deferente. Este reflejo de asa corta requiere la presencia de interneuronas que transmiten el mensaje de la descarga simpática de un conducto deferente a las neuronas histaminérgicas ganglionares contralaterales. En este trabajo, presentamos evidencia sobre la posible naturaleza peptidérgica, distribución y funcionamiento de estas interneuronas. Ratas *Sprague-Dawley* fueron sometidas a axotomía selectiva inducida por capsaicina, aplicada en el extremo prostático de los conductos deferentes. Para la descentralización selectiva, la capsaicina se aplicó localmente al nervio hipogástrico preganglionar. Después de la cirugía, los animales recibieron reserpina intraperitoneal para evaluar los cambios de la actividad simpática en los conductos deferente. Nuestros resultados demuestran que la axotomía postganglionar selectiva inducida por capsaicina, no afecta los niveles de noradrenalina o histamina en los conductos deferentes. La aplicación local de capsaicina al nervio simpático preganglionar ipsilateral, no alteró la actividad simpática en los conductos deferentes. La capsaicina aplicada localmente a un solo ganglio simpático provocó la facilitación de la actividad simpática en ambos conductos deferentes. La axotomía selectiva postganglionar inducida por capsaicina provocó la facilitación local de la actividad simpática en presencia de niveles normales de histamina. La facilitación de la actividad simpática inducida por la ganglionectomía quirúrgica, fue similar en magnitud a la inducida por la axotomía postganglionar selectiva con capsaicina. Aún más, la combinación de axotomía selectiva postganglionar inducida por capsaicina más ganglionectomía contralateral quirúrgica provocó una facilitación de la actividad simpática similar a la de cualquiera de los dos procedimientos, lo que sugiere que ambos componentes inhibidores del reflejo comparten el mismo mecanismo para amortiguar la actividad simpática en los conductos deferentes. Nuestros hallazgos indican que las neuronas neuropeptidérgicas periféricas, así como las histaminérgicas, son componentes importantes del reflejo compensatorio periférico de la actividad simpática, contribuyendo así a mantener la homeostasis de la actividad simpática.

Palabras clave: histamina, sustancia P, capsaicina, axotomia, ganglionectomia

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Introduction

Previous studies have shown an interaction between noradrenergic and histaminergic-containing neurons at the vas deferens sympathetic system (Campos, 1983, 1988; Campos and Briceño, 1992), via a decussation of neural pathways at the level of the sympathetic ganglionic clusters of both vas deferentia. Enhancement of sympathetic activity of one vas deferens causes activation of contralateral ganglionic histaminergic neurons whose nervous impulses, traveling toward the vas deferens with enhanced sympathetic activity, might inhibit the release of noradrenaline from sympathetic nerve terminal (Campos, 1988; Campos and Domínguez, 1995) via an H_3 inhibitory receptor (Campos, 1983), causing inhibition of the sympathetic discharge.

The novel peripheral reflex might be inhibitorily modulating the vas deferens sympathetic activity when the sympathetic discharge is enhanced. This histamine-containing neuronal system is involved in a peripheral short-loop reflex, in which noradrenergic neurons interact with contralateral sympathetic ganglionic histamine-containing neurons (Campos and Dominguez, 1995). The latter causes a contralateral reciprocal inhibitory modulation of vas deferens sympathetic activity (Campos and Briceño, 1992).

This peripheral modulation model of the sympathetic nervous system is contralateral, and it possibly operates through a regulatory neuron that is activated by a peptidergic sensory afferent pathway (Campos *et al.*, 1998). This short loop reflex requires the presence of interneurons which convey the message of the sympathetic discharge from on vas deferens to the contralateral ganglionic histaminergic neurons (**Figure 1**). In this work, we are presenting evidence related with the possible nature, distribution, and functioning of these interneurons.

Material and methods

Sprague-Dawley rats, 350-450 g body weight were subjected to axotomy induced by capsaicin applied at the prostatic end of the vas deferens. For this, animals were anesthetized with sodium pentobarbital (35 mg/kg, i.p.). Capsaicin was dissolved in 0.15 M sodium phosphate buffer, pH 7.0, containing 1% bovine serum albumin and 5% Tween 80. A strand of the cotton thread was soaked in either the vehicle or capsaicin solution and wound up 3 turns avoiding to block blood circulation, at the prostatic end of the vas deferens, 10 mm of the angle formed by the seminal vesicle and the vas deferens. The exposure to capsaicin

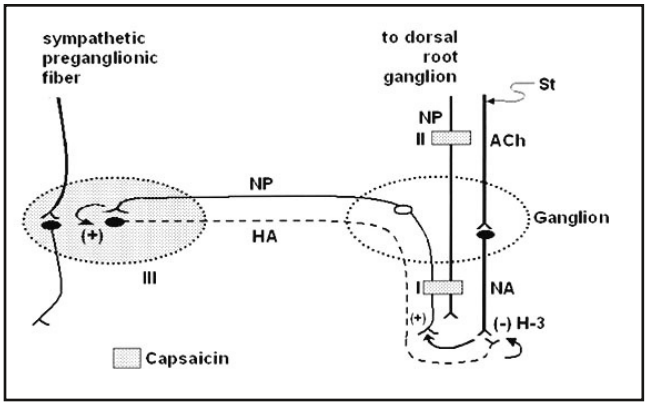

Figure 1. Diagram depicting the distribution of the neuronal pathways of the short loop reflex damping down the vas deferens sympathetic activity. The decussation of pathways is at the level of the sympathetic ganglionic clusters of vas deferens. NA: noradrenergic neurons. HA: histaminergic neurons. NP: neuropeptidergic neurons. Arrows indicate the direction of nerve impulses. For the sake of clarity, only one side of the reflex is presented.

was for 30 min. The highest capsaicin concentration was 5 mg/mL to assure a minimal diffusion from the site of action. Control animals were treated with vehicle.

For selective decentralization, capsaicin was locally applied to the preganglionic hypogastric nerve, in the abdomen, utilizing a 19 mm long wrested piece of cotton soaked in capsaicin solution and inserted around the nerve for 30 min (Campos, 1983). At half a period, 20 µl capsaicin solution was added to the cotton placed around the nerve. The control was treated with the vehicle. When capsaicin was locally applied to the ganglionic clusters of one vas deferens, a tiny cotton swab, about 2 mm in diameter, was built at one end of a toothpick and soaked either in capsaicin solution or vehicle. The toothpick was held in place by a stereotaxic instrument, kipping the swap at the angle formed by the seminal vesicle and the vas deferens, exerting a mild pressure on the area. Exposures time to capsaicin was also 30 min. At the end of the procedures, the area was rinsed with a saline solution, and the abdominal wall was repaired. 200.000 U procaine penicillin was administered subcutaneously. 6-8 days after the surgery the animals received reserpine (2 mg/kg, i.p.) for 3 or 1 ½ hr. At the end of this period, the rats were anesthetized with pentobarbital solution (60 mg/kg, i.p.) to remove the vas deferens. Catecholamine's (Anton and Sayre, 1962), and histamine (Schwartz *et al.*, 1970, modified and adapted by Campos *et al.*, 1999), were determined fluorometrically, in a Perkin Elmer LS 50B luminescence spectrometer, at 354 nm of excitation and 450 nm of emission, and the results were expressed in µg/g fresh tissue.

Statistical analysis

The data were expressed as mean ± standard error of the mean (S.E.M.) and were analyzed by one-way analysis of variance (ANOVA), and multiple comparisons were performed with the "Student t-test" for paired samples and Newman Keuls' test among the groups. A value of $p<0.05$ was considered significant. All statistical analysis performed was done using the IBM Pi-Stat program.

Results and discussion

To assess changes of sympathetic activity in the vas deferens, we have measured the degree of the disappearance of noradrenaline from the vas deferens 3 or 1 ½ hr after reserpine administration, since such disappearance is related to traffic of nervous impulses at the sympathetic nerve terminal (Karki *et al.*, 1959; Weiner*et al.*, 1962; Campos *et al.*, 1999). Moreover, electrical stimulation of the lumbar spinal cord causes the acceleration of noradrenaline depletion in the vas deferens of the reserpinized rat (Benmiloud and von Euler, 1963). In previous studies, we reported the facilitation of sympathetic activity in the vas deferens of the awake rat by using this technique (Campos and Briceño, 1992).

On the other hand, it has been shown in the rat sciatic nerve *in vivo* that capsaicin applied locally to the nerve stops the flow of neuropeptides such as substance P, calcitonin-gene-related peptide, somatostatin and neurokinin, but spares the flow of acetylcholine and catecholamine's (Gillespie and McGrath, 1974). These findings lead to the concept of selective axotomy induced by

capsaicin. Thus we have employed this technique by locally applying capsaicin to sites of the vas deferens sympathetic system to track down the possible distribution of neuropeptidergic pathways involved in the peripheral homeostatic mechanism downregulating sympathetic activity.

Our findings demonstrate that locally applied capsaicin, for selective postganglionic axotomy (**Figure 1, I**), does not affect noradrenaline or histamine levels in the vas deferens (**Figure 2**). This suggests that only capsaicin-sensitive neuronal pathways are to be damaged by capsaicin in the vas deferens as it occurs with the local application of capsaicin to the sciatic nerve (Gillespie and McGrath, 1974). Such damage could occur since, after the supposedly selective postganglionic axotomy, sympathetic activity is enhanced in the ipsilateral vas deferens as compared to the contralateral control or the one treated locally with the vehicle (**Figure 2**). This indicates, as postulated formerly (Campos and Briceño, 1992), that afferencies arise from the vicinity of sympathetic nerve terminals toward the ipsilateral sympathetic ganglion, where the body of capsaicin-sensitive neurons would be present (Gibson *et al.*, 1982). By contrast, the local application of capsaicin to the ipsilateral preganglionic sympathetic nerve does not alter sympathetic activity in the vas deferens (**Figure 1, II; Figure 2**). This strongly suggests that the known sensory afferencies toward the dorsal root ganglion are not involved in the neuronal system damping down the vas deferens sympathetic activity and leads support to the concept that the homeostatic mechanisms are peripheral in location.

Moreover, the hypothesis that capsaicin-sensitive pathways are crossing over to convey the message of enhanced sympathetic activity to the contralateral ganglionic histaminergic neurons is sustained by the finding showing that local application of capsaicin to only one sympathetic ganglion causes facilitation of sympathetic activity in both vasa deferentia (**Figure 1, III; Figure 2**). This effect occurs in the presence of normal levels of noradrenaline and histamine in both vasa deferentia, which indicates a selective action of capsaicin on neurons different from the ones containing noradrenaline or histamine. This agent would be touching the cross-roads of capsaicin-sensitive interneurons, therefore causing a bilateral interruption of the peripheral homeostatic mechanism, thus bilateral facilitation of sympathetic activity.

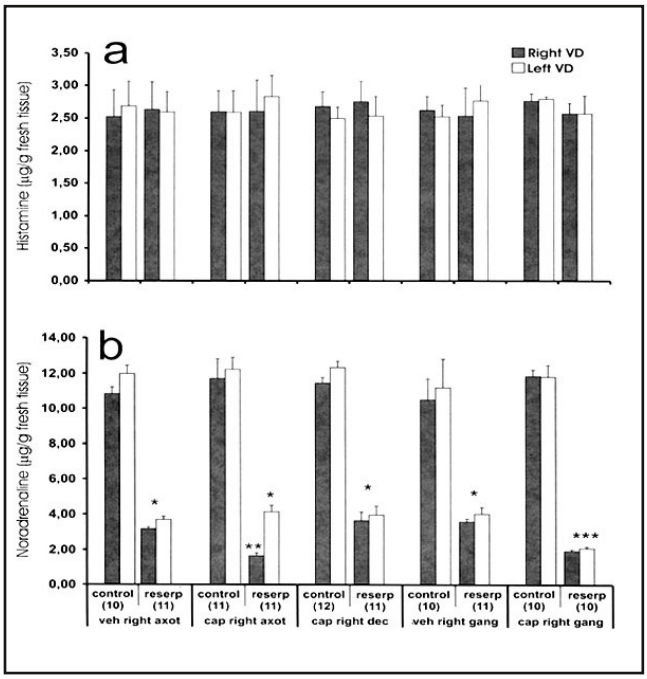

Figure 2. Changes of sympathetic activity (degree of reserpine-induced NA depletion) in both vasa deferentia due to capsaicin-induced selective postganglionic axotomy, decentralization, or ganglionectomy. Exposure time to reserpine was 3 hr. *p<0.05; **p<0.01 and ***p<0.001.

Since afferencies coming from the vicinity of the sympathetic nerve terminal appear to be at the initial step of the reflex, it is plausible to think that the link between noradrenergic and histaminergic neurons are the capsaicin-sensitive interneurons. On the other hand, contralateral surgical chronic sympathetic ganglionectomy causes histamine depletion in both vasa deferentia. This depletion is accompanied by the facilitation of contralateral sympathetic activity which could be reverted by histamine intravenous injection (Campos and Briceño, 1992). Notwithstanding capsaicin-induced postganglionic selective axotomy causes similar local facilitation of the sympathetic activity in the presence of normal histamine levels (Present results), one wonders whether both inhibitory components of the reflex are either dependent or independent from each other.

If they were independent of each other, facilitation of sympathetic activity induced by surgical ganglionectomy, which supposedly involves both neuropeptidergic and histaminergic neurons, would be greater than the one induced by capsaicin selective postganglionic axotomy, which supposedly acts only on neuropeptidergic neurons. This is not true, since both types of facilitation are of a similar order of magnitude (**Figure 3**). Furthermore, the combination of capsaicin-induced postganglionic selective axotomy plus surgical contralateral ganglionectomy causes similar facilitation on the sympathetic activity as either one alone (**Figure 3**). These findings suggest that both inhibitory components of the reflex are sharing the same mechanism to damp down sympathetic activity in the vas deferens as shown in **Figure 1**.

The facilitation of sympathetic activity caused by relative suppression of histaminergic or capsaicin-sensitive neurons in the vas deferens was extended to studies in the whole organism, showing that either inhibition of peripheral neuronal histamine biosynthesis (Campos *et al.*, 1996; Magaldi *et al.*, 1993) or neonatal destruction of capsaicin-sensitive neurons (Campos *et al.*, 1998), causes overall facilitation of sympathetic activity and hypertension in the awake rat. In this regard, it is important to note that both the hypertensive rat and humans show a reduction of substance P levels in comparison with normotensive controls (Mori *et al.*, 1993; Faulhaber *et al.*, 1983).

Furthermore, substance P-like immunoreactivity in the superior cervical ganglion, in a subpopulation of ganglion

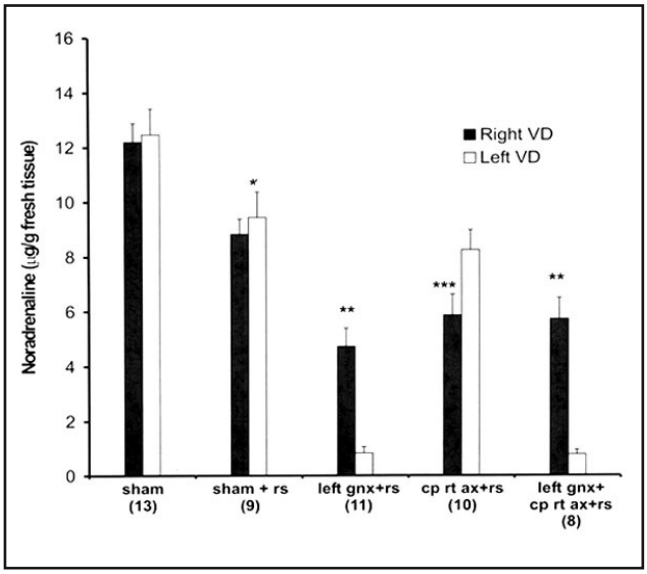

Figure 3. Changes of sympathetic activity (degree of reserpine-induced NA depletion) in vas deferens due to surgical left ganglionectomy, or capsaicin-induced selective right postganglionic axotomy, or the combination of both. Exposure time to reserpine was 1 1/2 hr. *p<0.05; **p<0.01 and ***p<0.001.

cells of genetically hypertensive Otago Wistar rats, was several times higher than in normotensive. As substance P has been reported to have an excitatory effect in sympathetic ganglia, intraganglionic release of substance P might contribute to the development of hypertension in the genetically hypertensive strain (Gurusinghe and Bell, 1989). This evidence suggests that substance P is a good candidate to be involved in the sequence of the peripheral reflex damping down sympathetic activity and arterial pressure, but this remains to be proven. Also, it was shown that intravenous administration of substance P swiftly brings down to normal levels pronounced hypertension and tachycardia caused by prolonged noise-induced stress in the rat (Gurusinghe and Bell, 1989). Moreover, associations between blood pressure and nociception are well documented (Bruehl, Carlson, McCubbin, 1992; Bruehl and Chung, 2004) and sensitivity deficits to heat or pain are respectively present in hypertensive rats and humans. In effect, pain sensitivity is diminished in hypertensive rats, clinical hypertension, as well as in healthy individuals with only moderately elevated blood pressure (Zamir and Shuber, 1980; Ghione, 1996; Myers *et al.*, 2001). Also, a reduction of blood histamine levels has been found in hypertensive humans as compared with normotensive control (Campos, 1999). Furthermore, Magaldi *et al.* (1993) demonstrated that in conditions of peripheral histamine deficiency induced by the irreversible inhibition of the enzyme histidine-decarboxylase with alpha-fluoro-methylhistidine results in sympathetic facilitation, which is associated with arterial hypertension and tachycardia, suggesting the presence of inhibitory action of neuronal histamine on the peripheral sympathetic nervous system.

Neuronal histamine appears to be reflexly released, as an overall compensatory phenomenon, during enhanced sympathetic activity. Histamine may be inhibiting noradrenaline release from sympathetic nerve terminals, and thus vascular responses due to stress, via H_3 inhibitory receptors, as shown when intraperitoneal administration of the histamine H_3 receptor antagonist, thioperamide, enhance vasopressor response to brief footshocks stress in the rat (Acuña *et al.*, 1998). This evidence suggests an action of endogenous histamine on inhibitory presynaptic H_3 receptors at the sympathetic nerve terminal to inhibit the release of noradrenaline from those terminals. Indeed, intravenous infusion of exo-genous histamine reverses the facilitation of sympathetic activity caused by chronic surgical interception of the vas deferens histamine containing pathways, while an H_1 or H_2 histamine receptor antagonist does not have any effect on the vas deferens sympathetic activity of the intact rat (Campos and Briceño, 1992). Moreover, histamine released to the blood circulation during footshocks stress in the rat depends upon the activation of sympathetic neurons (Campos and Montenegro, 1998). In addition, in the heart of the deafferented dog, removal of the left stellate sympathetic ganglion causes facilitation of the chronotropic responses induced by stimulation of the right sympathetic postganglionic nerve, also suggesting a peripheral contralateral inhibitory modulation of sympathetic activity at the heart (Campos and Briceño, 1992). In this direction, noradrenaline released from sympathetic nerve terminals might be the trigger of

the peripheral reflex since "auto-regulatory escape" from vasoconstriction occurs with intravascular inhibition of noradrenaline. It appears from all these findings that a peripheral inhibitory reflex is evoked when sympathetic activity is enhanced, thus contributing to maintaining homeostasis.

Finally, our studies indicate that peripheral neuropeptidergic, as well as histaminergic neurons, are worth to be considered within the context of the peripheral compensatory reflex of sym-pathetic activity in studies of the mechanism of primary arterial hypertension in both, animals and man.

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