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European Journal of Pharmacology 458 (2003) 313–318
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Role of voltage-gated cation channels and axon reflexes in the release of sensory neuropeptides by capsaicin from isolated rat trachea

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Received 9 August 2002; received in revised form 8 November 2002; accepted 15 November 2002

Abstract

In order to reveal the role of axon reflexes and sensory receptors in sensory neuropeptide release in response to capsaicin, liberation of substance P, calcitonin gene-related peptide and somatostatin from isolated rat tracheae was investigated in the presence of voltage-sensitive Na^+ and Ca^{2+} channel blocking agents. Neuropeptide release induced by capsaicin (10 nM) remained unchanged in the presence of 25 mM lidocaine, 1 μM tetrodotoxin or the N-type Ca^{2+} channel inhibitor, ω-conotoxin GVIA (100–300 nM). Peptide release by 100 pulses of 2 Hz field stimulation was prevented by lidocaine or tetrodotoxin. Omega-agatoxin TK (250 nM) significantly inhibited and Cd^{2+} (200 μM) prevented capsaicin-induced neuropeptide release. These results suggest that chemical stimulation-induced neuropeptide release does not involve activation of fast Na^+ channels or N- and P-type voltage-dependent Ca^{2+} channels, but contribution of Q-type Ca^{2+} channels is possible. Sensory neuropeptides are released by capsaicin from sensory receptors without axon reflexes.

Keywords: Capsaicin; Neuropeptide release; Substance P; CGRP (calcitonin gene-related peptide); Somatostatin; Tetrodotoxin

1. Introduction

Chemical or electrical stimulation of peripheral endings of the capsaicin-sensitive primary afferent neurones results in the release of sensory neuropeptides, such as substance P, calcitonin gene-related peptide (CGRP) and somatostatin (Maggi, 1995; Szolcsányi, 1996a,b). For the mechanism of the release process two theories has gained strong support. The axon reflex theory of Bayliss (1923) and Lewis (1927) was based on the prediction that the sensory receptors and the mediator releasing effector nerve terminals are distinct and axon reflex is required for the release of mediator(s) in response to nociceptive stimuli. Our earlier results with capsaicin have favoured the theory of dual sensory-efferent function for each nerve terminal of the peripheral axonal arborization (Szolcsányi, 1996a,b). Recently, the capsaicin receptor denoted as vanilloid VR_1 or $TRPV_1$ (Benham et al., 2002) receptor has been cloned and identified in a subset a primary afferent neurones being in fact a noxious heat-activated cation channel (Caterina et al., 1997) suitable for generation nociceptive signals. Therefore in order to decide whether sensory receptors could operate also as effector sites or operation of axon reflexes are necessary for the release process testings with capsaicin still seemed to be a useful approach. In this line the axon reflex theory was supported by some recent observations on the perfused guinea pig lung where the release of CGRP evoked by low concentration of capsaicin (10 nM) was abolished by the fast Na^+ channel blocker tetrodotoxin. High concentration of capsaicin, however, was shown to release substance P and CGRP in the presence of tetrodotoxin (Lou et al., 1992; Lundberg, 1996).

On the other hand, capsaicin-induced contraction of the guinea pig trachea at a concentration range of 33 nM–3 μM was resistent to tetrodotoxin, but in that study the release of sensory neuropeptides was not measured (Szolcsányi, 1983). Furthermore, ω-conotoxin GVIA, an inhibitor of voltage-gated N-type Ca^{2+} channels (Mc Cleskey et al., 1987), also failed to influence the Ca^{2+}-dependent release of the sensory neuropeptides, although the effect of electrical nerve stimulation was abolished by the toxin (Maggi, 1995;

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PII: S0014-2999(02)02794-2

Maggi et al., 1988). In theses studies, however, nanomolar concentrations of capsaicin were not tested and therefore the relevance of the conclusions has been questioned, since exposure of the guinea-pig isolated ureters to these high concentrations (1–10 μM) of capsaicin for 6 min caused neurotoxic axonal degenerations as established in specimens fixed 60 min later (Király et al., 1991).

The aim of the present study was to investigate these unsettled issues by measuring the release of three sensory neuropeptides (subtance P, CGRP and somatostatin) from the afferent nerve terminals of the rat isolated trachea in response to electrical field stimulation or capsaicin (10 nM) under control condition and in the presence of different Na^+ and Ca^{2+} channel blocking agents (tetrodotoxin, lidocaine, ω-conotoxin GVIA, ω-agatoxin TK and Cd^{2+}). It is worthy to mention that concomittant release of somatostatin with substance P and CGRP from sensory nerve endings has not yet been measured although all of them have functional relevance. Subtance P and CGRP are responsible for the local efferent responses (vasodilatation and plasma extravasation) and somatostatin for a systemic anti-inflammatory effect (Szolcsányi, 1996a; Szolcsányi et al., 1998).

2. Materials and methods

2.1. Release studies

Rats weighing 180–260 g of both sexes were anesthetized with sodium pentobarbitone (40 mg/kg, i.p.), then exsanguinated. The whole tracheae (dissected bellow the crycoid cartilage) together with the two main bronchi were removed and cleaned from fat and adhering connective tissues. In each experiment the tracheae from two animals were placed into an organ bath (1.8 ml) and perfused (1 ml/min) at 37 °C for 60 min (equilibration period) with pH-controlled (7.2), oxygenated (95% O_2 and 5% CO_2) Krebs solution. The composition of the Krebs solution (in mM) was the following: NaCl 119, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.5, KCl 4.7, $CaCl_2$ 2.5, glucose 11. After discontinuation of the flow, the solution was changed in the bath three times for 8 min to collect three separate fractions. The first was the basal (prestimulated) period without stimulation and test agent administration. Test compounds for blocking the respective cation channels were added into the organ bath at the beginning of the second 8-min incubation (stimulated) period. In the applied 25 mM concentration lidocaine was shown to abolish the tetrodotoxin-resistant impulses in nociceptive nerve terminals (Brock et al., 1998). For inhibition of the voltage-gated Ca^{2+} channels, ω-conotoxin GVIA in concentration range of 100–300 nM is suitable for selective action on N-type channels (Mc Cleskey et al., 1987), ω-agatoxin TK in 50 nM on P-type channels and above 200 nM also on Q-type channels (Teramoto et al., 1995; Wright and Angus, 1996). For antagonism of Ca^{2+} influx at all types of cation channels, Cd^{2+} was used in 20 and 200 μM concentrations (Mc Cleskey et al., 1987). Electrical field stimulation (40 V, 0.1 ms, 2 Hz, 50 s) or chemical challenge with capsaicin (10 nM) started after 5 min of this period to induce neuropeptide release from the tissue pieces. Stimulation and drug application were not performed in the third period (post-stimulated fraction). The whole volume of the organ bath was exchanged after each incubation period. The fractions were collected in ice-cold tubes to avoid enzymatic peptide degradation and the wet weight of the tracheae was measured. The average cumulative weight of two tracheas placed into one organ bath was 111.1 ± 7.9 mg. Concentrations of substance P, CGRP and somatostatin in the incubation medium were determined from 200 μl samples by specific an sensitive radioimmunoassay (RIA) methods developed in our laboratory as described (Németh et al., 1996, 1998a,b, 1999; Helyes et al., 1997) and were expressed as the released amount of peptide per tissue weight (fmol/mg). Detection limits of the RIA were 2 fmol/ml (substance P) and 1 fmol/ml (CGRP and somatostatin). Polypropilene tubes were used to reduce unspecific peptide binding.

2.2. Drugs

Sodium pentobarbitone (Nembutal) from Serva (Heidelberg, Germany) was used for anaesthesia. Capsaicin

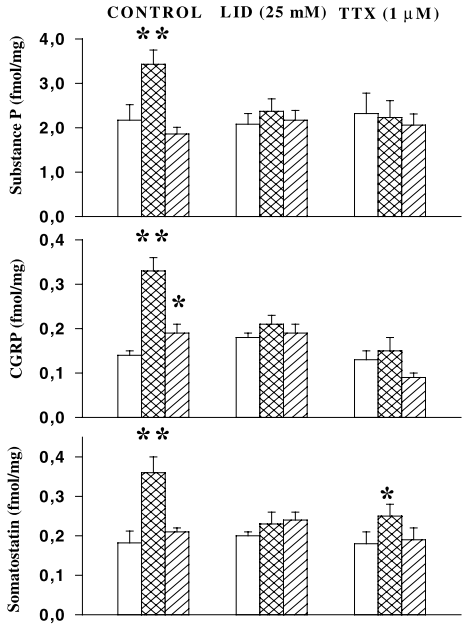

Fig. 1. Effect of lidocaine (LID) (25 mM) and tetrodotoxin (TTX) (1 μM) on the release of substance P, calcitonin-gene related peptide (CGRP) and somatostatin in response to electrical field stimulation (40 V, 0.1 ms, 2 Hz, 50 s) from rat tracheae in vitro. Peptide contents are shown in fmol/mg wet tissue and expressed as means ± S.E.M. of six experiments. Open columns indicate values of the pre-stimulation period, cross-hatched bars show the peptide release in response to stimulation and hatched columns refer to the post-stimulation fractions. Analysis of variance (repeated measures ANOVA) was used to compare fractions 2 or 3 to fraction 1; $*P<0.05$ and $**P<0.01$.

(8-methyl-*N*-vanillyl-6-nonenamide), $CdCl_2$, tetrodotoxin and somatostatin-14 were obtained from Sigma (St. Louis, MO, USA). Stock solution of capsaicin (1%) was dissolved in 10% ethanol, 10% Tween 80 (Reanal, Hungary), 80% isotonic NaCl solution and further diluted in isotonic saline. [Tyr^1]-somatostatin-14, rat α-CGRP, and Tyr-α-CGRP-(23–37) were purchased from Bachem (Bubendorf, Switzerland), Subtance P RIA tracer from Amersham (Little Chalfont, UK), lidocaine-chloride from Egis (Budapest, Hungary), ω-agatoxin TK from Alomone Laboratories (Jerusalem, Israel) and ω-conotoxin GVIA from Research Biochemicals International (RBI, Natick, MA, USA). Substance P antiserum was provided by Prof. G.J. Dockray, University of Liverpool, SOM and CGRP antiserum by Dr. T. Görcs, University Medical School of Budapest. ^{125}I-labelled Tyr-α-CGRP-(23–37) and ^{125}I-labelled [Tyr^1]-somatostatin-14 were prepared in our laboratory (Németh et al., 2002).

2.3. Statistical analysis

The results are expressed as means ± standard error of means (S.E.M.) of $n = 6$ experiments. Analysis of variance (repeated measures ANOVA) was used to determine statistically significant differences between the three fractions, $P < 0.05$ was regarded as significant.

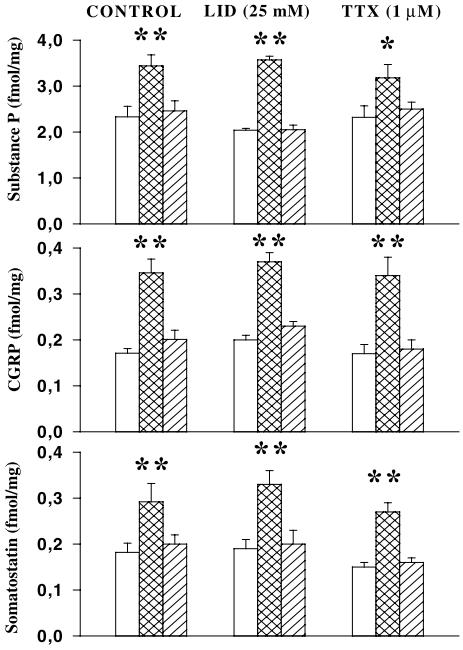

Fig. 2. Effect of lidocaine (LID) (25 mM) and tetrodotoxin (TTX) (1 μM) on the release of substance P, calcitonin-gene related peptide (CGRP) and somatostatin in response to capsaicin (10 nM) from rat tracheae in vitro. Peptide contents are shown in fmol/mg wet tissue and expressed as means of six experiments with vertical bars representing S.E.M. Open columns indicate values of the pre-stimulation period, cross-hatched bars show the peptide release in response to stimulation and hatched columns refer to the post-stimulation fractions. $*P < 0.05$ and $**P < 0.01$ show statistical significances compared to the pre-stimulation values (analysis of variance, repeated measures ANOVA).

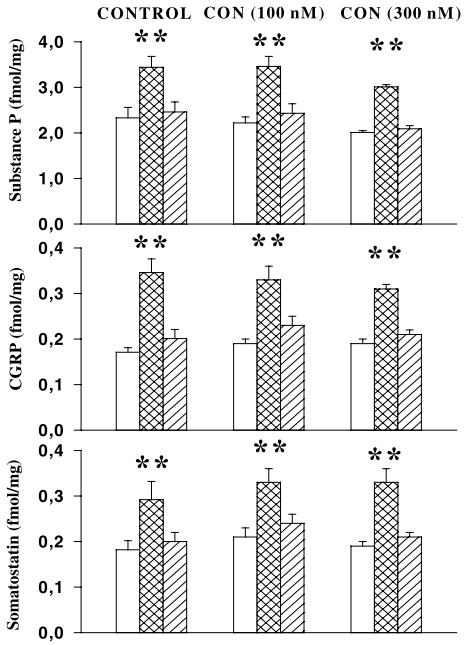

Fig. 3. Effect of ω-conotoxin GVIA (CON) (100 or 300 nM) on the liberation of substance P, calcitonin-gene related peptide (CGRP) and somatostatin in response to capsaicin (10 nM) from rat tracheae in vitro. Peptide contents are shown in fmol/mg wet tissue and expressed as means ± S.E.M. of six experiments. Open columns indicate values of the pre-stimulation period, cross-hatched bars show the peptide release in response to stimulation and hatched columns refer to the post-stimulation fractions. $**P < 0.01$ show statistical significances compared to the pre-stimulation values (analysis of variance, repeated measures ANOVA).

2.4. Ethics

The experiments performed in the present work conform to European Community guiding principles for the care and use of laboratory animals. The experimental protocol applied has been approved by the local ethical committee of Medical Faculty, University of Pécs, Hungary (BA02/2000-6/2001).

3. Results

3.1. Effect of lidocaine and tetrodotoxin on electrically induced substance P, CGRP and somatostatin release from isolated rat tracheae

In control samples electrical field stimulation (40 V, 0.1 ms, 2 Hz, 50 s) of the rat isolated tracheae evoked significant increase in the release of substance P (from 2.17 ± 0.35 to 3.43 ± 0.32 fmol/mg), CGRP (from 0.14 ± 0.01 to 0.33 ± 0.03 fmol/mg) and somatostatin (from 0.18 ± 0.03 to 0.36 ± 0.04 fmol/mg). The release of substance P, CGRP and somatostatin in response to field stimulation was strongly inhibited in the presence of 25 mM lidocaine by 76.08 ±

5.17%, 84.21 ± 13.7% and 83.33 ± 9.78%, respectively, and the remaining enhancements were non-significant. Tetrodotoxin (1 μM) completely abolished the field stimulation-induced release of substance P and decreased the release of CGRP by 89.47 ± 10.04% and somatostatin by 61.11 ± 8.65% (Fig. 1).

3.2. Effect of lidocaine and tetrodotoxin on capsaicin-induced substance P, CGRP and somatostatin release from isolated rat tracheae

Chemical challenge with capsaicin (10 nM) also caused marked elevation in the release of substance P (from 2.33 ± 0.23 to 3.44 ± 0.24 fmol/mg), CGRP (from 0.17 ± 0.01 to 0.35 ± 0.03 fmol/mg) and somatostatin (from 0.18 ± 0.02 to 0.29 ± 0.04 fmol/mg). Lidocaine or tetrodotoxin did not alter the release of the sensory neuropeptides in response to capsaicin (Fig. 2).

3.3. Effect of ω-conotoxin GVIA, ω-agatoxin TK and Cd^{2+} on capsaicin-induced substance P, CGRP and somatostatin release from isolated rat tracheae

Omega-conotoxin GVIA (100 and 300 nM) failed to influence the capsaicin-induced release of all the three neuropeptides (Fig. 3). Omega-agatoxin TK in a concentration of

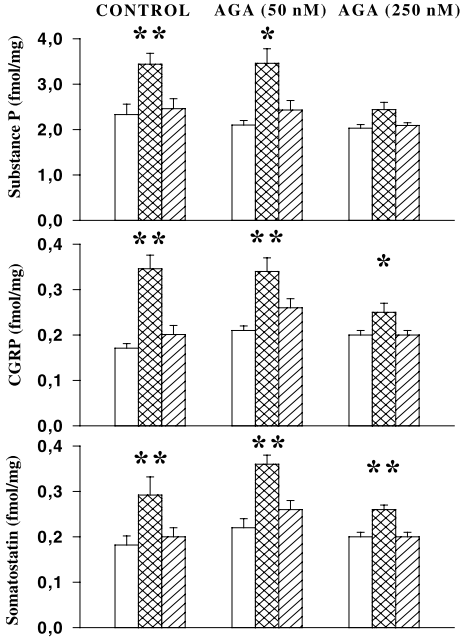

Fig. 4. Effect of ω-agatoxin TK (AGA) (50 or 250 nM) on the liberation of substance P, calcitonin-gene related peptide (CGRP) and somatostatin in response to capsaicin (10 nM) from isolated rat tracheae. Peptide contents are shown in fmol/mg wet tissue and expressed as means ± S.E.M. of six experiments. Open columns indicate values of the pre-stimulation period, cross-hatched bars show the peptide release in response to stimulation and hatched columns refer to the post-stimulation fractions. Analysis of variance (repeated measures ANOVA) was used to compare fractions 2 or 3 to fraction 1; $*P<0.05$ and $**P<0.01$.

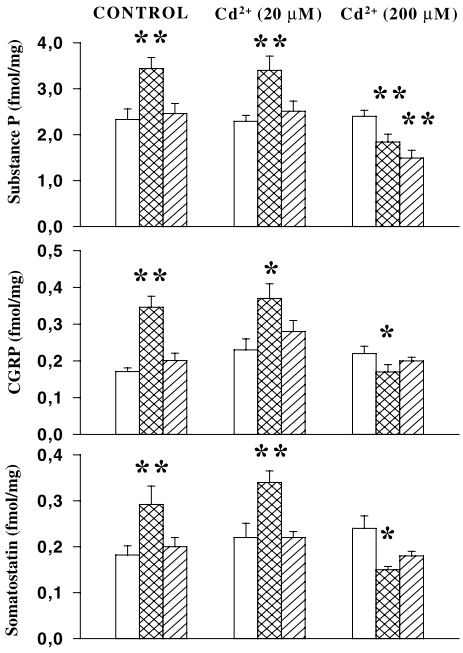

Fig. 5. Effect of Cd^{2+} (20 or 200 μM) on the release of substance P, calcitonin-gene related peptide (CGRP) and somatostatin in response to capsaicin (10 nM) from rat tracheae in vitro. Peptide contents are shown in fmol/mg wet tissue and expressed as means of six experiments with vertical lines refering to S.E.M. Open columns indicate values of the pre-stimulation period, cross-hatched bars show the peptide release in response to stimulation and hatched columns refer to the post-stimulation fractions. Analysis of variance (repeated measures ANOVA) was used to compare fractions 2 or 3 to fraction 1; $*P<0.05$ and $**P<0.01$.

50 nM was also without any effect, but 250 nM suppressed the release of substance P by 63.06 ± 8.95%, CGRP by 72.22 ± 10.84% and somatostatin by 45.45 ± 7.71% (Fig. 4). Cd^{2+} (200 μM) abolished the capsaicin-induced release of substance P, CGRP and somatostatin and the basal liberation was still reduced after wash-out. Cd^{2+} in a concentration of 20 μM did not alter the magnitude of the responses (Fig. 5).

4. Discussion

These results confirm our previous findings that both electrical field stimulation (100 pulses at low frequency) and capsaicin in a near threshold concentration of 10 nM elicit a significant release of substance P, CGRP and somatostatin from the isolated tracheobronchial segments of the rat in vitro (Helyes et al., 1997; Németh et al., 1998a,b). It has also been established that the field stimulation-induced release of these neuropeptides was abolished by tetrodotoxin or lidocaine suggesting the involvement of fast Na^{+} channels in activation of the release process. The capsaicin-induced neuropeptide release, however, remained resistant to the Na^{+} channel blockers (tetrodotoxin and lidocaine) as well as to the N-type voltage-gated Ca^{2+} channel inhibitor ω-conotoxin GVIA (Mc Cleskey et al., 1987; Maggi et al., 1988; Maggi, 1995). Lidocaine in the applied concentration

was sufficient to abolish tetrodotoxin-resistant impulses in nociceptive nerve terminals (Brock et al., 1998). The findings that another Ca^{2+} channel blocking agent ω-agatoxin TK in 50 nM concentration did not inhibit the capsaicin-induced neuropeptide release but evoked some inhibition in 250 nM, indicate that the P-type Ca^{2+} channels are not involved in the response, while the Q-type channels could participate (Teramoto et al., 1995; Wright and Angus, 1996). It should be noted, however, that effective concentrations of Ca^{2+} channel blocking agents at the soma and the nerve terminals might be different (Fischer and Bourque, 1995). Cd^{2+} is suitable for inhibiting all types of Ca^{2+} influxes through various cation channels. It completely abolished the capsaicin-induced neuropeptide release, indicating that it is a Ca^{2+}-dependent mechanism.

Lundberg et al. (Kröll et al., 1990; Lou et al., 1992; Lundberg, 1996) emphasized that low concentration of capsaicin (10 nM) induces sensory neuropeptide release which is abolished by tetrodotoxin or N-type Ca^{2+} channel blocking agents similarly to that seen after electrical antidromic vagal nerve stimulation and only high concentrations of capsaicin could release neuropeptides without axonal conduction. Taking into consideration that in vitro exposure of tissue specimens to high concentration of capsaicin (>1 μM) causes degeneration of the terminal arborization of sensory axons within minutes (Hoyes et al., 1981; Király et al., 1991), the tetrodotoxin-resistant release process at high concentration range might be due to these secondary toxic effects and not to opening the capsaicin-gated cation channels per se (Bevan and Szolcsányi, 1990; Szolcsányi, 1993; Caterina et al., 1997). The present results clearly show, however, that capsaicin in a concentration which does not cause axonal damage (Király et al., 1991) releases sensory neuropeptides without intervention of axonal conduction. Capsaicin induces cation influx into sensory neurones through vanilloid receptor-associated non-selective cation channels (Bevan and Szolcsányi, 1990; Szolcsányi, 1993; Caterina et al., 1997). The pronounced increase in Ca^{2+} concentration observed in the cell body of a subset of sensory neurones seems to operate also at the nerve terminals and is sufficient for the release of neuropeptides. On the other hand, the concommittant increase in Na^{+} concentration shares the major part for depolarization and spike generation at the same site (Szolcsányi, 1993, 1996b). Blocking the effect of N-type Ca^{2+} channels is suitable for the prevention of axon reflexes through action potential propagation, but this mechanism is not a prerequisite for the neuropeptide release by low concentration of capsaicin as suggested by Lundberg (1996) in the light of the classical work of Lewis (1927). It is worthy to mention that neurogenic inflammation of the skin and eye of the rat induced by irritants is not inhibited either by lidocaine or tetrodotoxin (Jancsó et al., 1968; Jancsó-Gábor and Szolcsányi, 1969). Furthermore the non-cholinergic neurogenic contraction of the guinea pig trachea provoked by capsaicinoids and piperine in a wide concentration range is also insensitive to tetrodotoxin (Szolcsányi, 1983). Consequently, it has been suggested that the release of sensory neuropeptides by low concentration of capsaicin can be prevented by tetrodotoxin or lidocaine under experimental conditions when diffusion of the neuropeptides from the sensory receptors to the perfusion fluid or to the vessels is hindered. Under these conditions involvement of axonal conduction between terminal boutons of the sensory nerve terminals is required (Szolcsányi, 1996a,b).

In summary, our results show that activation of the vanilloid VR_1/$TRPV_1$ "capsaicin-hot receptor" (Bevan and Szolcsányi, 1990; Caterina et al., 1997; Benham et al., 2002) by near threshold concentration of capsaicin leads to the release of substance P, CGRP and somatostatin, which is independent of the activation of voltage-dependent Na^{+} and Ca^{2+} channels. These biochemical data support further the concept that capsaicin-sensitive sensory nerve terminals are suitable for a dual "sensory-efferent" function without the intervention of any axon reflexes (Szolcsányi, 1996a,b).

Acknowledgements

This work was supported by the Hungarian Academy of Sciences and Hungarian Research Grants: OTKA T-039411, T-029428, TS-040753; ETT-T-04367/2000; NRDP 1/047/2001.

J. Németh was supported by István Széchenyi Fellowship and Zs. Helyes by János Bolyai Fellowship.

References

Bayliss, W.M., 1923. The Vaso-Motor System. Longmans Green, London.

Benham, C.D., Davis, J.B., Randall, A.D., 2002. Vanilloid and TRP channels: a family of lipid-gated cation channels. Neuropharmacology 42, 873–888.

Bevan, S., Szolcsányi, J., 1990. Sensory neuron-specific actions of capsaicin: mechanisms and applications. TIPS 11, 330–333.

Brock, J.A., Mc Lachlan, E., Belmonte, C., 1998. Tetrodotoxin-resistant impulses in single nociceptor nerve terminals in guinea-pig cornea. J. Physiol. 512, 211–217.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816–824.

Fischer, T.E., Bourque, C.V., 1995. Distinct ω-agatoxin-sensitive calcium currents in somata and axon-terminals of rat supraoptic neurones. J. Physiol. 489, 383–388.

Helyes, Zs., Németh, J., Pintér, E., Szolcsányi, J., 1997. Inhibition by nociceptin of neurogenic inflammation and the release of SP and CGRP from sensory nerve terminals. Br. J. Pharmacol. 121, 613–615.

Hoyes, A.D., Barber, P., Jagessar, H., 1981. Effect of capsaicin on intraepithelial axons of the trachea. Neurosci. Lett. 25, 19–24.

Jancsó-Gábor, A., Szolcsányi, J., 1969. The mechanism of neurogenic inflammation. In: Bertelli, A., Houck, J.C. (Eds.), Inflammation Biochemistry and Drug Interaction. Excerpta Medica, Amsterdam, pp. 210–217.

Jancsó, N., Jancsó-Gábor, A., Szolcsányi, J., 1968. The role of sensory nerve endings in neurogenic inflammation induced in human skin and in the eye and paw of the rat. Br. J. Pharmacol. 33, 32–41.

Király, E., Jancsó, G., Hajós, M., 1991. Possible morphological correlates of capsaicin desensitization. Brain Res. 540, 279–282.

Kröll, F., Karlsson, J.A., Lundberg, J.M., Persson, C.G.A., 1990. Capsaicin-induced bronchoconstriction and neuropeptide release in guinea-pig perfused lungs. J. Appl. Physiol. 68, 1679–1687.

Lewis, T., 1927. The Blood Vessels of the Human Skin and Their Responses. Shaw, London.

Lou, Y.-P., Franco-Cereceda, A., Lundberg, J.M., 1992. Different ion channel mechanisms between low concentrations of capsaicin and high concentrations of capsaicin and nicotine regarding peptide release from pulmonary afferents. Acta Physiol. Scand. 142, 119–127.

Lundberg, J.M., 1996. Pharmacology of cotransmission in the autonomic nervous system: integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. Pharmacol. Rev. 48, 113–178.

Maggi, C.A., 1995. Tachykinins and calcitonin gene-related peptide (CGRP) as co-transmitters released from peripherial endings of sensory nerves. Prog. Neurobiol. 45, 1–98.

Maggi, C.A., Patacchini, R., Santicioli, P., Lippe, J.T., Giuliani, S., Geppetti, P., Del-Bianco, E., Selleri, S., Meli, A., 1988. The effect of omega-conotoxin GVIA, a peptid modulator of the N-type voltage-sensitive calcium channels, on motor responses produced by activation of efferent and sensory nerves in mammalian smooth muscle. Naunyn-Schmiedeberg's Arch. Pharmacol. 338, 107–113.

Mc Cleskey, E.W., Fox, A.P., Feldman, D.H., Cruz, L.J., Olivera, B.M., Tsien, R.W., Yoshikami, D., 1987. Omega-conotoxin direct and persistent blockade of specific types of calcium channels in neurons but not muscle. Proc. Natl. Acad. Sci. U. S. A. 84, 4327–4331.

Németh, J., Helyes, Zs., Görcs, T., Gardi, J., Pintér, E., Szolcsányi, J., 1996. Development of somatostatin radioimmunoassay for the measurement of plasma and tissue contents of hormone. Acta Physiol. Hung. 83, 313–315.

Németh, J., Görcs, T., Helyes, Zs., Oroszi, G., Kocsy, T., Pintér, E., Szolcsányi, J., 1998a. Development of a new sensitive CGRP radioimmunoassay for neuropharmacological research. Neurobiology 6, 473–475.

Németh, J., Helyes, Zs., Oroszi, G., Thán, M., Pintér, E., Szolcsányi, J., 1998b. Inhibition of nociceptin on sensory neuropeptide release and mast cell-mediated plasma extravasation in rats. Eur. J. Pharmacol. 347, 101–104.

Németh, J., Szilvássy, Z., Thán, M., Oroszi, G., Sári, R., Szolcsányi, J., 1999. Decreased sensory neuropeptide release from trachea of rats with streptozotocin-induced diabetes. Eur. J. Pharmacol. 369, 221–224.

Németh, J., Oroszi, G., Jakab, B., Magyarlaki, M., Szilvássy, Z., Rőth, E., Farkas, B., 2002. ^{125}I-labeling and purification of peptide hormones and bovine serum albumin. J. Radioanal. Nucl. Chem. 251, 129–133.

Szolcsányi, J., 1983. Tetrodotoxin-resistant non-cholinergic neurogenic contraction evoked by capsaicinoids and piperine on the guinea-pig trachea. Neurosci. Lett. 42, 83–88.

Szolcsányi, J., 1993. Actions of capsaicin on sensory receptors. In: Wood, J.N. (Ed.), Capsaicin in the Study of Pain. Academic Press, London, pp. 1–26.

Szolcsányi, J., 1996a. Capsaicin-sensitive sensory nerve terminals with local and systemic efferent functions: facts and scopes of an unorthodox neuroregulatory mechanism. In: Kumazawa, T., Kruger, L., Mizumura, K. (Eds.), Progress in Brain Research, vol. 113. Elsevier, Amsterdam, pp. 343–359.

Szolcsányi, J., 1996b. Neurogenic inflammation: reevaluation of axon reflex theory. In: Geppetti, P., Holzer, P. (Eds.), Neurogenic Inflammation. CRC Press, Boca Raton, FL, pp. 35–44.

Szolcsányi, J., Helyes, Zs., Oroszi, G., Németh, J., Pintér, E., 1998. Release of somatostatin and its role in mediation of the anti-inflammatory effect induced by antidromic stimulation of sensory fibres of the rat sciatic nerve. Br. J. Pharmacol. 123, 936–942.

Teramoto, T., Niidome, T., Miyagawa, T., Nishizawa, Y., Katayama, K., Sawada, K., 1995. Two types of calcium channels sensitive to ω-agatoxin-TK in cultured rat hippocampal neurones. NeuroReport 6, 1684–1688.

Wright, F., Angus, T., 1996. Effects of N-, P- and Q-type neuronal calcium channel antagonists on mammalian peripheral neurotransmission. Br. J. Pharmacol. 119, 49–56.