Acute inflammation in the joint: Its control by the sympathetic nervous system and by neuroendocrine systems

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A R T I C L E   I N F O

Article history:
Received 18 October 2013
Received in revised form 3 January 2014
Accepted 6 January 2014

Keywords:
Inflammation
Joint
Sympathetic nervous system
Sympatho-adrenal system
Hypothalamo-pituitary-adrenal system

A B S T R A C T

Inflammation of tissues is under neural control involving neuroendocrine, sympathetic and central nervous systems. Here we used the acute experimental inflammatory model of bradykinin-induced plasma extravasation (BK-induced PE) of the rat knee joint to investigate the neural and neuroendocrine components controlling this inflammation. 1. BK-induced PE is largely dependent on the sympathetic innervation of the synovium, but not on activity in these neurons and not on release of norepinephrine. 2. BK-induced PE is under the control of the hypothalamo-pituitary-adrenal (HPA) system and the sympatho-adrenal (SA) system, activation of both leading to depression of BK-induced PE. The inhibitory effect of the HPA system is mediated by corticosterone and dependent on the sympathetic innervation of the synovium. The inhibitory effect of the SA system is mediated by epinephrine and β2-adrenoceptors. 3. BK-induced PE is inhibited during noxious stimulation of somatic or visceral tissues and is mediated by the neuroendocrine systems. The nociceptive–neuroendocrine reflex circuits are under powerful inhibitory control of vagal afferent neurons innervating the defense line (connected to the gut-associated lymphoid tissue) of the gastrointestinal tract. This inhibitory link between the visceral defense line and the central mechanisms controlling inflammatory mechanisms in body tissues serves to co-ordinate protective defensive mechanisms of the body. 5. The circuits of the nociceptive–neuroendocrine reflexes are under control of the forebrain. In this way, the defensive mechanisms of inflammation in the body are co-ordinated, optimized, terminated as appropriate, and adapted to the behavior of the organism.

1. Introduction

Inflammation is characterized by increased blood flow and vascular permeability, attraction of leukocytes and sensitization of primary afferent neurons. While the inflammation provides a protective response against infection and injury, it constitutes a positive feedback cascade that, if left unregulated, can result in tissue injury. For example, while approximately 50% of acute inflammatory arthritis/synovitis is self-limiting, resolving after ~12 months (Inaoui et al., 2004), persistent chronic synovial inflammation leads to joint destruction and the development of rheumatoid arthritis.

The autonomic nervous system regulates physiological systems by integrating afferent inputs from the internal and external environments with neuronal, endocrine and cell-mediated responses to maintain physiological homeostasis (Jänig, 2006). A key role of the autonomic system is the regulation of acute inflammatory responses at local and systemic levels. Multiple integrated mechanisms exist to maintain or to limit the magnitude of inflammation, involving the hypothalmo-pituitary–adrenal and sympatho-adrenal axes (3 and 4 in Fig. 1), afferent nociceptive neurons with unmyelinated (C-) fibers or small diameter myelinated (A-) fibers (2 in Fig. 1), and the sympathetic–neural system (1 in Fig. 1). These neural and neuroendocrine systems are orchestrated by the brain (spinal cord, brain stem and higher centers; see 6 to 8 in Fig. 1) and powerfully modulated by processes in the visceral body domain via vagal afferent neurons (5 in Fig. 1).

This topical review will summarize an integrated system, involving sympathetic, neuroendocrine, spinal and vagal sensory and central nervous systems, that acts in the control of an acute experimental inflammatory response in the rat knee joint. This close interaction between the nervous system, neuroendocrine and immune systems regulates peripheral inflammatory responses and may provide a link (1) to understand the mechanisms underlying the control of protective inflammatory processes by the brain under physiological conditions and (2) to understand this regulation in inflammatory diseases under pathophysiological conditions, e.g. chronic inflammation. We will use the experimental in vivo model of acute bradykinin-induced plasma extravasation (BK-induced PE) of the synovium in the rat knee joint to dissect out the neural and neuroendocrine components that are involved.
2. Peripheral mechanisms

2.1. Bradykinin-induced synovial plasma extravasation as a model

The synovium is innervated by both postganglionic sympathetic fibers and afferent C-fibers. Sympathetic postganglionic fibers constitute between half and two-thirds of the nerve fibers in the synovium (Hildebrand et al., 1991). These postganglionic fibers, which are closely associated with synovial blood vessels (Mapp et al., 1990), are involved in control of blood flow related to the joint including synovium and in control of synovial fluid secretion. Whether the terminals of sympathetic fibers are specifically arranged around the venules of the synovial vasculature is unknown (Eitner and Schaible personal communication). Resting and BK-induced PE are not dependent on the innervation of the joint capsule by unmynelinated afferent fibers (Corderre et al., 1989, see later) but about 60% of BK-induced PE is dependent on the innervation of the synovium by sympathetic post-ganglionic nerve fibers. Both resting and BK-induced PE are significantly reduced 7 to 14 days after surgical sympathectomy (Figs. 2D, and 3) (Miao et al., 1996a; Green et al., 1997).

Transecting the preganglionic axons, to acutely or chronically decentralize the lumbar sympathetic ganglia that contain the cell bodies of the postganglionic neurons to the rat hindlimb, does not significantly change the BK-induced PE in the knee joint capsule (Fig. 2D green). Similarly, acute interruption of the lumbar sympathetic chains during ongoing BK-induced PE does not reduce this plasma extravasation. Furthermore, blockade of conduction of the postganglionic terminals in the synovia by intraarticular co-perfusion of tetrodotoxin does not reduce the resting plasma extravasation and the BK-induced PE. Finally, increased synovial plasma extravasation by intraarticular perfusion of platelet activating factor, which acts directly on the endothelium, does not change after sympathectomy (removal of the paravertebral ganglia 4 or 14 days before the experiments). As expected, activation of the sympathetic postganglionic neurons by electrical stimulation of the lumbar sympathetic chain at frequencies of 0.2 to 5 Hz reduces both resting and BK-induced PE as well as plasma extravasation during infusion of platelet activating factor because the blood flow through the synovium is reduced (Miao et al., 1996a).

Quantitative analysis of the synovial plasma extravasation generated by different concentrations of bradykinin in the perfusate shows that the sympathetically mediated component is particularly large at bradykinin concentrations which have been measured in inflamed tissues (between $10^{-4}$ and $3 \times 10^{-7}$ M [black bar in Fig. 3]; Hargreaves et al., 1993; Swift et al., 1993) and almost undetectable at higher (pharmacological) concentrations ($\geq 10^{-6}$ M) as shown by Cambridge and Brain (1995), probably because bradykinin also acts directly on the endothelial cells at these high concentrations and because this direct endothelial...
The effect is maximal at pharmacological bradykinin concentrations (Fig. 3) (Miao et al., 1996b).

These types of experiments suggest that sympathetic postganglionic neurons innervating the joint capsule and its synovium have two functions (Fig. 4): first, to regulate blood flow (vasoconstrictor function), and second, to mediate vascular permeability. The first function occurs at the precapillary resistance vessels by vesicular release of transmitter(s) (norepinephrine, possibly ATP) which induces vasoconstriction.

**Fig. 2.** Bradykinin-induced plasma extravasation (BK-induced PE) in the synovia of the knee joint is largely dependent on the sympathetic innervation but not on activity in the sympathetic neurons. A. The rat knee joint was perfused with saline, bradykinin and/or other compounds. B. Degree of plasma extravasation of Evans Blue (ordinate scale) during saline infusion (first three 5 min samples) and during continuous infusion of BK (0.15 μM [160 ng ml⁻¹], n = 8) starting after 15 min and for the remainder of the experiment. C. Three preparations were used: intact lumbar sympathetic system; decentralized lumbar sympathetic system (preganglionic axons interrupted 7 days before the experiment by sectioning the ipsilateral white rami L2 and L3, the ipsilateral sympathetic chain between the ganglion L1 and L2, and the contralateral chain between the ganglia L1 and L2 and the ganglia L2 and L3, leaving in this way the postganglionic neurons intact); surgical sympathectomy 4 or 14 days before the experiment (surgical removal of the paravertebral ganglia L2–L4 bilaterally (see Baron et al., 1988)). D. Increase of plasma extravasation induced by BK in control rats (open circles, n = 12 knees). This increase was significantly smaller 14 days after sympathectomy (closed red squares, n = 12 knees; p < 0.01 two-way ANOVA) but was not significantly different from control after decentralization (closed green circles, n = 12 knees). Inset: Baseline plasma extravasation over 15 min preceding infusion of BK was also lower in animals 4 days and 14 days after sympathectomy than in control animals. *p < 0.05, #p < 0.01 t-test. The ordinate scale in B and D is absorbance of light by the knee joint perfusate. Mean + SEM. D modified from Miao et al. (1996a).

**Fig. 3.** Concentration-dependent bradykinin-induced plasma extravasation of the knee in control rats (n = 8) and in surgically sympathectomized rats (n = 8). Bradykinin was added cumulatively from 10⁻⁸ to 10⁻⁵ M to the perfusate. The range of bradykinin concentrations which have been measured in inflamed tissue is indicated by the black bar. In sympathectomized rats, the extravasation is significantly reduced at 3 × 10⁻⁸ to 3 × 10⁻⁷ M bradykinin (p < 0.05 two-way ANOVA). At higher concentrations, no effect of sympathectomy can be measured because the effect of bradykinin is generated via other than the sympathetically-mediated pathways. Ordinate scale same as in Fig. 2B,D. Mean + SEM (indicated for one in 6 measurements). Modified from Miao et al. (1996b).
Superfused by norepinephrine increase production of superoxide (Deo et al., 1992), blood mononuclear cells (Chaves et al., 2011). Furthermore, superfusion of synovial cells (e.g., synovial fibroblasts) to release a pro-inflammatory mediator (e.g., interleukin). For example, in vitro studies show that in addition to release of pro-inflammatory mediators, such as the cytokine interleukin 1 beta from sympathetic neurons (Freidin et al., 1992), blood mononuclear cells superfused by norepinephrine increase production of superoxide (Deo et al., 2013). Nitric oxide (NO) is known to contribute to synovial inflammation (Chaves et al., 2011). Furthermore, superfusion of synovial cells in vitro by epinephrine or norepinephrine increases the number of macrophage-like synovial cells (Mishima et al., 2001), and these cells are a major source of inflammatory cytokines (de Lange-Brokaar et al., 2012). The sympathetically mediated component of neurogenic inflammation is an entirely peripheral function of the sympathetic terminals. Whether both functions of the sympathetic postganglionic neurons are represented in the same class of postganglionic neuron or in distinct ones remains to be studied.

2.3. The afferent innervation in the control of bradykinin-induced plasma extravasation of the synovium

Afferent neurons with C-fibers innervating the knee joint synovium also contribute to neurogenic inflammation. Electrical stimulation of the posterior articular nerve innervating the knee joint synovium increases plasma extravasation mainly by release of substance P (Ferrell and Russell, 1986; Maggi, 1995a, 1995b; for review see Donaldson, 2009). Perfusion of substance P and another C-fiber mediator, calcitonin gene-related peptide (CGRP), through the rat knee joint enhances plasma extravasation (Green et al., 1992) whereby CGRP acts at the precapillary resistance vessels inducing vasodilation (Häbler et al., 1999). However, synovial plasma extravasation produced by bradykinin is not dependent on afferent C-fibers, as it is not affected by chemical destruction of unmyelinated primary afferents (by neonatal treatment in rats with a neurotoxic dose of capsaicin) (Jancsó et al., 1987).

The activity of post-ganglionic sympathetic fibers may alter the excitability of afferent C-fibers (Jänig, 2009). The synovium is well-innervated with sympathetic and afferent C-fibers that are both located perivascularly (Kidd et al., 1990). Importantly, during chronic inflammation these are marked sprouting of both sympathetic and C-fiber neurons (Jimenez-Andrade and Mantyh, 2012) with sympathetic neurons being wrapped around peptidergic fibers in the synovium in chronically inflamed rat knee joints (Longo et al., 2013). These findings might suggest that sympathetic-sensory neuron coupling, which is considered to be an underlying peripheral mechanism of sympathetically maintained pain (Jänig, 2009, 2013), also plays a role in the pathophysiology of chronic synovial inflammation. However, a close proximity of sympathetic and afferent terminals in peripheral tissues does not allow making predictions about sympathetic-afferent coupling. Chen et al. (2010) have shown in rats that increase in hindpaw volume and decrease of mechanical paw withdrawal threshold generated by intraplantar injection of bee venom are attenuated by surgical or chemical sympathectomy or by intraperitoneal injection of an α-adrenoceptor blocker. This may imply that peptidergic afferents are excited by sympathetic neurons in this model leading to venular plasma extravasation via release of substance P and CGRP.

To understand better the role of peptidergic afferent neurons in acute and chronic joint inflammation and the functional relationship between sympathetic and afferent C-fibers in the synovium, electrophysiological studies of dorsal root afferents supplying the synovium would have to be performed. After all bradykinin infused into synovial cavity should activate nociceptive afferents leading to release of neuropeptides and plasma extravasation. A clean study of the interaction of the two neural components in the synovium, avoiding global pharmacological interventions or global interventions like application of neurotoxic doses of capsaicin in neonates to chemically ablate the peptidergic afferent neurons, has never been done (see Häbler et al., 1997). This study should include the role of mast cells and cells related to the immune system (Fig. 4).
innervation occurs at the venular site of the vasculature and is independent of its function to regulate blood flow through the synovium (vasoconstrictor function). The cellular processes underlying the sympathetically mediated venular plasma extravasation are unclear. It is hypothesized that they are related to release of a prostaglandin (E2 or I) from the sympathetic terminals or from cells associated with them. This has to be proven.

BK-induced PE is not dependent on the innervation of the synovium by peptidergic primary afferent neurons which have nociceptive function. However, activation of this afferent innervation also leads to venular plasma extravasation by the release of substance P leading to arteriolar vasodilation. The mechanism of interaction between the sympathetically-dependent plasma extravasation and the afferent-mediated plasma extravasation is unclear and has to be explored.

The results obtained from these experiments on the rat knee joint raise some questions:

- Are noradrenergic sympathetic neurons also involved in the maintenance of inflammatory processes in other tissues of the body (e.g., the skin, the viscera, the deep somatic tissues)?
- Do inflammatory mediators other than bradykinin (such as serotonin, released from platelets present in synovial microvasculature (Boilard et al., 2012)) generate increases in permeability of postcapillary venules via the sympathetic noradrenergic varicose terminals (Pietruck et al., 2003)?
- Is there interaction between sympathetically mediated neurogenic inflammation and peptidergic (afferent)-mediated neurogenic inflammation?
- What are the molecular mechanisms of interaction of bradykinin, sympathetic terminal, venule and other cellular components in the generation of plasma extravasation? Thus how are the peripheral cellular and neural components involved in acute inflammation integrated?

3. Central control of bradykinin-induced plasma extravasation

BK-induced PE in the rat knee joint is under central control involving the hypothalamo-pituitary–adrenal (HPA) system and the sympatho-adrenal system. We have worked out two nociceptive–neuroendocrine reflex circuits involving the two final neuroendocrine pathways. The activation of the two reflex circuits by stimulation of nociceptors attenuates synovial plasma extravasation. This attenuation is enhanced after subdiaphragmatic vagotomy (Pietruck et al., 2003). In the following text these two reflex circuits will be described separately including their modulation by activity in vagal afferent neurons.

Cutaneous afferent neurons innervating the hindpaw (or sometimes the forepaw) contralateral to the perfused knee joint were activated in two ways:

1. The plantar/palmar skin, contralateral to the perfused knee joint, was stimulated electrically via a pair of stainless steel needles inserted transversely into the skin. The afferent fibers were activated continuously by 0.25 ms pulses of 25 mA at frequencies of 0.0625 to 3 Hz. At this stimulus strength afferent C-fibers as well as A-fibers are activated. This mode of afferent stimulation activates preferentially the HPA system (see below).

2. Afferent fibers of the plantar skin (sometimes also the palmar skin), contralateral to the perfused knee joint, were activated by capsaicin (CAP) at doses 3, 10 and 30 μg injected in volumes of 10 μl into the skin. Higher doses of CAP (100 and 300 μg) were not used since they had systemic effects. CAP excites nociceptive C-afferents and a few nociceptive Aδ-afferents. This mode of afferent stimulation activates preferentially the sympatho-adrenal system in the inhibition of BK-induced PE (see below).

3.1. Control of bradykinin-induced plasma extravasation via the hypothalamo-pituitary–adrenal (HPA) system and the sympatho-adrenal system and its inhibitory control involving vagal afferents

Continuous transcutaneous electrical stimulation of afferent C-fibers in the plantar skin of the hindpaw at 1 to 3 Hz reduces BK-induced PE. This afferent-induced depression of BK-induced PE has the following characteristics (Green et al., 1995):

- The decrease of BK-induced PE develops slowly reaching its maximum of about 30–40% of its baseline in ~50 min after the start of the C-fiber stimulation (Fig. 5, normal triangles). It recovers by ~50 to 60 min after termination of stimulation of the afferent nerve fibers. The depression of BK-induced PE is graded with respect to the stimulus frequency starting at about 0.06 Hz and being maximal at 1 Hz (Fig. 6).
- The depression of BK-induced PE occurs during continuous stimulation of afferent C-fibers. Electrical stimulation of A-fibers has either no or only small (statistically not significant) effects.
- After transection of the spinal cord at the segmental level thoracic T2 (distal to the afferent inflow from the foreleg to the spinal cord), performed the day before, depression of BK-induced PE generated by stimulation of the forepaw skin is unchanged. This argues that the sympatho-adrenal system is not involved in depression of BK-induced PE under these experimental conditions (i.e. electrical stimulation of afferents).
- The depression of BK-induced PE generated by C-fiber stimulation is unchanged after acute interruption of the lumbar sympathetic chain between the paravertebral ganglia L1 and L2 as well as L2 and L3 (which does not affect the innervation of the adrenal medullae). Thus the depression of BK-induced PE (1) is not mediated by activity in the sympathetic neurons innervating the synovium and (2) is not generated by decrease of blood flow generated by reflex activation of vasoconstrictor neurons innervating blood vessels in the knee joint.
- After removal of the adrenal glands, BK-induced PE is no longer depressed during stimulation of afferent C-fibers (Fig. 5, inverted triangles).
- Stimulation of the C-afferents does not depress plasma extravasation induced by platelet activating factor (which acts directly on the vascular endothelium), supporting the idea that the depression of BK-induced PE during stimulation of C-afferents is not generated by decrease of blood flow through the synovium, but by a mechanism upstream of the endothelium that integrates local cellular signals and remote signals.
- BK-induced PE is not depressed during afferent C-fiber stimulation in hypophysectomized rats (Fig. 5B), indicating that the HPA system is involved.

These results strongly support the idea that the depression of BK-induced PE during electrical stimulation of afferent C-fibers is mediated by activation of the HPA system. This depression is enhanced in rats in which the vagus nerve is transected bilaterally below the diaphragm (performed immediately before the experiments). The time–effect curve of depression of BK-induced PE by electrical stimulation of afferent C-fibers is shifted to lower frequencies of stimulation in vagotomized rats compared to sham vagotomized rats (Fig. 6) (Miao et al., 1997a). These results show that activity in vagal afferents innervating abdominal organs and projecting to the nucleus of the solitary tract modulates the impulse transmission in the nociceptive–neuroendocrine reflex pathway to the pituitary gland in an inhibitory way (more see below).

Is the inhibitory effect of the activation of the HPA axis on BK-induced PE mediated by corticosterone and does this inhibitory effect require the presence of the sympathetic postganglionic terminals at the site of plasma
extravasation in the knee joint? Experiments testing these ideas are clearly supportive (Green et al., 1997):

1. Corticosterone infused intravenously at a rate of 5 μg/min mimics the inhibition of BK-induced PE generated by electrical stimulation of afferent C-fibers in normal rats with intact innervation of the joint by sympathetic postganglionic neurons (compare Fig. 7A1 with Fig. 7B1).

2. Neither electrical stimulation of cutaneous nociceptive afferent C-fibers nor intravenous infusion of corticosterone inhibits the residual BK-induced PE in sympathectomized rats (removal of the lumbar paravertebral ganglia 7 days before the experiments) (Fig. 7A2, B2).

3. Corticosterone infused intravenously does not inhibit plasma extravasation generated by intraarticular infusion of platelet activating factor. To emphasize, platelet activating factor acts directly on the endothelial cells, i.e. downstream to the local integration of endocrine and neural signals.

4. Depression of BK-induced PE generated by intravenous infusion of corticosterone is not changed by an intra-articular co-perfusion of the α-adrenoceptor blocker phentolamine (1 μM) or of the β2-

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**Fig. 5.** Bradykinin-induced synovial plasma extravasation (BK-induced PE) is inhibited during transcutaneous electrical stimulation of afferent C-fibers. This inhibition is mediated by the hypothalamo-pituitary-adrenal (HPA) axis. A. Inhibition of BK-induced PE during continuous transcutaneous electrical stimulation of afferent C-fibers in the plantar region of the hindpaw (25 mA pulses of 0.25 duration at 3 Hz) is abolished after surgical removal of the adrenal gland 7 days before the experiments (compare inverted triangles [n = 7] with normal triangles [n = 6]). Closed circles, BK infusion alone with no electrical stimulation of the hindpaw (n = 8). B. Inhibition of BK-induced PE during transcutaneous electrical stimulation is abolished in rats in which the pituitary gland was removed 2 weeks before the experiments (compare inverted triangles [n = 6] with normal triangles [n = 6]). Closed circles, BK infusion alone same as in A. Ordinate scale same as in Fig. 2B,D. Mean ± SEM. Modified from Green et al. (1995).
and its action on effect of corticosterone is independent of release of norepinephrine innervation of the synovium by sympathetic postganglionic neurons released by the adrenal cortex and that the inhibitory effect of corticosterone was infused intravenously (5 μg/min i.v.) for the remainder of the experiment. Open black circles (n = 8 in A1, n = 15 in A2): BK-induced PE in control groups of rats without C-fiber stimulation. Closed red circles (n = 6 in A1, n = 14 in A2): BK-induced PE in rats with electrical C-fiber stimulation, B1, B2. Effect of intravenous infusion of corticosterone on BK-induced PE extravasation in normal knee joints (B1) and in knee joints in rats in which the hindlimb was sympathectomized 7 days before the experiments. Control groups of rat (no corticosterone infusion; open black circles; n = 8 in B1; n = 15 in B2). In rats with corticosterone infusion (closed red circles; n = 12 in B1, n = 9 in B2), bradykinin was added after the first three baseline samples, and then 40 min after beginning knee perfusion, corticosterone was infused intravenously (5 μg/min i.v.) for the remainder of the experiment. Ordinate scale same as in Fig. 2B,D. Mean ± SEM. Modified from Green et al. (1997).

3. Summary

3.1. Control of bradykinin-induced plasma extravasation via the sympathetic-adrenal (SA) system

Stimulation of cutaneous nociceptors in the hindpaw by the injection of CAP (3–30 μg) into the plantar skin (of the hindlimb contralateral
Stimulation of cutaneous or peritoneal nociceptors by intradermal or intraperitoneal capsaicin leads to depression of BK-induced synovial PE. This depression is mediated by the sympathetic-adrenal system (adrenal medullae). The signal to the synovium is most likely epi-
ephrine. The mechanism in the synovium by which epinephrine generates a depression of synovial plasma extravasation is unknown, but involves the β2-adrenoceptor (Miao et al., 1999). We need direct evidence for the involvement of epinephrine and for the location of the β2-adrenoceptors. It is unlikely that the depression is generated by decrease in blood flow in the knee joint. Finally synovial plasma extravasation produced by platelet activating factor (0.1 μM) infused into the knee joint cavity is not decreased by activation of the nociceptive–neuroendocrine reflex circuit (Green et al., 1999).

2. Stimulation of deep nociceptive afferents is particularly powerful in the depression of BK-induced PE by activation of the SA-system: 100 ng/kg capsaicin in sham-vagotomized rats and 1 ng/kg capsaicin in vagotomized rats produces already a depression of BK-induced PE (Fig. 8B). The nociceptive afferents stimulated are visceral nociceptive ones (e.g., those innervating the urinary bladder (Bahns et al., 1986) or the gastrointestinal tract (Blumberg et al., 1983)) and nociceptive afferents associated with the parietal peritoneum. The latter afferents belong to the group of deep somatic afferents. The density of nocicep-
tive afferents innervating deep somatic or visceral tissues is much lower than the density of nociceptive afferents innervating the skin (Jänig and Morrison, 1986; Bielefeldt and Gebhart, 2013).

3. The nociceptive–neuroendocrine reflex pathway is normally inhibited by activity in vagal afferents. The plasma concentration of epinephrine increases 2- to 3-fold after subdiaphragmatic vagotomy (e.g., on day 3 after vagotomy from 290 pg/ml to 720 pg/ml) (Khasar et al., 2003)). The vagal afferents involved project through the celiac branches of the abdominal vagus nerves which innervate the small intestine, distal duodenum and proximal colon (Prechtl and Powley, 1985, 1990). Experiments have shown that transection of the celiac branches has the same dis-inhibiting effect on the nociceptive–neuroendocrine reflex activated by intraperitoneal injection of capsaicin as subdiaphragmatic vagotomy. Transection of the gastric and/or hepatic branches has no effect (Miao et al., 1997b). These results are supported by experiments in which part of the upper gastrointestinal tract is removed or stimulated by distension: Acute gastrectomy has no effect on the nociceptive–neuroendocrine reflex, activated by stimulation of cutaneous nociceptors, in controlling the synovial plasma extravasation, whereas resection of the duodenum has the same effect as sectioning the celiac branches of the vagus nerve. Finally, fasting of rats produced the same effect as section of the celiac branches (Miao et al., 2004).

The following conclusions are drawn from these experiments:

1. BK alone
2. CAP & sham vagotomy
3. CAP & acute vagotomy
4. CAP & acute adrenal denervation
5. CAP & acute adrenal denervation & adrenal denervation

stimulates the parietal peritoneum and viscera by intraperitoneal injection of capsaicin (3–30 μg) is enhanced after acute vagotomy (compare closed circles [n = 16] and open circles [n = 16]) and attenuated after acute denervation of the adrenal medulla (compare closed and open circles with open [n = 8] and closed triangles [n = 16], respectively). Crosses, BK infusion alone with no intradermal injection of capsaicin (n = 16). B. Inhibition of BK-induced PE during graded stimulation of peritoneal nociceptors by intraperitoneal injection of capsaicin (10−6–10−3 mg/kg) is en-

hanced after subdiaphragmatic vagotomy (compare closed circles [n = 8] with open circuits [n = 8]) and attenuated after denervation of the adrenal medulla (compare closed triangles [n = 8] with closed circles and open triangles [n = 8] with open circles). Crosses, BK infusion alone with no injection of capsaicin (n = 8). Ordinate scale is normalized with respect to maximal plasma extravasation before start of intracutaneous or intraperitoneal injection of capsaicin. Mean + SEM. Modified from Miao et al. (2000).

After acute subdiaphragmatic vagotomy, depression of BK-induced PE generated by intraplantar or intraperitoneal injection of capsaicin is strongly enhanced. This inhibition is now already significant at 3 μg capsaicin injected intradermally or at 10−6 mg/kg capsaicin injected intraperitoneally (compare closed circles with open circles in Fig. 8A, B). In vagotomized animals with denervated adrenal medullae, stimulation of nociceptors by capsaicin injected in the hindpaw does not signifi-
cantly depress BK-induced PE (Fig. 8A) and depression of BK-induced PE during stimulation of nociceptors by capsaicin injected intraperite-

rionally is significantly attenuated (Fig. 8B). The depression of BK-

induced PE generated during stimulation of visceral nociceptors by intraperitoneal injection of capsaicin may also additionally be mediated by the activation of the HPA system. This would explain that denervation of the adrenal medulla is not followed by a complete attenuation of BK-induced PE.

The vagal afferents involved in the inhibitory control of BK-induced PE project through the celiac branches of the abdominal vagus nerves which innervate the small intestine, distal duodenum and proximal colon (Prechtl and Powley, 1985, 1990). Experiments have shown that transection of the celiac branches has the same dis-inhibiting effect on the nociceptive–neuroendocrine reflex activated by intraperitoneal injection of capsaicin as subdiaphragmatic vagotomy. Transection of the gastric and/or hepatic branches has no effect (Miao et al., 1997b). These results are supported by experiments in which part of the upper gastrointestinal tract is removed or stimulated by distension: Acute gastrectomy has no effect on the nociceptive–neuroendocrine reflex, activated by stimulation of cutaneous nociceptors, in controlling the synovial plasma extravasation, whereas resection of the duodenum has the same effect as sectioning the celiac branches of the vagus nerve. Finally, fasting of rats produced the same effect as section of the celiac branches (Miao et al., 2004).

The following conclusions are drawn from these experiments:

1. Stimulation of cutaneous or peritoneal nociceptors by intradermal or intraperitoneal capsaicin leads to depression of BK-induced synovial PE. This depression is mediated by the sympato-adrenal system (adrenal medullae). The signal to the synovium is most likely epithe-

lirine. The mechanism in the synovium by which epinephrine generates a depression of synovial plasma extravasation is unknown, but involves the β2-adrenoceptor (Miao et al., 1999). We need direct evidence for the involvement of epinephrine and for the location of the β2-adrenoceptors. It is unlikely that the depression is generated by decrease in blood flow in the knee joint. Finally synovial plasma extravasation produced by platelet activating factor (0.1 μM) infused into the knee joint cavity is not decreased by activation of the nociceptive–neuroendocrine reflex circuit (Green et al., 1997)!

2. Stimulation of deep nociceptive afferents is particularly powerful in the depression of BK-induced PE by activation of the SA-system: 100 ng/kg capsaicin in sham-vagotomized rats and 1 ng/kg capsaicin in vagotomized rats produces already a depression of BK-induced PE (Fig. 8B). The nociceptive afferents stimulated are visceral nociceptive ones (e.g., those innervating the urinary bladder (Bahns et al., 1986) or the gastrointestinal tract (Blumberg et al., 1983)) and nociceptive afferents associated with the parietal peritoneum. The latter afferents belong to the group of deep somatic afferents. The density of nocicept-
tive afferents innervating deep somatic or visceral tissues is much lower than the density of nociceptive afferents innervating the skin (Jänig and Morrison, 1986; Bielefeldt and Gebhart, 2013).

3. The nociceptive–neuroendocrine reflex pathway is normally inhibited by activity in vagal afferents. The plasma concentration of epinephrine increases 2- to 3-fold after subdiaphragmatic vagotomy (e.g., on day 3 after vagotomy from 290 pg/ml to 720 pg/ml) (Khasar et al., 2003)). The vagal afferents involved project through the celiac branches of the abdominal vagus nerves which innervate the small intestine, distal duodenum and proximal colon. The physiological nature of these vagal afferent neurons is unknown although the experiments conducted by Miao et al. (2004) suggest that they are mechanosensitive being activated by distension of the duodenum and small intestine. These afferents must have ongoing activity that maintains centrally an ongoing inhibition of the nociceptive–neuroendocrine reflex
circuits (see below) and leads to their disinhibition when the vagal afferents are interrupted.

4. The receptor terminals of these vagal afferents may be located at the defense line to the environment, in the duodenum and small intestine, closely associated with the gut-associated lymphoid tissue. Thus these vagal afferent neurons may belong to a neural defense system which protects tissues of the body against damage and connects the defense system of the gastrointestinal tract with the defense systems of the somatic tissues which include neural circuits in the spinal cord, brain stem and hypothalamus (see below and Fig. 9), efferent neuroendocrine systems and sympathetic (postganglionic) neurons.

5. The powerful inhibitory effect of activity in vagal afferent neurons projecting through the celiac branches of the abdominal vagus nerve on the reflexly generated inhibition of BK-induced PE is also shown in the following experiments: Intrathecal application of nicotine at the lower lumbar segments, which is believed to activate spinal ascending neurons belonging to the nociceptive system, leads in sham vagotomized rats dose-dependently to depression of BK-induced PE. The effective dose 50 (ED50) of nicotine injected intrathecally is in the range of 2 × 10−6 mg/kg. The depression of BK-induced is markedly attenuated after hypophysectomy or after blockade of the glucocorticoid receptors. After acute subdiaphragmatic vagotomy or after partial vagotomy (interruption of the celiac branches of the abdominal vagus nerve) the depression of BK-induced PE by intrathecal nicotine is strongly potentiated by 3 to 4 orders of magnitude. The ED50 of nicotine is now in the range of 10−8 mg/kg (Miao et al., 1994, 1996c, 1997b). This suggests that vagal afferents projecting through the coeliac branches powerfully regulate HPA-mediated suppression of BK-induced synovial PE. We suggest that the gastrointestinal immune system is integrated, via these vagal afferent neurons, with a systemic inflammatory regulatory system.

3.2.1. Co-activation of the HPA and SA system in the control of BK-induced PE

We have demonstrated that an acute inflammatory (protective) response of the knee joint is controlled by both the HPA system and the SA system. Synchronous activation of afferent C-fibers by continuous transcutaneous electrical stimulation, which also activates large and small diameter afferent A-fibers, activates preferentially the HPA system leading to suppression of BK-induced PE. Stimulation of cutaneous nociceptive C-fibers and some small-diameter cutaneous A-fibers or spinal visceral afferents by capsaicin activates preferentially the SA system in the suppression of BK-induced PE. While stimulation of nociceptors by capsaicin is specific and selective for peptidergic nociceptive afferents and is similar to the physiological activation of nociceptors, transcutaneous electrical activation of afferents at C-fiber strength is not. This type of electrical stimulation activates all functional types of C-fibers (such as various types of nociceptive, non-nociceptive cold-sensitive, non-nociceptive mechanosensitive ones) and A-fibers. We do not know and have not further investigated why transcutaneous electrical stimulation of cutaneous afferents and stimulation of cutaneous afferents by capsaicin preferentially activated the HPA system or the sympathetic-adrenal system.

Under which physiological or pathophysiological conditions of tissue injury (accompanied by acute or chronic inflammatory pain) is the HPA system or the SA system activated? Or are both systems always activated together in normal in vivo conditions during stimulation of nociceptors? If so what is the nature of the co-operative activity of the two neuroendocrine systems in the control of acute and chronic inflammation?

3.2.2. Summary

Activation of the SA system decreases BK-induced PE mediated by the release of epinephrine and by β2-adrenoceptors. This decrease of plasma extravasation is not generated by decrease of blood flow through the synovium. The cellular mechanisms by which epinephrine leads to decrease of PE are unexplored but may involve cells of the immune system (Bjerknes et al., 1991; Strausbaugh et al., 1999). Nociceptive systems (cutaneous, visceral) and the sympathetic-adrenal system form nociceptive-neuroendocrine reflex circuits in the neural control of BK-induced PE. Stimulation of cutaneous or visceral nociceptors by capsaicin inhibits BK-induced PE by activation of the SA system.

Vagal afferent neurons projecting through the celiac branches of the abdominal vagus nerves and innervating the distal duodenum, small intestine and proximal colon have powerful inhibitory effects on the
nociceptive–neuroendocrine reflex pathway to the SA system. Transection of the vagal celiac nerve branches enhances the inhibition of BK-induced PE generated by noxious stimulation (by central disinhibition). The inhibition exerted by activity in the vagal afferent neurons occurs in the spinal cord (for the nociceptive–neuroendocrine reflex pathway to the SA system, Fig. 9) and probably elsewhere in the lower brain stem. The way that the HPA system and the SA system interact and are centrally integrated in regulating the BK-induced PE of the synovium remains unexplored. Finally, we do not know why quasi-physiological stimulation of nociceptors by capsaicin activates preferentially the SA system and transcutaneous electrical stimulation of cutaneous afferents the HPA system. Exploring this puzzle will also contribute to our understanding of the integration of the HPA-system and the SA-system in the regulation of BK-induced PE.

3.3. Organization of the nociceptive neuroendocrine reflex circuitry related to the sympatho-adrenal system in the spinal cord and lower brain stem

We used the model of the control of BK-induced PE by the SA system to study the neural circuits in the spinal cord and lower brain stem that mediate the effects of stimulation of cutaneous nociceptors by capsaicin in BK-induced synovial PE. For this purpose we stimulated the cutaneous nociceptors of the left hindpaw (and sometimes of the left forepaw) by capsaicin and recorded the BK-induced PE in the right knee (Fig. 9). We used several groups of rat in which spinal lesions were performed to interrupt ascending and/or descending spinal tracts 8 days before the experiments (inset in Fig. 9): unilateral transection of the dorsolateral funiculus (DLF) ipsi- or contralateral to the nociceptive input from the hindpaw at segmental levels T8/ T9 or C7/C8; transection of the DLF ipsilateral to the nociceptive input from the hindpaw at segmental level L2/L3; unilateral lesion of the DLF either ipsi- or contralateral to the nociceptive input from the forepaw at the segmental level T8/T9; hemisection of the spinal cord at the segmental level C5/C6 either ipsi- or contralateral to the nociceptive input from the hindpaw; bilateral lesion of the dorsal funiculi at the segmental level T4/T5; complete spinal transection at the segmental level T12/L1 or T3/T2 (Miao et al., 2000, 2001).

1. The central pathways activated by nociceptive afferent input and leading to reflex activation of the preganglionic neurons innervating the adrenal medulla have been postulated by us to consist of an excitatory spino-bulbo-spinal pathway and a spinal pathway (see circuits in violet in Fig. 9). The excitatory spino-bulbo-spinal pathway is a positive feedback circuit that enhances the synaptic transmission of activity from the nociceptive afferent neurons to neurons located in lamina I of the spinal dorsal horn that activates either directly or via interneurons the preganglionic neurons innervating the adrenal medulla. Such pathways have been shown to exist in experiments in which activity in the adrenal nerve (which contains the preganglionic axons innervating the chromaffin cells of the adrenal medulla) or release of catecholamines from the adrenal medulla have been measured. Stimulation of cutaneous nociceptive afferents activates the preganglionic neurons to the adrenal medulla and leads to release of catecholamines from the adrenal medulla in rats with intact spinal cord and in spinalized rats (Araki et al., 1984; Ito et al., 1984; Kurosawa et al., 1985; for review see Sato, 1987; Sato et al., 1997).

2. For the nociceptive–neuroendocrine reflex connected to the hindpaw, we postulate a spinal pathway from the lumbar segments L4 and L5 (which receives the afferent input from the plantar skin of the hindpaw) to the preganglionic neurons in the segments T4 to T12 which innervate the adrenal medulla (Strack et al., 1988). Spinalization at the segmental level T12/L1 (which separates the afferent inflow from hindpaw and the efferent outflow to the adrenal medulla) entirely abolishes the depression of BK-induced PE which is normally generated by noxious hindpaw stimulation. In animals spinalized at the segmental level T1/T2 some depression is present which is similar in degree as in normal animals with intact vagus nerves (Miao et al., 2000). Activation of the adrenal medulla via this spinal reflex is normally weak.

3. After transection of the dorsolateral funiculus at the spinal cord contralateral to the afferent nociceptive input at the segmental spinal levels cervical C5/C6 or thoracic T8/T9, the depression of BK-induced PE is almost totally abolished in rats with intact vagus nerves as well as in vagotomized rats. This shows that the reflex activation of the preganglionic neurons requires an excitatory spino-bulbo-spinal positive feedback circuit and that the ascending limb of the postulated spino-bulbo-spinal reflex pathway projects through dorsolateral funiculus of the spinal cord contralateral to the afferent nociceptive input from the hindlimb (1 in Fig. 9).

4. The location of the descending excitatory limb of the spino-bulbo-spinal positive feedback circuits (2 in Fig. 9) is unknown. However, it does project through the dorsal quadrants of the spinal cord. It does either project through both DLFs or also through the contralateral DLF alone, but not through the ipsilateral DLF alone.

5. After transection of the dorsolateral funiculus ipsilateral to the afferent nociceptive input at the segmental spinal level cervical C5/C6 or thoracic T8/T9 or lumbar L2/L3 in vagus-intact rats, the depression of BK-induced PE is quantitatively the same as in vagotomized rats. The transection of the ipsilateral dorsolateral funiculus at the spinal segmental lumbar level L2/L3 was most powerful in its effect since descending axons of sympathetic premotor neurons in the brain stem (e.g., located in the rostroventrolateral medulla or in the raphe nuclei of the medulla oblongata) activating preganglionic neurons innervating the adrenal medulla (Fig. 9) have not been interrupted. This shows that the inhibition maintained by the activity in vagal afferents is mediated by a descending pathway ipsilateral to the afferent nociceptive input from the skin and occurs at the spinal level, possibly in the dorsal horn at the segmental level of the activated nociceptive afferent input (3 in Fig. 9).

6. Preliminary experiments show that intrathecal application of the α1-adrenoceptor antagonist phenolamine and/or the opioid-receptor antagonist naloxone at the lumbar spinal level L4–L6 have the same effect as vagotomy or transection of the DLF ipsilateral to the nociceptive afferent input (Miao, Jasmin, Jänig & Levine, unpublished observations). However, these pharmacological interventions do not prove which descending system is involved in generating the vagus-maintained inhibition.

7. Various other types of spinal lesions were performed (hemisection of the spinal cord, section of the dorsolateral funiculi at various segmental levels, section of the dorsal funiculi) to support the key conclusions as graphically illustrated in Fig. 9.

8. The central nociceptive–neuroendocrine reflex pathways controlling inflammation and their inhibitory modulation by activity in vagal afferents are under the control of the periaqueductal gray (PAG). However, PAG–induced inhibition and afferent vagus–induced inhibition are relayed through central descending pathways which are not identical but overlap (Miao et al., 2003).

9. The multiple reflex circuits in the spinal cord and lower brain stem connecting the nociceptive afferent inputs and the vagal afferent inputs with the HPA system and the SA system and constituting the nociceptive–neuroendocrine reflexes, are under control of the hypothalamus and telencephalon (supramedullary control in Fig. 9). We hypothesize that these basic reflex machineries connected to the neuroendocrine systems are “used” by higher brain centers to control inflammation of peripheral tissues. Thus the nociceptive neuroendocrine reflexes are integrated in the control of inflammation by the forebrain. The forebrain centers involved are probably the same as those controlling nociception and pain (Treede & Apkarian, 2009; Apkarian et al., 2013).
The hypothesis explaining the results of the spinal lesion experiments (as outlined in Fig. 9), the evidence supporting the existence of the spino-bulbo-spinal reflex positive feedback loop, the evidence supporting the descending inhibitory pathway linked to vagal afferents and the evidence supporting the lateralization of the ascending excitatory and the descending inhibitory pathways have been extensively discussed by Miao et al. (2001; see literature here). The functional characteristics of the vagal afferents which are involved are unknown although they may be polymodal, i.e. activated by mechanical and chemical stimuli, or several functional types of vagal afferent neurons may be involved (Miao et al., 2004). Details about the neural circuits connected to the preganglionic neurons that innervate cells in the adrenal medulla releasing epinephrine are unknown. They involve sympathetic pre-motor neurons in the rostral ventrolateral medulla and in the raphe nuclei of the lower brain stem which are not under control of the arterial baroreceptors and different from those connected to sympathetic vasoconstrictor pathways or other sympathetic pathways (Bacon and Smith, 1988; Strack et al., 1989; Wesselingh et al., 1989; Morrison and Cao, 2000; Verberne and Sartor, 2010). They are specifically activated by hypoglycemia (neuroglucoprivation) leading to mobilization of glucose (Morrison and Cao, 2000; Verberne and Sartor, 2010). Our results suggest a special form of integration between the central nociceptive systems and the central circuits regulating the sympat-ho-adrenal system releasing epinephrine in the control of BK-induced inflammation.

3.4. Summary

We used the inhibition of the experimental inflammatory model of BK-induced PE in the knee joint synovia produced by noxious stimulation (called here nociceptive neuroendocrine reflex) to study spinal and supraspinal circuits mediating this reflex. (1) An excitatory spinal and an excitatory spino–bulbo–spinal feedback reflex pathway are involved. (2) The ascending limb of this reflex loop projects through the dorsolateral spinal funiculus contralateral to the spinal nociceptive input and the descending limb through the dorsal spinal quadrants. (3) The nociceptive–neuroendocrine reflex pathway is inhibited by activity in vagal afferent neurons. This inhibition occurs in the spinal cord, most likely in the spinal segments close to the nociceptive afferent input. (4) The descending inhibitory pathway, activated by activity in vagal afferents, projects through dorsolateral spinal funiculus ipsilateral to the nociceptive spinal input. (5) Concluding, the excitatory spino-bulbo-spinal pathway activated by the nociceptive input, establishes an endogenous positive feedback loop between nociceptive afferent input and sympathetic preganglionic output to the adrenal medulla, which is under inhibitory control from the viscera via activity in vagal afferent neurons.

4. Synopsis

While acute inflammation has a protective function in combating infection and facilitating tissue repair, unregulated inflammation can lead to tissue destruction and chronic inflammatory diseases, such as arthritis, inflammatory bowel disease, inflammatory airway disease, inflammatory skin disease. Here we used the acute experimental model of bradykinin-induced plasma extravasation (BK-induced PE) of the knee joint synovium in rat to demonstrate which neural and neuroendocrine systems are involved in the synovial plasma extravasation, the emphasis being on the sympathetic nervous system. BK-induced PE is a component of an acute inflammation that occurs under physiological conditions and has tissue-protective function. We believe that it is necessary to understand the neural regulation of this tissue protective reaction under physiological condition in order to have access to the mechanisms underlying the neural and neuroendocrine control in chronic inflammatory diseases. BK-induced PE in the rat knee joint depends on the sympathetic innervation but not on activity in sympathetic neurons and not on release of norepinephrine. It is regulated by the CNS via the HPA and the SA systems. Activation of both systems inhibits BK-induced PE via corticosterone or epinephrine. Nociceptive systems from skin, visceras and probably deep somatic tissues form nociceptive neuroendocrine reflex circuits with both neuroendocrine systems. The nociceptive–neuroendocrine reflex circuit to the sympatho-adrenal system is spinal and spino-bulbo-spinal.

The nociceptive–neuroendocrine reflex circuits controlling BK-induced PE are under powerful inhibitory control of vagal afferents projecting though the celiac branches of the abdominal vagus nerves. The vagal afferent neurons involved in the inhibitory control of the nociceptive–neuroendocrine reflex circuits probably are mecha-no- and chemosensitive and monitor toxic and/or dangerous events related to invading microorganisms at the defense line of the gastrointestinal tract. They probably are associated with the gut-associated lymphoid tissue (GALT) and connect this body defense line with the neural centers that activate and coordinate the defensive mechanisms in the body, including particularly the immune tissue (see Cervi et al and Sharkey and Savidge this issue of Autonomic Neuroscience).

The neural reflex circuits in the spinal cord and brain stem mediating the activation of the neuroendocrine final pathways and their inhibitory control by the vagal afferent neurons innervating the gastrointestinal tract form the neural machinery by way of which the forebrain controls and orchestrates the protection of somatic and visceral tissues following injury. The component parts of this control system, part of which have been described in this topical review, have to be seen in this functional context. Based on this concept we hypothesize that any behavioral and positive or negative psychological events (positive or negative “psychological stress”) can influence via this orchestrated control system the healing of tissues after injury, either enhancing or delaying it and resulting in chronic inflammatory states. Interestingly, the nociceptive system, activation of which leads to pain, appears to be under a similar neural control involving the vagal afferent neurons and the SA-system. Activation of the SA-system after vagotomy generates a long-lasting sensitization of cutaneous nociceptors for mechanical stimulation (Khasar et al., 1998a, 1998b; Jänig et al., 2000). This sensitization is mediated by epinephrine and β 2-adrenerceptors (Khasar et al., 2003).

Our reasoning is based on the results obtained in one experimental model of acute inflammation and applies to the physiological situation. Whether this can be applied to other inflammatory models and whether we can translate the mechanisms of neural control of inflammation to humans has to be investigated although it appears unlikely that there exist fundamental differences between the rat and the human in this field. Finally our experiments are the beginning of the research on the integrative neural control of inflammation involving the sympathetic nervous system, neuroendocrine systems, nociceptive systems and central integrative systems. They should help paving the way to understand the mechanisms underlying the neural dysregulation of chronic inflammation.

Acknowledgments

We are grateful to Elspeth McLachlan for her comments and for critically reviewing this manuscript. WJ was University of California Regent’s Professor.

References


