Opioid involvement in experimental peritonitis: minireview of comparative studies

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Abstract

Experimental peritonitis is a convenient model for investigations of the opioid action in the focus of inflammation, as both exudatory fluid and cells are easy for quantitative retrieval from the inflamed peritoneal cavity. During the course of inflammation the changes of Met-enkephalin, beta-endorphin, and dynorphin may be followed in the fluid while mRNAs for the precursor molecules of opioid peptides as well as opioid receptors may be detected in the inflammatory leukocytes. Endogenous opioid peptides deriving from the leukocytes recruited to the inflamed peritoneal cavity may participate in the control of visceral pain by the binding to the opioid receptors on the local endings of sensory neurons thus the pain symptoms are restricted to the early stages of peritonitis. Binding of opioid receptors by exogenous opioid, morphine, co-injected with the inflammation-inducing agent (zymosan), abolishes pain symptoms already at the low dose of morphine, while the high dose of morphine additionally inhibits intraperitoneal influx of leukocytes in the several strains of mice (except of CBA) and in the two fish species (salmon and goldfish), but not in the investigated frogs and toads; the putative reasons of the exceptions are being elucidated.

Key words: *opioid peptides, opioid receptors, morphine, inflammation, peritonitis, leukocyte, mice, fish, amphibians*

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An involvement of endogenous opioids in the inhibition of inflammatory pain is investigated in detail in the model of paw inflammation in rats by the team of Christoph Stein. In the elegant series of experiments they proved that after inoculation of Freund's complete adjuvant, opioid-containing leukocytes are recruited to the inflamed paw tissue, and, concurrently, opioid receptors are upregulated on the local peripheral endings of sensory neurons. Inflammatory cells locally liberate opioid peptides that bind to opioid receptors inducing peripheral analgesia [1-3].

Opioids in zymosan-induced peritonitis in mice

We wish to draw attention to zymosan-induced experimental peritonitis as a convenient model for investigations of participation of leukocyte-derived opioid peptides in the pain control. The convenience of this model relies on the possibility of a precise quantification of inflammation-related cells and soluble factors in the samples of exudatory fluid quantitatively retrieved from the control and inflamed peritoneal cavity. An early increase of vascular permeability during peritonitis is connected with a massive release of mast cell-derived histamine and mainly by macrophage-derived leukotrienes [4]. The intraperitoneal influx of blood proteins, including albumin quantitatively bound to the tail vein-injected Evans blue, with a peak by 30 minutes after zymosan injection [4-5], is accompanied and/or followed by the waves of proinflammatory cytokines (TNF-α, IL-1, IL-6) and inflammatory leukocytes. The polimorphonuclear leukocytes (PMNs) with a peak at 6 hours of peritonitis are followed by mononuclear cells, which dominate one week after zymosan injection [5].

The early stages of peritonitis in mice are accompanied by behavioural changes including characteristic body writhes considered to be the visceral pain symptoms. Their

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frequency is strain-dependent but in general they are restricted mainly to the first half-an-hour after zymosan injection [6-7], despite the inflammatory process induced by the dose of zymosan applied routinely here (2 mg/ml, 0.5 ml/g body weight) lasts for at least two weeks. By analogy to the model of adjuvant-induced paw inflammation we may assume that the visceral analgesia during zymosan-induced peritonitis may be induced both by the central mechanisms and by endogenous opioids activating opioid receptors on the local sensory nerve endings. In fact, the opioid peptides (Met-enkephalin, beta-endorphin, and dynorphin) accumulating in peritoneal fluid may derive both from the recruited inflammatory leukocytes and from the distal neurohormonal centres [8-9]. In particular, it has been shown that the amount of Met-enkephalin in peritoneal fluid raises rapidly after zymosan injection, concurrently with its drop in the inflammatory leukocytes, inguinal lymph nodes, and distal neurohormonal centres: striatum and hypothalamus [8-9]. The local changes concern all the components of the endogenous opioid systems, as inflammatory leukocytes recruited to peritoneum contain the opioid peptides and elevated levels of mRNAs for the precursor molecules of proopiomelanocortin (POMC), proenkephalin (PENK) and prodynorphin (PDYN) systems, as well as for the opioid receptors of mu and kappa type. Despite strong efforts, the delta type of opioid receptors were so far undetected in the leukocytes retrieved from the Swiss mice peritoneal cavity [10-11]. Leukocyte-derived opioid analgesic peptides may participate in a local anti-nociception while opioid receptors on the leukocytes may be involved in the regulation of leukocyte recruitment to the focus of inflammation. The latter statement is based on the evidences that under *in vitro* conditions the specific binding of leukocyte opioid receptors causes heterologous desensitisation of their receptors for some chemotacting factors [12-13]. Under *in vivo* condition such a desensitisation may inhibit intraperitoneal influx of leukocytes and participate in physiological mechanisms of resolution of inflammation.

Effects of morphine on zymosan-induced peritonitis and endogenous opioid system

In the light of a crucial involvement of systems of endogenous opioids and their receptors in the control of inflammatory pain it seems obvious that exogenous morphine should affect their mutual interplay. In fact, intraperitoneal injection of zymosan supplemented with morphine completely abolishes a visceral pain already at the low doses of this well-known analgesic agent. Moreover, the supplementation of zymosan with the high dose of morphine, besides its analgesic effect, additionally inhibits intraperitoneal influx of leukocytes in some investigated animals. Anti-inflammatory effects of morphine are present in the four out of five investigated strains of mice (i.e. in Swiss, C57C3H, Balb/c, and C57BL strains, but not in CBA), in fish (Atlantic salmon and goldfish), but not in the three investigated species of anuran amphibians (edible frogs, common toads, and fire-bellied toads) [6-7, 14-17].

In animals susceptible to anti-inflammatory effects of morphine, the limited influx of leukocytes corresponds with the decreased amount of chemotactic factors in blood plasma and peritoneal fluid [15-16]. Moreover, during recent studies on the Swiss mice, that are susceptible to anti-inflammatory effects of morphine, we recorded significant differences in the pattern of activation of the endogenous opioid system between the animals co-injected with zymosan plus morphine and their counterparts injected with zymosan only. In general the binding of opioid receptors by morphine exerts an analgesic effect and changes the kinetics of production/release of their natural ligands [9, 11]. On the other hand, *in vitro* incubation of leukocytes with morphine inhibits their subsequent migration towards zymosan-activated serum perhaps due to desensitisation of the leukocyte receptors for some chemotactic factors, perhaps mainly components of activated complement cascade [18]. Under *in vivo* conditions such morphine-induced desensitisation of leukocytes in animals co-injected with zymosan plus morphine may be responsible for the limited influx of leukocytes into the focus of inflammation [6, 15-17, 19].

In attempts to find out the main cell types connected with anti-inflammatory effects of morphine we focus on the involvement of the resident macrophages and mast cells. In the Swiss mice, clondronate-induced macrophage depletion enhances and prolongs intraperitoneal accumulation of polimorphonuclears, perhaps due to depletion of anti-inflammatory IL-10 of macrophage origin, but it happens in both the animals injected with zymosan only or with zymosan supplemented with morphine. It indicates that the macrophage-derived factors are not responsible for morphine-induced inhibition of inflammation [17]. In contrast, depletion of mast cell-derived factors in Balb/c mice by the animal pre-treatment with a potent mast cell degranulator, compound 48/80, causes inhibition of zymosan-induced peritonitis and the lack of further anti-inflammatory effects exerted by the morphine co-administration [19]. It suggests that the mast cell-derived factors, maybe of chemotactic activity, might participate in anti-inflammatory effects of morphine in concert with plasma-derived complement components.

Animals resistant to anti-inflammatory effects of morphine

Despite several efforts it was impossible to inhibit inflammation by morphine co-injection with proinflammatory agent in the edible frogs (*Rana esculenta*), common toads (*Bufo bufo*), and fire-bellied toads (*Bombina*

bombina) [14, 17], what corresponded with a lack of an in vitro morphine-induced inhibition of leukocyte chemotaxis to zymosan-activated serum [18]. We assume that the amphibian resistance to the anti-inflammatory effects of morphine might be connected with the abundance of amphibian-specific endogenous opioids such as dermorphins and deltorphins [20].

Even more puzzling was the lack of anti-inflammatory effects of morphine in the CBA strain of mice. It turned out, however, that the CBA mice possess the highest number of peritoneal mast cells among investigated strains (CBA>Balb/c>C57BL>Swiss) [21]. Moreover, in comparison with the mast cells of Swiss mice, the CBA mast cells are highly prone to degranulation by morphine [22] and resistant to cromolyn, the well-known mast cell stabiliser [23]. Evidently this is a reason that an injection of CBA males with only morphine induces stronger peritoneal inflammation than the negligible one recorded in Swiss mice after morphine treatment [22]. Therefore we concluded that a unique sensitivity of CBA mast cells to morphine-induced degranulation and induction of inflammation might dominate over morphine-induced anti-inflammatory effects [22].

Conclusions and further plans

Local administration of exogenous opioid to the focus of inflammation, e.g. during planned surgeries, may be of therapeutic importance due to its dual effects, both analgesic and anti-inflammatory, the mechanisms of which should be elucidated in detail. The model of experimental peritoneal inflammation seems to offer special advantages for investigations of opioids in peritoneal fluid and the components of the opioid systems in particular populations and subpopulations of inflammatory cells, what is a goal of our further experiments. Moreover, we received preliminary evidences of the systemic effects of the experimental peritonitis, as the increased uveal mast cell number was recorded in the Swiss mice with zymosan-induced peritoneal inflammation [24]. On the other hand, murine peritoneal cavity may be used as a sensitive sensor of the distal inflammatory processes, as the inflammation-related changes were recorded in the peritoneal exudate of the mice injected with zymosan into the hind-paw [24]. Finally, we shall conclude that the involvement of opioid peptides and receptors in the experimental peritonitis is a small part of the network of multidirectional interactions between the immune system with neurohormonal systems of the body, therefore it is worth to study from the both practical and theoretical point of view.

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