**Background and aim:** Morphine co-injection has anti-inflammatory effects on zymosan-induced peritonitis in several strains of mice except that of CBA. As peritoneal mast cells (pMCs) are much more numerous in CBA mice than in SWISS mice, the role of pMCs in morphine-modulated zymosan peritonitis is compared in CBA and SWISS males.

**Methods:** pMCs were treated *in vitro* with morphine or C48u80 for comparison of histamine release. *In vivo* accumulation of leukocytes and histamine in peritoneal exudate were recorded after intraperitoneal injection with morphine, zymosan, or zymosan plus morphine.

**Results and conclusion:** Morphine induces histamine release by pMCs from CBA mice but not SWISS mice. *In vivo* morphine-induced peritonitis is stronger in CBA mice than SWISS mice. Corollary, morphine anti-inflammatory effects on zymosan peritonitis are reversed in CBA mice by its pro-inflammatory action through CBA pMCs.

**Key words:** Peritoneal inflammation, Peritoneal mast cells, Histamine, Morphine, C48u80

**Introduction**

Experimental peritonitis induced in mice by intraperitoneal (i.p.) zymosan injection makes it possible to easily monitor behavioural symptoms of pain and precisely quantify inflammation-related cells and soluble factors in samples of peritoneal exudate. For these reasons such a model of inflammation is convenient for investigations of a modulatory action of various factors on the course of inflammation, including effects of morphine, a well-known antinociceptive drug. We have previously shown that characteristic body writhes, considered to be pain symptoms in zymosan-injected individuals, are completely absent in mice co-injected with morphine. The low dose of morphine (5 mg/kg of body weight) attenuated pain in all the investigated strains of mice, while only the high dose of morphine (20 mg/kg of body weight) additionally inhibited the early stages of i.p. accumulation of exudatory leukocytes in most strains (C57C3H, SWISS, Balb/c, C57BL/6)\(^1,2\) but not CBA.\(^3\) Anti-inflammatory effects of morphine might be advantageous during planned surgeries, but we should know why such effects are not universal even among various strains of the same species.

The main aim of the present study is to find out the reason(s) for the insensitivity of CBA mice to anti-inflammatory effects of the high dose of morphine. During previous experiments on the murine peritonitis we recorded that the strains investigated by our team differed in the number of peritoneal mast cells (pMCs), which were more numerous in the order SWISS < OUTBRED < C57BL < Balb/c < CBA.\(^3\) In the light of the critical role of mast cells in inflammation,\(^4\) those results prompted us to study the effects of mast cell depletion on the subsequent zymosan-induced peritonitis in animals with the extreme low and high numbers of pMCs (i.e. in SWISS and CBA strains, respectively). After a single i.p. injection of mast cell degranulator, compound 48/80, a subsequent zymosan-induced peritonitis was inhibited in SWISS mice (as previously described by Ajuebor *et al.*\(^5\)) but enhanced in CBA mice. In the latter strain, C48/80-induced mast cell degranulation was accompanied by concomitant mast cell influx and/or local proliferation, leading to drastic inter-strain differences in the peritoneal microenvironment at the time of zymosan injection.\(^3\) Therefore we put forward a hypothesis that the inter-strain differentiation in pMC numbers and characteristics might also be responsible for different responses to supplementation of peritonitis-inducing agent with morphine. It turned out that, indeed, in contrast to the pMCs of SWISS mice, the CBA pMCs are not only much more numerous, but also very sensitive to morphine-induced histamine release and the induction of inflammatory response.

**Methods**

Swiss males and CBA males (6–8 weeks old, 25–28 g) were purchased from the Animal Department of...
Collegium Medicum (Kraków, Poland). All mice were housed four per cage in the laboratory room with fixed light–darkness conditions (12:12 h), with water and standard diet ad libitum. The experiments were conducted according to license no. 16/OP/2001 from the Local Ethical Committee.

Swiss mice and CBA mice were either untreated (intact controls) or i.p.-injected with morphine (M group, 20 mg/kg of body weight; morphine chloride; Polfa, Kutno, Poland), with zymosan (ZM group, 2 mg/ml, 0.5 ml/mice; Sigma Chemical, St Louis, MO, USA), or zymosan supplemented with morphine (Z group). Animals were killed by cervical dislocation at time 0 (controls) or after 30 min, 4 h or 8 h after i.p. injection, and their peritoneal exudate was retrieved as described previously. Following centrifugation, exudatory fluid was used for measurement of histamine content by enzyme-linked immunosorbent assay (ICM Pharmaceuticals, Inc., Cost Mesa, CA, USA), while Türk-stained exudatory cells were counted in a hemocytometer and used for differential counts on MGG-stained cytospin preparations.

Peritoneal cells from some intact animals were prepared for an in vitro incubation of either a total pool of PTLs or purified pMCs. In both instances the cells were adjusted to approximately 70,000 pMCs/ml and incubated in tubes with various concentrations of C48/80 or morphine (see later Fig. 2). After 45 min incubation and centrifugation (10 min at 400 × g) the cell degranulation was assessed morphologically according to Levi-Schaffer et al. on safranine-stained cytospin preparations, while the percentage of histamine released to supernatant was assessed and calculated according to Verbsky et al.’s formula:

\[
\text{percent histamine released} = \left( \frac{\text{histamine released by the inducer/total histamine content}}{100} \right) \times 100
\]

The results of the experiments presented here show that the SWISS and CBA mice differ not only in the number of pMCs, but also in the quality of these cells. In contrast to the SWISS pMCs, which are relatively more stable, the pMCs of the CBA strain are susceptible to morphine-induced as well as spontaneous degranulation and histamine release. The mast cells of the CBA animals are perhaps more mobile.
than those of the SWISS mice, as indicated by the fast i.p. mast cell influx after a morphine injection in the former strain. Comparative studies on leukocyte mobility and on the quantity and quality of chemotactic factors operating in these two strains are in progress. So far we may conclude that morphine acts as a strong pro-inflammatory agent in the CBA mice, since it induces pMC degranulation and histamine release, which result in a morphine-induced peritonitis much more pronounced than that evoked in the SWISS mice by the same treatment. Such a CBA-specific pro-inflammatory action of morphine may at least partly explain the lack of its anti-inflammatory action when added to an irritant (zymosan) intraperitoneally injected in animals of this particular strain of mice. The anti-inflammatory effects of morphine recorded so far not only in all the other strains of mice, but also in fish, Atlantic salmon and goldfish, seem to be connected with the inhibition of leukocyte migration into the focus of inflammation caused by the desensitization of their receptors to some chemotactic factors. It seems that

FIG. 1. Comparison of early stages of peritonitis in males of CBA and SWISS mice intraperitoneally injected with zymosan (Z group), zymosan supplemented with morphine (ZM group) or morphine only (M group). Numbers of peritoneal polymorphonuclear leukocytes (PMNs), mast cells (MC) or histamine levels at time 0 (controls) or 30 min, 4 h and 8 h after injection. Data presented as mean ± standard error (n = 4–6). Values with the same letter (e.g. ‘A’ and ‘A’, ‘b’ and ‘b’) do not differ significantly within the group, while values with different letters (e.g. ‘A’ versus ‘B’, ‘a’ versus ‘b’) vary significantly within the group at p < 0.05. Values significantly different between the groups: *p < 0.05, **p < 0.01, ***p < 0.001.

FIG. 2. In vitro effects of various doses of morphine or C48:80 on the percentage of histamine released from peritoneal mast cells of males from CBA and SWISS mice. Data presented as mean ± standard error (n = 4). Values sharing at least one letter (e.g. ‘A’ and ‘AB’, ‘a’ and ‘a’, ‘d’ and ‘dc’) do not differ significantly within the group, while values with different letters (e.g. ‘A’ versus ‘B’, ‘c’ versus ‘d’) only vary significantly within the group at p < 0.05.
in the case of the CBA strain such anti-inflammatory effects are efficiently counterbalanced by morphine pro-inflammatory effects realized mainly through the highly morphine-sensitive mast cells of this strain.

The lack of anti-inflammatory effects of morphine on experimental peritonitis has been recorded also in several species of anuran amphibians (toads and frogs). This may be due to the high levels of their unique endogenous opioids, deltorphins and dermorphins. On the other hand, strain-specific differences in morphine effects on various components of the murine immune system have also been recorded by other investigators. Therefore we may conclude that the final results of morphine administration on the immune system are dependent on the balance of various (often contradictory) genetic, internal, and exogenous factors.

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