

Comparison of the influence of NSAIDs with different COX-selectivity on histamine release from mast cells isolated from naïve and sensitized rats

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Abstract

Mast cell stimulation leads to an early response with histamine release and prostaglandin (PGD₂) production but attempts to link these two events have been contradictory. In IgE-mediated mast cell activation, a late-phase PGD₂-production is caused by increased cyclooxygenase-2 (COX-2) expression whereas a COX-2 involvement in the early response is uncertain. The present study compares the influence of four COX-inhibitors (NSAIDs) on the histamine release of mast cells from naïve and actively sensitized rats. NSAIDs of different COX-1 vs. COX-2 selectivity were used, i.e. acetylsalicylic acid (ASA), piroxicam, meloxicam, and NS-398, a selective COX-2-inhibitor. All could inhibit antigen-induced histamine release, with 64%, 34%, 27% and 85% inhibition by ASA (5 mM), piroxicam (100 μM), meloxicam (100 μM) and NS-398 (100 μM), respectively. Similar inhibition was found with compound 48/80 without calcium added to the medium whereas compound 48/80 with calcium was affected less by ASA and NS-398 and unaffected by the oxicams. Only small differences between the two kinds of mast cells were found, except with NS-398 which was a significantly more effective inhibitor of naïve than sensitized cells when exposed to compound 48/80 with calcium present. The results do not show any consistent relationship between the influence of the NSAIDs and their COX-2-selectivity. The high NSAID-concentrations required for inhibition cast doubt about an involvement of COX-inhibition and indicate additional or other targets. The results seem to exclude toxic effects on mast cell energy production but are consistent with an interference with the calcium disposition.

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1. Introduction

Mast cells are important effector cells in allergic and inflammatory reactions [1–3]. In allergic conditions of the immediate type the ability of mast cells to release histamine is considered of greatest significance but in

addition mast cells are able to produce and release a great variety of substances such as cytokines and eicosanoids which contribute to development and regulation of the inflammatory response [4].

Much effort has been invested in order to clarify the mechanisms involved in the production and release of mast cell mediators. In addition, functional links between the different mediators as well as individual pathways for their production have been investigated with a variety of

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approaches, sometimes with contradictory results, e.g. of the influence of non-steroidal anti-inflammatory drugs (NSAID) on histamine release from rat peritoneal mast cells with findings of a pronounced inhibition [5], a variable inhibition [6,7], and of no inhibition despite a substantial inhibition of prostaglandin D₂ (PGD₂) synthesis [8].

Mast cell activation gives rise to cyclooxygenase (COX) mediated conversion of arachidonic acid to prostanoids, with PGD₂ as the major product irrespective of the stimulus employed [9,10]. Following the immediate response mast cells produce a late-phase generation of PGD₂ [11–14] and under certain conditions also of PGE₂ [15].

The prostanoids thus formed function as mediators in inflammatory responses and may also contribute to regulation of mast cells as indicated by observations with exogenous prostanoids and mouse [16,17] and rat [18] mast cells as well as by demonstration of specific prostanoid receptors on rat mast cells [17,19,20]. In addition, the cyclooxygenase pathway may be functionally linked to other pathways and endogenously derived prostanoids serve modulatory functions in mast cell responses.

The complexity of these circuits is emphasized by findings that mast cells in addition to the constitutive COX-1 enzyme also express the COX-2 isoform. The prostaglandin synthesis taking place in the immediate mast cell response seems to be due to COX-1 activation whereas the late-phase production is the consequence of induction of COX-2 [11,12,14,17]. In these studies the resting level of COX-2 is reported to be below the detection limit. In passively sensitized RBL-2H3 mast cells, however, COX-2 is considerably expressed even in unstimulated cells [21], and it takes part in the initial reactions of an allergic rat rhinitis model [22]. Moreover, an enhanced COX-2 expression can be detected in mast cells of asthmatic airways [23], indicating a possible involvement of COX-2 also in early responses.

It is thus of interest to explore whether active sensitization leads to functional changes of mast cells which can be ascribed to altered COX-2 levels. In the present investigation this is tested by the use of selected NSAIDs with different selectivity towards COX-1 and COX-2, i.e. acetylsalicylic acid (ASA) which has greatest potency against COX-1, NS-398 which is a selective COX-2 inhibitor, and, in addition, the two structurally closely related NSAIDs piroxicam and meloxicam, the former with some selectivity for COX-1, the latter with a modest preference for COX-2. The aim was to establish to which extent they affect the rapid response of histamine

release and to determine from their influence on mast cells from sensitized and naïve rats whether differences can be ascribed to COX-2 expression after sensitization.

2. Materials and methods

2.1. Isolation of mast cells

Mast cells from male Wistar rats (200–500 g) were collected from the abdominal and thoracic cavities and isolated on Ficoll, as previously described [24]. Sensitized mast cells were collected from rats sensitized to horse serum 3–8 weeks before [24], naïve mast cells from rats sacrificed immediately after leaving the barrier. The cells were washed and incubated in Sørensen phosphate buffer (6.7 mM, pH 7.0) containing NaCl (131 mM), KCl (2.4 mM), and bovine serum albumin (0.5 mg/ml). Unless otherwise stated, calcium was included in the phosphate buffer as CaCl₂ (1 mM).

2.2. Incubation of mast cells

All samples were run in duplicate with $3\text{--}5 \times 10^3$ mast cells per sample. The incubations were carried out at 37 °C in 0.5 ml of the incubation medium and were terminated by placing the tubes in an ice-bath followed by the addition of 1.5 ml of the buffer. The composition of the incubation medium and the experimental conditions are described in the legends to the figures. The concentration of antigen (i.e. dialyzed horse serum) used for incubation was 0.75% (v/v).

All values presented have been corrected for spontaneous histamine release. Piroxicam, meloxicam and DFU interfered with the histamine assay and consequently appropriate samples were included for correction. Further details of the experimental procedures and the determination of histamine release are given in [24].

2.3. Stock solutions

Piroxicam, meloxicam, NS-398, and DFU were dissolved as stock solutions in DMSO (20 mM), with protection as far as possible from light. Stock solutions of acetylsalicylic acid (ASA) and salicylic acid were made in DMSO (1 M).

2.4. Materials

All chemicals were of analytical grade, and all solutions were made using redistilled water. Piroxicam, salicylic acid, and bovine serum albumin were obtained from Sigma (St. Louis, Mo., USA), and NS-398 (*N*-(2-cyclohexyloxy-4-nitrophenyl)-methanesulfonamide) from Biomol Res. Lab. (PA, USA). Acetylsalicylic acid was kindly supplied by Nycomed DAK (Roskilde, Denmark), meloxicam by Boehringer Ingelheim (Ingelheim, Germany), and DFU (5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2 (5H)-furanone) by Merck Frosst (Quebec, Canada).

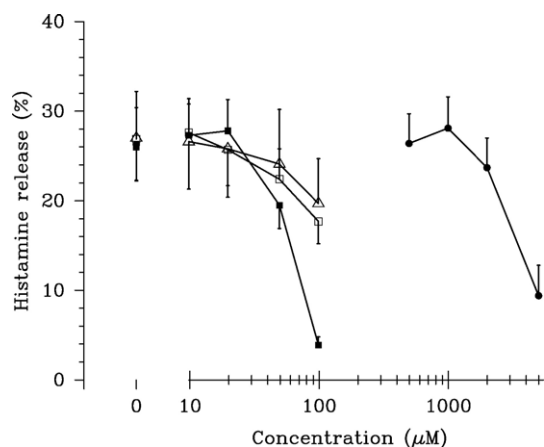


Fig. 1. The influence of ASA (●), piroxicam (□), meloxicam (△), and NS-398 (■) on the histamine release induced by antigen. The mast cells were preincubated with the test drug for 10 min, followed by addition of antigen and incubation for a further 10 min. Mean±S.E.M. of six (solid symbols) and five (open symbols) experiments.

2.5. Statistical analysis

The results were analyzed by use of Student's *t*-test.

3. Results

3.1. Influence of ASA, piroxicam, meloxicam, and NS-398 on antigen-induced histamine release; dependence on preincubation; influence on mast cells

Piroxicam, meloxicam and NS-398 inhibited antigen-induced histamine release at concentrations up to 100 μM, with almost complete inhibition by NS-398 (Fig. 1). At concentrations of piroxicam and meloxicam above 100 μM the

Table 1
Inhibition (%) by NSAID of histamine release induced by antigen

	ASA (5 mM)	Piroxicam (100 μM)	Meloxicam (100 μM)	NS-398 (100 μM)
Low control response ^a	71 (18/3)	40 (20/2)	49 (15/2)	87 (18/3)
High control response ^a	61 (35/3)	32 (31/3)	21 (35/3)	84 (34/3)
Mean (all) ^b	64	34	27	85

Mast cells were preincubated with test drug for 10 min, followed by addition of antigen and incubation for a further 10 min.

The table presents the per cent inhibition by the highest concentration of test drug studied.

^a The data are grouped according to the magnitude of the control response in low and high, respectively, as shown in brackets together with the number of experiments.

^b The mean inhibition of the pooled experiments ($n=5-6$; cf. Fig. 1).

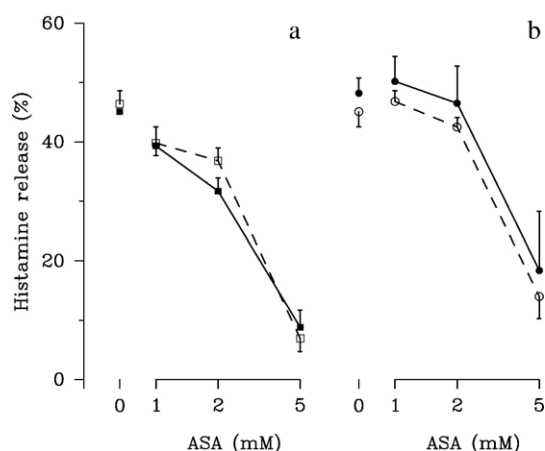


Fig. 2. The influence of ASA on the histamine release induced by compound 48/80 in a medium without added calcium (a) or with calcium (b). Mast cells from naïve (open symbols) or sensitized (filled symbols) rats were preincubated with ASA for 10 min, followed by addition of compound 48/80 (15 ng/ml (□) or 20 ng/ml (●)) and incubation for a further 10 min. Mean±S.E.M. of three (●) to five (□) experiments.

histamine assay was excessively disturbed. ASA was capable of a considerable inhibition but at concentrations in the mM-range (Fig. 1).

In general, the magnitude of mast cell responses to antigen is variable (cf. S.E.M. in Fig. 1). A grouping of the results according to the magnitude of the control histamine release reveals (Table 1) that NS-398 was an equally effective inhibitor of low and high responses whereas ASA and piroxicam tended to be more active against low responses.

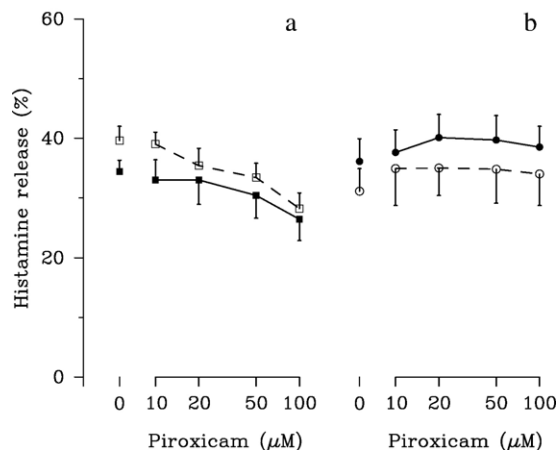


Fig. 3. The influence of piroxicam on the histamine release induced by compound 48/80 in a medium without added calcium (a) or with calcium (b). Mast cells from naïve (open symbols) or sensitized (filled symbols) rats were preincubated with piroxicam for 10 min, followed by addition of compound 48/80 (15 ng/ml (a) or 20 ng/ml (b)) and incubation for a further 10 min. Mean±S.E.M. of three (■) or four experiments.

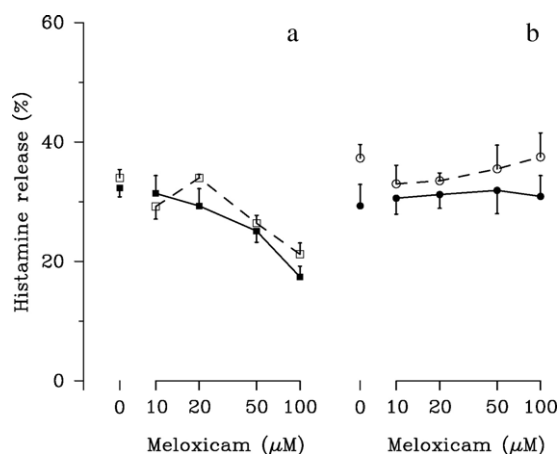


Fig. 4. The influence of meloxicam on the histamine release induced by compound 48/80 in a medium without added calcium (a) or with calcium (b). Mast cells from naïve (open symbols) or sensitized (filled symbols) rats were preincubated with meloxicam for 10 min, followed by addition of compound 48/80 (15 ng/ml (a) or 20 ng/ml (b)) and incubation for a further 10 min. Mean $\pm$ S.E.M. of three (a) or four (b) experiments.

The efficacy of meloxicam was significantly greater against the low responses to antigen ($p < 0.01$).

Piroxicam, meloxicam and NS-398 were also able to inhibit histamine release when the mast cells were exposed simultaneously to antigen and the drug (preliminary results, not shown).

Only ASA affected the mast cells on its own resulting in a 2–5% increase of the spontaneous histamine release.

3.2. Influence of DFU and salicylic acid on histamine release

DFU at concentrations up to 10 μ M did not inhibit histamine release induced by antigen or compound 48/80, irrespective of the presence of calcium (results not shown). Occasionally, a slight enhancement of the response was observed. Concentrations of DFU above 10 μ M caused too pronounced interference with the assay to permit reliable results.

Salicylic acid at concentrations up to 5 mM did not affect the mast cell response to compound 48/80 and was only slightly inhibitory against antigen-induced release (preliminary results, not shown).

3.3. Influence of ASA on compound 48/80-induced histamine release with and without calcium; comparison of naïve and sensitized mast cells; effect on energy production

ASA was an efficient inhibitor of histamine release induced by compound 48/80 (Fig. 2). It was somewhat more potent in the absence of added calcium (Fig. 2a vs. b), with 21–30% inhibition at an ASA-concentration of 2 mM (Fig. 2a) whereas higher concentrations were necessary for inhibition in the presence of calcium (Fig. 2b). There was no difference

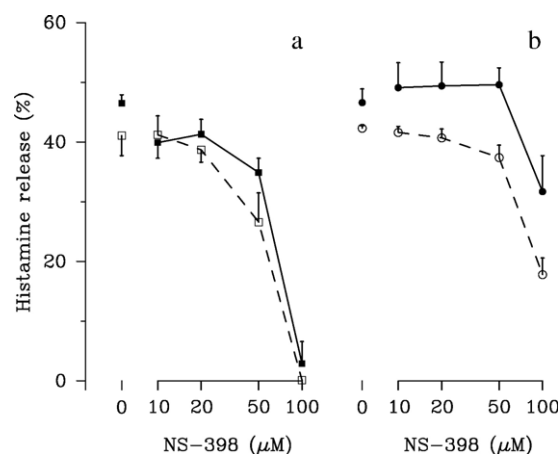


Fig. 5. The influence of NS-398 on the histamine release induced by compound 48/80 in a medium without added calcium (a) or with calcium (b). Mast cells from naïve (open symbols) or sensitized (filled symbols) rats were preincubated with NS-398 for 10 min, followed by addition of compound 48/80 (15 ng/ml (a) or 20 ng/ml (b)) and incubation for a further 10 min. Mean $\pm$ S.E.M. of three ($\square$, $\bullet$) or four ($\blacksquare$, $\circ$) experiments.

between the influence of ASA on mast cells from naïve and sensitized rats (Fig. 2).

The possibility of toxic effects of ASA on oxidative energy production was tested by including glucose (5 mM) in the incubation medium. The inhibition by ASA of compound 48/80-induced histamine release was unaffected by glucose (results not shown).

3.4. Influence of piroxicam and meloxicam on compound 48/80-induced histamine release with and without calcium; comparison of naïve and sensitized mast cells

The influence of piroxicam (Fig. 3) and meloxicam (Fig. 4) on mast cell responses to compound 48/80 were quite similar and did not differ much between naïve and sensitized cells.

Table 2

Summary of the inhibition (%) by NSAID of histamine release induced by compound 48/80

	ASA (5 mM)	Piroxicam (100 μ M)	Meloxicam (100 μ M)	NS-398 (100 μ M)
Naïve–Ca	85	29	38	95
Sens–Ca	80	23	46	94
Naïve+Ca	69	–9 ^a	0	58
Sens+Ca	62	–7 ^a	–5 ^a	32

Mast cells from naïve or sensitized (sens) rats were preincubated with test drug without or with added calcium for 10 min, followed by addition of compound 48/80 (15–20 ng/ml) and incubation for a further 10 min.

The table presents the per cent inhibition by the highest concentration of test drug studied (cf. Figs. 2–5).

^a Negative values indicate enhancement.

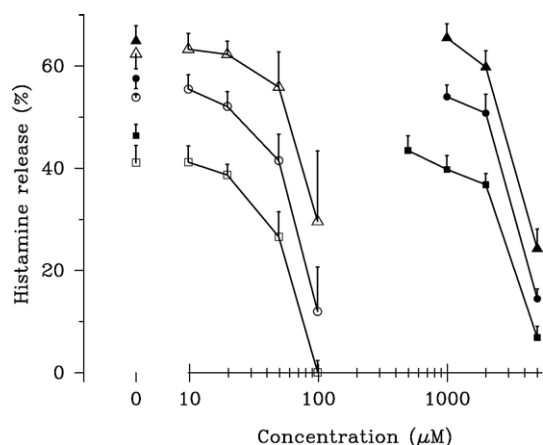


Fig. 6. The influence of ASA and NS-398 on the histamine release induced by different concentrations of compound 48/80 in a medium without added calcium. Mast cells from naïve rats were preincubated with NS-398 (open symbols) or ASA (filled symbols) for 10 min, followed by addition of compound 48/80 (15 ng/ml (□), 20 ng/ml (○) or 30 ng/ml (△)) and incubation for a further 10 min. Mean $\pm$ S.E.M. of three (NS-398) or four to five (ASA) experiments. The data obtained with compound 48/80 (15 ng/ml) are identical with those shown in Figs. 2a and 5a.

Without calcium included in the medium, histamine release was inhibited to a somewhat greater extent by meloxicam than by piroxicam (ca. 40% vs. 25%; Figs. 3a and 4a), with the greatest difference observed with sensitized cells. In the presence of calcium, the histamine release induced by compound 48/80 was unaffected by the two drugs in the concentration range studied (Figs. 3b and 4b).

3.5. Influence of NS-398 on compound 48/80-induced histamine release with and without calcium; comparison of naïve and sensitized mast cells

The response to compound 48/80 without calcium (Fig. 5a) was inhibited by NS-398 (50 μ M) by 35% with naïve and by 25% with sensitized mast cells and the inhibition was virtually complete at 100 μ M. NS-398 was a considerably less potent inhibitor of the response to compound 48/80 in the presence of calcium (Fig. 5b), with a notable difference in the influence on naïve and sensitized mast cells ($p < 0.1$ at 100 μ M).

3.6. Comparison of the ability of the four NSAIDs to inhibit compound 48/80-induced histamine release; dependency of the concentration of compound 48/80

The results presented in Figs. 2–5 are summarized in Table 2, with the highest concentration of the test drugs. The table emphasizes the similarity between the influence of piroxicam and meloxicam on the one hand and of ASA and NS-398 on the other.

The results presented (Figs. 2–5 and Table 2) were obtained with concentrations of compound 48/80 of 15–20 ng/ml giving rise to histamine release of an intermediate magnitude of 30–

45%. The experiments also comprised higher concentrations of compound 48/80 (up to 30 ng/ml) which caused histamine release up to ca. 65%. The NSAIDs were less effective inhibitors under these conditions as exemplified with ASA and NS-398 (Fig. 6) with inhibition dropping from 85 to 63% and from 95 to 53%, respectively.

4. Discussion

The present study searches for a functional role of COX-activity in the regulation of histamine release from rat mast cells. In particular, it explores the possibility of an involvement of the inducible COX-isoform, COX-2, by comparing the influence of a selection of NSAID on the response of mast cells from naïve and actively sensitized rats.

The results reveal only small differences between the two kinds of mast cells, as evaluated with NSAIDs of different selectivity for COX-1 and COX-2. Thus, the histamine release induced by compound 48/80 in the absence of calcium was affected to the same extent with both cell types when exposed to ASA and NS-398, the two test drugs with the greatest difference in COX-1/COX-2 selectivity. Under these conditions piroxicam tended to be less and meloxicam more inhibitory against the response of sensitized as compared with naïve mast cells which is consistent with a possible disposition of COX-2 after sensitization. This seems, however, to be in conflict with the slightly greater efficacy of piroxicam compared with meloxicam in antigen-induced histamine release.

The most striking findings relate to the response to compound 48/80 in the presence of calcium where NS-398 was a considerably more effective inhibitor of naïve than sensitized mast cells, which seems in conflict with a greater role of COX-2 after sensitization. Moreover, both ASA and NS-398 were less effective in the presence of calcium, and both piroxicam and meloxicam left the compound 48/80-response unaffected. This latter finding cannot be due to the presence of calcium in the medium per se since both oxicams are able to inhibit the antigen-induced histamine release which also takes place in a calcium-containing medium.

These apparently conflicting results may be a reflection of the inconsistencies in previous reports of the influence of NSAID on histamine release from rat mast cells [5–8]. Mast cells do produce PGD₂ in connection with histamine release [8,9] but there does not seem to be a firm correlation between the release of these two mediators in terms of neither time course or magnitude of response relative to intensity and nature of stimulus [9,10,25,26] nor susceptibility to COX-

inhibitors [8,27–29]. In the latter context it is relevant to consider various aspects of the pharmacology of NSAID. Both selectivity and specificity are of particular concern when the activity of NSAIDs takes place at concentrations as high as observed in the present as well as in other mast cell studies [5–7,30].

NS-398 was selected as a probe for an involvement of COX-2 because it reportedly is a highly selective COX-2 inhibitor [31]. The COX-selectivity of NSAIDs has, however, a tremendous variation in the values obtained by the use of different assays, e.g. with IC_{50} -ratios of NS-398 for COX-1 vs. COX-2 of 10, >100, and >10,000 [31–33]. The activity of NS-398 exhibits a similar degree of variation with IC_{50} s for COX-2-inhibition ranging from below nM [34] up to μ M [35] concentrations. Moreover, both the potency and the selectivity of NS-398 towards COX-2 are time-dependent [36–38] which may further contribute to the complexity of the issue.

In addition to NS-398, the highly selective COX-2 inhibitor DFU [39] was included in order to substantiate results related to an involvement of COX-2. DFU was without influence on the mast cell responses, despite the use of concentrations more than ten times higher than those effective in other systems [39–41]. Without a positive control for activity of DFU in our system, however, it is not possible to distinguish between lack of mast cell-effects of DFU and lack of COX-2 involvement in the mast cell responses.

NS-398 is a very potent inhibitor of the delayed mast cell prostaglandin generation [11,13,14], with maximal effect at 1 μ M or lower, i.e. at concentrations more than 100 times less than found here. The inhibition of PG-synthesis [11,13,14] took place over hours and it is thus possible that short term preincubation does not allow the drug to access relevant intracellular domains, as also indicated by earlier data of no effect on the PGD_2 synthesis in relation to histamine release [8]. Our preliminary observations with NS-398 of inhibition even without preincubation support this possibility. Therefore, the present finding of inhibition of histamine release is likely to be the consequence of NS-398-interactions with targets other than COX, in accordance with observations of distinct effects at 100- μ M concentrations in other cells [42–44].

Our findings with piroxicam agree quite well with earlier rat mast cell data [6] where antigen-induced histamine release was inhibited ca. 25% by a concentration of 100 μ M whereas the release induced by compound 48/80 in the presence of calcium was unaffected. ASA was also included at concentrations up to 0.5 mM which caused a similar inhibition [6]. On the other hand, piroxicam and ASA (both up to 100 μ M) did not affect anti-IgE-induced histamine release from human synovial mast cells [28]

although the PGD_2 production was effectively inhibited with IC_{50} s of less than 1 μ M and ca. 20 μ M, respectively. Moreover, μ M concentrations of ASA can inhibit PGD_2 -production of murine mast cells [11,13], both the early and the delayed response, even at 5 μ M [13].

Piroxicam and meloxicam were selected for the present investigation because their similar chemical structure combined with a reportedly distinct COX-1/COX-2-selectivity [45] could provide a suitable tool. They exhibit, however, a considerable variation in their COX-1 vs. COX-2 potency depending on the assay used, with IC_{50} ratios of 0.0017 to 100 for piroxicam [46,39] and 14 to 300 for meloxicam [45,39]. In addition, it appears that only small changes in the oxycam structure alter not only the selectivity for COX-isoforms but also the pharmacological effects in an unpredictable manner [47]. The latter may reflect our observations of apparently inconsistent differences between piroxicam and meloxicam and these drugs are thus of modest utility for distinction between COX-1 and COX-2 involvement.

Salicylic acid is an active metabolite of ASA and possesses equal in vivo and in vitro anti-inflammatory potency and efficacy but is an extremely weak COX-inhibitor. ASA and salicylic acid share, however, other targets of relevance for inflammation, e.g. by inhibiting the activation of certain transcription factors equally well [48]. Salicylic acid has been found to inhibit histamine release [30,49], with inhibition even by μ M concentrations [49], in contrast to the present findings of virtually no influence. Hence, our observations with salicylic acid do not conflict with the possibility of ASA-inhibition being caused by COX-inhibition. On the other hand, our inconsistent results with the NSAIDs used taken together with the high concentrations required for activity call for caution and indicate other or additional targets than COX.

Besides being COX-inhibitors, NSAIDs are able to affect oxidative energy production [50]. This is particularly evident for ASA whose high-dose human toxicity is due to inhibition of all levels of mitochondrial function. The inhibition of histamine release found here is not, however, caused by energy deprivation since it is unaffected by the presence of glucose and hence energy provision by glycolysis, in agreement with findings with other NSAIDs [5]. Others found, however, a considerable reduction of the cellular ATP-content along with inhibition of histamine release by a series of NSAID [30]. The reason for this discrepancy is not clear.

NSAIDs have long been recognized to interfere with the biological role of calcium in an apparently antagonistic manner [51]. The ability of NSAIDs of fenamate structure to inhibit calcium influx in human leukocytes [52] and colon carcinoma cells [53] has been implicated

in their anti-inflammatory and antiproliferative activity. Moreover, carboxylic acid NSAIDs inhibit calcium accumulation in rat liver mitochondria and microsomes [54]. These interactions with calcium homeostasis seem to be unrelated to inhibition of COX-activity but may be amplified if COX-inhibition gives rise to accumulation of arachidonic acid which on its own can inhibit calcium movements [55]. Obviously, such disturbances in the calcium disposition will have profound influence on cell responses. There are indications that mechanisms relating to calcium signalling contribute to the effect of NSAIDs on rat mast cells [5,6] since their inhibition of ionophore A23187- [5] and antigen-induced [6] histamine release could be reversed by increased extracellular calcium concentrations. Our findings of considerably less inhibition of the mast cell response to compound 48/80 with calcium present than without are in agreement with these earlier studies. It is conceivable that the cells become much more sensitive to the inhibitory effects of NSAIDs under conditions where the participation of intracellular calcium is crucial.

The present comparison of the influence of a selection of NSAIDs with different selectivity for COX-1 and COX-2 do not permit conclusions about neither an involvement of COX-mediated prostaglandin synthesis nor a functional significance of a sensitization-induced expression of COX-2 in the regulation of mast cell histamine release. The influence of the NSAIDs does, however, reveal some differences between the two kinds of mast cells. The NSAIDs are able to inhibit histamine release, with ASA and NS-398 exhibiting a very high efficacy, but the concentrations required are much higher than generally reported for their COX-inhibition. Among additional or alternative mechanisms for the inhibitory action of the NSAIDs, effects on the energy production do not seem to be involved whereas the results strongly support an interference with calcium disposition. It is tempting to suggest that inhibition of mast cell histamine release contributes to the beneficial effect of NSAIDs, e.g. at sites of inflammation where the establishment of high intracellular drug concentrations is facilitated by the acidic environment.

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