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SHORT COMMUNICATION

Differential Effect of Ebselen on Compound 48/80- and Anti-IgE-induced Histamine Release from Rat Peritoneal Mast Cells

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ABSTRACT. 2-Phenyl-1, 2-benzisoselenazol-3-(2H)one (ebselen), a nontoxic seleno-organic compound, exhibits anti-inflammatory activity through the inhibition of many enzymes involved in inflammation. In view of the role played by histamine in the pathophysiology of inflammation, we looked at the effect of ebselen on histamine secretion by rat peritoneal mast cells. It inhibited compound 48/80-induced histamine release in a concentration-dependent manner. Half-maximal and maximal (100%) inhibitory response occurred at 5.10^{-7}M and 10^{-5}M, respectively. In contrast, ebselen was without any effect on histamine release induced immunologically. Prevention of the inhibitory effect of ebselen by GSH suggests that it interacts with critical thiols involved in the compound 48/80 activation pathway. BIOCHEM PHARMACOL **56**;11:1525–1528, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. ebselen; *N*-ethylmaleimide; alkylating reagent; mast cells; histamine; inflammation

Ebselen (2-phenyl-1, 2-benzisoselenazol-3-(2H)one) is a nontoxic seleno-organic compound with antioxidant and anti-inflammatory activity [1]. It catalyses a glutathione peroxidase-like reaction, i.e. the reduction of H_2O_2 and organic hydroperoxides by glutathione [2]. It has been postulated that the antioxidative properties of the drug may be, at least in part, responsible for the anti-inflammatory properties of the compound [1]. Nevertheless, it exhibits anti-inflammatory activity through the inhibition of some enzymes involved in the cascade of arachidonic acid metabolism. These enzymes include 5-lipoxygenase, 12-lipoxygenase, and cyclooxygenase [3]. Ebselen has also been shown to inhibit the production of prostaglandins [4, 5] and to stimulate the isomerisation of leukotriene B4, a compound with inflammatory properties, to a biologically inactive compound [6]. Ebselen preferentially inhibits the inducible form of nitric oxide synthase which participates in the inflammatory process, e.g. by producing the dangerous peroxynitrite [7].

Histamine that is released from mast cells is also involved in the pathological process of inflammation [8]. Histamine is chemotactic to inflammatory cells and may therefore amplify the inflammatory reaction [8]. The effect of ebselen on histamine release from mast cells is not known. In view of the multifaceted role played by ebselen in inflammation and mindful of its possible therapeutic application as anti-inflammatory drug, we investigated its effects on histamine released from rat peritoneal mast cells.

MATERIALS AND METHODS

Materials

GSH, NEM,§ and compound 48/80 were obtained from Sigma. Chelerythrine was from Calbiochem. Ebselen was from Biomol. Rat IgE (rat myeloma) and goat anti-rat IgE were obtained from Chemicon. Phosphatidyl serine was from Sigma. Polypropylene tubes (12 × 75 mm) were from Falcon.

Mast Cell Isolation, Cell Incubation, and Histamine Release Assay

Isolation of rat peritoneal mast cells was performed as described by Mousli *et al.* [9]. Purified mast cells (2.10^4 / 0.1 mL) were equilibrated at 37° for 10 min into polypropylene tubes and pretreated with ebselen before stimulation with compound 48/80. Incubations were stopped 10 min later by chilling the tubes in a cold bath (0–4°). Controls were run simultaneously without ebselen. After centrifugation (4°, 180 *g*, 2 min), the supernatants were collected and decanted into other tubes for histamine determination. In the case of IgE-dependent release, purified mast cells were passively sensitised by incubation with myeloma-derived monoclonal IgE (15 μg/mL) for 2 hr at 37°. Thereafter, cells were washed twice and preincubated for 5 min at 37°

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§ *Abbreviation:* NEM, *N*-ethylmaleimide.

with 30 μg/mL of phosphatidyl serine (to give a maximal response [10]) prior to exposure to anti-IgE for 20 min. Incubations were stopped and supernatants decanted as described above. Histamine was assayed fluorometrically according to Shore *et al.* [11] without extraction steps. Curves were fitted to experimental data using regression wizard in SigmaPlot (version 4.0).

RESULTS

Ebselen was tested for its ability to modulate histamine release induced by compound 48/80. As can be seen from Fig. 1A, the prior exposure of mast cells to 1 μM ebselen for 15 min strongly inhibited the release of histamine. In contrast, histamine release induced immunologically was not affected by ebselen (Fig. 1, B and C) even after 25 min incubation (Fig. 1C, inset). Maximal inhibition of histamine release induced by compound 48/80 (0.25 μg/mL; 10 min) occurred after 5 min of treatment with 1 μM ebselen (Fig. 1C, inset). This inhibitory effect (about 80%) was effective at all compound 48/80 concentrations. It should be pointed out that in the range of ebselen concentrations tested, this compound did not by itself induce histamine release or inhibit basal histamine secretion (i.e. secretion in the absence of any stimuli). A dose-response relationship for the effect of ebselen showed that its activity occurred between 0.1 and 10 μM. The maximal inhibitory effect, obtained at 10 μM, was 100% (Fig. 1C). The half-maximal inhibitory response occurred at approximately 5.10^{-7} M. To investigate the origin of these differential effects of ebselen, we looked at the contribution of its known major biochemical activity (i.e. thiol alkylation) to the inhibition of the compound 48/80 pathway of mast cell activation. We tried to elucidate a possible contribution of such activity by investigating the effect of free thiol groups on the inhibitory activity of ebselen. If the inhibition of mast cell degranulation occurred through events involving thiol alkylation, then free thiol groups would compete with the putative target of ebselen. This would result in a reduction in the inhibitory level of ebselen. GSH did not affect the passive or the induced histamine release. However, it did prevent, in a concentration-dependent manner, ebselen-induced inhibition of histamine release by compound 48/80 (Fig. 2). For instance, the inhibitory effect of 1 μM ebselen dropped from about 80% to approximately 25% and 5% in the presence of 1 and 10 μM GSH, respectively. When GSH was added to the bath 5 min after ebselen, it did not reverse the inhibitory effect of ebselen. Moreover, when 1 μM ebselen was incubated with 10 μM GSH for 15 min before addition to the bath, ebselen completely lost its inhibitory activity (data not shown). These results suggest that ebselen probably inhibits histamine release by interaction with thiol groups. Washing the cells with fresh buffer after exposure to 1 μM ebselen did not restore the histamine release capacity of compound 48/80 (data not shown). The effect of ebselen was mimicked by NEM, another thiol modifying agent.

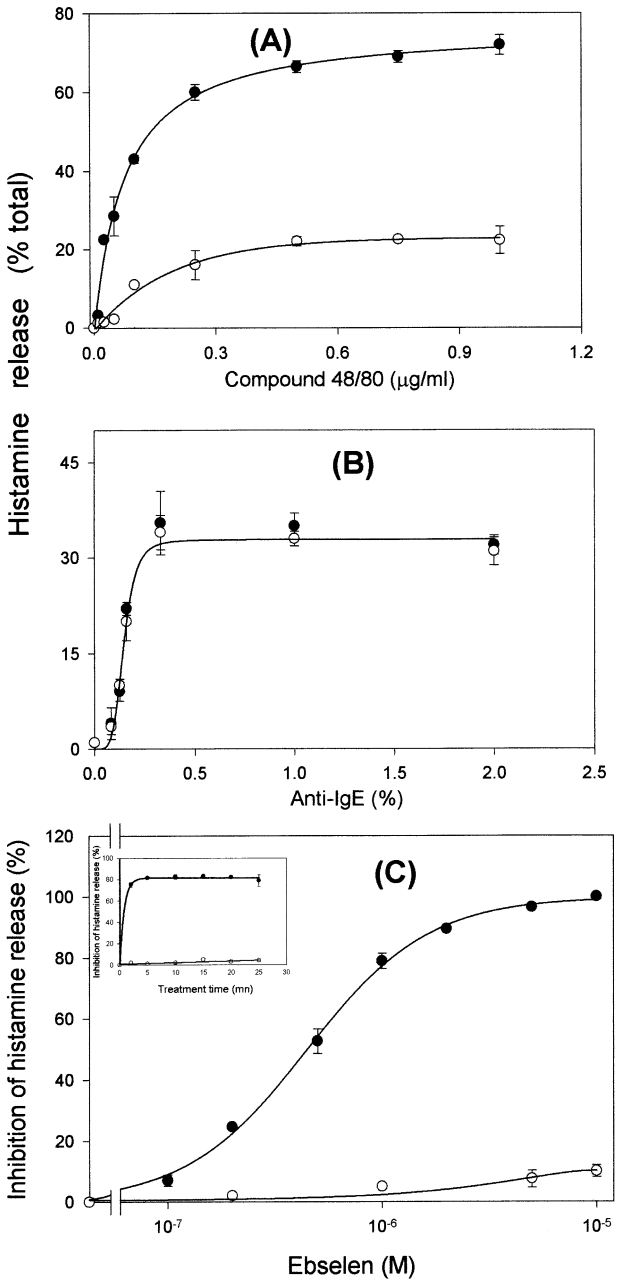

FIG. 1. (A and B) Effect of ebselen on compound 48/80- and anti-IgE-induced histamine release. The cells were incubated for 15 min with (○) or without (●) 1 μM of ebselen before exposure to increasing concentrations of compound 48/80 for 10 min (A) or anti-IgE for 20 min (B). (N = 4). (C) Time- and concentration-dependent effect of ebselen on compound 48/80- and anti-IgE-induced histamine release. The cells were treated with 1 μM of ebselen for various periods of time (inset) or with various concentrations of ebselen for 15 min before exposure to 0.25 μg/mL of compound 48/80 (●) for 10 min or 0.35% anti-IgE (○) for 20 min (N = 4).

NEM inhibited histamine release induced by compound 48/80 in a concentration-dependent manner. A maximal inhibition of 100% was obtained at 100 μM. In contrast, NEM was without any effect on anti-IgE-induced histamine release (Fig. 3).

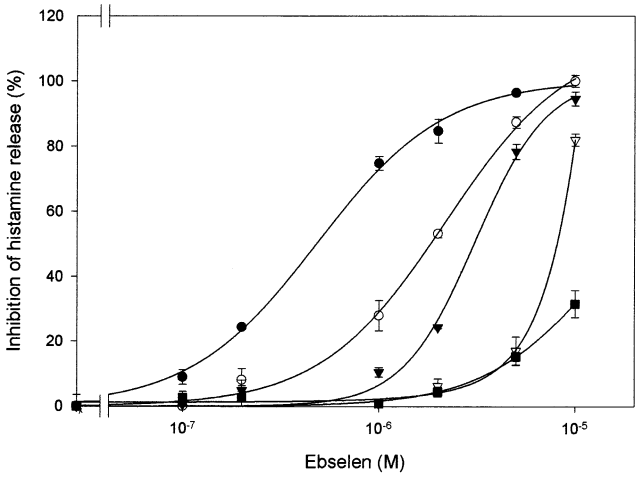

FIG. 2. Prevention by free thiols of ebselen-induced inhibition of histamine release. The cells were treated for 15 min with various concentrations of ebselen in the presence of 0 μM (●) or 1 μM (○) or 10 μM (▼) or 100 μM (▽) or 1 mM (■) GSH. GSH was added to the cell suspension 5 min before ebselen. Thereafter, the cells were challenged (for 10 min) with 0.25 μg/mL of compound 48/80 (N = 5).

DISCUSSION

Ebselen is described as an anti-inflammatory drug with a number of activities which can be regarded as advantageous in inflammation. Although it has been studied under many inflammatory conditions, its action on histamine release from mast cells is unknown. Results presented in this report show that ebselen is a potent inhibitor of compound 48/80- but not IgE-induced histamine release from rat peritoneal mast cells. We tried to follow up the mechanism of this inhibition.

The catalytic cycle of ebselen involves a selenoxide or hydroperoxide derivative as oxidant. The formation of a selenoxide derivative is dependent on low thiol and high hydroperoxide concentrations, unlikely to resemble biological conditions. Under the experimental conditions described here (excess of thiol), the organic hydroperoxide reacted to yield the selenic acid derivative that is sequentially reduced by thiol to give ebselen selenenylsulphide derivatives and regenerate ebselen selenol [12]. Based on its unique GSH peroxidase-like activity [13], removal of peroxides has been proposed as a possible mechanism of the anti-inflammatory action of ebselen [2, 14]. It shares this property with ebselen selenenylsulphides, which are formed during its catalytic cycle [1]. For example, ebselen is able to react with a number of thiols including GSH to give ebselen selenenylsulphides such as ebselen GSH selenenylsulphide, which retained the antiperoxidase activity of ebselen [15]. Since ebselen lost its inhibitory activity when it was preincubated with GSH before addition to cell suspension, it is clear that the mechanism of inhibition of histamine release is independent of its antiperoxidase activity. A protective effect by free thiol reagents against ebselen-induced inactivation of several enzymes has been described [1]. It has been postulated that ebselen reacts with a thiol group which is essential for the catalytic activity of the enzymes. Consistent with this, free thiol groups compete with the putative target containing thiol for the reaction with ebselen, resulting in the trapping of ebselen as nonreactive selenenylsulphides. Since GSH prevents, in a concentration-dependent manner, inhibition by ebselen of compound 48/80-induced histamine release, we suggest that the inhibitory mechanism proceeds through the reaction pathway postulated above, i.e. that ebselen targets an essential thiol involved in exocytosis induced by compound 48/80. Proteolysis of membrane protein is required in membrane fusion to remove charge or steric inhibition to membrane association. Since rat peritoneal mast cells have several proteases which contain cysteine residues, it is likely that these enzymes are candidates for inactivation through sulfhydryl modification.

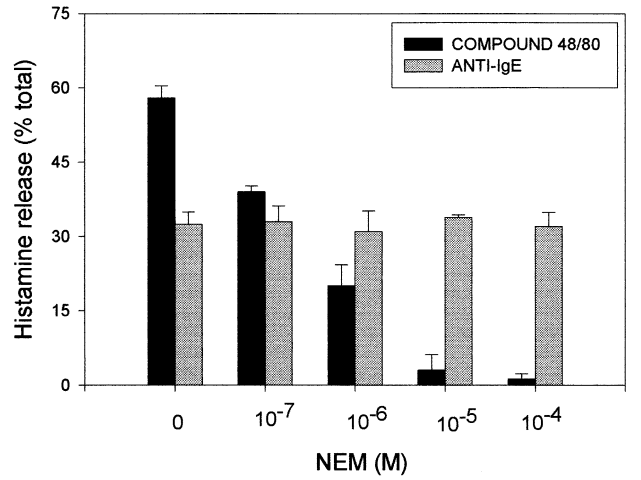

FIG. 3. Effect of NEM on compound 48/80- and anti-IgE-induced histamine release. The cells were treated with various concentrations of NEM for 15 min before exposure for 10 min to 0.25 μg/mL of compound 48/80 or for 20 min to 0.35% anti-IgE (N = 4).

In this regard, ebselen should also have inhibited anti-IgE-induced histamine release. The lack of effect of ebselen on histamine release induced by anti-IgE is the most intriguing aspect of this study. These differential effects of ebselen are very difficult to explain. An increase in intracellular calcium is obligatory for exocytosis. Since the molecular mechanism of events leading to exocytosis of mast cells activated by compound 48/80 or anti-IgE is the same after Ca^{2+} entry, the explanation for these differential effects could be found in the steps upstream of Ca^{2+} entry. In these steps, the biochemical events leading to calcium entry are different when the mast cells are activated by compound 48/80 or by anti-IgE. Bridging of IgE receptors on rat mast cell plasma membranes induces phospholipid methylation and raises cyclic AMP. This methylation leads to calcium influx and histamine release [16]. Therefore, the calcium influx induced by anti-IgE is independent of thiol-mediated interactions and cannot be inhibited by compounds such as ebselen. In contrast, compound 48/80-

induced histamine release is dependent on inositol (1,4,5)-triphosphate generation. The resulting elevation of free intracellular calcium initiates an influx of Ca^{2+} and the release of vesicular contents into the extracellular space. It has been reported that the inositol (1,4,5)-triphosphate receptor contains disulfide bridges as well as free sulfhydryl groups that are essential for both inositol (1,4,5)-triphosphate binding and calcium release [17, 18]. More interestingly, it has been shown that ebselen prevents inositol (1,4,5)-triphosphate binding to its receptor and thereby prevents calcium release from intracellular pools [19]. Therefore, the prevention of calcium release from intracellular pools could be a logical explanation for the inhibitory effect of ebselen on compound 48/80-induced histamine release from rat peritoneal mast cells. If it is very tempting to attribute the observed inhibitory effects of ebselen to an interaction with critical thiol groups, sulfhydryl alkylating agents must also be able to inhibit histamine release from rat mast cells. This hypothesis is consistent with our observations that NEM, a sulfhydryl-modifying agent, is a potent inhibitor of histamine release induced by compound 48/80 but not by anti-IgE. Since it binds to plasma proteins [20], ebselen may not display the effect described above *in vivo*. Nevertheless, it may be used as a pharmacological tool for the study of signal transduction after mast cell activation.

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