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Captopril-induced enhancement of fMet-Leu-Phe-activated enzyme secretion from neutrophils

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Abstract

Captopril enhanced exocytotic enzyme secretion from rabbit peritoneal neutrophils activated by sub-optimal concentrations of fMet-Leu-Phe. For optimal enhancement the presence of Ca^{2+} was required. Captopril did not induce enzyme secretion by its own, and there was little effect on PMA-induced exocytosis. Cyclic GMP level of neutrophils was transiently enhanced by captopril. Methylene blue inhibited both captopril-induced enhancement of enzyme release and of cGMP levels. From our studies and from reports in the literature it appears that cGMP level-enhancing compounds may have both activating or inhibitory properties.

Introduction

Captopril is a potent hypotensive agent, and possesses anti-inflammatory activities through as yet unknown mechanisms [1]. The drug improved symptoms in patients with rheumatoid arthritis [2]. The drug has, in addition, a number of other effects [1].

On a cellular level captopril shows inhibitory and activating properties. Recently, it was found that glutathione, and drugs such as penicillamine and tiopronin have a potentiating effect on both migration and exocytosis by neutrophils [3, 4]. The molecular structures of these compounds resemble that of captopril in that all agents possess a thiol group, and a carboxyl group. Because a modulation of neutrophil functions might be related to the anti-inflammatory properties of the drug we decided to study the effect of captopril on neutrophil exocytosis.

Materials and methods

Isolation of neutrophils

Neutrophils were isolated from the peritoneal cavity of the rabbit [4]. Four hours after intraperitoneal injection with 200 ml isotonic saline containing 1.5 mg/ml glycogen, the exudate was collected by flushing the peritoneal cavity with isotonic saline containing citrate (0.4%, pH 7.4). The cells were centrifuged and washed with medium. The medium used consisted of 140 m*M* NaCl, 5 m*M* KCl, 10 m*M* glucose and 20 m*M* Hepes pH 7.3. The final cell suspension during the experiments contained 3×10^6 neutrophils per ml.

Exocytosis

Exocytosis was measured as the release of the granule-associated enzyme, lysozyme, in the ab-

sence of a significant release of the cytoplasmic enzyme, lactate dehydrogenase (LDH). Lysozyme and LDH were measured as described previously [5]. Under the conditions of our experiments LDH release was negligibly low (less than 5%). Enzyme release was expressed as a percentage of a maximum value, obtained by treating the cells with 0.05% Triton X-100.

Cyclic GMP assay

Cyclic GMP was determined as described previously [3, 4], using the radioimmunoassay kit of Amersham (Amersham, England).

Results

To study the effect of captopril on the exocytotic response of neutrophils, exocytosis was induced by sub-optimal concentrations of secretagogue. For this purpose the following agents were used: fMet-Leu-Phe, at a "low" concentration (0.5 n*M*) in the presence of Ca^{2+} or at a "normal" concentration (10 n*M*) in the absence of Ca^{2+}; PMA (which is a relatively weak activator of exocytosis in peritoneal cells); or a low concentration of ionophore A23187 in the presence of Ca^{2+}. Captopril had a significant enhancing effect on exocytosis induced by fMet-Leu-Phe in the presence of Ca^{2+} (Table 1). In the absence of Ca^{2+} the enhancing effect was less than in the presence of Ca^{2+}. There was little effect on PMA-induced exocytosis, and in different experiments there was a small, but not significant enhancement of A23187-induced exocytosis.

The effect of captopril on fMet-Leu-Phe-induced exocytosis was concentration-dependent. The effect was maximal at a captopril concentration of 100 μ*M*. The potentiating effect of captopril on fMet-Leu-Phe-induced lysozyme release was most pronounced when the cells were only a short time in contact with captopril before adding fMet-Leu-Phe. The potentiating effect decreased when the preincubation time with captopril increased, but the degree of decrease was highly variable. For that reason activator and captopril were added together in most experiments.

Captopril caused a transient increase of cGMP levels in neutrophils. The maximum occurred 1 min after addition of captopril to the cells, after which the response declined again. Methylene blue, an inhibitor of cGMP accumulation in other cell types, inhibits captopril-induced enhancement of cGMP level, and the effect of captopril on enzyme release.

Table 1
Modulation of exocytosis by captopril.

	Lysozyme release (%) in presence of	
	medium	100 μ*M* captopril
Ca^{2+}	6±1	4±1*
Ca^{2+}, 1 μ*M* CB+0.5 n*M* FMLP	38±4	65±2**
EGTA, 5 μ*M* CB+10 n*M* FMLP	33±3	39±3***
Ca^{2+}, 0.05 μ*M* A23187	42±4	46±3*
Ca^{2+}, 0.2 μ*M* PMA	35±4	29±2***

Cells were incubated without or with 100 μ*M* captopril, in the presence of the reagents indicated, for 15 min at 37°C. FMLP: fMet-Leu-Phe; A23187: calcium ionophore A23187; PMA: phorbol myristate acetate; CB: cytochalasin B. Values given are the means of four experiments ± SEM ($n=4$). Comparison between the release by captopril-treated cells and non-treated cells (Students *t*-test): * not significant; ** $P<0.001$; *** $P<0.05$.

Discussion

The results show that captopril is able to potentiate neutrophil exocytosis, induced by fMet-Leu-Phe, but not by other agents. This suggests that potentiation occurs at a stage where receptor interactions are linked to the other stages of signal transduction. The target of captopril appears not only dependent on the interaction of fMet-Leu-Phe with its receptor, but also on calcium ions.

Agents that increase intracellular cGMP level have generally been found to potentiate exocytosis. For that reason cGMP was supposed to have a positive modulating effect on exocytosis [6]. The results reported here agree with this concept.

Recently, however, Schröder et al. [7] found that some vasodilators caused an increase of cGMP level in neutrophils, but an inhibition of exocytosis. They suggested that cGMP was an inhibitory second messenger in neutrophils. Wenzel-Seifert et al. [8] found a differential inhibition and potentiation by analogues of cGMP. The effect of the analogues depended on the type of secretagogue. Taken together, these data suggest that the effect on exocytosis of agents which increase cGMP level depend on the type of activator and the experimental conditions, and that both positive and negative modulation is possible.

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