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The effect of apomorphine on exocytosis and metabolic burst of polymorphonuclear leukocytes

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1 In rabbit polymorphonuclear leukocytes (PMNLs) apomorphine at 10–100 μM inhibits fMet-Leu-Phe and A23187-induced exocytosis, and the phorbol myristate acetate- and fMet-Leu-Phe-induced activation of the metabolic burst. The secretory response was not restored by washing the cells after pretreatment with apomorphine.

2 The inhibitory effect of apomorphine was not prevented by the dopamine receptor antagonists haloperidol and pimozide, nor did dopamine itself inhibit fMet-Leu-Phe-induced exocytosis. It therefore seems unlikely that effects are mediated via dopamine receptors. However, sulphydryl reagents reduced the inhibitory effect of apomorphine, suggesting that it may depend upon interaction with susceptible sulphydryl groups, the intactness of which is required for exocytosis and other functions of PMNLs.

Introduction

Apomorphine is a well known agonist of dopamine. It causes sedation and behavioural changes in mammals and it has a therapeutic effect in some neurological and psychiatric conditions, although the emetic action of the drug prevents the use of high concentrations. These effects are thought to be due to an interaction with the dopamine receptor (Di Chiara *et al.*, 1978).

Recently it was found that apomorphine has an inhibitory effect on several types of secretory cells. For example it inhibits insulin secretion from rat pancreas (Joost *et al.*, 1983), transmitter release evoked by 5-hydroxytryptamine from the sympathetic nerves of the perfused rabbit heart (Carr & Fozard, 1982), and histamine release by exocytosis from mast cells (Mietto *et al.*, 1984; Batistella *et al.*, 1986).

Exocytosis is an important function of the polymorphonuclear leukocyte (PMNL). The granules of the PMNL contain hydrolytic and proteolytic enzymes which may contribute to the destruction of invading microorganisms or cause tissue damage in inflammatory reactions (Weissmann *et al.*, 1980). Exocytosis may be induced with soluble activators many of which also stimulate the metabolic burst. This comprises a series of reactions, including the uptake of molecular oxygen and its conversion into toxic metabolites (Fantone & Ward, 1982).

This report concerns a pharmacological study of the effect of apomorphine on exocytosis and metabolic burst in rabbit PMNLs.

Methods

Polymorphonuclear leukocytes

Rabbit peritoneal polymorphonuclear leukocytes (PMNLs) were obtained as described previously (Elferink & Deierkauf, 1987). The cells were suspended in a medium consisting of (mM): NaCl 140, KCl 5, glucose 10 and HEPES 20, pH 7.3; 1 mM Ca^{2+} was included in the medium, except where indicated. The final cell suspension during the experiments contained 3×10^6 PMNLs per ml.

Exocytosis

Exocytosis was measured as the release of granule-associated enzyme lysozyme in the absence of significantly increased release of the cytoplasmic enzyme lactate dehydrogenase (LDH). Cells were preincubated with or without apomorphine for 10 min at 37°C, after which an exocytosis-inducing activator was added. After incubation for 20 min at 37°C the cells were centrifuged and enzyme release in the supernatant was measured as described previously (Elferink & Deierkauf, 1984). LDH release, which was measured as the conversion of NADH into NAD^+ during the conversion of pyruvate into lactate, was always lower than 5%. Enzyme release was expressed as a percentage of the total cellular lysozyme content,

obtained by lysing the cells with 0.2% Triton X-100. To cause exocytosis, either the combination of fMet-Leu-Phe (10^{-8} M) + cytochalasin B (5×10^{-6} M), or ionophore A23187 (5×10^{-7} M) was used.

Metabolic burst

The metabolic burst was measured in terms of increased NBT reduction (Elferink, 1984) by including 0.4 mM (Nitro)B(lue)T(etrazolium) in the reaction mixture. After preincubation of the cells with or without apomorphine for 10 min at 37°C an activator of the metabolic burst, fMet-Leu-Phe(10^{-8}M) + cytochalasin B (5×10^{-6} M), or phorbol myristate acetate (PMA)(100 ng ml^{-1}), was added, followed by incubation for 15 min at 37°C. Subsequently 5 ml 0.5 M HCl was added, the mixture was centrifuged, and the residue was dissolved in 2 ml pyridine in a boiling waterbath. The absorbance of the pyridine solution was measured spectrophotometrically at 510 nm, and the results were expressed as nmol NBT reduced per 3×10^6 PMNLs per 15 min.

ATP level

After incubation of PMNLs with or without apomorphine or another inhibitor for 20 min at 37°C, an equal volume of ice-cold tricholoroacetic acid (10%) was added to the reaction mixture. After 10 min the solution was neutralized with sodium acetate (1.5 M) and the ATP content was determined by the luminescence technique, using luciferin-luciferase from firefly tails, as described by Strehler (1968). In this experiment glucose was omitted from the medium.

Results

Apomorphine inhibited fMet-Leu-Phe-induced lysozyme release in the concentration range of 10–100 μM. Neither in the absence nor in the presence of apomorphine was there significant release of LDH, indicating that lysozyme release is a direct measure of the degree of exocytosis (Figure 1). Ionophore A23187-induced lysozyme release was inhibited by apomorphine in a similar fashion. Similarly, apomorphine inhibited the metabolic burst induced with either fMet-Leu-Phe in the presence of cytochalasin B, or with two doses of phorbol myristate acetate (PMA) at the same concentration (Figure 1). PMA was used to activate the metabolic burst, and ionophore A23187 for exocytosis, because in rabbit PMNLs, PMA is a strong activator of the metabolic burst but gives little exocytosis, whereas the reverse applies to A23187.

The inhibitory effect of apomorphine on fMet-Leu-Phe-induced lysozyme release persists even if the PMNLs are washed after pretreatment with apomor-

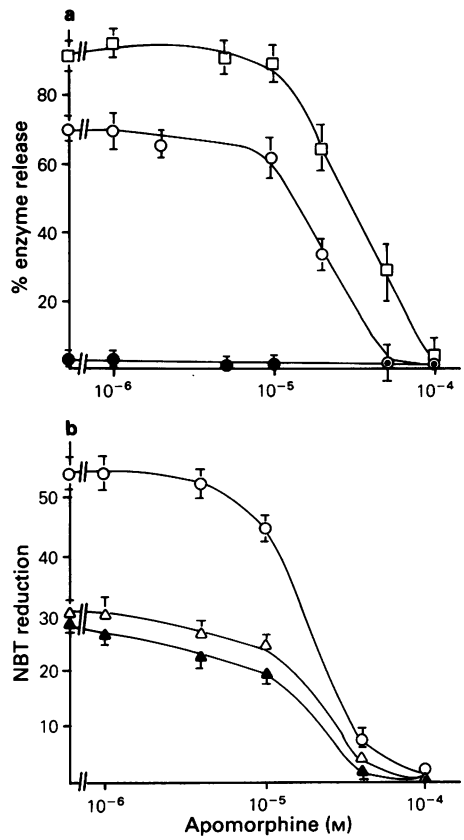

Figure 1 Effect of apomorphine on enzyme release and NBT (Nitro Blue Tetrazolium) reduction by activated PMNLs. Cells were preincubated with the given concentration of apomorphine for 10 min at 37°C, after which the activator was added and incubation was carried out for 20 min (enzyme release, a) or 15 min (NBT reduction, b) at 37°C. Activators of enzyme release: (○) fMet-Leu-Phe (10^{-8} M) + cytochalasin B (5×10^{-6} M), lysozyme release; (●) fMet-Leu-Phe + cytochalasin B, LDH release; (□) ionophore A23187 (5×10^{-7} M), lysozyme release. Activators for NBT reduction: (○) fMet Leu-Phe + cytochalasin B; (△) phorbol myristate acetate (PMA) 100 ng ml^{-1}; (▲) PMA, 5 ng ml^{-1}. The values given are the mean of three experiments with s.d. shown by vertical lines. At a concentration of 10 μM apomorphine there was a significant inhibition ($P < 0.05$) of fMet-Leu-Phe-induced lysozyme release but not of A23187-induced enzyme release (Student's t test). Inhibition of NBT reduction at this concentration was significant with fMet-Leu-Phe and phorbol myristate acetate (PMA, 5 ng ml^{-1}) ($P < 0.005$) and with PMA (100 ng ml^{-1}) ($P < 0.05$).

Table 1 Absence of reversibility of inhibition of chemotactic peptide-induced exocytosis by apomorphine

	% enzyme release			
	Procedure A		*Procedure B*	
	LDH	*Lys*	*LDH*	*Lys*
—	4 ± 1	83 ± 5	7 ± 1	63 ± 4
Apomorphine 50 μM	3 ± 1	2 ± 3	6 ± 0	8 ± 3
Apomorphine 100 μM	3 ± 0	0 ± 3	5 ± 2	9 ± 3

Procedure A: 3×10^6 cells per ml were exposed to the indicated amount of apomorphine for 10 min at 37°C. Then activator (10^{-8} M fMet-Leu-Phe + 5×10^{-6} M cytochalasin B) was added, followed by incubation for 20 min at 37°C. Procedure B: 3×10^6 cells per ml were exposed to the indicated amount of apomorphine for 10 min at 37°C. Then the mixture was centrifuged and the supernatant discarded. The pelleted cells were washed with 10 ml medium and centrifuged. The cells were resuspended in Ca^{2+}-containing medium and activator were added to a total volume of 1 ml, and the mixture was incubated for 20 min at 37°C Lys: lysozyme.

The values given are the means of four experiments ± s.d.

Table 2 Effect of pimozide and haloperidol on inhibition of fMet-Leu-Phe-induced lysozyme release by apormorphine

	% lysozyme release in the presence of:				
	Control	*Pimozide*		*Haloperidol*	
		10 μM	20 μM	10 μM	20 μM
—	77 ± 5	75 ± 3	53 ± 2	76 ± 3	79 ± 6
Apomorphine 20 μM	22 ± 5	22 ± 3	17 ± 2	21 ± 4	14 ± 7
Apomorphine 50 μM	1 ± 1	7 ± 4	8 ± 2	3 ± 2	5 ± 2

Cells were preincubated with the agents indicated for 10 min at 37°.
Then 10^{-8} M fMet-Leu-Phe + 5×10^{-6} M cytochalasin B was added followed by incubation for 20 min. LDH release was in all experiments less than 5%. The values given are the mean of four experiments ± s.d.

phine (Table 1). Activation of washed cells in the absence of apomorphine resulted in a slightly lower lysozyme release and a higher LDH release as compared with control cells.

Apomorphine is a known dopamine receptor agonist and to establish the possible involvement of this receptor in the mechanism of inhibition, the effect of dopamine and of the dopamine receptor antagonists pimozide and haloperidol were investigated. Dopamine (10–100 μM) had no inhibitory effect on exocytosis, and indeed slightly potentiated fMet-Leu-Phe-induced lysozyme release from 49 ± 4% to 59 ± 2% in the presence of 100 μM dopamine, without significant LDH release.

Pimozide and haloperidol did not prevent apomor-

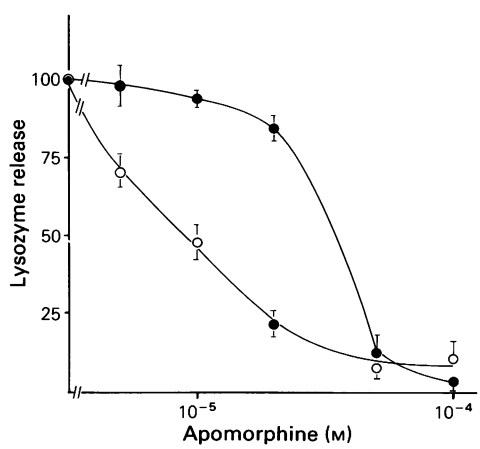

Figure 2 Effect of albumin on inhibition of fMet-Leu-Phe-induced lysozyme release by apomorphine. Cells were preincubated with the given concentration of apomorphine, in the presence or absence of 0.5% rabbit serum albumin, for 10 min at 37°C. Then activator (fMet-Leu-Phe (10^{-8} M) + cytochalasin B (5×10^{-6} M) were added followed by incubation for 20 min at 37°C. Values given are expressed as a percentage of lysozyme release in the absence of apomorphine, and are the mean of four experiments with s.d. shown by vertical lines: (○) lysozyme release in the absence of albumin; (●) lysozyme release in the presence of 0.5% albumin.

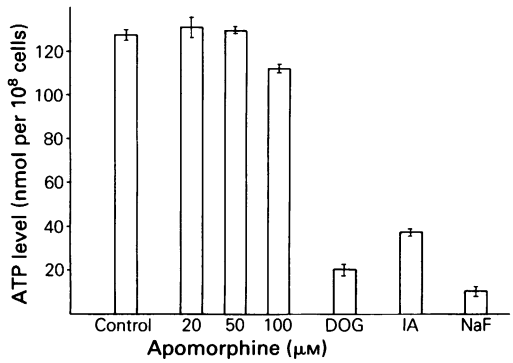

Figure 3 Effect of apomorphine and some inhibitors of glycolysis on the ATP level of resting PMNLs. Cells were incubated with the given concentration of apomorphine or with one of the the other inhibitors for 20 min at 37°C, after which the ATP level was determined as described in Methods. Values given are the mean of three experiments with s.d. shown by vertical lines. DOG: 2-deoxyglucose, 10 mM; IA: iodoacetate, 0.5 mM; NaF: sodium fluoride, 20 mM.

phine-induced inhibition of exocytosis (Table 2). However, pimozide itself at 20 μM inhibited exocytosis.

Albumin, which is often included in cell suspension media, interferes with the inhibitory effect of apomorphine. In the presence of albumin the inhibition of fMet-Leu-Phe-induced exocytosis by apomorphine is shifted to higher concentrations (Figure 2). Note that the extent of inhibition of exocytosis by apomorphine is to some degree dependent on the batch of cells, as can be seen by comparison of Figures 1 and 2.

Because an interference with cellular metabolism could provide an explanation for the inhibitory action of apomorphine, the effect on cellular ATP level was considered. Whereas the classical inhibitors of glycolysis (2-deoxyglucose, iodoacetate and sodium fluoride) strongly reduced the ATP level of resting cells, high concentrations of apomorphine had little effect (Figure 3).

Inhibition of exocytosis by apomorphine is substantially reduced by pretreatment of apomorphine with sulfphydryl compounds. In this regard glutathione was especially effective (Table 3).

Table 3 Interference of sulphydryl compounds with apomorphine inhibition of exocytosis

	% lysozyme release in the presence of	
	0	100 μM Apomorphine
	49 ± 4	1 ± 1
Dithiothreitol 100 μM	64 ± 4	36 ± 6
Glutathione 100 μM	70 ± 3	48 ± 2
Mercaptoethanol 100 μM	62 ± 2	39 ± 2
2,3-Dimercapto-propanol 100 μM	65 ± 5	35 ± 2
Cysteine 100 μM	56 ± 6	28 ± 7

Medium containing the indicated amounts of apomorphine and sulphydryl compound were left in contact for 10 min at 37°C. Then cells were added and preincubation was carried out for 10 min at 37°C. Subsequently activator (10^{-8} M fMet-leu-Phe + 5×10^{-6} M cytochalasin B) was added, followed by incubation for 20 min at 37°C.
Values given are the mean of four experiments ± s.d.

Discussion

Apomorphine inhibits exocytosis and the metabolic burst in PMNLs at about the same concentrations. Previously we found that phagocytosis was inhibited by slightly lower concentrations than required for inhibition of exocytosis and metabolic burst (Elferink & Deierkauf, 1987). Taken together these results suggest that apomorphine interferes with a cellular target which is of importance for all PMNL functions studied.

It is unlikely that the inhibitory effect of apomorphine on PMNL functions is due to an interaction with dopamine receptors because: (a) inhibition of PMNL functions occurs in the micromolar concentration range, whereas the receptor mediated actions occur in the nanomolar range (Di Chiara *et al.* (1978); (b) the apparent irreversibility of apomorphine-induced inhibition does not support the view that receptor binding is involved; (c) the agonist dopamine does not inhibit PMNL exocytosis at concentrations at which apomorphine inhibits completely; (d) the dopamine receptor antagonists pimozide and haloperidol do not prevent the inhibitory effect of apomorphine. However, the latter results are not definitive because pimozide and haloperidol possess anaesthetic-like properties, and this class of drugs is known to interefere non-specifically with PMNL functions (Goldstein *et al.*, 1977). For example pimozide at the highest concentration tested inhibited exocytosis by itself.

Exocytosis is supposed to be an energy-requiring process, and an interference with glycolysis, the main source of energy in the PMNL, (Becker & Showell, 1974), might provide an explanation for the inhibitory effect of apomorphine. The ATP level of resting PMNLs is strongly reduced by inhibitors of glycolysis, such as iodoacetate, deoxyglucose and fluoride. Apomorphine does not interfere with cellular ATP

level and thus an interference with metabolism as a basis for the inhibitory effects seems unlikely.

Exocytosis and other PMNL functions are inhibited by a wide range of sulphydryl reagents, indicating that vulnerable sulphydryl groups play a predominant role in these functions (Giordano & Lichtman, 1973); Elferink & Riemersma, 1980; Yamashita, 1983). The inhibitory effect of apomorphine was substantially reduced by pretreatment of the drug with compounds containing sulphydryl groups. Apparently apomorphine may act as a sulphydryl reagent. It seems likely that the inhibitory effect of apomorphine is due to its ability to react with vulnerable sulphydryl groups that are required for PMNL functions susceptible to apomorphine.

The PMNL contains in its cytoplasm a very high concentration (4 mM) of glutathione (Oliver *et al.*, 1976) which might be expected to inactivate apomorphine should it penetrate into the cell. The vulnerable sulphydryl groups involved are thus probably located on proteins of the PMNL plasma membrane.

The effect of apomorphine was also reduced in the presence of albumin. This could be related to the presence of a sulphydryl group in albumin. Because of the high concentration of albumin in serum it is unlikely that inhibition of PMNL function by apomorphine is of importance *in vivo*.

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