Naunyn-Schmiedeberg's Arch Pharmacol (1990) 341:455–461

Naunyn-Schmiedeberg's
Archives of Pharmacology
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Characterization of histamine H_2-receptors in human neutrophils with a series of guanidine analogues of impromidine

Are cell type-specific H_2-receptors involved in the regulation of NADPH oxidase?

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Received October 13, 1989/Accepted January 6, 1990

Summary. Human neutrophils possess an NADPH oxidase which catalyzes superoxide (O_2^-) formation and is activated by chemotactic peptides. Histamine inhibits O_2^-formation via H_2-receptors (Burde et al. 1989). We characterized the neutrophil H_2-receptor with a series of new guanidine-type H_2-agonists structurally derived from impromidine. Histamine inhibited O_2^- formation with an IC_{50} value of 6.7 ± 1.2 μM. Five aryloxy- and arylthioalkylguanidines were less potent and effective than histamine. Several arpromidine-like phenyl(pyridylalkyl)guanidines were either full or partial H_2-agonists. Some guanidines possess a three-membered carbon chain connecting the aromatic rings and the guanidine group; they were similarly potent and effective as histamine. Shortening or elongation of the carbon chain substantially decreased the potency and intrinsic activity of the guanidines. Halogenation of the phenyl ring did not substantially affect the potency and intrinsic activity of the compounds in comparison to the non-substituted parent compound. The H_2-antagonist, famotidine, competitively antagonized inhibition of O_2^- formation caused by the guanidine, arpromidine, with a pA_2 value of 6.84. The H_2-antagonist, cimetidine, differentially counteracted inhibition caused by partial and full H_2-agonists. Partial H_2-agonists antagonized the effects of histamine. The inhibitor of phosphodiesterases, 3-isobutyl-1-methylxanthine, additively enhanced the inhibitory effects of histamine and guanidines. The properties of the neutrophil H_2-receptor were compared with literature data concerning properties of the H_2-receptor of the guinea pig atrium. In the latter system, guanidines are full H_2-agonists with potencies of up to 125-fold of that of histamine. Our data indicate that guanidines inhibit O_2^- formation in human neutrophils via H_2-receptors. The structure/activity relationship for the neutrophil H_2-receptor substantially differs from the one for the H_2-receptor in the guinea pig atrium, suggesting that the neutrophil H_2-receptor has cell type-specific properties. Other possibilities to explain the differences between H_2-receptors in these systems are discussed.

Key words: Superoxide formation – Human neutrophils – Arpromidine – Histamine – H_2-receptors

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Introduction

Human neutrophils possess an NADPH oxidase which catalyzes superoxide (O_2^-) formation and is activated by the chemotactic peptide, N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMet-Leu-Phe) (Rossi 1986; Seifert et al. 1989a, b, c). Agents which increase the intracellular cAMP concentration, e.g. prostaglandins, adenosine, β-adrenergic agonists and the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX), inhibit O_2^- formation (Rossi 1986; Seifert et al. 1989a, b, c; Burde et al. 1989). In addition, histamine plays an important role as intercellular signal molecule in the pathogenesis of inflammatory processes (Gespach and Abita 1982; Seligmann et al. 1983; Burde et al. 1989). Human neutrophils possess histamine H_2-receptors, whose agonist occupation leads to an increase in cAMP and to inhibition of aggregation, lysosomal enzyme release and O_2^- formation (Gespach and Abita 1982; Seligmann et al. 1983; Burde et al. 1989). In human neutrophils, the H_2-agonist, impromidine, is about four-fold more potent than histamine with respect to inhibition of O_2^- formation and about 15-fold more potent than histamine with regard to cAMP accumulation (Gespach and Abita 1982; Burde et al. 1989). However, in the guinea pig atrium, impromidine is almost 50-fold as potent as histamine with respect to positive chronotropy (Durant et al. 1978). In contrast, impromidine is a partial agonist in the isolated guinea pig papillary muscle, in the isolated stomach of the rat, in cultured calf-aorta smooth muscle cells and in HL-60 leukemic cells (Parsons and Sykes 1980; Luchins and Makman 1980; Bertaccini and Coruzzi 1981;

Gespach et al. 1982). In addition, there is evidence that H_2-receptors in murine lymphocytes, human platelets and HL-60 cells possess properties different from those of H_2-receptors in other cell types, suggesting that H_2-receptors in blood cells may be different from those in other cell types (Gespach et al. 1986; Khan et al. 1987; Mitsuhashi et al. 1989).

Recently, a series of guanidines structurally related to impromidine and possessing H_2-agonistic properties has been synthesized (Buschauer 1988a, b, c, 1989). The structural formulae of the aryloxy- and arylthioalkylguanidines 1−5, and of the phenyl(pyridylalkyl)-guanidines 6−17 are shown in Table 1. The latter group of compounds represents a new class of very potent positive inotropic agents (Buschauer 1989). In addition, phenyl(pyridylalkyl)guanidines possess H_1-antagonistic properties (Buschauer 1989). All these results prompted us to study the effects of guanidines 1−17 in human neutrophils and to compare their H_2-agonistic activity with literature data concerning their activity in a standard model for the analysis of H_2-receptors, the guinea pig right atrium (Black et al. 1972). We here report that the H_2-agonistic activity of guanidines 1−17 in human neutrophils substantially differs from the one in the guinea pig atrium, suggesting that neutrophil H_2-receptors are different from H_2-receptors in other cell types.

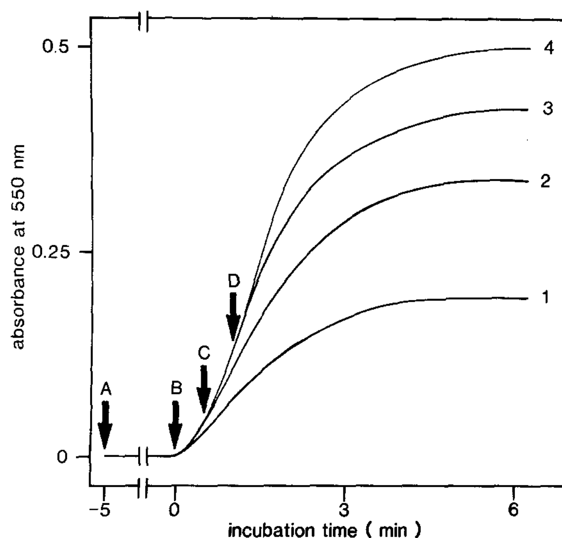


Fig. 1. Kinetics of O_2^- formation in human neutrophils. The effect of guanidine 9 (arpromidine) on fMet-Leu-Phe-induced O_2^- formation was studied. Superimposed original registrations from one representative experiment are shown. **A**, **B**, **C** and **D** indicate the addition of fMet-Leu-Phe (1 μM) or compound 9 (1 mM) at different times. Trace 1: **B**, fMet-Leu-Phe; **A** and **B**, compound 9. Trace 2: **B**, fMet-Leu-Phe; **C**, compound 9. Trace 3: **B**, fMet-Leu-Phe; **D**, compound 9. Trace 4: **B**, fMet-Leu-Phe. Similar results were obtained in three experiments carried out with different preparations of neutrophils

Materials and methods

Materials. Dimaprit, (R)-α-methylhistamine and guanidines 1−17 were synthesized as described recently (Kartinos 1964; Gerhard and Schunack 1980; Buschauer 1988a, b, c, 1989). Stock solutions of these compounds (100 mM each) were prepared in distilled water and were stored at −20°C. Sources of other materials have been described elsewhere (Seifert and Schächtele 1988; Seifert et al. 1989a, b, c; Burde et al. 1989).

Isolation of neutrophils. Human neutrophils were isolated from buffy coat preparations obtained from the local blood bank by dextran sedimentation and centrifugation through Ficoll-Hypaque (Seifert et al. 1989b). Cell preparations consisted of more than 98% viable neutrophils as judged by trypan blue dye exclusion and Pappenheim-stained smears.

Assay for superoxide formation. O_2^- formation was monitored by continuous measurement of ferricytochrome C reduction inhibitable by superoxide dismutase, using an Uvikon 810 dual-beam spectrophotometer (Kontron, Eching, FRG) (Seifert et al. 1989a, b, c; Burde et al. 1989). Reaction mixtures (0.5 ml) contained 1.0×10^6 neutrophils, 100 μM ferricytochrome C and a buffer consisting of (mM) 138 NaCl, 6 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 5.5 glucose and 20 Hepes/NaOH, pH 7.4. Reaction mixtures were preincubated for 5 min at 37°C in the presence of the substances indicated. Reactions were initiated by the addition of fMet-Leu-Phe (1 μM). The absolute amounts of O_2^- generated were calculated.

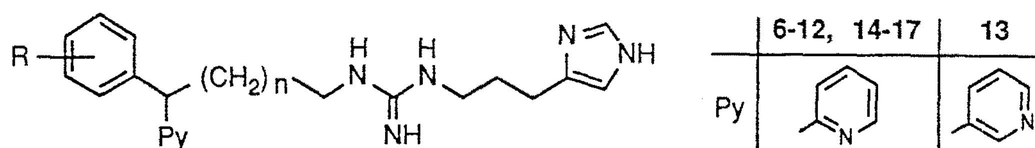
Results

The effect of guanidine 9 (arpromidine) on O_2^- formation in human neutrophils was studied. Figure 1 shows superimposed kinetic registrations of O_2^- formation. fMet-Leu-Phe reversibly activated O_2^- formation which ceased after 6 min (trace 4). When added 5 min prior to or simultaneously with fMet-Leu-Phe, compound 9 at 1 mM inhibited O_2^- formation by about 60% (trace 1). In addition, compound 9 rapidly terminated O_2^- formation when added 30 s or 1 min after fMet-Leu-Phe (traces 2 and 3). Similar results as with compound 9 were obtained with the H_2-agonists, dimaprit and impromidine (data not shown). These data show that H_2-agonists of various chemical classes rapidly inhibit O_2^- formation as is the case for histamine (Burde et al. 1989).

The effects of various H_2-agonists at concentrations between 100 nM and 1 mM on O_2^- formation were studied. Similarly to histamine, impromidine and the weak partial H_2-agonist, β-histine (Burde et al. 1989), the H_2-agonists analyzed in our present study inhibited O_2^- formation with sigmoid concentration response functions, reaching a plateau at 0.1 − 1 mM (data not shown). None of the H_2-agonists studied per se induced O_2^- formation (data not shown). Table 1 summarizes the IC_{50} values and intrinsic activities of compounds 1−17, histamine being the reference. In addition, the effects of dimaprit, and of the potent H_3-agonist and weak partial H_2-agonist, (R)-α-methylhistamine (Arrang et al. 1987), on O_2^- formation were studied. Histamine inhibited O_2^- formation with an IC_{50} value of 6.7 μM. In agreement with a recent report, dimaprit was less potent but similarly effective as histamine to inhibit O_2^- formation (Seligmann et al. 1983). (R)-α-methylhistamine was considerably less potent than histamine and acted as a partial agonist at the neutrophil H_2-receptor. The guanidines 1, 4 and 5 were much less potent and effective inhibitors of O_2^- formation than histamine. The phenylthioalkylguanidines 2

Table 1. Inhibition by various H_2-agonists of fMet-Leu-Phe-stimulated O_2^- formation in human neutrophils: Comparison with H_2-agonistic activity in the isolated guinea-pig right atrium

Compound	A	R	X	Chain	Inhibition of fMet-Leu-Phe-stimulated O_2^- formation in human neutrophils		H_2-agonism in the isolated guinea pig right atrium[a]	
					IC_{50} (μM)[b]	i.a.[c]	rel. pot.[d]	i.a.[c]
1	CH	H	O	$CH_2CH(OH)CH_2$	178 ± 47.3	0.37	3.9	1.0
2	CH	H	S	CH_2CH_2	13 ± 6.4	0.56	5.0	1.0
3	CH	Cl	S	CH_2CH_2	9 ± 4.9	0.65	4.0	1.0
4	N	H	S	CH_2CH_2	170 ± 51.4	0.49	3.2	1.0
5	N	H	S	$CH_2CH_2CH_2$	250 ± 2.0	0.70	12.6	1.0



Compound	R	n	Human neutrophils			Guinea-pig atrium	
			IC_{50} (μM)[b]	rel. pot.[d]	i.a.[c]	rel. pot.[d]	i.a.[c]
6	H	1	145 ± 5.0	(0.05)	0.39	2.2	1.0
7	H	2	4.0 ± 0.4	1.68	0.79	24.5	1.0
8	4-CH_3	2	8.2 ± 3.4	0.82	0.77	51.3	1.0
9 (Arpromidine)	4-F	2	10 ± 1.6	0.67	0.95	102.3	1.0
10	4-Cl	2	2.7 ± 0.9	2.48	0.92	69.2	1.0
11	4-Br	2	4.4 ± 0.4	1.52	0.89	30.2	1.0
12	4-CF_3	2	5.2 ± 1.1	1.29	0.94	57.5	0.9
13	4-F	2	8.2 ± 1.8	0.82	0.84	123.0	1.0
14	3,4-F_2	2	3.5 ± 1.6	1.91	0.88	131.8	1.0
15	3,5-F_2	2	3.5 ± 2.3	1.91	0.86	112.2	1.0
16	2,4-Cl_2	2	7.4 ± 2.7	(0.91)	0.64	52.5	0.9
17	H	3	183 ± 30	(0.04)	0.44	4.1	1.0
Histamine			6.7 ± 1.2	1.00	1.00	1.0	1.0
Dimaprit			23 ± 4.6	0.29	0.89	0.71	1.0
Impromidine[e]			2.0 ± 0.2	3.35	1.00	48.1	1.0
(R)-α-Methylhistamine			188 ± 2.3	(0.04)	0.70	0.01	0.8

The effects of various H_2-agonists on O_2^- formation induced by fMet-Leu-Phe in human neutrophils were studied. H_2-agonists or distilled water (control) were added to reaction mixtures 5 min prior to fMet-Leu-Phe (1 μM). In the absence of H_2-agonists, neutrophils generated 5.1 ± 0.6 nmoles of $O_2^-/10^6$ cells. [a]data for the relative potencies and intrinsic activities of compounds 1–17, dimaprit, impromidine, histamine and (R)-α-methylhistamine in the guinea pig atrium were compiled from Parsons et al. (1977); Gerhard and Schunack (1980); Arrang et al. (1987) and Buschauer (1988a, b, c, 1989); the EC_{50} value for histamine in the guinea pig atrium is 1 μM; [b] data shown are the means ± SE of four to seven experiments carried out with different preparations of neutrophils; [c] intrinsic activity, referred to histamine = 1; [d] relative potency, referred to histamine = 1, in parentheses the relative potencies for H_2-agonists with intrinsic activity < 0.75 are given; [e] data for the relative potency and intrinsic activity of impromidine were compiled from Burde et al. (1989)

and 3 possessed similar potencies as histamine but were partial agonists, too. The H_2-agonistic activity of the arpromidine-like phenyl(pyridylalkyl)guanidines in human neutrophils strongly depended on the length of the carbon chain connecting the aromatic rings with the guanidine group. Compounds 6 and 17 are characterized by two- and four-membered carbon chains, respectively, and were much less potent and effective than histamine. In contrast, guanidines 7–16 possess a three-membered carbon chain and were similarly potent and effective as histamine. Halogenation of the phenyl ring did not substantially affect the potency of guanidines (compare guanidine 7 versus guanidines 9–12 and 14–16). However, except 2,4-dichlorination, halogenation slightly increased the intrinsic activity (compare compounds 7 versus compounds 9–11).

The interaction of arpromidine (9) with the potent H_2-antagonist, famotidine (Schunack 1987; Fagot et al. 1988), was studied (Fig. 2). Famotidine per se at the concentrations studied did not affect O_2^- formation (data not shown). Famotidine at fixed concentrations ranging from 0.3–10 μM shifted the concentration-response

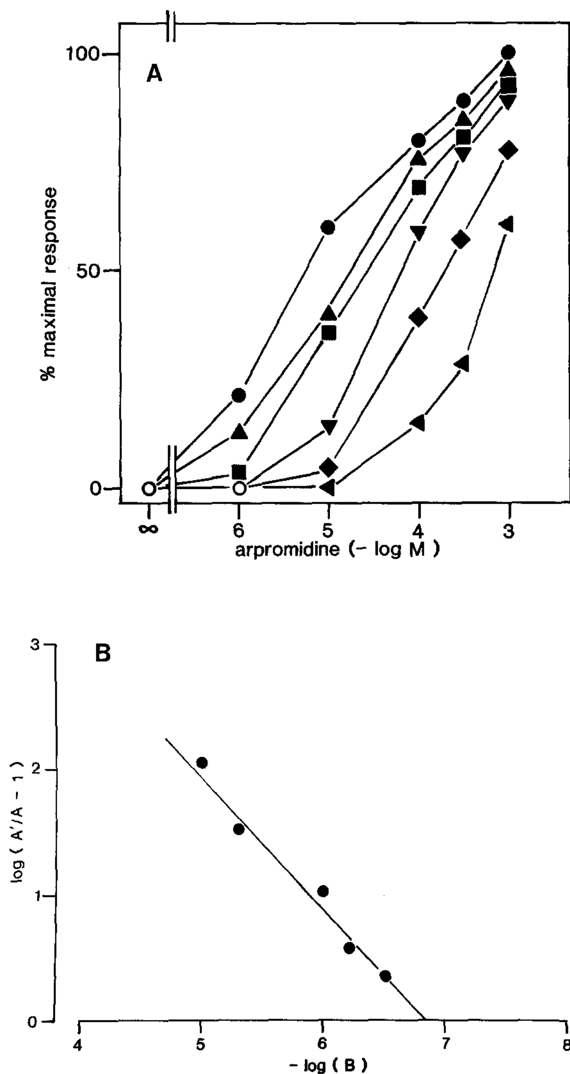


Fig. 2A, B. Effect of the H_2-antagonist, famotidine, on inhibition of fMet-Leu-Phe-induced O_2^- formation caused by guanidine 9 (arpromidine) in human neutrophils. Panel **A**: The effect of famotidine at various fixed concentrations on inhibition of O_2^- formation caused by compound 9 was studied. Compound 9 and famotidine or distilled water (control) were simultaneously added to reaction mixtures. After a preincubation period of 5 min, O_2^- formation was initiated by the addition of fMet-Leu-Phe (1 μM). The concentrations of famotidine were as follows: 0 μM (●), 0.3 μM (▲), 0.6 μM (■), 1 μM (▼), 5 μM (◆), 10 μM (◀). Data shown are the means of assay triplicates of an experiment performed with one preparation of neutrophils; these varied by less than 5%. Similar results were obtained in two experiments carried out with two different preparations of neutrophils. Panel **B**: Schild plot analysis of the data from Panel **A**. The agonist dose ratios A′/A were calculated, A being the concentration of compound 9 producing a given effect in the absence of famotidine and A′ being the concentration of compound 9 producing a given effect in the presence of famotidine at a concentration B. The regression line and the pA_2 value (intercept of the graph with the *abscissa*) were calculated as described (Tallarida and Jacob 1987)

curves for compound 9 to the right (panel A). These data were transformed into a Schild plot (Schild 1947; Tallarida and Jacob 1987) (panel B). The equation for the regression line is $y = -1.08 \times +7.41$ and the intercept with the abscissa (i.e. the pA_2 value for famotidine) is 6.48. These data indicate that famotidine competitively antagonizes the effects of guanidine 9 at neutrophil H_2-receptors.

The interaction of various other guanidines and histamine with the H_2-antagonist, cimetidine (Buschauer et al. 1989), was studied (Table 2). Cimetidine at 10 μM per se did not affect O_2^- formation but abolished the inhibitory effect of histamine at an equimolar concentration. In addition, cimetidine almost completely antagonized inhibition of O_2^- formation caused by guanidine 8 and attenuated the effects of compounds 3, 7, 10 and 11.

As a variety of guanidines were found to be partial agonists at neutrophil H_2-receptors (e.g. compounds 1–6, 16 and 17), we addressed the question whether they would act as partial antagonists in the presence of the full agonist, histamine. In *pars pro toto*, the interaction of

Table 2. Antagonism by cimetidine against inhibition of fMet-Leu-Phe-induced O_2^- formation in human neutrophils caused by histamine and by guanidines 3, 7, 8, 10 and 11

H_2-agonist	O_2^- formation (in % of control) H_2-antagonist	
	control	cimetidine
None	100	98[b]
Histamine	61	98[a]
Compound 3	80	87[a]
Compound 7	73	89[a]
Compound 8	76	94[a]
Compound 10	60	83[a]
Compound 11	64	85[a]

The effect of various H_2-agonists (10 μM) on O_2^- formation induced by fMet-Leu-Phe (1 μM) in the absence and presence of cimetidine (10 μM) was studied. H_2-agonists and cimetidine or distilled water (control) were added to reaction mixtures 5 min prior to fMet-Leu-Phe. In the absence of H_2-agonists and cimetidine, neutrophils generated 4.8 ± 0.6 nmoles of $O_2^-/10^6$ cells. Data shown are the means of five experiments carried out with different preparations of neutrophils; these varied by less than 10%. The statistical significance of the effects of cimetidine was assessed using the Wilcoxon test. [a] $p < 0.01$; [b] not significant

Table 3. Effect of aryloxyalkylguanidine 1 and phenyl(pyridylalkyl)guanidine 17 on fMet-Leu-Phe-induced O_2^- formation in human neutrophils: Antagonism against histamine

Addition	O_2^- formation (in % of control)		
	control	compound 1 (1 mM)	compound 17 (1 mM)
None	100	73	70
Histamine (100 μM)	16	59	72

The effects of guanidines 1 and 17 and of histamine at the indicated concentrations or combinations of these agents on O_2^- formation induced by fMet-Leu-Phe (1 μM) were studied. Histamine and/or compounds 1 or 17 or distilled water (control) were added to reaction mixtures 5 min prior to fMet-Leu-Phe. In the absence of the above substances, fMet-Leu-Phe induced the formation of 4.5 ± 0.5 nmoles of $O_2^-/10^6$ cells. Data shown are the means of four experiments carried out with different preparations of neutrophils; these varied by less than 10%

Table 4. Interaction of histamine and compounds 1, 10, 12, and 17 with IBMX on fMet-Leu-Phe-induced O_2^- formation in human neutrophils

H_2-agonist (1 mM)	O_2^- generation (in % of control)	
	control	IBMX
None	100	84*
Histamine	49	19*
Compound 1	73	55*
Compound 10	54	24*
Compound 12	43	20*
Compound 17	65	47*

The effects of various H_2-agonists in the absence and presence of IBMX (10 μM) on O_2^- formation induced by fMet-Leu-Phe (1 μM) were studied. H_2-agonists and IBMX or distilled water (control) were added to reaction mixtures 5 min prior to fMet-Leu-Phe. Data shown are the means of six experiments carried out with different preparations of neutrophils; these varied by less than 10%. The statistical significance of the effects of IBMX was assessed statistically using the Wilcoxon test. $^*p < 0.01$

guanidines 1 and 17, which possess very low intrinsic activity, with histamine was studied (Table 3). In fact, compounds 1 and 17 at 1 mM antagonized, at least in part, inhibition of O_2^- formation caused by histamine at 100 μM.

Finally, the interactions of IBMX with full and partial H_2-agonists were studied (Table 4). IBMX at a submaximally effective concentration (10 μM) inhibited O_2^- formation by 16%. In addition, IBMX additively enhanced the inhibitory effects of the full H_2-agonists, histamine, compounds 10 and 12, and of the partial H_2-agonists, guanidines 1 and 17, on O_2^- formation.

Discussion

We studied the effects of a series of guanidines on O_2^- formation in human neutrophils and found that these compounds are either full (e.g. compounds 9–12) or partial (e.g. compounds 1–6, 16 and 17) H_2-agonists (see Tables 1 and 3). The full H_2-agonists are similarly potent as histamine, and the partial H_2-agonists are either similarly potent as histamine (compounds 2, 3, and 16) or are less potent (e.g. compounds 1, 4 and 5). H_2-antagonists counteract the effects of partial and full H_2-agonists, and IBMX potentiates the effects of partial and full H_2-agonists on O_2^--formation (see Fig. 2 and Tables 2 and 4). In addition, the potent H_3-agonist and weak partial H_2-agonist, (R)-α-methylhistamine, is much less potent than histamine (see Table 1). All these data are in agreement with recent results showing that human neutrophils possess H_2-receptors (Gespach and Abita 1982; Gespach et al. 1982; Seligmann et al. 1983; Burde et al. 1989; Mitsuhashi et al. 1989).

Substantial evidence has been accumulated that H_2-receptors are heterogenous in various cell types (Luchins and Makman 1980; Parsons and Sykes 1980; Bertaccini and Coruzzi 1981, 1983; Gespach et al. 1982, 1986; Khan et al. 1987; Buschauer 1989; Mitsuhashi et al. 1989). Dissociations in the relative potency and intrinsic activity of H_2-agonists and differences in the potency of H_2-antagonists in various cell types suggest that subtypes of H_2-receptors exist (Bertaccini and Coruzzi 1983; Gespach et al. 1986). In order to answer the question of whether neutrophils possess cell-type specific H_2-receptors, we compared the structure/activity relationship of H_2-agonists in these cells with that in the guinea pig right atrium. The data concerning the properties of the H_2-receptor in the guinea pig atrium were taken from the literature and were obtained by us or by other groups of investigators (see Table 1). A disadvantage of our approach may be the fact that data with receptor agonists are less relevant than data with receptor antagonists with regard to the characterization of H_2-receptor subtypes (Bertaccini and Coruzzi 1983). Unfortunately, studies with H_2-antagonists on NADPH oxidase regulation are hampered by the fact that certain compounds containing imidazole structures, e.g. cimetidine, at concentrations above 10 μM per se may inhibit O_2^- formation, possibly due to interference with cytochrome b_{-245} (data not shown) (Ozaki et al. 1984; Iizuka et al. 1985). In addition, the guanidinothiazole, famotidine, at concentrations above 30 μM inhibits O_2^- formation (data not shown).

The data for the H_2-agonistic activities of dimaprit and (R)-α-methylhistamine in neutrophils are in agreement with the ones for the guinea pig atrium (Parsons et al. 1977; Gerhard and Schunack 1980; Arrang et al. 1987). However, there are several dissociations between the effects of guanidines 1–17 in these systems. First, guanidines 1–17 are all full agonists in the guinea pig atrium, but in the neutrophil, guanidines 1–6, 16 and 17 are only partial agonists. Second, guanidines 1–17 are 2–130-fold more potent than histamine in the guinea pig atrium. In contrast, in the neutrophil, guanidines 1, 4–6 and 17 are less potent than histamine, and the other guanidines are only up to 2.5-fold as potent as histamine. Third, in the neutrophil but not in the guinea pig atrium, shortening or elongation of the carbon chain connecting the aromatic rings with the guanidine group in phenyl-(pyridylalkyl)guanidines 6 and 17 is associated with a decrease in intrinsic activity. In contrast, the order of potency of guanidines is similar in both model systems ($6 < 7-16 > 17$). Fourth, in the guinea pig, the potency of phenyl(pyridylalkyl)guanidines is considerably increased by halogenation or by dihalogenation of the phenyl ring. In the neutrophil, halogenation of the phenyl ring slightly increases the intrinsic activity but not the potency of guanidines. Thus, structure/activity considerations for impromidine-like H_2-agonists in the guinea pig atrium reveal that these molecules may be varied in a wide range with considerable increase in potency and without decrease in intrinsic activity. In the neutrophil, however, the structural requirements of H_2-agonists appear to be more stringent. Interestingly, the differences between both test models are much less pronounced for relatively small molecules, i.e. histamine, dimaprit and (R)-α-methylhistamine, and are more prominent for guanidines with bulky lipophilic groups, e.g. diarylalkyl substituents.

Recently, Mitsuhashi et al. (1989) reported that H_2-agonists activated both phospholipase C and adenylyl cyclase in differentiated HL-60 cells through cholera toxin-sensitive guanine nucleotide-binding proteins. Histaminergic stimulation of phospholipase C would be expected to be associated with activation of NADPH oxidase (Rossi 1986), whereas histaminergic activation of adenylyl cyclase would lead to inhibition of O_2^- formation (Seligmann et al. 1983; Burde et al. 1989). Thus, the unique profile in potency and intrinsic activity of guanidines on NADPH oxidase may be due to the fact that these compounds differentially activate stimulatory and inhibitory signal transduction pathways in neutrophils through H_2-receptors. Further studies will have to address this hypothesis in detail.

The pA_2 values for famotidine in the neutrophil also deserve attention. The pA_2 values for famotidine in human neutrophils amount to 6.8 – 7.5 and are lower than the pA_2 values for famotidine in other systems (7.5 – 8.5) (see Fig. 2) (Schunack 1987; Burde et al. 1989). In addition, cimetidine at an equimolar concentration almost completely antagonized the effects of histamine and compound 8 on O_2^- formation, whereas the effects of the partial H_2-agonist, compound 3, and of the full agonists, guanidines 7, 10 and 11, were only partially antagonized by cimetidine (see Table 2). All these data support the concept that H_2-receptors are heterogenous, and we suggest that human neutrophils possess a cell-type specific subtype of H_2-receptors. Another interesting approach to characterize H_2-receptors in the guinea pig atrium and in the neutrophil would be the comparison of the potencies of guanidines to elicit biological effects and their affinity in binding studies. In this context it may be interesting to note that neutrophil purino- and pyrimidinoceptors also possess structural requirements for agonists which are different from those of nucleotide receptors in other cell types (Seifert and Schultz 1989; Seifert et al. 1989c).

However, other possibilities than H_2-receptor subtypes to explain the differential effects of H_2-agonists in the guinea pig atrium and in neutrophils cannot be ruled out. First, species-specificities of H_2-receptors may contribute, at least in part, to these differences. Second, the receptor reserves for histamine and synthetic H_2-agonists may be different (Rising and Steward 1986). Third, the metabolism of H_2-agonists may be different in the two systems. In addition, the tissue/cell type-specificity of the effects of H_2-agonists may be due to physicochemical differences in the microenvironment of the affinity-conferring domains of H_2-receptors (Khan et al. 1987; Buschauer 1989). Furthermore, differences in the kinetics of distribution of H_2-agonists in the guinea pig atrium and in neutrophils may contribute to differences in the effects of compounds 1 – 17. For example, the inhibitory effect of arpromidine (9) in neutrophils is evident immediately after its application (see Fig. 1), but in the guinea pig atrium, the effects of 9 are slow in onset (Buschauer 1989). This difference may be due to the fact that unlike in an intact tissue, diffusion barriers do not limit the accessibility of compound 9 for H_2-receptors in suspended neutrophils. Finally, it should be noted that in the guinea pig atrium, H_2-agonists per se exert stimulatory effects on the sinus node, whereas in the neutrophil H_2-agonists per se do not possess stimulatory effects but inhibit stimulated O_2^- formation. Thus, inhibition of fMet-Leu-Phe-induced O_2^- formation by H_2-agonists is an indirect method to assess H_2-receptors.

Regardless of whether H_2-receptor subtypes are or are not involved in the differential activity of H_2-agonists in the guinea pig atrium and in neutrophils, the relative selectivity of compounds 1 – 17 for cardiac tissue in comparison to neutrophils may be of therapeutical relevance. For example, a positive inotropic effect of H_2-agonists in congestive heart failure may be achieved without compromising O_2^- formation and hence the function of neutrophils in host defense. Conversely, our data raise the possibility that neutrophil-specific H_2-agonists may be developed. Inhibition of neutrophil functions by H_2-agonists may be of therapeutical value in a variety of pathological conditions associated with neutrophil activation, e.g. myocardial infarction, rheumatoid arthritis and nephritis (Malech and Gallin 1987).

Acknowledgements. The authors are grateful to Dr. G. Schultz for helpful suggestions, to Dr. E. Keller, Blutbank des Deutschen Roten Kreuzes Berlin, for providing buffy coat preparations and to Dr. W. Schunack for providing (R)-α-methylhistamine.

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