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Effects of Inhibitors on Chicken Polymorphonuclear Leukocyte Oxygenation Activity Measured by Use of Selective Chemiluminigenic Substrates

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Chicken heterophil polymorphonuclear leukocytes (CPMNLs) have NADPH oxidase activity, but lack myeloperoxidase (MPO). Stimulation of CPMNLs by phorbol 12-myristate 13-acetate or chicken opsonified zymosan results in luminol-dependent chemiluminescence (CL) activity, which is small relative to that of human peroxidase-positive neutrophils (HPMNLs), as well as lucigenin-dependent CL, comparable to HPMNL responses. Inhibitors were used to investigate and characterize the CL activity of CPMNLs. Inhibition constants were calculated, using Dixon inhibition analysis, or were reported as the concentration producing 50% inhibition of the magnitude of CL responses.

Azide and cyanide are effective inhibitors of luminol CL in HPMNLs, although these peroxidase inhibitors do not inhibit either luminol or lucigenin CL of CPMNLs. Since these agents also inhibit eosinophil peroxidase, lack of inhibition of CPMNL CL indicates that the small percentages of peroxidase-positive eosinophils in CPMNL preparations are not responsible for the luminol CL observed. Iodoacetate and fluoride, pre-oxidase and pre-peroxidase inhibitors of glycolytic metabolism, effectively inhibit lucigenin and luminol CL activities in CPMNLs. Superoxide dismutase competitively inhibits lucigenin and luminol CL in CPMNLs, but catalase is an ineffective inhibitor. Although luminol is efficiently dioxygenated by a MPO-dependent mechanism in HPMNL, use of peroxidase-deficient CPMNLs indicates that this substrate does not exclusively measure peroxidase activity.

Chicken polymorphonuclear (heterophil) leukocytes (PMNLs) have NADPH oxidase but lack myeloperoxidase (MPO) activity (1). Chemiluminescence (CL) is an energy product of the oxygenation activity following chicken PMNL activation by chemical or phagocytic stimuli (1-4). The sensitivity for detecting these microbicidal oxygenation activities is increased several magnitudes by introduction of high quantum yield chemiluminigenic substrates (CLSs) (5). These CLSs, such as luminol (5-amino-2, 3-dihydro-1, 4-phthalazinedione) and lucigenin (dimethyl biacridinium (DBA^{2+}) dinitrate), have different reactivities and measure different pathways of PMNL metabolism (5). Both CLSs have been used to measure respiratory burst activities (i.e., hexose monophosphate shunt metabolism and oxygen (O_2) consumption) of human and chicken PMNLs (1-9).

The DBA^{2+} luminescence reaction measures functional superoxide-producing oxidase activity of stimulated human and chicken PMNLs (1, 5, 9-16). At neutral pH, DBA^{2+} reacts with radical reducing agents and superoxide ($\cdot O_2^-$) to yield light (5, 7, 9, 17). Under more basic conditions, DBA^{2+} can directly react with hydrogen peroxide (H_2O_2) to yield luminescence (9).

Luminol is an efficient CLS for measuring peroxidase activity (5-9, 17). However, luminol dioxygenation and luminescence can also occur by peroxidase-independent pathways (5-9, 17). Stimulated chicken PMNLs produce a luminol-dependent luminescence that is small relative to that of human PMNLs (1). Although chicken PMNLs are devoid of MPO (18-23), chicken eosinophils contain an eosinophil peroxidase that is capable of producing luminol luminescence. However, a previous study indicated that luminol-dependent activity of chicken PMNLs was not well correlated with the presence of eosinophils (1).

An inhibitor-based approach to characterizing the oxidase and peroxidase activities of human PMNLs has been reported (10, 17, 24, 25). Azide and cyanide are inhibitors of MPO activity, but do not inhibit NADPH oxidase activity (12). Superoxide is a product of NADPH oxidase, and hydrogen peroxide arises by acid or enzymatic disproportionation of superoxide (5, 9, 26). As such, H_2O_2 is a secondary product of NADPH oxidase. Superoxide dismutase (SOD), an enzyme that converts superoxide to H_2O_2 and oxygen (O_2), is expected to inhibit superoxide-dependent reactions, such as the DBA^{2+} luminescence reaction, but is not expected to inhibit H_2O_2-dependent reactions. Catalase, an enzyme that converts H_2O_2 to water and O_2, is expected to inhibit H_2O_2-dependent reactions, such as the luminol luminescence reaction catalyzed by MPO, but is not expected to inhibit DBA^{2+}-dependent superoxide reactions. General inhibitors of glycolytic metabolism, such as sodium iodoacetate (IAA) and sodium fluoride (NaF), block reduction of $NADP^+$ to NADPH, depriving NADPH oxidase of its substrate, thus preventing generation of the H_2O_2 required for MPO activity in human PMNLs (17, 25).

The peroxidase-deficient PMNLs of chickens are ideally suited for investigating the relative roles of oxidase and peroxidase in PMNL oxidative microbicidal metabolism. In the study reported here, we used a battery of inhibitors to investigate the oxygenation activities of MPO-deficient rooster PMNLs in a manner that allowed comparison with the results of a previous study of MPO-containing human PMNLs (17).

Materials and Methods

Animals: This animal research was performed at an accredited

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institution following approval by an IACUC committee. Twelve adult Peterson roosters (Lester Hatchery, Gonzales, Texas) were studied. All animals were allowed ad libitum food (Purina chicken chow; Ralston Purina Co., St. Louis, Mo.) and water. Each rooster was allowed a 14-day acclimatization period prior to experimentation. Animals were housed individually in metal cages (30 × 30 × 32.5 in.) with rubber floor matting to prevent foot injury to the roosters.

Design of study: Rooster PMNLs were obtained as described (1). Approximately 20 ml of heparinized blood was obtained from each rooster (body weight > 5.4 kg) approximately every 10 to 14 days from a prominent wing vein. This phlebotomy schedule resulted in no apparent change in hematocrit over the course of these experiments.

The rooster whole blood was subjected to dextran sedimentation of erythrocytes followed by isopycnic centrifugation of the leukocyte-rich plasma as described (1) to achieve rooster PMNL preparations used in this study. Leukocyte differential counts were determined, and the PMNL concentration was adjusted to 5×10^5/ml as described (1).

Reagents: Luminol, 5-amino-2, 3 dihydro-1, 4 phthalazinedione (Sigma Chemical Co., St. Louis, Mo.), and lucigenin, dimethyl biacridinium, DBA^{2+}, (bis-N methylacridinium) nitrate (Aldrich Chemical Company, Inc., Milwaukee, Wis.), were prepared and stored as described (1). Appropriate dilutions of both CLSs were prepared in distilled water daily to achieve assay luminol concentrations of 10 and 2.5 μ*M*, and DBA^{2+} concentrations of 240 and 60 μ*M*. The CLS concentrations were verified spectrophotometrically as described (1).

Chemical (phorbol 12-myristate 13-acetate, PMA [Sigma Chemical Co.]) and particulate (chicken opsonized zymosan [cOZ]) stimuli were also prepared as described (1). Due to the low magnitude of the rooster PMNL luminol-dependent CL following cOZ stimulation (1), this CLS-stimulus combination was not investigated by use of certain inhibitors in this study. Inhibition assays were performed using 5×10^4 rooster PMNL (100 μl of a 5×10^5 PMNL/ml cell suspension) in phosphate buffered saline containing 0.1% (w/v) human albumin and 0.1% (w/v) glucose (PBS-HAG) buffer defined previously (1). The PMNLs were added to 8 ml siliconized borosilicate glass scintillation vials (Beckman Instruments, Inc., Palo Alto, Calif.) containing 1.7 ml of veronal (barbital) buffer (1), 100 μl of specified CLS, and 100 μl of inhibitor at one of eight concentrations tested. Chemiluminescence was determined in a single photon counter operated in a cyclic mode (each vial repeatedly counted sequentially at approximate 5-min intervals). Two luminescence determinations were obtained on each vial prior to stimulation to determine the basal activity of the PMNL preparations. The luminescence of each specimen was monitored for an additional 120-min period following stimulation.

Inhibitors: The inhibitors included sodium azide (NaN_3), sodium cyanide (NaCN), NaF, sodium iodoacetate ($C_2H_2IO_2Na$), SOD (source: bovine erythrocyte, activity: approx. 5,000 U/mg of protein (27), and catalase (source: *Aspergillus niger*, activity: approximately 6,000 U/mg of protein). All inhibitors were purchased from Sigma Chemical Co. The range of concentration for each inhibitor tested is shown in Table 1.

Analysis of data: Where the inhibition data were consistent with Dixon inhibition analysis, the inhibitor constant (K_i) was determined graphically from the plots (28). In cases where the inhibition was not linear over the entire concentration range of inhibitor tested, a subset of the range was used to calculate the degree of inhibition. For complex inhibition data that did not allow Dixon analysis, the concentration of inhibitor producing 50% inhibition (I_{50}) is reported.

Results

Unlike peroxidase-positive mammalian PMNLs, chicken PMNLs are devoid of MPO. As such, chicken leukocytes provide a model for investigation of the role of MPO in microbicidal oxygenation reactions. As described (1), the luminol luminescence response of rooster PMNLs is several orders of magnitude lower than that observed for human PMNLs. In contrast, the DBA^{2+} luminescence of rooster PMNLs is similar in magnitude to that of human PMNLs (1). We investigated the effects of nonspecific metabolic inhibitors and specific enzyme inhibitors of luminol and DBA^{2+} respiratory burst luminescence in rooster PMNLs. Table 1 presents the inhibition results, including approximate inhibition constant (K_i) or concentration of inhibitor producing 50% inhibition (I_{50}) when appropriate, for each inhibitor tested.

Azide, a potent inhibitor of MPO, selectively inhibits luminol-dependent luminescence in peroxidase-positive human PMNLs (17). At concentrations ranging from 0.015 to 1 μ*M*, sodium azide does not have inhibitory effects on either the luminol or DBA^{2+} luminescence responses of PMA-stimulated rooster PMNLs. The

Table 1. Summary of the effects of inhibitors on stimulated rooster polymorphonuclear leukocyte (PMNL) luminescence

CLS	Stimulus	Inhibitor Name	Inhibitor Concentration Range	K_i	I_{50}	Inhibition Type
Luminol	PMA	Sodium azide	0.015 - 1 μ*M*			None
Luminol	cOZ	Sodium azide	0.015 - 1 μ*M*			None
DBA^{2+}	PMA	Sodium azide	0.015 - 1 μ*M*			None
DBA^{2+}	cOZ	Sodium azide	0.015 - 1 μ*M*			None
Luminol	PMA	Sodium cyanide	0.39 - 25 μ*M*			None
DBA^{2+}	PMA	Sodium cyanide	0.39 - 25 μM			None
Luminol	PMA	Sodium fluoride	0.04 - 2.4 m*M*		1.8-2.4 m*M*	Mixed
Luminol	cOZ	Sodium fluoride	0.04 - 2.4 m*M*		2-2.4 m*M*	Mixed
DBA^{2+}	PMA	Sodium fluoride	0.04 - 2.4 m*M*		0.6-1.2 m*M*	Mixed
DBA^{2+}	cOZ	Sodium fluoride	0.04 - 2.4 m*M*		1.2-2.0 m*M*	Mixed
DBA^{2+}	PMA	Sodium iodoacetate	0.39 - 100 μ*M*		25 μ*M*	Uncompetitive
DBA^{2+}	cOZ	Sodium iodoacetate	0.39 - 100 μ*M*		10 μ*M*	Non-competitive
Luminol	PMA	Catalase	00.2 - 150 g/ml			None
DBA^{2+}	PMA	Catalase	00.2 - 150 g/ml			None
DBA^{2+}	cOZ	Catalase	00.2 - 150 g/ml			None
Luminol	PMA	SOD	0.03 - 125 U/ml	0.02 U/ml	0.1-0.2 U/ml	Competitive
Luminol	cOZ	SOD	0.03 - 125 U/ml	0.02 U/ml	0.05-0.2 U/ml	Competitive
DBA^{2+}	PMA	SOD	1.95 - 125 U/ml	35 U/ml	30-40 U/ml	Competitive
DBA^{2+}	cOZ	SOD	1.95 - 125 U/m	11 U/ml	20-40 U/ml	Competitive

CLS = chemiluminigenic substrate; PMA = phorbol myristate acetate; cOZ = chicken opsonized; DBA^{2+} = lucigenin; SOD = superoxide dismutase

Dixon plots of the data are presented in Fig. 1 where the abscissa (x-axis) presents the concentration of inhibitor (i.e., azide, in μM), and the ordinate (y-axis) presents the reciprocal of the maximal luminescence velocity (i.e., 1/megaphotons/min). Each concentration of inhibitor was tested at two concentrations of CLS, resulting in the two curves shown in each Dixon plot. In Figs. 1A and 1B, the CLSs are DBA^{2+} (60 and 240 μM) and luminol (2.5 and 10 μM), respectively. The zero-degree slopes of both curves in each plot indicate an absence of azide inhibition for either DBA^{2+} (Fig. 1A) or luminol (Fig. 1B). In addition, azide inhibition of either DBA^{2+} or luminol luminescence is not observed from cOZ-stimulated rooster PMNLs (data not shown). In direct contrast with the inhibition of luminol luminescence in peroxidase-positive human PMNLs, azide had no inhibitory effect on stimulated peroxidase-deficient rooster PMNLs. Likewise, cyanide, another inhibitor of MPO, had no effect on either luminol or DBA^{2+} luminescence of PMA-stimulated rooster PMNLs (Table 1). The effect of cyanide on the cOZ-stimulated luminol and DBA luminescence was not assessed.

Two scavenger enzymes also were used as inhibitors. Catalase, which catalyzes the breakdown of H_2O_2 to water and O_2, did not have an inhibitory effect on rooster PMNL luminescence within a concentration range from 0.2 to 150 μg/ml with either CLS. (Table 1). However, SOD, which catalyzes the disproportionation of superoxide to H_2O_2 and O_2, produced significant inhibition of DBA^{2+} and luminol luminescence of rooster PMNLs. Contrary to the effect of SOD on human PMNLs (17), SOD strongly inhibited the luminol luminescence of rooster PMNLs. The PMA-stimulated luminol luminescence was inhibited approximately 50% at a SOD concentration of 0.12 U/ml. Similar inhibition of PMA-stimulated DBA^{2+} luminescence required SOD concentration > 30 U/ml. The Dixon plots of Figs. 2A and 2B indicate kinetic patterns consistent with competitive inhibition by SOD (i.e., inhibition is overridden by high concentrations of CLS). Similar competitive inhibition of cOZ-stimulated rooster PMNLs by SOD was observed for luminol and DBA^{2+}-dependent CL (data not shown). The 50% inhibition value (I_{50}) and the graphically determined K_i for this inhibitor are shown in Table 1 for each CLS-stimulant combination.

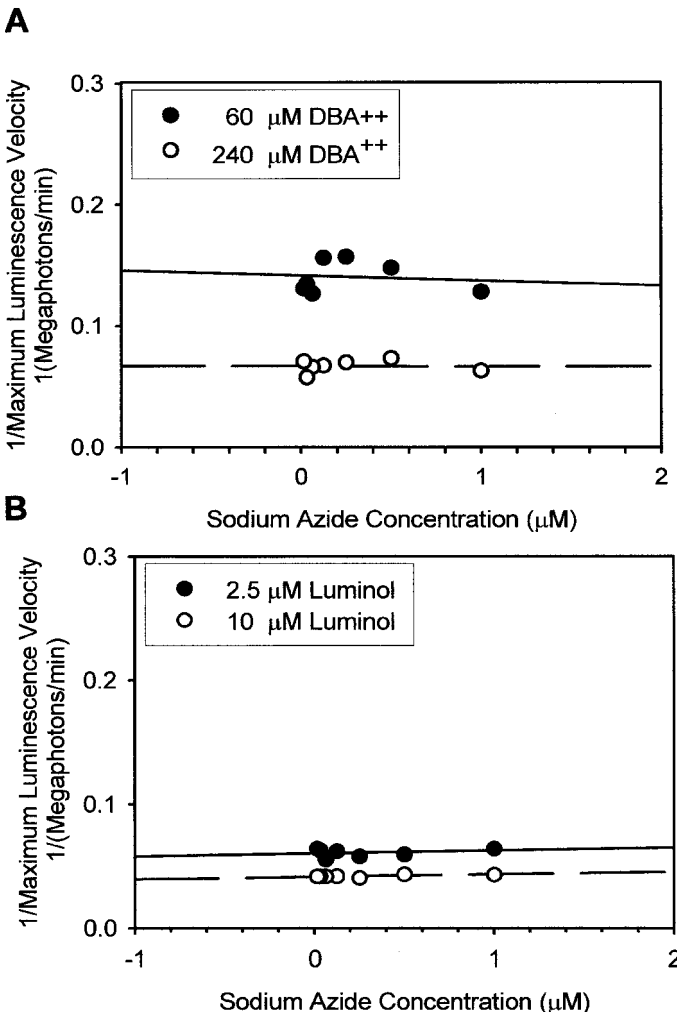

Figure 1. Dixon plot of the effect of sodium azide on maximal luminescent velocity of phorbol myristate acetate (PMA)-stimulated chicken heterophils in the presence of either lucigenin (DBA^{2+}) (plot A) or luminol (plot B). Two concentrations of each chemiluminigenic substrate (CLS) were used in each plot: 60 μM (●) and 240 μM (○) DBA^{2+} in plot A and 2.5 μM (●) and 10 μM (○) luminol in plot B.

Two general metabolic inhibitors, fluoride and iodoacetate, were investigated. Using concentrations ranging from 0.3 to 2.4 mM, sodium fluoride inhibits DBA^{2+}-dependent rooster PMNL luminescence (Table 1). However, with luminol as CLS, inhibition required > 1.2 mM NaF. The degree of NaF inhibition with luminol as CLS was approximately the same for both stimuli (Table 1). Fig. 3 depicts a Dixon plot showing the inhibition of cOZ-stimulated DBA^{2+} luminescence by NaF. The plots of the data clearly document inhibition at the higher concentrations of NaF tested. However, the curves are non-linear, and as such, the data cannot be interpreted on the basis of classical Dixon inhibition kinetics. The data shown in Fig. 3 are described by the power relationship: $1/Vmax = a + b\,[NaF]^c$. The value of the exponential c in this equation is > 1 for both curves. The R^2 for both curves of this plot exceeds 0.998. Similar non-linear NaF inhibition characteristics also were observed for PMA-stimulated luminol CL as well as cOZ- and PMA-stimulated DBA^{2+} luminescence (data not shown). The complex nature of NaF inhibition suggests that NaF may act at multiple levels, including formation of an ATP-Mg^{2+} complex with fluoride.

As anticipated, iodoacetate was a more effective general metabolic inhibitor. At concentrations as low as 4.2 μM, iodoacetate inhibited the DBA^{2+}-dependent luminescence response of rooster PMNLs to either stimuli. Dixon plots of iodoacetate inhibition of DBA^{2+} luminescence from PMA- and cOZ-stimulated rooster PMNLs are shown in Figs. 4A and 4B, respectively. This inhibitor appears to have somewhat different inhibition kinetics dependent on the stimuli used. A non-competitive pattern of IAA inhibition is observed for cOZ-stimulated DBA^{2+} luminescence (Fig. 4B). The pattern of IAA inhibition for PMA-stimulated DBA^{2+} luminescence (Fig. 4A) more closely resembles uncompetitive inhibition (non-intersecting curves). The I_{50} for cOZ-stimulated DBA^{2+} luminescence is estimated at a concentration of 10 μM iodoacetate and about 25 μM for DBA^{2+} luminescence stimulated with PMA. The effects of iodoacetate on luminol-dependent luminescence of rooster PMNLs were not examined.

Discussion

We reported that the respiratory burst metabolism of MPO-deficient rooster PMNLs could be monitored by measurement of CLS-dependent luminescence (1). The absence of MPO makes

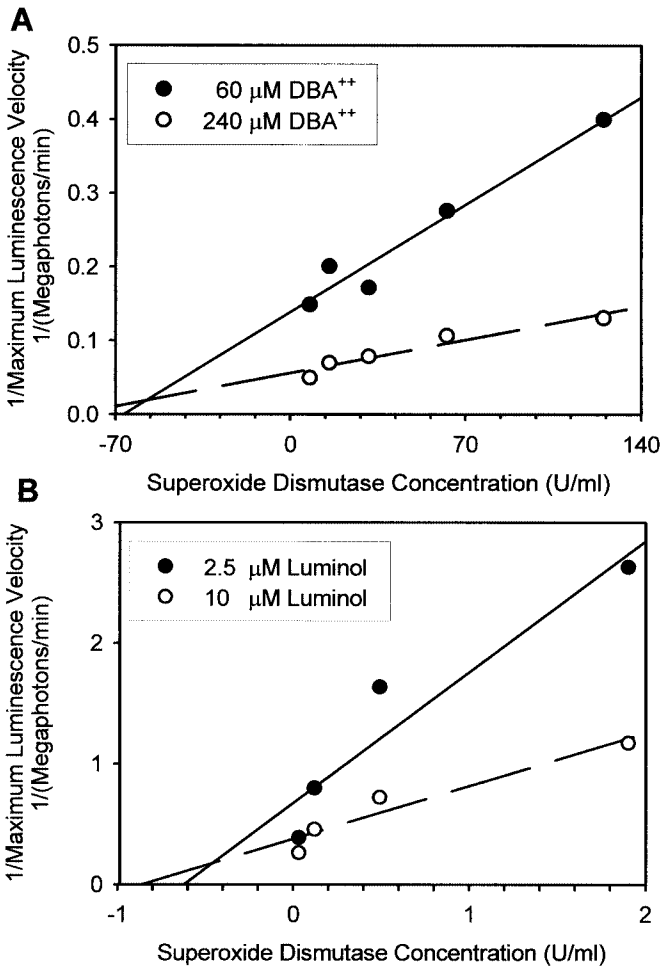

Figure 2. Dixon plot of the effect of superoxide dismutase on maximal luminescent velocity of PMA-stimulated chicken heterophils in the presence of either lucigenin (DBA^{2+}) (plot A) or luminol (plot B). Two concentrations of each CLS were used in each plot: 60 μ*M* (●) and 240 μ*M* (❍) DBA^{2+} in plot A and 2.5 μ*M* (●) and 10 μ*M* (❍) luminol in plot B.

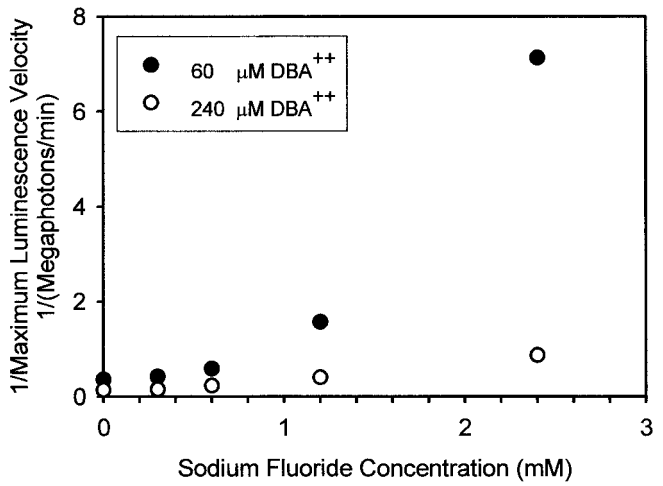

Figure 3. Dixon plot of the effect of sodium fluoride on maximal luminescent velocity of chicken cOZ-stimulated chicken heterophils in the presence of lucigenin (DBA^{2+}). Two concentrations of this CLS were used in this plot: 60 μ*M* (●) and 240 μ*M* (❍) DBA^{2+}.

the rooster PMNL an ideal model for investigating the role of MPO-independent redox reactions in the luminol and DBA^{2+} luminescence reactions (1). We used selective inhibitors to further elucidate these non-MPO mechanisms of PMNL oxygenation. The luminol luminescence responses of rooster PMNLs following stimulation with either PMA or cOZ are small relative to those of human PMNLs. However, the luminol luminescence responses of these peroxidase-deficient cells are highly significant relative to background, and reflect a non-peroxidase reaction mechanism. Contrary to the inhibitory effects on human PMNLs (17), azide and cyanide do not exert inhibitory action on the luminol-dependent luminescence of rooster PMNLs. These observations support the conclusion that MPO-dependent mechanisms are largely responsible for the luminol luminescence activity of human PMNLs, and also document that rooster PMNLs can produce luminol dioxygenation and luminescence by a peroxidase-independent pathway.

The findings of our previous study (1) did not exclude the possibility that a few eosinophils in the PMNL preparations might contribute to the luminol luminescence observed. In that study, significant correlation was not observed between eosinophil contamination and luminol-dependent CL (1). Azide and cyanide are potent inhibitors of luminol-dependent CL responses of human PMNLs. Likewise, eosinophil peroxidase is susceptible to azide and cyanide inhibition. The failure of azide or cyanide to inhibit the luminol luminescence responses of stimulated rooster PMNLs is consistent with lack of MPO and eosinophilic peroxidase activities.

Consistent with previous observations using human PMNLs (17), the DBA^{2+} luminescence responses of rooster PMNLs are not inhibited by either azide or cyanide. These inhibitors are not active against NADPH oxidase. Thus, azide and cyanide are not expected to inhibit superoxide formation and subsequent superoxide-dependent DBA^{2+} luminescence reactions.

The inhibitory action of iodoacetate and NaF on PMNL luminescence are of a general metabolic character. Both inhibitor mechanisms involve pre-oxidase and pre-MPO metabolism. As such, iodoacetate and NaF are expected to inhibit the DBA^{2+} and luminol luminescence activities of rooster PMNLs. Sodium fluoride can complex with divalent cations, and thereby, inhibit glycolysis by interaction with the Mg^{2+}-ATP complex. Iodoacetate inhibits glycolysis by a different mechanism involving reactions with the sulfhydryl groups of the glycolytic enzymes. Both of these inhibitors influence metabolism and cell viability. Inhibitors of glycolysis block generation of reducing equivalents and deprive the oxidase of its substrate, NADPH. As such, the subsequent production of superoxide and H_2O_2 by PMNLs is inhibited. On a molar basis, rooster PMNL metabolism and CLS-dependent luminescence are inhibited to a greater extent by IAA than by NaF.

Although not specific for superoxide, DBA^{2+} is selective in measuring PMNL oxidase activity. Peroxidases, including MPO, do not catalyze DBA^{2+} luminescence (9). One electron reduction of DBA^{2+} yields the radical $\cdot DBA^{+}$. This intermediate radical can react directly with superoxide via radical-radical, anion-cation annihilation to yield the unstable moloxide DBA-O_2 that disintegrates, yielding one electronically excited N-methylacridone and one ground state N-methylacridone. The excited N-methylacridone relaxes to ground state by photon emission. The overall reaction is a reductive dioxygenation yielding chemiluminescence (5, 9).

Superoxide is a reductant and can not directly react with luminol to yield light. One electron oxidation of luminol yields the radical ·luminol. This type of reaction can be catalyzed by classical

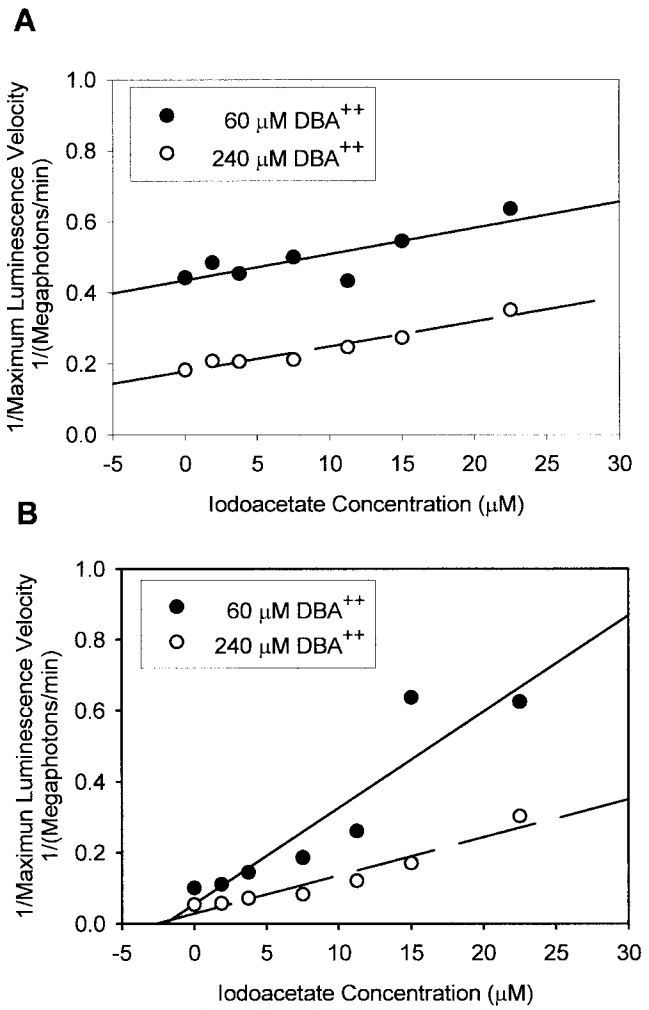

Figure 4. Dixon plot of the effect of iodoacetate on maximal luminescent velocity of PMA-stimulated (plot A) or cOZ-stimulated (plot B) chicken heterophils in the presence of lucigenin (DBA^{2++}). Two concentrations of DBA^{2++} were used in each plot: 60 μ*M* (●) and 240 μ*M* (❍).

peroxidases. Hydroxyl radical (·OH) and other radical oxidants can also catalyze this univalent oxidation. The intermediate ·luminol radical can react directly with superoxide via radical-radical annihilation to yield electronically excited aminophthalate and N_2. The excited aminophthalate relaxes to ground state by photon emission. The overall reaction is simple dioxygenation (5). Whereas the superoxide-dependent luminescence reaction with DBA^{2+} requires prior radical reduction of the CLS, the superoxide-dependent luminescence reaction with luminol requires prior radical oxidation (one electron dehydrogenation) of the CLS. The relative efficiency of non-peroxidase luminol dioxygenation yielding luminescence is low, as reflected by the minimal luminol CL of the peroxidase deficient rooster PMNLs.

Hydrogen peroxide is the secondary product of NADPH oxidase. It is generated by the acid- or SOD-catalyzed disproportionation of superoxide. Under alkaline conditions, H_2O_2 can directly react with DBA^{2+} to yield luminescence. However, the lack of catalase inhibition suggests that a H_2O_2 reaction pathway does not contribute significantly to either the DBA^{2+} or luminol luminescence in rooster PMNLs.

Superoxide dismutase inhibition of DBA^{2+} luminescence is consistent with previous observations using human PMNLs (17). Human and rooster PMNLs have functional oxidase activity. Super-oxide dismutase consumes superoxide, yielding H_2O_2 and O_2. The SOD competes with the radical intermediates $\cdot DBA^{+}$ and ·luminol for available superoxide, thereby exerting an inhibitory action. The competitive inhibition patterns obtained by Dixon analysis of SOD inhibition of DBA^{2+} and luminol luminescence are consistent with this mechanism. By contrast, in peroxidase-positive human PMNL, SOD only exerts a potent inhibitory action on DBA^{2+} luminescence and is an ineffective inhibitor of human PMNL luminol luminescence.

The participation of superoxide in the luminol luminescence of rooster PMNL is supported by the observed SOD inhibitor potency. The DBA^{2+} luminescence requires about 30 U of SOD/ml for 50% inhibition, whereas luminol luminescence requires 200-fold less SOD for comparable inhibition. The relative inefficiency of the superoxide-dependent luminol dioxygenation may render this process more susceptible to the action of SOD.

In conclusion, use of inhibitors of various enzymes and general metabolism has been successfully applied to the study of respiratory burst oxygenation activities of peroxidase-deficient rooster PMNLs. All data are consistent with the position that CLS-dependent luminescence directly results from the activity of rooster PMNL oxidase. Peroxidase inhibitors, such as azide and cyanide, did not exert significant inhibition of CLS luminescence. The results also support the position that peroxidase-positive rooster eosinophils that are susceptible to the action of these inhibitors do not contribute to the luminescence of the PMNL preparations.

Glycolytic inhibitors, such as iodoacetate and fluoride, were effective inhibitors of rooster PMNL metabolism, as measured by CLS luminescence. Superoxide dismutase was a potent competitive inhibitor of rooster PMNL metabolism, as measured by CLS luminescence. By destroying superoxide, the product of oxidase activity, SOD removes this reactant preventing its reaction with a CLS to yield luminescence. By contrast, catalase, an enzyme that destroys the secondary product of oxidase activity (i.e., H_2O_2) was not effective as an inhibitor of rooster PMNL metabolism, as measured by CLS luminescence.

Although luminol is preferentially dioxygenated by a peroxidase-based mechanism in peroxidase-positive leukocytes, this CLS does not exclusively measure peroxidase-dependent activity. Luminol can also be dioxygenated by less efficient oxidase-dependent mechanisms.

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