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HETEROGENEITY OF CIRCULATING AND EXUDATED POLYMORPHONUCLEAR LEUKOCYTES IN SUPEROXIDE-GENERATING RESPONSE TO CYCLIC AMP AND CYCLIC AMP-ELEVATING AGENTS

INVESTIGATION OF THE UNDERLYING MECHANISM

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Abstract—It has been found that cyclic AMP and cyclic AMP-elevating agents inhibit formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated superoxide production from polymorphonuclear leukocytes (PMNs). The quantitative differences of this inhibitory effect on human and rabbit blood versus human salivary and rabbit peritoneal (tissue) PMNs were investigated. PMNs from all sources showed the same pattern of fMLP-stimulated superoxide generation, although it was slightly higher in tissue PMNs. However, treatment with salbutamol differentially blunted fMLP-stimulated superoxide production from blood PMNs compared with tissue PMNs in both human and rabbit. While it could inhibit production from blood PMNs by 30–60%, it had only a negligible effect on generation from tissue PMNs. Similarly, forskolin, phosphodiesterase IV inhibitor Ro-201724, and dibutyryl cyclic AMP showed significantly higher inhibitory effects on superoxide generation from blood PMNs than tissue PMNs in both species. β-Adrenergic receptors, cyclic AMP accumulation, and protein kinase A activity were investigated in blood versus tissue PMNs to clarify the mechanism underlying the above-mentioned differences. At the β-adrenergic receptor level, no significant changes were detected in the number or the binding affinity of the receptors in tissue versus blood PMNs of human and rabbit. On the other hand, cyclic AMP accumulation was significantly higher in response to salbutamol and Ro-201724 in fMLP-stimulated blood versus tissue PMNs in human and rabbit. At the same time, blood PMNs showed significantly higher cyclic AMP-dependent protein kinase A activity than tissue PMNs in human and rabbit. We concluded that tissue PMNs are less responsive to the effect of cyclic AMP-elevating agents in terms of fMLP-stimulated superoxide inhibition. This is due to differences, at least, at two levels. The first is lower accumulation of cyclic AMP and the second is lower protein kinase A activity in tissue versus blood PMNs.

Key words: polymorphonuclear leukocytes; cyclic AMP; blood neutrophils; peritoneal neutrophils; salivary neutrophils; cyclic AMP-elevating agent

PMNs† take part in host defense against infection and in inflammatory and allergic conditions like asthma. To fulfill this role, they migrate from blood to various tissues. Many structural and functional changes have been found to take place in PMNs in association with this migration. Structurally, peritoneal PMNs have been reported to contain 10-fold more glycogen than blood PMNs in guinea pigs [1]. In rabbits, peritoneal PMNs are more fragile than blood PMNs under low osmotic pressure [2]. Functionally, enzyme activities and release of enzymes such as gelatinase and azocaseinase [3], migration [4] and adhesion properties [5] have changed in peritoneal PMNs versus blood PMNs. These differences between blood and tissue PMNs have also been observed in the context of drug actions in a number of reports. For example, duroquinone stimulates superoxide generation significantly more in peritoneal PMNs than in blood PMNs [3].

PMNs, whether circulating in blood or localized in tissues, have been shown to experience a characteristic biochemical event during phagocytosis or in response to stimulation by many endogenous or exogenous factors such as fMLP. This biochemical event is called the respiratory burst and is characterized by increased oxygen consumption, enzyme release and superoxide generation [6]. Superoxide generation is one way that PMNs combat invasive pathogens, but there are instances in which it plays a role in the pathogenesis of some disease states.

It has been demonstrated that, in human blood PMNs, fMLP-induced superoxide generation is inhibited by a β-adrenoceptor agonist [7] and an inhibitor of cyclic AMP phosphodiesterase type IV

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† Abbreviations: PMNs, polymorphonuclear leukocytes; fMLP, formyl-methionyl-leucyl-phenylalanine; SOD, superoxide dismutase; and HBSS, Hanks' Balanced Salt Solution containing 10 mM HEPES, pH 7.4.

[8] through the elevation of the intracellular cyclic AMP level. The transformation of this response after PMN migration to the tissues was investigated in this work. We studied the inhibitory effects of cyclic AMP and cyclic AMP-elevating agents on fMLP-induced superoxide generation in human and rabbit blood PMNs versus salivary and peritoneal PMNs in the same species. The mechanism behind the differences was investigated and discussed at different levels of cyclic AMP formation and action.

MATERIALS AND METHODS

Chemicals. Salbutamol, fMLP, propranolol, ferricytochrome *c* and Histopaque were purchased from Sigma (U.S.A.). Forskolin and SOD were purchased from Wako (Japan). Ro-201724, [^{3}H]-CGP-12177 (1.85 TBq/mmol), dextran (mol. wt 208,000), HEPES and dibutyryl cyclic AMP were purchased from Funakoshi (Japan), New England Nuclear (U.K.), Nacalai (Japan), DOJIN (Japan) and Boehringer Mannheim GmbH (Germany), respectively. Radioassay systems for cyclic AMP and protein kinase A were purchased from Amersham plc (U.K.) and Gibco (U.S.A.), respectively.

Preparation of PMNs. Human blood PMNs were isolated as previously described [9] with minor changes. Briefly, venous blood collected in sodium citrate solution (3.8%) from healthy volunteers was centrifuged (110 *g*, 10 min), and the platelet-rich plasma was discarded. The remaining part of the blood was mixed (1:1, v:v) with a solution of 3% dextran in 0.9% sodium chloride solution in a plastic syringe and fixed vertically for 20 min at 25°. PMN-rich plasma was collected from the upper layer of the suspension and centrifuged (250 *g*, 10 min). The pellet was subjected to hypotonic lysis to destroy the remaining erythrocytes, centrifuged, and then suspended in HBSS. The suspension was cushioned carefully on Histopaque solution (d = 1.077) and centrifuged (420 *g*, 30 min) at 20°. The purified PMNs of the bottom pellet were finally resuspended in HBSS.

Rabbit blood PMNs were obtained from cardiac blood of Japanese white rabbits according to the same method as described above.

Human salivary PMNs were prepared by nylon mesh filtration followed by Histopaque centrifugation. Briefly, intensive mouth washings with saline of each normal donor were collected through nylon mesh in 50-mL centrifuge tubes, centrifuged (250 *g*, 10 min) and the pellets suspended in HBSS. Then the cell suspension was cushioned carefully on Histopaque (d = 1.083) and centrifuged (420 *g*, 30 min) at 20°. Pure salivary PMNs were collected from the interface between Histopaque and HBSS, washed and resuspended again in HBSS.

Rabbit peritoneal PMNs were obtained by collecting the PMN-rich exudate from rabbits 8–12 hr after injection of 150 mL/kg of sterile 0.2% (w/v) glycogen in 0.9% sodium chloride solution. PMNs were washed twice with HBSS and then suspended in HBSS.

The purity of the PMNs was greater than 95% in all the above preparations from the different sources. Cells were counted by a Coulter counter model ZM (Coulter, U.S.A.), and diluted in HBSS to the final needed concentrations and kept on ice until the time of the experiment. For good comparison, the latent time between getting the PMN-containing body fluids and the time of the experiments was adjusted to almost the same (about 4 hr including the time needed for the isolation of PMNs).

Superoxide measurement. The superoxide-releasing activity of PMNs was assessed by SOD-inhibitable reduction of ferricytochrome *c* [10]. Briefly, PMNs (1×10^6 cells/mL) were incubated with cyclic AMP or cyclic AMP-elevating drug for 10 min at 37°. After this incubation, 100 μM ferricytochrome *c* (horse ferricytochrome *c*, type IV) and fMLP were added simultaneously, and then they were incubated for another 15 min. The reaction was terminated by adding SOD under incubation in an ice bath. The tubes were centrifuged (1500 *g*, 10 min), and the supernatants were measured by a dual wavelength spectrophotometer (U-best-50, JASCO, Tokyo, Japan) at 550–540 nm. One millimolar extinction coefficient of reduced cytochrome *c* at 550 nm is 29.5.

Cyclic AMP measurement. Cyclic AMP was measured by the iodinated assay system of Amersham. The experiment was performed by exposing 1.4×10^6 PMNs/mL suspension to fMLP (100 nM) and salbutamol (1 or 10 μM) simultaneously. The reaction was terminated after 30 sec by the addition of 0.1 vol. of 20% ice-cold perchloric acid and incubation in an ice bath. In the Ro-201724 experiments, PMNs were incubated for 10 min at 37° with Ro-201724 (10 μM); fMLP was added after this incubation. The reaction was terminated, by the same method as in the salbutamol experiments, after 30 sec or 3 min of the fMLP addition. Cyclic AMP content was determined in 100-μL aliquots of the supernatant by radioimmunoassay [11].

Protein kinase A activity measurement. This was achieved by using the radioassay system of protein kinase A from Gibco. PMNs were sonicated and then centrifuged at 100,000 *g* at 4° for 60 min to separate the cytosolic fraction. Protein kinase A-rich fraction was incubated with the synthetic protein kinase A substrate (Kemptide: Leu-Arg-Arg-Ala-Ser-Leu-Gly) and [^{32}P]-ATP and cyclic AMP (0.01 to 10 μM). Phosphorylated Kemptide was separated by using the binding paper (phosphocellulose disk paper). The binding paper was washed, and radioactivity of the paper was measured by a liquid scintillation counter (Beckman LS 6500, U.S.A.).

Binding assay study. The specific binding of the radiolabelled β-adrenergic ligand to cells was determined as published elsewhere [12]. The experiments were performed by incubating the PMNs (1×10^6 cells/mL) with the hydrophilic β-adrenergic ligand [^{3}H]-CGP-12177, which binds only surface receptors and cannot enter the cells [13], at 25° for 30 min. Then the mixture was filtrated under reduced pressure (400 mbar) using the Cell Harvester System (Inotech, Switzerland) over G-10 glass filters (Inotech, Switzerland). The filters were washed with ice-cold, calcium-free HBSS and were dried and counted in 4 mL of scintillation fluid using a scintillation counter. The specific binding of [^{3}H]-CGP-12177 was calculated from the difference

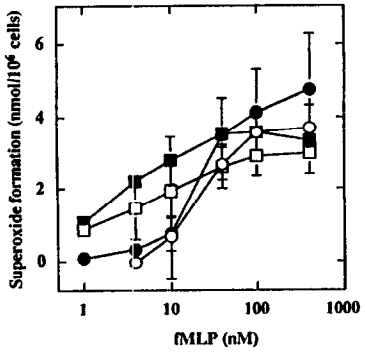

Fig. 1. Concentration-dependent fMLP-stimulated superoxide generation in human blood (○), human salivary (●), rabbit blood (□) or rabbit peritoneal (■) PMNs. Data are the means ± SD of 3 independent experiments.

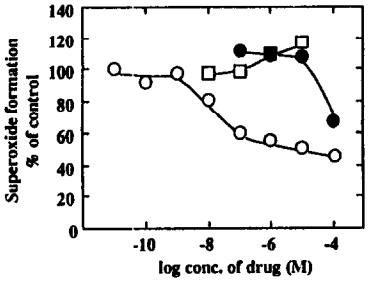

Fig. 2. Effects of different concentrations of salbutamol (○), propranolol (□), or the combination of different concentrations of salbutamol with 1 μM propranolol (●) on fMLP (100 nM)-stimulated superoxide generation in human blood PMNs. Results are the means of 2 independent experiments.

between the counts in the presence and absence of 10 μM propranolol.

Statistics. Results are expressed as means ± SD. Statistically significant differences were determined by using Student's two-tailed unpaired *t*-test.

RESULTS

fMLP-induced superoxide generation. Different concentrations of fMLP (1–1000 nM) were used to stimulate superoxide generation from human blood and salivary PMNs, and rabbit blood and peritoneal PMNs. Superoxide generation from tissue PMNs, i.e. human salivary and rabbit peritoneal PMNs, was higher than from blood PMNs in both species. However, the pattern of the concentration-response curve and the concentration of fMLP needed for maximal stimulation appeared to be almost the same (Fig. 1).

Effects of drugs on superoxide generation in human PMNs. Salbutamol (0.01 to 100 μM) inhibited fMLP (100 nM)-stimulated superoxide generation from human blood PMNs in a concentration-dependent manner. This inhibition could be blocked by the β-adrenergic blocker propranolol (1 μM), as shown in Fig. 2. In separate comparative experiments, salbutamol (10–1000 nM) did not elicit any inhibitory effect on fMLP-induced superoxide production from human salivary PMNs, but it inhibited about 60% of the fMLP-induced superoxide production from human blood PMNs. The inhibition rates between human salivary and blood PMNs were significantly different (Fig. 3A).

Although forskolin (1–100 μM) produced some inhibitory effect (1–32%) on fMLP-induced superoxide generation in salivary PMNs, this effect was still much smaller than the effect on blood PMNs (24–70%). The differences between the two types of PMNs were statistically significant (Fig. 3B).

The specific phosphodiesterase IV inhibitor Ro-201724 (0.01 to 10 μM) showed some inhibitory effect (0–50%) on fMLP-induced superoxide generation in salivary PMNs, but this inhibitory effect was much smaller than the inhibition produced by the same concentrations in blood PMNs (15–90%). The differences were statistically significant (Fig. 3C).

Dibutyryl cyclic AMP at a concentration of 1 mM completely inhibited fMLP-induced superoxide production in human blood PMNs. It also inhibited superoxide production in salivary PMNs (7–61%) but to a lesser extent than in blood PMNs, and the differences were statistically significant (Fig. 3D).

Effects of drugs on fMLP-induced superoxide generation in rabbit PMNs. The effect of salbutamol (0.1 to 10 μM) on rabbit blood and peritoneal PMNs exhibited a pattern similar to that on human blood and salivary PMNs. Salbutamol (10 μM) inhibited by about 30% fMLP-induced superoxide generation in rabbit blood PMNs, whereas it had no effect on peritoneal PMNs (Fig. 4A).

Like salbutamol, forskolin (1–100 μM) also inhibited fMLP-induced superoxide production more in blood than in peritoneal PMNs (Fig. 4B).

Ro-201724 (0.01 to 10 μM) inhibited superoxide production from peritoneal PMNs only 30%. It inhibited fMLP-stimulated superoxide generation from rabbit blood PMNs (about 50%) but not as much as that from human blood (90%). Inhibition of superoxide generation in rabbit blood PMNs was significantly greater than in peritoneal PMNs in the range of the concentrations examined (Fig. 4C).

The inhibition pattern of fMLP-induced superoxide generation by dibutyryl cyclic AMP in rabbit blood and peritoneal PMNs looks like that found in the human. However, a statistically significant difference was observed at only one concentration (0.4 mM) (Fig. 4D).

Cyclic AMP responses to drugs in human PMNs. In response to salbutamol (1 or 10 μM), cyclic AMP levels in salivary PMNs increased only slightly (about 5%). This increase in cyclic AMP was significantly higher in human blood PMNs than in salivary PMNs. The level of cyclic AMP at 1 μM salbutamol was almost comparable to that at 10 μM (Fig. 5A).

Ro-201724 caused a small accumulation of cyclic AMP in salivary PMNs, whereas in blood PMNs this compound elicited a significantly higher increase

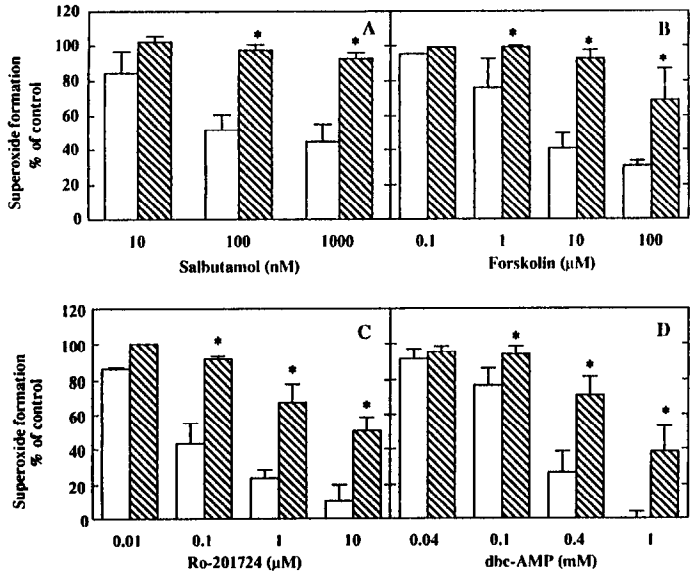

Fig. 3. Effects of different concentrations of salbutamol, forskolin, Ro-201724 or dibutyryl cyclic AMP on fMLP-induced superoxide generation in human blood (□) and human salivary (▨) PMNs. Results are the means ± SD of 3–6 independent experiments. Key: (*) significant difference ($P < 0.05$) from human blood PMNs.

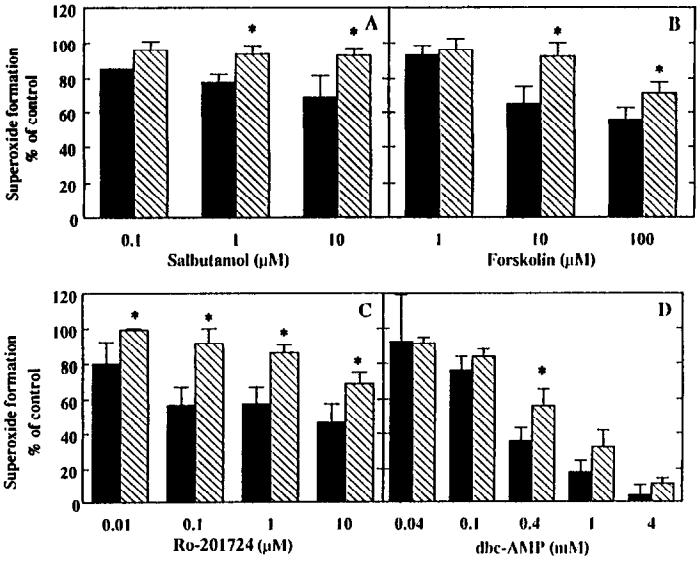

Fig. 4. Effects of different concentrations of salbutamol, forskolin, Ro-201724 or dibutyryl cyclic AMP on fMLP-induced superoxide generation in rabbit blood (■) and peritoneal (▨) PMNs. Results are the means ± SD of 3–6 independent experiments. Key: (*) significant difference ($P < 0.05$) from rabbit blood PMNs.

(about 3 times). This change was similar at both 30 sec and 3 min after the start of the reaction by adding fMLP (Fig. 5).

Cyclic AMP responses to drugs in rabbit PMNs. The picture of differentiation in cyclic AMP formation between rabbit peritoneal and blood PMNs was roughly similar to that between human salivary and blood PMNs. Salbutamol (1 or 10 μM) increased cyclic AMP twice as much in blood PMNs as in peritoneal PMNs. In the same manner, Ro-201724 increased cyclic AMP in blood PMNs 2–3 times more than in peritoneal PMNs (Fig. 6).

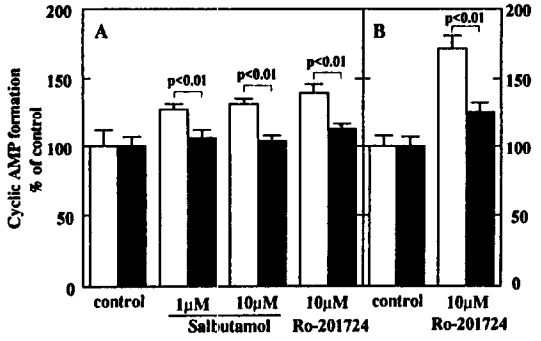

Fig. 5. Effect of salbutamol or Ro-201724 on fMLP (100 nM)-stimulated cyclic AMP formation in human blood (□) or salivary (■) PMNs. (A) The experiment was terminated 30 sec after the addition of fMLP. Salbutamol was added simultaneously with fMLP, whereas in the Ro-201724 experiments PMNs were incubated with Ro-201724 for 15 min before the addition of fMLP. Control values were 597.6 ± 67.3 fmol/10^6 cells for blood PMNs and 659.1 ± 42.9 fmol/10^6 cells for salivary PMNs. (B) The experiment was terminated 3 min after the addition of fMLP. PMNs were incubated with Ro-201724 for 15 min before the addition of fMLP. Control values were 516.0 ± 40.4 fmol/10^6 cells for blood PMNs and 620.4 ± 42.0 fmol/10^6 cells for salivary PMNs. Results are the means ± SD of 4 independent experiments.

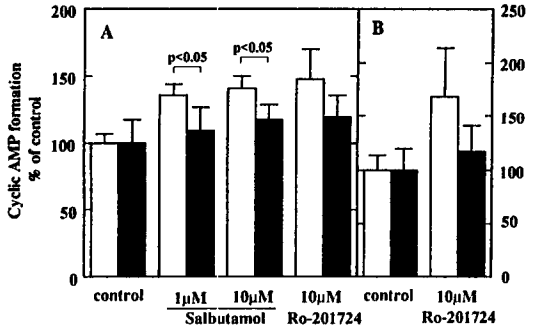

Fig. 6. Effect of salbutamol or Ro-201724 on fMLP (100 nM)-stimulated cyclic AMP formation in rabbit blood (□) or peritoneal (■) PMNs. (A) The experiment was terminated 30 sec after the addition of fMLP. Salbutamol was added simultaneously with fMLP, whereas in the Ro-201724 experiments PMNs were incubated with Ro-201724 for 15 min before the addition of fMLP. Control values were 728.1 ± 92.8 fmol/10^6 cells for blood PMNs and 572.4 ± 187.4 fmol/10^6 cells for peritoneal PMNs. (B) The experiment was terminated 3 min after the addition of fMLP. PMNs were incubated with Ro-201724 for 15 min before the addition of fMLP. Control values were 770.2 ± 188.2 fmol/10^6 cells for blood PMNs and 526.1 ± 203.7 fmol/10^6 cells for peritoneal PMNs. Results are the means ± SD of 4 independent experiments.

Protein kinase A activity in human PMNs. Stimulation of protein kinase A in the cytosolic fractions by different concentrations of cyclic AMP showed different patterns between blood and salivary PMNs. Blood PMNs had significantly higher cyclic AMP-dependent protein kinase A activity (Fig. 7A).

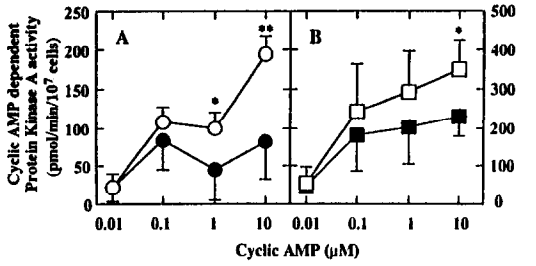

Fig. 7. (A) Cyclic AMP-dependent protein kinase A activity in human blood (○) and human salivary (●) PMNs. (B) Cyclic AMP-dependent protein kinase A activity in rabbit blood (□) and peritoneal (■) PMNs. Results are the means ± SD of 4–5 independent experiments. Key: (*) and (**) indicate significant differences from salivary PMNs at $P < 0.05$ and $P < 0.01$, respectively.

Table 1. Characterization of [^{3}H]-CGP-12177 binding to the β-adrenergic receptors in blood or tissue PMNs

		N	B_{max} (fmol/10^6 cells)	K_d (nM)
Human	Blood	3	1.02 ± 0.26	0.21 ± 0.03
	Salivary	3	1.51 ± 0.42	0.26 ± 0.08
Rabbit	Blood	3	1.06 ± 0.36	0.27 ± 0.09
	Peritoneal	3	1.21 ± 0.23	0.39 ± 0.21

Values are the means ± SD of 3 independent experiments in duplicate.

Protein kinase A activity in rabbit PMNs. In the same manner as in human PMNs, rabbit blood PMNs showed higher cyclic AMP-dependent protein kinase A activity than peritoneal PMNs. This difference was statistically significant at a cyclic AMP concentration of 10 μM (Fig. 7B).

Binding study of β-adrenergic receptors in human and rabbit PMNs. The binding study of the hydrophilic β-adrenergic antagonist [^{3}H]-CGP-12177 showed no decrease in the binding capacity (B_{max}) in tissue PMNs in comparison to blood PMNs. Nor was there a significant difference in the dissociation constants (Table 1 and Fig. 8) of either type of PMN.

DISCUSSION

Intracellular formation of cyclic AMP is a main inhibitory signal to many responses induced by various stimulators acting on different sites of PMNs. It inhibits migration of PMNs [14], production and secretion of eicosanoid and platelet-activating factor (PAF) [15, 16], aggregation [17] and exocytosis [17–19]. Inhibition of some of these responses is mediated probably through phospholipase D and A_2 inhibition [15, 16, 20, 21]. Cyclic AMP can inhibit some responses to fMLP [15, 17, 22], ionophore A23187 [15], C5a [17], interleukin-8 [18, 23], neutrophil-

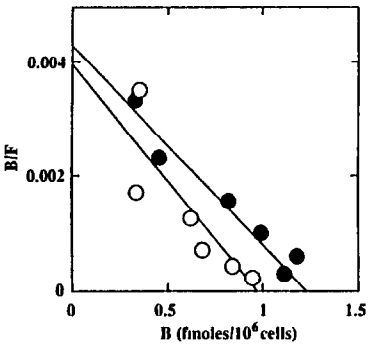

Fig. 8. Scatchard plots of [^{3}H]-CGP-12177 binding to the β-adrenoceptor in human blood (○) or human salivary (●) PMNs.

activating peptide 2 [18], granulocyte-macrophage colony-stimulating factor (GM-CSF) [19], tumor necrosis factor (TNF) [19] and PAF [24]. In light of the wide involvement of cyclic AMP mentioned above, we can perceive that cyclic AMP plays an important role in regulating PMN responses. In most of these studies, experiments have been performed using circulating PMNs.

Cyclic AMP-modulating drugs have been widely used in clinics for the treatment of many diseases. They have been used in conditions where superoxide or PMN dysfunction has been blamed for playing a role in the pathogenesis of, for example, asthma, ischemic diseases and hypertension [25]. These drugs include β-adrenergic agonists and antagonists, phosphodiesterase inhibitors, adenosine receptor agonists and antihistamines. There are also many endogenous factors that can modulate the cyclic AMP level in PMNs. These factors include circulating ones, such as catecholamines and glucagon [26, 27], or locally produced factors, such as prostaglandins, histamine and adenosine [28–30]. Based on these facts, it is important to know the effects of cyclic AMP and its modulators on PMN function.

In accord with their location, blood and tissue PMNs are engaged differently in immune responses, and some of these differences have been investigated. Blood PMNs differ from tissue PMNs in their adhesion properties [6], migration [5] and granule enzymes [4]. Yamamoto *et al.* [31] have found that salivary PMNs generate more superoxide than blood PMNs in response to fMLP. We have found the same difference between blood and tissue PMNs, whether they were isolated from an oral or a peritoneal cavity, though to a lesser extent than found by Yamamoto (Fig. 1). This is probably due to the fact that they ignored the difference in the latent period between the sampling time of the body fluid and measurement of superoxide production. They suggested that salivary PMNs have higher superoxide-generating responses than blood PMNs, attributing it to the fact that salivary PMNs had been already primed in association with exudating into the oral cavity. Our unpublished observation that G-CSF and TNF-α failed to prime tissue PMNs agrees with their conclusions.

β-Adrenergic agonists like isoproterenolol [32], the adenylate cyclase stimulant forskolin [16], phosphodiesterase inhibitors [8] and dibutyryl cyclic AMP [33] have been reported to inhibit fMLP-stimulated superoxide production and some other responses (mentioned earlier) of blood PMNs. These results are in agreement with ours. The mechanism of this effect remains to be clarified, but some studies suggested the inhibition of phospholipase D as one possible mechanism of action [20, 21]. However, a study in which the effects of such cyclic AMP-elevating agents would be compared between blood and tissue PMNs was needed. We chose fMLP as a stimulant and investigated superoxide formation as a response by employing some drugs that act on the different steps of cyclic AMP synthesis and action.

The β-agonist salbutamol, which acts on surface receptors, showed only negligible inhibition of superoxide production from fMLP-stimulated tissue PMNs originating from the human oral cavity and the rabbit peritoneal cavity, whereas in human and rabbit blood PMNs it produced a noticeable inhibition of about 40%. This effect could be blocked by propranolol. In accordance, salbutamol only produced a minimal increase of cyclic AMP in tissue PMNs as compared with blood PMNs from both human and rabbit. By studying the specific binding of [^{3}H]-CGP-12177 to β-adrenergic receptors, we could not demonstrate any decrease in the binding density or a significant change in the dissociation constant in tissue PMNs compared with blood PMNs. It is concluded that the failure of salbutamol to inhibit superoxide generation in tissue PMNs is not due to any changes in the β-adrenergic receptors. It may be due, in part, to less accumulation of cyclic AMP by salbutamol.

Forskolin (100 μM) inhibited superoxide production by about 25% in tissue PMNs. However, this inhibition was significantly lower than in blood PMNs in both species (Figs. 3 and 4). Forskolin acts directly on adenylate cyclase to increase cyclic AMP. These results confirm our conclusion that the different effects of salbutamol on blood and tissue PMNs are due to a difference at the post-receptor site.

The specific phosphodiesterase IV inhibitor Ro-201724 produced significantly more inhibition of the fMLP-stimulated superoxide production from human or rabbit blood PMNs than from human salivary or rabbit peritoneal PMNs. It has been found that fMLP can cause a transient increase in cyclic AMP [33]. This increase has been shown to be due to stimulation of adenylate cyclase, probably by endogenously produced adenosine [34]. After incubation of PMNs with Ro-201724 (10 μM) and stimulation with 100 nM fMLP, cyclic AMP increase was higher in blood than in tissue PMNs in both human and rabbit (Figs. 5 and 6). Generally speaking, the increase of cyclic AMP accumulation reflects an increase in adenylate cyclase activity and/or a decrease in cyclic AMP breakdown by phosphodiesterase. In this experiment, phosphodiesterase was inhibited by a high concentration of Ro-201724, but the differences in cyclic AMP

accumulation between blood and tissue PMNs still exist. This may indicate that adenylate cyclase activity is higher in blood PMNs than in tissue PMNs, giving an explanation for the difference in superoxide generation between tissue and blood PMNs in response to Ro-201724, salbutamol and forskolin.

Dibutyryl cyclic AMP (a lipid soluble analog of cyclic AMP) showed a concentration-dependent (0.04 to 1 or 4 mM in human and rabbit PMNs, respectively) inhibition of fMLP-stimulated superoxide production in both tissue and blood PMNs (Figs. 3 and 4). However, its effect was significantly higher on blood than on tissue PMNs. The usual target site of cyclic AMP is protein kinase A. Huang *et al.* [35] found that rabbit peritoneal neutrophils, unlike human blood neutrophils, have protein kinase AII activity but no protein kinase AI activity. In their study, they did not exclude the species difference as a cause of this finding. We demonstrated that blood PMNs have higher cyclic AMP-dependent protein kinase A activity than tissue PMNs in human and rabbit. This can explain, in part, the difference in dibutyryl cyclic AMP effect on superoxide production between blood and tissue PMNs. However, we cannot exclude other differences beyond protein kinase A at this moment.

In this study, we demonstrated that cyclic AMP and cyclic AMP-elevating agents inhibit superoxide production from tissue PMNs less effectively than from blood PMNs. This difference was due to at least two factors: first, the decrease in the accumulation of cyclic AMP, which may be due to a decrease in adenylate cyclase activity, and second, a decrease in protein kinase A activity, although the differences at other levels have not been excluded. If we take into consideration the role of cyclic AMP in modulating PMN responses, we can imagine the area of responses that may be covered by this difference. However, this needs to be evaluated further. Also, this difference between blood and tissue PMNs may represent a forward step in the preparation to defend the body against invading microorganisms or antigens.

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