Augmentation of the Neutrophil Respiratory Burst Through the Action of Advanced Glycation End Products

A Potential Contributor to Vascular Oxidant Stress

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An accelerated accumulation of advanced glycation end products (AGEs) occurs in diabetes secondary to the increased glycemic burden. In this study, we investigated the contribution of AGEs to intravascular oxidant stress by examining their action on the neutrophil burst of reactive oxygen species (ROS); this may be a significant donor to the overall vascular redox status and to vasculopathy. AGEs exerted a dose-dependent enhancement on the neutrophil respiratory burst in response to a secondary mechanical stimulus (up to 265 ± 42%, P = 0.022) or chemical stimulation with formyl-methylleucylphenylalanine 100 nmol/l (up to 218 ± 19%, P < 0.001), although they possessed no ability to augment the neutrophil respiratory burst alone. This phenomenon was both immediate and reversible and depended on the simultaneous presence of AGEs with the additional stimulus. It appeared to work through an upregulation of the neutrophil NADPH oxidase, the enzyme responsible for ROS generation, as seen by a diphenyleneiodonium-dependent suppression of basal and augmented ROS output. Moreover, this action of AGEs was found to be complementary to that of neutrophil priming agents, also known to upregulate neutrophil ROS production, implying the presence of distinct intracellular transduction pathways mediating the effect of these two classes of agents. *Diabetes* 51:2846–2853, 2002

Advanced glycation end products (AGEs) are irreversible adducts resulting from the nonenzymatic reaction of reducing sugars with the amino groups of proteins. Their formation is partly dependent on prevailing sugar and protein concentrations, accounting for their increased accumulation in patients with diabetes, in whom they have been implicated as mediators of a spectrum of pathologies (1,2). This may relate to their ability to covalently cross-link proteins (3) causing structural changes to tissues, but there has also been realization that AGEs are able to effect a host of direct cellular responses through interaction with cellular receptors recognizing AGE ligands, of which the receptor for AGE (RAGE) is the most well characterized (2). Induced responses may include cytokine induction, adhesion molecule expression, smooth muscle and fibroblast proliferation, and chemoattraction of inflammatory cells, which may influence vascular tissue remodeling (1,2).

Another key to the progression of much AGE-related pathology may be via induction of oxidative stress, reflecting an excess production of highly reactive oxygen species (ROS), including free radicals and peroxides, compared with countering antioxidant defenses. Indeed, oxidative stress has been reported in the context of diabetes (4), whereas RAGE ligation by AGEs has been shown to deplete intracellular antioxidant defenses (5), and extraneous addition of antioxidant compounds may ameliorate the downstream effects of AGE/RAGE interaction (6,7).

Free radical production is an important component of an organism's defense against microbial invasion, but inappropriate production may have detrimental sequelae, resulting in molecular and tissue damage (8,9). Additionally, ROS have been recognized to possess messenger roles themselves within certain biological pathways (10). Their function within such pathways may again result in deleterious cellular responses when overactivated. Through such mechanisms, AGEs and oxidative stress may contribute to a range of pathological phenomena, which may be especially pertinent to the pathogenesis of macrovascular disease.

The possibility of directly visualizing cellular ROS production resulting from AGE exposure in real time was investigated in this study. Neutrophils are one of the main producers of ROS within the vascular compartment, and their main pathway to ROS production involves the NADPH oxidase. This enzyme acts through the one-electron reduction of molecular oxygen to form superoxide (O_2^-) (11), which in turn undergoes dismutation by superoxide dismutase (12), thus generating hydrogen peroxide as well. In the current study, we have investigated the effect of AGEs on neutrophil ROS generation in the

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AGE, advanced glycation end product; DPI, diphenyleneiodonium; fMLP, formyl-methylleucylphenylalanine; HBSS, Hank's balanced salt solution; HSA, human serum albumin; MM-albumin, minimally modified albumin, PAF, platelet activating factor; RAGE, receptor for AGE; RLU, relative light unit; ROS, reactive oxygen species; TC199, tissue culture media 199; TNF-α, tumor necrosis factor-α.

context of a potential contribution to the vascular oxidative burden.

RESEARCH DESIGN AND METHODS

Materials. Bis-*N*-methylacridinium nitrate (Lucigenin), diphenyleneiodonium (DPI), formyl-methylleucylphenylalanine (fMLP), glucose, platelet activating factor (PAF), percoll, tissue culture media 199 (TC199), and tumor necrosis factor-α (TNFα human recombinant) were all purchased from Sigma-Aldrich, and 20% endotoxin-free human serum albumin (HSA) was obtained from BioProducts Laboratory.

Preparation of AGE-albumin. AGE-albumin and minimally modified albumin (MM-albumin) were prepared by preincubation of endotoxin-free HSA (20%) with 1 or 30 mmol/l glucose, respectively, at 37° for 12 weeks in 100 mmol/l phosphate (pH 7.4). At the end of the incubation, AGE-albumin/MM-albumin were dialysed against PBS for 24 h and 0.9% sodium chloride for 12 h. Nonglycated HSA was used as a negative control.

Neutrophil isolation. Neutrophils were prepared according to the method of Baron et al. (13). After informed consent was received, 20 ml fresh citrated blood was obtained from healthy adult volunteers, added to Dextran solution (300 mg in 5 ml Hank's balanced salt solution [HBSS] containing 140 mmol/l NaCl, 4 mmol/l $NaHCO_3$, 0.3 mmol/l Na_2HPO_4 [anhydrous], 5 mmol/l KCl, 0.4 mmol/l KH_2PO_4 [anhydrous], 5.5 mmol/l glucose, 0.4 mmol/l $MgSO_4$, and 4.7 mmol/l H_2O), and allowed to sediment for 30 min. Plasma (containing platelets and leukocytes) was removed and centrifuged for 10 min at 482*g*. The cell pellet was vortexed with dilute HBSS for 10 s to lyse contaminating erythrocytes, and then osmolarity was restored by the addition of excess HBSS. A mixed leukocyte pellet was then obtained by further centrifugation at 482*g* for 10 min and layered onto a prepared continuous density gradient of Percoll (65% Percoll, 10% 10 × TC199, 1.6% 1 mol/l HEPES, 0.4% 5 mol/l NaCl, and 23% sterile water, pH 7.4, and centrifuged at 21,982*g* for 15 min at 4°). The neutrophil band was recovered, washed in TC199, and incubated at 37° for 20 min before use. The viability of neutrophils isolated by this method was 95%, as determined by the trypan blue dye exclusion test.

Detection of ROS. ROS detection was based on the chemiluminescent technique described by Liu et al. (14), with chemiluminescence recorded on an EG&G Berthold microplate luminometer LB96V. All experiments were performed at 37°. Lucigenin (50 μmol/l) was made up in a balanced salt solution containing 140 mmol/l NaCl, 15 mmol/l HEPES, 5 mmol/l KCl, 5 mmol/l glucose, 1.8 mmol/l $CaCl_2.2H_2$ 0, and 0.8 mmol/l $MgSO_4.7H_2O$ (pH 7.4). All experiments were performed in triplicate with 10^5 cells per well.

Neutrophil stimulation. Mechanical stimulation of neutrophils was by multiple passages through a standard 1-ml pipette tip. The number and intensity of passages were equal for each experiment, although it was found that with more than one passage, the same amplitude of neutrophil ROS production was induced whatever the actual number or intensity of passages (data not shown). Such a plateau effect of shear stress on neutrophil activation parameters has been previously observed (15) when neutrophils were subject to a certain range of shear stresses. The chemical stimulus used in this study was fMLP (100 nmol/l), a bacterial cell-wall peptide that activates neutrophils via specific G-protein–coupled cell-surface receptors (16).

Coating of microplate wells. Microlite-2 wells (Dynex) were filled with 100 μl PBS containing 500 μg AGE-albumin/albumin, covered, and left overnight on a plate shaker. They were then washed three times with PBS and left to dry before use.

Data analysis. Chemiluminescent values are presented as relative light units (RLUs) per minute and means ± SE. For the analysis of total ROS production (RLU), we calculated the area under the curve (cut off at 6 min). We used two-tailed *t* tests for comparisons of two groups and one-way ANOVA with Tukey's post hoc analysis for between-group analyses involving more than two groups (SPSS).

RESULTS

AGE-albumin cooperates with a secondary stimulus resulting in enhancement of the neutrophil respiratory burst. The direct effect of AGEs on neutrophil ROS production was assessed through the addition of AGE-albumin or albumin control (50–800 μg/ml) to 5×10^5 neutrophils, which had been allowed to settle onto microplate wells. The subsequent integrated ROS production over 15 min was measured and compared with baseline ROS production (also over 15 min), as measured by Lucigenin-enhanced chemiluminescence. Although addition of both AGE-albumin and albumin resulted in slight increases in neutrophil ROS production over basal levels, there was no significant difference between them (comparing the two groups over the given dose range, $P = 0.774$).

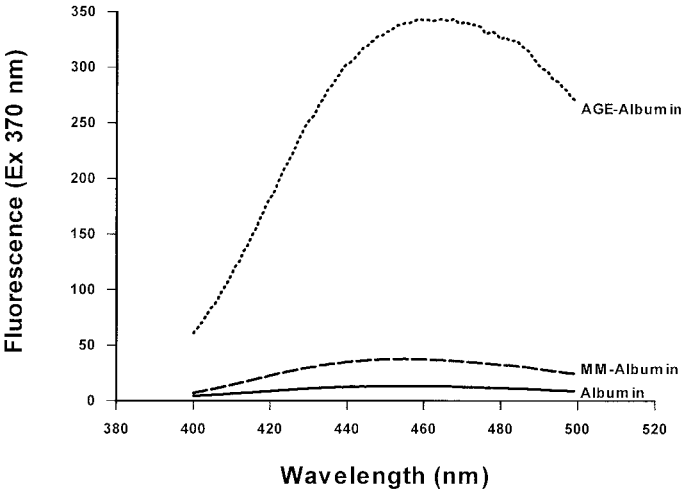

FIG. 1. Fluorescence spectra of AGE-albumin, MM-albumin, and albumin. The excitation wavelength is 370 nm.

However, neutrophils subjected to a secondary stimulus (mechanical or chemical) in the presence of AGE-albumin responded with a respiratory burst that was significantly enhanced compared with activation in the presence of control albumin, with the magnitude of enhancement dependent on the AGE concentration. Both mechanical and fMLP stimulation resulted in a two-phase neutrophil ROS burst, a primary peak, and a secondary trough phase, with AGE-albumin able to enhance both of these aspects. The intensity of the fluorescence spectra of the albumin and AGE-modified albumin samples used in this study give some quantitative idea to the relative degrees of AGE modification of these entities (Fig. 1). MM-albumin was able to augment neutrophil ROS release with much reduced gain compared with AGE-albumin (Fig. 2*A* and *B*). At a concentration of 400 μg/ml (the optimum dose for effect of MM-albumin), AGE-albumin and MM-albumin enhanced peak ROS production in response to mechanical stimulation by an average of 362% ($P < 0.001$) and 157% ($P = 0.011$), respectively, and total ROS production by an average of 282% ($P < 0.001$) and 169% ($P = 0.009$), respectively ($P < 0.001$ between groups in both cases) (Fig. 2*C* and *D*). With fMLP stimulation, AGE-albumin and MM-albumin increased peak ROS production by an average of 177% ($P < 0.001$) and 119% ($P = 0.021$), respectively, but only AGE-albumin enhanced total ROS production (by an average of 145%, $P < 0.001$).

Even within each class of AGE modification there existed a dose-response relationship. For AGE-albumin, optimum responses were achieved at concentrations of AGE-albumin ≥200 μg/ml for both mechanical and fMLP stimulation (Fig. 3*A* and *B*) (for both forms of stimulation, $P < 0.001$ between groups). At progressively higher doses of AGE-albumin, this relative difference was maintained, although the absolute chemiluminescent intensity from the samples diminished, probably due to impairment of light transmission by higher protein concentrations (17).

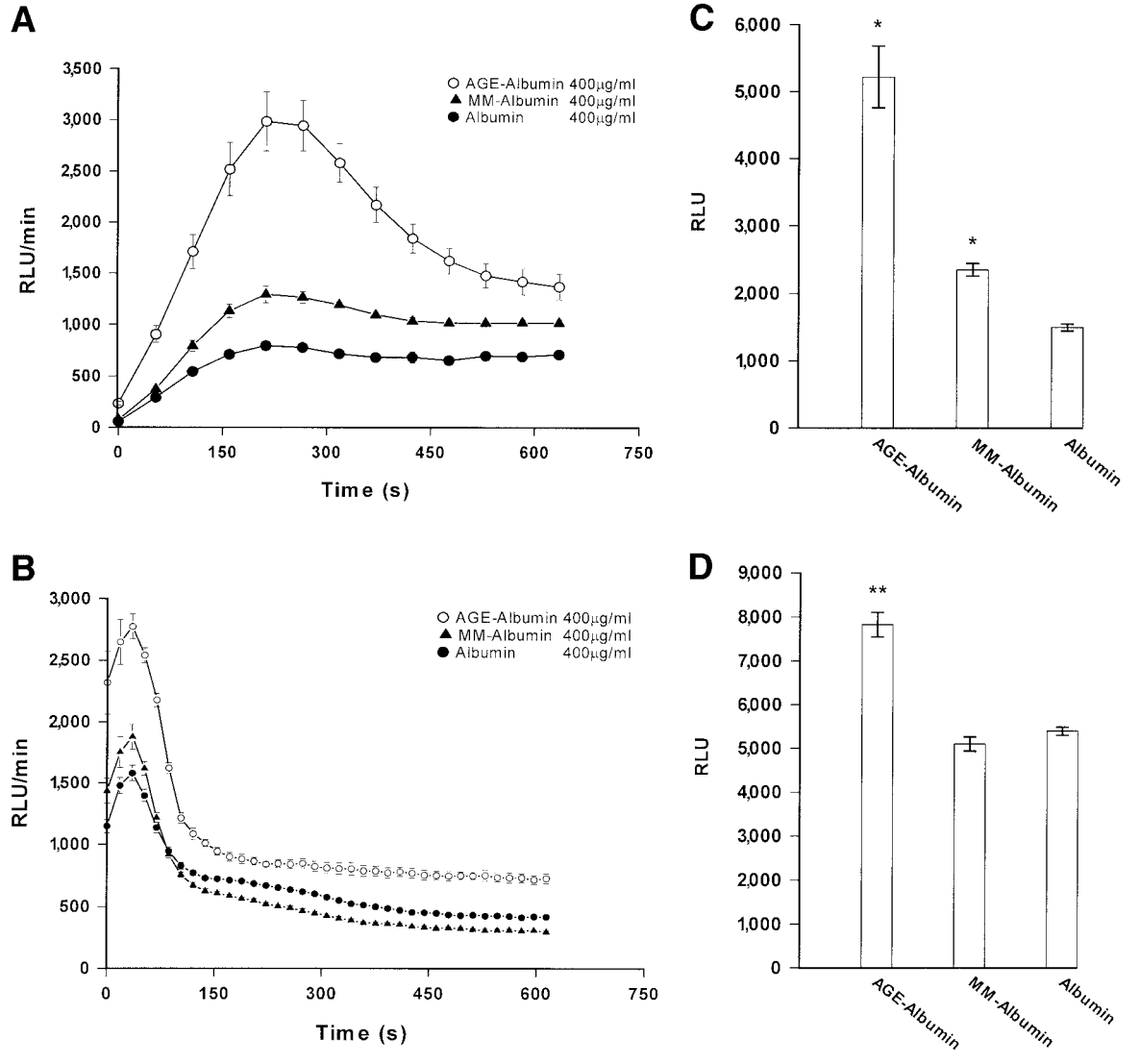

FIG. 2. Acute neutrophil ROS production as detected by lucigenin-enhanced chemiluminescence in the presence of AGE-albumin, MM-albumin, or albumin, all at 400 μg/ml. *A*: Response to a mechanical stimulus ($P < 0.001$ between groups by ANOVA). *B*: Response to stimulation with fMLP ($P < 0.001$ between groups by ANOVA). *C*: Total ROS production after mechanical stimulation ($P < 0.001$ between groups by ANOVA). For comparisons with control, *$P < 0.001$ and **$P = 0.009$ (post hoc Tukey's analysis). *D*: Total ROS production after stimulation with fMLP ($P < 0.001$ between groups by ANOVA). For comparisons with control, **$P < 0.001$ (post hoc Tukey's analysis between groups). Results are representative of multiple experiments and each result represents the mean of three separate aliquots of 10^5 cells recorded simultaneously. Where error bars are not seen, SE is less than the size of the symbol.

The dose response for MM-albumin was much less marked, although still significant for both forms of stimulation (Fig. 3*C* and *D*) (for both, $P < 0.001$ between groups). There was little response below a concentration of 400 μg/ml of albumin, but at higher concentrations (800 μg/ml and above) it was also not possible to detect any small differences in ROS production due to the increasing chemiluminescent quenching caused by the high protein load (Fig. 3*C* and *D*). There appeared to be no requirement for significant preincubation of neutrophils with AGE-albumin to elicit this response; immediate suspension of cells in AGE-albumin before stimulation was as effective as preincubation with AGE-albumin for longer periods (data not shown).

We also investigated the effect of temporally reversing the two processes of AGE-albumin addition and mechanical stimulation. When neutrophils had been mechanically stimulated first, before the subsequent addition of AGE-albumin or control, there was no differential enhancement of ROS generation (total or peak) in the cells exposed to AGE-albumin (Fig. 4*A*) ($P = 0.603$ comparing total ROS production). Likewise, mechanically stimulating neutrophils and then releasing them onto AGE-albumin or albumin-coated microplate wells also did not result in any major difference in neutrophil ROS production (total or peak) (Fig. 4*B*) ($P = 0.319$ comparing total ROS production). Therefore, there appears to be a prerequisite for the presence of AGEs in the immediate neutrophil milieu, immediately before or simultaneously with the mechanical stimulus so that augmentation of the neutrophil ROS burst may take place.

It is well recognized that the cellular enzyme NADPH oxidase is responsible for phagocytic ROS production (12), and it could be inferred that any increase in neutrophil ROS output is attributable to increased NADPH oxidase activity. As confirmation, neutrophils were

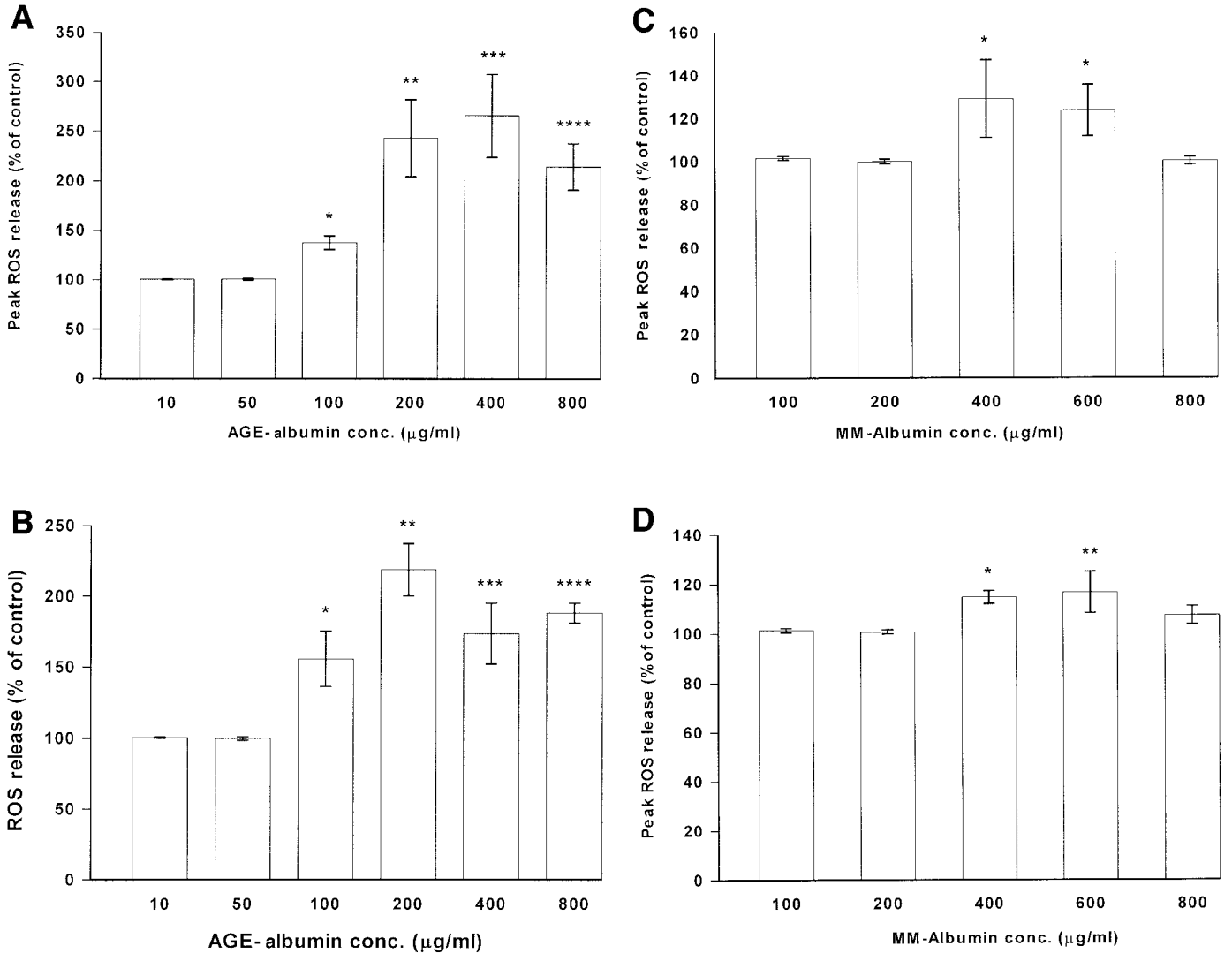

FIG. 3. The dose response of AGE-modified albumin on its relative enhancement of the peak neutrophil ROS release over control. *A*: Peak ROS release after mechanical stimulation in the presence of varying doses of AGE-albumin. $P < 0.001$ by ANOVA between groups. With post hoc Tukey's analysis comparing with control, *$P = 0.047$, **$P = 0.013$, ***$P = 0.022$, and ****$P = 0.002$. *B*: Peak ROS release after stimulation with fMLP in the presence of varying concentrations of AGE-albumin. $P < 0.001$ by ANOVA between groups. With post hoc Tukey's analysis comparing with control, *$P = 0.03$, **$P < 0.001$, ***$P = 0.003$, and ****$P = 0.032$. *C*: Peak ROS release after mechanical stimulation in the presence of varying doses of MM-albumin. $P < 0.001$ by ANOVA between groups, with post hoc Tukey's analysis comparing with control, *$P < 0.001$. *D*: Peak ROS release after stimulation with fMLP in the presence of varying concentrations of MM-albumin. $P < 0.001$ by ANOVA between groups. With post hoc Tukey's analysis comparing with control, *$P = 0.009$ and **$P < 0.001$. The results for each dose are the mean results from 10 different sets of neutrophils.

preincubated with the flavoprotein inhibitor DPI (18) for 30 min and at various concentrations before the addition of AGE-albumin/albumin (200 μg/ml) and application of a mechanical or chemical stimulus, all in the continuing presence of DPI. The result was a dose-dependent abrogation of ROS release, albeit maintaining the relative differential production of ROS between experimental and control groups (Fig. 5*A* and *B*) (for both, $P < 0.001$ between groups). In contrast, the specific mitochondrial flavoprotein inhibitor rotenone (100 nmol/l, 10 μmol/l) had little effect on neutrophil ROS output in this situation (data not shown). Therefore, it would appear that the action of AGEs on neutrophils requires a functioning NADPH oxidase and that the most likely mechanism of action involves an upregulation of the activity of the NADPH oxidase.

The effect of AGE-albumin on the neutrophil respiratory burst is a reversible process. We examined the reversibility of the AGE effect on neutrophils as an extension of the notion that AGEs must be present simultaneously with another stimulus to generate enhanced neutrophil ROS production. Neutrophils were incubated in AGE-albumin or control albumin (both 200 μg/ml) for a period of 30 min before being washed and then activated immediately with either mechanical or chemical (fMLP) stimuli. With both stimuli, the differential enhancement of ROS production previously induced by AGE-albumin was abrogated ($P = 0.71$ and $P = 0.12$, respectively, for total ROS production; data not shown). It is therefore apparent that the effect of AGE on neutrophils is a reversible phenomenon with no imprinting of any previous interaction on cell memory and response.

The effect of AGE-albumin is complementary to the effect of neutrophil priming agents in enhancing neutrophil respiratory burst capacity. A variety of

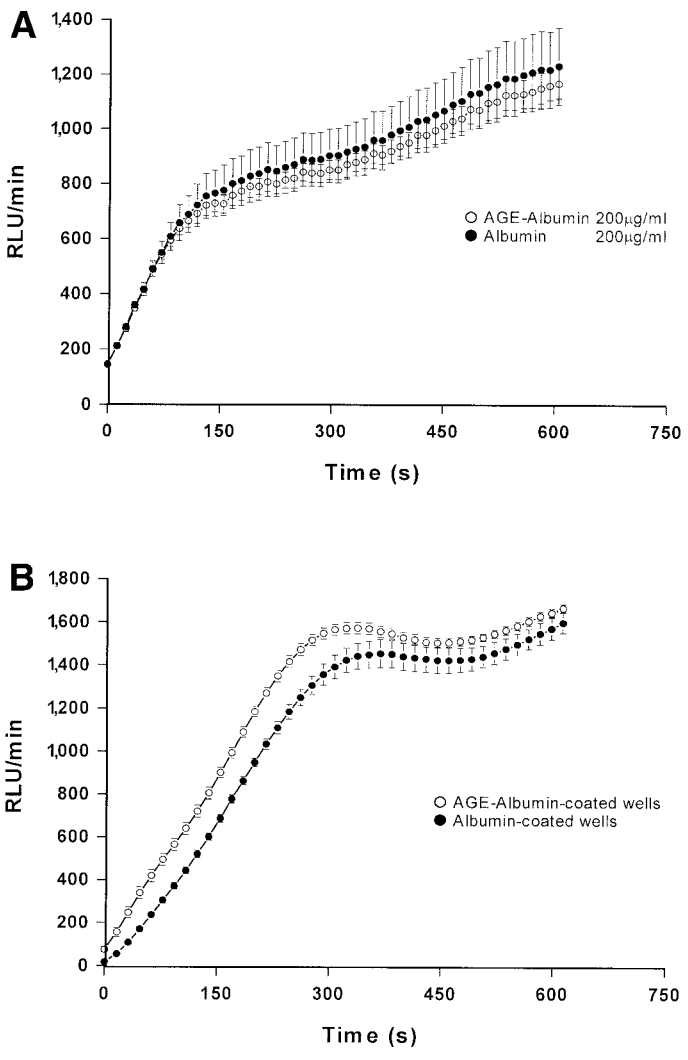

FIG. 4. Acute neutrophil ROS production detected by lucigenin-enhanced chemiluminescence when subjected to a reversal in the order of AGE-albumin addition and mechanical stimulation. *A*: Neutrophils were subjected to a mechanical stimulus first, before subsequent addition of AGE-albumin or albumin. *B*: Neutrophils were mechanically stimulated and then released onto microplate wells coated with AGE-albumin or albumin. Results are representative of multiple experiments, and each result is the mean of three aliquots of 10^5 cells recorded simultaneously. Where error bars are not seen, SE is less than the size of the symbol.

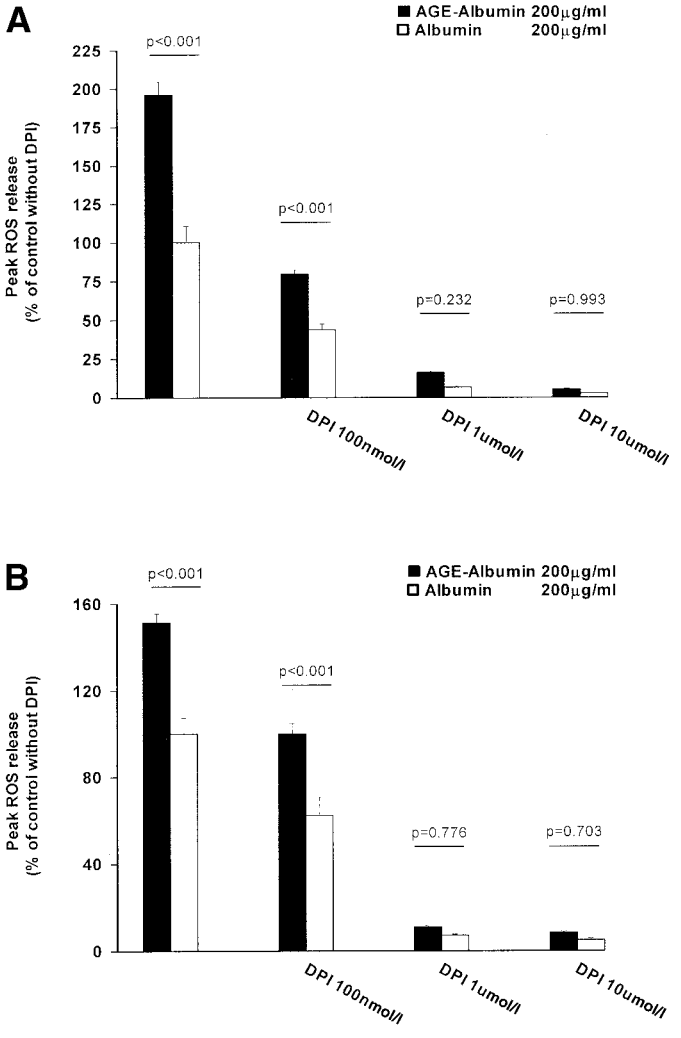

FIG. 5. Peak neutrophil ROS release with exposure to AGE-albumin or control albumin under conditions of increasing DPI concentration. *A*: ROS release after mechanical stimulation ($P < 0.001$ between groups by ANOVA). *B*: ROS release after stimulation with fMLP ($P < 0.001$ between groups by ANOVA). Peak ROS release is expressed as a percentage of peak ROS release with albumin control and without addition of DPI.

other agents have also been documented to possess the capacity for increasing neutrophil ROS production. One such group of agents, collectively termed "neutrophil priming agents" (19) and including two well-studied agents, TNF-α and PAF, differs from AGE-albumin in requiring significant preincubation time with cells in order to achieve an effect. In investigating the possibility of shared cellular upregulation mechanisms between AGEs and priming agents, we studied the effects of combined exposure to these agents. Neutrophils were preincubated with TNF-α, PAF, or control vehicle for the required priming time (30 min) with the subsequent addition of AGE-albumin or albumin (both at 200 μg/ml) and further stimulation of the cells either mechanically or by fMLP (Fig. 6*A–D*). Both TNF-α and PAF appeared to exert even more potent augmentation effects on the neutrophil respiratory burst than AGE-albumin alone, and the effects appeared to be complementary when AGE-albumin was combined with them. For mechanical stimulation, there was an ~10-fold increase in the absolute values of total and peak ROS production in both AGE-albumin and albumin groups when TNF-α was added (data not shown), generally maintaining the differential increase in ROS production between the AGE-albumin and albumin groups at 150–200%, whether assessed by peak or total ROS production (Fig. 6*A*). With a combination of TNF-α priming and fMLP stimulation, there was an ~6-fold increase in peak ROS and an 11-fold increase in total ROS production (data not shown), with a maintenance of the differential increase in ROS production (peak or total) between 150 and 160% (Fig. 6*B*).

PAF priming led to a lesser increase in ROS production than TNF-α. Allied to mechanical stimulation, there was an approximate twofold increase in peak ROS and a 1.5-fold increase in total ROS production (data not shown), maintaining a 200–230% differential increase (in peak or total ROS production) in AGE-albumin compared with albumin

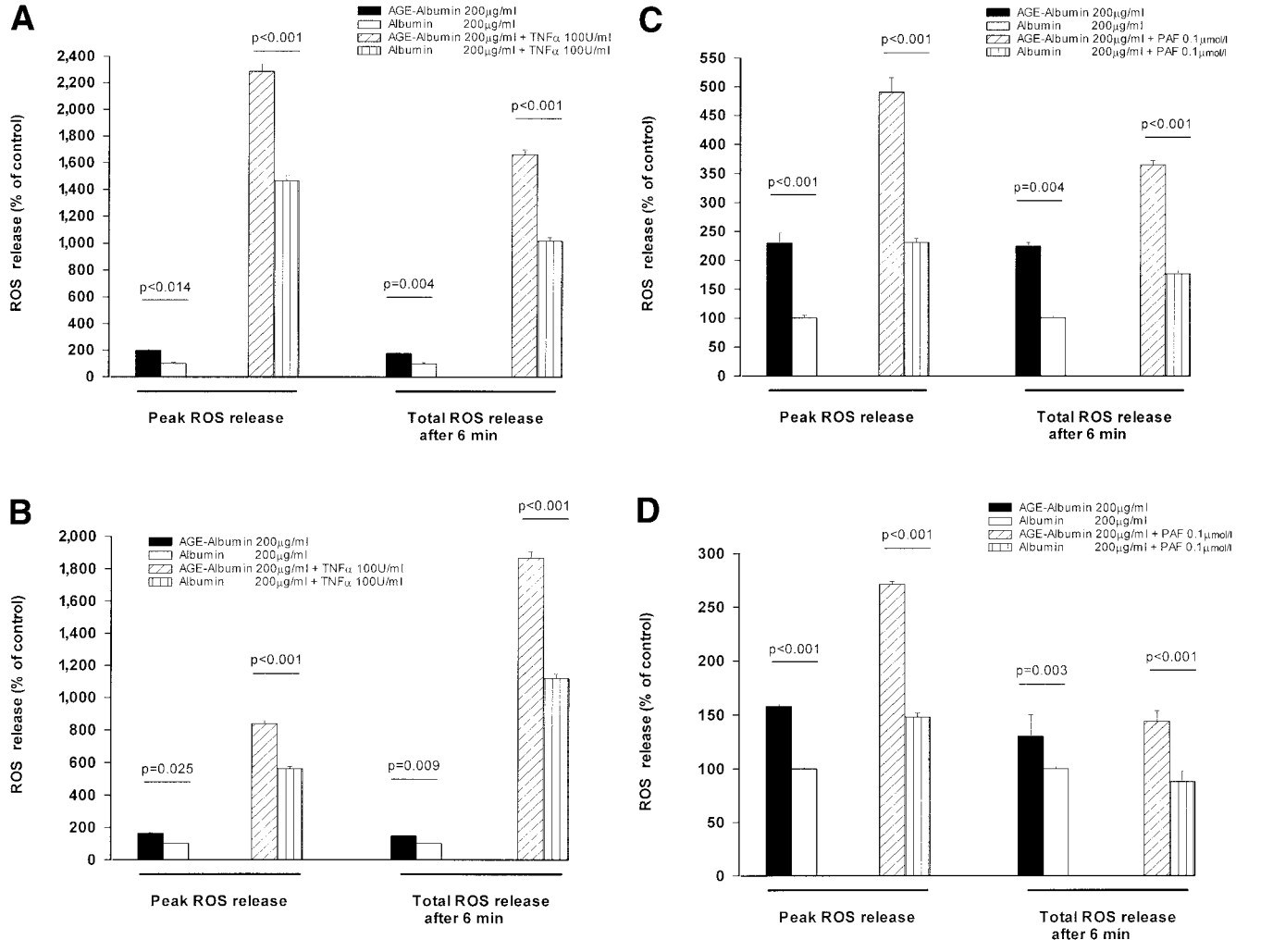

FIG. 6. AGE-albumin complements both TNF-α and PAF to further augment neutrophil ROS production. Neutrophils were preincubated with TNF-α (100 units/ml), PAF (0.1 μmol/l), or DMSO vehicle for 30 min before addition of AGE-albumin or albumin (both at 200 μg/ml). They were then subjected to a mechanical stimulus or activation by fMLP in the continuing presence of TNF-α (*A* and *B*, respectively) or PAF (*C* and *D*, respectively). Each recording is the mean of three separate aliquots of 10^5 cells measured simultaneously, and the results are representative of multiple experiments. For *A–D*, $P < 0.001$ between groups by ANOVA.

groups (Fig. 6*C*). With fMLP stimulation, there was only a 1.5-fold increase in peak ROS production but no real change in total ROS production, principally caused by lower secondary trough phase ROS production in the PAF groups, negating the effect of the higher peak ROS values in these groups (data not shown). There was nonetheless still a differential increase in peak and total ROS production to the level of 130–180% in the AGE-albumin compared with albumin groups (Fig. 6*D*).

DISCUSSION

Premature macrovascular disease is a prevalent accompanying pathology in diabetes (20) and in renal failure (21), with the increased rate of accumulation of AGEs in these conditions recognized as a potential contributor to this pathology (22). Oxidative stress may play a vasculopathic role (23) by neutralization of the natural vasodilator nitric oxide (NO) (8) and augmentation of plaque development through LDL oxidation, rendering it more susceptible to uptake by scavenger macrophages (24). The possibility that AGEs may significantly contribute to this vascular oxidative burden has been examined in this study. For ROS detection, we used a chemiluminescent assay in which the reaction of free radical species with a chemical probe (Lucigenin) generates light. The application of this technique to the biological substrates involved in this study has previously been validated (15,25). Moreover, such an assay was pertinent to this situation because of its great sensitivity and ability to allow observation of ROS production in real time.

A state of increased oxidative stress has been reported previously upon ligation of RAGE by AGEs (5–7,26). Most of this evidence has been indirect inference, through observing the activation of intracellular pathways normally regulated by oxidant stress or through the abrogation of AGE/RAGE responses by antioxidants (6,7). There has also been one report of the direct detection of hydrogen peroxide release when AGEs were allowed to interact with the RAGE receptor on endothelial cells. Although peroxide release was a direct response in this case, it occurred as a slow, integrated accumulation measured over a period of 60 min (26).

In the current study, further evidence for AGE-induced oxidant stress has been outlined. As physiological ROS

producers in antimicrobial defense, neutrophils were chosen for investigation because they may also contribute significantly to intravascular oxidant stress. Their role in reperfusion damage after ischemic events is already acknowledged (12). Evidence also suggests that even controlling for other factors, a higher blood leukocyte count and especially the granulocytic component, predicts a greater likelihood of future vascular events. Infection is similarly often found preceding the development of ischemic events (27,28). In comparing neutrophils from healthy individuals with those from patients with stable and unstable coronary artery disease, there exists a gradation of progressive neutrophil activation that may provide a pathophysiological milieu for the progression of stable to unstable coronary artery disease. Activated neutrophils release toxic chemicals including ROS and proteolytic enzymes that damage the endothelium and basement membrane. ROS release also causes lipid oxidation and initiates platelet activation/aggregation; neutrophils may thus be involved in the primary pathogenesis and progression of occlusive vascular disease (27,28).

In this study there was no evidence for a direct, rapid induction of oxidant stress upon the exposure of neutrophils to AGE-albumin. However, the presence of AGEs allowed for a dose-dependent enhancement of the ROS burst imparted by a secondary stimulus, whether it was mechanical or chemical, occurring with rapid kinetics. Allied to the prompt reversibility of this effect on removal of AGEs from the neutrophil milieu, we conclude that AGEs may play an as yet undescribed role as neutrophil "coagonists." As coagonists, AGEs are distinct from priming agents, which also enhance cellular function but require significant interaction time with cells to bring about their response (19). There is in vivo relevance to the coagonist actions of AGEs, because leukocyte passage through the vasculature generates enough shear stress to potentially act as a secondary mechanical agonist (29). In vivo chemical agonists, on the other hand, may be provided by pathogen exposure (fMLP is a bacterial derivative [16]), and some acute vascular events have already been correlated with infective episodes (30).

The complementary effect on ROS production gained by combining AGEs with the previously characterized priming agents PAF and TNF-α illustrates potential in vivo synergism and also further distinguishes AGE coagonist action from that of neutrophil priming agents. Complementarity implies that there are distinct intracellular pathways governing upregulation of the neutrophil response (31), of which the common end point is the activation of the neutrophil NADPH oxidase. That the NADPH oxidase enzyme is central to both the basal production of ROS by neutrophils and the AGE-augmented production of ROS is suggested by the dose-dependent inhibition of ROS output with DPI. This flavoprotein inhibitor is effectively regarded as an NADPH oxidase inhibitor, especially in neutrophils where NADPH oxidase is the dominant flavoprotein (18); moreover, there was a comparative lack of effect of the specific mitochondrial flavoprotein inhibitor rotenone on suppressing this ROS output.

Many candidate pathways exist for mediation of the effect of AGEs on the neutrophil respiratory burst. The key trigger probably involves the interaction of AGEs with a neutrophil transmembrane receptor of which several candidates have been identified (2). Indeed, the presence of RAGE has been recently demonstrated in neutrophils (32). Whatever the subsequent pathway of signal transduction, its end result provides for a mechanism that is able to augment NADPH oxidase activity triggered by a heterogeneous group of stimuli.

The AGE dosages in this study bear similarity to those validated in previous receptor-binding studies (33). Relating these to actual pathological levels in diabetic patients, our unpublished data shows that AGE-albumin doses in this study were ~10-fold greater than the concentration in serum from diabetic patients with macrovascular complications, as assessed by AGE fluorescence. The latter was already threefold greater than serum from healthy control subjects. This difference between in vitro and in vivo intensities does not necessarily render our experimental dosages irrelevant. In vitro pharmacological effects generally occur at greater dosages than are required in vivo, and the difference here is barely one order of magnitude. Additionally, AGE concentrations in vivo are not uniform throughout the body. AGEs are often closely allied to the locations of atherosclerotic lesions (2,34), although whether this is cause or effect has not been definitively answered; indeed both elements may be present. Nevertheless, in the vicinity of atherosclerotic plaques, AGEs may have greater local compared with circulating concentrations resulting in locally enhanced neutrophil ROS production. This may also facilitate localized accelerated AGE formation, thus further perpetuating this cycle (35).

This nonhomogenous distribution of AGEs in the body may account for the apparent paradox between an increase in AGE-mediated neutrophil ROS generation and the observation that diabetic patients, especially those with poor metabolic control, appear to have a greater frequency of infective complications (36). The reversibility of the AGE effect may lead to neutrophils being only transiently in an activated state at sites of heavy AGE accumulation and reverting to a nearer normal state on relocation to other parts of the circulation or nonvascular compartments. Also, hyperglycemia per se is cytotoxic, impeding effective neutrophil functioning (37). Thus, neutrophil function under conditions of acute hyperglycemia differs from that during times when metabolic control is closer to physiological parameters but when the legacy of past metabolic indiscretions has led to accelerated AGE accumulation.

The evidence from this study therefore adds further insight to possible pathogenic effects of AGEs in conditions such as diabetes and uremia. Through their action as unique neutrophil coagonists, it has been demonstrated that AGEs could play a key role in the induction of a state of increased oxidative stress through augmentation of neutrophil ROS production, and this may be in part responsible for the acceleration of vascular disease noted in these conditions.

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