Role of NO and PAF in the impairment of skeletal muscle contractility induced by TNF-α

GIUSEPPE ALLOATTI,1,2 CLAUDIA PENNA,1 FILIPPO MARIANO,3 AND GIOVANNI CAMUSSI3
1Laboratorio di Fisiologia Generale, Dipartimento di Biologia Animale e dell’Uomo,
2Istituto Nazionale per la Fisica della Materia and 3Dipartimento di Medicina Interna,
Università degli Studi di Torino, 10123 Torino, Italy

Address for reprint requests and other correspondence: G. Alloatti,
Dipartimento di Biologia Animale e dell’Uomo, Università degli
Studi di Torino, Via Accademia Albertina 13 10123 Torino, Italy
(E-mail: alloatti@dba.unito.it).

Role of NO and PAF in the impairment of skeletal muscle contractility induced by TNF-α. Am J Physiol Regulatory Integrative Comp Physiol 279: R2156–R2163, 2000.—The role of platelet-activating factor (PAF) and nitric oxide (NO) as mediators of the effects of tumor necrosis factor-α (TNF-α) on skeletal muscle contractility was studied in guinea pig extensor digitorum longus (EDL) muscle. TNF-α (5–10 ng/ml) reduced contractility at every stimulation frequency (1–200 Hz) and shifted the force-frequency relationship to the right. The role of NO and PAF as mediators of TNF-α was suggested by the protective effect of Nω-nitro-arginine methyl ester (L-NAME; 1 mM), but not of Nω-nitro-arginine methyl ester (D-NAME; 1 mM), and by the inhibitory effect of the PAF-receptor antagonist WEB-2170 (3 μM). TNF-α increased the production of PAF and NO. Similar to TNF-α, both S-nitroso-N-acetylpenicillamine (0.5–1 μM), an NO-generating compound, and PAF (10–20 nM) reduced EDL contractility. L-NAME, but not D-NAME, blocked the negative effect of PAF. Blockade of phospholipase A2, which is required for PAF synthesis, significantly reduced the effects of TNF-α.

Role of NO and PAF in the impairment of skeletal muscle contractility induced by TNF-α. Am J Physiol Regulatory Integrative Comp Physiol 279: R2156–R2163, 2000.—The role of platelet-activating factor (PAF) and nitric oxide (NO) as mediators of the effects of tumor necrosis factor-α (TNF-α) on skeletal muscle contractility was studied in guinea pig extensor digitorum longus (EDL) muscle. TNF-α (5–10 ng/ml) reduced contractility at every stimulation frequency (1–200 Hz) and shifted the force-frequency relationship to the right. The role of NO and PAF as mediators of TNF-α was suggested by the protective effect of Nω-nitro-arginine methyl ester (L-NAME; 1 mM), but not of Nω-nitro-arginine methyl ester (D-NAME; 1 mM), and by the inhibitory effect of the PAF-receptor antagonist WEB-2170 (3 μM). TNF-α increased the production of PAF and NO. Similar to TNF-α, both S-nitroso-N-acetylpenicillamine (0.5–1 μM), an NO-generating compound, and PAF (10–20 nM) reduced EDL contractility. L-NAME, but not D-NAME, blocked the negative effect of PAF. Blockade of phospholipase A2, which is required for PAF synthesis, significantly reduced the effects of TNF-α.

Role of NO and PAF in the impairment of skeletal muscle contractility induced by TNF-α. Am J Physiol Regulatory Integrative Comp Physiol 279: R2156–R2163, 2000.—The role of platelet-activating factor (PAF) and nitric oxide (NO) as mediators of the effects of tumor necrosis factor-α (TNF-α) on skeletal muscle contractility was studied in guinea pig extensor digitorum longus (EDL) muscle. TNF-α (5–10 ng/ml) reduced contractility at every stimulation frequency (1–200 Hz) and shifted the force-frequency relationship to the right. The role of NO and PAF as mediators of TNF-α was suggested by the protective effect of Nω-nitro-arginine methyl ester (L-NAME; 1 mM), but not of Nω-nitro-arginine methyl ester (D-NAME; 1 mM), and by the inhibitory effect of the PAF-receptor antagonist WEB-2170 (3 μM). TNF-α increased the production of PAF and NO. Similar to TNF-α, both S-nitroso-N-acetylpenicillamine (0.5–1 μM), an NO-generating compound, and PAF (10–20 nM) reduced EDL contractility. L-NAME, but not D-NAME, blocked the negative effect of PAF. Blockade of phospholipase A2, which is required for PAF synthesis, significantly reduced the effects of TNF-α.

Role of NO and PAF in the impairment of skeletal muscle contractility induced by TNF-α. Am J Physiol Regulatory Integrative Comp Physiol 279: R2156–R2163, 2000.—The role of platelet-activating factor (PAF) and nitric oxide (NO) as mediators of the effects of tumor necrosis factor-α (TNF-α) on skeletal muscle contractility was studied in guinea pig extensor digitorum longus (EDL) muscle. TNF-α (5–10 ng/ml) reduced contractility at every stimulation frequency (1–200 Hz) and shifted the force-frequency relationship to the right. The role of NO and PAF as mediators of TNF-α was suggested by the protective effect of Nω-nitro-arginine methyl ester (L-NAME; 1 mM), but not of Nω-nitro-arginine methyl ester (D-NAME; 1 mM), and by the inhibitory effect of the PAF-receptor antagonist WEB-2170 (3 μM). TNF-α increased the production of PAF and NO. Similar to TNF-α, both S-nitroso-N-acetylpenicillamine (0.5–1 μM), an NO-generating compound, and PAF (10–20 nM) reduced EDL contractility. L-NAME, but not D-NAME, blocked the negative effect of PAF. Blockade of phospholipase A2, which is required for PAF synthesis, significantly reduced the effects of TNF-α.

Role of NO and PAF in the impairment of skeletal muscle contractility induced by TNF-α. Am J Physiol Regulatory Integrative Comp Physiol 279: R2156–R2163, 2000.—The role of platelet-activating factor (PAF) and nitric oxide (NO) as mediators of the effects of tumor necrosis factor-α (TNF-α) on skeletal muscle contractility was studied in guinea pig extensor digitorum longus (EDL) muscle. TNF-α (5–10 ng/ml) reduced contractility at every stimulation frequency (1–200 Hz) and shifted the force-frequency relationship to the right. The role of NO and PAF as mediators of TNF-α was suggested by the protective effect of Nω-nitro-arginine methyl ester (L-NAME; 1 mM), but not of Nω-nitro-arginine methyl ester (D-NAME; 1 mM), and by the inhibitory effect of the PAF-receptor antagonist WEB-2170 (3 μM). TNF-α increased the production of PAF and NO. Similar to TNF-α, both S-nitroso-N-acetylpenicillamine (0.5–1 μM), an NO-generating compound, and PAF (10–20 nM) reduced EDL contractility. L-NAME, but not D-NAME, blocked the negative effect of PAF. Blockade of phospholipase A2, which is required for PAF synthesis, significantly reduced the effects of TNF-α.

Role of NO and PAF in the impairment of skeletal muscle contractility induced by TNF-α. Am J Physiol Regulatory Integrative Comp Physiol 279: R2156–R2163, 2000.—The role of platelet-activating factor (PAF) and nitric oxide (NO) as mediators of the effects of tumor necrosis factor-α (TNF-α) on skeletal muscle contractility was studied in guinea pig extensor digitorum longus (EDL) muscle. TNF-α (5–10 ng/ml) reduced contractility at every stimulation frequency (1–200 Hz) and shifted the force-frequency relationship to the right. The role of NO and PAF as mediators of TNF-α was suggested by the protective effect of Nω-nitro-arginine methyl ester (L-NAME; 1 mM), but not of Nω-nitro-arginine methyl ester (D-NAME; 1 mM), and by the inhibitory effect of the PAF-receptor antagonist WEB-2170 (3 μM). TNF-α increased the production of PAF and NO. Similar to TNF-α, both S-nitroso-N-acetylpenicillamine (0.5–1 μM), an NO-generating compound, and PAF (10–20 nM) reduced EDL contractility. L-NAME, but not D-NAME, blocked the negative effect of PAF. Blockade of phospholipase A2, which is required for PAF synthesis, significantly reduced the effects of TNF-α.

Role of NO and PAF in the impairment of skeletal muscle contractility induced by TNF-α. Am J Physiol Regulatory Integrative Comp Physiol 279: R2156–R2163, 2000.—The role of platelet-activating factor (PAF) and nitric oxide (NO) as mediators of the effects of tumor necrosis factor-α (TNF-α) on skeletal muscle contractility was studied in guinea pig extensor digitorum longus (EDL) muscle. TNF-α (5–10 ng/ml) reduced contractility at every stimulation frequency (1–200 Hz) and shifted the force-frequency relationship to the right. The role of NO and PAF as mediators of TNF-α was suggested by the protective effect of Nω-nitro-arginine methyl ester (L-NAME; 1 mM), but not of Nω-nitro-arginine methyl ester (D-NAME; 1 mM), and by the inhibitory effect of the PAF-receptor antagonist WEB-2170 (3 μM). TNF-α increased the production of PAF and NO. Similar to TNF-α, both S-nitroso-N-acetylpenicillamine (0.5–1 μM), an NO-generating compound, and PAF (10–20 nM) reduced EDL contractility. L-NAME, but not D-NAME, blocked the negative effect of PAF. Blockade of phospholipase A2, which is required for PAF synthesis, significantly reduced the effects of TNF-α.
were mounted on an apparatus for in vitro physiological studies on muscle preparations, which was detailed in a previous study (1) and continuously perfused with oxygenated (100% O₂) Tyrode solution at 37°C with d-tubocurarine (10 μM) added. Transmembrane potentials were recorded by means of standard glass microelectrodes. To study the force-frequency relationship, muscles were stimulated at different rates (1, 15, 30, 50, 67, 80, 100, 150, 200 Hz) with a pair of electrodes connected to a 302 T Anapulse Stimulator via a 305-R Stimulus Isolator (WP Instruments, New Haven, CT) operating in constant current mode. Tetanic stimulations lasted 500 ms and were separated by 120 s; in selected experiments, muscles were stimulated at 67 Hz for 20 s to perform fatigue tests. At the beginning of the experiments, muscles were lengthened incrementally, until maximal twitch tension was obtained. Stimulation voltage (30% higher than that producing maximal twitch tension) and stimulus duration (0.5 ms) were maintained constant during all the experiments. Baseline control isometric force, recorded during maximal activation after stabilization in Tyrode solution, was 16.8 ± 1.6 N/cm². Baseline control values for isometric twitches were time-to-peak tension 11.2 ± 1.2 ms; half-relaxation time 7.5 ± 0.8 ms. Fatigue half time, measured during stimulation at 67 Hz for 20 s, was 15.7 ± 2.2 s. No significant differences were present in baseline control parameters among the groups. The electrical and mechanical activities of EDL muscles were recorded onto magnetic tape by a 3964 A Hewlett-Packard recorder (Palo Alto, CA), visualized on a Tektronix 2211 digital storage oscilloscope, and reproduced for data analysis by means of a Hewlett-Packard 7470A plotter.

**Experimental protocol.** EDL muscles were equilibrated in Tyrode solution for at least 30 min before each challenge. All solutions containing TNF-α (5 and 10 ng/ml; Sigma Chemical, St. Louis, MO) or the other drugs were prepared immediately before the experiments and were not recirculated. N⁴-nitro-L-arginine methyl ester (L-NAME; 1 mM; Sigma) was applied for 2 h before challenge with TNF-α (10 ng/ml) or PAF (20 nM) to block the synthesis of NO (19). The biologically inactive enantiomer of L-NAME, N⁵-nitro-D-arginine methyl ester (D-NAME; 1 mM; Sigma), was used as control. WEB-2170 (3 μM; Boehringer Ingelheim), a PAF-receptor antagonist (12), was used to block PAF receptor; WEB-2170 was administered to EDL muscles starting 15 min before and during the entire period of treatment with TNF-α (10 ng/ml). PAF (Bachem Feinchemikalien, Bubendorf, Switzerland) was first dissolved in physiological solution containing 0.25% bovine serum albumin (Sigma), and then the appropriate aliquots of the stock solution were added to the Tyrode solution to reach the concentrations of 10 and 20 nM. S-nitroso-N-acetylpenicillamine (SNAP; 0.5–1 μM; Sigma) was used as a donor of NO. Two main pathways of PAF synthesis are known: the so-called de novo pathway, which is responsible for the basal production of PAF, is not inducible and is mainly involved in the synthesis of PAF in the nervous system and in the renal medulla, and a remodeling pathway of membrane phospholipids, which is inducible and represents the main pathway of PAF synthesis from inflammatory cells (34). The activation of phospholipase A₂ (PLA₂) is a key step in the synthesis of PAF in this latter pathway (34). We used 4-bromophenacyl bromide (4-BPB; 20 μM; Sigma), a PLA₂ inhibitor (22), to study the role of PLA₂ in the synthesis of PAF induced by TNF-α; 4-BPB was administered to EDL muscles starting 15 min before and during all the period of treatment with TNF-α (10 ng/ml) or PAF (20 nM). Treatment with TNF-α, PAF, or SNAP lasted 30 min, and then the perfusion was switched to control, drug-free Tyrode solution, to study the reversibility of the effect.

**Nitrite assay.** To measure nitrite production by isolated EDL muscles, small aliquots (150 μl) of perfusate were mixed with an equal volume of 1% sulfanilamide-1% N-(1-naphthyl) ethylenediamine dihydrochloride in 2% phosphoric acid free acid (Greiss acid; Sigma) at room temperature for 10 min (24). Nitrite concentrations were calculated by comparison with optical density of standard solution of sodium nitrite prepared in Tyrode solution. All values were background corrected for nitrite values obtained in nonconditioned Tyrode solution. Optical density at 550 nm was measured using a Microplate Reader model 450 (BioRad Laboratories, Hercules, CA). To assess the effects of TNF-α and the role of PAF on the synthesis of NO, we measured nitrite production from EDL muscles challenged with 10 ng/ml TNF-α, without or after pretreatment with L-NAME (1 mM); WEB-2170 (3 μM), or 4-BPB (20 μM); further experiments were performed in EDL muscles challenged with 20 nM PAF.

**PAF assay.** PAF was extracted and purified from EDL muscles as previously described in detail (1, 18). PAF bioactivity was tested by bioassay on washed rabbit platelets (4, 18) after extraction and purification by thin layer chromatography (TLC) and HPLC and was characterized by comparison with synthetic PAF according to the following criteria: induction of platelet aggregation by a pathway independent of both ADP and arachidonic acid/thromboxane A₂; specificity of platelet aggregation as inferred from the inhibitory effect of PAF receptor antagonist WEB-2170 (5 μM); and TLC and HPLC chromatographic behavior and physicochemical characteristics such as inactivation by strong bases and 5 min heating in boiling water.

**Statistical analysis.** Data are expressed as the means ± SE. The experimental groups were compared using two-way analysis of variance. If a significant F resulted from the analysis of variance (P < 0.05), the Newman-Keuls multiple-range test was applied to determine where differences were located among the groups.

**RESULTS**

**Effect of TNF-α on EDL mechanical performance.** In preliminary experiments, after the equilibration period of 30 min, EDL muscles were further maintained in Tyrode solution for 60 min to study the stability of the preparation. In these conditions, the amplitude of contractions recorded after tetanic stimulation at 67 Hz declined only to 93.8 ± 1.1% of the control value. As shown in Fig. 1, perfusion of EDL with Tyrode solution containing TNF-α induced concentration-dependent effects on mechanical properties of EDL. One nanogram per milliliter TNF-α had no effect on EDL (not shown), whereas the higher concentrations (5 and 10 ng/ml) shifted the force-frequency relationship to the right and reduced developed force. Ten nanograms per milliliter TNF-α reduced the maximal twitch tension at 1 Hz to 28.2 ± 6.1% of the control value, whereas no significant difference was observed for the time-to-peak tension (105.5 ± 1.9%) and the half-relaxation time (108.0 ± 8.8%). The reduction of contractility induced by TNF-α was not accompanied by significant changes of the resting membrane potential (−83.8 ± 1.6 and −83.3 ± 1.7 mV before and after treatment with TNF-α; 5 experiments, at least 10 measurements for each), the overshoot (+23.8 ± 3.4 and +25.0 ± 2.7
mV), the maximum rate of depolarization (635 ± 39 and 622 ± 47 V/s), and the action potential duration (at 50% of repolarization, 1.6 ± 0.3 and 1.5 ± 0.2 ms), as well as of excitability. Fatigue half time, recorded during stimulations at 67 Hz for 20 s, was reduced to 86.0 ± 5.0% of the control value. In muscles treated with 5 or 10 ng/ml TNF-α and subsequently washed for 20–30 min, mechanical tension recorded during tetani recovered, respectively, to 88.6 ± 7.7 or 80.4 ± 15.6% of the control and the force-frequency relationships were highly significantly different (P < 0.01) from those recorded after TNF-α treatment (Fig. 1). However, after 10 ng/ml TNF-α, the recovery was not complete and the force-frequency relationship remained significantly lower (P < 0.05) than control, pretreatment values.

Role of NO in contractile failure induced by TNF-α. Several studies indicate that the effects of TNF-α on other muscle types, such as cardiac (1, 9, 11) and smooth muscle (25), are, at least in part, mediated by NO. To study whether NO plays a similar role also in skeletal muscle, the effects of TNF-α (10 ng/ml) were studied after 2 h pretreatment with the NO-synthase inhibitor L-NAME (1 mM) or with its biologically inactive enantiomer D-NAME (1 mM). In accordance with other studies in which NO-synthase inhibitors were used (10, 14), the incubation of EDL with L-NAME shifted the force-frequency relationship to the left and increased contractile force. Pretreatment of EDL muscles with L-NAME abolished the shift of the force-frequency relationship and reduced the contractile failure induced by TNF-α at every tested frequency (Fig. 2). In contrast, pretreatment with d-NAME, which was ineffective per se, did not alter the mechanical responses of EDL to TNF-α (Fig. 2).

Further experiments were performed to compare the effects of NO with those induced by TNF-α. As shown in Fig. 3, SNAP (0.5–1 μM), used to produce exogenous NO, exerted dose-dependent effects on the force-frequency relationship that resembled those induced by TNF-α. Moreover, similar to TNF-α, SNAP reduced the contractile force in a dose-dependent manner. The effects of SNAP were completely reversed after 15- to 20-min washout. The role of NO as mediator of TNF-α was further studied by measuring nitrite production from EDL muscles stimulated with this cytokine. TNF-α (10 ng/ml) enhanced the basal nitrite production to 183.7 ± 9.3% of the control value. This effect was completely abrogated by pretreatment of EDL muscles with the NO synthase inhibitor L-NAME, but not by d-NAME (Fig. 4).

Role of PAF in contractile alterations induced by TNF-α. Several studies indicate that TNF-α stimulates the synthesis and release of PAF by various cell types (5, 18, 28), including cardiac muscle (1). It was sug-
gested that in cardiac muscle, the negative inotropic effect of TNF-α is mediated by PAF (1). Therefore, we tested the possibility that PAF mediates some of the effects of TNF-α in skeletal muscle. For this purpose, EDL muscles were pretreated with WEB-2170 (3 μM), a PAF-receptor antagonist, before the challenge with TNF-α (10 ng/ml). As shown in Fig. 5, treatment of EDL muscles with WEB-2170 shifted the force-frequency relationship to the left; moreover, WEB-2170 significantly increased contractile force. In the presence of the PAF receptor antagonist, the effects of TNF-α on the force-frequency relationship and on the contractile force of EDL muscles were completely abrogated. Moreover, the synthesis of PAF by EDL muscles was evaluated. Small amounts of PAF were present in EDL homogenates in basal conditions (PAF concentration = 2.9 ± 0.5 pg/g tissue; n = 4), whereas PAF was not detectable in the perfusate. After stimulation with TNF-α (10 ng/ml), an increased amount of PAF was recovered both in the EDL homogenate (PAF = 19.2 ± 4.4 pg/g tissue; P < 0.01 vs. control) and in the perfusate (1.7 ± 0.8 pg/ml, corresponding to 3.1 ± 1.5 pM; n = 4).

The role of PAF in mediating the mechanical effects of TNF-α was further studied in experiments in which exogenous PAF (10 and 20 nM, corresponding to 5.5 and 11 ng/ml, respectively) was added to the bathing solution. Similar to TNF-α, PAF induced a dose-dependent shift in the force-frequency relationship and reduced contractile force. These effects were completely reversed after a 15- to 20-min washout of PAF (Fig. 6).

Relationship between PAF and NO. Our results indicate that both the generation of NO and production of PAF contribute to the development of mechanical alterations induced by TNF-α. To evaluate whether a relationship exists between PAF and NO production, we compared the effects of PAF administration to control EDL muscles with those caused by PAF in muscles pretreated with L-NAME (1 mM) or D-NAME (1 mM). When PAF (20 nM) was administered to EDL muscles pretreated for 2 h with L-NAME, both the reduction of contractility and the shift of the force-frequency relationship induced by PAF in control muscles were abrogated. However, D-NAME completely failed to protect EDL muscles against the negative effect of PAF (Fig. 7).

The role of PAF as intermediate mediator of NO synthase (NOS) stimulation by TNF-α was confirmed by the observations that pretreatment of EDL muscles with WEB-2170 completely blocked the enhancement of NO production induced by TNF-α (Fig. 4) and that PAF (20 nM) stimulates NO production (Fig. 8).

Role of PLA₂ in mechanical alterations induced by TNF-α. Several studies indicate that stimulation of TNF-α receptors leads to activation of PLA₂ (31). Activation of this enzyme represents a primary step in the biosynthesis of PAF (34), suggesting that PLA₂ plays...
an important role in the synthesis of PAF induced by TNF-α. To test this hypothesis, EDL muscles were pretreated with the PLA₂ blocker 4-bromophenacyl bromide (4-BPB) (20 μM) before the challenge with TNF-α (10 ng/ml). Pretreatment with 4-BPB, which had no significant effect per se (Figs. 4 and 9), completely blocked the effects caused by 10 ng/ml TNF-α on the force-frequency relationship. Data are expressed as the means ± SE % of the maximal contractile force recorded during tetani in control, untreated muscles. Statistical analysis was performed to compare the effects of WEB-2170 vs. control (***P < 0.01; n = 5) or those induced by TNF-α on untreated vs. WEB-2170-pretreated muscles (††P < 0.01; n = 5 for each group).

DISCUSSION

Our study demonstrates that, in the isolated guinea pig EDL muscle, 1) TNF-α markedly alters the contractile activity, 2) the effects induced by TNF-α are mediated both by PAF and NO, and 3) the production of NO induced by TNF-α is consequent to the synthesis and release of PAF.

Effect of TNF-α on skeletal muscle contractility. A deleterious effect of TNF-α on skeletal muscle contractility was previously reported in vivo by Wilcox et al. (33) as well as in vitro in an isolated hamster diaphragm preparation (32). The latter effect, however, was evident only at very high doses (500 ng/ml) of TNF-α, whereas, at 0.1 ng/ml, a concentration comparable to those measured in serum during sepsis (15),
TNF-α had no effect on this preparation. In our experiments, 5 ng/ml TNF-α induced a significant reduction of contractility, suggesting that guinea pig EDL muscle is more sensitive than the hamster diaphragm to this cytokine. Differences in sensitivity to TNF-α observed in EDL muscle, compared with the experiments by Wilcox et al. (32) on hamster diaphragm, which are composed of 98 versus 60% of type II muscle fibers (14), respectively, suggest that fast-twitch muscles have much higher sensitivity to TNF-α. This may also depend on differences of NOS activity between diaphragm and EDL, which contains a significantly higher amount of NOS activity (14). The concentrations of TNF-α used in our experiments (1–10 ng/ml) are higher than serum levels of TNF-α detected in patients with septic shock (15) or cardiac failure (30), but comparable with those measured in other pathophysiological conditions, such as acute rejection and viral/bacterial infection after renal transplant (26), or acute peritonitis (17). However, the study by Torre-Amione et al. (30) shows that measurement of TNF-α in serum underestimates the local concentration of the cytokine present in the interstitium among cells. Moreover, it should be considered that the use of an in vitro preparation, in which the perfusion occurs via the external bathing solution instead of the capillary network, reduces the delivery of substances to muscle cells (32). This is particularly important for chemical messengers with a high molecular weight, such as TNF-α.

Several reports suggest an important role of TNF-α in different pathophysiological events, including septic shock, tissue injury, allograft rejection, reperfusion injury, chronic renal failure, cancer, and human immunodeficiency virus infection (28). The impairment of skeletal muscle contractility, a commonly reported event in these pathophysiological conditions, was attributed to muscle wasting due to muscle protein breakdown (6) and alterations in vascular tone (13). The use of an in vitro isolated preparation suggests that an acute administration of TNF-α exerts direct effects on skeletal muscle, independent from impairment in neuromuscular impulse propagation or alterations in vascular tone and blood supply. Tracey and colleagues (29) reported decreases in muscle transmembrane potential after TNF-α treatment of isolated rat EDL and soleus muscles. In the present experiments, however, TNF-α had no significant effect on membrane potential, suggesting that the negative inotropic effect of this cytokine is independent of alterations of the electrical activity. Indeed, in cardiac muscle, TNF-α exerts a marked negative inotropic effect without altering the resting membrane potential (1).

Fig. 8. NO synthesis (pmol·min⁻¹·mg tissue⁻¹) induced by PAF in EDL muscle. The enhancement of nitrite production induced by PAF (20 nM; **P < 0.01 vs. baseline) was blocked by pretreatment of EDL muscles with l-NAME (1 mM) or WEB-2170 (3 μM) (**P < 0.01 vs. PAF in untreated muscles), but not by 4-BPB. l-NAME significantly reduced baseline nitrite production (**P < 0.05), whereas D-NAME, WEB-2170, and 4-BPB had no significant effect. Values are expressed as the means ± SE.

Fig. 9. Role of phospholipase A₂ on contractile alterations induced by TNF-α. The effects caused by 10 ng/ml TNF-α or 20 nM PAF in control EDL muscles are compared with those induced in muscles pretreated with 4-BPB (20 μM). Data are expressed as the means ± SE % of the maximal contractile force recorded during tetani in control, untreated muscles. Statistical analysis was performed to compare the effects of TNF-α on untreated muscles with those induced after pretreatment with 4-BPB (**P < 0.05, ***P < 0.01; n = 5 for each group). No significant change was induced by pretreatment with 4-BPB, nor was a significant response to PAF found between untreated muscles and those treated with 4-BPB.
This discrepancy may depend on the concentration of TNF-α used by Tracey and colleagues (29), which was significantly higher (~25-fold) than that employed in our experiments.

Effects of PAF on skeletal muscle and its role as mediator of TNF-α. The present study supports the hypothesis that, as previously shown in cardiac muscle (1), in skeletal muscle PAF acts as a mediator of the negative inotropic effect of TNF-α. Indeed, pretreatment of EDL muscle with both a PAF-receptor antagonist or an inhibitor of PAF synthesis completely blocked the effects of TNF-α. PAF was found to act as a secondary mediator of TNF-α in several other experimental conditions (4, 18). Recent studies in our laboratory indicate that in cardiac muscle, the negative inotropic effect of TNF-α is due to the synthesis of PAF (1). In the present report, we show for the first time that TNF-α induces PAF synthesis in isolated EDL muscle and, similar to TNF-α, PAF impairs skeletal muscle contractility. The concentrations of PAF used in our experiments are comparable to those released by inflammatory cells after stimulation with TNF-α (5); at these concentrations, PAF significantly reduces contractility of isolated cardiac preparations (16). Moreover, the observations that in control conditions detectable amounts of PAF are present within skeletal muscle and that treatment with a PAF receptor blocker increases contractile force, strongly suggest that PAF synthesized may modulate contractile properties of skeletal muscle also under basal conditions.

Role of NO as secondary mediator of TNF-α and PAF. Previous experiments indicate that in the heart, the negative inotropic effects of TNF-α and PAF depend on the generation of NO (1, 9, 11). Skeletal muscle cells express both the inducible NOS (iNOS) and constitutive NOS (cNOS) isoforms (8, 10, 14). The results of the present study indicate that TNF-α and PAF induce early production of NO. This evidence suggests an involvement of cNOS, rather than the iNOS, in NO production triggered by TNF-α and PAF. However, in pathological conditions such as septic shock, a persistent production of TNF-α and PAF occurs; therefore, it is possible that in these conditions, iNOS contributes to the NO generation. Indeed, in experimentally induced endotoxin septic shock, an activation of iNOS within skeletal muscle was observed in guinea pigs (10) and rats (8). Moreover, in the latter animal species, the induction of iNOS caused by infusion of endotoxin was accompanied by upregulation of both the endothelial and neuronal NOS (8). NO generation is critical in mediating the negative inotropic effect of TNF-α and PAF, because L-NAME, but not D-NAME, prevented the mechanical alterations triggered by these mediators. Several lines of evidence demonstrate that NO modulates skeletal muscle contraction. The study of the force-frequency relationship from different skeletal muscle types shows an inverse correlation between NO activity and force development (14). Moreover, treatment with NOS inhibitors enhanced skeletal muscle contractility (10, 14, 20) and reduced the decline of contractile force induced by endotoxin (8) or ischemia and reperfusion injury (23). NO-producing substances, such as SNAP (21) or sodium nitroprusside (14), induce frequency-dependent reduction of contractile force. The finding that both inhibitors of NO synthesis (10, 14) and a PAF receptor antagonist shift the force-frequency relationship and increase contractile force suggests that skeletal muscle in basal conditions produces both these mediators. Indeed, Balon and Nadler (2) reported that resting skeletal muscle releases significant amounts of NO. Our experiments support the hypothesis that TNF-α induces NO generation through the synthesis of PAF rather than directly. These results are in agreement with the finding that the angio-genic effect of TNF-α also depends on the production of PAF and on PAF-induced NO generation (18). Previous studies have shown that PAF contributes to the induction of NOS by bacterial lipopolysaccharides (LPS). Indeed, it has been shown that PAF receptor antagonists inhibit the induction of calcium-independent NOS in the lungs of rats treated with LPS, but does not interfere with the in vitro activity of the enzyme (27). These experiments suggest that the synthesis of PAF induced by LPS stimulates subsequent production of NO. Moreover, it has been shown that the vasoactive and hypotensive effects of PAF are dependent on NO generation (7, 27). In conclusion, the results of the present experiments indicate that TNF-α acutely impairs skeletal muscle contractility, as a result of NO production, which may be largely dependent on the synthesis of PAF.

Perspectives

The results of the present study indicate that TNF-α exerts a negative effect on skeletal muscle contractility, via generation of secondary mediators such as PAF and NO. In light of our knowledge that TNF-α is involved in several pathophysiological conditions, such as endotoxic/septic shock, uremia, and cardiac failure, which include symptoms related to muscle weakness, such studies can be designed to inhibit some of the biological effects of this cytokine by blocking the action of PAF and/or NO. Because it has been recognized that TNF-α also possesses beneficial properties, such as protection against infection, and TNF-α inhibition may be, in some cases, detrimental to the organism, it may be useful to investigate therapeutic strategies designed to interfere only with the negative effects of this cytokine. This could be achieved with greater knowledge of the secondary mediators involved in the different biological actions of TNF-α.

This study was supported by grants of the Ministero dell’Università e della Ricerca Scientifica, Istituto Nazionale per la Fisica della Materia, Consiglio Nazionale delle Ricerche (target project on Biotechnology) and Istituto Superiore di Sanità (Pathology, Clinic and Therapy of AIDS, Grant 30.B.10).

REFERENCES