Tumour necrosis factor- α , but not septic plasma depresses cardiac myofilament contraction

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Purpose: In sepsis, myocardial depression may be caused by mediators released as part of the inflammatory reaction. Tumour necrosis factor alpha (TNF α) is one mediator that may contribute to this depression. In the present study, we contrasted the effects of TNF α and septic plasma fraction (SP) obtained from an *E. coli* model on contractile tension in intact and skinned canine ventricular trabecular (VT) preparations. The objectives were to determine whether SP or TNF α could impair contractile tension at the level of the myofilaments, and to determine the extent to which TNF α may account for myocardial depression found in *E. coli* sepsis.

Methods: Measurements of isometric tensions were made after TNF α and SP (10,000 to 30,000 MW fraction) were added to respective intact or skinned canine VT preparations. In the skinned preparation, trabeculae were chemically skinned with Triton X-100.

Results: Septic plasma caused a decrease in contraction in the intact preparation compared with pre-septic plasma (50 ± 7 vs $33 \pm 7\%$, $P < 0.05$), but had no effect in the skinned preparation. On the other hand, TNF α ($30 \text{ ng}\cdot\text{ml}^{-1}$) caused an approximately 50% reduction in tension ($29 \pm 2 \text{ mg}$ vs $16 \pm 5 \text{ mg}$) in the skinned preparation ($P < 0.05$), but had no effect in the intact preparation.

Conclusion: These results suggest that TNF α and SP act through different mechanisms. While SP requires an intact membrane, TNF α impairs function by a direct effect on the myofilaments.

Objectif : Dans les états septiques, la dépression myocardique peut être causée par des médiateurs provenant de la réaction inflammatoire. Le facteur de nécrose tumorale alpha (FNT α) est un des médiateurs qui peut contribuer à cette dépression. Dans cette étude, nous avons comparé les effets de FNT α et de la fraction septique du plasma (SP), obtenus d'un modèle d'*E. Coli*, sur la tension contractile de préparations de muscle trabéculaire ventriculaire (VT) de chien, intact ou sans endothélium. Les buts étaient de déterminer si SP ou FNT α pouvaient altérer la tension contractile au niveau des myofilaments, et de déterminer la responsabilité du FNT α dans la dépression myocardique de la septicémie à *E. Coli*.

Méthodes : Des mesures de tension isométrique ont été réalisées après ajouts de FNT α et de SP (fractions de PM de 10,000 à 30,000) à des préparations de VT de chien, intact ou sans endothélium. Dans les préparations sans endothélium, ce dernier était éliminé des muscles par traitement au Triton X-100.

Résultats : Le plasma septique entraîne une dépression de la contraction de la préparation intacte par rapport au plasma présepticémie (50 ± 7 vs $33 \pm 7\%$, $P < 0,05$), mais n'a pas d'effet sur la préparation sans endothélium. D'autre part, le FNT α ($30 \text{ ng}\cdot\text{ml}^{-1}$) entraîne approximativement 50% de réduction sur la tension ($29 \pm 2 \text{ mg}$ vs $16 \pm 5 \text{ mg}$) de la préparation sans endothélium ($P < 0,05$), mais est sans effet sur la préparation intacte.

Conclusion : Ces résultats suggèrent que le FNT α et le SP agissent par des mécanismes différents. Alors que le SP requiert un endothélium intact, le FNT α altère la fonction par un effet direct sur les myofilaments.

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REVERSIBLE depression of left ventricular (LV) contractility has been demonstrated to be an important component of circulatory collapse in sepsis.^{1,2} Some have attributed this cardiac dysfunction to mediators released as part of the inflammatory reaction.²⁻⁷ Among the many substances released, tumour necrosis factor alpha (TNF α), a cytokine produced by monocytes and macrophages, has been acclaimed as a putative mediator which could lead to myocardial depression in sepsis.⁸⁻¹⁴

Although TNF α is often cited, it is likely that a number of other substances released during sepsis may also play an important role in causing myocardial depression. In a canine model of *Escherichia coli* sepsis, we previously showed that myocardial depression could be ascribed to a circulating substance of molecular weight (MW) between 10,000 to 30,000. We further showed that removal of this substance by continuous arteriovenous haemofiltration was associated with an improvement in cardiac function in sepsis.^{2,5} By protein separation techniques, this substance did not appear to be TNF α .¹⁵ In subsequent studies, Jha *et al.*⁵ examined the mechanism by which septic plasma (SP) caused myocardial depression. These results suggested that SP caused depression by interfering with the transport of calcium from the sarcoplasmic reticulum (SR) uptake-site to the release-site or in the SR release-site itself. However, Jha *et al.*⁵ also indicated that the SR changes caused by SP could be of secondary importance, while the primary defect may reside in a decrease in myofilament responsiveness to calcium.

Whether TNF α and SP are the same substance and whether either could cause cardiac depression at the level of the contractile apparatus are not clear. To examine these questions in the present study, we contrasted the acute effects of SP and TNF α on contractile tension in canine intact and skinned trabecular preparations. A depression in contractility in the intact trabecular preparation would indicate that an intact membrane system would be required for its mechanism of action. On the other hand, in the skinned preparation, the sarcolemmal, sarcoplasmic reticulum, and mitochondrial membranes are chemically disrupted.^{18,19} Depression in isometric tension in this preparation would indicate that the mechanism of action was related to a direct interaction with the myofilaments to inhibit contraction.

Methods

Intact right ventricular preparation

All of the procedures used in this study were approved by Central Animal Care Committee at the University of Manitoba. Our intact right ventricular (RV) trabecular

preparation has been previously described.^{2,5} Trabeculae are obtained from the RV rather than the left ventricle for the intact preparation and skinned preparation (see below) because it is possible to obtain thinner muscles from the RV, and also because trabeculae obtained from both ventricles were shown to produce essentially identical changes in sepsis.^{2,5}

The methodology for the intact preparation was as follows. Mongrel dogs (3 to 10 kg) were anaesthetised with pentobarbitone. Their hearts were removed and placed in ice-cold Krebs-Henseleit solution (KH). The coronary vessels were flushed with 50 ml of cold KH. Thin trabeculae (<1 mm diameter) obtained from the RV were tied at each end with 6-0 silk thread. Each thin muscle was suspended in a vertical constant temperature bath (5 ml) containing KH (in mM: NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.4; NaHCO₃, 25; and dextrose¹¹). The solution was bubbled with O₂ 95% and CO₂ 5% and maintained at 37°C. Isometric contractions at optimum length were recorded with a force transducer (Grass FTO3C) which was connected to a polygraph (Grass Model 7). The muscle was stimulated electrically via platinum bipolar field electrodes with rectangular pulses (1 msec duration) at an intensity of 100% above threshold delivered at intervals of 2000 msec.

Septic plasma isolation and protocol

E. coli sepsis was produced in six dogs (20–25 kg) as previously described.^{2,5} The animals were anaesthetised with 30 mg·kg⁻¹ pentobarbitone, the trachea intubated with an endotracheal tube, and the lungs ventilated at 15 ml·kg⁻¹ (Harvard Apparatus model 607, South Natick, MA) to maintain PCO₂ and pH at $\approx$ 35 mmHg and 7.35, respectively. Sepsis was induced by infusion of 10¹⁰ colony-forming units (CFU) of live *E. coli* (0111:B4) which were suspended in normal saline and given over one hour. Approximately 5 $\times$ 10⁹ CFU·hr⁻¹ of *E. coli* were then infused for three hours.

In each experiment, plasma samples (30 ml) were obtained before (*baseline*) and after four hours of sepsis (*sepsis*). The samples were sequentially passed through filters (Amicon) of different pore sizes to isolate the 10,000–30,000 dalton fraction.^{2,5,15} The effects of pre vs post sepsis plasma fractions (0.05 to 1 ml) on isometric tension were determined in separate trabeculae. The difference between pre and postsepsis values was used as an index of SP depressant activity.^{2,5} In nine other time-control animals, plasma samples were obtained at baseline and after four hours of sham sepsis in which the animals had been infused with normal saline.

After measurements were obtained in the intact RV preparation, the plasma samples were then stored in at

-70°C in a freezer. The fractions were then examined in the skinned muscle preparation (see further below) within the next two days, since depressant activity is not lost over this interval.^{2,5}

Tumour necrosis factor- α protocol

Tumour necrosis factor- α (Human recombinant, Sigma Corporation) was supplied as a sterile-filtered solution in 1 ml phosphate buffered saline which contained bovine serum albumin (BSA) 1% that was tested to be devoid of endotoxin. In the intact RV preparation, the effects of TNF α solution (range: 3 to 600 ng·ml⁻¹ TNF α) and equivalent concentrations of BSA alone on isometric contraction were examined in six experiments. Three trabeculae were obtained from the RV of each of the six dogs examined. Of the three trabeculae, one was used to determine the effect of the TNF α solution; the second to determine the effect of the BSA solution alone; and the third to examine the effect of time on the preparation in which KH solution was placed into the bath.

Additionally, in two of the six TNF α experiments, concentrations of ionised calcium were measured when either TNF α solution or BSA alone were added to their respective baths. The objective was to determine whether any changes in tension could be attributed to changes in the bath concentration of calcium.

Skinned RV trabecular experiments

Trabeculae $\approx$ 5 mm long and $\approx$ 300 μ m wide were excised from the right ventricle in a manner described for the intact experiments. Trabeculae were tied at each end with 8-0 silk thread (Matuda) and mounted horizontally between two stainless steel hooks. One hook was fixed to a metal stand, while the other was glued to an AE 801 force displacement transducer (Sensonor, Norway) which was mounted on a micromanipulator.¹⁹ The transducer signal was amplified and was recorded on a Fisher Recordall Series 5000 oscillograph (Fisher Scientific). The preload tension was adjusted to $\approx$ 15 mg, and all experiments were performed at 22°C.¹⁹

The trabeculae could be immersed in one of six small (1 ml) chambers. The chambers were milled on a plexi-glass plate that could be moved vertically as well as horizontally. After the trabecula was mounted on the hooks, it was immersed into a chamber which contained relaxing solution (see below) and Triton X-100 1% for 60 min.¹⁹⁻²¹ The disruption of the membranous system was verified by the ability of the muscle to contract in a low Ca solution which did not cause contraction with intact membranes. Relaxing solution consisted (in mM) of Mg 3, MgATP 3, phospho(enol)pyruvate 6, K 80, Na 30, imidazole 30, total EGTA 10, dithiothreitol (DTT) 9,

and pyruvate kinase 10 units ml⁻¹; imidazole and acetic acid were used to adjust the pH of solutions to 7.0.

Activating solutions were made by the provision of different concentrations of free calcium in the form of EGTA-calcium buffer to the relaxing solution. Free calcium concentrations were calculated based on the computer program of Fabiato and Fabiato¹⁶ and the binding constants used by Fabiato.¹⁷ Stock solutions of K₂EGTA and CaK₂EGTA were used to obtain a range of free calcium concentrations (pCa) = -log [Ca⁺²]. Activating solutions with pCa values = 7.08, 6.54, 6.11, 5.76, 5.38, 5.19 were used to obtain pCa-force relationships in the skinned preparation. In preliminary experiments, pCa-force relationships were obtained in which the entire range of calcium concentrations was used. No change in this relationship was observed for at least five hours, while experiments usually lasted for about three hours (see below).

Skinned muscle protocols

In both SP and TNF α studies, measurements were initially made without intervention (baseline). At baseline, active tensions were measured after exposure to at least three pCa values. The activating solution with the lowest concentration of free calcium (i.e., highest pCa) that caused contraction was first determined (termed: lowest calcium concentration), and this solution was usually in the range of pCa of 6 to 7. Tensions were then obtained in the presence of the next two to three higher calcium concentrations. A plateau in tension was reached by ten minutes.

In the TNF α experiments, after baseline measurements were obtained, three concentrations of TNF α were examined (experimental condition). The TNF α concentrations chosen were 3 ng·ml⁻¹, 30 ng·ml⁻¹, and 300 ng·ml⁻¹ which were comparable to those used in whole animal experiments.^{10,14} In a given experiment, activating solutions of identical pCa values were used during the baseline and experimental conditions. During the experimental condition, the skinned muscle was initially bathed in relaxing solution which contained 3 ng·ml⁻¹ of TNF α for a duration of 30 min. Tensions were then determined after 3 ng·ml⁻¹ of TNF α were added to each of the activating solutions in which exposure was for ten minutes. After measurements with 3 ng·ml⁻¹ were obtained, the concentration was increased to 30 ng·ml⁻¹. The skinned muscle was placed into relaxing solution containing 30 ng·ml⁻¹ TNF α for 30 min. Measurements were repeated at the same pCa values as those used for the lower TNF α concentration. In approximately one-half the experiments, the concentration of TNF α was increased to 300 ng·ml⁻¹. As an additional part of the TNF α protocol, the skinned trabecula was then washed free of TNF α for 30

min with relaxing solution. Measurements were then repeated without TNF α to determine whether the depressant effect of TNF α could be reversed by washing.

In separate experiments ($n = 4$), the effects of time and bovine serum albumin (BSA) on this preparation were examined in which the protocol was similar to that described in the TNF α study. After baseline measurements were determined, BSA 1% was added to the relaxing solutions. The concentration of BSA examined was equal to the highest value used in the TNF α experiments. Measurements were compared before and after BSA was added to the activating solutions.

In the SP skinned muscle experiments ($n = 6$), baseline measurements were obtained as described for the TNF α experiments. Then the skinned muscle was bathed in relaxing solution which contained pre-septic plasma fraction (100 μ l) for 30 min. This quantity represented the same relative concentration as that used in the intact preparation.^{2,5} The skinned muscle was then bathed in three activating solutions which contained 100 μ l of pre-septic plasma fraction for 10 min after which respective tensions were obtained. The muscle was then washed in relaxing solution for 30 min. It was then bathed for 30 min in relaxing solution which contained 100 μ l of post-sepsis plasma fraction. The sequence of steps was then repeated as for the pre-sepsis sample.

Skinned muscle analysis

In the skinned muscle experiments, the results were analysed in two ways. In one analysis, the absolute tensions obtained with the respective pCa were compared between baseline and experimental conditions. In about one-half of the experiments, moreover, as increasing amounts of calcium were added to the bath, a plateau in tension was attained which indicated that maximal tension was achieved. From the pCa *vs* tension curve, pCa₅₀ was defined as the calcium concentration that produced 50% of maximal tension.¹⁹ A decrease in pCa₅₀ would indicate a decrease in sensitivity of the myofilaments to calcium.¹⁹

Tumour necrosis factor- α Assay

The extent to which TNF α may be present in the septic plasma fraction to affect our results was also examined. Canine TNF α shows 92% homology with the human putative mature TNF α protein, and a monoclonal antibody against human TNF α cross-reacts with canine TNF α in Western blot and ELISA analyses.²² An ELISA analysis was used to assay for TNF α which is sensitive down to 10 pg·ml⁻¹ (Dupont Products, NEK-066, Boston, MA). Concentrations were compared pre and post-sepsis in three dogs.

Statistics

Two way analysis of variance for two repeated measures with missing values (within-within ANOVA, NWA Statpak, Portland, OR) was used to assess the effects of TNF α and SP on isometric tension in the intact ventricular preparation and also in the skinned muscle experiments when multiple pCa were compared. Within group analysis was determined by one-way repeated measures ANOVA. A Duncan's multiple comparison range test was used when multiple means were compared. Results are reported as mean $\pm$ 1SE.

Results

In the intact trabecular preparation, SP (Figure 1 upper panel) caused a reduction in contractile tension as compared to pre-septic plasma fraction. On the other hand, there were no changes in isometric tension in the time-control group when KH was placed into the bath (Figure 1 lower panel). In control dogs in which sham sepsis was produced, depressant activity was unchanged when baseline *vs* four hour plasma fractions were added to the preparation.

On the other hand, when BSA and TNF α were examined in the intact trabecular preparation, there were no differences in depressant activity found between the two substances. At 3 ng·ml⁻¹, 30 ng·ml⁻¹, 300 ng·ml⁻¹, or 600 ng·ml⁻¹ TNF α , the respective mean ($\pm$ SE) decreases in isometric tension (TNF α *vs* BSA) for an equivalent concentration of carrier solution were $2 \pm 1\%$ *vs* $-4 \pm 1\%$, $-10 \pm 2\%$ *vs* $-10 \pm 5\%$, $-15 \pm 7\%$ *vs* $-20 \pm 5\%$, and $-37 \pm 4\%$ *vs* $-36 \pm 3\%$ ($P = 0.31$ between groups).

Moreover, this reduction in contraction with TNF α and BSA was not due to an effect of time on the preparation because the time control group showed no change over the same interval (Figure 1: lower panel). However, in both the TNF α and BSA experiments, tissue bath calcium concentrations decreased slightly, from $1.98 \pm .007$ mM L⁻¹ to $1.71 \pm .001$ mM L⁻¹ with TNF α and from $1.98 \pm$ to $1.75 \pm .06$ mM L⁻¹ with BSA.

The results of a skinned muscle experiment in which TNF α was added to the bath is shown in Figure 2. The mean tensions obtained in the skinned muscle preparation are shown in Figure 3. The three highest pCa (termed lowest, middle, and high calcium concentrations) were compared among the different conditions, since at least three values could be obtained in each experiment. These three pCa values averaged 6.40 ± 0.10 , 6.05 ± 0.12 , and 5.79 ± 0.12 , respectively.

In Figure 3, at 3 ng·ml⁻¹ of TNF α , the percent decreases at the three calcium concentrations were 24%, 18%, and 38%, respectively; at 30 ng·ml⁻¹, the decreases in tension were 51.4%, 40%, and 24%, respectively. In the six experiments obtained with 300 ng·ml⁻¹ of

FIGURE 1 In the upper panel, the effects (mean $\pm$ SE; $n = 6$) of pre and postsepsis plasma fractions (10,000 to 30,000 Da) on isometric tension found in the intact RV preparation are shown. Septic plasma (SP) caused a decrease in active tension compared with that observed with the presepsis plasma fraction. $*P = 0.0225$, by two way ANOVA with repeated measures.

In the lower panel, there were no effects of plasma fractions observed in the sham sepsis group or in the time-control group in which Krebs-Henseleit was placed into the trabecular bath.

TABLE I Effect of TNF α on tension and myofilament sensitivity to calcium (pCa_{50} ; mean $\pm$ SE).

	Tension (mg)	pCa_{50}
Baseline ($n = 6$)	13.7 ± 1.7	$6.23 \pm .23$
3 ng·ml $^{-1}$ ($n = 6$)	$6.8 \pm 1.4^*$	$6.46 \pm .22$
30 ng·ml $^{-1}$ ($n = 5$)	$5.8 \pm 1.6^*$	$6.22 \pm .30$

$*P < 0.01$ vs baseline by repeated measures ANOVA and Duncan's multiple comparison test.

pCa_{50} were not different between conditions ($P = 0.31$).

TABLE II Effect of septic plasma on isometric tension in skinned trabeculae (mean $\pm$ SE; $n = 6$).

pCa	Tension (mg)		
	Baseline	Presepsis plasma	Postsepsis plasma
6.0 ± 0.6	5.3 ± 1.2	7.0 ± 1.2	6.5 ± 1
$5.79 \pm .08$	11.3 ± 3.0	10 ± 1.7	10.6 ± 2
$5.4 \pm .08$	19.8 ± 7.3	20.1 ± 8.5	22.2 ± 9.6

Tensions were not different between conditions ($P = 0.97$).

FIGURE 2 pCa vs active tension relationship obtained with different concentrations of TNF α in one experiment. Compared with baseline values, tensions were reduced in the presence of TNF α . pCa_{50} , defined as the calcium concentration which caused one-half maximal contraction, was unchanged with TNF α (see Results).

TNF α , the decreases in tension measured at the three calcium concentrations averaged 9%, 28%, 49%, respectively compared with baseline values ($P < .05$). There was a nonsignificant increase in tension when the muscles were washed for 30 min (see Figure 3).

The changes in tension shown in Figure 3 were not due to the effects of time or BSA. In time control BSA experiments, at the lowest calcium concentration (mean $\pm$ SE: $pCa = 6.2 \pm 0.29$), baseline tension averaged 5.8 ± 2 mg vs 5.8 ± 2.2 mg with BSA; at the middle calcium concentration ($pCa = 5.9 \pm 0.22$), baseline tension averaged 7.2 ± 1.25 mg vs 13.4 ± 4.3 mg with BSA; and at the highest calcium concentration ($pCa = 5.5 \pm 0.14$) baseline tension averaged 31.5 ± 8.5 vs 34.5 ± 9.1 mg with BSA ($P = 0.53$ between groups).

In the skinned muscle experiments, pCa_{50} was also used as an index of myofilament sensitivity to calcium.¹⁹ In those experiments, maximal tensions measured with TNF α decreased compared with the baseline value, but pCa_{50} did not change (see Table I).

In contrast to the TNF α experiments, tension did not change when SP was added to the skinned preparation at the different pCa examined (see Table II).

Concentrations of TNF α were also measured in pre and postsepsis plasma samples to determine whether concentrations were increased at the four hour period. In the postsepsis plasma fraction, there was no increase in TNF α observed compared with the pre-sepsis value. Mean TNF α found in presepsis plasma fraction averaged 13.3 ± 11 pg·ml $^{-1}$ vs 8 ± 6 pg·ml $^{-1}$ in the postsepsis plasma fraction ($P = 0.426$).

FIGURE 3 Skinned trabecular preparation showing the effects of different concentrations of TNF α . Active tensions (mean $\pm$ SE) are shown for three calcium concentrations examined in which identical concentrations were used in the baseline and experimental conditions. Measurements were obtained at baseline ($n = 12$), after TNF α 3 ng·ml $^{-1}$ ($n = 12$) and TNF α 30 ng·ml $^{-1}$ ($n = 11$) were added to the activating solution, and after the TNF α was removed from the activating solution ($n = 8$) [n = number of experiments performed in which one skinned trabecula was obtained from each heart examined]. Tensions obtained at 3 ng·ml $^{-1}$ and 30 ng·ml $^{-1}$ TNF α were different from baseline value * $P < 0.01$ by two way ANOVA for repeated measures and Duncan's test.

Discussion

In the intact ventricular trabecular experiments, the results showed that SP caused a reduction in isometric tension as compared with the presepsis fraction (see Figure 1). These findings are similar to those described in previous studies.^{2,5} In the postsepsis sample, the increase in depressant activity found postsepsis appears to be caused by a protein since activity is heat labile, is found in the acetone-insoluble fraction of the plasma, and is eliminated by incubation with the proteolytic enzyme-proteinase K.⁵ On the other hand, in the pre-sepsis sample, the background depressant activity (BDS) observed is heat stable, is not abolished by proteinase K, and appears to be a lipid moiety.⁵ Thus, the increase in depressant activity found postsepsis does not represent a doubling of BDS activity, but it appears to be due to the formation of a different substance that develops over the four hour course of the study.^{2,5}

In contrast to SP, there was no effect of TNF α on isometric tension in the intact preparation. Both TNF α and BSA caused a reduction in tension to the same extent, and the mechanism appeared to be due to a decrease in trabecular calcium concentration. Such an effect would occur in the TNF α experiments because albumin is available to bind with the calcium ion in the bath. On the other hand, in the SP experiments, albumin was removed by the filtration process. In a previous study, we showed that trabecular calcium concentration did not decrease when postsepsis plasma was added to this preparation.²

The lack of an acute effect of TNF α in the intact ventricular preparation in the present study is at variance with some studies,^{9,13} but in agreement with others.^{10,23} Some investigators^{9,13} showed that TNF α caused acute myocardial depression (within two to 20 min) in isolated hamster papillary and adult heart preparations, respectively. In contrast, *in vivo* canine preparations,^{10,23} TNF α has shown little,²⁴ if any,^{10,23} depression over a five to six hour period. Although the explanation for the different findings is not clear, our results indicate that acute exposure to TNF α does not result in a reduction in isometric tension in this intact preparation.

The effects of TNF α and SP were also different in the skinned muscle preparation. Whereas septic plasma produced no change in tension, TNF α caused an impairment in myocardial function at the level of the contractile apparatus. The fact that maximal tension was reduced with TNF α , but pCa₅₀ was unchanged indicates that the TNF α molecule may occupy binding sites on the myofilament preventing actin-myosin interaction, while there is no decrease in sensitivity of the myofilaments to calcium.¹⁹

Our results suggest there is a dose response effect of TNF α in the skinned preparation. In SP, significant quantities of unbound TNF α were not found, and TNF α averaged 8 pg·ml⁻¹ which is close to the value found in normal subjects (mean $\pm$ SD: 3.1 $\pm$ 1.2 pg·ml⁻¹).¹¹ In SP, it is possible that TNF α was not filtered because it circulates in the form of trimer of $\approx$ 55,000 molecular weight.²⁵ Alternatively, TNF α appears in the circulation within minutes following endotoxin injection, and then rapidly declines in concentration, since the half-life of TNF α is approximately six minutes.^{26,27} Thus, in subjects with acute hypotension in sepsis, Pinsky *et al.*¹¹ found that mean ($\pm$ SD) TNF α averaged 273 $\pm$ 648 pg·ml⁻¹ which is close to the 3 ng·ml⁻¹ value that caused myofilament depression in the present study. However, after 12 and 48 hr, Pinsky *et al.*¹¹ found that TNF α declined to 60 $\pm$ 76 and 30 $\pm$ 39 pg·ml⁻¹, respectively.

Our results indicate that TNF α caused an acute reduction in tension in the skinned, but not in the intact preparation. Then, how do we reconcile the present findings with other studies that indicate that depressant effects following TNF α infusion, particularly in canine preparations^{10,23} may take many hours to be achieved? As previously indicated, the mechanism by which TNF α causes depression in contractility is controversial. TNF α may cause depression by induction of a myocardial calcium-independent NO synthase¹² which would increase cyclic GMP and may therefore impair calcium entry into the cell.²⁸⁻³² In addition, TNF α may initiate the release of other cardiodepressant mediators, such as the interleukins.^{7,9,20,24,33} For instance, the depressant activity found in SP that develops over four hours may be related to TNF α mediated cytokine release in this model.

Alternatively, it is well known that TNF α binds to specific cell surface receptors and is rapidly internalised.^{34,35} This occurs by receptor mediated endocytosis, after which intracellular metabolism of TNF α is observed. Selective formation of TNF α degradation products may contribute to TNF α mediated cytotoxicity.^{35,36} Accordingly, our results suggest that once TNF α binds to cell surface receptors and is internalised, it is likely that there is interaction with TNF α at the level of the myofilament which interferes with contraction. Since this internalisation process may take two to five hours to occur,³⁵ an acute depression in tension with TNF α would not be observed in the intact trabecular preparation, but would be observed in the skinned preparation.

In this study, we found that SP caused a decrease in isometric tension in the intact, but not in the skinned preparation. The mechanism of action of SP probably

involves a step in the excitation-contraction process involving the translocation of calcium from the uptake site to the release site in the sarcoplasmic reticulum.⁵ We have ruled out direct inhibition of the contractile apparatus by SP, whereas a depressant-effect of TNF α could be demonstrated. We would agree that many mediators may be released in sepsis and that each may cause myocardial depression in different ways. These results suggest that our previously described filterable cardiodepressant substance in sepsis and TNF α are different substances,^{2,5} and that the former requires an intact membrane system for its action.

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